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THERMAL DESTRUCTION OF ERYTHROCYTE SPECTRIN: RHEOLOGY, DEFORMABILITY, AND STABILITY WITH RESPECT TO DETERGENTS

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By means of blood heating in the region of the thermal denaturation transition of spectrin (50°C) the relationship between the stability of erythrocytes with respect to detergents and their deformability and rheological parameters of blood was studied.

Introduction. Under the actual conditions of the blood flow, rheological properties of blood at low shear velocities are determined, in addition to hematocrit, by the concentration of fibrinogen and other plasma proteins, and the structural status of erythrocytes constituting the suspension. Data can be found in the literature on the effect of rigidity (deformability) of erythrocytes on rheological properties of blood [1]. Erythrocyte deformability depends on the cytoskeleton status that, in its turn, is a function of various factors: temperature, pH, and ionic strength of the medium. The main protein of the membrane cytoskeleton that determines the shape and deformability of the erythrocytes is the protein of the band 1 or 2, or spectrin [2]. Spectrin undergoes the thermal denaturation transition within the narrow temperature region in the vicinity of 50°C [3], which is connected with the huge activation energy of the process: about 200 kcal/mole [4]. When cells are heated in the vicinity of this temperature in an isotonic salt buffer, pH 7.4, waves are created at the rim of the discocyte, they grow, and, as a result, in several tenths of a second spectrin-free vesicules separate from the erythrocyte [5, 6]. Our objective was to find out how variations in mechanical properties of the membrane and deformability of erythrocytes taking place upon the denaturation transition of spectrin correlate with variations in rheological properties of blood.

Materials. Experiments were carried out with donor blood conserved with the Glugicir blood preservative. Rheological properties and deformability were studied on whole blood, and stability with respect to detergents was studied using an erythrocyte suspension (ES) in the isotonic buffer (IB = 148 mM NaCl + 5 mM sodium phosphate buffer, pH 7.4). To prepare the ES we washed plasma off the whole blood in an OPN-2 desktop centrifuge with a bucket rotor for 10 min at 1000 *g*, and two more times in the IB removing supernatant. Then the erythrocyte paste was diluted 5 times by the IB (hematocrit Ht = 20%). Each of the samples of whole donor blood was heated for 10 min at temperatures 46, 48, 50, 52, and 54°C, and then was cooled to room temperature. For experiments on the stability with respect to detergents blood samples heated in this manner were washed three times and suspended in the IB. In studies of the stability of erythrocytes with respect to detergents we used sodium dodecyl sulfate detergent (SDS) purchased from Sigma.

Methods. Rheological measurements were carried out on whole blood using a coaxial cylindrical viscosimeter with a step motor, which was designed in our laboratory. All the experiments were carried out at the temperature 37 ± 0.2° in the working unit of the viscosimeter within the range of shear velocities $1-10\ \text{sec}^{-1}$. The parameters of the Caisson model

$$\sqrt{\tau} = \sqrt{\tau_0} + \sqrt{\eta\dot{\gamma}}\,,$$

were determined by the method of least squares; here τ is the shear stress; τ_0 is the limiting shear stress; η is the Caisonian viscosity; and $\dot{\gamma}$ is the shear velocity.

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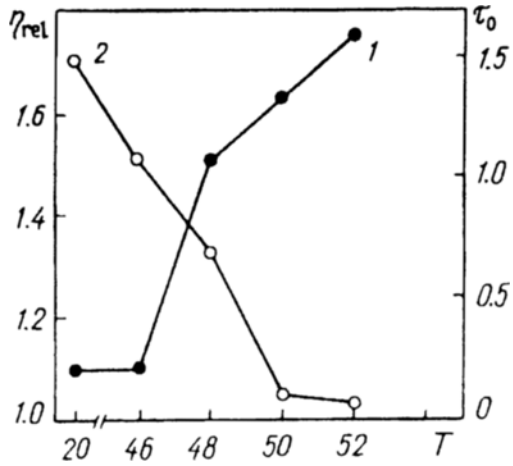

Fig. 1. Dependence of the relative viscosity (1) and the limiting shear stress (2) on the temperature of blood preheating. τ_0, mPa; T, °C.

In studies of deformability of erythrocytes we used the method developed by Ham et al. [7] and modified by Kostin [8]. Capillaries with closed lower end were filled with blood samples and placed into the adapter of the rotor of the centrifuge. The centrifuge was rapidly speeded up to 1500 rpm. Periodically, after t = 5, 10, 15, 25, and 35 min from the start of centrifugation the centrifuge was stopped, the samples were taken out of the centrifuge adapters, and the height of the erythrocytes column l_e was measured. Then the samples were placed back in the cells of the centrifuge and the centrifugation was continued for 35 min total time. The parameter measured was the so-called current hematocrit

$$H = 100 l_e / l_0 ,$$

where l_0 is the hight of the column of the liquid in the capillary.

Simultaneously we measured the reference hematocrit value under conditions of tough centrifugation on an MGTs-8 multipurpose device (H_0). The deformability index of erythrocytes ΔH is determined in percent as follows:

$$\Delta H = 100 (H - H_0)/H_0 .$$

Dependences of ΔH on the centrifugation time were processed by the method of least squares as exponentials:

$$\Delta H = H_0 + A \exp(-Kt) .$$

A decrease in the rate constant of erythrocytes shrinkage K means a decrease in their deformability.

In studies of stability of erythrocytes with respect to detergents we added 0.1 ml SE to the cell with 2.4 ml 350 μM SDS solution in IB at the temperature 37°C. Kinetics of hemolysis of erythrocytes was measured on a JY3CS spectrofluorimeter. The intensity of light scattered by the suspension of erythrocytes at the wavelength 670 nm was recorded at the angle 90°. Hemolysis of erythrocytes by SDS has two stages [9]. Inasmuch as the hemolysis degree at the first stage h_1 decreases with increasing erythrocyte concentration, to compensate for small oscillations of the concentration resulting from washing and subsequent dilution of erythrocytes to the original volume in the IB, the result of the experiment was expressed by the quantity $D_1 = h_1 C$, where C is the optical density of the erythrocyte sample obtained by addition of 0.1 ml SE to 2.4 ml IB.

Results and Discussion. Heating of whole blood at 50°C increases the hematocrit by ca. 10%. This is explained by spherulation of erythrocytes and fusion of vesicles and increasing rigidity of erythrocytes, which leads to variation in packing of erythrocytes during measurements of hematocrit Ht. We carried out additional experiments on determination of the functional dependence of parameters of the rheological model employed on Ht. It was found out that within the region of hematocrit values under investigation (30–50%) this dependence is linear. Therefore, in processing of experimental data we calculated the viscosity value corresponding to the

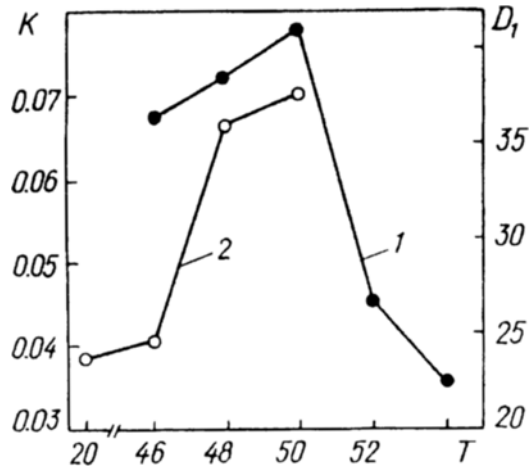

Fig. 2. Dependence of the rate constant of squeezing of erythrocytes (1) and the degree of hemolysis by SDS at the first stage (2) on the temperature of blood preheating. K, min^{-1}; D_1, %; T, °C.

hematocrit of the sample, and the relative quantity η_{rel} was used as a final result of rheological experiments (Fig. 1, curve 1). As follows from Fig. 1, the sharp variation in the relative viscosity takes place in the region 46–48°C, which corresponds to preliminary stages of the denaturation transition of spectrin. Within the same temperature region, up to 50°C, the limiting shear stress decreases ($\tau \to 0$), i.e., a change in the type of the rheological model takes place (Fig. 1, curve 2). The limiting shear stress is usually connected in the literature with the ability of erythrocytes of whole blood to form structures (to aggregate into unified kinetic units with participation of fibrinogen). It is natural to treat the decrease in τ_0 as a loss in the aggregation ability of erythrocytes, probably due to spectrin denaturation; however, other mechanisms cannot be ruled out.

Figure 2 presents variations in the rate constant of squeezing of erythrocytes upon heating of blood in the region of the temperature of spectrin denaturation (curve 1). It is known that within the temperature region 48–50°C erythrocytes change their shape: spicules appear on the surface (rim) of erythrocytes from which thereafter spectrin-free vesicules separate [5]. In this case deformability of erythrocytes varies just slightly, namely, somewhat increases. Then, in the region of 50–54°C vesicles separate from erythrocytes, which results in a decrease in dimensions of the latter; in addition, spherulation of erythrocytes takes place, which likely increases their rigidity. Indeed, the deposition rate constant drops sharply in this region.

Figure 2 (curve 2) shows that the stability of erythrocytes with respect to hemolysis by the anionic SDS detergent is also a parameter sensitive to processes that take place within the erythrocyte upon spectrin denaturation. As is evident from the Figure, the degree of hemolysis increases sharply just in the region of 46–50°C, i.e., in the region where dramatic changes in the arrangement of the protein in the membrane (aggregation) and echinocytosis take place.

Thus, all three of these methods that we used for probing the erythrocyte status from the viewpoint of its mechanical properties are sensitive to variations in erythrocytes upon denaturation of the cytoskeleton protein spectrin. However, the comparison of the results obtained shows that these methods are sensitive to different stages of the process that takes place upon heating of blood past 50°C. Rheological parameters and stability with respect to the detergent are sensitive to initial stages, whereas the parameters of squeezing of cells in the centrifuge are sensitive to the final stage, the release of vesicles.

Investigation of the stability of erythrocytes with respect to detergents seems to be a promising method in diagnostics of diseases connected with disorders in blood rheology as a result of variation of the ability of erythrocytes to form structures. We also expect much of this method in estimating the ability of the organism to sustain overheating (hyperthermia, labor in heat-treating departments and deep-lying mines).

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