CHANGES IN THE INTERNAL ORGANS FOLLOWING HEMODILUTION DURING HYPERBARIC OXYGENATION

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The possibility of maintaining the viability of an organism in which oxygen transport is carried out without erythrocytes, but in the form of gas physically dissolved under increased barometric pressure, has been demonstrated experimentally [4].

The study of morphological changes in the tissue systems of organs functioning under these conditions and determination of the factors responsible for their development, which are essential steps in the effective and safe use of "bloodless" perfusion in surgery and othe branches of medicine, were the principal aims of the investigation described below.

EXPERIMENTAL METHOD

Experiments were carried out on 62 dogs weighing from 5 to 10 kg. After morphine premedication and under hexobarbital anesthesia, the animals' blood was replaced by a colloidal preparation of dextran (polyglucin). In the experiments of series I (35 dogs) 100% of the circulating blood volume was replaced until the final hemoglobin concentration was 2-2.5 g%; in series II (27 dogs) maximal hemodilution was obtained (0.5-0.8 g% hemoglobin). All the animals were kept in a pressure chamber in oxygen under a pressure of 3 atm for 2 h. After this some of the dogs were killed by air embolism, the rest at various times (from 1 to 30 days) after decompression and retransfusion of blood.

The motor cortex of the brain, heart, liver, kidneys, and lungs were studied. Sections were stained with hematoxylin and eosin and by Nissl's method (brain). Histochemical reactions were carried out for nucleic acids (by Einarson's method), for protein (tetrazolium reaction), for glycosaminoglycans (with toluidine blue), and the PAS reaction also was performed. Activity of succinate dehydrogenase (SDH), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH), and aspartate aminotransferase (AST) was determined. The concentrations of lactic and pyruvic acids in the blood were determined by biochemical methods [6].

Material for electron-microscopic investigation was fixed in glutaraldehyde, postfixed in 2% buffered OsO_4 solution, processed in the usual way, and embedded in Durcupan. Ultrathin sections were studied and photographed in the IEM-100B electron microscope.

EXPERIMENTAL RESULTS

In the course of hemodilution under hyperbaric oxygenation (HBO) conditions a microvascular response developed in the internal organs, of the same type in animals in both series of experiments. The intensity varied considerably depending on organ specificity and was increased in the experiments with maximal hemodilution. In the heart, liver (Fig. 1a), kidneys, and lungs the PAS reaction of the basement membranes of the vessel walls was intensified, perivascular and interstitial edema infiltrated with leukocytes mainly of the lymphoid series, developed, degranulation of the mast cells of the connective tissue was observed and their glycosaminoglycan content was reduced. Changes in the ultrastructure of the lung capillaries

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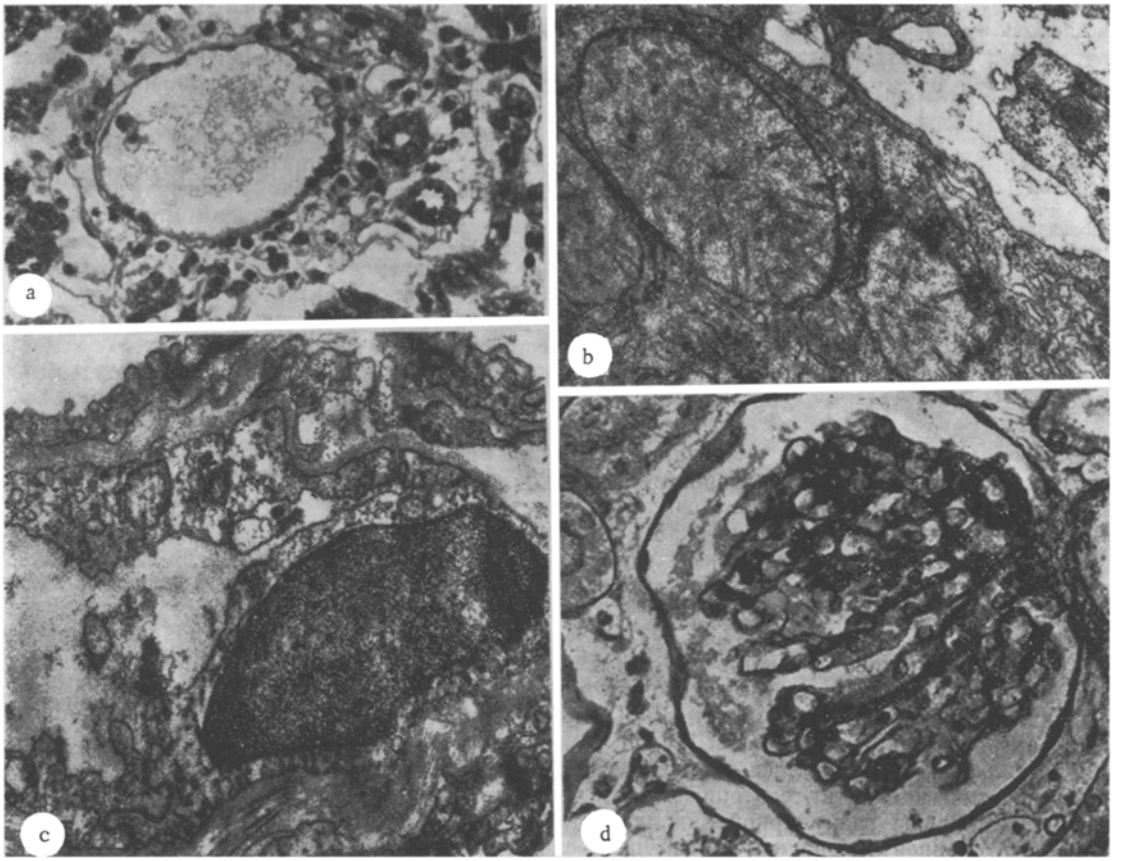

Fig. 1. Morphological changes in the organs under the influence of hemodilution (0.5-2.5 g% hemoglobin) under HBO conditions (3 atm) for 2 h. a) Liver: perivascular edema and leukocytic infiltration of interlobular connective tissue. Hematoxylin and eosin, 252 ×; b) lungs: nonhomogeneous electron density of general basement membrane of endothelium and alveolar epithelium, intensification of pinocytosis of cytoplasm of capillary endothelium, 22,000 ×; c) liver: marked dilation of tubles of smooth endoplasmic reticulum of hepatocyte, 40,000 ×; d) kidneys: protein effusion in dilated lumen of capsule of renal corpuscle. PAS reaction, 252 ×.

took the form of edema of the cytoplasm of the endothelium, increased microvesiculation in the cytoplasm, the formation of apical and basal outgrowths from the membrane surface, variable in shape and size, and loosening and thickening of the basememt membranes in which regions of nonhomogeneous electron density of the ground substance appeared (Fig. 1b). In the motor cortex changes in the microvessels were limited to vasodilation and swelling of the endothelium, evidently due to impermeability of the blood–brain barrier for dextran preparations.

The vascular response was accompanied by morphological and functional changes in the cells of the internal organs, the same in principle in the animals in the two series of experiments. Hypo- and hyperchromic neurons appeared in the cerebral cortex. Hypochromic nerve cells predominated in the upper layers of the cortex, and were distinguished by a low concentration of cytoplasmic RNA and total protein. Signs of hyperchromatosis were mainly confined to neurons in the lower layers of the cortex and took the form of a sharp increase in the concentrations of RNA and total protein. The morphological picture of moderate hypochromatosis is known to reflect excitation of nerve cells, whereas the morphological picture of hyperchromatosis, on the other hand, reflects their inhibition [1, 5]. Consequently, exposure to the experimental factors modified the relations and interaction between these processes in different layers of the motor cortex.

Evidence of focal granular degeneration of the cardiomyocytes appeared in the heart, with a decrease in their glycogen content and AST activity and an increase in their SDH, MDH, and LDH Activity.

TABLE 1. Dynamics of Blood Lactic and Pyruvic Acid Concentrations during Hemodilution under HBO Conditions (M ± m)

Acid, mg %	No. of observations	Initial value	After exposure for 2 h	
			2—2,5 g % Hb	0,5—0,8 g % Hb
Lactic	9	11,4±2,3	27,4±3,1 $P<0,01$	45,5±3,4 $P_1<0,05$
Pyruvic	9	0,66±0,08	1,14±0,14 $P>0,1$	1,13±0,16 $P_1>0,2$

Legend. P) Significance of differences compared with initial values; P_1) significance of differences compared with values after exposure of 2 h with hemoglobin concentration 2-2.5 g%.

In the liver the tubules of the smooth endoplasmic reticulum of the hepatocytes were sharply dilated (Fig. 1c). Tubules of the rough endoplasmic reticulum were concentrated mainly close to the mitochondria, with a moderately translucent matrix. The dimensions of the nuclei and nucleoli were increased, the concentration of nuclear and cytoplasmic RNA and of total protein was raised, G6PDH and LDH activity was greatly strengthened, SDH and MDH activity showed a more moderate increase, and the glycogen concentration was slightly reduced. The combination of changes mentioned above has been shown to reflect hyperfunction of the liver cells [2], stimulation of which during hemodilution under HBO conditions was evidently caused by a sharp decrease in the protein concentration in the blood plasma.

In the kidneys the lumen of the capsules of the renal corpuscles was dilated and a protein-containing effusion appeared in them (Fig. 1d); the lumen of both proximal and distal portions of the nephrons contained debris. The epithelium of the tubules was flattened in some places, granular degeneration developed in it, and activity of succinate and malate dehydrogenases was reduced.

After retransfusion of the blood and decompression of the animals the functional state and structure of the cells and the permeability of the microvessels in the various organs studied returned to normal between the 7th and 14th days.

It must be concluded from this investigation that with degrees of hemodilution incompatible with life under ordinary conditions, and under an oxygen pressure of 3 atm for 2 h, the morphological functional changes in the organs become mainly compensatory-adaptive in character and are reversible. It can be tentatively suggested that changes in the cells of the internal organs were largely due to the development of secondary tissue hypoxia. The main factor complicating transcapillary diffusion of oxygen was evidently increased permeability of vessels of the microcirculatory system which, according to Chernukh et al. [3], disturbs the spatial organization of transport pathways within the tissues. The increase in vascular permeability and switching of energy formation toward activation of glycolysis, as reflected by an increase in the blood concentrations of lactic and pyruvic acids (Table 1), simultaneously with an increase in the degree of hemodilution, demonstrate that the leading factor causing disturbances of the microcirculation was the dextran infusion. Consequently, correction of the composition of the plasma substitute to stabilize the permeability of the tissue—blood barrier may significantly reduce the functional stress on tissue systems resulting from the action of hemodilution under HBO conditions and may permit the more effective use of the method in clinical practice.

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ELECTRON-MICROSCOPIC INVESTIGATION OF UPTAKE OF LOW-DENSITY LIPOPROTEINS BY PERICARDIAL MACROPHAGES FROM PATIENTS WITH ATHEROSCLEROSIS

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In atherosclerosis in man and experimental atherosclerosis in animals cells of the mononuclear phagocyte system take up lipids and are converted into "foam cells." These cells are found in organs of the reticuloendothelial system and also actually in the atherosclerotic focus in the vessel wall [1, 2, 10]. Experiments *in vitro* on albino rat macrophages have shown that foam cells can be formed by accumulation of products of lysosomal degradation of low-density lipoproteins (LDLP) [6]. The existence of a similar mechanism in man has also been postulated.

This paper describes an electron-microscopic study of LDLP uptake by pericardial macrophages of patients with atherosclerosis based on cell culture in medium containing homologous lipoprotein particles.

EXPERIMENTAL METHOD

Macrophages obtained from the pericardial fluid of seven men aged from 36 to 57 years, undergoing open heart operations for coronary atherosclerosis in the Clinic of the Surgical Faculty of the First Leningrad Medical Institute were used.

LDLP were isolated in the Department of Biochemistry (Head, Academician of the Academy of Medical Sciences of the USSR A. N. Klimov), Research Institute of Experimental Medicine, from blood donors' plasma within the density range 1.019-1.063 by the method of Havel et al. [9].

The cells were cultured immediately after removal from the patient directly in pericardial fluid at 37°C, with the addition of LDLP in a final concentration of between 0.5 and 3.6 mg/ml, which is within the limits of variation of the LDLP level in human lymph and blood plasma [7, 11]. In the control series of experiments macrophages of three patients were cultured without the addition of LDLP. After culture for 5 and 10 min the pericardial fluid was mixed with an equal volume of aldehyde fixative (2% paraformaldehyde and 2.5% glutaraldehyde), made up in 0.1 M cacodylate buffer (pH 7.2). The cells were fixed for 1 h at 4°C and then sedimented in a centrifuge. Electron-microscopic detection of LDLP in the macrophages was based on the method [14] which, as has been shown in the case of the developing hen's egg [12], gives a clear picture of intracellular lipoprotein particles. In the present investigation the material was processed as follows: After sedimentation in a centrifuge the cell clot was washed in 0.1 M cacodylate buffer for 24 h at 4°C, postfixed in a 2% solution of OsO_4 in 0.1 M cadoylate buffer for 1.5 h at 4°C, etched in 2% tannin solution for 2 h at 4°C, and washed for 10 min in a 1% solution of sodium sulfate at room temperature. After dehydration in alcohols of increasing strength the cells were embedded in Araldite. Sections were cut on the LKB-III Ultrotome, stained with lead citrate, and examined in the JEM-7A electron microscope.

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