The fact will be noted that "zones of mixed blood supply" between territories of the principal cerebral arteries [3, 4, 8] coincide with the cortical projection areas. The parietal association cortex, formed in carnivores as a single, structurally differentiated formation [2], is located in the distal part of the ramification of the middle cerebral artery.

In our view the results of this investigation reveal a close connection in the organization of the cortex and pial system and demonstrate their functional unity during unilateral injury to cortical areas, promoting compensation and restoration of disturbed functions.

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BLOOD AGGREGATION STATE AND THE MICROCIRCULATORY SYSTEM IN EXPERIMENTAL ATHEROSCLEROSIS AND ITS SPONTANEOUS REGRESSION AND DURING HEMOPERFUSION

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KEY WORDS: atherosclerosis; hemoperfusion; blood aggregation state; microcirculatory system.

Changes in the rheologic properties and aggregation state of the blood not only affect the onset and course of diseases such as atherosclerosis and ischemic heart disease, but they are also a fundamental stage in the pathogenesis of these diseases [2, 6, 7]. According to some investigators [4], the rheologic parameters of the blood flow in the microcirculatory system depend on the hematocrit index, the deformability of erythrocytes, the size and stability of blood aggregates, dimensions of blood vessels, viscosity of plasma, and possible changes in its composition.

The aim of this investigation was to study the aggregation state of the blood and the microcirculatory system in rabbits during development of experimental atherosclerosis and its spontaneous regression, and after hemoperfusion of the animals.

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TABLE 1. Biochemical and Biophysical Parameters of Blood during Development of Experimental Atherosclerosis (M ± m; n = 15)

Parameter	Background	Time of development of atherosclerosis, months				
		1	2	3	4	5
ChS, mg%	64±2	360±29*	663±42*	724±35*	704±34*	911±82*
Triglycerides, mg%	62±6	135±18*	172±15*	248±23*	—	—
Apo-B-LP, mg%	150±20	736±73*	1233±100*	1103±102*	1258±125*	1816±305*
ChS/PL ratio of erythrocytes, moles/moles	0,84±0,03	1,01±0,04*	1,08±0,05*	1,07±0,04*	1,34±0,08*	1,14±0,09*
Hematocrit index, %	32±6	29±5	28±6	29±4	25±2*	25±2*
Erythrocytes, g/liter	3,5±0,05	3,2±0,1*	3,2±0,15*	3,2±0,1*	2,9±0,2*	2,7±0,25*
Viscosity, cP	2,9±0,07	3,3±0,2*	3,4±0,2*	3,5±0,16*	3,8±0,6*	4,1±0,5*
Aggregation of erythrocytes, %	56,6±6,4	61,8±3,65	79,5±3,9*	67,7±9,5	—	—
Aggregation of platelets, %	50,4±11,0	52,4±15,2	82,0±19,6*	62,8±36,0	—	—
Parameter of order S of 5NS probe of erythrocytes	0,640±0,03	—	—	0,684±0,03*	—	—
Parameter of order S of 5NS probe of platelets	0,619±0,018	—	—	0,662±0,002*	—	—

Note. Here and in Table 2 asterisks indicate $P < 0.05$ compared with initial values.

EXPERIMENTAL METHODS

Experiments were carried out on 75 male chinchilla rabbits weighing 2.5-3.5 kg and aged 9-12 months. Experimental atherosclerosis was induced by feeding the rabbits daily with cholesterol (ChS) in a daily dose of 0.25 g/kg body weight with vegetables. Animals of group 1 (n = 15) received the ordinary animal house diet for 5 months. Rabbits of group 2 (n = 25) were kept on an atherogenic diet for 5 months. Animals of group 3 (n = 15) received an atherogenic diet for 3 months, after which they were transferred to the ordinary animal house diet for 2 months. Animals of group 4 (n = 20) were kept on the atherogenic diet for 3 months, transferred to the animal house diet, and then underwent hemoperfusion 3 times with intervals of 7 days. Hemoperfusion was carried out with an arteriovenous circuit without a pump, and using MINKh-2 alumosilicate adsorbent (N. N. Belov). In the course of the experiment the hematocrit index, erythrocyte and platelet counts, were determined on the animals' blood, plasma ChS, triglyceride, and apo-B-containing lipoprotein (apo-B-LP) levels, the ChS/phospholipid (ChS/PL) ratio of the erythrocytes, and microviscosity of erythrocyte and platelet membranes (by measuring the parameter of order S of a 5NS probe by the EPR method — this investigation was conducted jointly with O. A. Azizova and E. A. Gorbatenkova) were determined. At different times of the investigation and at the end, the microcirculatory bed and the state of the blood flow of the animals were studied in the mesentery of the small intestine by intravital microscopy. The morphometric analysis of the microcirculatory bed was undertaken on total preparations of membranes (mesentery of the small intestine, pericardium) and sections cut from the myocardium after treatment with silver nitrate.

RESULTS

The data given in Tables 1-3 show that the development of experimental atherosclerosis was accompanied by the following changes: 1) elevation of blood ChS, triglyceride, and apo-B-LP levels and the ChS/PL ratio of the erythrocytes; 2) lowering of the hematocrit index and erythrocyte count, increased viscosity of whole blood and aggregating properties of erythrocytes and platelets; 3) increased microviscosity of erythrocyte and platelet membranes; 4) reduction of capillary networks, of the tortuousness of the microvessels, and deformation of their contours; 5) reduction in the diameter of precapillaries and capillaries, an increase in the mean diameter of postcapillaries and venules, reduction of the number of capillaries 6.1-8 μ in diameter, accompanied by a relative increase in the proportion of capillaries with small diameter (1-6 μ); 6) slowing of the blood flow in all parts of the microcirculatory bed. Adhesions of leukocytes and platelets to the inner surface of the vessel wall, and aggregation of erythrocytes and platelets were intensified in all microvessels.

The biochemical, biophysical, and morphological investigations thus confirmed that the development of experimental atherosclerosis is accompanied by considerable changes in the rheologic properties and aggregation state of the blood, and in the structure and function of the microcirculatory bed. All these changes also take place in patients with atherosclerosis, ischemic heart disease (IHD), and familial hyperlipoproteinemia. As long ago as in 1967 Dintenfass [9] described an "increased viscosity syndrome" in patients with coronary insufficiency. In our view the disturbance of the blood aggregation state in atherosclerosis and IHD is

TABLE 2. Parameters of Vessels of Microcirculatory Bed of Rabbits during Spontaneous Regression of Atherosclerosis Treated by Hemoperfusion (M ± m; n = 10)

Test object	Ateriole				Capillary								Venule			
	control	5 months of hypercholesterolemia	3 months of hypercholesterolemia + 2 months of regression	3 months of hypercholesterolemia + 3 perfusions	control		5 months of hypercholesterolemia		3 months of hypercholesterolemia + 2 months of regression		3 months of hypercholesterolemia + 3 hemoperfusions		control	5 months of hypercholesterolemia	3 months of hypercholesterolemia + 2 months of regression	3 months of hypercholesterolemia + 3 hemoperfusions
					D	R	D	R	D	R	D	R				
Myocardium	15±0,6	13±0,7*	13±0,6*	13,7±0,7	4,1±0,1	3,1±0,2	3,8±0,1	6,1±0,4*	4,0±0,7*	4,4±0,3*	4,1±0,4	3,8±0,2*	11±0,4	12±0,3	11,9±1	11,4±0,3
Pericardium	27±1,2	24±1*	26±1	26±1	11,4±0,2	—	7,6±0,2*	—	8,5±0,3*	—	8,9±0,3*	—	37±1,5	41±2	42±1,1*	40±1,9
Mesentery of small intestine	24±1,2	21±1,2*	22±1,0	23±1,1	6,4±0,2	—	5,5±0,2*	—	5,8±0,1*	—	6,0±0,3	—	27±1,2	30±1,4	28±0,8	27,5±1,0

Note. D) Diameter of capillaries (in μ); R) radius of diffusion of capillaries (in μ).

TABLE 3. Biochemical and Biophysical Parameters of Blood during Spontaneous Regression of Atherosclerosis and Treatment by Hemoperfusion (5 months after beginning of experiment)

Experimental conditions	ChS, mg%	Triglycerides, mg%	Apo-B-LP, mg%	ChS/PL ratio of erythrocytes, moles/moles	Hematocrit index, %	Erythrocytes, g/liter	Viscosity, cP
Control (n=15)	74±10	59±12	133±64	0,99±0,05	35±4	3,6±0,25	3,2±0,27
Spontaneous regression (n=15)	233±41*	130±24*	621±24*	1,14±0,05*	30±4	3,2±0,2	3,7±0,3
Hemoperfusion (n=20)	107±27	65±7	326±86*	0,87±0,06	30±4	3,5±0,18	2,7±0,4

Note. Asterisks indicate $P < 0.05$ relative to control series of experiments; n) number of animals.

largely connected with membrane effects of ChS. Experiments in vitro and on animals have shown that ChS or a change in its ratio with PL has a direct effect on the aggregation properties of erythrocytes and platelets and on the deformability of erythrocytes [1, 3, 8]. Similar results have been obtained inclinical practice in the case of patients with IHD [2] and with familial hyperlipoproteinemia [5].

Spontaneous regression of atherosclerosis was accompanied by the following changes: 1) a fall of the ChS, triglyceride and apo-B-LP levels and an increase in the ChS/PL ratio in erythrocyte membranes; 2) an increase in the hematocrit index and erythrocyte count, a very small decrease in viscosity of whole blood, and a very small decrease in the aggregation power of the blood cells; 3) stabilization of the microviscosity of erythrocyte and platelet membrane at a level corresponding to 3 months of hypercholesterolemia; 4) slowing of the negative trend in the characteristics of the microcirculatory bed; 5) a further decrease in diameter of the arterioles and dilatation, congestion, and varicosis of the venous portions of the microcirculatory bed, and a decrease in the number of plasmatic capillaries; 6) the blood flow in all parts of the microcirculatory bed remained slowed, and adhesion of leukocytes and platelets in the venular portion remained increased.

Discontinuation of the high cholesterol diet for 2 months thus was not followed by normalization of the aggregation state of the blood or the structure of the microcirculatory bed. It merely led to a decrease in the serum lipid concentration but had virtually no effect on the membrane pool.

After hemoperfusion had been performed 3 times on the rabbits the following changes were observed in the parameters tested: 1) normalization of the plasma ChS, triglyceride, and apo-B-LP levels and of the ChS/PL ratio in the erythrocyte membrane; 2) normalization of the hematocrit index, erythrocyte count, and blood viscosity and a reduction in the aggregation powers of the erythrocytes and platelets; 3) a reduction in microviscosity of the erythrocyte and platelet membranes; 4) a decrease in the radius of diffusion of the capillaries, giving indirect evidence of an improvement in the blood supply of the organs; 5) an increase in the diameter of the arterioles and a decrease in the diameter of the venules, and an increase in the number of capillaries 6.1-8 μ in diameter; 6) acceleration of the microcirculatory blood flow, a reduction of adhesion of leukocytes and platelets to the endothelium, and a decrease in aggregation of erythrocytes and platelets.

The results described above show that hemoperfusion has a marked effect not only on the serum lipids, but also on the lipid composition of the membranes, and it largely normalizes the aggregation state of the blood. Ultimately this leads to restoration of the normal structure of the microcirculatory bed.

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STRUCTURAL CHANGES IN THE LIVER PARENCHYMA OF RATS DURING LONG-TERM FEEDING ON DIETS DIFFERING IN PROTEIN CONTENT

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The liver is a metabolic center where substances taken in with the diet are transformed into a utilizable form to meet the energy and plastic requirements of the body. Individual food preferences, ecologic and economic factors determining the composition and quantity of food consumed, and also restrictions of diet in connection with disease may have a substantial influence on structural and functional parameters of the liver.

The aim of this investigation was to study the effect of diets differing in their content of food components on the structural organization of the liver.

EXPERIMENTAL METHODS

Male Wistar rats were kept for 21 days on diets of the following composition: standard (25% protein, 53% carbohydrate, 22% fat), low-protein (6% protein, 73% carbohydrate, 21% fat), high-protein (60% protein, 27% carbohydrate, 13% fat) [9]. After alkaline dissociation of samples of liver, fixed in formalin [1], films were made from cell suspensions and stained with hematoxylin and eosin; the ratio of the number of binuclear hepatocytes to the total number of hepatocytes was determined inpromille. The distribution of hepatocytes by ploidy classes were studied in liver films stained by Feulgen's method. The measurements were done on an IKÉM-1 cytophotometer [4]. Samples of liver from five animals in each group were fixed in 1% OsO_4 in phosphate buffer and embedded in Epon for electron microscopy. Epon sections 1 μ thick were stained with toluidine blue and used for morphometry, which was done in accordance with the recommendations in [11]. Differences between the mean values compared were taken to be significant at the $P < 0.05$ level (Student's test).

RESULTS

In animals kept on a low protein diet the weight of the liver decreased by 34% but its relative weight was unchanged; the number of diploid hepatocytes and the index of binuclear hepatocytes were increased (Fig. 1, Table 1). Together with an increase in the relative volume of the parenchyma, this can be taken as evidence of activation of proliferation [3]. The increased glycogen concentration (Table 2) may perhaps be connected with slowing of its phosphorylation on account of depressed glucose-6-phosphatase activity [10].

The number of attached ribosomes and the surface area of the membranes of the rough endoplasmic reticulum (RER) were slightly reduced (Fig. 2; Table 2). Mainly secretory proteins are synthesized on attached ribosomes of the hepatocyte RER [13], and under conditions of protein insufficiency their synthesis is restricted [5]. On that account, evidently, synthesis of transport proteins also was depressed, and this led to accumulation of lipids (Table 1).

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