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DISTURBANCE OF THE BLOOD FLUIDITY AS A RESULT OF AN EXPERIMENTAL INSULT

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The biomechanical properties of the blood of rats with insult caused by an insufficient blood supply of the cerebrum or a hemorrhage have been experimentally investigated. The hemorheological characteristics at the initial stages of the disease and at the stages of its correction have been obtained. The hemorheological status in the case of local cerebral ischemia has been determined.

One of the most serious diseases of modern society is insult, which often leads to a fatal outcome. It is known that the main reason for an insult is an insufficient blood supply of some cerebrum zones, i.e., ischemia leading to the death of nerve cells [1, 2]. The blood supply depends substantially on the biomechanical (rheological) properties of the blood (hemorheological properties). However, the role of hemorheological disturbances in the development of insult is not understood. In particular, the time of their appearance is an open question, even though it is known that the blood fluidity is adversely affected as an ischemic insult develops [3–5]. There are only a few experimental investigations devoted to the hemorheological disturbances arising when limited areas of the cerebrum are affected [6, 7]. The rheological properties of the blood in the case of hemorrhagic insult caused by a cerebral hemorrhage [8] were practically not investigated [8]. These problems are the subject of our experimental investigation.

Materials and Methods. In all series of experiments, we investigated the biomechanical properties of the venous blood of rats, stabilized with trilon B (0.002 g/ml), at 25°C. Below is the procedure of determining the parameters desired.

The apparent viscosity of the blood was estimated by the results of investigation of samples in a coaxial-cylindrical rotational viscosimeter (thickness of the spacing 1.5 mm, rate of shear 2–130 $\sec^{-1}$) with the use of the Casson approximation $\tau^{1/2} = \tau_0^{1/2} + k\dot{\gamma}^{1/2}$. The quantity k^2 was used as the viscosity coefficient [9].

The main determinant of the blood viscosity Ht was estimated by the standard method after the centrifugation of the blood in an MTsG-8 centrifuge (8000 ± 800 $\min^{-1}$, 6 min).

The deformational properties of the erythrocytes were investigated in the process of filtration of 250 μl of their diluted suspension through nucleoporous filters (3 μm); the coefficient of erythrocyte stiffness was calculated by the formula $IR = [(T_1 - T_2)/(T_2 \cdot Htc)] \cdot 100$.

The kinetics of erythrocyte aggregation was determined by the intensity of backward light scattering I measured in a coaxial-cylindrical aggregometer (blood-layer thickness 0.9 mm, rate of shear 0–610 $\sec^{-1}$). The characteristic time of spontaneous aggregation T_a was estimated by the change in I for the time t with the use of the formula $T_a = t/(1/I)$, i.e., the hyperbola $I(t)$ was straightened, and the cotangent of the angle between the straight line $1/I$ and t was calculated. The hydrodynamic-strength coefficient β of aggregates was determined by the measured dependence of I on the rate of shear $\dot{\gamma}$ and by the formula $\beta = \dot{\gamma}/\ln(I)$ [10].

The rheological parameters of the blood were measured in four series of experiments.

In the first series of experiments, the dynamics of hemorheological disturbances arising in the process of development of ischemic insult was investigated in 15 rats of the Wistar population, in which an extensive ischemic insult was caused by the 50% narrowing of one of the carotic arteries and the ligation of the other one. The blood of some animals (n = 6) was investigated within 2 h and the blood of the other animals (n = 7) was investigated within 24 h after the operation. The control samples were the blood samples of nine rats, in which one of the carotic arteries was ligated and the other artery was not narrowed.

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In the second series of experiments, a limited area of the cerebrum of rats (n = 8) was acted on, which led to a local ischemic insult in them. For this purpose, the middle meningeal artery and one of the carotic arteries were ligated [11]. The control animals were animals (n = 6) subjected to the whole operation process without a final stage — ligation of the middle meningeal artery. Moreover, the damage of the cerebrum tissue was controlled by the method of histochemical coloring with 2,3,5-triphenyltetrazolium chloride after the decapitation of the animals [12]. The damage was calculated by the MOCHA program after the scanning of each colored section.

The rheological properties of the blood in the case of hemorrhagic insult were investigated in rats of the KM line. These animals are predisposed to epileptiform fits arising as a result of the action of sound and accompanied by a massive cerebral hemorrhage. The degree of the fit was estimated by a number scale (from one to four) [13]. At the end of an experiment, after the decapitation of the animals and fixation of the cerebrum in a 10% solution of formalin, the hemorrhage area on the cerebrum surface was measured. The hemorheological properties of the blood of the rats subjected to the sound action (n = 13), the control animals (not subjected to any action, n = 11), and the rats of an individual group (n = 11) were investigated. To level the possible hemorheological disturbances in the animals of the third group, we trained them before the sound action for a short-term oxygen deficiency (a hypoxia arising at a height of 5000 m) in a barostat with rarefied air.

The regime of hypoxia training was preliminarily investigated in rats of the Wistar population. Some animals were control (n = 28) and others were subjected to the hypoxia training under the conditions of a continuous oxygen deficiency for 2 h (n = 10) or for three 40-min cycles of hypoxia exposure, in each of which the 5-min hypoxia conditions alternated with the normal conditions [14] in the pulsed regime ("interval hypoxia," n = 13).

Statistical analysis was performed using the SPSS program 10.0.5 for Windows. The average values of the data obtained $\pm$ a standard deviation were considered. The data distributions were estimated using the Shapiro–Wilk test. The results obtained were further processed using nonparametric criteria or criteria for normally distributed data. The reliability of the averaged data obtained in the first and third experimental series was estimated by the data of dispersion analysis of independent samples (One-Way ANOVA). When these data were found to be different, they were further compared (Post Hoc Comparisons) using the Sheffe criterion. Nonparametric dispersion analysis was performed using the Kruscal–Wollis criterion. The reliability of the average data obtained for two groups in the second series of experiments was estimated by the unpaired Student t-criterion or the Mann–Whitney unpaired criterion. The data were considered as reliable when the difference between them was less than 0.05.

Results and Discussion. *Disturbances of the blood fluidity in the process of development of extensive ischemic insult.* The viscosimetric measurements have shown that, at the early stages of the disease, the Caisson viscosity of the blood (3.12 $\pm$ 0.79 mPa·sec after 2 h and 2.68 $\pm$ 50 mPa·sec after 24 h) does not differ from its control values (3.30 $\pm$ 0.65 mPa·sec); the same is true for the hematocrit, which was 44 $\pm$ 6% after 2 h and 42 $\pm$ 4% after 24 h (control value 43 $\pm$ 3%). There is only a tendency toward an increase in the critical shear stress (23.7 $\pm$ 9.9 mPa after 2 h and 36.7 $\pm$ 21.1 mPa after 24 h; control value 20.2 $\pm$ 11.2 mPa). However, the microrheological properties of the blood were disturbed. Within 24 h of the disease, the rate of erythrocyte aggregation increased by four times and the strength of the erythrocyte aggregates increased by two times (Fig. 1).

Thus, the disturbances of the rheological properties of the blood characteristic of ischemic insult [3–5, 15] arise at the early stage of the disease. The microrheological properties of the solid blood elements are disturbed evidently by the biochemical processes arising in the process of ischemic insult and leading to a hypoxia, an increase in the fibrinogen concentration, the concentration of the acute-inflammation proteins, and the number of activated leukocytes, and an intoxication of the blood [16]. As a result, the deformation and aggregation ability of erythrocytes changes and the blood fluidity decreases, which can further deteriorate the microcirculation of blood and the supply of the affected tissue with oxygen and, in doing so, can provoke further development of the disease.

The significant change in the rheological properties of the blood, detected after 24 h elapsed from the beginning of ischemic insult, determined the time of testing the hemorheological properties of blood in the next series of experiments on a more local ischemic insult.

Rheological properties of the blood in the case of local ischemic insult. In the blood of experimental animals we detected a significant increase (by three times) in the critical shear stress (see Fig. 2), which indirectly points to the fact that the aggregation processes are enhanced in this case and the erythrocyte aggregates are strong: they were broken down at a rate of shear of 105 $\pm$ 53 sec^{-1} in the experimental group of animals and 32 $\pm$ 23 sec^{-1} in the

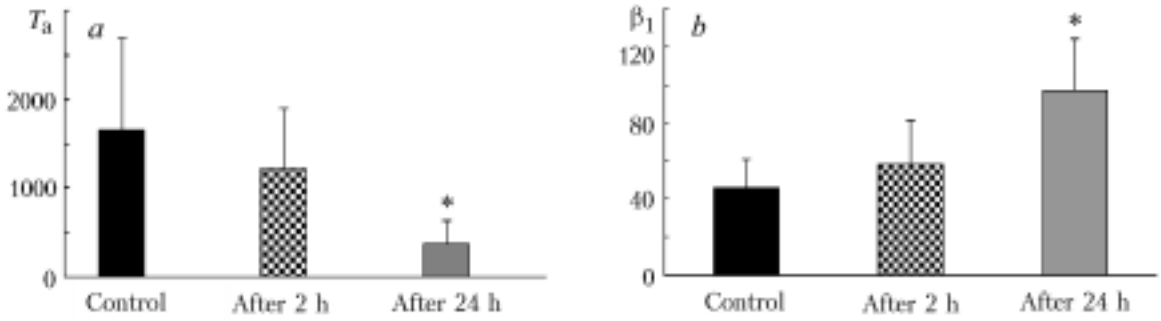

Fig. 1. Aggregation properties of the erythrocytes of control rats and rats with an extensive ischemic insult developed for 2 h and 24 h: a) characteristic time of spontaneous erythrocyte aggregation (T_a); b) strength of the large erythrocyte aggregates in a shear flow (β_1); *) $p < 0.05$, comparison with the control data.

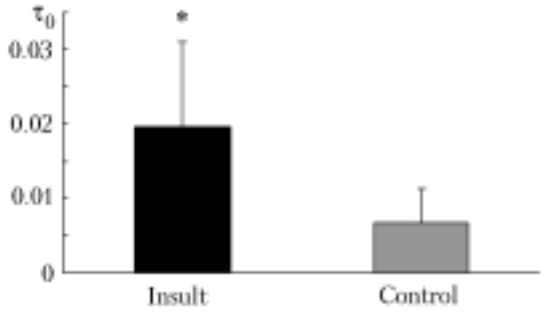

Fig. 2. Critical shear stress (τ_0) of the blood of rats with a local ischemic insult and of control animals; *) $p < 0.05$.

control group of animals ($p < 0.05$). Thus, when the regulation functions are depressed (one carotic artery is ligated), even a local ischemic action on the cerebrum can significantly deteriorate the hemorheological status. The model constructed is close to the actual clinical pattern of a local ischemic insult [17]. The data obtained can be used to determine the degree of cerebrum ischemia at which hemorheological disturbances appear.

It has been established that even a fairly local ischemic insult leads to a decrease in the blood fluidity. As a rule, the hemorheological status deteriorates as the disease develops and, by contrast, normalizes in the process of treatment of the disease [5, 16]. The fact that the clinical pattern of a local ischemic insult changes in parallel with the rheological properties of the blood allows us to suggest that the hemorheological factors influence the development of the disease and that a preliminary action on an organism can be used for prophylaxis of ischemic disturbances. Such an action can be a short-term training of an organism for hypoxia [18]. In this case, a natural question on the optimum regime of this training arises.

Influence of the regime of training for hypoxia on the hemorheological parameters. The ischemic state was corrected by a short-term training for hypoxia in a continuous regime (120 min) or a periodic regime including three periods of duration 40 min each.

A continuous action of an oxygen deficiency increased the rigidity of the erythrocytes: the "index of erythrocyte rigidity" of the animals trained for 120 min was 147.4 $\pm$ 25.2% and exceeded the control IR = 98.5 $\pm$ 7.6% and the IR determined after the periodic hypoxia action (95.6 $\pm$ 10%). As is seen from Fig. 3, the continuous training increased the rate of spontaneous aggregation of erythrocytes, while the periodic training decelerated this process.

The periodic training for hypoxia improved not only the microrheological parameters (Fig. 3) but also the integral parameters. After the training including three periods of duration 40 min each, the critical shear stress of the blood was 0.00262 $\pm$ 0.00029 Pa, which is much ($p < 0.05$) smaller than the control values of this parameter (0.00525 $\pm$ 0.00096 Pa) and the values obtained after the continuous hypoxia action (0.00507 $\pm$ 0.00152 Pa).

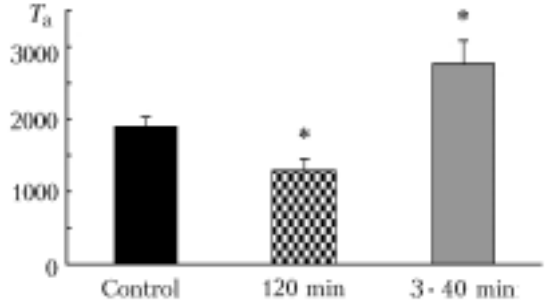

Fig. 3. Characteristic time of spontaneous erythrocyte aggregation (T_a) in the blood of control rats and rats subjected to a short-term training for hypoxia in a continuous regime (120 min) or a periodic regime including three periods of duration 40 min each; *) $p < 0.05$, comparison with the control data.

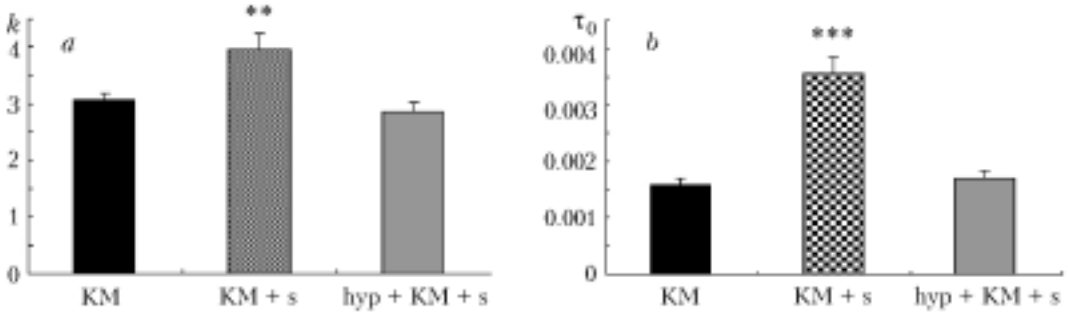

Fig. 4. Rheological properties of the blood of KM-line rats that were not subjected to any action and of rats subjected to a sound action or a preliminary periodic training for hypoxia and a subsequent sound action: a) apparent viscosity (k); b) critical shear stress (τ_0) of the blood; **) $p < 0.01$ and ***) $p < 0.001$, comparison with the control data.

Thus, the study of the different regimes of training has shown that a continuous hypoxia action does not improve but somewhat deteriorates the hemorheological status. By contrast, the periodic hypoxia, realized in our experiments, increased the blood fluidity. It is probable that the training accompanied by a favorable change in the state and properties of erythrocytes is realized in response to the appearance/disappearance of a hypoxia stimulus, which makes it possible to activate the protective forces and block the possible negative effect of a future hypoxia. The results obtained determined the parameters of the periodic training for hypoxia conducted in the next series of experiments for correction purposes.

The rheological properties of the blood in the case of hemorrhagic insult. Sound action on rats of the KM line increased the viscosity of their blood (which exceeded the control values by 1.3 times) and the critical shear stress (which exceeded the control values by 2.2 times; see Fig. 4, KM + sound and KM groups). The decrease in the blood fluidity was caused by an increase in the hematocrit (45 $\pm$ 1.4%; compare with the control values 37.8 $\pm$ 0.7%, $p < 0.001$) and a significant increase (by 2.3 times) in the strength of small erythrocyte aggregates (6441 $\pm$ 1125 rel. units; compare with the control values 1562 $\pm$ 467 rel. units, $p < 0.05$).

The hemorheological disturbances correlated with a clinical pattern. For example, 100% of the KM-line rats subjected to a sound action had critical disturbances of the locomotive functions and massive cerebral hemorrhages (of mean area 55% of the total cerebrum area). Frequently a sound action led to the death of animals (in 55% of the cases). A preliminary training for hypoxia substantially improved the clinical pattern: after this training, the sound action did not lead to a fatal outcome, the critical disturbances of the locomotive functions disappeared completely, and the hemorrhage area decreased to 5%.

When animals trained preliminarily for a hypoxia action were subjected to a sound action, the viscosity and the critical shear stress of the blood became normal (compare the hypoxia + KM + sound group with the control KM

group in Fig. 4). The volume concentration of erythrocytes in the blood of the trained animals (38.5 ± 0.9%) was also equal to the control one (37.8 ± 0.7%). As a result of the hypoxia training, the index of erythrocyte rigidity decreased by 1.6 times as compared to the control one (138 ± 21% for the group of trained animals and 225 ± 56% for the control group; $p < 0.05$). The aggregation properties of the erythrocytes were also improved: the rate of erythrocyte aggregation decreased by 1.5 times (the characteristic time of erythrocyte aggregation was 6855 ± 741 rel. units for the trained rats and 4698 ± 454 rel. units for the control animals, $p < 0.05$) and the rate of decomposition of large aggregates increased by 1.5 times (the coefficient of erythrocyte-aggregate strength was 33.2 ± 5.5 rel. units for the rats subjected to the training and 48.9 ± 4.9 rel. units for the control animals, $p < 0.05$).

Thus, the results of this series of experiments made it possible, first of all, to characterize the rheological properties of the blood in the case of hemorrhagic insult. It is known that a blood-supply deficiency — an ischemia of a cerebrum tissue — can arise as a result of an insult caused by a hemorrhage [19]. We were the first to establish that the hemorheological status of the KM-line rats with a hemorrhagic insult accompanied by epileptiform fits is deteriorated, which can adversely affect the ischemic state and provoke further development of the disease, and that a preliminary short-term periodic training for a hypoxia action normalizes the macro- and microrheological parameters. A comparison of this data with the data of clinical analysis has shown that the hemorheological parameters of rats change in parallel with the clinical pattern of the disease. This points to the fact that hemorheological disturbances can be responsible for the development of a hemorrhagic insult.

CONCLUSIONS

1. The rheological properties of the blood of rats subjected to an experimental extensive ischemic insult deteriorated within 24 h after the beginning of the disease: the rate of erythrocyte aggregation decreased by 77% and the strength of large erythrocyte aggregates increased by 52%.

2. A local ischemic insult in rats adversely affected the hemorheological properties of their blood — the critical shear stress was increased by 66% and the strength of the erythrocyte aggregates was increased by 70%.

3. After a continuous 2-h oxygen deficiency, the rate of erythrocyte aggregation increased by 31% and, as a consequence, the rheological properties of the blood deteriorated. A pulsed 2-h hypoxia action improved the hemorheological parameters, since the critical shear stress decreased by two times and the rate of erythrocyte aggregation decreased by 46% in this case.

4. The blood fluidity of the KM-line rats with a massive cerebral hemorrhage, caused by a sound stress, was decreased. In this case, the apparent viscosity of the blood was increased by 29% and its critical shear stress was increased by two times. A preliminary periodic training for hypoxia improved both the clinical pattern and the biochemical properties of the blood.

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NOTATION

Ht, hematocrit number, volume concentration of erythrocytes in the blood, %; Htc, volume concentration of erythrocytes in a diluted suspension of erythrocytes; I, intensity of backward scattering of light, rel. units; IR, index of erythrocyte "rigidity", %; k^2, Caisson viscosity, mPa·sec; n, number of blood samples or animals studied; T_1, time for which an erythrocyte suspension flows through a filter, sec; T_2, time for which a pure solvent flows through a filter, sec; T_a, characteristic time of aggregation, rel. units; t, time, sec; β, coefficient of hydrodynamic strength of the erythrocyte aggregates, rel. units; $\dot{\gamma}$, rate of shear, sec^{-1}; τ, shear stress, Pa; τ_0, critical shear stress, Pa.

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