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CHANGES IN SURFACTANT ACTIVITY AND ULTRASTRUCTURE OF THE AIR–BLOOD BARRIER IN EXPERIMENTAL ALCOHOL POISONING

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In alcoholism, ethanol acts on the system of local and general defense [2], but there is no information in the literature on the state of the lung surfactant (LS) system in alcohol poisoning, although changes in the surface activity of that system play an important role in the pathogenesis of various diseases of the bronchopulmonary system [1, 3].

The aim of this investigation was accordingly to study changes in the surface-active properties of LS and in the ultrastructure of the air–blood barrier (ABB) in experimental alcohol poisoning.

EXPERIMENTAL METHOD

The lungs of 26 Wistar albino rats of both sexes, weighing from 290 to 350 g, in which acute and chronic poisoning with ethyl alcohol was induced, were subjected to physicochemical [determination of maximal and minimal surface tension (ST_{min}) of the surface-active fraction of lung extracts from the animals on Wilhelmy scales], biochemical (determination of total lipids and phospholipids in LS followed by thin-layer chromatography of the latter), and electron-microscopic investigation. Acute poisoning was produced by intragastric administration of 50% ethyl alcohol, in a dose of 7.5 ml pure ethanol/kg body weight, divided into fractions and given at 5-min intervals. Group 1 consisted of six such animals. Chronic ethanol poisoning was produced by giving 50% ethyl alcohol by gastric tube in a dose of 4 ml/kg body weight in a single dose at 24-hourly intervals. All the animals were killed by decapitation under thiopental anesthesia 1.5 months (group 2 – five observations), 3 months (group 3 – five), 6 months (group 4 – three), and 12 months (group five – seven) after the beginning of the experiment. The lungs of five healthy animals were used as the control.

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EXPERIMENTAL RESULTS

The results of a study of the surfactant properties of LS are given in Table 1. In animals in which acute ethanol poisoning was induced, the surface activity of LS decreased. This was shown by an increase in ST_{min} from 14.6 ± 1.0 mN/m in the control to 25.5 ± 1.2 mN/m ($p < 0.001$), accompanied by a sharp fall in the phospholipid level in LS to 0.018 ± 0.004 mM (0.041 ± 0.004 mM in the control; $p < 0.02$). The phosphatidylcholine fraction, with the highest surface activity, accounted for only 9.4 ± 3.1% of the phospholipids of LS, significantly less than in the control observations (27.4 ± 3.1%; $p < 0.01$).

This inhibition of the surface activity of LS may be due, in our opinion, to two factors: first, the direct action of ethanol in damaging the surface-active film and the structure of the tubular myelin, for being a lipotropic substance, ethanol can pass easily through cell membranes and, consequently, it can enter the lumen of the alveolus through structures of the ABB; second, protein components of the blood plasma, which enter the lumen of the alveolus because of increased permeability of the components of ABB, may also have an inactivating effect on LS.

These suggestions were confirmed by electron-microscopic study of the lungs of this group of animals. Analysis of the state of the ultrastructure of the components of ABB revealed congestion with signs of stasis in the capillaries of the alveolar septa (Fig. 1a). The cytoplasmic processes of the endothelial cells, which on the whole preserved their characteristic structure, contained large numbers of micropinocytotic vesicles. The interstitial space was widened (Fig. 1b). The type I alveolocytes appeared edematous and had pale cytoplasm, which contained a few organelles. The cytoplasmic processes of these cells over a large area of ABB formed saillike evaginations, projecting into the alveolar lumen (Fig. 1c). The type II alveolocytes showed much less marked changes. As a rule these cells contained a complex of well developed organelles, among which osmiophilic lamellar bodies (OLB) could be distinguished, numbering on average 12-14 per cell. Signs of edema were visible only in some type II alveolocytes in the form of regular translucencies of the cytoplasm, swelling of the mitochondria, and dilatation of the tubules of the cytoplasmic reticulum. Serous fluid, undeveloped OLB or their fragments, and disorganized membrane formations of LS were found in the alveolar lumen of the lungs of this group of animals (Fig. 1d).

As Table 1 shows, during long exposure of the albino rats to ethanol the surface-active properties of LS also were inhibited, as shown by a significant ($0.001 < p < 0.05$) increase in the values of ST_{min} and a fall in the level of phospholipids of the surface-active fraction of the lung extracts; the content of phosphatidylcholine, moreover, the fraction with the greatest surface activity, in the composition of these extracts was reduced. These changes were most marked in animals exposed to the action of ethanol for 12 months. The highest level of ST_{min} (28 ± 1.5 mN/m) and the lowest value of Clements' stability index (0.71 ± 0.06) were noted in this group. The level not only of phospholipids, but also of total lipids (0.87 ± 0.03 g/liter; 1.08 ± 0.04 g/liter in the control; $p < 0.02$) was lowered in the composition of the surface-active fraction of the animals of this group. The phosphatidylcholine fraction in the composition of LS amounted to only 15.1 ± 2.8%.

Ultrastructural changes in the components of ABB during long-term ethanol administration included moderately severe edema, affecting mainly cells of the alveolar epithelium and, in particular, the type I alveolocytes. Foci of translucency of the cytoplasm and swelling of the mitochondria were observed in these cells, and the cytoplasmic processes in some places formed outgrowths and evaginations projecting into the lumen of the alveoli. Edema also spread to some type II alveolocytes, in which, besides foci of translucency of the cytoplasm and swelling of the mitochondria, tubules of the cytoplasmic reticulum were dilated, and this was accompanied by a reduced number of ribosomes in its rough portion. The apical surface of the type II alveolocytes in these cases was smoothed, and there was a sharp decrease in the number of microvilli on it. Meanwhile, the overwhelming majority of type II alveolocytes, especially in animals poisoned with ethanol for 6 and 12 months, showed signs of increased functional activity. The cytoplasm of these cells contained many mitochondria of normal size with a moderately dense matrix, tugbules of the rough cytoplasmic reticulum, rich in ribosomes, were hypertrophied, and the number of OLB reached 14-16 or more per cell. Type II alveolocytes, which were in a state of increased functional activity (Fig. 2a), secreted osmiophilic material, which was found in the alveolar lumen in the form of concentrations of undeveloped OLB (Fig. 2b).

Comparison of the results of the electron-microscopic study of the animals' lungs with those of the study of the surface active properties of LS revealed a paradox: most cells responsible for LS production (type II alveolocytes) were in a state of enhanced functional activity, but the results of the physicochemical and biochemical tests revealed inhibition of the surface activity of LS.

TABLE 1. Values of ST, Clements' Stability Index (SI), Total Lipid and Phospholipid Levels of LS and Their Qualitative Composition in Surface-Active Fraction of Lung Extracts of Albino Rats with Acute and Chronic Ethanol Poisoning ($M \pm m$)

Group of observations	ST_{min}, mN/m	Clements' SI	Total lipid content, g/liter	Phospholipid content		
				total, mM	introducing	
					phosphatidylcholine %	phosphatidylethanolamine, %
1 (acute ethanol poisoning; n = 6)	25,5±1,2*	0,9±0,06*	1,06±0,02	0,018±0,004*	9,4±4,3*	31,2±3,1
2 (ethanol poisoning for 1.5 months; n = 5)	20,2±0,6*	0,87±0,03*	0,90±0,005*	0,028±0,006	None*	25,3±1,7*
3 (ethanol poisoning for 3 months; n = 5)	28,3±3,8*	0,78±0,16*	1,01±0,02	0,059±0,024	23,3±1,9	32,5±3,7
4 (ethanol poisoning for 6 months; n = 3)	24,8±1,1*	0,92±0,04*	1,05±0,01	0,029±0,002*	24,5±4,3	19,9±5,5*
5 (ethanol poisoning for 12 months; n = 7)	28,5±1,5*	0,71±0,06*	0,87±0,03*	0,039±0,013	15,1±2,8*	38,6±4,2
Control (n=5)	14,6±1,0	1,2±0,03	1,08±0,04	0,041±0,004	27,4±3,1	35,5±2,4

Legend. Differences compared with control significant ($0.001 < p < 0.05$).

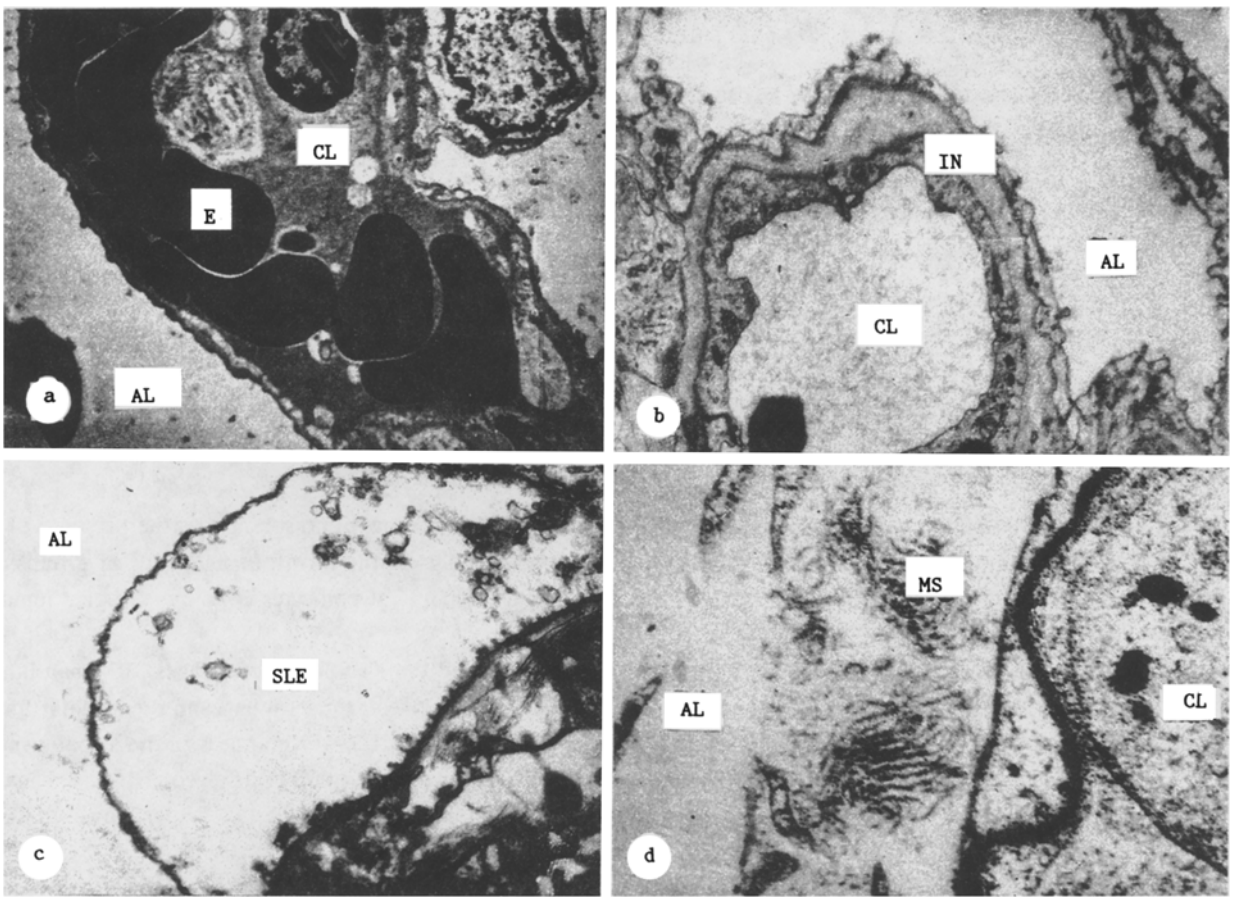

Fig. 1. Changes in ultrastructure of components of ABB in acute experimental ethanol poisoning. a) Stasis of erythrocytes (E) in capillary of alveolar septum; CL) capillary lumen; AL) alveolar lumen. 11,000×; b) Widening of interstitial space (IN). CL) Capillary lumen; AL) alveolar lumen. 11,000×; c) Sail-like evagination (SLE) of cytoplasmic process of type I alveolocyte. AL) Alveolar lumen. 13,000×; d) disorganization of membrane structures (MS) of tubular myelin. AL) Alveolar lumen; CL) capillary lumen. 60,000×.

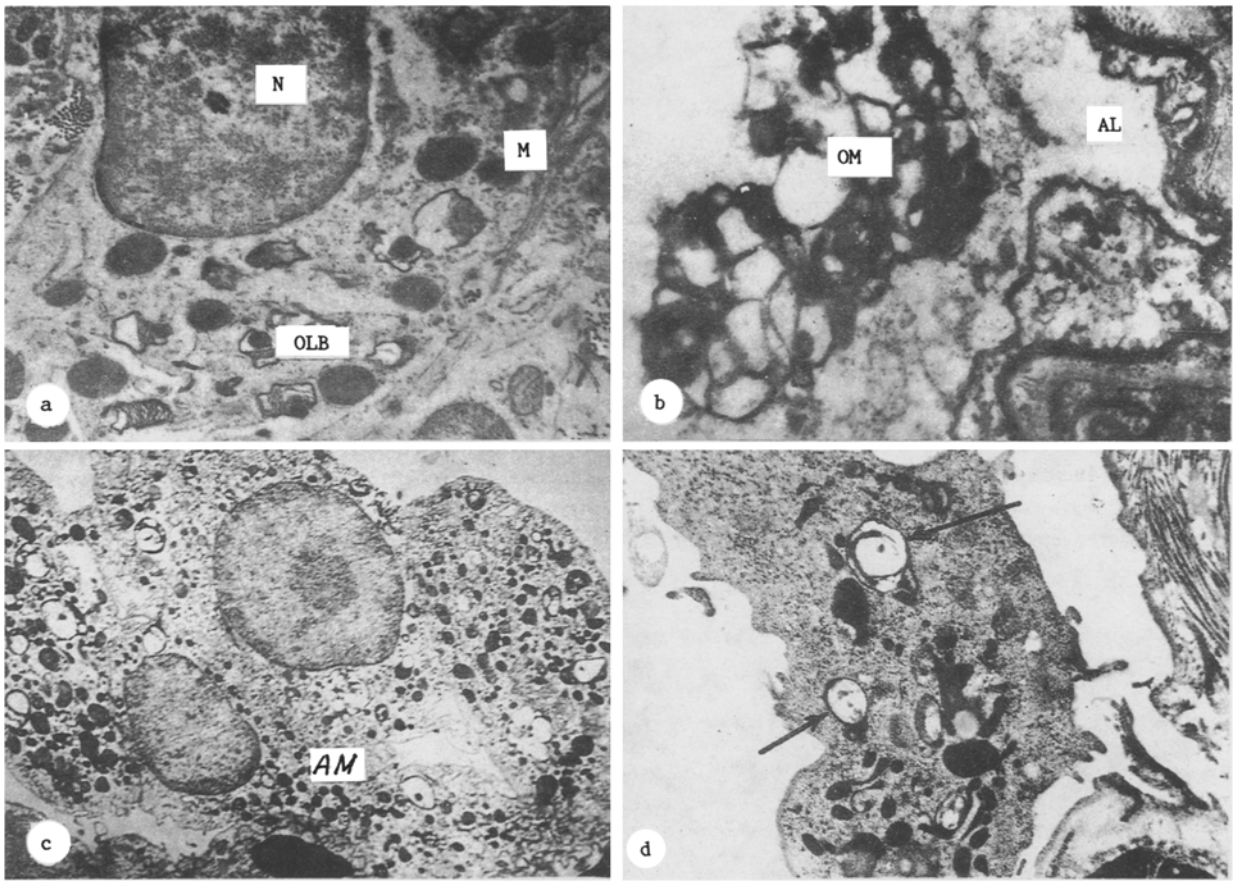

Fig. 2. Changes in ultrastructure of components of ABB in chronic experimental ethanol poisoning. a) Fragment of type II alveolocyte in state of enhanced functional activity. N) Nucleus; M) mitochondria; OLB) osmiophilic lamellar bodies. 26,000×; b) Osmiophilic material (OM) in alveolar lumen (AL). 40,000×; c) concentration of alveolar macrophages (AM) in alveolar lumen. 11,000×; Cytoplasm of alveolar macrophage contains phagocytosed osmiophilic material (arrow). 13,000×.

The most characteristic feature of the lungs of the albino rats in chronic ethanol poisoning was the presence of numerous alveolar macrophages. Alveolar macrophages, found in nearly every alveolus in the form of single cells or groups of them (Fig. 2c) actively phagocytosed the osmiophilic material secreted by the type II alveolocytes into the alveolar lumen. This phagocytosed material could be found in most cells (Fig. 2d).

Close correlation exists between the state of surface activity of LS and the alveolar macrophages [6]. Quantitative and qualitative changes in phospholipids of LS lead to an increase in the number of alveolar macrophages in the alveolar lumen [5]. Moreover, one function of the alveolar macrophages is phagocytosis of "superfluous" LS, which appear in the alveolus in various pathological states [4]. In this connection it can be postulated that the increase in the number of alveolar macrophages in the alveolar lumen in chronic ethanol poisoning is connected with the need for phagocytosis of the "superfluous" LS, which is secreted into the alveoli by type II alveolocytes in a state of enhanced functional activity.

Thus under the influence of ethanol the surface-active properties of LS of the surfactant fraction of lung extracts of albino rats are inhibited. In acute ethanol poisoning this is connected with the harmful effect of ethanol, directly damaging the surface-active film, and with inactivation of LS by plasma proteins penetrating into the alveolar lumen because of edema of the components of ABB. In chronic alcohol poisoning inhibition of the surface activity of LS is due to an increase in its catabolism by alveolar macrophages.

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MORPHOMETRIC CHARACTERISTICS OF HEPATOCYTE MITOCHONDRIA FOLLOWING PERORAL ADMINISTRATION OF WATER CONTAINING BORON

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Boron compounds are widely distributed in nature, and they invariably occur in the composition of both ordinary drinking waters and of mineral waters; they may reach high concentrations, moreover, in the latter [4, 8]. If boron-containing waters are taken internally, they can modify coupling of oxidation and phosphorylation [5] and can intensify processes of preparative regeneration [2]. Meanwhile many aspects of the biological and therapeutic action of boron-containing waters remain to be studied.

The aim of this investigation was to study changes in hepatocyte mitochondria and the boron content in the liver of animals taking internally the natural Semigor'e and artificially prepared boron-containing waters (NW and AW respectively), containing equal concentrations of boron, but differing in their ionic salt basis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-250 g. The experimental animals were given perorally 3 ml of sodium chloride-hydrocarbonate NW with a boron concentration of 250 mg/liter (chemical composition of the water: $M_{10.6} = (HCO_3 \cdot 63Cl \cdot 34)/Na \cdot 96$ (pH 8.0) and AW in the form of an aqueous solution of sodium tetraborate with a boron concentration of 250 mg/liter (pH 8.0). The daily dose of boron was 3.75 mg/kg. Animals taking piped water served as the control. The course consisted of 24 procedures. The animals were killed by decapitation after 15 and 24 procedures, and also on the 6th day after the end of the procedures. The boron content in the liver was determined by a spectrophotometric method with H-resorcin [6]. Pieces of liver tissue for electron microscopic investigation were fixed in 3% glutaraldehyde, made up in phosphate buffer (pH 7.37), and then post-fixed in OsO_4. The material was dehydrated in alcohols of increasing concentrations and propylene oxide, and embedded in a mixture of Epon and Araldite. Ultrathin sections were examined in the IEM-100C electron microscope. For quantitative estimation of the state of the mitochondria we used stereometric system analysis [1] and automated morphometry, with the aid of an IBAS-2 television image analyzer ("Opton," West Germany). Measurements were made automatically under IBAS-2 control, using a program developed in the Laboratory of Cytology, Research Institute of Physicochemical

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