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Studies on acute lung lymphatic edema

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The outflow from lymphatic vessels running along the right cardinal bronchus was interrupted in six dogs by the removal of the corresponding hilar lymph nodes. After 6—8 days the right lung showed slight subpleural areas of atelectasis and edema. Edema of endothelial cells of the lung septal capilaries was observed electron-microscopically. This lesion is similar to the findings in cases of mitral stenosis.

Low (7) was the first to use electron microscopy in studies of the lung. The application of this method opened a new stage for investigations in the field of pathology of the interalveolar septum. Increased vascular permeability is known to be one of the most frequent reactions of the "blood-air barrier" to a variety of stimuli (10). It is related to the disturbed lymphatic drainage of the lung (2). Electron microscopy, used in studies of conditions accompanied by lung edema, revealed various types of damage of the structures of the interalveolar septum. Despite a certain similarity these conditions differ. In the case of administration of oxygen in high concentration, it was the vascular endothelium which developed the most striking lesions up to necrosis (6). The interspace seems to be the first zone of fluid accumulation in the case of hemodynamic lung edema. In turn, the edema resulting from alloxan intoxication involves both the endothelium and alveolar epithelium (3). Because of these differences it was our purpose to study the interalveolar septum in the course of acute lymphatic edema and to compare its morphology with that found in other conditions with pulmonary edema.

Material and method

The studies were carried out with 11 mongrel dogs, weighing 11—20 kg. Six experimental animals were operated under general Eunarcon anesthesia 0.4 ml/kg. An intubation probe was introduced into the trachea to ensure adequate ventilation. After checking that the spontaneous respiration was unimpeded the dog was stretched on its left side, and the chest was opened on the right side. When pneumothorax occurred controlled respiration was secured manually with an Ambu set. Positive inspiration pressure was maintained within the limits of 15—20 cm H_2O. In this way the respiratory volume was from 10 to 20 ml/kg of body weight. After exposure of the right main bronchus all tissues with the lymphatic vessels were ligated and cut. All lymph nodes visible in the hilus and below the tracheal bifurcation were ligated and removed. Attention was paid to avoid the narrowing of blood vessels. Finally, the lymphatic vessels on the bronchial external surface were compressed by a loose silk ligature. The lung was decompressed and the chest closed with a suture. Penicillin (1200,000 u) was administered into the wound. The effect of anesthesia gradually diminished towards the end of the procedure. The intubation probe was removed after re-appearance of defense reflexes. The left lung served as control.

In five dogs which constituted the control group thoracotomy was performed as described above. After opening of the chest the lung was compressed with towels, and the hilar area with sterile gauze plugs for 90 minutes (mean period of experiment), the thorax was closed thereafter.

All dogs were killed on the 6th — 8th day after surgery by rapid, intravenous injection of concentrated potassium chloride solution. Immediately after the arrest of circulation and respiration the chest was opened and the position of the lung determined in situ. Thereafter, the lungs were distended through the trachea by means of the Ambu sac, all organs of the thorax were removed in block and the hilar blood vessels were ligated.

For histological studies the lungs were fixed in toto in neutral formaline buffered with phosphate buffer (pH 7.2). Twenty-four hours later specimens were taken from all lung segments. They were embedded in paraffin, and 2—3 μm thick sections were stained with hematoxylin and eosin, Azan, Astra blue at pH 0.25, and silver-impregnated according to Gomori.

The specimens for electron microscopy were taken immediately after decompression of the lungs from the areas which seemed grossly unchanged as well as from those which appeared to be abnormal. A part of the specimens (derived from 4 animals subjected to blockade of lymphatic vessels) were transferred to the ruthenium red solution and the Luft reaction was carried out (8). Other specimens were fixed in 3.6% glutaraldehyde in cacodil buffer for two hours, and then transferred to 1% osmic acid for one hour. All specimens were dehydrated in a rising gradient of ethyl alcohol and acetone, and embedded in Epon. Blocks were cut on a Reichert type II microtome, stained with lead citrate and uranyl acetate, and examined in a JEM-7 electron microscope.

Results

After opening of the chest the lungs of all animals slightly collapsed. In the group of dogs with ipsilateral blockade of peribronchial tissues of the right bronchus, the lateral lung surface showed dense, airless, flat subpleural areas of the diameter from 1×1 to 5×5 cm, up to 1.5 cm thick. They showed rather distinct network of connective tissue delineating particular lung lobules. Other segments of the parenchyma of the right and left lung, as well as the lungs of control animals, failed to show any morphological lesions, either at the macroscopic

or electron-microscopic level. There was no passive lung hyperemia. In lungs subjected to blockade some perivascular and peribronchial spaces seemed to be slightly dilated.

Light-microscopic examination of changed fragments revealed atelectasis, mainly of peripheral lung alveoli (Fig. 1), alveolar ductules and respiratory bronchioles remaining partly aerated. In the poorly aerated parts a small amount of edema fluid was present on the epithelium surface, there was, however, no advanced edema. In some segments of partly atelectatic areas the alveolar epithelium was cuboidal (Astra blue staining was negative). Subpleurally, dilated fluid-filled spaces were visible in the interlobular spaces (Fig. 2). In perivascular and peribronchial spaces clefts in connective tissue filled with protein-rich fluid were present sometimes at various levels. This phenomenon was, however, infrequent.

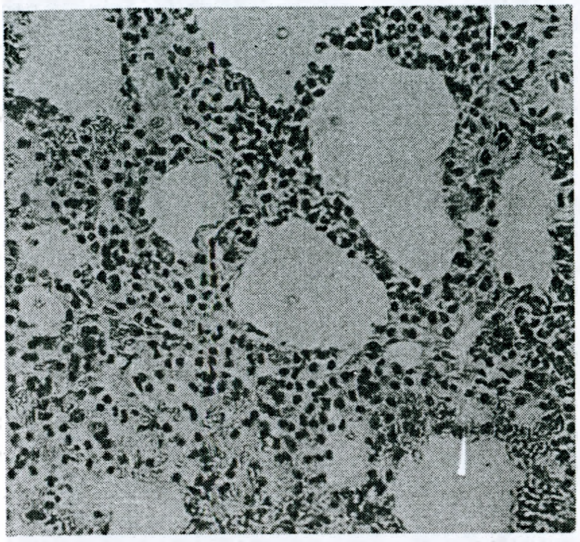

Fig. 1. Blockade of lymphatic vessels. Lung atelectasis. H. E., magn × 300.

Fig. 2. Dilated subpleural space. H. E., magn. × 250.

Electron microscopy of changed fragments confirmed the presence of markedly advanced atelectasis (Figs 3, 4, 5). A slight amount of fluid of rather high electron density was present on the surface of respiratory epithelium, on the contact surface with respired air (alveolar ductules and respiratory bronchioles). Some destroyed osmophilic as well as Ruthenium red-bound fragments were floating on the surface of the former (R, Figs 3, 6). Edema fluid was observed at all levels of the "blood-air" barrier. It was particularly pronounced in the endothelium. The cytoplasm of endothelial cells was transparent and comprised vacuoles of variable diameter (up to 600 nm and more), bulging into the capillary lumen. Sometimes the endothelial cells were swollen to such an extent that the plasmatic rim around the red blood cell was hardly noticeable (Fig. 4—7). The zona occluda was rather distinct, but unchanged. The endothelial cells were not detached from the basement membrane.

The basement membranes were unchanged except for a slight thickening and clear areas in places where they adhered to one another what seemed to correspond to fluid accumulation. If present in the interspace, the collagenous fibers were "dispersed" owing to accumulation of fluid of low electron density between them. The type I epithelial cells also showed cytoplasmic clarifications,

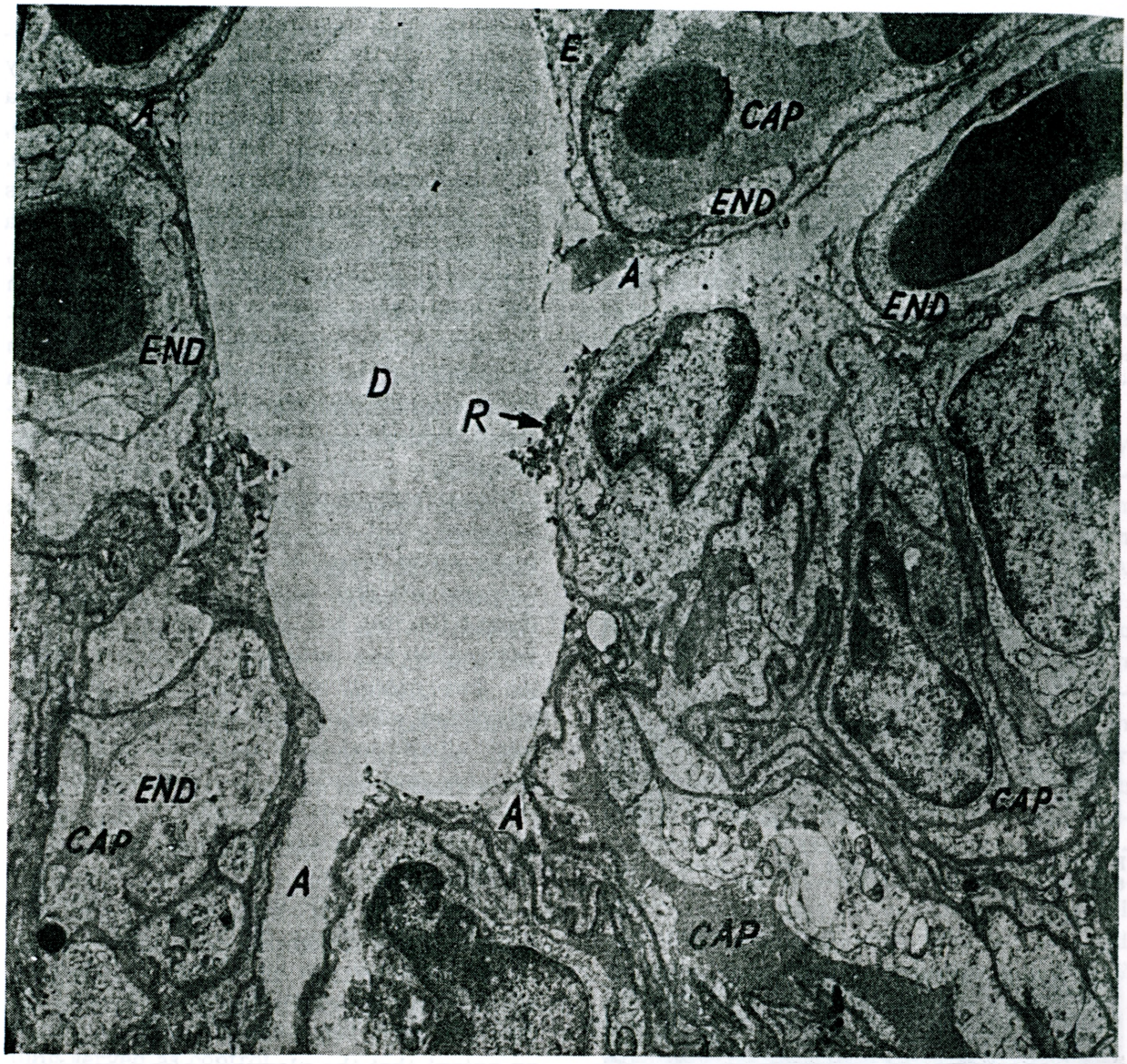

Fig. 3. Electron microscopy of pulmonary lesions. A — atelectasis, D — aerated space, END — swollen endothelial cell with clarified cytoplasm. CAP — capillary lumen, R — deposit on the surface of respiratory epithelium. Ruthenium red. Magn. × 5,400.

but not as pronounced as the endothelial ones. The alveolar epithelium did not desquamate from the basement membrane. The type II (granular) cells gave two types of reaction. In those rich in lamellar structures disappearance of osmophilic deposit was observed, and the reaction with Ruthenium red was of relatively low intensity. The others exhibited proliferation (Fig. 8). The proliferating cells adhered to one another, and were not edematous. Their lamellar structures were only scanty, the same was true of the osmophilic or Ruthenium red-positive material. The cell membrane facing the alveolar lumen was poorly developed, without microvilli. Single desquamated type II cells without deposit in the lamellar structures were present in the lumen of aerated ducts: they were hardly discernible from the macrophages.

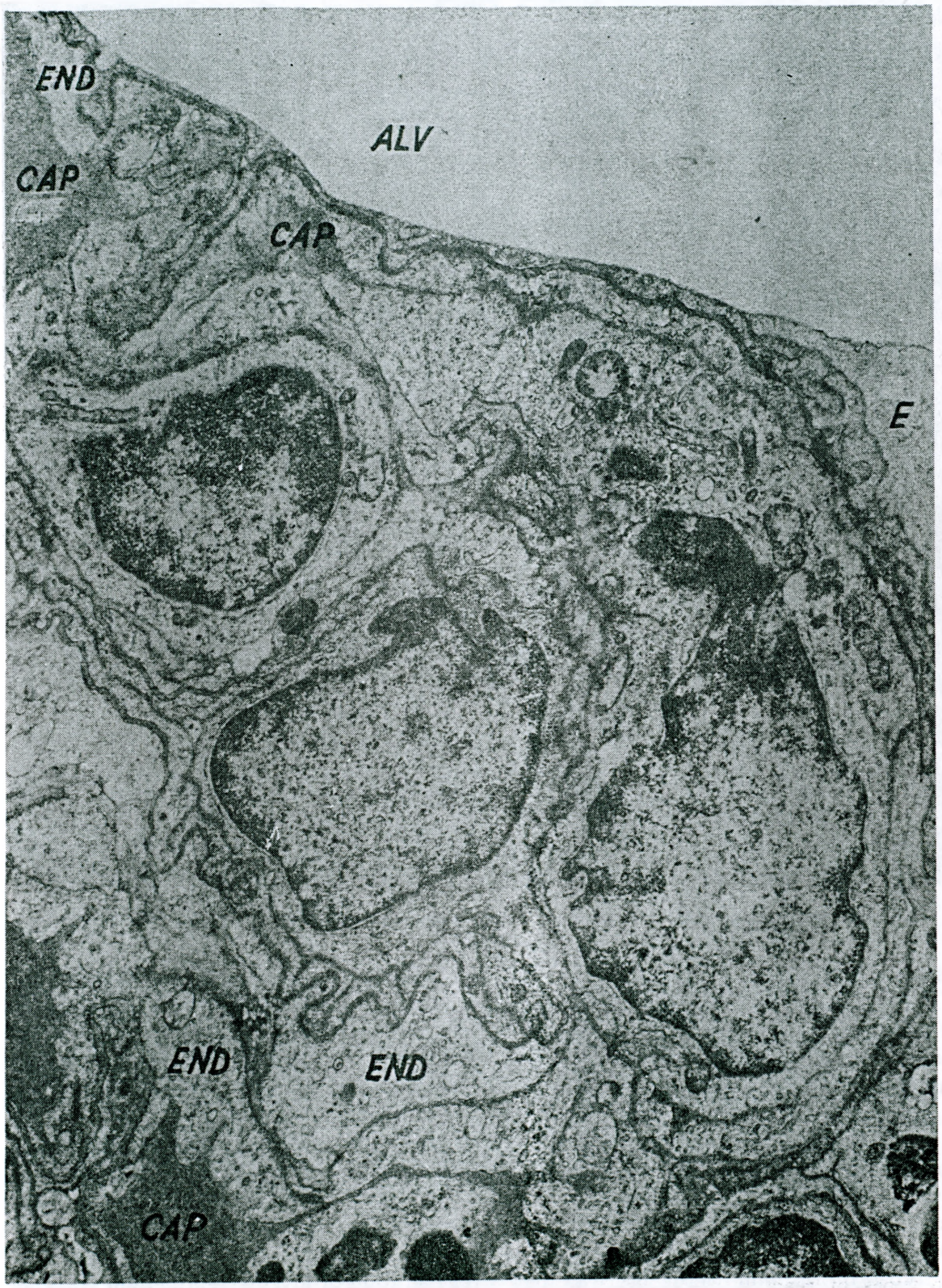

Fig. 4. Changed interalveolar septum. ALV — alveolar lumen, E — edema fluid. Other symbols as in Fig. 3. Fixation with glutaraldehyde and osmium tetroxide. Magn. × 6,200.

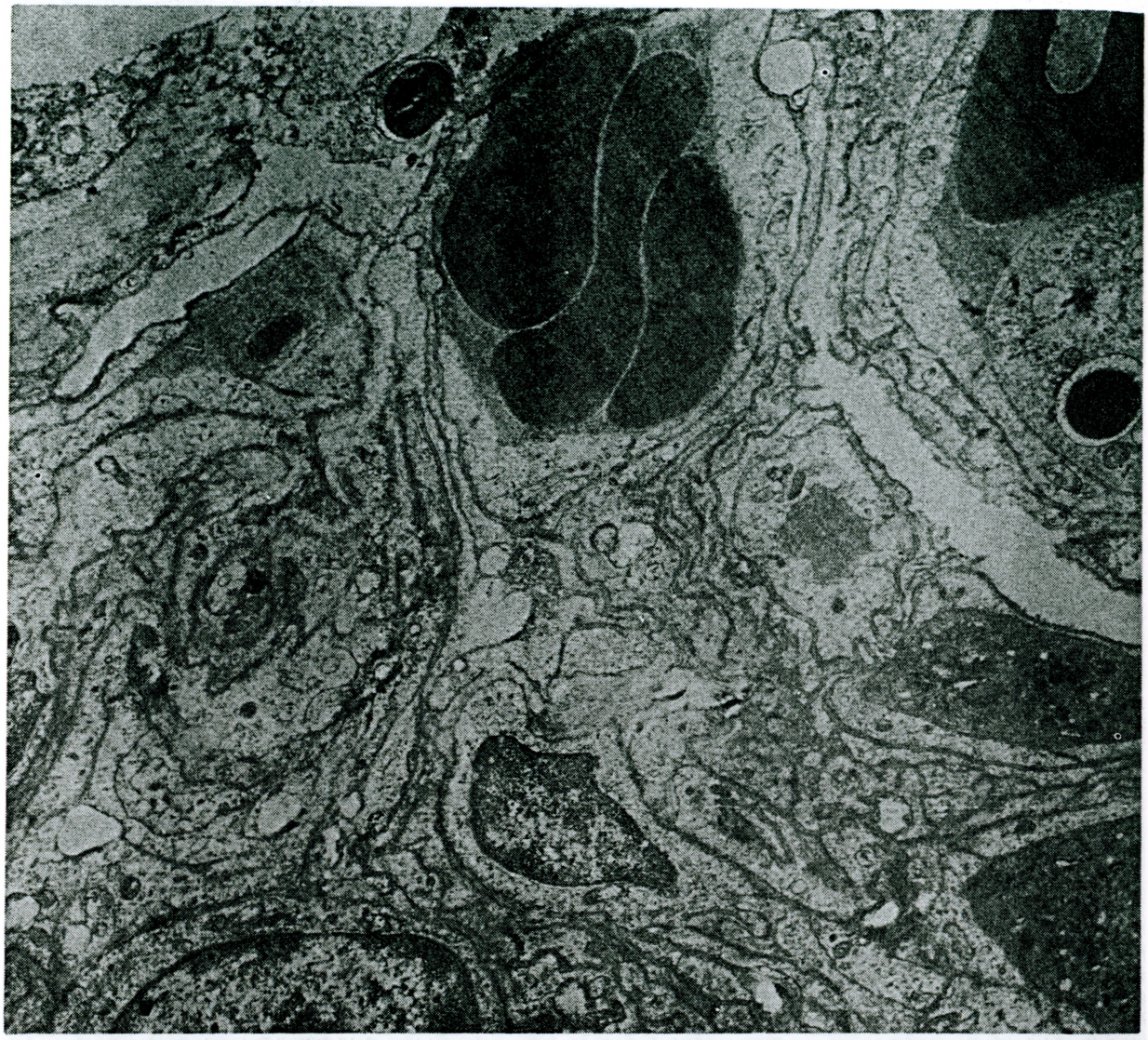

Fig. 5. Atelectasis and edema. Fixation withglutar aldehyde and osmium tetroxide. Magn. × 5,400.

Discussion

The described pulmonary lesions constitute one of the forms of lung edema. The edema of endothelial cells makes this form really particular. It has been described in the literature only in mitral stenosis (5). Edema of the interspace or epithelial cells, namely, is found in many entities as e.g. uremic lung or acute hemodynamic edema (3, 11).

Two types of reaction were observed in type II epithelial cells. The disappearance of lipid (no osmium-positive material) and mucopolysaccharide deposit, negligible, if any, reaction with Ruthenium red from the lamellar structures can result from the exhaustion of the surface tension-regulating mechanisms. Attention has been called to this finding also in light-microscopical studies, since the type II cells did not stain with Astra blue (4, 11). On the other hand, the proliferation of cells rather poor in lamellar structures can reflect the trend to regeneration of alveolar epithelium (1). These attempts to regeneration seem to occur

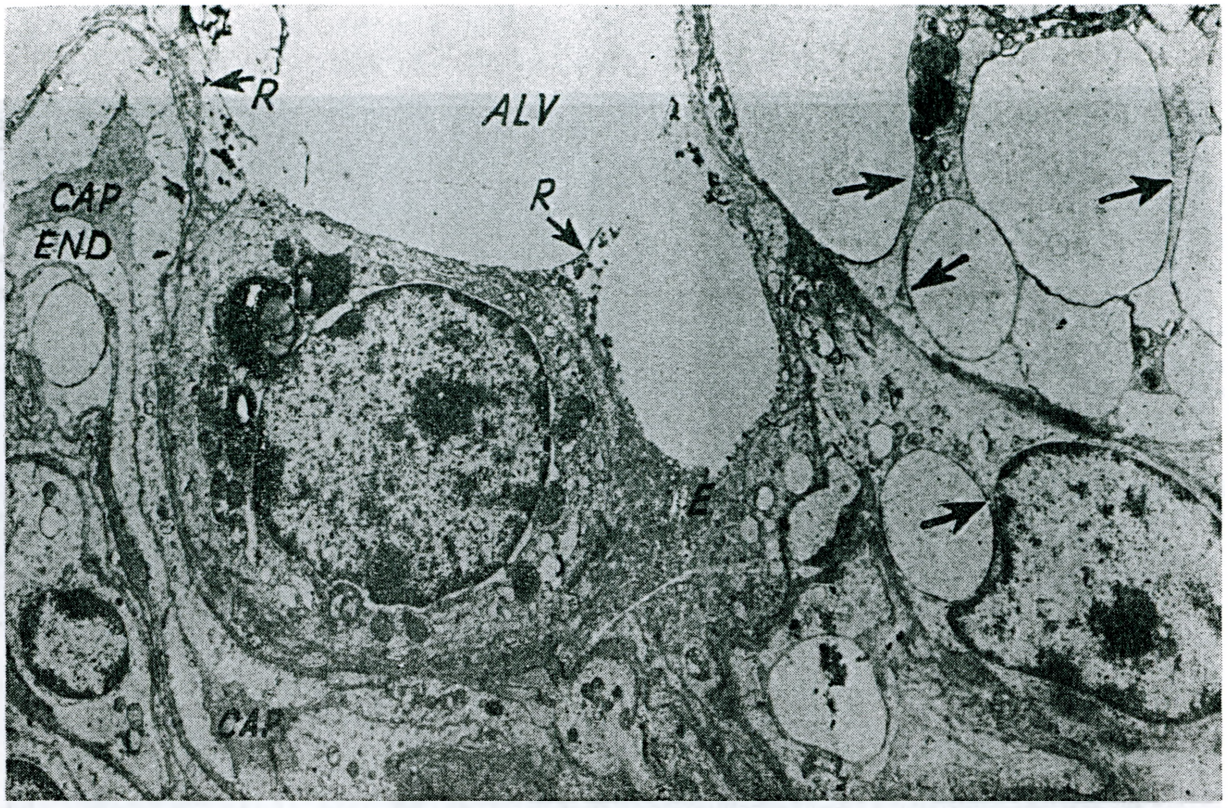

Fig. 6. Type II cell with lamellar structures: on its surface partly Ruthenium red-positive material. Edema of endothelium with vacuolization (arrows, right). Other symbols as in previous figures. Magn. × 6,000.

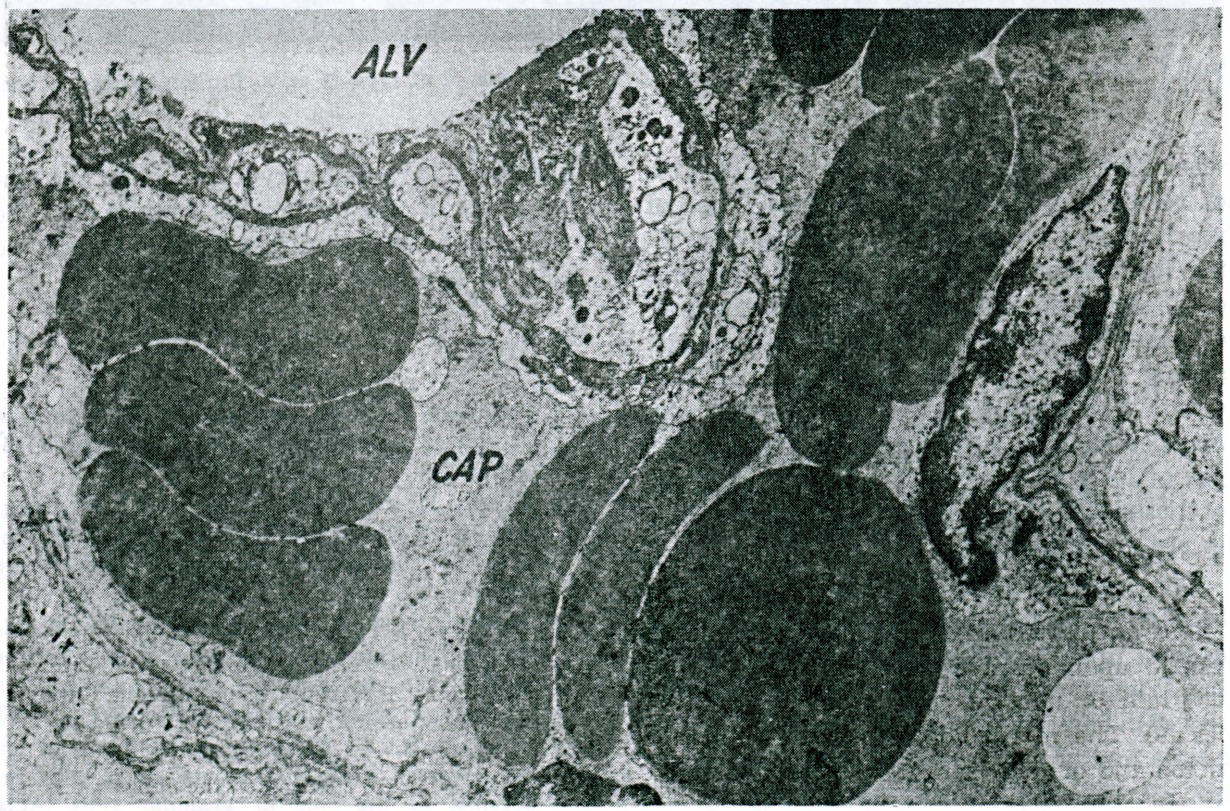

Fig. 7. Septal capillary with moderate endothelial edema. Fixation in glutaraldehyde and osmium tetroxide. Magn. × 8,000.

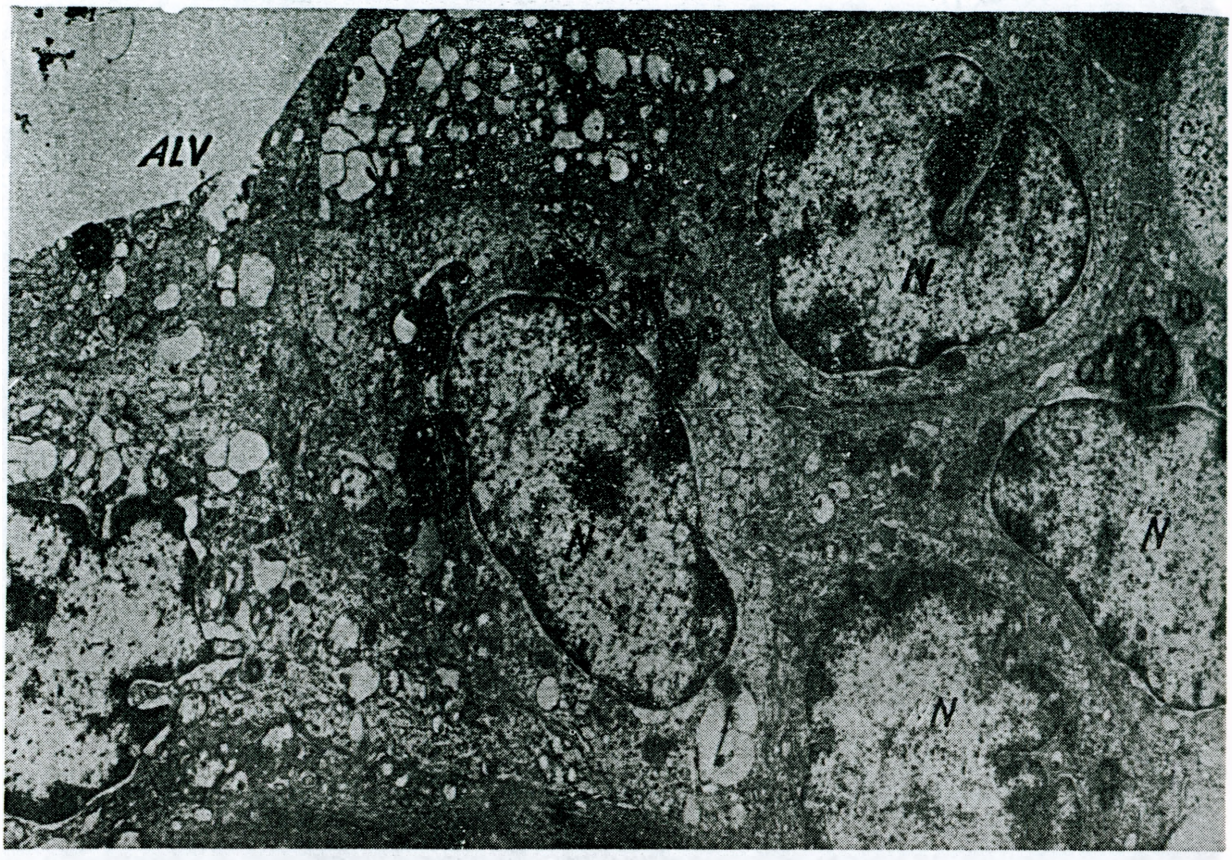

Fig. 8. Proliferation of type II cells, on septal surface. N — nuclei of type II cells. Ruthenium red. Magn. × 6,000.

when the pulmonary parenchyma is capable of respiratory function. Thus, two types of reaction may occur around the 8th day after ligation of lymphatic vessels: the first one related to the disturbance of the mechanism, regulating surface tension, the second reflecting restoration of this function. The regeneration reaction is also nonspecific and can be seen in various types of lung alterations (1). A better outflow of tissue fluid seems to be its prerequisite in our experiment.

The presence of atelectatic — edematous lesions on limited areas of lung tissue might depend upon possible collateral flow of tissue fluid or upon its reflux to the capillaries (9). The morphology of lung lymphatic edema and of that in mitral stenosis is similar. In the latter it can be related to increased transsudation in the course of chronic stasis in pulmonary capillaries, but impairment of the outflow of lymph into the veins with increased blood pressure should also be taken into consideration.

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