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ULTRASTRUCTURAL STUDY ON THE ALVEOLAR-CAPILLARY INJURY WITH PULMONARY EDEMA INDUCED BY OLEIC ACID IN DOGS*)

By

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ABSTRACT

Oleic acid induced pulmonary edema was subjected as an experimental model of ARDS. Three adult mongrel dogs were injected with 0.09 ml/kg of oleic acid into the pulmonary artery and sacrificed 2 hours later. Light and electron microscopic studies were carried out on the pulmonary specimen. The light microscopic findings showed bronchovascular cuffs and alveolar flooding. The electron microscopic findings of the mildly to moderately damaged lesions showed that the interstitial tissues were thickened with edema fluid and alveolar spaces were filled with plasma exudate containing erythrocytes, strands of fibrin, macrophages, and electron dense material that surfactant and lamellar bodies resembled, while the capillary endothelium and alveolar epithelium appeared normal. The electron microscopic findings of severely damaged lesions showed that both endothelium and epithelium were degenerative.

It was concluded that ultrastructural changes were more characteristic of permeability than of hemodynamic edema.

INTRODUCTION

The Adult Respiratory Distress Syndrome (ARDS) may be defined as a clinical and pathophysiologic condition in which there is a severe impairment of gas exchange at the alveolar level. Numerous different etiologic factors are known to trigger the disesse. Regardless of the etiology, however, the underlying alteration of the lung structure shows a rather uniform pattern. The syndrome may be initiated by a sudden respiratory failure due to a fulminant pulmonary edema of noncardiac origin, paticularly on the structural alteration taking place in the alveolar-capillary barrier that demonstrates increased permeability[1].

Typical experimental examples of permeability edema are those induced by alphanaphtyl thiourea in rats[2], alloxan and oliec acid in dogs[3,4], and pseudomonas bacteremia in sheep[5]. Pulmonary edema induced by administration of oleic acid has proved to be a useful model

*) 宮沢輝臣，中川仁，平本光英，有田健一，平本雄彦，西田修実：イヌオイレン酸肺水腫における肺胞-毛細血管障害の超微形態学的観察

for studying the ARDS[4,6,7].

Our experiment was designed to study the ultrastructural alteration of the alveolar-capillary injury associated with pulmonary edema induced by oleic acid in dogs.

METHOD

Three adult mongrel dogs (mean weight 14± 0.7 kg) were injected with 0.09 ml/kg of oleic acid into the pulmonary artery and sacrificed 2 hours later. Tissues for electron microscopy were fixed in 2.5% glutardehyde solution in 2.0%OsO_4 for 2 hours. They were subsequently dehydrated in graded ethanols and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate, and were observed under a Hitati H-500 electron microscope.

RESULTS

The clinical course was remarkably consistent. Rapid shallow breathing occurred immediately after injection of oleic acid. Inspiratory and expiratory efforts increased with the passage of time and a grunting type of expiration occurred. The tongue became markedly cyanotic.

A) Gross aspects

At autopsy, the lung were edematous, weighting 2 to 3 times their normal weight. The surfaces were covered with blotchy hemorrhages. The trachea and main bronchi usually contained moderate to large amount of bloody froth.

B) Light microscopy

The light microscopic findings showed striking perivascular cuff. Edema fluid are filled with the alveoli (Fig. 1).

C) Electron microscopy

1) The alveolar space was filled with plasma exudate containing erythrocytes. Endothelial and epithelial cells were intact. Capillary lumen contained polymorphonuclear leukocytes. The vacuolation of lamellar inclusion bodies was found in type II epithelial cells (arrow) (Fig. 2).

2) Electron dense material of which surfactant and lamellar bodies resembled within the alveoli was seen (arrow) (Fig. 3).

3) The interstitium of the alveolar-capillary membrane was thickened by accumulation of edema fluid, and collagen fibers were spread apart by fluid. (*) Intra-alveolar fibrin was observed (arrow) (Fig. 4).

4) The alveolar space was filled with plasma exudate containing erythrocytes, strands of fi-

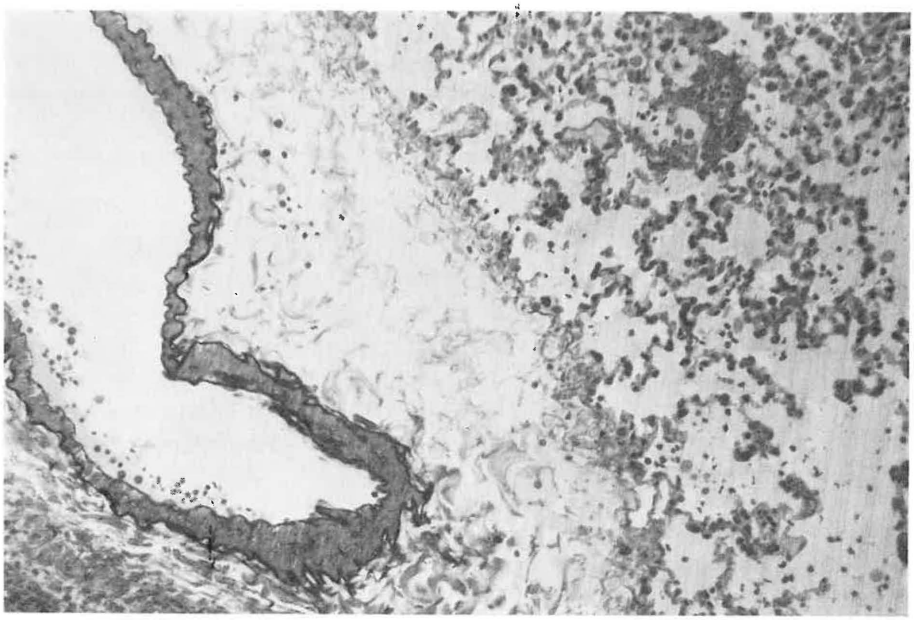

Fig. 1. The light microscopic finding showed striking perivascular cuff and intra-alveolar edema. (×200)

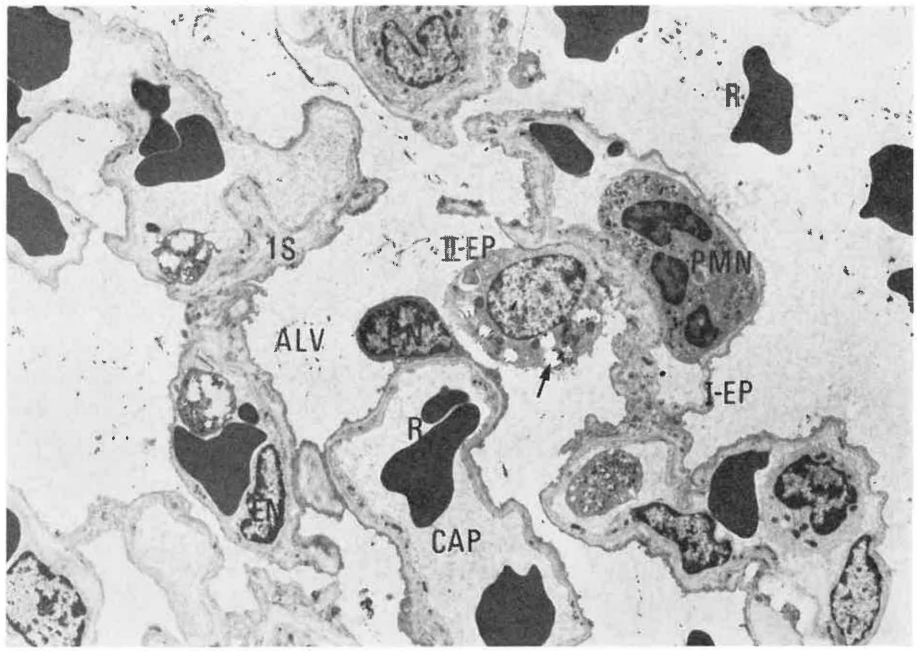

Fig. 2. capillary (CAP) alveolar space (ALV) endothelium (EN) interstitium (IS) type I epithelium (I-EP) erythrocyte (R) polymorphonuclear leukocyte (PMN) vacuolation of lamellar inclusion bodies (arrow) (×2000)

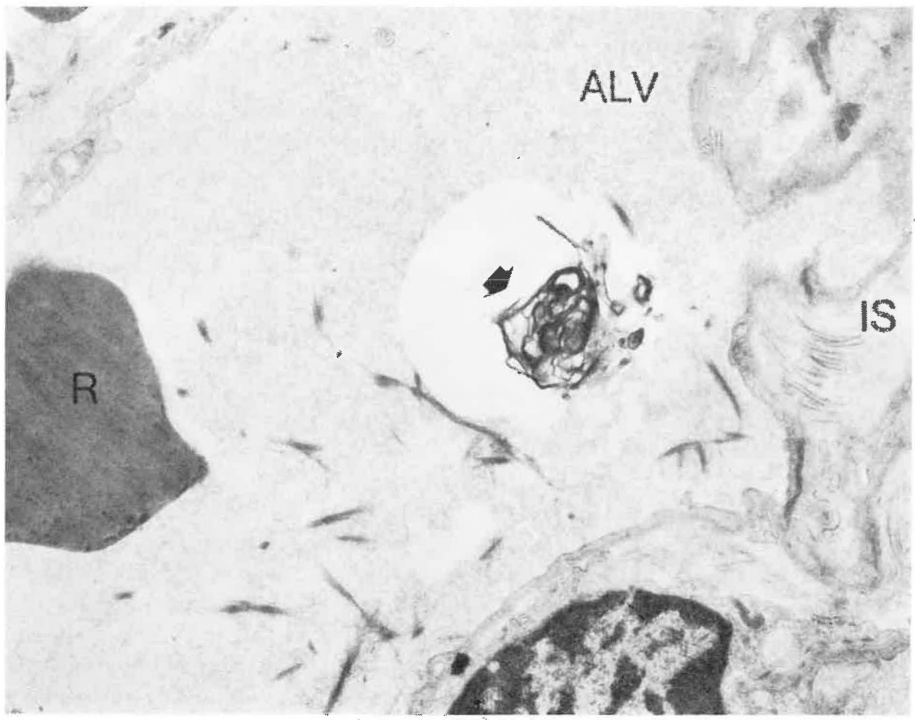

Fig. 3. electron dense material reminiscent of surfactant (arrow) alveolar space (ALV) interstitium (IS) erythrocyte (R) (×6000)

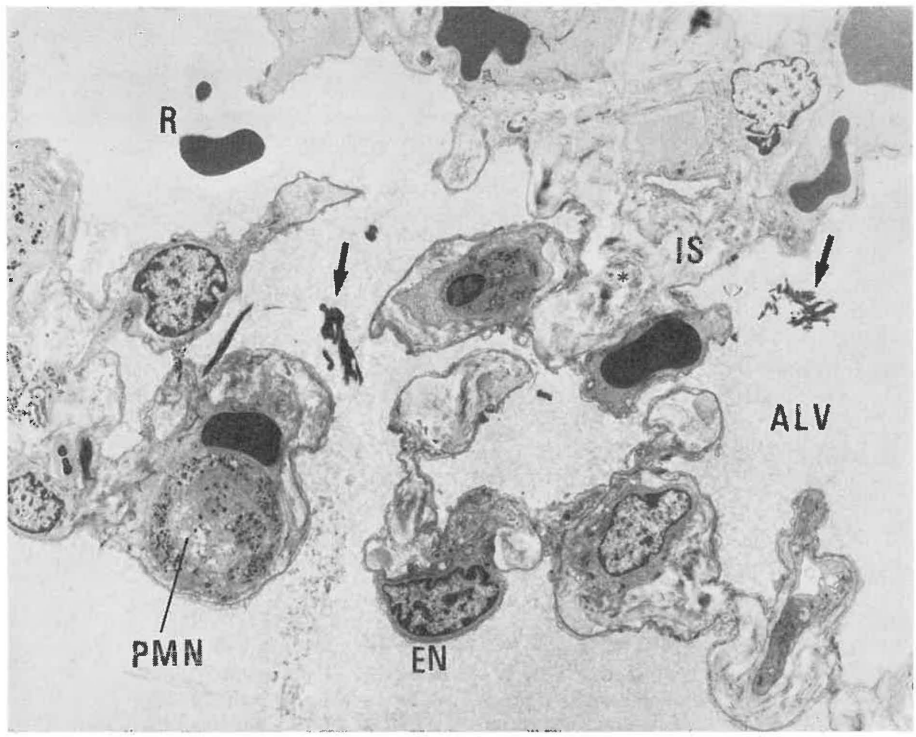

Fig. 4. swelling and widening of the interstitium (*)
fibrin (arrow) alveolar space (ALV)
endothelium (EN) erythrocyte (R)
polymorphonuclear leukocyte (PMN) (×2000)

brin and macrophages. Both endothelium and epithelium were not markedly degenerated (Fig. 5).

5) Fatty degeneration was found in type II epithelial cell (arrow) (Fig. 6).

6) Type I epithelial cell was swollen (arrow) (Fig. 7).

7) The electron microscopic findings of severely damaged lesion showed that the endothelium was degenerative and the epithelium was desquamated. The capillary was filled with fat-plasma complex (arrow) and erythrocytes (Fig. 8).

DISCUSSION

Ashbaugh and Uzawa[4] described that administration of oleic acid in dogs caused a clinical, physiological and pathological illness which was closely similar to ARDS.

When oleic acid lodges in the pulmonary vessels, it damages the capillary-alveolar cells by direct toxicity and causes an increase in cell permeability[8]. Hamilton et al.[9] suggested that oleic acid inhibited surfactant activity.

1) Endothelial cell injury

The early ultrastructural investigations by Schultz and others[10], showed severe microvascular endothelial and alveolar epithelial damages in permeability edema characterized by cell swelling, vacuolization, bleb formation, and general membrane dissolution. The recent studies of Cottrell and his associates[11] seemed to support this report, although others have not found such wholesale destruction. These latter reports are more in tone with clinical experience wherein permeability edema may be a limited and reversible process. Hovig et al.[12] produced acute and massive permeability edema in isolated, perfused rabbit lungs, but by electron microscopy they could not find any obvious structural changes in the microvascular endothelium, including the intercellular junctions. Meyrick et al.[2] confirmed their results.

Staub et al.[13,14] discovered that only modest loosing of intercellular junctions was required, when they used their multiple equivalent pore

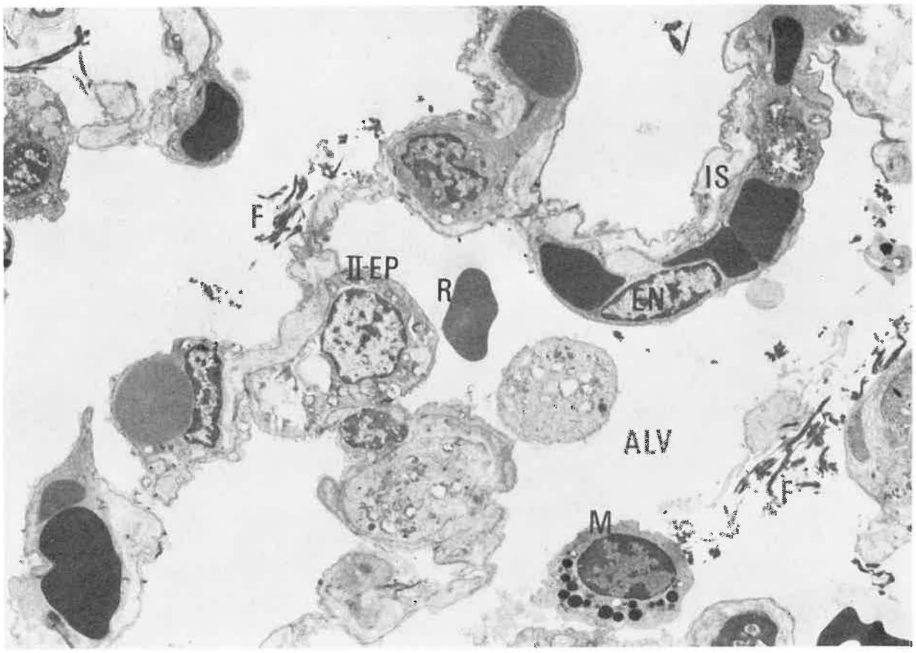

Fig. 5. abundant fibrin- and cell-rich alveolar exudate alveolar space (ALV) erythrocyte (R)
fibrin strands (F) alveolar macrophage (M) endothelium (EN) typeII epithelium (II-EP) interstitium (IS) (×2000)

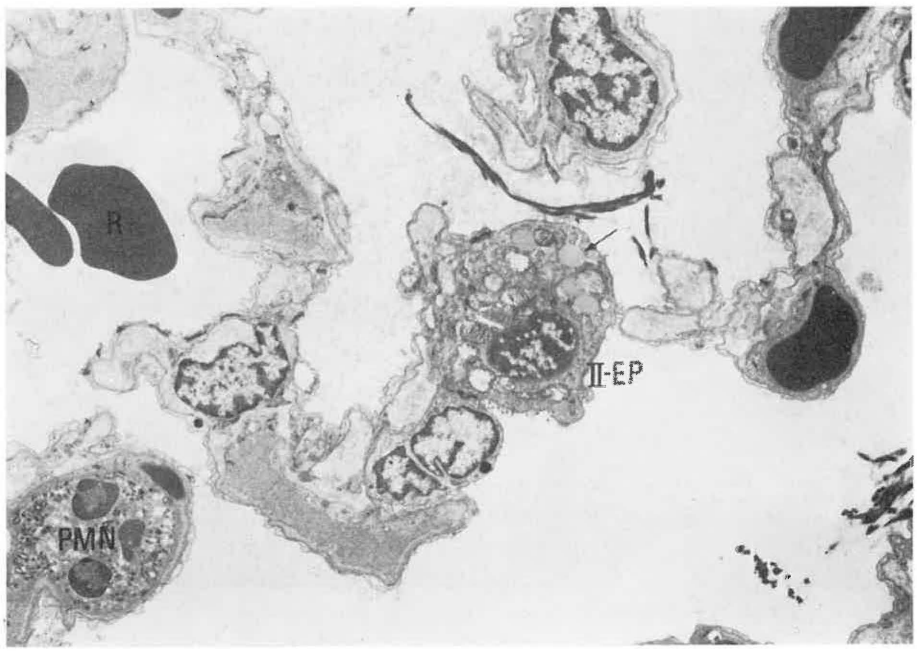

Fig. 6. fatty degeneration (arrow) of type II epithelium (II-EP) erythrocyte (E) polymorphonuclear leukocyte (PMN) (×3000)

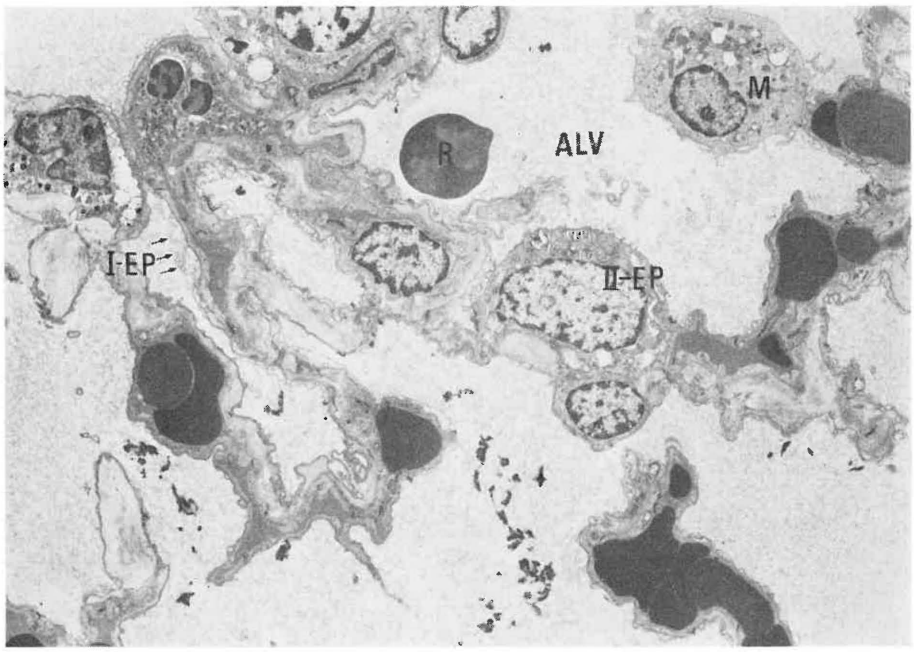

Fig. 7. marked swelling of type I epithelium (arrows)
alveolar space (ALV) erythrocyte (R)
type II epithelium (II-EP) (×2000)

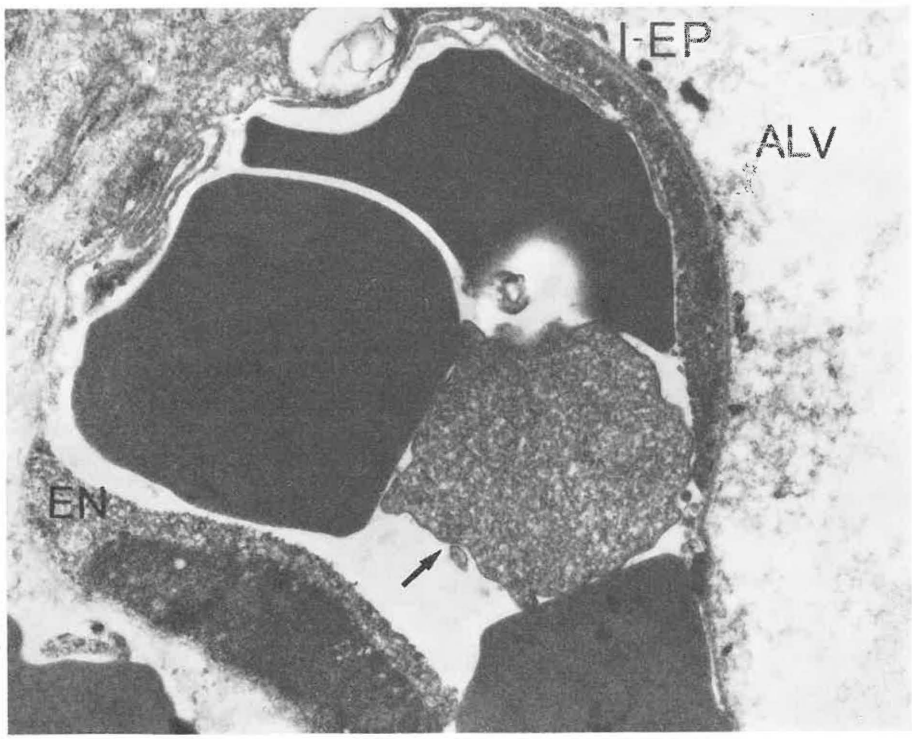

Fig. 8. fat-plasma complex (arrow)
degenerative changes of endothelium (EN) and type I epithelium (I-EP)
alveolar space (ALV) (×8000)

model to explain the animals' condition during the steady state period of maximum increased microvascular permeability. In their opinion, the predicted changes in small and intermediate pore sizes would be difficult to detect even by quantitative electron microscopy. Therefore the old notion that permeability injury was due to wholesale destruction of endothelium turned out to be incorrect.

In our study, the capillary endothelium was degenerative in the most severely damaged lesion, while the capillary endothelium appeared normal in the mildly to moderatly damaged lesions.

Teplitz[15] showed that ultrastructural distinctions could be made between hemodynamic and permeability types of pulmonary edema in man by cellular changes. In permeability edema, endothelial cells were focally damaged, whereas in hemodynamic edema, cellular changes were uncommon.

2) Interstitial fluid cuff

In the electron-microscopic appearance of pulmonary interstitial edema, the connective tissue space between capillary endothelium and alveolar lining epithelium was widened. In contrast, the thin segment of the blood-air barrier, with its attenuated endothelial and epithelial cellular layers fused by a common basement membrane remained thin.

Weibel and co-workers[16] suggested that the design of the alveolar septum displayed a number of features that appeared to favor the maintenance of a dry air-blood barrier in the interest of efficient gas exchange. They explained that the septa had an extensive system of drip channels that lead directly into interstitial fluid sumps: the free interstitial space of the septum surrounded the septal connective tissue fiber strands; because these were anchored in the peripheral and axial coarse-fiber systems. This drip-channel system was apparently most efficient in draining any excess interstitial fluid toward the sumps. In most cases of interstitial edema the excess fluid was first observed to accumulate in the sumps, appearing as widened cuffs around small bronchioles and vessels, whereas the septal interstitial spaces remained dry at first.

In our study, striking bronchovascular cuffs, swelling and widenings of the intraalveolar septal connective tissue spaces without alveolar flood were seen in the mildly damaged lesion. In both types of edema (permeability, hemodynamic) interstitial swelling was prominent and affected predominantly the thick portion.

3) Alveolar flood

Both physiologically and by ultrastructural analysis, the alveolar epithelium was a severe barrier not only to macromolecules but even to crystalloids[17-19].

Hayward[20] suggested that in pulmonary edema the alveolar epithelium somehow lost its barrier function.

Although the phenomenon was well-documented, the mechanism by which tight epithelial junction suddenly gave a way and permitted free access of interstitial edema fluid and protein to the alveolar space in controversial. No one has actually seen the pathway by which the fluid flows.

In permeability edema, Staub[14,21] suggested that both alveolar and airway epithelial intercellular junctions might be disrupted and allowed alveolar flooding to proceed.

In our study, the epithelium was very swollen and focally desquamated in the severely damaged lesion. The vacuolation or the disappearance of lamellar inclusion bodies was found

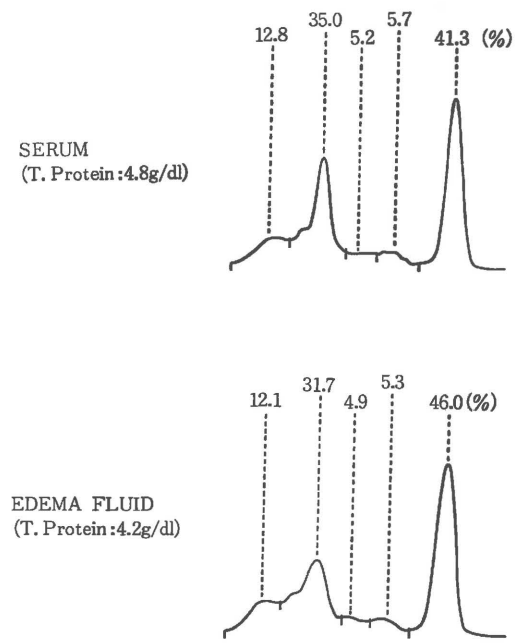

Fig. 9. The serum and edema fluid, collected from a dog with oleic acid-induced pulmonary edema, showed almost identical electrophoretic patterns.

in alveolar type II epithelial cells. These changes of lamellar inclusion bodies may lead to decrease the surfactant activities on alveolar walls. The alveolar space was filled with plasma exudate containing erythrocytes, strands of fibrin and macrophages.

Teplitz[15] showed that the presence of intra-alveolar fibrin was more characteristic of permeability than of hemodynamic edema.

The serum and edema fluid, collected from a dog with oleic acid-induced pulmonary edema, showed almost identical electrophoretic patterns (Fig. 9). It was therefore inferred that pulmonary vascular permeabilities not only to fluid but also to proteins might be increased in this experimental pulmonary edema.

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