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CORRELATION BETWEEN CHANGES IN BLOOD RHEOLOGIC PROPERTIES AND MICROCIRCULATORY DISTURBANCES IN EARLY AND LATE STAGES OF EXPERIMENTAL HYPERLIPOPROTEINEMIA

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Clinical and experimental investigations have yielded evidence of the effect of hyperlipoproteinemia (HLP) on the rheologic properties of the blood and on the microcirculation (MC) [1-3, 5, 10-12]. We know that HLP is one of the leading risk factors of atheroschlerosis and its complications: ischemic heart disease (IHD) and myocardial infarction. According to some authorities changes in blood rheologic properties are similar in character whether atherosclerotic changes are present in the large arteries or not [7]. Accordingly, the study of the mechanisms of disturbance of the rheologic properties of the blood and of MC in the early stages of development of HLP, in the preclinical stage of the disease, becomes particularly important.

This paper describes a comparative study of the rheologic properties of the blood and MC in the early and late stages of experimental HLP.

EXPERIMENTAL METHOD

Twenty male Chinchilla rabbits weighing 2.3-2.8 kg were used. Experimental HLP was induced by Anichkov's method in Yushchenko's modification: 0.5 g/kg cholesterol (Ch) in 100 g of carrot. Blood for testing was taken from the auricular vein of the rabbits before Ch loading, 15 h after a single Ch loading, and also after the animals had been kept for 1, 3, 9, 30, 60, and 90 days on an atherogenic diet (AGD). Total Ch (TCh) (by the method of Girard and Assous), chylomicrone (ChM), and low (LDL), very low (VLDL), and high (HDL) density lipoproteins (LP) [8] were determined in the blood serum.

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TABLE 1. Changes in Hematocrit Index, Osmotic Resistance of RBC, and Blood Viscosity of Rabbits Kept on AGD

Parameter studied (n = 10)	Control (1)	Single loading, 15 h (2)	Time animals were kept on AGD						
			1 day (3)	3 days (4)	9 days (5)	1 month (6)	2 months (7)	n	3 months (8)
Hematocrit, %	35,48±0,88	34,50±0,88 $P_{1-2}>0,1$	31,79±0,84 $P_{1-3}<0,01$ $P_{2-3}<0,05$	30,25±1,05 $P_{1,2-4}<0,01$ $P_{3-4}>0,1$	32,43±0,71 $P_{1-5}<0,02$ $P_{2-5}>0,05$ $P_{3,4-5}>0,1$	33,60±0,50 $P_{1,3-6}>0,05$ $P_{2,5-6}>0,1$ $P_{4-6}<0,01$	31,90±0,46 $P_{1-7}<0,01$ $P_{2-7}<0,02$ $P_{3,4,5-7}>0,1$ $P_{6-7}<0,05$	4	24,40±4,90 $P_{1-8}>0,05$ $P_{4-8}>0,1$
Osmotic resistance of RBC: minimal	0,56±0,008	0,55±0,019 $P_{1-2}>0,1$	0,55±0,016 $P_{1,2-3}>0,1$	0,56±0,018 $P_{1,2,3-4}>0,1$	0,60±0,009 $P_{1-5}<0,01$ $P_{2-5}<0,02$ $P_{3-5}<0,02$ $P_{4-5}>0,05$	0,62±0,007 $P_{1,2,3-6}<0,001$ $P_{4-6}<0,01$ $P_{5-6}>0,1$	0,61±0,012 $P_{1-7}<0,01$ $P_{2,4-7}<0,05$ $P_{5,6-7}>0,01$ $P_{3-7}<0,02$	4	0,61±0,003 $P_{1-8}<0,001$ $P_{2-8}<0,02$ $P_{3-8}<0,01$ $P_{4-8}<0,05$ $P_{5,6-8}>0,1$
maximal	0,42±0,013	0,42±0,010 $P_{1-2}>0,1$	0,40±0,012 $P_{1,2-3}>0,1$	0,40±0,005 $P_{1,3-4}>0,1$ $P_{2-4}>0,05$	0,40±0,013 $P_{1,2,3,4-5}>0,1$	0,44±0,008 $P_{1,2-6}>0,1$ $P_{3,5-6}<0,02$ $P_{4-6}<0,001$	0,456±0,005 $P_{1-7}<0,05$ $P_{2,3,5-7}<0,01$ $P_{4-7}<0,001$ $P_{6-7}>0,1$	4	0,41±0,018 $P_{1,2,3-8}>0,1$ $P_{4,5,6-8}>0,1$ $P_{7-8}>0,05$
Blood viscosity, cP	2,08±0,097	2,19±0,160 $P_{1-2}>0,1$	1,67±0,030 $P_{1,2-3}<0,001$	1,52±0,100 $P_{3-4}>0,1$ $P_{1,2-3}<0,001$	2,00±0,170 $P_{1,2-5}>0,1$ $P_{3-5}>0,05$ $P_{4-5}<0,05$	2,10±0,080 $P_{1,2,5-6}>0,1$ $P_{3,4-6}<0,001$	1,789±0,075 $P_{1,2-7}<0,05$ $P_{3,5-7}>0,1$ $P_{4-7}>0,05$ $P_{6-7}<0,02$	4	2,52±0,200 $P_{1,5-8}>0,05$ $P_{3,4-8}<0,01$ $P_{2,6-8}>0,1$ $P_{7-8}<0,02$

Legend. n) Chislo Zhivotnykh.

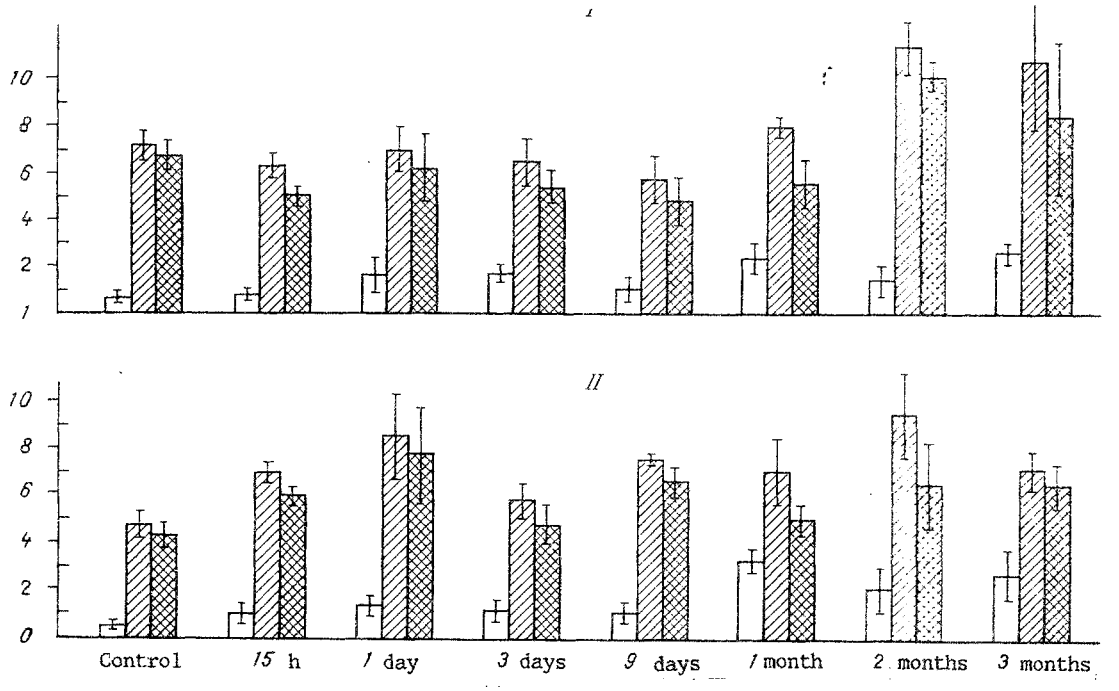

Fig. 1. Dynamics of ABCS of rabbits with experimental HLP. Abscissa, time animals were kept on AGD; ordinate, time (in min). I) Group 1; II) group 2. Unshaded columns indicate beginning of blood clotting; obliquely shaded columns, end of blood clotting; cross-hatched columns, duration of blood clotting. Each group contained five rabbits.

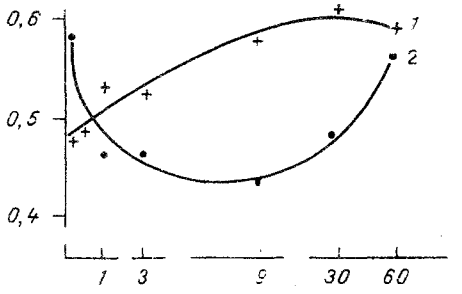

Fig. 2. Dynamics of sedimentation rate of single RBC in glass capillary tubes in experimental HLP. Abscissa, time (in days); ordinate, sedimentation rate (in μ/sec). 1) RBC with initially low sedimentation rate; 2) RBC with initially high sedimentation rate. Each cross or dot corresponds to mean for 80-100 cells.

The hematocrit index was determined *in vitro* by means of an MTsG-8 microhemocentrifuge. The dynamic viscosity of the blood was measured with a "Copley" viscosimeter in the writers' modification. The dynamic viscosity of the blood, the shear velocity, and the shear stress were calculated by equations given in [9]. The dynamic viscosity of the blood was estimated at a shear stress of 3.68 dynes/cm^2 and at a temperature of 35°C. The osmotic resistance of the red blood cells (RBC) was determined by the macroscopic method of Limbeck and Ribier, in the writers' modification. The time course of parameters of the blood clotting system (beginning, end, and duration of blood clotting) was recorded on the N-333 coagulograph. The sedimentation rate of single RBC was studied in glass capillary tubes 20-40 μ in diameter. The ultrastructure of the aorta, coronary arteries, and microvessels of the myocardium was investigated by light (Goldman's method) and electron microscopy (the usual method). Numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Elevation of the TCh level by 53% (control 28.2 ± 2.8 mg %) and of the atherogenic LP (ChM, LDL, VLDL) level by 132% (control 98.2 ± 7.2 mg %) was observed in the rabbits' peri-

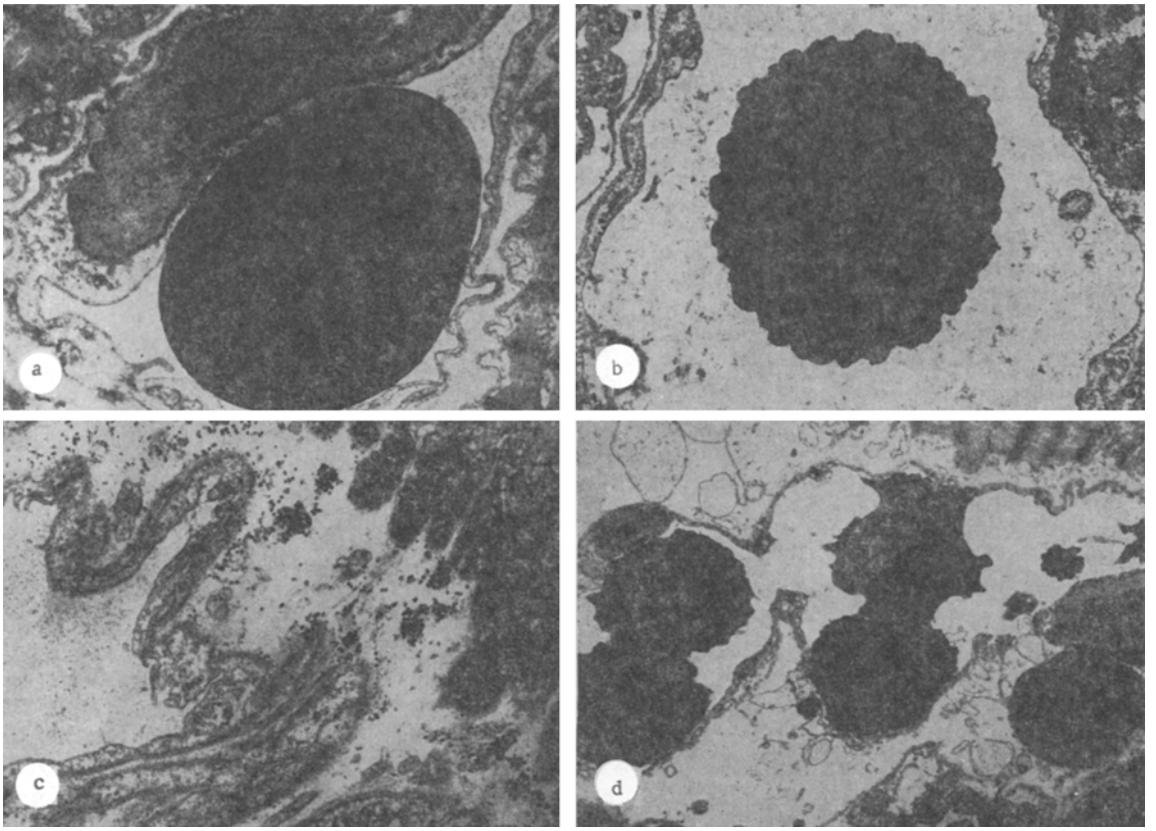

Fig. 3. Structural changes in myocardial microvessels of rabbit with experimental HLP. a) After 15 h of experiment: hydration of endotheliocytes, reduction of micropinocytosis, subsarcolemmal edema, unwinding of myofilaments (RBC with unchanged membrane in capillary lumen); b) after 48 h of experiment: RBC with modified membrane in lumen of dilated venule; c) after 15 h of experiment: endothelium of venule is edematous, structure of basement membrane loosened, concentrations of electron-dense particles can be seen in the widened perivascular space; d) after 1 month of experiment: aggregation of RBC with modified surface in capillary lumen. Magnification: a, b, c) 7500, d) 3500.

pheral blood 24 h after administration of Ch to the animals *per os*. Later the TCh and atherogenic LP levels rose progressively to reach a maximum after 60 days on the AGD. The level of antiatherogenic LP (HDL) was increased after 9 days on AGD by 255% (control 75.7 ± 10.2 mg %) and continued to rise subsequently. After 90 days on the AGD a tendency was noted for the TCh concentration in the peripheral blood to fall and there was a significant decrease in the concentrations of atherogenic LP.

Elevation of the blood TCh and atherogenic LP levels was accompanied by disturbances of the rheologic properties of the blood. The dynamic viscosity of the blood was lowered 24 h after administration of Ch (Table 1). The reduction of the blood viscosity was due, besides other factors, to a fall of the hematocrit index at this same time (Table 1). Changes in the hematocrit index and blood viscosity were fluctuating in character. After the 9th day on AGD a decrease of the minimal osmotic resistance of the RBC membrane, which was evidently one cause of the decrease in the hematocrit index, was observed, and later persisted. The maximal osmotic resistance of RBC did not fall until the late stage of HLP, after 2 months on the AGD.

In the early stages of development of experimental HLP (15 and 24 h)changes took place in activity of the blood clotting system (ABCS). The trend of this reaction depended on the initial level of ABCS, and the experimental animals were accordingly divided into two groups (Fig. 1). Group 1 included animals with an initially low ABCS, group 2 those with an initially high activity. In the animals of group 1, 15 h after Ch administration ABCS increased, whereas in the animals of group 2 the opposite reaction was observed (reduction of ABCS). Later, the time of ending and the duration of blood clotting showed fluctuating changes. The time

of beginning of blood clotting increased in the animals of both groups during the course of HLP and was connected with changes in lipid metabolism.

Similar changes were observed in the sedmentation rate of single RBC in the early stage of HLP (15 and 24 h). Cells with an initially low sedimentation rate began to sediment more rapidly under the influence of Ch, whereas if the initial sedimentation rate of the RBC was high, it fell (Fig. 2). In the later stages of AGD (after 1-2 months) the reaction of RBC became similar in both groups and was characterized by an increase in their sedimentation rates.

Structural changes in the microcirculatory bed (MCB) of the myocardium were found in the first hours [4, 6] after administration of a single dose of Ch to the animals (group 1) and they progressed on transfer of the animals to the AGD (group 2). Besides evidence of increased functional activity of the endotheliocytes of MCB, edema developed, micropinocytosis decreased, and the structure of the basement membrane became looser. After 15-24 h, intra- and extravascular changes were observed in the animals of group 1, indicating increased permeability and disturbance of the rheologic properties of the blood (Fig. 3). The microvessels were dilated, especially the venules. Concentrations of electron-dense particles from 32 to 69 nm in diameter were seen at different distances from the basement membrane (Fig. 3c). Since formations of this kind were absent in the control animals, they were identified as LP-complexes. Aggregation of RBC and stasis were observed in the lumen of the microvessels and some RBC had a modified surface (Fig. 3b). With an increase in the duration of the experiment (30 days) the number of modified RBC increased, they underwent fragmentation, these RBC formed aggregates (Fig. 3d), and marked dystrophic changes were observed in the microvessels. Changes of a similar kind were found by the writers when studying the action of a risk factor such as hypercholesterolemia, and also in the MCB of other organs [4, 6], from which it can be concluded that the reaction of MCB to this factor is generalized. Atherosclerotic changes in the aorta and coronary arteries of the animals of group 2 appeared after 2-3 months.

The results of this investigation suggest that disturbances of the hemorheology and microcirculation discovered under the influence of HLP begin to appear in the early stages of disturbance of lipid metabolism. Individual differences in the primary response of the blood clotting system and of RBC were discovered under these conditions. Starting with the first rise of the blood levels of TCh and atherogenic fractions of LP, damage to the endothelium of the myocardial microvessels and of other components of the vessel wall was observed, and the action of HLP on RBC produced an echinocytic effect. Correlation between the hemorheologic and microcirculatory disorders and the structural changes in MCB in the early stages of disturbance of lipid metabolism is evidence of the important role of disturbances of intercellular relations in the development of changes in the blood rheologic properties. Early correction of the rheologic and microcirculatory disturbances in HLP may evidently be one method of pathogenetic prophylaxis of IHD.

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