sity was not as great as was described previously. However, the fact that there was a change in the vascular network in an investigation conducted by the method of examination of preparations which we adopted, confirms the previous results. Our data indicate some specific structural changes in the vessels in hypertension. The structural changes observed can evidently be explained by two processes: a decrease in caliber of the vessels and complete closing of some arterioles and capillaries. The change in the vascular network is an important fact which must evidently affect the pathogenesis of hypertension.

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CHANGES IN PULMONARY MICROVESSELS OF RATS WITH BRONCHIOLO-ALVEOLAR FIBROSIS INDUCED BY INTRABRONCHIAL INJECTION OF TRYPSIN

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The interest of research workers has recently been drawn to interstitial diseases of the lungs (IDL), which are characterized by diffuse septo-alveolar fibrosis and alveolitis [6, 10]. All IDL are characterized not only by immunopathological processes of various kinds [6, 9], but also by systemic lesions of the microcirculation and the development of an alveolar-capillary block, which is the cause of the patients' death [8, 10]. Meanwhile the histogenesis of these lesions and, in particular, relations between specific (immunopathological) and nonspecific (regenerative) changes in the microvessels in IDL has not been adequately studied.

It was accordingly decided to study changes in the microvessels of the lungs in experimental bronchiolo-alveolar fibrosis induced by intrabronchial injection of trypsin.

EXPERIMENTAL METHOD

Experiments were carried out on 35 noninbred albino rats of both sexes weighing 260-320 g, into which 0.5 ml of a solution of trypsin (from Spofa, Czechoslovakia) in a concentration of 50 mg in 1 ml of isotonic sodium chloride solution was injected intrabronchially under ether anesthesia by tracheotomy. The animals were killed 1, 3, 5, 8, 10, and 15 days after the injection (five to seven rats at each time). Pieces of the lungs were fixed for light and electron microscopy by the usual methods [5, 7]. Serial frozen sections 5-7 μm thick for immunomorphological investigations were stained by the direct Coons' method with luminescent serum against rat globules, and also by the method of Goldwasser and Shepard to reveal complement [2]. The vascular system of the lungs was injected with a mixture of ink and gelatin in two or three animals at each time post mortem [5].

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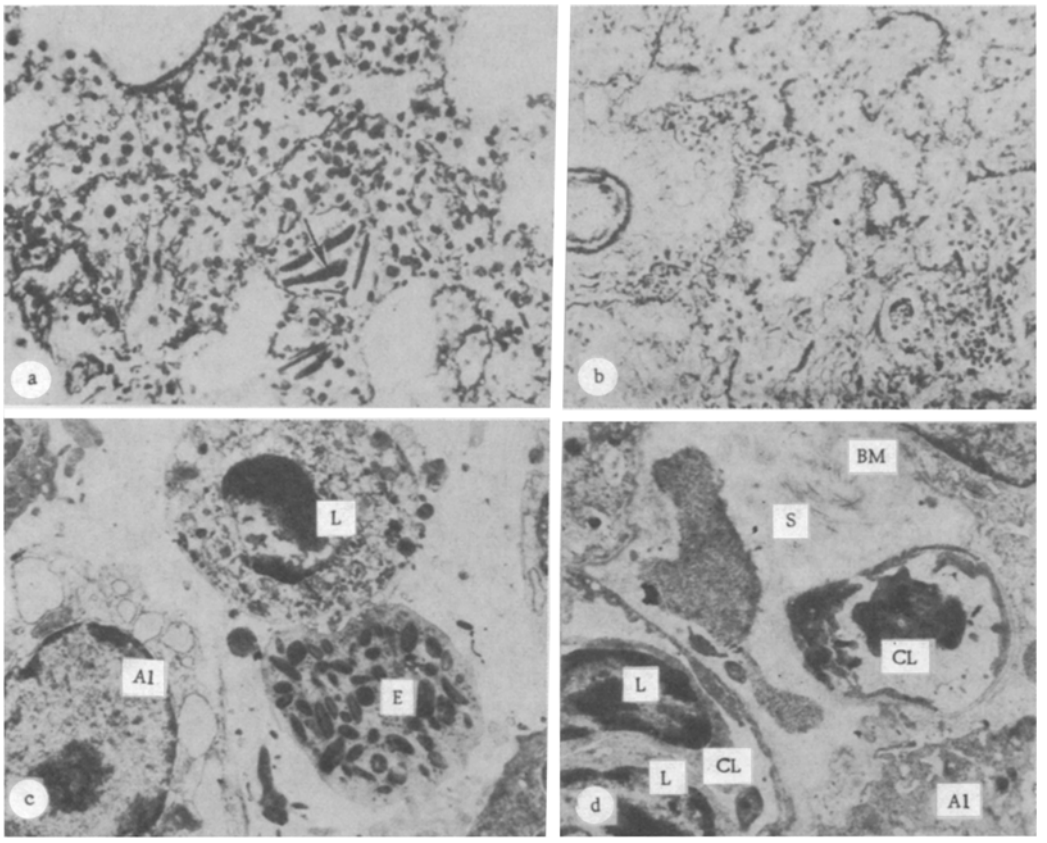

Fig. 1. Morphology of rat lung tissue on first days after intrabronchial injection of trypsin. a) First day of experiment: alveoli slightly collapsed, round cells (alveolar macrophages and single polymorphs) in their lumen and what are evidently trypsin crystals also visible (arrow). Impregnation with silver by Gomori's method. 250×; b) 3rd day of experiment: an eosinophil (E), a leukocyte (L), a degenerating alveolocyte (Al), and finely granular material (albuminous fluid) visible in alveolar lumen. Transmission electron microscopy (TEM). 3500 ×; c) 5th day of experiment: lung tissue moderately collapsed, marked edema present around artery at level of intralobular bronchus. Silver impregnation by Gomori's method. 100 ×; d) alveolar septum (S) swollen and thickened due to edema of interstitial substance, basement membrane (BM) of alveolocytes swollen, two leukocytes (L) visible in lumen of one capillary (CL). Al) Alveolocyte. TEM. 3500 ×.

Sections for light microscopy were stained with hematoxylin and eosin, with fuchselin by Weigert's method and counterstaining by Van Gieson's method, impregnated by Gomori's method, and treated by the PAS reaction with amylase control. Ultrathin sections were studied on the Tesla BS-500 electron micrsocope.

EXPERIMENTAL RESULTS

Traces of yellowish fluid were found in the pleural cavities of the rats 24 h after injection of trypsin. The lungs were enlarged, doughy in consistency, with multiple dark red areas up to 1-2 μm in diameter. Examination with the light microscope revealed focal atelectasis, and the lumen of the alveoli contained erythrocytes, pinkish fluid, desquamated epithelial cells and macrophages, and trypsin crystals (Fig. 1a). Eosinophilic masses resembling hyaline membranes were seen on the alveolar walls. Marked interstitial edema was present with a few polymorphs in the edematous connective tissue (especially peribronchial and perivenous). On electron microscopy the alveolar lumen was seen to contain granular masses, eosinophils, polymorphs, macrophages, and desquamated alveolocytes (Fig. 1b). The type I alveolocytes were swollen, with marked edema and focal destruction of their cytoplasm, as well as vesicular dilatation of the tubules of the endoplasmic reticulum.

On the 3rd day the lungs remained variegated: small lobular and acinar bluish-red areas alternated with red and pale pink areas. Microscopically, concentrations of large round cells of alveolar macrophage type and

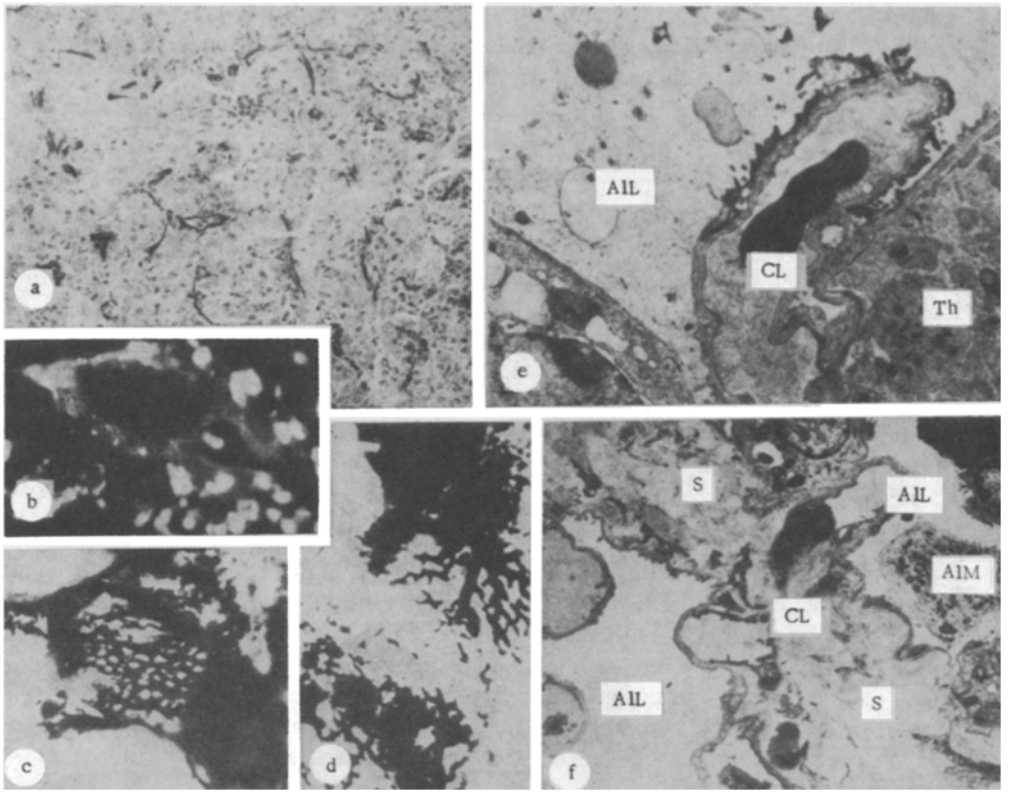

Fig. 2. Morphology of rat lung tissue at end of 1st week and during 2nd week after intrabronchial injection of trypsin. a) Alveolar lumen filled with complexes of elongated cells (8th day). Silver impregnation by Gomori's method. 250 ×; b) fluorescence of immunoglobulins absent in wall of interlobular vein (V), immunoglobulins present only in cells in perivenous connective tissue. Direct Coons' method. 250 ×; c) capillary network of alveolar wall of lung of control rat. Ink in gelatin solution. Intravascular injection. 250 ×; d) reduction of capillaries of alveolar wall of rat lung on 15th day after intrabronchial injection of trypsin. Same method as in Fig. 2c. 250 ×; e) thrombosis (Th) of area of capillary network of alveolar wall and ectasia of neighboring area. TEM, 3500 ×; f) fragment of alveolar septum (S), containing collagen bundles, with swelling of capillary loops (Cl and arrows). AlM) Alveolar macrophages, AlL) alveolar lumen.

a certain number of polymorphs were visible in the lumen of the alveoli and respiratory bronchioles. On silver impregnation slight collapse of the connective-tissue framework of the lungs could be detected, i.e., on the whole the picture observed was one of acute desquamative alveolitis (Fig. 1c). Electron-microscopically, besides an exudative reaction, thickening of the alveolar septa on account of swelling of the epithelial and endothelial basement membranes and of the interstitial ground substance was discovered (Fig. 1d).

On the 5th-8th day large segmental and subsegmental bluish-red foci were found in some animals. In others the lungs were variegated on account of small cyanotic, red, or dark red areas with a rust-colored staining of the pleura above them. Under the light microscope, complexes and symplasts of elongated cells were visible, together with alveolar macrophages, in the lumen of the alveoli (Fig. 2a). Electron-microscopically, against the background of marked desquamative alveolitis there were signs of regeneration of the alveolar epithelium: On the surface of the alveoli large cells with large nuclei and with loosely structured, hypertrophied nucleoli were present.

On the 10th-15th day multiple small areas, collapsing in places, cyanotic or whitish in color, were visible macroscopically on the pleural surfaces of the lungs. The lung tissue was doughy to the touch. Staining with picrofuchsine revealed thickening of the alveolar septa due to delicate bronchiolo-alveolar fibrosis. Ultrastructurally, the septa were thickened (up to 7-9 μm, normally 4.5 ± 0.4 μm), and coarse bundles of collagen and

elastic fibers could be identified in them, mingled with fibroblasts. Occasionally plasma cells and macrophages were seen. Highly constricted alveolar passages and collapsed alveoli were present. In the latter the alveolocytes contained large granules of average electron density in their cytoplasm and resembled secretory cells. After intrabronchial injection of trypsin solution, desquamative alveolitis thus developed, going on to bronchiolo-alveolar fibrosis.

Changes in the lung vessels deserve particular attention. On immunomorphological investigation at all times of the experiment, fixation of globulins and complement in the walls of the arteries, veins, and capillaries was not demonstrated. Fluorescence of globulins was observed only in cells of the peribronchial and perivenous connective tissue (Fig. 2b). Individual cells fixing globulins were discovered in the alveolar septa. A study of histological preparations, including thick (up to 50-70 μm) se ctions through the lungs, the vascular network of which had been injected with ink in gelatin, reduction of the capillary network of the interalveolar septa was observed. This reduction developed parallel with the development of bronchiolo-alveolar fibrosis and was clearly visible in the 2nd-3rd week after injection of trypsin. Capillaries of the interalveolar septa, filled with ink, formed a dense network in the control animals (Fig. 2c). In rats with bronchiolo-alveolar fibrosis ink filled the arterial and venous collectors and also regions of the capillary network adjacent to the arteriole. Meanwhile reduction of certain segments of the capillary network of the alveolar septa was observed (Fig. 2d), since they did not fill with ink in gelatin.

Electron-microscopically, in the first 3 days after injection of trypsin capillaries with a swollen (thickened) basement membrane were present in the thickness of the swollen alveolar septa. Sometimes leukocytes were present in the lumen of the microvessels. Thrombosed capillary loops were seen, and adjacent to them capillary segment were swollen and dilated (ectatic (Fig. 2e). With progression of the bronchiolo-alveolar fibrosis, ectasia and swelling of the capillary loops in the alveolar lumen became increasingly obvious (Fig. 2f). Under these circumstances ectasia was combined with obliteration of capillaries in the thickness of the alveolar septa, and it was evidently compensatory. No signs of capillarities were found at any stage of the experiment.

After injury to the bronchiolo-alveolar lining, acute desquamative alveolitis thus develops, and terminates as bronchiolo-alveolar sclerosis, with reduction of individual segments of the capillary network of the alveolar walls and ectasia of other segments of this network.

When the mechanism of these changes is interpreted, absence of fixation of immunoglobulins in the vessel walls must be taken into consideration. This reorganization of the microvascular network of the lungs is evidently nonimmune and not specific. It reflects the reaction of the microvessels to injury of the alveolar lining, disturbance of the gas exchange as a result of alveolitis, and processes of fibrosis and regeneration in the alveolar septum. The results shed some light on the interpretation of clinical data and, in particular, the dispute between investigators who associate reorganization of the pulmonary microvessels in cryptogenic fibrosing alveolitis (one form of IDL) with damage to the alveolar lining [1], and those who regard this alveolitis as productive capillaritis [8]. In human biopsy material the writers discovered [4] a combination of both types (specific and nonspecific) of lesions of the pulmonary microvessels in IDL. In particular, besides changes in the microvessels similar to those found experimentally, changes of endoarteriolitis, periarteriolitis, and pericapillaritis were found, with accumulation of immunocompetent cells around the vessels and in the thickness of their walls, reflecting the presence of immunopathological processes [3].

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