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Mathematical Analysis of the Effects of Geometric Parameters and Mechanical Properties of Erythrocytes on the Filterability of Nonuniform Suspensions

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Approaches to determination of the pattern of erythrocytes distribution with regard to the rates of their passage through pores (3 μm in diameter) of a membrane filter by processing the data on changes in the flow rates of erythrocyte suspensions with time (filtration curves) are discussed. We considered the case when the suspension consisted of two subpopulations of erythrocytes differing in a single parameter. Using a model describing the erythrocyte passage through a pore and a model describing filtration of a nonuniform suspension, we analyzed the dependences of filtration kinetics of such suspensions on the relative contents of the subpopulations and their rheological characteristics. It has been shown that the filtration rates of the major subpopulation and the minor abnormal subpopulation, and their relative contents can be determined from the analysis of filtration curves. This can be done when the filtration rate of cells from the minor subpopulation is at least one order of magnitude lower than the filtration rate of cells from the major subpopulation. Thus we can register the presence of the minor subpopulation in the range of 0.5–1%. If filtration rates are recorded at different osmolalities, their analysis makes it possible to determine the surface area, intracellular viscosity, and membrane rigidity of cells of the major subpopulation and, in certain cases, the same parameters for the cells of the minor subpopulation.

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The ability of erythrocytes to pass through narrow capillaries plays an important role in the performance of their transport function. Therefore, investigation of the filterability of erythrocytes is of high diagnostic significance. It has been established that numerous pathological states lead to the

appearance in blood of abnormal erythrocytes that differ from the major cell population in their filterability [1]. Filtrational methods prove to be the most convenient and informative for diagnostic purposes and studies of erythrocyte properties. At present, one of the techniques for evaluating the filterability of erythrocytes consists in measuring the flow rate of dilute suspensions of washed erythrocytes through membrane filters with cylindrical pores the diameter of which is close to that of capillaries in microcirculation (3–5 μm). The best reproducible results are obtained using filtrometers based on measurement of the passage time of small volumes of erythrocyte suspensions (about 50–100 μl) under the action of gravitation force [2]. The filterability of suspensions is usually expressed as $F = Ht \cdot t_b/(t_s - t_b)$, where t_b and t_s are the times of passage through a filter of the resuspending medium (buffer) and the suspension under study, respectively; Ht is the hematocrit of suspension.

Interpretation of the results of such measurements encounters appreciable difficulties that are primarily related to heterogeneity of studied suspensions. If erythrocyte suspensions were composed of identical cells, the filtrational method based on measurement of the passage time of a fixed volume of suspension through a filter would allow estimation of the rate of an individual erythrocyte passage through a filter pore. Carrying out measurements at different osmolalities of resuspending medium (sweep over osmolality), one could determine the ratio of the surface area to the cell volume, 'intracellular viscosity' and mechanical characteristics of the cell membrane [3]. However, real suspensions of erythrocytes are always heterogeneous and contain cells that differ in many parameters, including geometric size and density [4]. When erythrocytes are distributed in suspension according to a certain index, the rates of their passage through pores may be different. In this situation, one cannot consider that the results obtained by means of filtration of a fixed suspension volume characterize a certain 'average' cell, i.e. the erythrocytes making the greater part of suspension. Indeed, the rate of suspension filtration is determined by slow, 'bad' erythrocytes [5]. The point is that under standard conditions of filtrational studies ($Ht > 1\%$; volume of filtered suspension, 50–250 μl) the quantity of erythrocytes on the filter in the process of single measurement is much (nearly two orders of magnitude) greater than the number of pores in the filter. Therefore, the time of passage through the filter of a definite volume of suspension containing a minor admixture of poorly filtering cells may differ appreciably from the passage time of homogeneous suspension.

Filtrational experiments may be modified to replace a single measurement of the passage time of a fixed erythrocyte suspension volume by measurements with adequate resolution of the dependence of the erythrocyte suspension flow rate on time (filtration curve). During the initial period of suspension flow, when nearly all pores are vacant, cells of the major

subpopulation pass through a filter (namely because their quantity is much greater than that of other cells). For this reason, in a given filter region the rate of suspension flow is determined by the passage rate of cells from the major subpopulation, i.e. the 'average' cells. Knowledge of the suspension passage rate at the initial filtration stages will possibly allow estimation of the rate of 'average' cell.

The aim of our work was to study the influence of the distribution of cells in suspension with regard to their rates of passage through a membrane filter pore on the process of suspension flow through the filter. We used mathematical modelling to study the simplest case, when a suspension of erythrocytes contains cells of two types. Using a model of single erythrocyte passage through a capillary [3] and a model of heterogeneous suspension filtration [6, 7], we investigated the dependence of the erythrocyte suspension flow kinetics on the ratio and rheological properties of its constituent subpopulations. This analysis made it possible to develop the experimental protocol for deriving information on the pattern of cells distribution in real suspensions over the rates of passage through a filter and determining parameters of different cell types that determine deformability of cells.

MATHEMATICAL MODEL

A mathematical model of the dynamics of passage through a filter of a suspension composed of two cell types. Let us consider the simplest case of a suspension consisting of two different subpopulations. Assume that the following suggestions are fulfilled: (a) all pores in the filter are identical; (b) when a cell is in a pore no buffer passes through this pore; the consequence of this suggestion is that the suspension hematocrit (the ratio of the overall volume of cells to the suspension volume) above the filter does not change; (c) only one cell is in the pore at any time moment; (d) the rate of cell passage through the pore is constant; (e) the rate of buffer flow through the pore is much greater that the passage rate of cells.

The rate of erythrocyte suspension flow through a filter is determined by the processes of cell delivery onto the filter and the release of cells from the membrane pores. The rate of the cell influx to the filter depends on the suspension flow rate on the whole and on the concentration of cells. The release rate of cells from the filter is dependent on the number of pores occupied by cells and the rate of cell passage through a pore. A stationary quantity of cells in the filter pores is reached when the rate of cells influx onto the filter (the number of cells transferred onto the filter per time unit) becomes equal to the rate of cells efflux from the filter (the number of cells released from the filter per time unit).

The rate of change in the number of pores occupied by each cell type in the process of filtration is described by the following equations:

$$dP_1/dt = (T-P_1-P_2)n_1w_b - w_1/V_1P_1,$$

$$dP_2/dt = (T-P_1-P_2)n_2w_b - w_2/V_2P_2, \quad (1)$$

where T is the total number of pores in the filter; P_1 and P_2 are the numbers of pores occupied by erythrocytes of the major and minor subpopulations, respectively; n_1 and n_2 are the concentrations of each type of cells in suspension; w_b is the bulk rate of buffer flow through a pore (in our case it is more convenient to measure the rate of buffer flow and the rate of erythrocyte passage through a pore as the volume of buffer or erythrocytes that passes through a pore per time unit); w_1 and w_2 are the volumetric rates of passage through a pore of cells from the major and minor populations, respectively; V_1 and V_2 are the volumes of cells of the first and second subpopulations, respectively.

The bulk rate of suspension flow through a filter is described by the following equation:

$$Q = (T-P_1-P_2)w_b. \quad (2)$$

The above equations were derived in studies [6, 7]. For the general case, analytical solution for the given model may be readily obtained. The dependence of the bulk flow rate of suspension on time is expressed by the following equation:

$$Q(t) = Q_{st} + A_1\exp(-t/t_1) + A_2\exp(-t/t_2), \quad (3)$$

where $Q_{st} = Tw_b - A_1 - A_2$.

$$A_1 = (k_1+k_0)(k_2+k_0)/(2k_0)t_1Tw_b,$$

$$A_2 = -(k_1-k_0)(k_2-k_0)/(2k_0)t_2Tw_b,$$

$$t_1 = 1/(k_3+k_0),$$

$$t_2 = 1/(k_3-k_0),$$

$$k_0 = (0.25(n_1w_b + n_2w_b + w_1/V_1 + w_2/V_2)^2$$

$$- (n_1 w_b w_2/V_2 + n_2 w_b w_1/V_1 + w_1/V_1 w_2/V_2))^{1/2},$$

$$k_1 = 0.5(n_1 w_b + n_2 w_b + w_1/V_1 - w_2/V_2),$$

$$k_2 = 0.5(n_1 w_b + n_2 w_b - w_1/V_1 + w_2/V_2),$$

$$k_3 = 0.5(n_1 w_b + n_2 w_b + w_1/V_1 + w_2/V_2).$$

Dependence of the rate of cell passage through a filter pore on geometric and mechanical parameters of erythrocytes. A theory considering the passage of single erythrocyte through a pore was proposed in an earlier report [3]. This theory related geometric and mechanical properties of erythrocyte to the rate of its passage through a pore. The use of this model for a homogeneous suspension of erythrocytes has shown that knowing the dependence of erythrocyte rate w on the medium osmolality u, one can assess certain geometric and mechanical parameters of erythrocytes. Let us consider briefly the basic results of work [3].

At a given surface area, an erythrocyte can pass through a pore when its volume is smaller than a certain critical value V_{cr}. The value of critical volume is related to both the size of filter pores and geometric parameters of erythrocytes. In the case of a sufficiently wide pore, an erythrocyte of discoid shape can pass through it by rolling into a tube and becoming slightly flattened [8]. The increasing erythrocyte volume leads to the situation when it fills the entire pore volume. Let us first consider the case when the erythrocyte volume is larger than the pore volume and only part of the erythrocyte fills the entire pore volume. An erythrocyte will no longer pass through the pore when the membrane sites free of contact with the pore walls become spherical surfaces. For a given surface area, the minimal volume at which the erythrocyte cannot pass through the pore (i.e. critical volume) corresponds to a dumbbell formed by the portion of the erythrocyte situated in the pore and two spheres of equal diameters protruding on both sides of the filter. The larger the cell surface area and the pore diameter the greater the critical volume [3].

If a pore is sufficiently long to accommodate the whole erythrocyte in the channel, its critical volume will correspond to the form of a cylinder restricted by two semi-spheres. Nonetheless, all reasoning relative to the dependence of critical erythrocyte volume on the cell surface area and geometric parameters of the pore remains valid.

It was shown earlier [9] that upon changes in the medium osmolality an erythrocyte starts to behave as an osmometer. In this case the dependence of

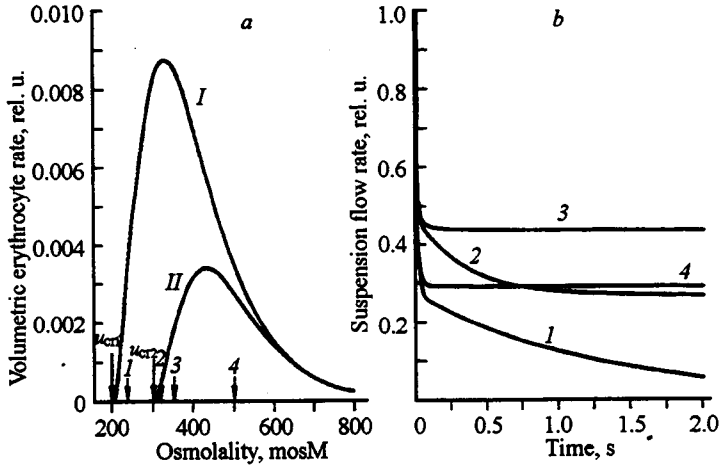

Figure 1. Filterability of a suspension consisting of two subpopulations of cells with different surface area through a 10-μm-thick filter with pores 3 μm in diameter. The suspension contains 95% of cells with the surface area S_1 = 155 μm^2 and 5% of cells with the surface area S_2 = 130 μm^2. *a*, Dependence of the volumetric rate of passage of a single erythrocyte from the major (*I*) and minor (*II*) subpopulations on osmolality. The values of erythrocyte rate are normalized relative to the volumetric rate of buffer flow through a pore. The dependence was calculated by formula (4) at $H = 33\times10^{-12}$ g, f = 13 osM μm^3, a_0 = 2.63, $b_0 = 7.24\times10^3$ μm$^{4.5}$ for both subpopulations of erythrocytes. *b*, The time course of the erythrocyte suspension flow rate at different osmolalities. The values of the suspension flow rate were normalized relative to the volumetric flow rate of buffer through the same filter. The pressure of 6-cm H_2O above the filter was 5.9×10^{-1} g/(μm s^2). Viscosity of buffer solution $q = 7\times10^{-7}$ g/(μm s). Hematocrit of suspension Ht = 1%. Osmolality of buffer solution (mosM): 240 (*1*), 310 (*2*), 350 (*3*) and 500 (*4*). These osmolalities are indicated in Fig. 1*a* by the arrows with numerals corresponding to the nos. of curves in Fig. 1*b*.

erythrocyte volume on osmolality is described by the formula $V = H/Hb_0 + flu$, where H is the quantity of hemoglobin in the cell, Hb_0 is the maximum possible concentration of hemoglobin in the cell, f is the coefficient of osmotic sensitivity that characterizes the sensitivity of erythrocyte volume to osmolality variation.

The value of osmolality at which the cell reaches its critical volume was called critical osmolality u_{cr}. At the osmolality smaller than u_{cr} for a given erythrocyte, it will not pass through a pore, i.e. its rate becomes zero. As the osmolality grows, the erythrocyte volume decreases. At the medium osmolality exceeding u_{cr}, an erythrocyte will start to pass through the pore. The smaller is the erythrocyte volume compared to its critical value the less

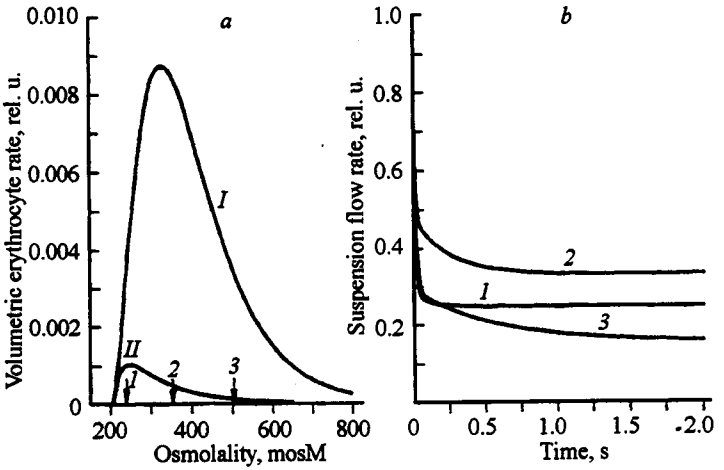

Figure 2. Filterability of a suspension consisting of two subpopulations of cells with different a_0 coefficients (viscosity of intracellular contents). The suspension contains 95% of cells with a_{01}= 2.63 and 5% of cells with a_{02} = 78.9. Normalization, filter parameters and filtration conditions are the same as in Fig.1. *a*, Dependence of the volumetric rate of passage of a single erythrocyte from the major (*I*) and minor (*II*) subpopulations on osmolality. $S = 155\ \mu m^2$, $H = 33\times10^{-12}$ g, $f = 13$ osM μm^3, $b_0 = 7.24\times10^3\ \mu m^{4.5}$ for both subpopulations of erythrocytes. *b*, The time course of the erythrocyte suspension flow rate at different osmolalities. Osmolality of the buffer solution (mosM): 240 (*1*), 350 (*2*) and 500 (*3*). These osmolalities are indicated in Fig. 2*a* with numerals corresponding to nos. of curves in Fig. 2*b*.

significant role play the geometric characteristics of the cell in the erythrocyte passage through the pore. The viscosity of intracellular contents is also dependent on medium osmolality. The intracellular concentration of hemoglobin increases with the higher osmolality and the corresponding decrease in erythrocyte volume. This leads to a higher viscosity of intracellular contents which increases exponentially with the growing hemoglobin concentration in the cell [10]. With the increasing viscosity of intracellular contents at higher osmolality the passage of an erythrocyte through a pore will slow down. Thus, upon variation of medium osmolality the effects of corresponding changes in the volume and viscosity on the rate of erythrocyte passage through a pore are opposite. The dependence of w on medium osmolality has a bell-like shape and is described by the following formula:

$$w = w_b/(a_0 \exp(y_1 Hu/f) + b_0/(V_{cr} - H/Hb_0 - f/u)^{1.5}), \qquad (4)$$

where $y_1 = 3.6 \times 10^{12}$ μm³/g; $Hb_0 = 0.694 \times 10^{-12}$ g/μm³; a_0 is the coefficient characterizing the effect of viscosity of intracellular contents on the cell rate; b_0 is the coefficient characterizing the rigidity of the cell or its membrane [3].

At the given length and diameter of pores, the critical volume is clearly related to the cell surface area. The greater the surface area the larger the critical volume of the cell and the lower the critical osmolality. (Fig. 1*a*). The decreasing quantity of hemoglobin in the cell and the smaller coefficient of osmotic sensitivity *f* also lead to a lower level of critical osmolality. Variation of the coefficient a_0 does not affect the critical osmolality. Its increase is conducive to a lower rate of erythrocyte passage predominantly in the hypertonic region (Fig. 2*a*). Variation of the coefficient b_0 has no effect on critical osmolality, either. At the same time, its increase leads to a lower rate of erythrocyte mostly in the hypertonic region.

RESULTS

Dynamics of the flow of suspension consisting of two cell types through a filter. When a suspension is composed of two types of cells, its flow rate is described by two exponential functions. Let us consider the time course of the suspension flow rate for two subpopulations. The first subpopulation is regarded as the major well filtering and the second subpopulation – as the minor poorly filtering one ($w_1 > w_2$, $n_1 > n_2$). Coefficients A_1, t_1 and A_2, t_2 characterize the rapidly and slowly decaying components of the suspension flow rate, respectively. Figure 3*a* shows the dependence of suspension flow rate on time for the suspensions containing different quantities of poorly filtering cells. It is seen that even when the content of 'bad' cells increases to 50%, the initial segment of the filtration curve changes insignificantly. In this case, major differences in the curve shape are manifested in the segment of slow decrease: with the growing content of 'bad' cells the drop of flow rate in this segment increases, while the time required for reaching the stationary suspension flow rate decreases. Figure 3*b* shows changes in the dependence of the suspension flow rate on time upon variation of the rate of poorly filtering cells. Like in the preceding case, the initial segment of the filtration curve is not virtually altered. Major changes occur in the region of slow decay. With the diminishing rate of poorly filtering cells the decrease in the suspension flow rate is larger in this region. Herewith, the time necessary for reaching the stationary suspension flow rate increases. As seen in Fig. 3, the values of A_1, t_1 and A_2, t_2 coefficients are determined primarily by properties of the major well filtering and the minor poorly filtering subpopulations, respectively. Indeed, in some cases this result is obvious. For example, at $w_2 \ll w_1$, $n_2 \ll n_1$, using formula (3) one obtains:

$$A_1 = Tw_b n w_b / (n w_b + w_1 / V_1),$$

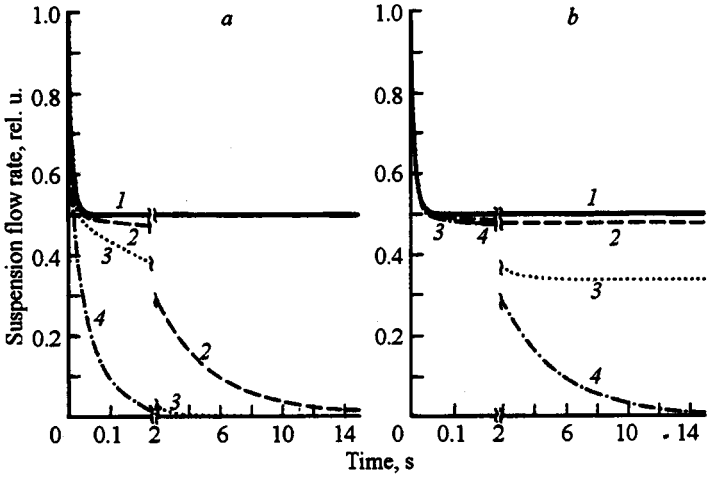

Figure 3. The time course of the flow rate of suspension consisting of two cell types for different contents of poorly filtering cells (*a*) and different rates of poorly filtering cells (*b*). Normalization, filter parameters and filtering conditions are the same as in Fig. 1. The rate of erythrocyte from the well filtering subpopulation $w_1 = 0.01\, w_b$. The isotonic volume of erythrocytes of the well and poorly filtering subpopulations $V_1 = V_2 = 90$ μm^3. *a*, Ratio of the poorly filtering to the well filtering cells $w_1/w_2 = 0.0001$; percentage of poorly filtering cells in 1% suspension (%): 0 (*1*), 1 (*2*), 5 (*3*), 50 (*4*). *b*, Percentage of poorly filtering cells in 1% suspension; the rate of poorly filtering cells w_2: w_1 (*1*), $0.1\, w_1$ (*2*), $0.01\, w_1$ (*3*), 0 (*4*).

$$A_2 = Tw_b(n_2 w_b w_1/V_1 - w_2^2/V_2^2)(w_1/V_1)/[(nw_b + w_1/V_1) \times (nw_b w_2/V_2 + n_2 w_b w_1/V_1 + (w_1/V_1)(w_2/V_2))],$$

$$t_1 = 1/(nw_b + w_1/V_1),$$

$$t_2 = (nw_b + w_1/V_1)/[nw_b w_2/V_2 + n_2 w_b w_1/V_1 + (w_1/V_1)(w_2/V_2)], \quad (5)$$

where $n = n_1 + n_2$ is the total concentration of cells in suspension.

Qualitatively, the character of the filtration curve for a suspension consisting of two subpopulations may be explained in the following manner. At the initial time moment, only buffer passes through a filter, so the flow rate of suspension is equal to that of buffer. If there are few cells of the poorly

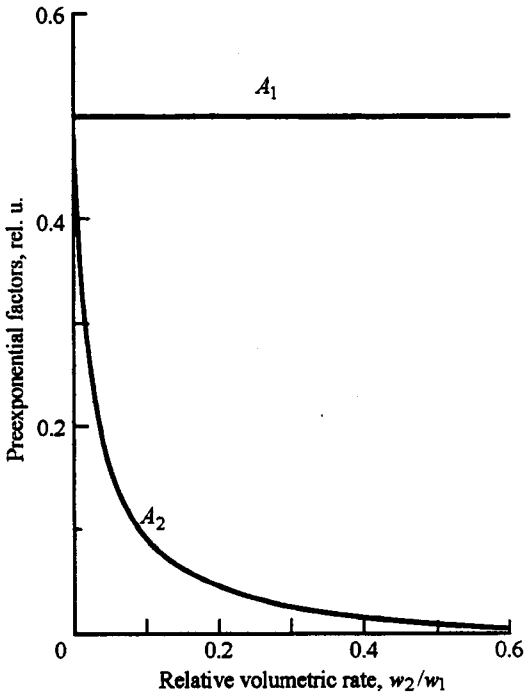

Figure 4. Dependence of preexponential factors in the formula describing the flow rate of a suspension consisting of two cell types on the rate of erythrocytes from the poorly filtering subpopulation. Normalization, filter parameter and filtration conditions are the same as in Fig.1. The rate of an erythrocyte from the poorly filtering subpopulation $w_1 = 0.01 w_b$. The isotonic volume of erythrocytes of the poorly and well filtering subpopulations $V_1 = V_2 = 90\ \mu m^3$. Percentage of poorly filtering cells in suspension, 5%.

filtrating subpopulation, then at first, the cells of the major subpopulation enter the filter. In such a system, a quasi-stationary suspension flow rate determined by the major subpopulation cells is established. In this situation, the flow rate of suspension is larger by magnitude A_1 than that of buffer. The magnitude of A_1 the larger the lower the rate of major subpopulation cells (see formula (5)). (According to our estimates based on the results of studies [11, 12], the rate of passage of the major subpopulation of erythrocytes from normal blood under physiological conditions through a 3-μm pore of the filter is about 100 times as low as that of the buffer flow rate). At the rate of major subpopulation cells $w_1 = 0.01\ w_b$ and the hematocrit of suspension $Ht = 1\%$, the value of A_1 is approximately twice as small as the buffer flow rate through the filter. In this case, under the pressure of 6 cm of H_2O above the filter, the

characteristic time of the onset of a quasi-stationary suspension flow rate is about 10 ms. The cells of minor subpopulation begin to occupy the filter pores later than the cells of major subpopulation. The time necessary for the onset of the stationary state relative to the number of pores occupied by such 'bad' cells depends on the rate of their passage through a pore (w_2) and their concentration (n_2). The characteristic time of the onset of this stationary state will be the longer the lower the rate of cells of this type and the smaller their concentration. The magnitude of A_2 is also dependent on the rate and concentration of poorly filtering cells. At w_2 different from zero, A_2 is the larger the lower the rate of these cells and the higher their concentration: if $w_2 = 0.0001\,w_b$, then at $n_2 = 0.05\,n$ the magnitude of A_2 makes up 0% of the buffer flow rate through the filter. The characteristic time t_2 required for reaching the stationary state is 0.5 s. At $n_2 = 0.005\,n$, A_2 makes up 1% of the rate of buffer flow through the filter, whereas t_2 increases to 1.4 s. In this situation, the stationary suspension flow rate is different from zero. If the rate of 'bad' cells is equal to 0, then the suspension flow rate will obligatorily drop to 0 because the 'bad' cells will plug up all of the filter pores regardless of the concentration of these cells. When the rate of 'bad' cells $w_2 = 0$ and $n_2 = 0.05\,n$, the characteristic time of the transient process t_2 is ~ 0.7 s. At $n_2 = 0.005\,n$, this time increases to 7 s.

If the rates of 'good' and 'bad' cells are close (of the same order of magnitude), the coefficient A_2 is close to 0 (Fig. 4). At equal rates of cells the dependence of the suspension flow rate on time (curve ($Q(t)$) is described by a single exponent $Q = Q_{st} + A_1 \exp(-t/t_1)$. If w_1 and w_2 differ appreciably (by more than one order of magnitude), the coefficient A_2 differs substantially from 0, and a segment of slow decrease appears in the filtration curve $Q(t)$.

Irrespective of the extent of difference between the rates of 'good' and 'bad' cells (w_1 and w_2), knowing the A_1 value, one can derive an approximate ratio of the rate of cells belonging to the major subpopulation to their volume V_1 (see formula (5)):

$$w_1/V_1 = n \times w_b \times (T \times w_b - A_1)/A_1, \qquad (6)$$

where n is the total number of cells in suspension per unit volume.

It is more convenient to use the rate to volume ratio since it seems to be the natural normative factor of the rate. It indicates the number of cells that have passed through the filter per 1 s.

When the initial segment of the filtration curve is unknown, one can estimate the rate w_1 for the cells of major subpopulation in the following way. After the decay of the 'fast' exponent (for the hematocrit $Ht = 1\%$ and the cell rate $w_1 \sim 0.01\,w_b$, this corresponds to a time of ~30 s) the filtration curve is described by the equation

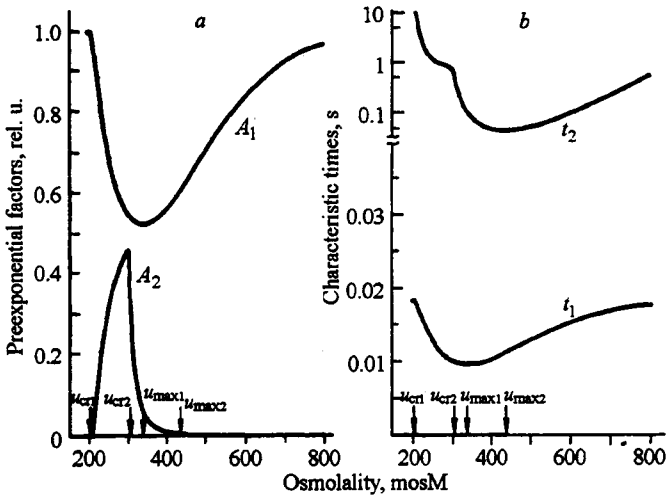

Figure 5. Dependence of preexponential factors (*a*) and characteristic times (*b*) in the formula describing the flow rate of suspension consisting of two cell types on osmolality. All suspension parameters and normalization are the same as in Fig. 1.

$$Q(t) = Q_1 - A_2 + A_2 \exp(-t/t_2), \tag{7}$$

where Q_1 is the stationary flow rate of homogeneous erythrocyte suspension composed of only the major cells. In this case, $A_1 = Tw_b - Q_1$. Then using formula (6), one can derive the ratio of the rate of major subpopulation cell w_1 to its volume V_1.

If after the decay of the 'fast' exponent the region of slow decay is distinguishable (A_2 is not equal to 0), then one can find A_2 and t_2 by appropriate selection of parameters. Assuming that the concentration of 'bad' cells n_2 is much lower than the concentration of cells in suspension n, one may determine the rate of 'bad' cells w_2 and their concentration n_2. If by these times the suspension flow rate has already reached the stationary level, the suspension may be regarded as homogeneous: $n_2 = 0$.

In principle, the rate of major subpopulation cells w_1 can also be estimated when a suspension contains a larger number of subpopulations. In this case, the slow decay segment of the filtration curve is no longer described by a single exponent. Nonetheless, extrapolation of the segment of $Q(t)$ curve in the time range 70–200 s to the zero time moment provides an estimate of the flow rate of suspension composed of cells of major population Q_1.

The basic conclusion from the above analysis consists in the following: having information about the behaviour of filtration curve at times of the

order of 100 ms (exceeding the time necessary for reaching the quasi-stationary flow rate of major subpopulation), one can always estimate at least the rate of major subpopulation.

The possibility of determination of geometric and mechanical parameters of erythrocytes from the curves of the time dependence of flow rate of suspension consisting of two cell types. Let us go back to considering the case when the cells of two subpopulations in suspension differ in a single parameter – the cell surface area. The rate of cells with a large surface area w_1 (let us call them 'good' cells) is higher than the rate of cells with a smaller surface area ('bad' cells). The cells of these two subpopulations are characterized by different values of critical osmolality: u_{cr1} and u_{cr2}, respectively ($u_{cr1} < u_{cr2}$). Let the concentration of the 'bad' cells be less than that of the 'good' cells ($n_2 < n_1$). Since there is a reverse dependence of parameters A_1, t_1 on the rate of major cells, the increase in osmolality from the u_{cr1} level, the values of these parameters pass through a minimum (Fig. 5). For both parameters this minimum is reached in the vicinity of osmolality u_{max1} at which the rate of 'good' cells is maximal. This means that at a given osmolality the quasi-stationary flow rate of 'good' cells is established faster than at other osmolality levels, while the quasi-stationary flow rate of whole suspension is maximal. The second preexponential coefficient A_2 is noticeably different from zero, when the rates of cells differ appreciably; therefore, it appears in the range from u_{cr1} to u_{cr2} and drops rapidly to zero upon osmolality increase above u_{cr2} (see Fig. 5*a*). The parameter t_2 is determined by properties of the subpopulation of 'bad' cells and reaches its maximum at the osmolality u_{max2} at which the rate of 'bad' cells is maximal (see Fig. 5*b*).

Variation of parameters A_1, t_1, A_2, t_2 at different osmolality values determines the dependence of the character of filtration curves on osmolality (Fig. 1*b*). This figure shows that at $u_{cr1} < u < u_{cr2}$ (curves *1*, *2*) a distinct segment of slow decay appears in the filtration curve. As the rates of cells approximate with the increasing u, the slow segment disappears, and the dependence $Q(t)$ looks like a single exponent (curves *3*, *4*). Thus, using the osmotic sweep, one can reveal the presence of 'bad' cells in suspension in the osmolality range $u_{cr1} < u < u_{cr2}$.

We have considered the case when two subpopulations of cells in suspension differ in the surface area of erythrocytes assuming that other characteristics (H, f, a_0, b_0) are identical. For the cells differing only by the quantity of hemoglobin or the coefficient of osmotic sensitivity f, the slow decay segment of the filtration curve will also appear in the hypertonic region. For the cells differing by the coefficients a_0 (Fig. 2*b*) or b_0, this segment will appear in both the hypotonic and hypertonic regions.

The possibility to reveal differences in parameters of cells depends on the accuracy of measurements, temporal resolution and osmolality step. It would be more appropriate to analyze the influence of the precision of measuring

equipment using a concrete apparatus. However, we do not have such a possibility. Therefore, let us choose arbitrarily these parameters for illustrating the influence of measurement accuracy on results.

Assume the accuracy of experimental measurements to be 5%, time step of 5 ms and osmolality step of 20 mosM. At the osmolality step of 20 mosM, we can determine 5% differences in the area of erythrocyte surface S. For the 25% difference in the hemoglobin quantity per cell H, the difference in the critical osmolalities amounts to 25 mosM. At the 20-mosM osmolality step we can detect the presence of 'bad' cells and determine approximately 10% differences in the values of osmotic sensitivity coefficient f. The shape of filtration curves is little sensitive to variation of parameters a_0 and b_0. At an experimental accuracy of 5% we can reveal the presence of the second subpopulation of cells if the a_0 and b_0 coefficients of cells belonging to different subpopulations differ 50 times. But if these differences exist, we will reveal them in a wider osmolality range embracing both the hypotonic and hypertonic regions.

DISCUSSION

Analysis of the model has shown that investigation of the dependence of suspension flow rate on time allows determination of the filtration rate of major subpopulation of cells, the rate of admixture filtration and its percentage. This may be done with a sufficient accuracy, if the filtration of the cells of 'bad' subpopulation is tenfold worse than that of the cells of 'good' subpopulation. Herewith, the method proves to be rather sensitive and can detect 0.5–1.0% admixture of poorly filtering cells. The rate of passage of major subpopulation cells through a filter pore can also be determined when the 'bad' cells are heterogeneous and have different filtration rates. The only condition consists in that the filtration of all these cells must be tenfold worse than that of major subpopulation cells.

The existence in suspension of two subpopulation of cells that differ in mechanical and geometric parameters affecting their filterability is not always manifested in the character of suspension flow through a filter. However, if the filtration curves are obtained at different osmolality levels, it is always possible to find the osmolality range in which differences in the filtration rate of cells can be revealed, i.e. to detect the presence in suspension of cells different from those belonging to the major subpopulation.

Assume that the experimental results imply that the filtration curve may be described by two exponents at some osmolality levels and by a single exponent at some other osmolalities. If the filtration curve is described by two exponents in the hypotonic region and by one exponent in the hypertonic region, then there are three possibilities (assuming that suspended cells differ in one parameter): the cells differ in their area S, quantity of hemoglobin per

cell H or coefficients of osmotic sensitivity f. When two exponents appear within the segment embracing both hypotonic and hypertonic regions, one can say that the cells differ in coefficients a_0 or b_0.

The results discussed above have been obtained for the case when suspension consisted of two subpopulations of erythrocytes differing in one parameter. In reality, erythrocytes are continuously distributed in blood over a number of parameters: volume, surface area, and density, all these parameters being interdependent [4, 13, 14]. In some pathologies there appear in blood minor (~1%) admixtures of strongly altered, poorly filtrating erythrocytes [1]. Therefore, special studies are in order for elucidating the effects of continuous distributions on the above-cited results.

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