ELECTRON-MICROSCOPIC STUDY OF THE PATHOGENESIS OF EXPERIMENTAL LIPOID PNEUMONIA

V. V. Erokhin and H. Eckert

UDC 616.24-002-092.2

Stages of development of lipoid pneumonia induced in albino mice by means of mineral oil were studied by electron microscopy and histochemistry. The first changes were most marked in the blood capillaries. Ingestion of foreign material by interstitial mononuclear cells and alveolar macrophages took place actively. However, active digestion of the material was observed only in the cytoplasm of the alveolar macrophages, which were especially numerous in the early granuloma. Later, in the interstitial tissues and small granulomas fibroblasts were predominant and could cause the development of atelectasis and pneumosclerosis.

KEY WORDS: lipoid pneumonia; electron microscopy.

The widespread use of technical oils in industry and of inhalations of oily emulsions in medicine has increased the risk of development of lipoid pneumonias. The diagnosis of exogenous lipoid pneumonias can be very difficult, and often a correct diagnosis is made only by histological analysis of areas of the lung removed surgically. Pathomorphological investigations of this disease, including experimental studies, revealing structural and metabolic features of the pathological and defensive reactions arising in lung tissues are accordingly of great scientific interest [3, 8, 11, 12]. However, the pathogenesis of lipoid pneumonias has not yet been adequately studied, although information on this subject is essential for the successful development of preventive and therapeutic measures. The present investigation was devoted to a study of some aspects of the pathogenesis of lipoid pneumonias.

EXPERIMENTAL METHOD

Experiments were carried out on albino mice which inhaled an aerosol of mineral oil daily for 6 h in a special chamber. The animals were killed after 1, 4, 7, and 11 days and 1 and 2 months. In order to study the earliest reaction of the alveolar wall to inhalation of mineral oil a group of animals was sacrificed 30, 60, 120, and 180 min after the beginning of the experiment. Material for electron microscopy was prepared in the usual way. Ultrathin sections, after staining, were examined in the JEM-100B electron microscope. Parallel histological and histochemical (for ATPase) studies of lung tissue and also of semithin sections stained with toluidine blue also were carried out.

EXPERIMENTAL RESULTS

Inhalation of mineral oil caused an inflammatory reaction with diffuse involvement of the lung tissue, an increase in vascular permeability, edema, and focal hemorrhages. These changes could be seen as early as during the first week and they were accompanied by a sharp fall in ATPase activity, which was observed after 1 h and reached a maximum by the 4th day. Activity of the enzyme then began to recover. Infiltration and proliferation led to thickening of the alveolar septa and the accumulation of alveolar "foam" macrophages, and to the formation of small granulomas by the 11th day.

Electron-microscopic investigation showed changes in the fine structure of the lungs as early as 30 min after entry of the mineral oil into the respiratory tract. They were most marked in the capillaries, but by the end of the first day they had spread gradually and relatively widely. In the cytoplasm of the endothelial cells the number of microvesicles

---

Central Research Institute of Tuberculosis, Ministry of Health of the USSR. Research Institute of Lung Diseases and Tuberculosis, Ministry of Health of the German Democratic Republic. (Presented by Academician of the Academy of Medical Sciences of the USSR L. K. Bogush.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 3, pp. 362-366, March, 1980. Original article submitted June 26, 1979.

0007-4888/80/8903-0381$07.50

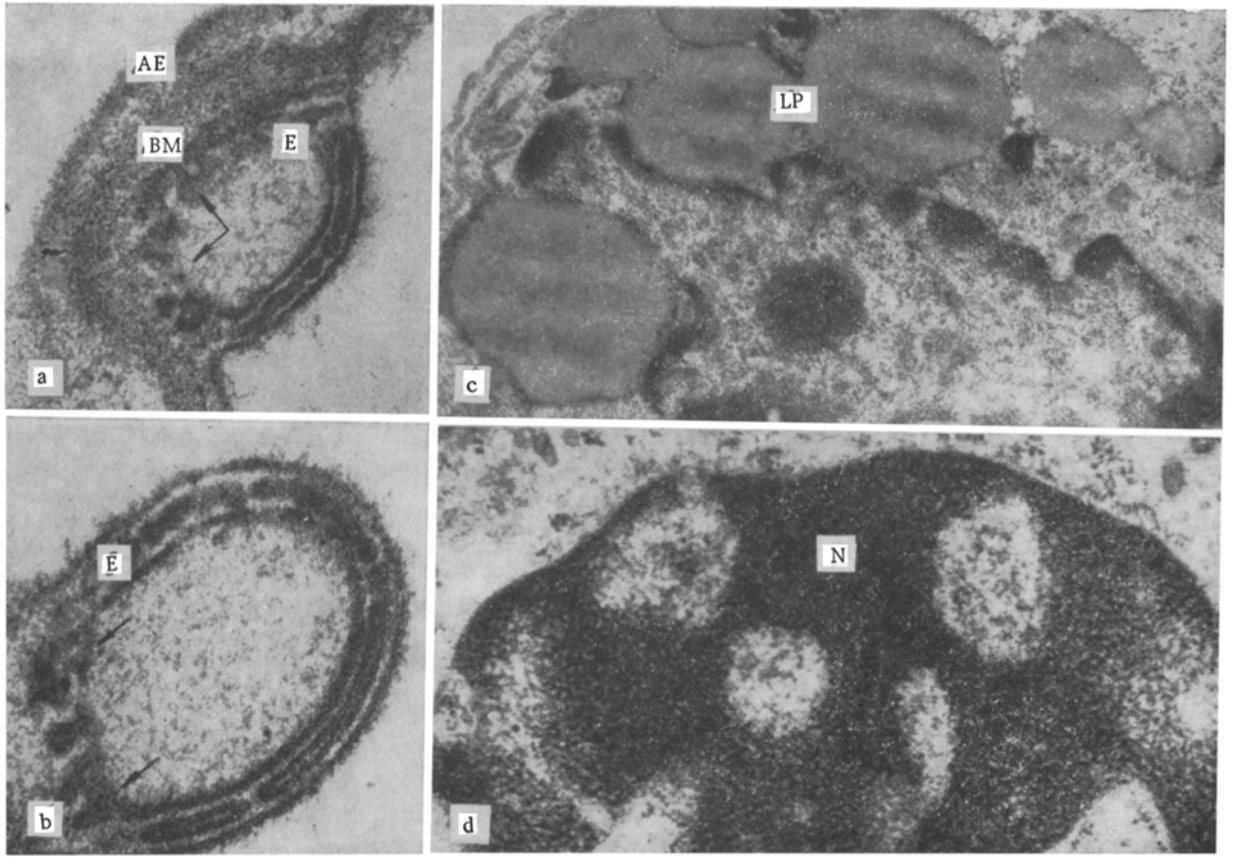

Fig. 1. Response of blood capillaries and accumulation of lipids in interstitial tissue of alveolar wall of lung during first hours of experiment: a, b) 60 and 120 min of experiment. Swelling of alveolar epithelium (AE), edema and destruction (arrows) of endothelium (E), loosening of basement membrane (BM); c) 180 min of experiment. Accumulation of lipids (LP) in interstitial tissue and cytoplasm of a histiocyte; d) nucleus (N) of histiocyte with localized translucencies in chromatin. Magnification: a) 65,000, b) 90,000, c) 1700, d) 60,000×.

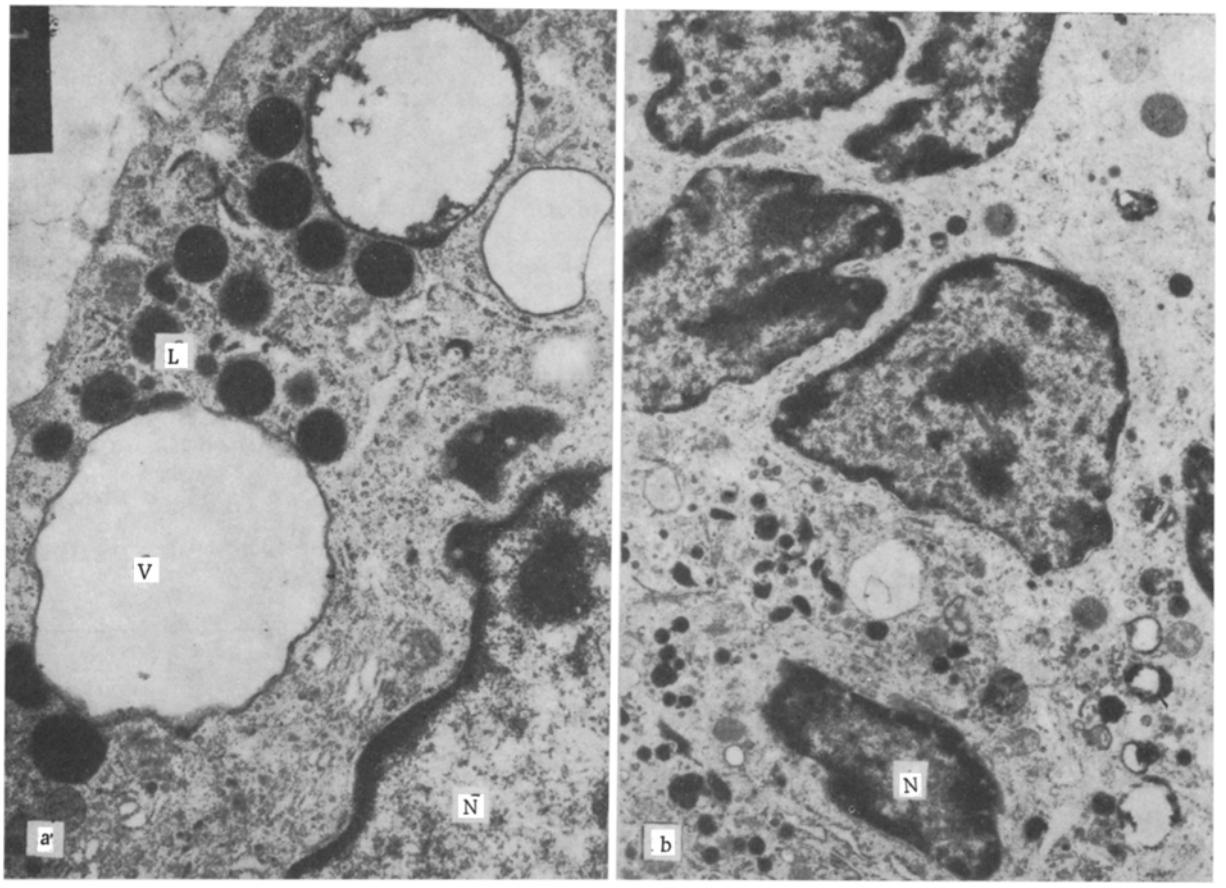

Fig. 2. Further development of inflammation of the lungs. a) 7th day of experiment. Part of alveolar macrophage: contact and fusion of lysosomes (L) with large vacuole (V) (28,400×); b) after 1 month of experiment. Multinuclear cell: nuclei (N) in center, some of them with microfocal translucencies in chromatin at periphery; granuloma (14,000×).

increased, large vacuoles appeared, and subendothelial edema developed, with disturbance of the integrity of the cell in the region of the basal plasmalemma. The endothelial cells were expressed into the lumen of the capillary and detached from the basement membrane. The latter was loosened in structure and lost its clear outlines (Fig. 1a, b). The changes in the alveolar epithelium were less marked. In the type II alveolocytes the volume of the lamellar component of the osmiophilic bodies were reduced and the number of microvilli on the surface of the type I alveolocytes was increased. Type II alveolocytes with a well developed ultrastructure were found. From the first stages of the experiment, accumulation of lipids was observed in the interstices and histiocytes, and they merged to form large drops of average electron-optical density (Fig. 1c). Meanwhile focal translucencies appeared in the chromatin of some nuclei (Fig. 1d). The ultrastructure of the histiocytes was poorly developed and the lipids contained in them did not undergo hydrolysis.

This process continued intensively in the alveolar macrophages (lipophages), the number of which rose sharply until the 4th-7th day. The course of hydrolysis of the lipids ingested by them could be observed: from contact of lysosomes with them to the formation of vacuoles and fusion of the vacuoles to form a giant cavity (Fig. 2a). Individual vacuoles contained highly osmiophilic granular material, probably the residual hydrolysis product of the mineral oil. Some lipophages were destroyed. Accumulations of alveolar macrophages, together with interstitial cells, formed the basis of the newly developing oil granuloma.

During further development of the inflammation (1 month) diffuse and focal thickening of the alveolar septa took place on account of concentrations of lymphohistiocytes and proliferation of fibroblasts. Fewer "foam" cells could be seen in the granulomas and processes of fibrosis were now predominant, although multinuclear cells with a well-developed ultrastructure could be seen among the cell population (Fig. 2b). Numerous lysosomes, phagosomes,

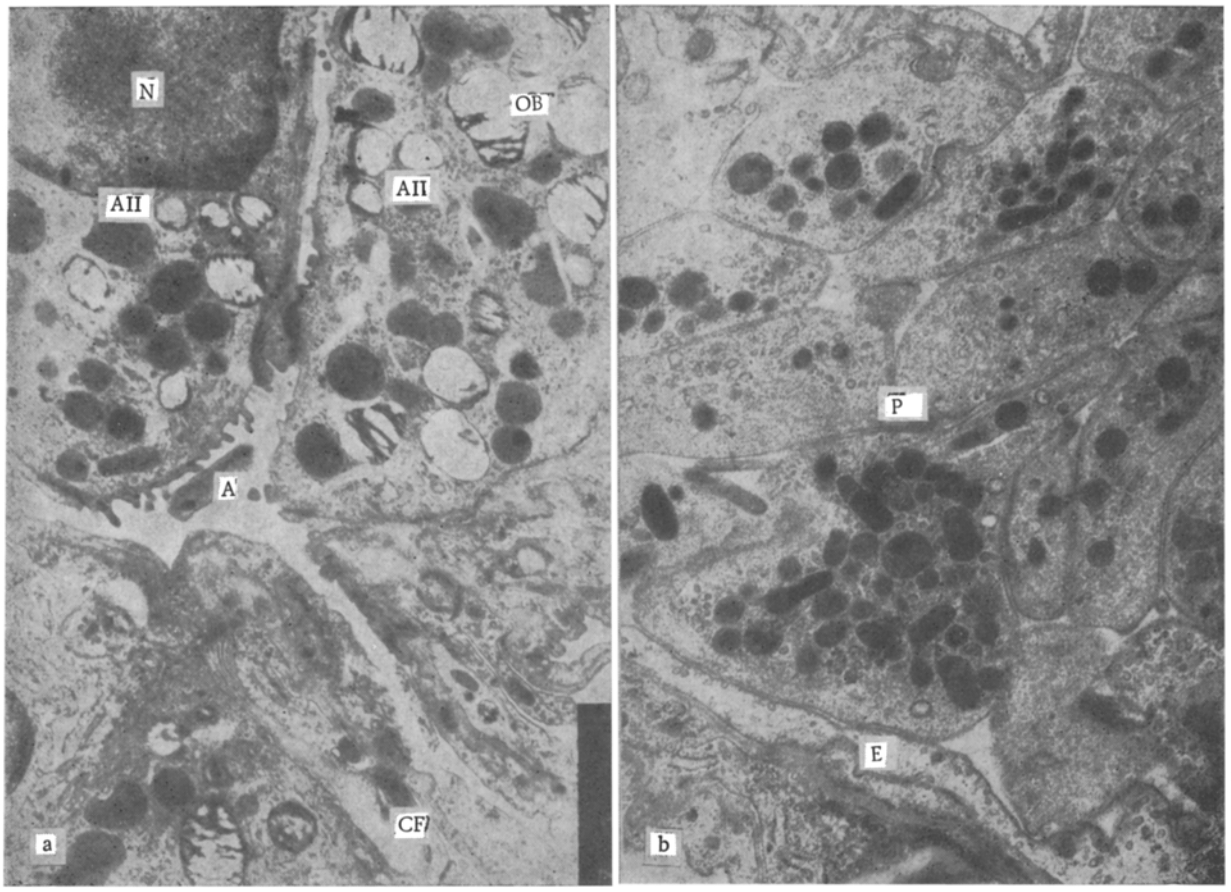

Fig. 3. Atelectasis of the lung. a) After 2 months of experiment. Slit-like lumen of alveolus (A); disintegration and reduction of lamellar material in osmiophilic bodies (OB) is observed in type II alveolocytes (AII) (13,500×); b) after 2 months of experiment. Concentration of platelets (P) in blood capillary; numerous collagen fibrils (CF) visible in interstitial connective tissue (20,000×). Remainder of legend as in Figs. 1 and 2.

vacuoles, and other inclusions could be seen in the cytoplasm of the giant cells. Nuclei were concentrated in the central part of the cell, and in some of them numerous microfocal translucencies could be seen in the chromatin around the periphery.

By the 2nd month of the experiment, besides a decrease in the intensity of infiltration and proliferation, the formation of atelectases and development of pneumosclerosis could be observed in the lungs. Electron-microscopic investigation of the atelectatic areas showed considerable disturbances of capillary ultrastructure with the formation of microthrombi and with activation of fibroblast function. Some capillaries were in a collapsed state while others, on the contrary, were wide open, with very thin portions of the air–blood barrier and with erythrocytes in their lumen. Disorganization and reduction of the lamellar material in the osmiophilic bodies was observed in the type II alveolocytes (Fig. 3).

Injection of mineral oil into the respiratory tract thus induces an inflammatory reaction with damage to the fine structure of the alveolar wall, activation of the macrophagal system, the formation of granulomas, and development of pneumosclerosis. Atelectases may appear and subsequently undergo fibrosis. Small droplets of oil, on reaching the alveoli, pass through the epithelial lining into the interstitial connective tissue, where lipids accumulate. A special role, in the writers' opinion, in this process is played by the basement membrane of the capillaries, which acts as a fine filter, probably passing only the soluble fractions of the oil. The rapid development of edema and injury to the endothelium may be a reflection of this process, and also of the toxic action of products of the mineral oil. The epithelial basement membrane is more resistant to injury, as one of us (V.V.E.) has shown to be the case in tuberculosis also [2]. Accumulation of lipids in the intestices act as an irritant for the cells. Mononuclear cells appearing in this region ingest the oil, but

active hydrolysis of lipid inclusions is observed only in the alveolar macrophages. Mononuclear infiltration of the lungs precedes an increase in the number of alveolar macrophages, which confirms the common source of origin of these cells, which most workers associated with the bone marrow [6-8]. Hydrolysis of the lipids in the interstitial mononuclear cells is at a low level of intensity, which indicates that the cells need to be prepared for this process.

The results of the investigation are evidence of the development of fatty infiltration of the cells. The oil permeates the membranes, penetrates into the cytoplasm and nuclei, and causes injury to them. Destructive changes in the capillaries are the cause of the hemorrhages and microthrombi which, in turn, cause the conditions of function of the cells and tissues to deteriorate sharply and lead to the formation of atelectases together with obstructive lesions of the terminal respiratory passages. The state of the microcirculation plays a leading role also in the development of pneumosclerosis, just as in other lung diseases [1, 4, 5, 10]. An important place in the general combination of factors acting on the inflammatory process evoked in the lungs by a suspension of mineral oil must therefore be ascribed to measures aimed at overcoming the effects of injury to the microcirculation.

LITERATURE CITED

1. Y. D. Batsura, Byull. Éksp. Biol. Med., No. 11, 13 (1970).
2. V. V. Erokhin, "Subcellular morphology of the lungs in experimental tuberculosis," Author's Abstract of Doctoral Dissertation, Moscow (1974).
3. V. V. Erokhin and H. Eckert, Probl. Tuberk., No. 7, 71 (1978).
4. V. V. Serov et al., Arkh. Patol., No. 4, 52 (1972).
5. A. I. Strukov and I. M. Kodolova, Chronic Nonspecific Diseases of the Lungs [in Russian], Moscow (1970).
6. A. Ya. Fridenshtein, Arkh. Anat., No. 4, 3 (1974).
7. D. H. Bowden and I. J. R. Adamson, Am. J. Path., 83, 123 (1976).
8. H. Eckert and S. Jerochin, Z. Erkr. Atm., 150, 34 (1978).
9. R. Furth and J. Thompson, Ann. Inst. Pasteur, 120, 337 (1971).
10. S. F. Ryan, Am. Rev. Resp. Dis., 105, 776 (1972).
11. E. F. Stula and B. K. Kwon, Am. Indust. Hyg. Assoc. J., 39, 393 (1978).
12. I. Weber, Prax. Pneum., 31, 989 (1977).

MORPHOLOGY OF EXPERIMENTAL ACUTE PANCREATITIS DURING TREATMENT WITH 5-FLUOROURACIL

Kh. T. Nishanov and R. I. Kaem

UDC 616.37-002.1-092.9-085.277.3:547.854.4]-091

In experimental pancreatitis induced by a modified method (Arai, 1965) 5-fluorouracil has a marked therapeutic action, preventing the development of necrosis of the acini and suppurative liquefaction of the gland tissue. The authors attribute the therapeutic effect of 5-fluorouracil to its inhibitory action on RNA synthesis and blockade of the liberation of secretion from the exocrine part of the pancreas.

KEY WORDS: acute pancreatitis; 5-fluorouracil.

The treatment of acute pancreatitis still remains a difficult problem in abdominal surgery. The mortality is still extremely high [1, 2]. Several workers [4, 5] have suggested that acute pancreatitis be treated by 5-fluorouracil (5-FU) which, as an inhibitor of RNA synthesis, in the opinion of Martin et al. [6], prevents synthesis in the pancreatic exocrine cells and inhibits secretion of enzymes.

Department of Abdominal Surgery and Department of Surgical Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 3, pp. 366-368, March, 1980. Original article submitted June 6, 1979.

0007-4888/80/8903-0385$07.50