STATE OF THE MICROCIRCULATION IN RATS AFTER COMPLETE EXCHANGE TRANSFUSION WITH A BLOOD SUBSTITUTE BASED ON PERFLUOROTRIBUTYLAMINE

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Further improvements in blood-replacing solutions will follow the lines of creation of blood substitutes capable of performing the respiratory function of blood in the body [2, 3, 9, 10]. One of the most promising developments in this field is work on a blood substitute and oxygen carrier based on emulsions of fluorocarbon compounds, which will dissolve high concentrations of gases [8, 10, 11]. In the blood stream, fluorocarbon compounds can become saturated with O_2 and CO_2 and can give them up when the partial pressure falls, i.e., they can perform a gas-transporting function [6-8].

A model of combined blood substitute and O_2 carrier based on a finely dispersed (particle size about 0.22 μ) emulsion containing 12% perfluorotributylamine by volume, has been developed at the Central Institute of Hematology and Blood Transfusion. As stabilizers for the emulsion, 7% solutions of Soviet surface-active substances – proxanols (block copolymers of ethylene oxide and propylene oxide) – are used. The composition of the blood substitute also includes electrolytes and colloids to create the necessary colloid–osmotic gradient and pH.

This paper gives the results of an investigation of the state of the microcirculation in rats after complete exchange transfusion with the model blood substitute and oxygen carrier.

EXPERIMENTAL METHOD

Experiments were carried out on 20 Wistar rats weighing 150-200 g. Under pentobarbital anesthesia (3 mg/100 g) catheters were introduced into the common carotid artery and external jugular vein of the animals. Heparin was injected into the blood stream in a dose of 50 units/100 g. The rats breathed pure O_2. The blood substitute was then injected into the external jugular vein of the rats of the experimental group (12 animals) at the rate of 0.5 ml/min/100 g, and blood was withdrawn from the carotid artery at the same rate. The exchange transfusion continued until the hematocrit index fell to 2-3%. In the control group of animals (8 rats) an arteriovenous shunt was created during this time, through which the autologous blood was passed.

During the exchange transfusion, the general arterial pressure (GAP) was measured by a Barovar electromanometer (France). The state of the microcirculation was investigated by intravital microscopy of the mesenteric vessels [1, 4, 12, 14]. The preparation was stabilized with Ringer-gelatin solution, pH 7.4, at 37.5°C and the rate of perfusion was 2-3 ml/min. For the biomicroscopic investigation of the blood vessels, an apparatus mounted on the MBI-6 microscope was used [5]. The sensitivity of the microvessels to local application of adrenalin (0.1 ml) was investigated by determination of threshold concentrations [13]. For this purpose a test scale of different concentrations of adrenalin – from 32 to 0.12 μg/ml – in Ringer-gelatin solution was prepared. During the investigation the scale was kept at a constant temperature of 38°C. The test was considered positive if slowing of the venous blood flow and contraction of the precapillaries took place not later than 30 sec after application.

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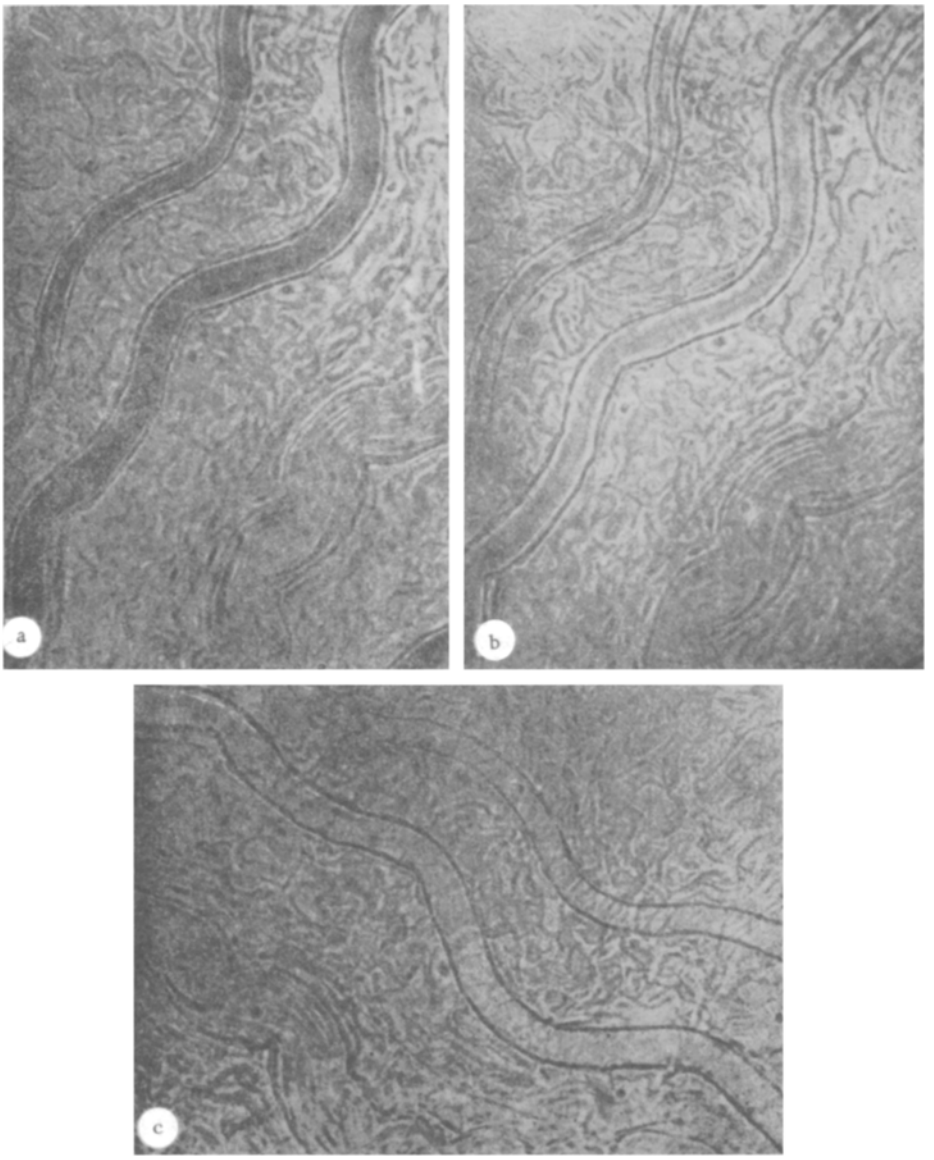

Fig. 1. Area of vascular system during replacement of blood by perfluorotributylamine emulsion: a) initial state of blood flow; b) replacement to hematocrit 10%; c) total replacement of blood. 160×.

TABLE 1. Arterial Pressure in Rats during Exchange Transfusion (M ± m)

Group of animals	Initial state	Time after beginning of exchange transfusion, min				
		30	60	90	120	150
Control	128±5	125±5	120±5	118±4	115±4	115±5
Experimental (blood replacement)	130±5	110±6	105±6	95±4*	95±5*	85±5*

*Values for which $P < 0.05$.

EXPERIMENTAL RESULTS

Replacement of blood by the emulsion of perfluorotributylamine continued for 40-50 min. During the exchange the animal received altogether 20-25 ml emulsion/100 g body weight. The erythrocyte count fell to 150,000-300,000 cells/μl, and the hematocrit index to 2-3%. When these levels were reached the exchange was regarded as complete.

TABLE 2. Sensitivity of Microvessels to Adrenalin (in μg/ml)

Group of animals	Initial state	Time after beginning of exchange transfusion, min				
		30	60	90	120	150
Control	2—4	2—4	2—4	2—4	4—8	4—8
Experimental (blood replacement)	2—4	2—4	2—4	2—4	1—2	1—0,5

The mean GAP level fell somewhat during the exchange transfusion, and toward its end was 105 ± 6 mm Hg. After exchange transfusion, an additional plethoric injection of blood substitute was required to stabilize GAP. The maintenance infusion of the substitute was given at the rate of 0.02-0.03 ml/min/100 g through the period of observation. In this way GAP could be stabilized at the 95 ± 4 mm Hg level (Table 1). The length of survival of the animals after exchange transfusion was 3.2 ± 1.2 h.

Investigation of the microcirculation during blood replacement showed that, parallel with hemodilution, there was a gradual increase in the velocity of the blood flow in the microvessels. Dilatation of the arterioles by 20-25% of their initial diameter and an increase in the number of functioning capillaries from 2-3 to 3-5 per field of vision were observed. The structure of the blood flow in the microvessels also changed: the axial flow of erythrocytes was narrowed and the juxtamural layer, free from cells, widened. However, the laminar motion of the blood and axial orientation of the erythrocytes still continued (Fig. 1a, b). In individual post-capillary and collecting venules, sedimentation of platelets of the vessel walls could be detected.

The character of the functional mobility of the microvessels observed in some cases – vasomotor contraction of the arterioles – showed a rhythm which corresponded to their normal physiological state. The duration of the phase of dilatation was 45-60 sec and the duration of the phase of constriction 10-15 sec.

The state of reactivity of the microvessels, determined by their sensitivity to local application of adrenalin, showed no significant changes (Table 2).

During the period of total replacement of blood by the model substitute the microvessels were perfused with emulsion containing single blood cells (Fig. 1c). The rate of the circulation of emulsion was significantly higher than the original velocity of the blood flow. The walls of the microvessels were free from blood cells. Moderate dilatation of the arterioles and an increased number of functioning capillaries still remained. The normal rhythm of functional mobility of the microvessels was maintained. The sensitivity of the microvessels to adrenalin was at the physiologically normal level (Table 2).

The state of the microcirculation worsened 1.5-2.5 h after replacement of blood by the perfluorotributylamine emulsion. The velocity of circulation of the emulsion was slowed. Constriction of the arterioles took place. The number of functioning capillaries was reduced to one or two per field of vision. The sensitivity of the microvessels to adrenalin increased considerably (Table 2).

In the animals of the control group, during shunting of the blood flow and in the subsequent periods of observation no significant changes took place in the state of the microcirculation. Such changes as were observed did not go outside the bounds of phenomena due to fixation of the animals, anesthesia, and exposure of the mesentery to the series of factors connected with intravital microscopy [14].

The experimental results thus showed that during complete replacement of blood by perfluorotributylamine emulsion the microcirculation is actively perfused with the blood substitute. The velocity of circulation of the blood substitute is higher than the original velocity of the blood flow, a high level of perfusion of the nutritive vessels is thereby maintained, and the physiological parameters of reactivity and functional mobility of the microvessels are preserved. It can be tentatively suggested that under these circumstances favorable conditions are also provided for transcapillary exchange. The period of active circulation of the blood substitute in the microcirculation measures 1.5-2.5 h. At the end of this time the conditions of perfusion of the microvessels deteriorate, probably on account of the escape of components of the blood substitute from the blood vessels, thus reducing the volume of circulating blood substitute and changing its composition.

LITERATURE CITED

1. A. M. Guseinov, Tr. Inst. Norm. Patol. Fiziol. Akad. Med. Nauk SSSR, 12, 188 (1969).
2. V. B. Koziner, Patol. Fiziol., No. 1, 84 (1978).

3. G. Ya. Rozenberg, Probl. Gematol., No. 10, 40 (1977).
4. V. P. Smolichev and V. M. Volodin, Patol. Fiziol., No. 3, 72 (1968).
5. A. M. Chernukh, P. N. Aleksandrov, and O. V. Alekseev, The Microcirculation [in Russian], Moscow (1975).
6. J. L. C. Clark, S. Kaplan, and F. Becattini, J. Thorac. Cardiovasc. Surg., 60, 757 (1970).
7. J. L. C. Clark, E. P. Wesseler, S. Kaplan, et al., Fed. Proc., 34, 1468 (1975).
8. R. P. Geyer, New Engl. J. Med., 289, 1077 (1973).
9. R. P. Geyer, Fed. Proc., 34, 1525 (1973).
10. H. A. Sloviter and T. Kamimoto, Nature, 216, 458 (1967).
11. H. A. Sloviter, Fed. Proc., 34, 1484 (1975).
12. B. W. Zweifach, Anat. Rec., 120, 277 (1954).
13. B. W. Zweifach and D. B. Metz, Ergebn. Anat. Entwickl.-Gesch., 35, 176 (1956).
14. B. W. Zweifach, Microvasc. Res., 5, 363 (1973).

STRUCTURAL AND FUNCTIONAL CHANGES IN ERYTHROCYTE MEMBRANES IN EXPERIMENTAL ATHEROSCLEROSIS

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Changes in the functional state of cell membranes in the development of atherosclerosis have attracted the attention of many research workers. According to one hypothesis [5, 12], one of the early stages in the development of atherosclerosis, namely proliferation of the smooth muscle cells of the aorta, may be due to a decrease in membrane activity and in the activity of membrane-bound enzymes as the result of an increase in the content of nonesterified cholesterol (Ch) in the membrane.

The object of this investigation was to study changes in Na,K-ATPase activity and in the structural characteristics of the membranes of erythrocytes which, like smooth muscle cells of the aorta, are mesenchymal in origin [1], and also activity of the enzyme in homogenates of the aorta of animals with alimentary atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 28 chinchilla rabbits. Experimental atherosclerosis was induced in 13 rabbits by feeding them daily for 4 months with Ch in a dose of 0.2 g/kg body weight. In 8 rabbits total involvement of the thoracic aorta was found at autopsy, and in 5 rabbits there were single lipid plaques; 15 rabbits served as the control. Blood was collected in tubes containing heparin (1000 units to 5 ml blood). Erythrocyte membranes were isolated by centrifugation at 28,000g after hemolysis of the erythrocytes in 10 mM histidine solution [2]. The protein content was determined in the suspension of membranes by the method in [10], and after extraction with a mixture of chloroform and methanol (2:1), the Ch content was determined on a Technikon A II automatic analyzer and the phospholipid content by Svannborg's method [14]. Activity of Na,K-ATPase was determined by the concentration of inorganic phosphate [13] after incubation in a medium containing 150 mM NaCl, 20 mM KCl, 30 mM $MgCl_2$, and 30 mM ATP [7]. The reaction was stopped by the addition of 1 ml acetate buffer, pH 4.7.

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