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ANALYSIS OF THE GEOMETRIC PARAMETERS AND MECHANICAL PROPERTIES OF ERYTHROCYTES BY THE METHOD OF MEMBRANE NUCLEAR FILTRATION 1. MATHEMATICAL MODEL*

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A mathematical model has been constructed and investigated quantitatively describing the rate of passage of erythrocytes through the pores of nuclear membrane filters on filtration of a dilute suspension of erythrocytes on exposure to constant hydrostatic pressure. In constructing the model, account was taken of the following main factors: geometric constraints linking the surface area of the erythrocyte membrane, the volume of the erythrocyte and the geometric parameters of the filter pores; and the mechanical characteristics of the erythrocyte membrane and the viscosity of the intracellular contents. Investigation of the model suggests the possibility of obtaining independent information on all the above listed characteristics of the erythrocyte on the basis of experimental curves of the dependence of the filtration rate of the erythrocyte suspension on the osmotic capability of the extracellular medium.

The ability of the erythrocyte to pass through narrow capillaries is determined by its ability to deform readily, assuming the form of the channel it which it finds itself. Because of this the deformability of the erythrocyte is a most important characteristic determining the rheological properties of blood [1, 2]. The deformability of the erythrocyte is very sensitive to disturbances of practically all the important metabolic systems of this cell. Therefore, the informative value of the methods of measuring deformability is very high and filtration methods are the simplest and most convenient. They are widely used in the diagnosis of different pathologies and may find wide application for estimating the functional state of erythrocytes [3, 4]. The main drawback of these methods is a consequence of their merits; high sensitivity to practically all disturbances of metabolism does not allow information to be obtained on where the defect is located.

This limitation of the methods of filtrability may be largely overcome by expanding the space (by increasing the number) of parameters varied in such experiments. The task of the present work is to analyse the influence of the geometric parameters and mechanical properties of erythrocytes on the process of passage of the erythrocyte through a narrow

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channel. The aim of the work is to find ways of determining these factors directly from filtrability experiments.

We shall consider a method in which the erythrocytes are filtered through nuclear membrane filters. The channels in such filters have the form of cylindrical apertures the geometric parameters of which vary insignificantly. This greatly simplifies the analysis providing a quantitative interpretation of the results of the experiments.

Usually in experiments nuclear filters 10 μm thick with a pore diameter 3–5 μm are used. The diameter of the erythrocyte disc is 8 μm and, therefore, to pass through the channel in the membrane the erythrocyte must change its form, i.e. be deformed. The force inducing deformation is determined by the pressure difference on the two sides of the membrane. Usually these are low pressures of the order 0.01 atm. The forces appearing at such pressures are not able to stretch the cell membrane [5].

Among the host of factors acting on the cell amenable to the experimenter, in our case the most convenient is change in osmotic pressure (osmotic capability) of the medium (u) in which the erythrocyte is present by changing the concentration of NaCl.

In the experiment it is convenient also to vary the pore diameter in the membrane (d), the length of the channel (thickness of the membrane) (l), the pressure difference (p) determining the fluid flow and the haematocrit of the erythrocyte suspension (n).

To evaluate the contributions of the factors to the rate of filtration of the erythrocyte suspension, a mathematical model of the process is required.

MATHEMATICAL MODEL

Postulates of the model

To describe the process of filtration of the erythrocyte we used the following postulates.

1. The speed of passage of the erythrocyte through the channel in the membrane (w) is constant.

2. The erythrocyte in the channel is acted upon by a constant force equal to the product of the pressure difference on the two sides of the membrane and the area of the cross section of the channel

$$F = p\pi d^2/4, \tag{1}$$

3. The erythrocyte membrane is unstretchable.

4. The force of friction of the erythrocyte against the channel wall is proportional to the speed of movement of the erythrocyte and consists of two components. The first component of the force of friction is due to the viscosity of the intracellular contents q_i

$$F_1 = a(d,l) \cdot q_i \cdot w, \tag{2}$$

where $at(d, l)$ is a parameter characterizing change in the shape of the erythrocyte leading to overflow of part of the cell contents. It is assumed that this redistribution of the intracellular contents changes little with change in volume and it may be taken as constant. This parameter of course, depends on the geometric parameters of the channel. However, it is very difficult to obtain the explicit form of this dependence.

The second component of the force of friction is connected with the concept of the limiting volume of the erythrocyte. As is known, for the greatest possible volume the erythrocyte is in the form of a sphere. Because of the unstretchability of the erythrocyte membrane, such a

sphere cannot pass through a channel (pore) the diameter of which is less than the diameter of the sphere. Evidently, through a specified channel with a diameter less than the diameter of the erythrocyte the cells will cease to pass for a certain limiting volume less than the greatest possible volume of the erythrocyte. As the volume of the erythrocyte approaches the value limiting for the given channel, the force of friction of the erythrocyte in this channel tends to infinity. To describe the changes in this force with increase in volume, as a first approximation we assume that these changes are determined by the difference between the limiting and ongoing value of volume (V):

$$F_2 = b(d,l) \cdot w / (V_k - V)^n, \tag{3}$$

where $b(d, l)$ is a parameter characterizing the flexural elasticity of the cell membrane or the cell, V_k is the limiting value of the volume of the erythrocyte, n is an exponent allowing adequate correspondence to be chosen between the deformation of the cell and the degree of change in volume.

5. The cell volume is inversely proportional to the osmotic capability of the medium.

The rate of movement of the erythrocyte in the channel according to these postulates will be constant when the force of pressure is equal to the forces of friction

$$F = F_1 + F_2, \tag{4}$$

whence

$$w = (p\pi d^2/4) / [a(d,l)\ q_i + b(d,l) / (V_k - V)^n]. \tag{5}$$

In this formula the viscosity (q_i) and the volume are functions of the osmotic capability of the medium. To obtain the dependence of the rate of filtration of the erythrocytes on osmotic capability, it is necessary to determine how the parameters q_i and V_k depend on it.

The link between the volume and osmotic capability of the medium (buffer) in which the erythrocyte is present is determined by the postulate No. 5, and is confirmed by the results of numerous measurements of the dependence of volume on the osmoticity of the incubation medium [6].

$$V = f/u + V_0, \tag{6}$$

where f is a coefficient.

V_0 is the minimal value of volume to which the erythrocyte is compressed when the osmotic capability of the buffer solution tends to infinity. As will be shown below, this value is determined by the haemoglobin content of the erythrocyte. The parameter f depends on the concentration of haemoglobin and many parameters of the ionic homeostasis of the cell. It cannot be determined as simply as V_0 and, therefore, we leave it as an empirically determined coefficient.

The dependence of the intracellular viscosity on the osmotic pressure of the medium is essentially determined by the concentration of haemoglobin in the erythrocyte. With rise in the concentration the viscosity grows exponentially [7]. The dependence is well described by the formula

$$q_i = q \cdot \exp\ [y_1 \cdot Hb\ /\ (1 - y_0 \cdot y_1 \cdot Hb)], \tag{7}$$

where Hb is the intracellular concentration of haemoglobin, $q = 0.7$ centipoises (viscosity of water), $y_0 = 0.4$, $y_1 = 0.0036$ g/l.

With change in the osmotic capability of the medium, the intracellular concentration of haemoglobin (but not its amount) will change with change in volume. Let H be the amount of haemoglobin in a separate erythrocyte. Then

$$H = Hb \cdot V.$$

With rise in osmotic capability the concentration of haemoglobin will grow until, according to equation (7), the viscosity of the intracellular contents tends to infinity. This determines the limiting concentration of haemoglobin in the erythrocyte (Hb_0) and the minimum cell volume

$$\begin{aligned} &y_0 \cdot y_1 \cdot Hb_0 = 1, \\ &Hb_0 = 1/(y_0 \cdot y_1) = 694{,}4 \text{ g/l}, \\ &V_0 = H/Hb_0, \end{aligned} \tag{8}$$

whence

$$q_i = q \cdot \exp\,[y_1 \cdot H/(V - V_0)]. \tag{9}$$

From relation (5) noting (6), (8) and (9) let us find the velocity of movement of the erythrocyte through the channel

$$w = \pi p d^2/4\,[a \cdot q \cdot \exp\,(y_1 \cdot H \cdot u/f) + b/(V_k - H/Hb_0 - f/u)^n]. \tag{10}$$

Geometric constraints on passage of the erythrocyte through the channel in the membrane figure in the formula for the rate of filtration of the erythrocyte as a critical value of the volume V_k. Let us see how it is linked with the size of the channel in the membrane and the geometric parameters of the erythrocyte.

In the case of a sufficiently wide channel, the erythrocyte of discoid shape may readily pass through it coiling into a "tube" or gently flattening as shown in Fig. 1 [8]. The first variant of passage is possible when the area of the central cross-section of the erythrocyte by a plane perpendicular to the disc does not exceed the area of the cross-section of the channel. Increase in the volume of the erythrocyte for a fixed surface area or reduction in the diameter of the channel means that the erythrocyte begins to fill the volume of the

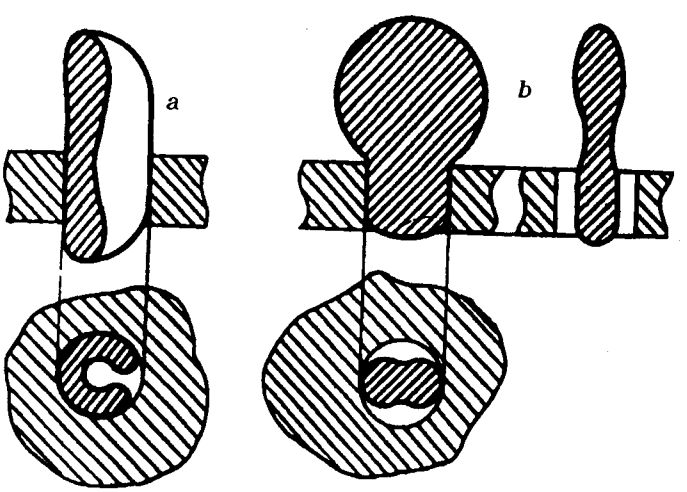

Fig. 1. Schematic representation of two variants of passage of the erythrocyte through the channel (pore) of the nuclear membrane filter: *a*, the erythrocyte coils into a tube; *b*, retains axial symmetry.

channel fully. The erythrocyte may be in the channel either as a whole (Fig. 2*a*) or in part "overflowing" from one side of the membrane to the other (Fig. 2*b*).

Obviously, the erythrocyte will cease to pass through the channel when the portions of the membrane not in contact with the channel walls become spherical surfaces. Simple analysis shows that the critical volume, i.e. the least volume at which the erythrocyte ceases to pass through the channel in the case depicted in Fig. 2*a*, corresponds to the form of a cylinder bounded by two hemispheres and in the case depicted in Fig. 2*b*, to a shape in which the radii of the spheres formed on the two sides of the membrane are equal between themselves. The limiting values of the volume of the erythrocyte (V_k) for the cases depicted in Fig. 2*a, b*, may be calculated from the following formulae

$$V_k = \begin{cases} ds/4 - \pi d^3/12, & S < \pi d^2 + \pi dh \\ \pi d^2 h/4 + (S - \pi dh)^2/3C - \pi d^2(S - \pi dh)/6C + d^2C/24, & S > \pi d^2 + \pi dh, \end{cases} \quad (11)$$

where $C = \{8\pi [s - \pi d(h + d/2]\}^{1/2}$.

If the speed of passage of the erythrocyte through the channel is measured as a function of the osmotic capability of the buffer solution, then in a certain region with reduction in osmotic pressure (which corresponds to rise in the volume of the erythrocyte) the filtration rate will diminish and become zero when the osmotic capability of the medium corresponds to the volume of the erythrocyte limiting for the channel. This limiting value of osmotic capability is determined only by geometric constraints and should not depend on the viscoelastic properties of the cell.

Figure 3 shows the link between the limiting volume of the erythrocyte and its surface area obtained from (11) for different channels with dimensions characteristic of those of the channels in membranes used for measurements of the filtrability of erythrocytes.

For a surface area less than the area at point *A*, the erythrocyte will pass through the channel whatever the volume confined by this surface. For each channel the values of the volumes lying above the corresponding curve apply to cells which cannot pass through the given channel.

For surface areas lying between the points *A* and *B* in the curves, the critical volume of the erythrocytes is determined by the first of conditions (11) and for values larger than at point *B* by the second of conditions (11). As Fig. 3 shows, for the channel with a diameter 5 μm the critical volume is determined by the second of conditions (11) for all reasonable values of the surface area of the erythrocyte.

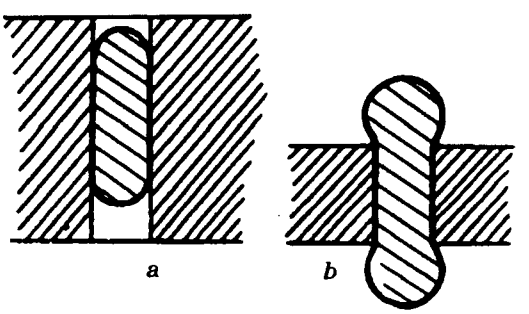

Fig. 2. Schematic representation of the erythrocyte present in the pore of the nuclear membrane filter: *a*, volume of erythrocyte is less than the pore volume; *b*, volume of erythrocyte greater than the pore volume.

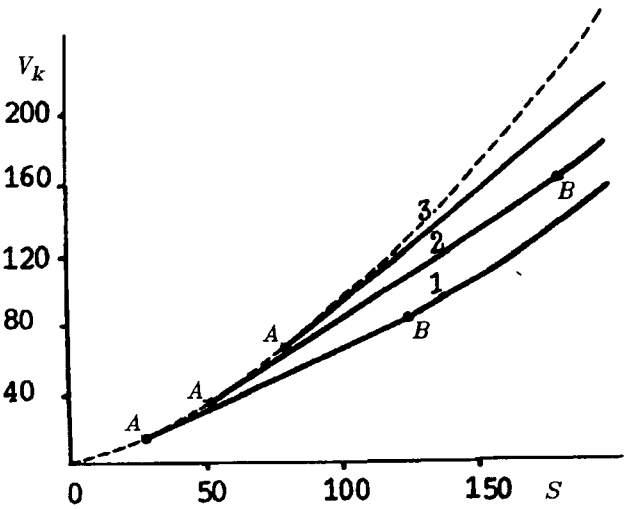

Fig. 3. Dependence of the limiting volume of the erythrocyte on its surface area for different diameters (μm) of the filter pores: *1*, 3; *2* , 4; *3*, 5 (length of channel $l = 10$ μm).

Microcharacteristics of the filtration process. Link between the velocity of passage of the erythrocyte through the channel in the membrane and the velocity of flow of the cell suspension

To characterize the deformability of the erythrocyte, it is necessary to measure the speed of its passage through the channel in the membrane. In the experiment another magnitude is measured — the flow time of a certain volume of the erythrocyte suspension through the filter and the index of deformability D or the index of rigidity I is calculated [9]:

$$D = 100/I = t_b h/(t_s - t_b), \tag{12}$$

where t_s is the flow time of a specified volume of the erythrocyte suspension, h is the haematocrit of the suspension, and t_b is the flow time of the same volume of buffer through the same filter.

To relate this index to the velocity of passage of the erythrocyte through a channel, let us consider this process in more detail. We make the following assumptions.

1. All the channels in the membrane are identical and have the diameter d.

2. When the erythrocyte is in the channel the buffer will not flow through this channel, i.e. the erythrocyte completely blocks it.

Let w be the mean volumetric velocity of passage of one erythrocyte through the channel in the membrane and V the volume of the erythrocyte.

$$t_e = V/w - \text{is the mean stay time of the erythrocyte in the channel} \tag{13}$$

Q is the filtrable volume of the suspension (buffer)

$$Qh = NV, \tag{14}$$

where N is the total number of erythrocytes in the suspension.

If M is the total number of channels in the membrane the volume of the suspension Q/M will flow through one channel.

The flow time of this volume is equal to

$$t_s = t_1 + t_2, \tag{15}$$

where t_1 is the flow time of the buffer solution in which the cells are suspended. The volume of this solution $Q(1-h)/M$.

$$t_1 = t_b\,[Q\,(1-h)\,M]\cdot\,(M/Q) = t_b\,(1-h), \tag{16}$$

t_2 is the time during which the channel is occupied by erythrocytes. Through one channel, on average, will pass the erythrocytes

$$N/M = Q\,h/(M\,V) \tag{17}$$

$$t_e = t_2\,M\,V/(Q\,h). \tag{18}$$

Substituting (15) and (16) into (18) we get

$$t_e = [(t_s - t_b\,(1-h)/Q\,h]\cdot M\,V. \tag{19}$$

The rate of filtration of the buffer w_b is linked with the number of channels

$$t_b = Q/(w_b\cdot M). \tag{20}$$

Let us find M from (20) and substitute it into (19) and from (13) find the rate of filtration of the erythrocyte

$$w = w_b\,t_b\,h/[t_e - t_b\,(1-h)], \tag{21}$$

$$w/w_b = t_b\,h/[t_s - t_b\,(1-h)]. \tag{22}$$

Here w_b, the flow velocity of the buffer solution through the cylindrical channel, is determined from the Poiseuille formula:

$$w_b = \pi\,p\,d^4/128\,l\,q, \tag{23}$$

where q is the viscosity of the buffer solution.

Comparison of relations (10) and (22) shows that for low haematocrit values the index of deformability corresponds to the ratio of the filtration rate of the erythrocyte to the rate of filtration of the buffer.

Relation (22) is violated if channels with different characteristics are present in the filter. It is known that in nuclear filters apertures are encountered, formed by two or more closed channels. It it is assumed that the fraction of such channels m/M and their area is equal to double the area of a single channel, then expression (22) for the velocity of movement of the erythrocytes through a single channel will assume the form

$$\begin{aligned} w/w_b = {} & [t_b\,(1+4\,m/M) - 4\,m/M\,t_s]\,h/ \\ & /t_s\,[1+4\,m/M\,(1-h)] - t_b(1-h)\,(1+4\,m/M) \end{aligned} \tag{24}$$

From this formula it follows that, if the rate of filtration of the erythrocyte through a single channel is equal to zero, the flow time of the suspension through the filter will not tend to infinity. It is equal to

$$t_{s_0} = t_b(1 + M/4m). \tag{25}$$

Analysis of formula (24) shows that even if the fraction of double channels is low, they may in practice completely determine the filtration time of the suspension for low velocity of passage of the erythrocytes through a single channel. Thus, if $m/M = 0.1$, then $t_{s_0} = 3.5t_b$. The index of deformability gives a badly distorted idea of the deformability of erythrocytes.

The link between the rate of filtration of the erythrocyte and the filtration time of the suspension is even more complicated if assumption 2 is violated. In this case the possibility of flow of buffer through the channel partially occupied by an erythrocyte may even change

the value of the haematocrit of the suspension passing through the filter. If the haematocrit on filtration changes little it may be considered that assumption 2 is fulfilled.

RESULTS

Bearing in mind the link between the velocity of passage of the erythrocyte through a single channel (10) and the index of deformability of the erythrocytes D (12) and (22), and the expression for the flow velocity of the buffer (23) for low values of the haematocrit, we obtain a formula describing the dependence of the index of deformability on the osmotic capability of the medium

$$D = w/w_b = 1/[a_1 \exp(y_1 H u/f) + b_1/(V_k - H/Hb_0 - f/u)^n], \quad (26)$$

where $a_1 = a/32 \cdot l$; $b_1 = b/128 \cdot l \cdot q$.

From formula (26), it follows that the index of filtrability D does not depend on the pressure difference p. The dependence of the index D on the geometric parameters of the channel cannot be expressed as long as this dependence for the parameters a and b is unknown.

Figure 4 depicts the dependence of the index of deformability on the osmotic pressure of the extracellular fluid calculated from the model for normal human erythrocytes for different values of the index n. It will be seen that, with rise in the osmotic capability of the medium (reduction in the erythrocyte volume), the curve moves sharply upwards, remaining linear in a fairly large region. The higher the index n, the longer the linear portion and the sharper the maximum in the curve. For large n, the curve may rise with "acceleration". The exact value of the index n in the model may be determined from the condition of the best fit of the theoretical curves to the experimental data on the dependence of the deformability of the erythrocytes on the osmotic capability of the medium. For a fixed value of n, the steepness of the rising portion in the dependence of deformability of the erythrocytes on the osmotic capability of the medium is determined by the elasticity of the cell or the cell membrane (Fig. 5). Decrease in the deformability of the erythrocytes for large values of the osmotic capability of the medium in the model is linked with increase in intracellular viscosity (Fig. 6).

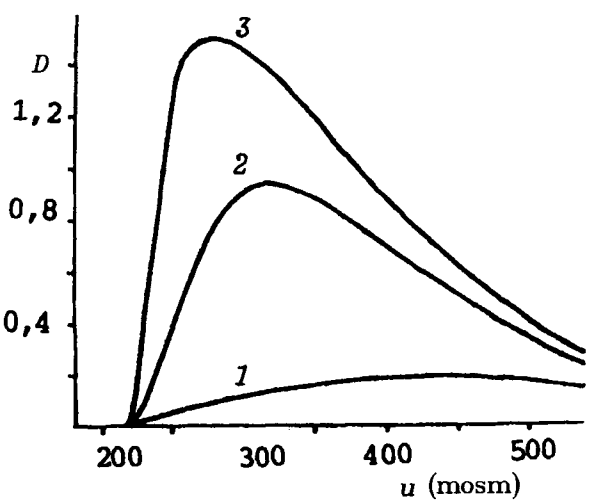

Fig. 4. Dependence calculated from the model of the deformability index D on the osmotic pressure of the medium u for different values of the index n: *1*, 1; *2*, 2.3; *3*, 3. ($a_1 = 0.1$, $b_1 = 143$, $V_k = 102.48$; $H = 25.33$, $Hb_0 = 0.6944$).

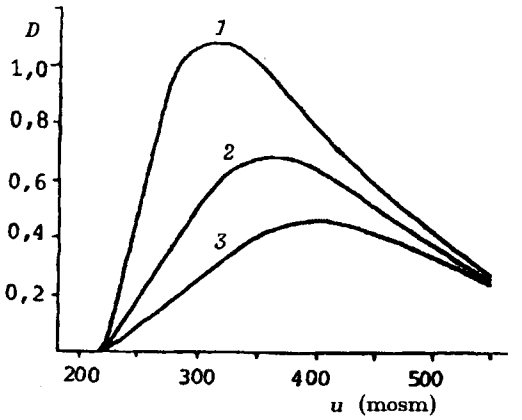

Fig. 5. Dependence calculated from the model of the deformability index D on the osmotic pressure of the medium u for different values of the parameter b_1 characterizing the elasticity of the cell and/or the cell membrane: *1*, 1285.7; *2*, 7285; *3*, 21857.1. ($a_1 = 0.1$; $V_k = 102.48$; $H = 25.33$; $Hb = 0.6944$).

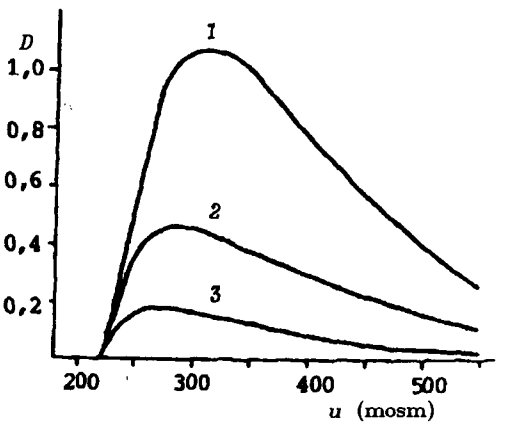

Fig. 6. Dependence calculated from the model of the deformability index D on the osmotic pressure of the medium u for different values of the parameter a_1 characterizing the contribution of viscous friction: *1*, 0.1; *2*, 0.3; *3*, 9. ($b_1 = 1285.7$; $V_k = 102.48$; $H = 25.33$; $Hb_0 = 0.6944$).

From expression (26) it follows that the osmotic pressure of the medium at which the erythrocyte has a critical volume corresponds to a zero value of the index of deformability.

DISCUSSION OF RESULTS

Of the utmost interest for checking the model considered here is investigation of the dependence of the filtrability of erythrocytes on the osmotic pressure of the extracellular medium. Study of this dependence for a different haemoglobin content of the erythrocytes with variation in the mechanical properties of the membrane and also for different initial values of the volume of the erythrocyte would make possible an exhaustive quantitative check on the correctness of the estimation of these factors in the model.

It is also important to investigate the dependence of the filtrability of erythrocytes on the hydrostatic pressure difference at the membrane and on the dimensions of the channels in the membrane. Such an investigation would give a fuller idea of the influence of the experimental conditions on the value of the index of deformability of the erythrocytes.

The predictions of the model concerning the critical volume are of special interest. For an osmotic pressure corresponding to the critical volume the filtration rate of erythrocytes is equal to 0. This means that the conclusions concerning the critical volume do not depend on most of the postulates of the model, for example, on the quantity and viscosity of haemoglobin, the index n, the ratios of the different components of the forces of friction b, etc. This parameter is determined only by the geometric characteristics of the erythrocyte and the channel. If the conclusions of the model on the critical volume receive quantitative confirmation in the experiment, then this model may give correct information even if a complete model description of the dependence of filtrability on osmoticity is in poor agreement with the experiment.

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