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FUNDAMENTAL LAWS OF THE DEFORMATIONAL BEHAVIOR OF ERYTHROCYTES IN SHEAR FLOW

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The dependences of the deformability parameter of erythrocytes on the shear stress in the Couette flow upon a change in their membrane rigidity or internal-content viscosity have been investigated by the diffractometric (ectacytometric) method. The results obtained suggest that the yield stress of cells measured by this method reflects the deformability of erythrocyte membranes, and the slope of the deformation curve rectified in the semilogarithmic coordinate system depends on the viscosity of the internal content of the cell. A parameter such as the limit deformation of erythrocytes is primarily determined by the viscosity of the membrane cytoskeleton.

Introduction. There are several methods for investigating the deformational behavior of erythrocytes: membrane drawing in a pipette and filtration of erythrocytes through microfilters, especially with the use of nucleopores with d = 5 μm (for human erythrocytes) [1–3]. Again, the centrifugation method is worth mentioning [4]. At present, the better-substantiated diffractometric method is successfully competing with them [5, 6]. This method relies on laser-beam diffraction by an erythrocyte suspension in the shear flow. Erythrocytes extended in the flow can be approximated with a good degree of accuracy by ellipsoids. A measure of erythrocyte deformation in the flow as a function of the shear stress is the deformability index defined by the relation DI = $(L - H)/(L + H)$. With the aid of this method, Morris and Williams [7] investigated the hemolysis of erythrocytes in the presence of a shear stress and associated its value with the degree of "membrane fatigue." Phillips [8] proposed a physical substantiation of the method and showed that an increase in the ellipsoid length in the shear flow for normal erythrocytes occurs in accordance with the dependence $\Delta L/L = 0.19 \ln(\tau/\tau_0)$, $\tau_0 = 0.38$ N/m^2.

Results are independent of the erythrocyte size [9], which was used to reveal the features of the deformability of erythrocytes of various mammals. In particular, it was shown that ellipsoid-like erythrocytes (e.g., in the llama) do not deform and are only oriented in the flow. In [10], a dramatic decrease in the erythrocyte deformability upon the formation of Heinz bodies in them was revealed. The investigation of the effect of oxidative stress on the erythrocyte deformability showed that the greatest damage is caused by hydrogen peroxide [11], which was confirmed later in [12].

In the rheology of erythrocytes, the problem of determining their "internal viscosity," introduced by Dintenfass as a deformability criterion [13], is still to be solved. While the methods for calculating the "internal viscosity" by means of viscosimetry were substantiated and recognized [14], it is still not known what contribution to this parameter is made by a change in the membrane properties and by a change in the internal content of the erythrocyte around which the membrane rotates in the shear flow [15].

The aim of the present work is to find out to what extent the dependence of the deformability parameter on the shear stress is determined by a change in the erythrocyte cytoskeleton and to what extent — by a change in its internal content.

Experimental. The constructional arrangement of our facility is based on the ectacytometer of [6] modified as in [16]. It is schematically represented in Fig. 1. The basic part consists of two coaxial cylindrical thin-walled cups 2 and 4 made from polished optical acrylic plastic. Alignment is achieved by moving the internal cup 2, which is fixed in a holder equipped with a coordinate helical mechanism of precision motion. The wall thickness of the cylindrical

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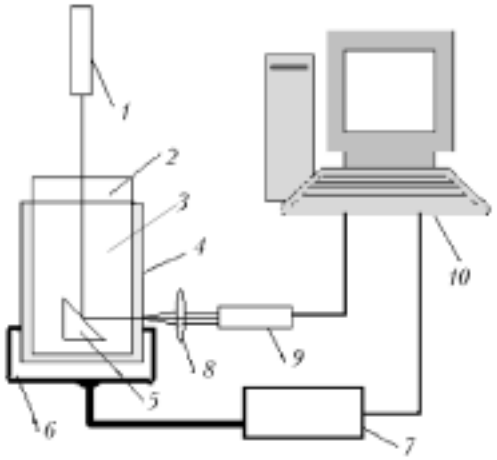

Fig. 1. Schematical representation of the facility: 1) He–Ne laser; 2) internal fixed cylinder; 3) suspension of erythrocytes; 4) external rotating transparent cylinder; 5) mirror; 6) holder; 7) reducer; 8) lens; 9) video camera; 10) computer.

cups is equal to 1 mm, and the clearance between them is 0.5 mm. The external cup 4 is fixed in a holder 6 that can freely rotate in the system of bearings. The rate of shear is defined as

$$\dot{\gamma} = \frac{4\pi R_1 R_2 \nu}{R_1^2 - R_2^2} = A\nu \; [c^{-1}] \,, \;\; \tau = \eta\dot{\gamma} \,, \;\; A = 232.4 \,.$$

The reducer 7 can vary the rate of shear over a wide range from 1 to 30,000 sec^{-1}. However, the results described in the present paper were obtained with the use of 16 points within the 23–4650-sec^{-1} range of change in the rate of shear, which corresponds to a change in τ from 40 to 6000 H/m^2.

The optical part of the facility consists of an LG-72 He–Ne laser 1 (wavelength of 632.8 nm, power of 1 mW), whose beam is reflected from the mirror 5 mounted on the bottom of the cylinder 2 and passes horizontally through the walls of the cylinders 2 and 4, the layer with the suspension of erythrocytes 3 situated between them, and the lens 8. On the clouded glass located at the focal length from the lens, a diffraction pattern corresponding to the size and shape of the size-averaged erythrocyte is observed. It was registered by video camera 9, and the image was analyzed on computer 10.

The composite diffraction pattern registered by the video camera from erythrocytes extended in the shear flow represents a superposition of zero diffraction maxima and two or three minima and maxima of higher orders and is visualized on the monitor display as a system of concentric circles corresponding to levels of different intensities. In the presence of a shear stress in the flow, it transforms to a system of ellipses turned by $\pi/2$ from the major axis of the erythrocyte subjected to a shear stress.

The concentration of the erythrocytic suspension is chosen so that at a given layer thickness of 0.5 mm single scattering of the laser beam occurs. This condition is fulfilled by diluting the whole blood sample 300 times, which we did with an isotonic solution of high-molecular polyethylene oxide at 0.5% concentration. In so doing, the solution viscosity was 13 mPa·sec. Such a solution practically has no scattering and is a Newtonian liquid. In making such measurements, some authors use, instead of polyethylenoxide, a solution of high-molecular dextran with a concentration of a few percent. Solutions of dextrans of such a concentration exhibit Newtonian properties and large light scattering.

The degree of deformation of erythrocytes was determined by the so-called ellipticity index of the diffraction pattern, EI = $(a - b)/(a + b)$, which corresponds, to an accuracy of 10%, to the deformability index of erythrocytes. Here a and b are the major and minor semiaxes of the ellipses corresponding to levels of equal intensities. The dependences of the deformability index on the shear-stress logarithm (hereinafter referred to as deformation curves for brevity) plotted in the semilogarithmic coordinate system are well rectified. In so doing, by the characteristic of each straight line obtained one can choose a point of its intersection with the shear-stress axis, which yields the critical

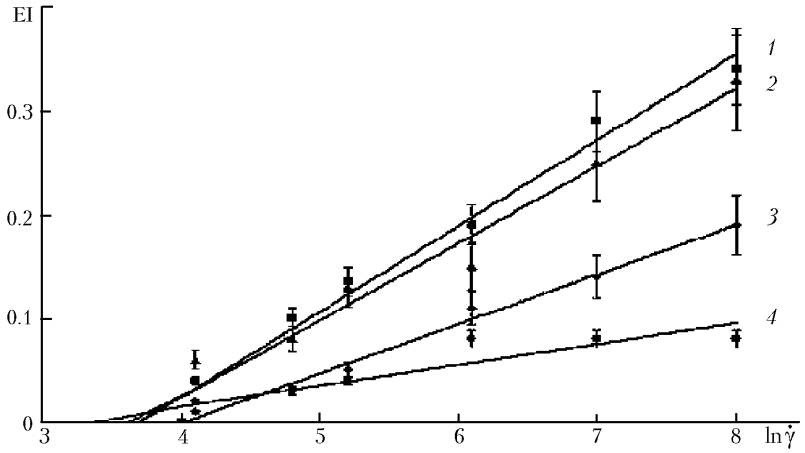

Fig. 2. Change in the deformation curves of erythrocytes with increasing concentration of NaCl: 1) norm; 2) 0.6; 3) 3.0; 4) 4.8%.

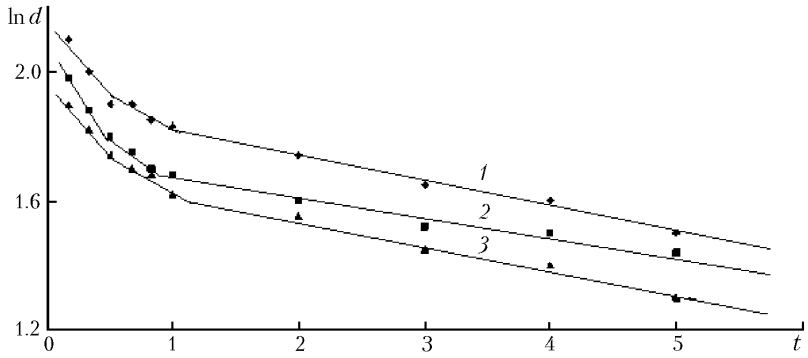

Fig. 3. Time dependence of the change in the size of erythrocytes (total time of the experiment is 20 min): 1), 2), 3) erythrocytes of different initial (intact) sizes: $d_1 > d_2 > d_3$. t, min.

shear stress at which deformation begins (i.e, the yield point of the membrane). The second characteristic is the slope of the straight line.

Results and Discussion. It is natural to assume that in the shear flow the deformation of erythrocytes depends on the mechanical properties of two components: membrane and internal content. Each of them makes a contribution to the deformation curve, and the internal content thereby has to be recognized as a viscous liquid, since it has been proved [17] that the membrane of the normal (not rigid) erythrocyte moving in the shear flow rotates around the liquid content at a rate depending on the latter's viscosity and the rate of shear.

To determine the contribution of each component, it is necessary to choose such actions that selectively change either the membrane properties or the hemoglobin-solution viscosity inside the erythrocyte. The latter is easy to achieve by placing cells in a hypertonic medium. Figure 2 shows the rectified deformation curves obtained for erythrocytes of one and the same blood sample suspended in a hyperosmotic medium of sodium chloride. In both cases, one type of the family of deformation curves is observed: all of them are characterized by close values of the yield stress (with an accuracy of 15%) and differ in slope. In this case, the yield stress of the erythrocyte membrane τ_0 is independent of the erythrocyte-content viscosity, whose change influences only the slope of the straight lines and limits the size of deformation.

Figure 3 shows three typical curves of change in the erythrocyte-diameter logarithm depending on the residence time in the hyperosmotic solution at an osmotic pressure of 2000 mOsmole/liter. The characteristic times for ten experiments in the approximation of the kinetics of water flowing out of the erythrocyte by exponents ($d \approx \sum_{i=1}^{2} \exp(t/t_i)$) are

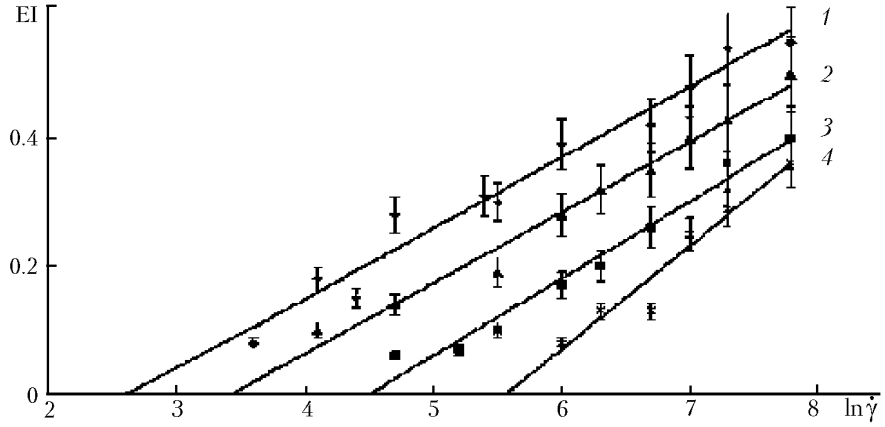

Fig. 4. Change in the deformation curves caused by a change in the concentration of camphocaine in the suspending medium: 1) norm; 2) 1; 3) 3; 4) 6%.

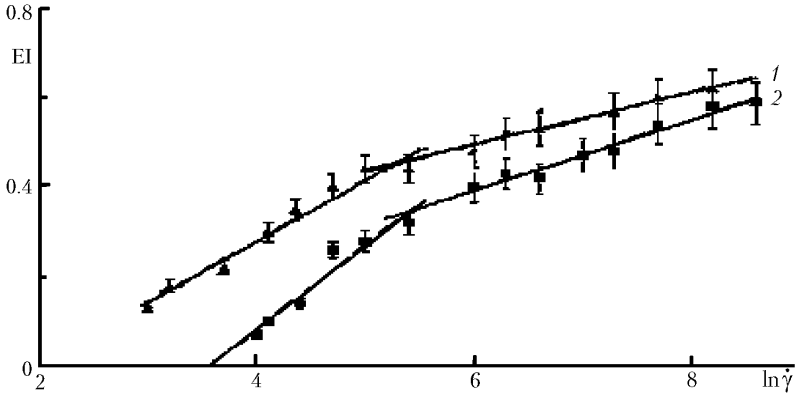

Fig. 5. Deformability index versus the logarithm of the rate of shear in storage of erythrocytes: 1) storage for 10 days; 2) 20 days.

t_1 = 2.5 ± 0.1 min and t_2 = 13 ± 1.5 min. A decrease in the volume of erythrocytes by 46% almost doubles the concentration of hemoglobin in it, which leads to a marked change in viscosity. In our experiments, the concentration of hemoglobin changed by a factor of 1.5.

To act on the erythrocyte membrane, we chose: leukocytic interferon, which, due to its lipotropy (as a suspension of lyophilized leukocytes), is nonspecifically bound by the membrane, and camphocaine — a water-soluble derivative of camphor with powerful surface-active properties. These substances are combined exclusively with the cell membrane and influence only the rigidity of the membrane cytoskeleton. Figure 4 gives typical families of deformation curves of erythrocytes under the action of various concentrations of camphocaine. Under the action on erythrocytes by interferon, similar families of curves are obtained. Both preparations change mainly the yield stress, but almost have no effect on the slope of the graphs.

The experiments performed suggest that the yield stress reflects mainly the deformability of erythrocyte membranes, and the slope of the deformation curve rectified in the semilogarithmic system of coordinates depends on the viscosity of the internal content of the cell.

The deformational behavior of erythrocytes in the shear flow is not exhausted by the dependence

$$(L - H)/(L + H) = k \ln \dot{\gamma} + C .$$

In some groups of experimental and clinical studies, deviations from the proportional dependences were observed. Often there is a kink of the graph at high rates of shear, which points to the switching on of some mechanisms, restricting further deformation. Such a type of deformation was observed in patients with dilatation cardiomyopathy (DCMP) and in storing erythrocytes (standard storing conditions at blood transfusion centers), as is shown in Fig. 5.

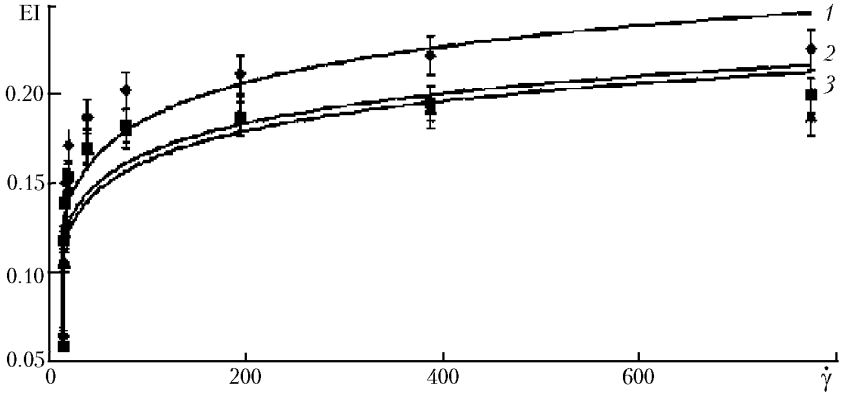

Fig. 6. Deformability index versus the rate of shear in erythrocytes of rats: 1) norm; 2) 90 min after the operation; 3) after 4 days.

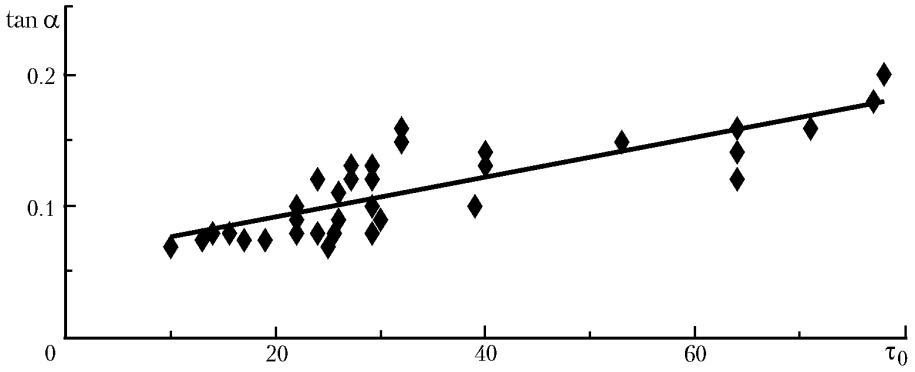

Fig. 7. Correlation between the yield point and the slope of the rectified deformation curve of erythrocytes in the shear flow. Each point represents one curve.

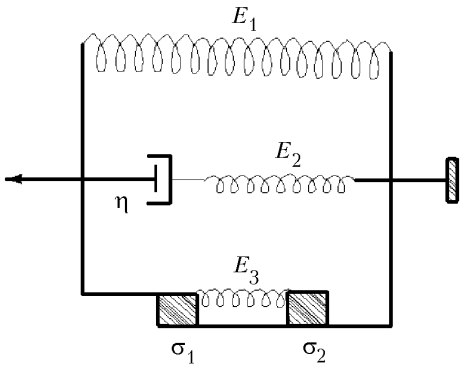

Fig. 8. Rheological model of erythrocytes.

It may be suggested that in the process of shear stress increase, there occurs a stepwise reversible change in the structure of the erythrocyte cytoskeleton followed by a rapid growth of cell ellipticity.

In addition to the two parameters characterizing the membrane and internal state of the erythrocyte, we can introduce the parameter "limit deformation of the erythrocyte." Figure 6 shows three deformation curves of erythrocytes of rats: before (upper curve) and after hypoxis resulting from the operation of pinching the carotid arteries. The asymptotic deformation values before and after the operation leading to hypoxis are different. This phenomenon is largely associated with the kink of the deformation curve presented in Fig. 5.

The presence of a correlation between the yield point and the slope of the graph given in Fig. 7 can partly confirm the validity of such a hypothesis. The interaction between the lipid membrane, spectrin, and hemoglobin was discussed in [18–20].

As the basis for the rheological model of the erythrocyte, we used the three-element model of [21] complemented by Saint Venant bodies (Fig. 8). The first body σ_1 determines the yield point of the membrane under stresses below which the body does not deform, and the second body σ_2 determines the position of the inflection point in Fig. 5. It may also be suggested that the nonlinearity of the deformation curve is associated with the nonlinear elastic element reflecting not so much the properties of the lipid layer of the membrane as the property of the membrane skeleton (spectrin network).

Conclusions. An increase in the viscosity of the internal content of erythrocytes decreases the slope of the curve of the diffraction-pattern ellipticity index versus the logarithm of the rate of shear. One can judge a change in the deformational behavior of precisely the membrane of erythrocytes by the so-called yield point obtained from the curve of the diffraction-pattern ellipticity index versus the logarithm of the rate of shear or the shear stress. Such a parameter as the limit deformation of erythrocytes is significant in investigating the effect of various medicines or diseases on the cell membranes.

NOTATION

A, dimensionless quantity, constant of the facility; a and b, major and minor axis of ellipses in the diffraction pattern, m; C, integration constant; d, diameter of nucleopores, μm; DI, deformability index; E_1, E_2, and E_3, elasticity of membrane components of erythrocytes; EI, ellipticity index; H, width of deformable cell, μm; k, numerical coefficient; L, length of deformable cell, μm; R_1, internal radius of the external cup, mm; R_2, outer radius of the internal cup, mm; t, time, sec; t_i, characteristic time of the process, sec; α, slope of the deformation curve–logarithm of the rate of shear; $\dot{\gamma}$, rate of shear, sec^{-1}; η, solution viscosity, mPa·sec; ν, frequency of relation of the external cup, sec^{-1}; σ_1 and σ_2, Saint Venant bodies; τ, shear stress, N/m^2; τ, critical shear stress, N/m^2. Subscripts: 0, fixed value of a quantity.

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