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ULTRASTRUCTURAL MORPHOLOGY OF THE AIR—BLOOD BARRIER AND SURFACTANT IN EXPERIMENTAL PNEUMONIA SUPERPOSED ON ALCOHOL POISONING

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Inflammatory diseases of the lungs in alcoholics are characterized by a tendency to run a protracted and complicated course, with predominance of suppurative changes resistant to treatment [4-6]. However, there have been only sporadic studies of changes in the ultrastructure of components of the air—blood barrier (ABB) in pneumonia superposed on alcohol poisoning [2, 5]. There have been virtually no investigations into changes in the state of the lung surfactant system (LSS) in pneumonia superposed on alcohol poisoning, although changes in surface activity of LSS are known to play an important role in the pathogenesis of diseases of the bronchopulmonary apparatus.

The aim of this investigation was to study changes in the ultrastructure of components of ABB and the state of surface activity of LSS in animals with experimental pneumonia superposed on alcohol poisoning.

EXPERIMENTAL METHOD

The material for this investigation consisted of the lungs of 21 male and female Wistar albino cats weighing from 290 to 350 g. As the control, the lungs of five healthy rats of both sexes which, like the experimental animals, were decapitated under thiopental anesthesia, served as the control. All the experimental animals were divided into three groups. Group 1 consisted of 7 healthy albino rats in which pneumonia was induced by the method in [1], by transtracheal insertion of a length of sterile fishing line. In the animals of groups 2 and 3, experimental alcohol poisoning was induced by intragastric injection of 50% ethyl alcohol through a tube in a dose of 4 ml/kg body weight in a single dose at 24-hourly intervals for one month. After this period a similar length of sterile fishing line was inserted by the same method as was described above. Later, the albino rats of group 3 continued to receive ethanol by the above schedule, whereas administration of ethanol to animals of group 2 ceased. All animals of groups 2 and 3 were decapitated 21 days after insertion of the sterile thread as described above. Fragments measuring 1 mm^3, free from large vessels and bronchi, were excised from the lungs for electron-microscopic investigation, fixed in 2.5% glutaraldehyde solution in phosphate buffer, and then postfixed in 1% OsO_4 solution. After dehydration the tissue was embedded in a mixture of Epon-812, DDSA, and MNA. Ultrathin sections were stained with lead citrate and examined in the UEMV-100K electron microscope. The state of surface activity of the LSS was studied by physicochemical (determination of surface tension — ST — of the surface-active fraction of lung extracts isolated from animals as described in [7], on Wilhelmy scales) and biochemical (determination of total lipid levels as in [9], phospholipids as in [10]) methods, and thin-layer chromatography of the phospholipids on "Silufol-UV-254" plates (Czechoslovakia) were used.

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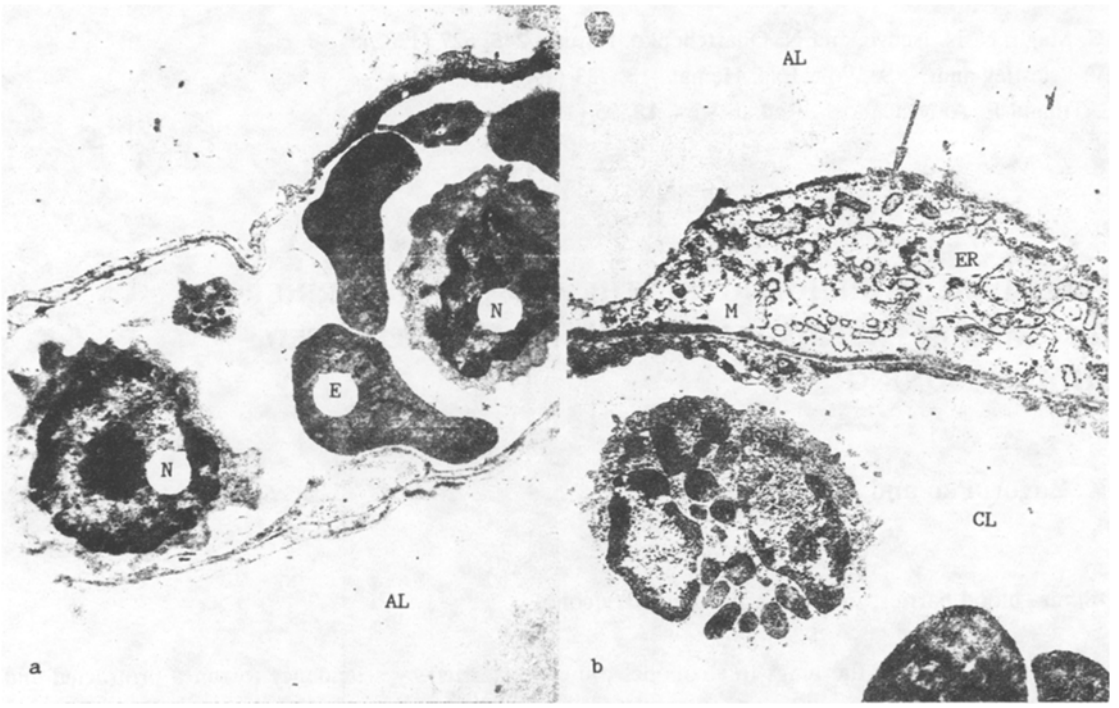

Fig. 1. Changes in ultrastructure of components of air–blood barrier in albino rats with pneumonia: a) congestion of capillaries in alveolar septa. E) erythrocyte, N) neutrophil, AL) alveolar lumen. 12,000×; b) edema of cytoplasm and of intracellular organelles of type I alveolocyte with formation of outgrowth into alveolar lumen (arrow). AL) alveolar lumen, CL) capillary lumen, M) mitochondrion, ER) endoplasmic reticulum.

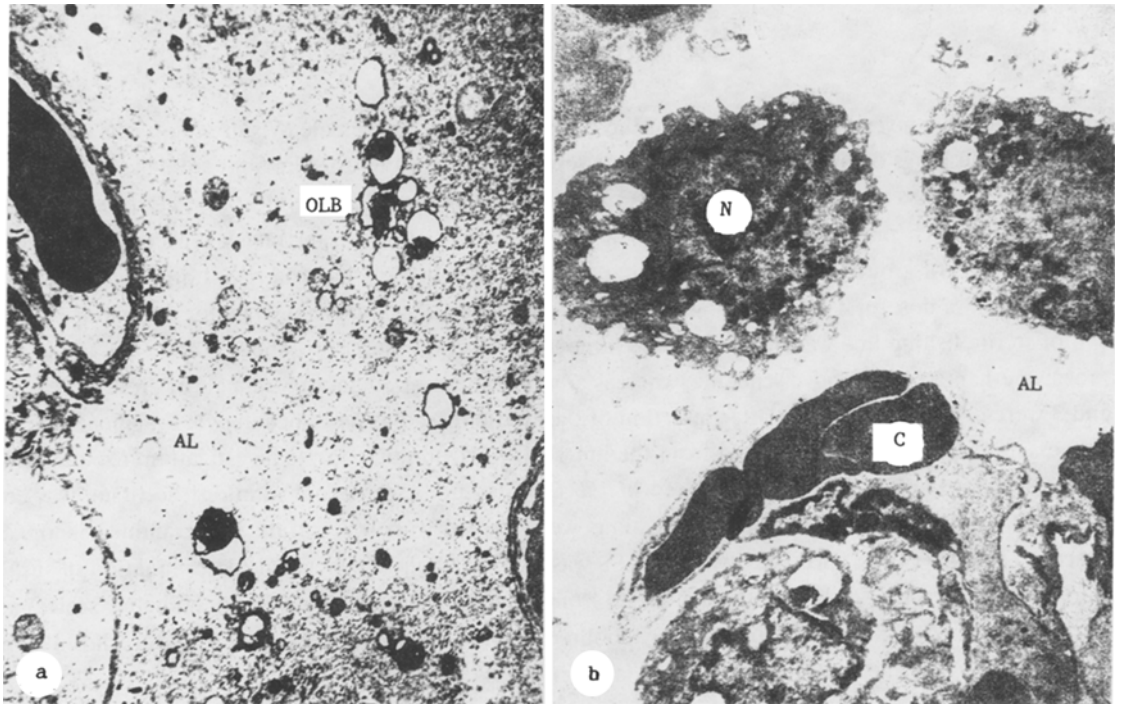

Fig. 2. Changes in ultrastructure of components of air–blood barrier during pneumonia superposed on alcohol poisoning. 8000×. a) nonunfolded OLB and their fragments in alveolar lumen (AL); b) alveolar macrophages in alveolar lumen (AL) have irregularly shaped nucleus (N) with chromatin condensed around periphery of nucleus. C) capillary.

TABLE 1. Surface Tension (ST), Stability Index (SI), Content of Total Lipids and Phospholipids, and Their Qualitative Composition in Surface-Active Fraction of Albino Rats (M ± m)

Group of animals	ST_{min}, mN/m	SI, conventional units	Cont. of total lipids, g/liter	Cont. of phospholipids, mmoles/liter		
				total	phosphatidylcholine	phosphatidylethanolamine
1- (n=7)	21,0±0,57	0,97±0,04	0,89±0,015	0,029±0,007	0,0082±0,0002	0,0082±0,0002
2- (n=7)	21,3±1,13	0,92±0,03	0,96±0,021	0,057±0,016	0,0141±0,0002	0,0111±0,0001
3- (n=7)	17,3±1,48	1,10±0,01	1,03±0,011	0,027±0,005	0,0064±0.0001	0,0087±0,0003
4- (n=5)	14,6±1,00	1.20±0,03	1,08±0,04	0.041±0,004	0,0146±0,0003	0,0123±0,0004

EXPERIMENTAL RESULTS

The results of electron-microscopic study demonstrate that marked changes took place in the lungs of the animals of group 1 with a model of pneumonia, affecting all components of the ABB. Capillaries in the alveolar septa were congested with blood, and contained many neutrophils (Fig. 1a). Cells of the vascular endothelium in places appeared swollen, due to which the lumen of some capillaries was greatly narrowed. The cytoplasm of the endothelial cells in the peripheral portions contained many micropinocytotic vesicles, which sometimes merged to form large vacuoles. In some places the cell cytoplasm appeared unevenly translucent. As a rule the basement membranes were well outlined, although in some places they appeared swollen. The interstitial spaces were widened, and contained areas of accumulation of amorphous material of average electron density. The most marked changes took place in cells of the alveolar epithelium. The following changes were characteristic of type I alveolocytes: the cell cytoplasm appeared unevenly translucent; the mitochondria were greatly swollen, with translucency of their matrix and reduction and disorientation of the cristae; the tubules of the endoplasmic reticulum were distended; the peripheral part of the type I alveolocytes formed outgrowths and evaginations in some places, facing the lumen of the alveoli (Fig. 1b). Signs of degeneration and destruction predominated in the type II alveolocytes; the chromatin content was reduced in the cell nuclei, leading to translucency of the central part of the karyoplasm; the mitochondria were greatly edematous and had a clear matrix with shortened and disoriented cristae; tubules of the endoplasmic reticulum were greatly distended, and some areas were deprived of ribosomes; the number of osmiophilic lamellar bodies (OLB) was reduced to not more than 4 or 5 per cell; the apical surface of the cells was smooth, it contained no microvilli, and in some cells the apical plasmalemma was destroyed, so that the cell contents were released into the lumen of the alveolus. Meanwhile, in some large alveolar cells signs of hyperfunction were observed; however, the number of these hyperfunctioning type II alveolocytes was small. A few erythrocytes, concentrations of leukocytes, and solitary alveolar macrophages could be seen in the lumen of the alveoli. As a rule the macrophages had a nucleus with festooned edges and clearly distinguishable nucleolus, as well as a complex of well developed organelles, among which small lysosomes could be predominantly distinguished, their number varying within wide limits, on average 34-48 per cell. Microscopic outgrowths on the surface of the alveolar macrophages were poorly developed. In the cytoplasm of these cells there were one or two secondary phagosomes.

Analysis of the results of an electron-microscopic study of the lungs of the animals of the two other groups, in which pneumonia was induced in animals poisoned with alcohol, indicates that changes in ultrastructure of the components of ABB in these cases differed significantly from those described above. The manifestations of edema and increased permeability of the components of ABB were common features. However, unlike in the animals of group 1, as a rule only serous fluid was found in the alveolar lumen of the lungs of the animals of groups 2 and 3, whereas single erythrocytes were relatively rare, and neutrophils virtually absent. Meanwhile, in the lumen of the alveoli there were many nonunfolded OLB and their fragments (Fig. 2a), and also many alveolar macrophages, which in some cases literally "stuffed" the alveoli, to occupy almost the whole of their space. The overwhelming majority of alveolar macrophages showed evidence of increased functional activity: the nuclei were irregular in shape, with outgrowths and invaginations of their karyoplasm, and the chromatin was condensed mainly near the nuclear membrane (Fig. 2b); these cells had a powerful lysosomal apparatus, for the number of lysosomes of different shapes and sizes reached 120, with on average 66-84 per cell; as a rule 5 or 6 secondary phagosomes were found in each cell, and in the overwhelming majority of cases they consisted of membranes of OLB or intact osmiophilic bodies.

Incidentally, whereas in the animals of group 2 the number of alveolar macrophages was 45.8 ± 3.6% of all cells of the respiratory part of the lung counted (the count included alveolocytes of types I and II and alveolar macrophages), in the rats of group 3 these cells numbered 72.7 ± 2.4%. It can be concluded from these findings that cessation of alcohol poisoning in the experimental animals was accompanied by a smaller degree of mobilization of the alveolar macrophages than in animals receiving alcohol in the postoperative period also. However, compared with group 1 (42.1 ± 2.7%), in the animals of group 2 and, in particular, those of group 3 the number of alveolar macrophages was nevertheless increased.

Compared with group 1, in the rats of groups 2 and 3 there were significantly more type II alveolocytes in a state of increased functional activity. At the same time, besides these hyperfunctioning type II alveolocytes, numbering 60-65% of all large alveolar cells, in some parts degeneratively changed type II alveolocytes were observed.

The results of this study of the surface-active properties of LSS of the experimental animals are given in Table 1. They show that the parameters characterizing surface-active properties of LSS of the animals of groups 1-3 different significantly from the corresponding parameters in the control group. This was particularly the case with the level of ST_{min} and of Clements' stability index (SI). In this case, although the values of SI differ from the control, they do not reach the critical level (0.75-0.70). It is also follows from Table 1 that the qualitative composition of phospholipids of LSS was significantly changed. In particular, the content bath of total phospholipids and of the phosphatidylcholine fraction, the most active fraction from the standpoint of surface activity ($p < 0.05$), was significantly reduced in the animals of groups 1 and 3 compared with the control ($p < 0.05$ to $p < 0.01$). The phosphatidylethanol amine fraction also was significantly reduced ($p < 0.05$).

It is a very important fact that in the animals of group 2 the content of phospholipids of LSS and also the content of their phosphatidylcholine and phosphatidylethanolamine fractions did not differ significantly from the control values. This state of affairs suggests that cessation of alcohol poisoning had a beneficial effect on the state of the LSS during pneumonia, whereas continued administration of ethanol to the animals postoperatively aggravated the LSS deficiency arising under the conditions of inflammation of the lungs.

It can be concluded from these results that in albino rats with experimental pneumonia the surface-active properties of LSS are depressed, and this is linked with changes in the ultrastructure of the components of ABB, the dynamics of which was described in detail previously [3]. As a background against which the inflammatory changes in the lung develop, alcohol poisoning aggravates depression of the surface-active properties of LSS. This is evidently associated with two circumstances. First, as a lipotropic substance, alcohol can dissolve the monomolecular film on the alveolar surface [11]. Consequently, alcohol poisoning preceding the development of pneumonia itself leads to changes in the LSS, as shown by the results of previous investigations [2]. As the response to the SLL deficit arising in connection with alcohol-induced damage the functional activity of the type II alveolocytes is enhanced, and these cells begin to produce an extra amount of surface-active material. Second, increased production of lung surfactant (LS) by the type II alveolocytes, under conditions of alcohol poisoning leads to the appearance of large numbers of alveolar macrophages, for according to existing data [5], close correlation exists between the number of alveolar macrophages and the quantity of LS on the alveolar surface. The alveolar macrophages undertake active phagocytosis of the LS secreted by the type II alveolocytes. Thus experimental inflammation of the lungs, induced against a background of alcohol poisoning, arises under conditions of an already existing LS deficiency. Reduction of alcohol poisoning during the development of experimental pneumonia is accompanied by some degree of improvement of the qualitative composition of the phospholipids of LS, first, because the number of mobilized macrophages decreases and, accordingly, the quantity of LS phagocytosed by them is reduced, and second, because many of the type II alveolocytes are in a state of hyperfunction, and are producing extra LS required to maintain the stability of alveoli under conditions of inflammation. Conversely, alcohol poisoning continuing after insertion of the sterile thread is accompanied by mobilization of an ever-increasing number of alveolar macrophages, which ingest the SL produced by the type II alveolocytes, and this aggravates the LS deficiency appearing as a result of inflammation of the lungs even more.

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EFFECT OF PROPER VAPOR LASER RADIATION ON MICRORELIEF AND ULTRASTRUCTURE OF GLANDULOCYTES OF THE GASTRIC MUCOSA

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The stimulating effect of low-intensity laser radiation (LILR) has accounted for the widespread use of different types of lasers in medicine. The helium-neon laser (HNL) has achieved the most widespread popularity [5, 7]. A series of investigations has shown that the structural basis of the stimulating effect of HNL is an increase in the rate of proliferation of cells combined with an increase in the rate of their differentiation [2-4]. LILR induces characteristic changes in intracellular structures, reflecting the intensification of intracellular processes. This is manifested as an increase in the volume of profiles of the endoplasmic reticulum, an increase in the number and size of the mitochondria, etc. [1, 6].

At the present time, to stimulate repair processes and, in particular, to accelerate the healing of gastric and duodenal ulcers, copper vapor lasers (CVL) are used. However, many of the structural aspects of the effect of LILR of CVL on the gastric mucosa remain unstudied.

EXPERIMENTAL METHOD

The microrelief and ultrastructure of cells of the fundal glands of 28 Wistar albino rats were studied. The power of the radiation at the object level was 8 mW, the diameter of the zone of irradiation 3 mm, the duration of the procedure 1, 3, and 5 min, and the doses 6.78, 20.34, and 33.909 J/cm^2, respectively. The animals were killed by instant decapitation 5-10 min and 1 h after irradiation. In the control group, similar manipulations were carried out with the animals, but without irradiation. Tissue samples, fixed with glutaraldehyde and postfixed with osmic acid, were dehydrated for scanning electron microscopy in alcohol and acetone, dried by the critical point method, and sprayed with gold (HCP-2 and IB-3 instruments, "Hitachi," Japan). For transmission electron microscopy, Epon-Araldite sections were subjected to double staining and examined in the "Hitachi" H-600 microscope. Semithin sections were stained with methylene blue and fuchsine.

EXPERIMENTAL RESULTS

Examination of semithin sections after intragastric irradiation of the gastric mucosa for 1 min revealed congestion of the microvessels of the gastric mucosa, and an increase in size of the cells of the fundal glands, especially the chief cells (Fig. 1a). Changes were observed in the surface of the gastric mucosa, with variation in the context of mucoid in the surface and pit cells (Fig. 1b, c). Changes in microrelief were seen particularly clearly during scanning electron microscopy. The ridges surrounding

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