EFFECT OF LONG-TERM EXPOSURE TO HYPERBARIC CONDITIONS ON LIPID PEROXIDATION AND ON ERYTHROCYTE STRUCTURE AND FUNCTION

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Exposure to long-term hyperbaric conditions presupposes the action of a combination of extremal factors, leading to the development of both adaptive and pathological reactions [3]. One manifestation of the pathological process arising under the influence of a hyperbaric helium–oxygen environment is the development of a "high pressure nervous syndrome," leading to the appearance of metabolic disturbances in nerve and other tissues [2, 3]. The main point of application of the compression and anesthetic action of inert gases, which are components of hyperbaric mixtures, is considered to be the biomembranes [14]. Meanwhile the leading role of free-radical processes and of biomembrane damage in the nonspecific response to the action of extremal and environmental factors is well known [8]. Consequently, the study of activity of lipid peroxidation (LPO) and of the structural and functional state of erythrocyte membranes under hyperbaric conditions is particularly interesting.

In the investigation described below the effect of a hyperbaric helium–oxygen mixture was studied on LPO parameters in the blood plasma and erythrocyte membranes, on activity of enzymes of the erythrocyte antioxidative system, namely superoxide dismutase (SOD), catalase, and glucose-6-phosphate dehydrogenase (G-6-PD), and on the stability and structural properties of erythrocyte membranes, was studied.

EXPERIMENTAL METHOD

Albino mice weighing 50-70 g were used. The effect of a helium–oxygen (normoxic) environment under a pressure of 3.6 MPa (ambient temperature +32.5°C, humidity 65-70%) on animals was investigated. Compression lasted 5 h, neutral pressure 5 days, and decompression 18 h. Under hyperbaric conditions on this schedule the initial symptoms of a "high pressure nervous syndrome" were observed: mild tremor, disturbance of movement coordination, and changes in brain electrical activity. Intact animals and animals kept in the pressure chamber under normobaric atmospheric conditions for the same period of time as the experimental animals (chamber control), served as the control.

Blood from three or four animals was pooled into one sample. Erythrocyte membranes were isolated by the method in [7] and lipids were extracted from the blood plasma and erythrocyte membranes by the method in [11]. LPO activity was estimated from the concentrations of primary LPO products (conjugated dienes [5]) and of terminal fluorescent products (Schiff bases [5]). The content of total lipids was determined by the phosphovanillin method [6], cholesterol by Ilkes' method [7], phospholipids as in [10], total peroxidase activity (TPA) as in [9], SOD activity as in [12], catalase as in [13], and G-6-PD as in [4]. Protein was determined by a modified Lowry's method [15] and hemoglobin by the cyanhemoglobin method, using a standard kit for clinical determinations. The structural properties

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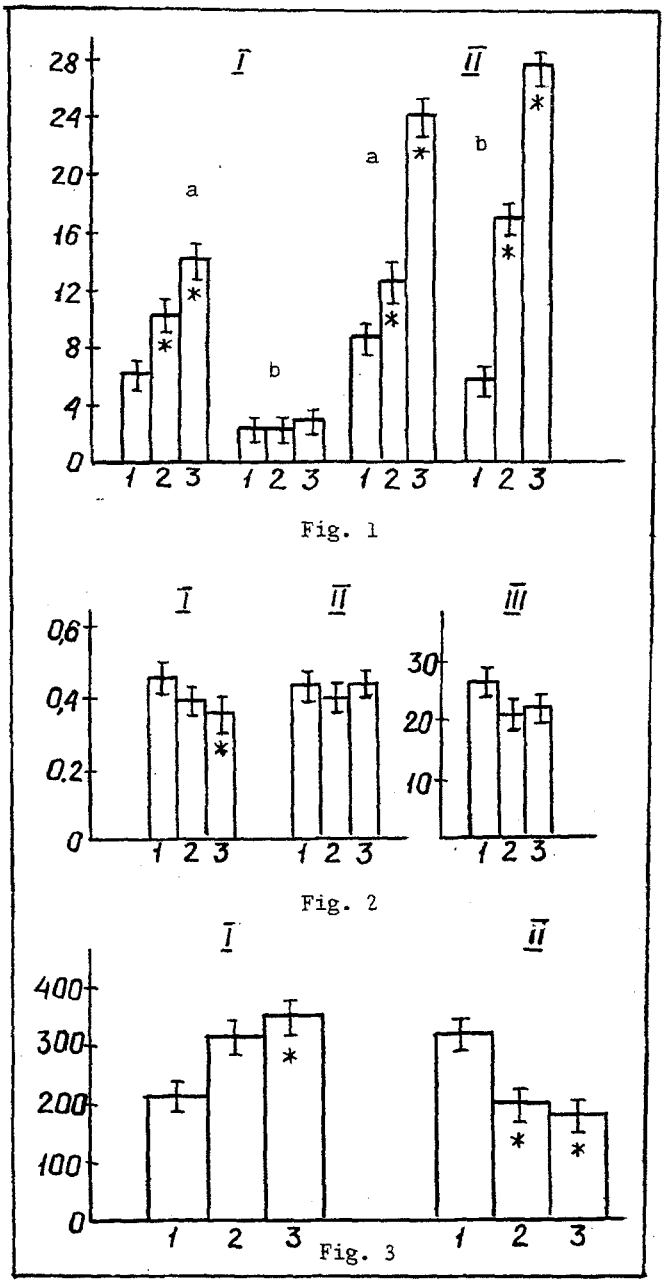

Fig. 1. Effect of long-term exposure to hyperbaric conditions on levels of conjugated dienes (a) and Schiff bases (b) in blood plasma (I) and erythrocyte membranes (II). Ordinate, level of LPO products: conjugated dienes (in nanomoles/mg lipid) and Schiff bases (in relative units/mg lipid). 1) Control, 2) chamber control, 3) hyperbaric conditions (3.6 MPa helium–oxygen normoxia, compression – 5 h, neutral pressure – 5 days, decompression – 18 h). *$p < 0.001$.

Fig. 2. Changes in fluorescence of pyrene in suspension of mouse erythrocyte membranes during exposure to long-term hyperbaric conditions. I, II) Coefficients of excimerization of pyrene (10 μM) at excitation wavelength of 334 nm [F_e/F_m (334)] and 287 nm [F_e/F_m (287)], III) quenching of fluorescence of proteins by pyrene – ΔF. Ordinate: I, II) F_e/F_m (334) and F_e/F_m (287), ΔF (in relative units). 1) Control, 2) chamber control, 3) hyperbaric conditions. *$p < 0.02$.

Fig. 3. Cholesterol concentration in erythrocyte membranes (I) and blood plasma (II) of mice under long-term hyperbaric conditions. Ordinate, cholesterol concentration (in μg/mg lipid). 1) Control, 2) chamber control, 3) hyperbaric conditions. *$p < 0.05$.

TABLE 1. Effect of Long-Term Exposure to Hyperbaric Conditions on Activity of Enzymes of Erythrocyte Antioxidative System and on Stability of Mouse Erythrocyte Membranes (M ± m)

Parameter	Control	Experimental conditions	
		chamber control	experiment
Erythrocytes			
SOD, activity units/mg hemoglobin	2,12±0,31 (8)	1,79±0,34 (6)	1,18±0,21* (4)
Catalase, activity units/mg hemoglobin	4,49±0,57 (4)	3,52±0,26 (7)	3,79±0,33 (4)
G-6-PD, nmoles NADPH/mg protein/min	3,12±0,14 (4)	2,92±0,24 (5)	2,52±0,15* (4)
Plasma			
TPA, activity units/mg protein	10,14±1,55 (4)	13,91±1,92 (4)	19,57±2,60* (4)
SOD, activity units/mg protein	0,100±0,015 (4)	0,114±0,028 (5)	0,367±0,036** (4)

Legend. Number of pooled blood samples shown in parentheses. *$p < 0.05$, **$p < 0.01$ compared with control.

of the erythrocyte membranes were studied with the aid of the fluorescent probe pyrene. The microviscosity of the lipid bilayer and zones of protein–lipid contact of the membranes was estimated with the aid of values of coefficients of pyrene excimerization F_e/F_m (334) and F_e/F_m (286), equal to the ratio of the intensity of fluorescence of its excimers (F_e) and monomers (F_m) at a wavelength of excitation of 334 nm and 286 nm [1]. In a viscous medium the degree of excimerization of pyrene is reduced [1]. Correspondingly, the parameters F_e/F_m (334) and F_e/F_m (286) are inversely proportional to the microviscosity. The degree of penetration of the lipid bilayer by the protein component was determined from the level of quenching of protein fluorescence by pyrene (ΔF) as a result of nonradiative transfer of energy from protein to pyrene [1].

EXPERIMENTAL RESULTS

Keeping the mice under long-term hyperbaric conditions led to activation of the initial stages of LPO in the blood plasma, in which the conjugated diene level rose by 141% (Fig. 1). Hyperbaric conditions led to a greater increase in the intensity of the process in the erythrocyte membranes in the final stages. The level of primary LPO products rose by 185%, and that of fluorescent end-products (Schiff bases) by 450%. Keeping the mice under normobaric atmospheric conditions in the pressure chamber (chamber control) also caused elevation of the blood LPO level, but much less so than in the experimental group. The increase in the intensity of LPO in the blood under the influence of a helium–oxygen environment under a pressure of 3.6 MPa may be due to different causes and, mainly, to the stressor action of hyperbaric factors and insufficiency of the erythrocyte antioxidative system.

An important role in maintenance of a stationary LPO level in the erythrocytes is played by SOD and catalase, and G-6-PD is a very important source of NADPH, which is necessary for the functioning of several antioxidative enzymes. Under hyperbaric conditions SOD and G-6-PD activity in mouse erythrocytes fell by 44% and 19% respectively (Table 1).

LPO activity during exposure to hyperbaric conditions is followed by increased permeability of the erythrocyte membranes. This is confirmed by an increase of 93% in TPA and an increase of 267% in plasma SOD activity, evidence of destabilization of the erythrocyte membrane under hyperbaric conditions (Table 1).

Uncompensated activation of LPO may be the cause of disturbance of the structural organization of biomembranes [1]. Under hyperbaric conditions the degree of excimerization of pyrene F_e/F_m (334) in erythrocyte membranes fell by 27% (Fig. 2), evidence of an increase in the relative microviscosity of the lipid bilayer. Under hyperbaric conditions an increase in the relative microviscosity of the lipid bilayer was accompanied by an increase in the concentration of cholesterol, the most important regulators of biomembrane stability, in the erythrocyte membranes and a fall in its plasma level (Fig. 3).

Structural parameters of erythrocyte membranes characterizing the relative microviscosity of zones of protein–lipid contact, namely F_e/F_m (286) and the degree of penetration of proteins into the lipid bilayer (ΔF) during exposure to hyperbaric conditions did not change significantly (Fig. 2).

The formation of hydrophilic peroxide clusters in the internal regions of the membrane and an increase in the concentration of internal membrane water [1, 8] during activation of LPO assumes special significance under the conditions of exposure to a hyperbaric helium–oxygen environment. Considering the increased solubility of helium in the aqueous phase [14], it can be tentatively suggested that under hyperbaric conditions, during induction of LPO, "water–helium corrosion" of the membranes takes place and is accompanied by disturbance of their permeability and of their structural and functional organization.

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