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NEW RHEOLOGICAL MODEL FOR ANALYZING THE AGGREGATABILITY AND DEFORMABILITY OF ERYTHROCYTES IN A NUMBER OF HEMATOLOGICAL PATHOLOGIES

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A new model of approximation of the blood-flow curve with five parameters independent of the hematocrit and the rate of shear is proposed. On the basis of the statistical analysis, we have shown the reliability of differences of the mean values of the independently measured aggregatability and deformability of erythrocytes and the parameters of the new rheological model of hematological patients (myeloma, erythremia, anemia, blood loss) from donors. The relation of two parameters of the model, γ_1 and γ_3, to the aggregatibility and deformability of erythrocytes, respectively, has been proved.

A great number of diseases exhibit a common feature characteristic of most pathologies — disturbance of the rheological indices of the blood. On the one hand, this is due to the change in the viscosity of the carrier medium — blood plasma. In particular, in the case of one of the pathologies chosen for the investigation — myeloma — the plasma viscosity increases by a factor of about one and a half compared to the norm because of the strong increase in the concentration in the plasma of proteins — immunoglobulins [1]. On the other hand, changes in the blood viscosity and relative viscosity of the medium η/η_{pl} are caused by a change in the microrheological properties of erythrocytes, their aggregatability and deformability.

The blood viscosity depends on the hematocrit and the rate of shear. The shape of the flow curve — viscosity as a decreasing function of the rate of shear (Fig. 1) — is assumed to be associated with the disaggregation of erythrocytes at low rates of shear and their deformation under the action of the shear stress at high rates of shear. This dependence can be approximated by a great variety of functions (hyperbola, exponent, power function) as is the viscosity–hematocrit dependence. But the function used has been substantiated only in Quemada's works (see, e.g., [2] and his other works). However, Quemada's model did not satisfy us either for the reason that its parameter depended on the hematocrit index. The other authors known to us used different empirical functions fairly well approximating flow curves and viscosity–hematocrit dependences.

To elucidate the changes in the flow curves, the model parameters (ideally) should be independent of both the hematocrit and the rate of shear. Unfortunately, the parameters of all models known from the literature turned out to be monotonically dependent on the hematocrit. Therefore, we have developed a new model which, as we see it, is more perfect than all the previous ones.

Materials and Methods. In our experiments, we used fresh blood samples of donors or patients preserved in heparin. The hematocrit index was measured on a hematocrit centrifuge.

The blood and plasma *viscosity* of healthy donors were measured at 37°C on a Low Shear 30 coaxially cylindrical viscosimeter (Switzerland) with a 0–130-sec^{-1} range of rates of shear, with a thermostated working cell.

Measurements of the *aggregatability* (Agr) of erythrocytes are based on the change and graphical recording of photometric indices upon the formation in the dynamics of cell aggregates (erythrocytes) in the autoplasma. Heparinized blood is centrifuged (1500 rpm, 15 min); the erythroconcentrate is drawn from the bottom and mixed with precentrifuged (3000 rpm, 15 min) autoplasma to Hct ≈ 0.25–0.27. The suspension is poured into a Goryaev chamber and placed in

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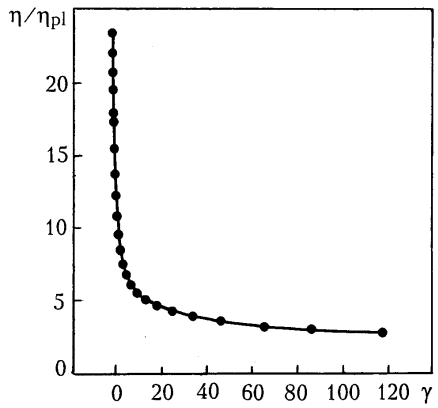

Fig. 1. Relative viscosity of blood as a function of the rate of shear. Donor blood, Hct_{meas} = 0.42. Solid line, data processing by formulas (2). γ, sec^{-1}.

a photometer. The recorder registers the maximum deviation of the curve of the change in the optical density in a fixed time interval (4 min). Normal values of aggregatability reach 100–105 arbitrary units [4].

The *rigidity index* (IR) of erythrocytes was measured on an IDA-1 apparatus. The principle of the method is based on the registration of the filtration time of a fixed volume of the erythrocyte suspension in a resuspending medium through a membrane filter with a mean diameter of the pores of 3 μm. We prepared a 2% suspension of erythrocytes twice washed-off (1500 rpm) in a physiological solution in a resuspending medium (HEPES-buffer). The hydrostatic pressure on the filter is 60 mm of H_2O. A sensing element recording the time of flow of a fixed volume (250 μl) of the suspension through the membrane filter t_s (base — 7 μm-thick polyethyleneterphthalate film) was placed on the column with the erythrocyte suspension. The rigidity index calculated by the formula

$$IR = (t_s - t_b)\,(50/t_b)\,,$$

is a characteristic of the *deformability* of the erythrocytes (inversely proportional dependence). Normal IR values reach 40–45 arbitrary units [5].

All calculations according to the model were performed with the use of software of the graphical program Sigma Plot for Windows.

Results and Discussion. In the model proposed in [3],

$$\ln(\eta/\eta_{pl}) = A\,Hct^{B}\,, \tag{1}$$

it has been shown that the parameters A and B remain constant at least within each of the three ranges of erythrocyte concentrations: $Hct < 0.3$, $0.3 < Hct < 0.7$, and $Hct > 0.7$. At the boundaries of these ranges, in the regions of $Hct \approx 0.3$ and $Hct \approx 0.7$, the values of the parameters A and B experienced a jump in a narrow range of erythrocyte concentrations ($\Delta Hct \approx 0.05$).

The parameters of (1), A and B, are functions of the rate of shear. To describe these dependences, we used the following formulas satisfactorily describing the flow curves (Fig. 1, solid line):

$$Hct = \Sigma\, V_{er} = b_0 Hct_{meas}\,,$$

$$A = 2.5 \exp(a_1 \exp(-\gamma/\gamma_1) + a_2 \exp(-\gamma/\gamma_2) + a_3 \exp(-\gamma/\gamma_3))\,, \tag{2}$$

$$B = 1 + \ln(2.5/A)\,,$$

It turned out that γ_1 and γ_2 linearly correlate between themselves ($P < 1.01$):

$$\gamma_2 = 2.03 + 3.41\gamma_1\,. \tag{3}$$

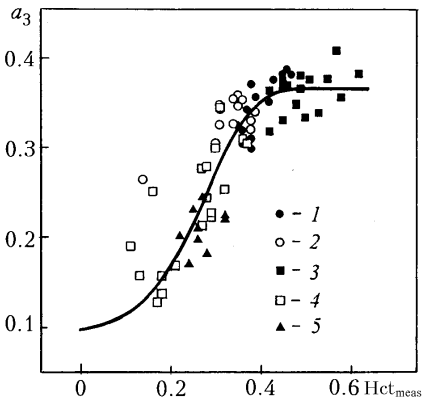

Fig. 2. Parameters a_3 of (2) as a function of the hematocrit index: 1) donors; 2) myeloma; 3) erythremia; 4) anemia; 5) blood loss. Solid line, results of approximation of experimental data by formula (4).

The parameter a_3 depends on the hematocrit index (Fig. 2) and is well approximated by the sigmoid function

$$a_3 = k_0 - k_1 \exp\left(-\left(\mathrm{Hct}_{meas} + 1 - \mathrm{Hct}_0\right)^c\right), \quad (4)$$

where k_0 = 0.36, k_1 = 0.27, Hct_0 = 0.29, and c = 11.21.

The other parameters of (2), b_0, a_1, a_2, γ_1, and γ_3, depended on neither the hematocrit nor the rate of shear ($P > 0.05$).

The rheological measurement data on the blood of donors and patients were processed by formula (2) in view of (3) and (4). The statistical analysis of the distinctions of the mean values of the five independent parameters of (2) for patients from the respective values for donors was carried out with the use of the Student criterion (see Table 1). It has been found that reliable differences between the mean values of the erythrocyte aggregatability (Agr) for patients and donors ($P < 0.05$) and the parameter of model (2) γ_1 are observed for the same pathologies (myeloma, erythremia, anemia for IR > 100, see Table 1), and for the rigidity index of erythrocytes (IR) and the parameter of model (2) γ_3 — for anemia and blood loss. The establishment of these facts is the main practical result of this work.

The effective rheological hematocrit is larger than the sum of the geometric volumes of the cells by the volume of the plasma carried away by the cells. With increasing rate of shear, the rheological hematocrit $\mathrm{Hct}_{eff} = \mathrm{Hct}^B$ tends to the true one ($B \to 1$, (2)). The parameter of (2) b_0 reflects the packing density of erythrocytes in measuring the hematocrit in the centrifuge. As is seen from Table 1, this value reliably changes for two pathologies — myeloma and erythremia. In myeloma, however, the parameter b_0 decreases, whereas in erythremia it increases. As mentioned above, in myeloma the content of immunoglobulins in the blood plasma increases. It may be suggested that on the membrane surface of erythrocytes in this pathology a spongy "coat" from proteins preventing close packing of cells during centrifugation is formed. But if the plasma viscosity increases due to the increase in the fibrinogen concentration, then the packing density and the value of the parameter b_0 increase [3], which very likely occurs in erythremia.

According to our ideas about the character of the processes proceeding in the blood flow with increasing rate of shear, the dimensional parameters of (2) are as follows:

a) $\gamma_1 = 0.39 \pm 0.08$ sec^{-1} — a characteristic rate of shear at which large aggregates of erythrocytes transform to "coin pillars" (see Table 1, data for donors);

b) $\gamma_2 = 3.37 \pm 0.69$ sec^{-1} — a characteristic rate of shear at which the "coin pillars" break down into separate erythrocytes (obtained from γ_1 by formula (3));

c) $\gamma_3 = 57.1 \pm 3.6$ sec^{-1} — a characteristic rate of shear at which under the action of shear forces the shape of erythrocytes changes (see Table 1, data for donors).

The correlation of γ_1 and γ_2 (3) becomes clear if we assume that both of them reflect the binding forces between erythrocytes.

Of all the parameters of (2), only a_3 was hematocrit-dependent (Fig. 2). Half-transformation of the sigmoid is attained at $\mathrm{Hct}_{1/2} = 0.26$ (4). Thus, the jump of the parameters of (1) in the region of concentrations of Hct $\approx$ 0.3,

TABLE 1. Difference of the Mean Values of the Independently Measured Rheological Parameters of Erythrocytes and the Parameters of Model (2) of Patients from Donors According to Student

Quantity	Donors ($N = 13$)	Myeloma ($N = 14$)	Erythremia ($N = 17$)	Anemia ($N = 6$, IR > 100)	Anemia ($N = 12$, IR < 100)	Blood loss ($N = 9$)
Agr ± s.e.	53.7 ± 2.2	117 ± 12 ($P < 0.05$)	72.8 ± 5.9 ($P < 0.05$)	72.0 ± 9.5 ($P < 0.05$)	49.4 ± 4.0 ($P > 0.05$)	68.2 ± 11.0 ($P > 0.05$)
IR ± s.e.	29.0 ± 2.2	35.1 ± 4.1 ($P > 0.05$)	37.4 ± 3.5 ($P > 0.05$)	4293 ± 2115 ($P < 0.05$)	61.1 ± 6.6 ($P < 0.05$)	45.8 ± 5.8 ($P < 0.05$)
η_{pl}, MPa·sec	1.33 ± 0.02	1.74 ± 0.07	1.53 ± 0.03	1.41 ± 0.07	1.22 ± 0.03	1.17 ± 0.04
$T = 37\ ^{o}$C		($P < 0.05$)	($P < 0.05$)	($P > 0.05$)	($P < 0.05$)	($P < 0.05$)
a_1	0.13 ± 0.01	0.09 ± 0.01 ($P < 0.05$)	0.10 ± 0.01 ($P < 0.05$)	0.10 ± 0.02 ($P > 0.05$)	0.19 ± 0.07 ($P > 0.05$)	0.11 ± 0.03 ($P > 0.05$)
a_2	0.22 ± 0.01	0.19 ± 0.01 ($P < 0.05$)	0.20 ± 0.003 ($P < 0.05$)	0.14 ± 0.03 ($P < 0.05$)	0.16 ± 0.02 ($P < 0.05$)	0.18 ± 0.03 ($P > 0.05$)
γ_1 ± s.e., sec^{-1}	0.39 ± 0.08	0.82 ± 0.17 ($P < 0.05$)	1.10 ± 0.14 ($P < 0.05$)	3.03 ± 1.68 ($P < 0.05$)	1.21 ± 0.44 ($P > 0.05$)	0.75 ± 0.26 ($P > 0.05$)
γ_3 ± s.e., sec^{-1}	57.1 ± 3.6	51.8 ± 5.2 ($P > 0.05$)	64.7 ± 3.9 ($P > 0.05$)	113 ± 33 ($P < 0.05$)	79.5 ± 8.2 ($P < 0.05$)	104 ± 16 ($P < 0.05$)
b_0 ± s.e.	0.94 ± 0.02	0.85 ± 0.02 ($P < 0.05$)	0.97 ± 0.01 ($P < 0.05$)	0.90 ± 0.06 ($P > 0.05$)	0.94 ± 0.02 ($P > 0.05$)	0.88 ± 0.04 ($P > 0.05$)

noted in [3], shows up exclusively due to the parameter a_3 of model (2) and is associated, as we supposed initially, with the property of deformability of erythrocytes. It turned out, however, that for hard particles (erythrocytes treated with acetaldehyde [6, 7]) exactly the same jump of the parameters A and B at Hct ≈ 0.3 was observed. Therefore, this phenomenon is likely to be due to the absence of collisions of suspension particles at Hct < 0.3 and the appearance of interaction at Hct > 0.3. In collisions, first, the conditions for the formation of the solvent shell of erythrocytes — the effective hematocrit Hct_{eff} — change and, second, there is an increase in the effective radius of the hydrodynamical drag of deformed erythrocytes, which from particles prolonged by the shear forces after an inelastic impact are transformed into the likeness of a deflated football with dents.

The values of the parameters of formulas (2) do not correlate with the aggregatability and deformability of erythrocytes measured by independent methods. This is not surprising if we remember that the quantity Agr characterizes the sizes of erythrocyte aggregates at rest, and γ_1 and γ_2 reflect the forces of interactions between erythrocytes in aggregates. The changes following the passage of erythrocytes through the filter pores are their twisting and folding, and the deformation under the action of the shear forces is their elongation. However, as is seen from Table 1, there is a change in the very reliabilities of the differences of the mean values of the above parameters of model (2) and the microrheological characteristics of erythrocytes (Agr and IR) for patients compared to the respective values for donors. Thus, to analyze the microrheological state of the blood, it is now enough to measure the blood and plasma flow curves, process them by formulas (2), and analyze the difference from the norm of the parameters of (2) γ_1 and γ_3, respectively.

Thus, a new model of approximation of the blood-flow curve with seven parameters, five of which are independent of the hematocrit and the rate of shear, is proposed. Statistical analysis of the reliability of the differences of the mean values of the independently measured aggregatability and deformability of erythrocytes and five independent parameters of the new rheological model of hematological patients (myeloma, erythremia, anemia, blood loss) from the respective values for donors has been performed. It has been proved that there is a relation between two parameters of the model (characteristic rates of shear γ_1 and γ_3) and the aggregatibility and deformability of erythrocytes.

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NOTATION

Hct, hematocrit index, volume concentration of erythrocytes; Hct_{eff}, effective hematocrit; Hct_{meas}, hematocrit index measured in a hematocrit centrifuge; V_{er}, erythrocyte volume, μm^3; η, suspension viscosity, Pa·sec; η_{pl}, plasma viscosity at high rates of shear $\gamma \approx 120\ sec^{-1}$, Pa·sec; γ, rate of shear, sec^{-1}; P, significance level; Agr, aggregatability

of erythrocytes; IR, rigidity index, deformability coefficient of erythrocytes; s.e, standard error; N, number of subjects in the group; t_s, time of flow of a fixed volume of erythrocyte suspension through the membrane filter, min; t_b, time of passage of the buffer through the filter under the same conditions; T, temperature, ^oC; A and B, parameters of formula (1); b_0, a_1, a_2, a_3, γ_1, γ_2, γ_3, parameters of formulas (2); k_0, k_1, Hct_0, c, parameters of formula (4). Indices: pl, blood plasma; eff, effective; er, erythrocyte; s, suspension; b, buffer; meas, measured.

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