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ULTRASTRUCTURE OF THE AIR–BLOOD BARRIER OF THE LUNGS IN DOGS TREATED FOR ACUTE HYPOXIA BY EXTRACORPOREAL MEMBRANE OXYGENATION

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KEY WORDS: lungs; hypoxima;extracorporeal membrane oxygenation; air–blood barrier; electron microscopy.

Treatment of acute respiratory failure (ARF) still remains an urgent problem in modern medicine [2, 5, 10]. The high mortality from ARF and the limited possibilities of methods of intensive treatment of respiratory failure make the development of new methods of oxygenation of the patient, one of which is extracorporeal membrane oxygenation (ECMO), particularly important. Accounts of experimental [3, 7, 8] and clinical [1, 11, 12] investigations and survey articles [4, 6, 9, 13], giving the results of the use of membrane oxygenators (MO) in the treatment. Yet the effect of new gas-exchange systems of membrane type on the structure of the lungs and other organs, which is particularly important in connection with the evaluation of the functional properties and qualities of MO, has received totally inadequate study.

The aim of this investigation was to study the ultrastructural changes in the air–blood barrier (ABB) of the lungs under conditions of severe hypoxia and treatment with MO.

EXPERIMENTAL METHODS

Experiments were carried out on 10 mongrel dogs of both sexes weighing 15-20 kg. Severe hypoventilation hypoxia was induced in all the animals by reducing the respiratory minute volume suddenly to 30% of normal. In five dogs of the control series, 40-90 min after the beginning of hypoxia, under general anesthesia (pentobarbital) and muscle relaxation (succinylchlorine), left thoractomy was performed and pieces of the lung removed for electron-microscopic investigation. In the main series of experiments (on five dogs) hypoxic animals were connected to a Soviet Sever-OMR membrane oxygenator, on the vein–oxygenator–vein principle. After the MO had been in operation for 3-3.5 h, under hypoventilation conditions material was taken for morphologic investigation in the same way as from animals of the control series. Material for electron microscopy of the lungs was treated by the usual method. Ultrathin sections were studied in the IEM-100CX electron microscope. In each series 10 samples of the lungs were studied. The lungs of four healthy intact dogs also were investigated electron-microscopically.

RESULTS

Electron-microscopic study of the lungs of dogs of the control series with severa hypoxia revealed considerable changes in all components of ABB, the most severe being disturbances of the microcirculation (Table 1). In 70% of lung samples studied the blood capillaries in the alveolar septa were grossly dilated and contained agglutinated erythrocytes (Fig. 1a), with at the same time unchanged erythrocytes and erythrocytes with a

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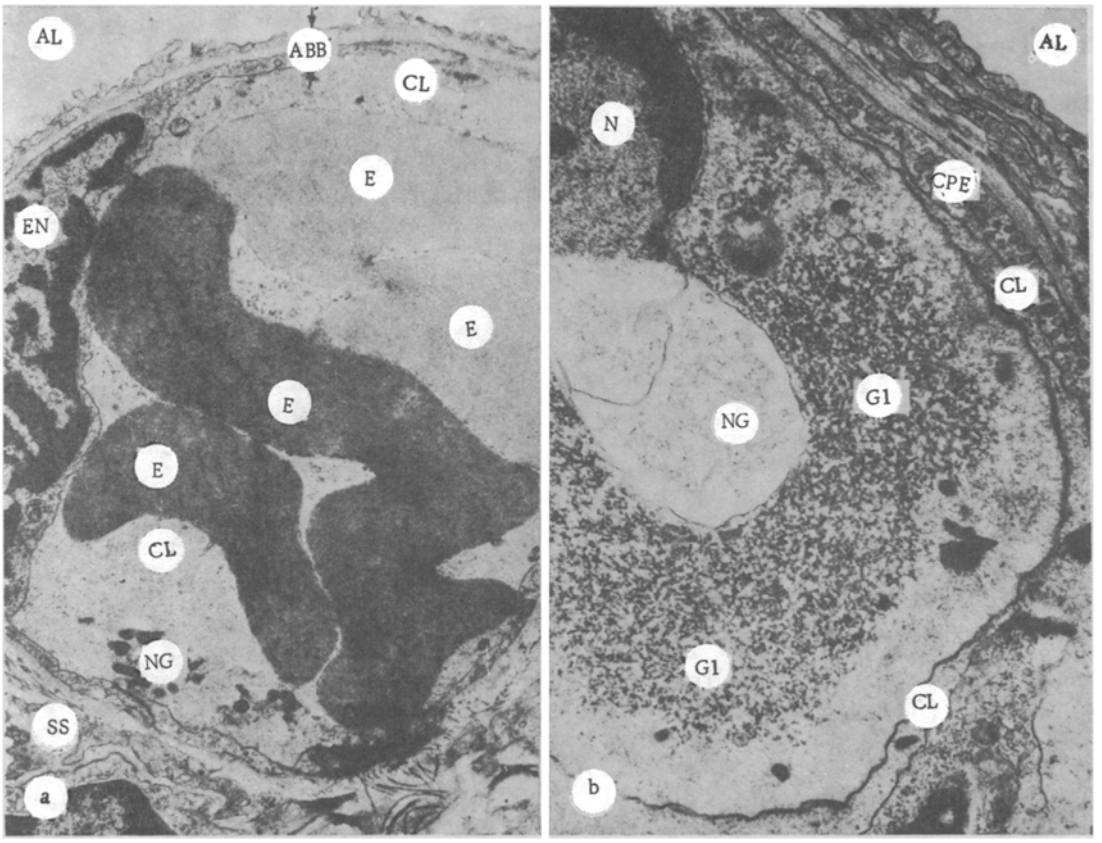

Fig. 1. Intravascular disturbances in ABB of the lungs of dogs with hypoxia. a) Agglutinated erythrocytes in capillary lumen (CL) in alveolar septum; erythrocytes (E) of varied electron density; 5000×. b) In capillary lumen (CL) with destructive changes, 10,000×. AL) Alveolar lumen, EN) endotheliocyte nucleus, NG) remnants of dying NG, SS) septal space, N) nucleus of NG, Gl) glycogen particles. Arrows indicate thin part of ABB.

greatly reduced hemoglobin content and ghost cells. Mixed groups of agglutinated erythrocytes, neutrophilic granulocytes (NG), and platelets were found in only 10% of specimens. In half of the specimens NG in different stages of fragmentation were present in the capillary lumen (Fig. 1b). Unlike in intact dogs, in animals with hypoxia the blood plasma contained fragments of disintegrated NG and cytoplasmic processes of endotheliocytes (CPE), vesicles, and myelin-like figures. Microcirculatory intravascular disturbances were accompanied by considerable ultrastructural changes in the endotheliocytes, more especially in their CPE. Focal destruction of the cytolemma of CPE over their luminal surface which, as a rule, was found in zones of adhesion of the CPE to erythrocytes or NG, was observed most frequently. In 40% of lung samples regions with completely destroyed CPE were observed. In the same blood capillary, regions denuded of endothelium alternated with zones in which its structure was normal (Fig. 2a). The most characteristic change in the basal layer of ABB was local loosening of its delicate fibrous structure, which was always accompanied by adhesion of erythrocytes to the luminal surface of CPE and by destruction of the latter. In the septal space focal edema, distintegration of collagen fibers, and large electron-translucent cavities were found. Changes in the cytoplasmic processes of the type I alveolocytes (CPA) were largely similar to those observed in CPE. Increased translucency of the matrix and the appearance of numerous micropinocytotic vesicles and vacuolated mitochrondira were observed in them (Fig. 2b). In one-third of samples total destruction of CPA was observed (Fig. 2c). The structure of the type II alveolocytes was characterized by greater polymorphism. Heterogeneity of the ultrastructure of these cells was manifested mainly in the structure of their lamellar bodies. In some lung samples most cells had numerous large "empty" lamellar bodies, sometimes fused together, and not containing osmiophilic material. In other animals the bodies were completely filled with osmiophilic lamellae which were arranged less compactly than in intact dogs. Destruction of the thin portion of ABB was observed in one dog, and regions with destroyed components of ABB alternated with zones with a normal structure. In 40% of samples finely granular blood plasma, micro-

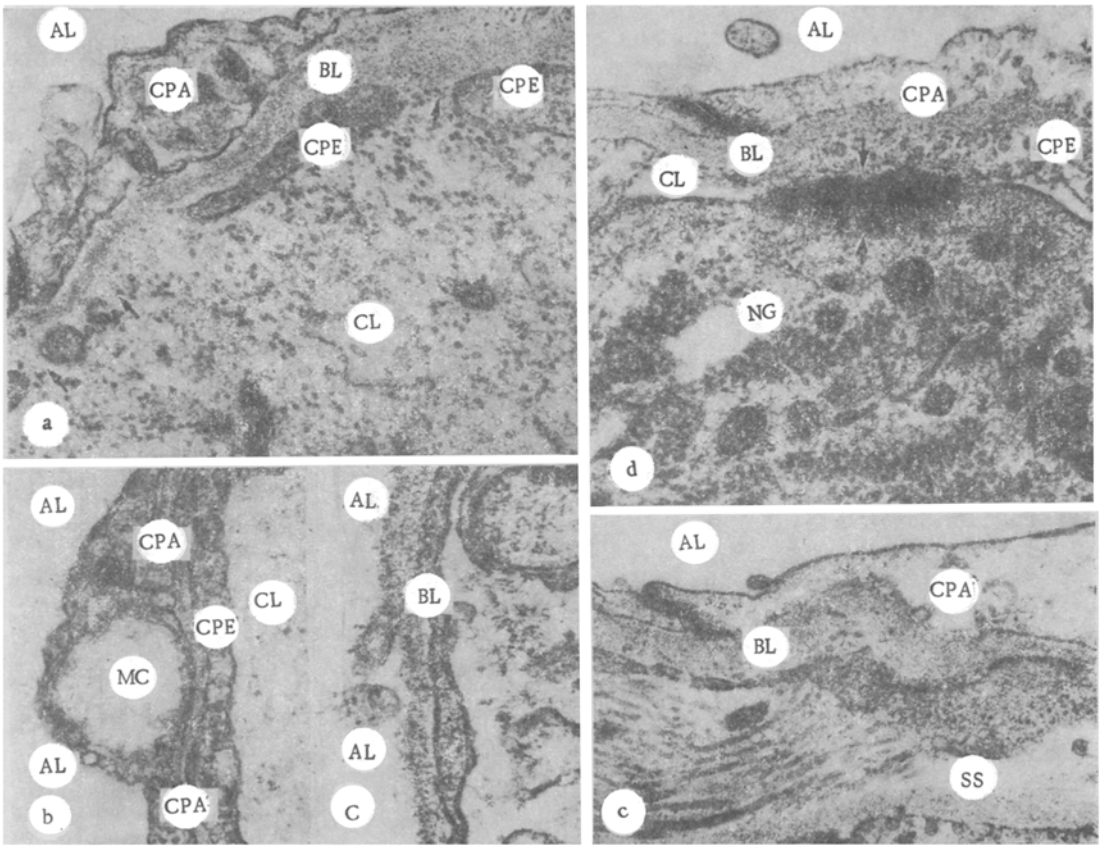

Fig. 2. Extravascular disturbances in ABB of lungs of untreated dogs with hypoxia (a, b, c) and dogs treated by ECMO (d, e). a) Segments of capillary (arrows) without CPE, 16,000×. b) Vacuolated mitochondrion (MC) in cytoplasmic process of type I alveolocyte (CPA), 16,000×. c) Region of ABB without CPA, exposure of basal layer (BL); 20,000×. d) Zone of adhesion of NG to CPE, focal destruction of cytolemma of these cells (arrow); 16,000×. e) Focal edema of CPA and septal space (SS); integrity of cytolemma of CPA undisturbed; 13,000×. CL) Capillary lumen, AL) alveolar lumen.

pinocytotic vesicles, solitary erythrocytes and alveolar macrophages, and networks of tubular myelin (surfactant) were found in the lumen of the alveoli.

In animals with extracorporeal oxygenation disturbance of the structure of the endotheliocytes mainly took the form of focal translucency of the matrix of CPE, disintegration and reduction of the number of intracellular organelles, and local destruction of the cytolemma along the luminal surface, which often was accompanied by adhesion of the blood cells to the endothelium (Fig. 2d). However, complete destruction of CPE and exposure of the basal layer were not observed in any of the samples. Ultrastructural changes in type I alveolocytes were ill defined and appeared mainly as focal edema of the cytoplasm (Fig. 2e). Local disturbance of the integrity of the cytolemma of CPA along the free border was rarely observed. No marked destructive changes were observed in the type I alveolocytes. In the cytoplasm of the type II alveolocytes in most lung samples the lamellar bodies typical of intact animals could not be found. Most frequently they appeared as large vacuoles containing residues of osmiophilic material. In 10% of samples the alveolar lumen contained fragments of destroyed cells and blood plasma. Local destruction of the thin part of ABB was not found in a single dog. Intravascular disturbances in ABB were characterized by the formation of groups of agglutinated erythrocytes or mixed erythrocytes and leukocytes (Fig. 3a, b), which were found in 80% of lung samples. In one-third of the samples only agglutinated leukocytes were seen (Fig. 3c). As a rule marked destructive changes were observed in the groups of agglutinated blood cells.

Comparative analysis of the results shows that in severe hypoventilation hypoxia the entire multicomponent system of the lung ABB is damaged, the microcirculation is disturbed, and capillary and cell permeability is increased. During extrapulmonary oxygenation of the

TABLE 1. Ultrastructural Changes in ABB of Lungs and Frequency of Their Discovery in Dogs with Hypoxia, Treated with ECMO

Character of ultrastructural changes	Number of lung samples with ultrastructural changes, % of total number of samples	
	Hypoxia	Hypoxia and ECMO
Intravascular agglutination of:		
erythrocytes	70	80
leukocytes	0	30
mixture of the two	10	80
destruction of cells in agglutinated groups	50	70
Capillary endotheliocytes:		
destruction of cytolemma of CPA	80	60
distingegration of intracellular organelles of CPE	40	40
total destruction of CPA	40	0
Basal layer:		
edema, lossening of structure, indistinctness of boundaries	60	50
destruction	10	0
Septal space:		
edema, disintegration of fibrous structures	40	40
Type I alveolocytes:		
edema of CPA	30	20
destruction of cytolemma of CPA	30	10
total destruction of CPA	30	0
Type II alveolotyces:		
predominance of vacuolated lamellar bodies	30	60
Destruction of thin part of ABB	10	0
Presence of contents in alveolar lumen	40	10

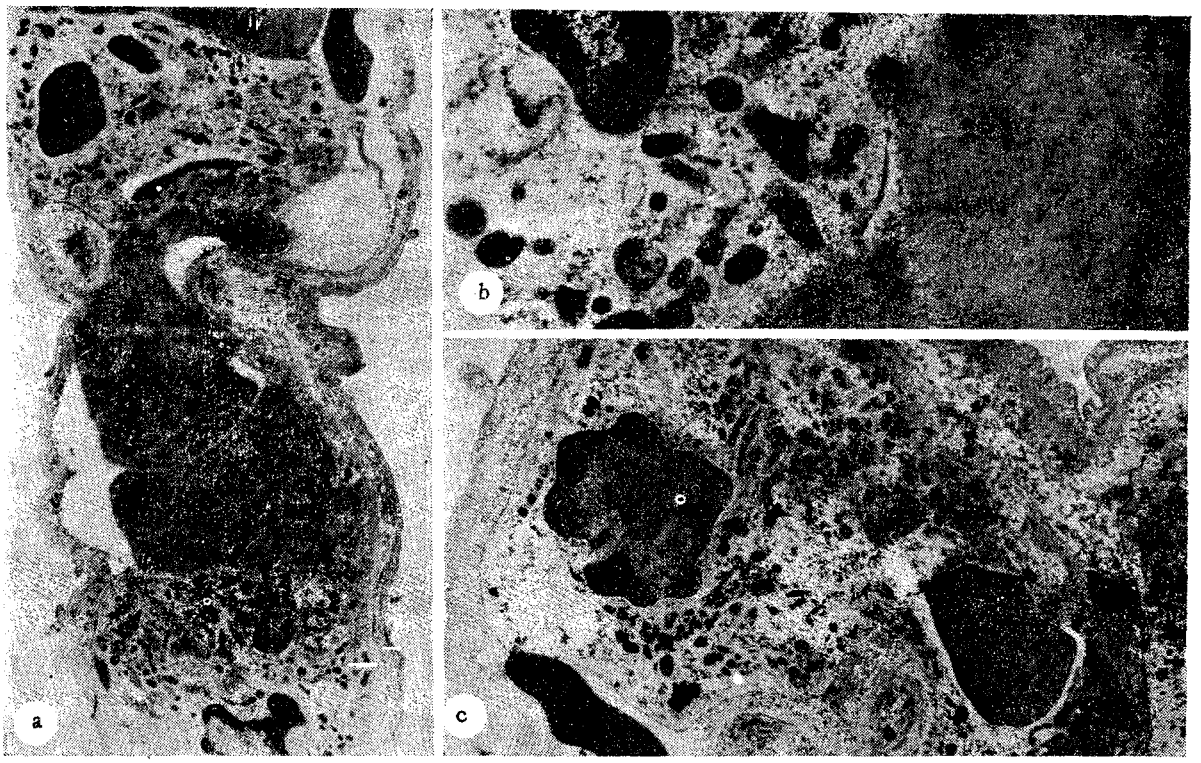

Fig. 3. Intravascular disturbances in ABB of lungs of dogs with hypoxia treated by ECMO. a, b) Agglutinated mixture of erythrocytes and leukocytes, c) agglutinated leukocytes in capillary lumen (CL) of alveolar septum. NG and erythrocytes (E) show destructive changes. AL) Alveolar lumen. Magnification: a) 5000×, b) 13,000×, c) 16,000× detail of Fig. 3a showing zones of adhesion (arrow) of erythrocyte (E) and NG; N) nucleus of NG. Letters and arrows omitted in Russian original figures — Editor.

blood total destruction of the cytolemma of CPE and CPA was observed less frequently than in the control, and total destruction of endotheliocytes, type I alveolocytes, and the thin part of ABB was never observed. Thus if severe hypoventilation hypoxia is treated by the "Sever-OMR" MO, the ultrastructural changes in ABB of the lungs are less profound and widespread in character, evidence of the beneficial effect of ECMO on the state of the lung tissue. However, when the "Sever-OMR" MO was used, a tendency was observed for disturbances of the rheologic properties of the blood to be increased, and this was reflected morphologically in the formation of multiple intravascular groups of agglutinated erythrocytes, leukocytes, or a mixture of both kinds of cells. Further research must therefore be undertaken in order to obtain new and improved types of gas-exchange systems.

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MORPHOMETRIC ANALYSIS OF CHANGES IN HEPATOCYTES OF RABBITS WITH EXPERIMENTAL HYPERCHOLESTEROLEMIA AND THEIR REVERSIBILITY

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KEY WORDS: morphometry; hypercholesterolemia; reversibility; hepatocytes.

Cholesterol is synthesized and converted into bile acids with high intensity in the liver [7, 9]. These processes are modified if intake of this steroid into the gastrointestinal tract is increased for a long period of time [9]. Considerable abnormalities are found under these circumstances both in the morphology and the metabolism of heaptocytes. This statement is confirmed by an increase in the lipid content, changes in karyometric parameters, and changes in the intensity of oxidation–reduction and of hydrolysis, evidence of deviations of many functions of the hepatocytes from normal [10]. Changes arising in experimental hypercholesterolemia in rabbits are similar in many of their parameters to those in patients with atherosclerosis [1]. It is now possible, in principle, largely to correct disturbances of lipid metabolism characteristic of atherosclerosis [6, 8]. The question accordingly arises whether normalization of lipid metabolism is followed by regression of the morphological changes developing under conditions of hypercholesterolemia.

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