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A MATHEMATICAL MODEL OF ERYTHROCYTE SEDIMENTATION IN CAPILLARIES

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A mathematical model of the process of erythrocyte sedimentation is developed that contains only two optimization parameters, namely, a rheological parameter and the hydrodynamic-resistance radius of a cell. It satisfactorily describes experimental data and can be used to judge the deformability of erythrocytes from their kinetic data and to determine the viscosity of suspensions without a viscosimeter.

Stokes's formula, which is ordinarily used to estimate the velocity of fall of a sphere in a viscous liquid (Re << 1):

$$v_0 = \frac{2gR^2\Delta\rho}{9\eta}, \tag{1}$$

overrestimates the result for an individual erythrocyte by an order of magnitude in comparison with experiments. The velocity of motion of the interface of the medium and the erythrocyte layer (the ESR) depends strongly on the erythrocyte concentration, which is neglected by Stokes's formula. The process of erythrocyte sedimentation is described rather thoroughly in [1]. A theoretical model is developed in Losev's works [2]. However, it is rather complicated and we do not know experimental works based on it and other models. Losev himself indicates that with the number of optimized parameters above two the search is arbitrary. It seems desirable, first, to simplify the formalism as much as possible and, second, to include real mechanical properties of the erythrocyte in the model (the shape, volume, surface area of the membrane, deformability); then, the range of practical applicability of the model will be expanded substantially. We published the first version of a model in [3]. In the present work we describe a mathematical model of the process of erythrocyte sedimentation that is refined for the chosen geometry of a centrifuge.

Experiments were carried out using donor whole blood sampled into a hemopreservative based on sodium citrate or using suspensions of washed erythrocytes in an isotonic buffer (IB) containing 148 mM of NaCl and 5 mM of Na-phosphate buffer, pH 7.4. The blood was washed three times in an isotonic solution of NaCl at 1000 *g* for 600 sec. It was washed a fourth time in the IB. A paste of washed erythrocytes was diluted with the IB in various proportions for variation of the hematocrit (H_0) indices. Similarly, the blood was centrifuged and the paste was diluted in native plasma.

Erythrocyte sedimentation was observed in glass capillaries with a soldered lower end, $d_{in} \cong 0.004$ m. According to preliminary experiments, at $d_{in} < 0.004$ m friction of the medium against the capillary walls makes a large contribution to the ESR, and the latter depends strongly on the diameter of the capillary; on the other hand, at $d_{in} > 0.004$ m, the ESR is almost independent of the diameter of the capillary. The height of the column of blood or the suspension of erythrocytes in the capillary is $h_0 \cong 0.09$ m. Capillaries filled with blood samples were placed in an OPN-2 table centrifuge with a basket rotor and centrifuged at 17, 25, or 50 rps, stopping the centrifuge at fixed intervals of time and measuring the height h of the column of settling erythrocytes. The instantaneous hematocrit was determined from the relation

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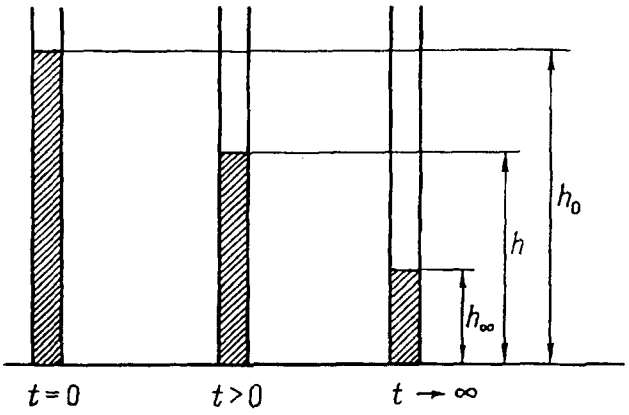

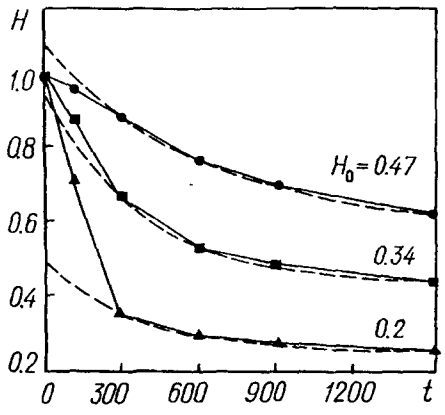

Fig. 1. Erythrocyte sedimentation in a capillary.

Fig. 2. Kinetics of erythrocyte sedimentation in the IB at 25 rps. Dashed lines are results of approximation of the curves by exponentials at $t >> 0$. t, sec.

$$H = \frac{h}{h_0}. \tag{2}$$

The hematocrit of a sample $H_0 = h_\infty / h_0$ was measured in an MGTs-8 high-speed hematocrit centrifuge (Fig. 1).

Erythrocyte sedimentation in the gravitational field (1 g) was measured in vertical capillaries at room temperature of about 20°C. The viscosity of the medium was corrected for temperature nonuniformity using a formula presented in [4].

The erythrocyte concentration distribution over the height of the column in the gravitational field was evaluated, using measuring pipets with d_{in} = 0.005 m fastened strictly vertically. A plastic tube with a clamp was put on the lower end of the pipet. Several pipets were filled simultaneously to maximum capacity (5 ml). In intervals of 1 h the contents of a successive pipet were poured, opening the clamp, into tubes, in 10 portions of 0.5 ml each. Then, distilled water (4.5 ml) was added to the portions up to full hemolysis, and the hemoglobin was measured by an optical method using light absorption at 540 nm. The obtained optical density D is proportional to the erythrocyte concentration in the sample. 4.5 ml of water was added to 0.5 ml of the initial erythrocyte suspension, and light absorption was measured to obtain D_0, proportional to the erythrocyte concentration in the initial suspension. The final result was the relative hemoglobin content in the samples, equal to the relative erythrocyte concentration:

$$\mathrm{Hb} = 100 \frac{D}{D_0}, \ \% .$$

Kinetics of erythrocyte sedimentation in the centrifuge for different hematocrits are shown in Fig. 2. At first glance they resemble exponential curves, which suggested to us and other researchers that they can be approximated by the formula

$$H = H_0 + (1 - H_0) \exp(-Kt). \tag{3}$$

However, a more careful analysis of the shape of the curves showed that the initial section of the kinetics did not satisfy relation (3), although at long times t the description is quite suitable (Fig. 2, dashed curves).

Erythrocyte sedimentation kinetics in native plasma and the gravitational field are somewhat different from their analogs in the IB. For the first ones, because of aggregation of erythrocytes at the very beginning of the experiment and a low ESR of individual cells in comparison with aggregates, a section of the curve with almost no slope (a lag-phase) is observed. The lag-phase is seen especially clearly at large erythrocyte concentrations, while the kinetics of erythrocyte sedimentation in the IB are rectilinear in at least the first hour of the experiment.

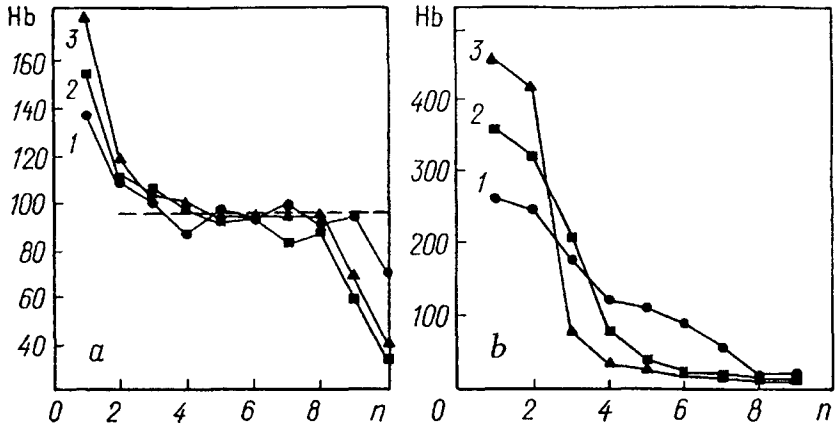

Fig. 3. Vertical distribution of the relative erythrocyte concentration in the case of sedimentation in the gravitational field at different sedimentation times: a) in the IB: 1) 2 h, 2) 3 h, 3) 4 h of sedimentation, b) in native plasma: 1) 1 h, 2) 2 h, 3) 4 h of sedimentation. Hb, %.

Profiles of the vertical variation of the density of erythrocyte suspensions in the gravitational field (d_{in} = 0.005 m) recorded at different times are shown in Fig. 3. A comparison of data for erythrocyte suspensions in the IB and plasma reveals three layers in the IB: an upper layer of the IB; a middle layer of erythrocytes with a concentration equal to the initial one (100%); a lower layer of settled erythrocytes with a very high concentration of cells. The boundary between the upper layer of the buffer and the layer of settling erythrocytes is clearcut (Fig. 3a). In the case of erythrocyte sedimentation in native plasma the situation is more complicated. As a result of aggregation of erythrocytes and, evidently, a substantial spread in the size of the aggregates, their velocities are different. Therefore, in the initial minutes and even hours of sedimentation, a clearcut boundary between the settling erythrocytes and the plasma is absent. The boundary is smeared, and the column density increases down the pipet. Thus, we can speak about the kinetics of the motion of the interface of the medium and the erythrocytes rather arbitrarily. A clearcut interface appears only upon attainment of a critical density of the suspension where not sedimentation, but further packing of settled cells in the column occurs (Fig. 3b).

Plots of the ESR at 1 g in capillaries in plasma (curve 1) and the IB (curve 2) versus the hematocrit are shown in Fig. 4. The length of the column of erythrocytes in plasma or the IB was measured after 1 h of sedimentation. Apart from errors of measurement of the length and time, the main source of errors is the fact (see Fig. 2) that the initial rate of the process, equal to the time derivative of the kinetics at $t = 0$, is always larger than the quantity measured, calculating $v_0 = \Delta h/\Delta t$ at the beginning of the curve. The error increases as the erythrocyte concentration decreases. In the case of sedimentation in plasma, the presence of the lag-phase makes a contribution to the error. However, experience shows that the error is almost always within 10%.

The mathematical model of the process is developed with the following assumptions:

1) sedimentation occurs as "a single front" without division into zones;

2) the erythrocyte concentration in the column is constant over the entire height, and at each given moment it depends only on the length of the column and the initial erythrocyte concentration;

3) the settling particles can be regarded as spherical;

4) for each individual erythrocyte or aggregate all the rest of the medium is continuous with some averaged values of the density $\Delta\rho(H_0) = \Delta\rho(1 - H_0)$ and viscosity $\eta(H_0) = \eta(1 + \alpha H_0^2)$;

5) the effects of concurrent entrainment of the plasma are neglected.

Using Stokes's formula, we write an equation for the velocity of the interface of the erythrocytes and the medium at the initial time:

$$v_0 = \frac{Aw(1 - H_0)}{1 + \alpha H_0^2}, \quad A = \frac{2gR^2\Delta\rho}{9\eta}, \quad w = \frac{4\pi^2\nu^2(0.065 + h_0 - h)}{g}. \tag{4}$$

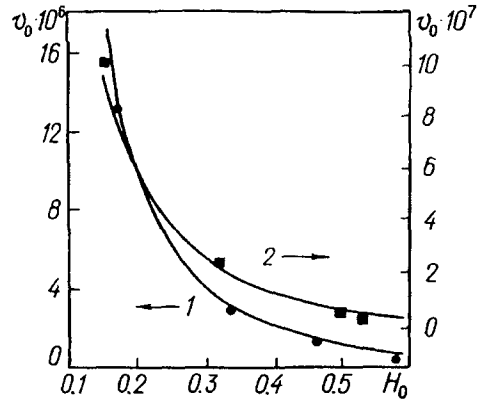

Fig. 4. Plots of the ESR in native plasma (1) and in the IB (2) in the gravitational field versus the hematocrit. v_0, m/sec.

First, we consider the simpler case of erythrocyte sedimentation in the gravitational field. In the course of the process their concentration in the column increases. At a certain moment t the velocity of the particles at the interface is expressed as

$$v(t) = \frac{A\left(1 - \frac{H_0 h_0}{h(t)}\right)}{1 + \frac{\alpha H_0^2 h_0^2}{h^2(t)}}. \tag{5}$$

Here the length of the plasma column is equal to

$$h_0 - h(t) = \int_0^t v(\tau)\, d\tau. \tag{6}$$

We substitute (5) into (6) and integrate the right- and left-hand sides. As a result of transformations we obtain a function of the height of the erythrocyte column in the left-hand side and a linear function of time in the right-hand side:

$$h - h_0 + H_0 h_0 (1 - \alpha) \ln\left(\frac{h - H_0 h_0}{h_0 (1 - H_0)}\right) - H_0 h_0 \alpha \ln\left(\frac{h}{h_0}\right) = -At. \tag{7}$$

Limiting cases will be considered. At the beginning of the process at $t \to 0$, the sedimentation kinetics is expressed by the linear equation

$$h = h_0 - At \tag{8}$$

in good agreement with (4). At long times $t \to \infty$, the relation becomes exponential and also agrees well with experiments:

$$H = H_0 + (1 - H_0) \exp(-Kt), \quad K = \frac{A}{H_0 h_0 (1 + \alpha)}. \tag{9}$$

It can be seen from formula (4) that the curve of the ESR versus the hematocrit is linear at small H_0; as the concentration of erythrocytes, increases it becomes hyperbolic. This is also in good agreement with experimental data (Fig. 4).

TABLE 1. Hydrodynamic Resistance Radius (R) and Rheological Viscosity Parameter (α) Calculated According to the Model

Experimental conditions		Calculated parameters	
medium	acceleration of sedimentation, g^*	$R \cdot 10^6$, m	α
IB	1	4.31	106.4
Plasma	1	30.7	166.3
IB	123	3.49	21.9
Plasma	123	4.37	18.2
IB	278	2.72	11.1
Plasma	278	3.39	17.6
IB	1107	1.48	2.9
Plasma	1107	3.03	11.9

For the cases of sedimentation in the centrifuge, the accelerations given correspond to the middle of the capillary.

TABLE 2. Verification of Conclusions of the Model (formula (9)). The Experiment on Erythrocyte Sedimentation Was Carried Out at 1 g in Native Plasma, $T = 23.5°C$

h_0, m	K, sec^{-1}	$K \cdot h_0$, $m \cdot sec^{-1}$
0.093	$5.12 \cdot 10^{-4}$	4.76
0.080	$5..87 \cdot 10^{-4}$	4.69
0.063	$7.25 \cdot 10^{-4}$	4.57
0.038	$13.25 \cdot 10^{-4}$	5.04

In experiments in the centrifuge, in which acceleration of sedimentation increases with the distance of the interface of the erythrocytes and the medium from the center of rotation, formula (7) becomes more complicated in form; however, nothing changes basically:

$$A_1 \ln (0.065 + h_0 - h) + A_2 \ln \left(\frac{h - h_0 H_0}{h_0 (1 - H_0)} \right) - A_3 \ln \left(\frac{h}{h_0} \right) = - A_4 t,$$

$$A_1 = - ((0.065 + h_0)^2 + \alpha h_0^2 H_0^2) \ln (0.065); \quad A_2 = \frac{h_0 H_0 (\alpha H_0 + 1)}{0.065 + h_0 (1 - H_0)};$$

$$A_3 = \frac{\alpha h_0 H_0}{0.065 + h_0}; \quad A_4 = \frac{4 A \pi^2 \nu^2}{g}.$$

As can be seen from (4) and (9), the initial ESR and the sedimentation rate constant K are proportional to the square of the average particle radius. When erythrocytes settle in plasma, this quantity is much larger than the average erythrocyte radius because of aggregation. This statement is confirmed by the large differences in the sedimentation rates in plasma and the buffer at the same hematocrit indices (Fig. 4). From results of experiments similar to those shown in Fig. 4, it is possible to calculate the average particle radius R and the rheological viscosity parameter α using the two-parameter optimization program. Such calculations were carried out (Table 1). The curves in Fig. 4 show dependences calculated in accordance with the model. They agree with the experimental data quite satisfactorily.

It is thought that in the case of sedimentation in the centrifuge erythrocytes do not aggregate. Consequently, the changes in v_0 and K are caused by the variability of the average erythrocyte radius due to deformation of the erythrocyte. However, this has not been proved so far. As can be seen from Table 1, in the case

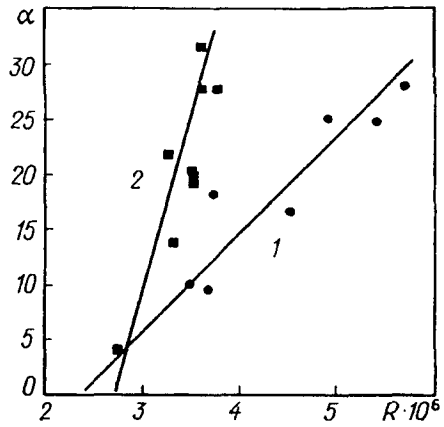

Fig. 5. Correlation of the rheological viscosity parameter α and the hydrodynamic resistance radius R calculated in accordance with the model in the case of erythrocyte sedimentation in plasma (1) and in the IB (2) for different rotational velocities of the centrifuge. R, m.

of erythrocyte sedimentation in the centrifuge, the calculated values of R do not exceed the dimensions of an individual erythrocyte known from the literature (the diameter of the discocyte is $\sim 8 \cdot 10^{-6}$ m, and the thickness of the rim is $\sim 2 \cdot 10^{-6}$ m [5]). As the acceleration of sedimentation increases, R decreases in accordance with the concepts of the process: the discocyte is stretched and becomes an ellipsoid of revolution with a smaller effective radius of resistance to the medium (Table 1). The parameter α decreases as the acceleration of sedimentation increases. It is known from the literature that as the shear rate increases, the dependence of the viscosity on the hematocrit of the erythrocyte suspension becomes flatter, i.e., the parameter α decreases [6].

The dependence of K on the height of the liquid column h_0 in the capillary was verified in experiments at 1 g in native plasma and at $H_0 = 0.2$ (Table 2). In accordance with the model concepts, the product Kh_0 should remain constant, other things being equal, which is confirmed by the experiments.

Making independent measurements of K and v_0 for normal and pathological blood samples and calculating R and α, we obtained an interesting result (Fig. 5): there is a correlation between them. According to Fig. 5, the larger the particle size with the same hematocrit, the higher the value of α, i.e., the steeper the dependence of the viscosity on the hematocrit, other things being equal.

The shape of the curve of the viscosity versus the hematocrit is determined by several factors. The deformability of the cells, depending on the form factor $F = S/V^{2/3}$ and their linear dimensions, and the shear rate γ, determining the deformation of an erythrocyte in a concrete situation, are the most important of them. In the literature a number of works are known whose authors tried to find a relation between the viscosity of an erythrocyte suspension and the linear dimensions of the cells. In most cases no correlation was found between the viscosity and the dimensions of the cells. Work [7] is an exception, where at very low shear rates (0.05 sec^{-1}) the viscosity of the suspensions increased with the erythrocyte dimensions. However, already at $\gamma = 0.5$ sec^{-1} the same suspensions did not show differences in viscosity. Conversely, in [6] it was found that the smaller the linear erythrocyte dimensions, the larger the value of α. It should be noted that in [6] the shear rate was about 100 sec^{-1}. These differences can easily be explained within the model presented. As the shear rate, like the acceleration of sedimentation in the centrifuge, increases, the erythrocytes become deformed, being stretched along the direction of motion and tending to the lowest resistance to the medium. Here the hydrodynamic resistance radius included in the model decreases. The deformability of the cells is higher, the larger the "reverse of area," i.e., the ratio F/F_S, but they cannot change their shape ad infinitum. At shear rates of about 0.05 sec^{-1} we deal with nondeformable erythrocytes, and the quantity α increases together with their linear dimensions. At 0.5 sec^{-1} erythrocytes are already partially deformed. It should be remembered that erythrocytes of different mammalian species, from the dog to the elephant, were taken for comparison. In a partially deformed state that is far from the deformability limit, erythrocytes take a shape that is optimum for the proposed conditions, and therefore their hydrodyanmic resistance radii R and, consequently, the volues of the parameter α became equal [7]. In [6] at 100

sec^{-1} erythrocytes were in the form of limiting deformation. Since large erythrocytes have a large relative form factor, under extreme conditions their hydrodynamic resistance radius can become smaller than that of small erythrocytes. This is an explanation of the decrease in α for larger erythrocytes and the increase in α for smaller ones found in [6] and the apparent contradiction with the results of [7]. Thus, the conclusions obtained by the present authors on the basis of the model concepts are confirmed experimentally by literature data, namely, the quantity α increases proportionally to the growth of the hydrodynamic resistance radius R; however, it should always be remembered that R depends on the shear rate and the acceleration of sedimentation.

The mathematical model of erythrocyte sedimentation suggested in the present work contains two optimization parameters in all, each of which has a real physical meaning. It satisfactorily describes experimental data both obtained by the present authors and reported in the literature. It was effectively used to explain some, at first glance contradictory, results of different authors largely because of an original approach, namely, the concept of an erythrocyte suspension as a continuum with some averaged parameters of density and viscosity and use of Stokes's formula for sedimentation of an individual erythrocyte in such a medium.

It seems necessary to enumerate some possibilities of practical application of the conclusions of the model. Additional experiments and a comparison of the speed of the centrifuge and the shear rate of the viscosimeter show that it is possible to study the rheology of erythrocyte suspensions using measured kinetics of erythrocyte sedimentation and calculated α. Experiments on erythrocyte sedimentation are rather simple and do not require an expensive viscosimeter. This may be especially important for our health-care institutions. From experiments on determination of the ESR in native plasma it is possible to calculate the average number of cells in erythrocyte aggregates from the formula $N = R^3/R_e^3$. Comparing R calculated for different speeds of the centrifuge, we can judge the deformability of the cells, which is a rather informative quantity for determination of the nature of different forms of anemia.

NOTATION

$g = 9.8$ m/sec^2, acceleration of gravity; Re, Reynolds number; D, optical density of the hemolyzed sample at 540 nm; D_0, optical density of a hemolyzed sample obtained from the initial erythrocyte suspension at 540 nm; Hb, relative erythrocyte concentration in the sample; n, number of the sample; R, hydrodynamic resistance radius of a sphere, an erythrocyte, or a cell aggregate; $\Delta\rho$, difference of the densities of a settling particle and the medium, for erythrocytes in plasma $\Delta\rho = 50$ kg/m^3, for erythrocytes in the IB $\Delta\rho = 100$ kg/m^3; η, viscosity of the medium; d_{in}, inner diameter of the capillary; w , acceleration of sedimentation measured in g; ν, rotational velocity of the centrifuge, rps; S, surface area of the membrane; V, volume of a cell; F, form factor; $F = 4.84$, form factor of a sphere; $R_e = 4$ μm, conventional erythrocyte radius; N, average number of erythrocytes in an aggregate; T, temperature; γ, shear rate; α, rheological parameter; t, time; H_0, erythrocyte concentration per unit volume, hematocrit; v_0, erythrocyte sedimentation rate (ESR); h_0, height of the liquid column in the capillary; h, height of the column of settling erythrocytes in the capillary; h_∞, limiting height of the column of settling erythrocytes; IB, isotonic buffer; K, erythrocyte sedimentation rate constant in the centrifuge.

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