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Kinetics of Filterability Loss in Ephazol-Treated Erythrocytes

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Abstract—The effect of a palladium-containing complex Ephazol on the rate of passage of an erythrocyte suspension through nuclear filters was studied by the constant-pressure filtration method. Ephazol was shown to decrease the erythrocyte filterability. The curves obtained by plotting the time necessary for a fixed volume of the erythrocyte suspension to pass through the filter versus the time of incubation with Ephazol or versus its initial concentration were typical of autoaccelerated processes. From analysis of the corresponding kinetic models, a conclusion was made that the effects observed were accounted for by the nonlinear dependence of the filtration rate of a suspension on the rate w of the passage of an individual erythrocyte through a pore, and by the Ephazol-induced changes in the erythrocyte distribution with respect to w. Several models describing these changes and the possible mechanisms relating the filtration kinetics to the incubation parameters were discussed.

Key words: erythrocyte suspension, filtration rate, Ephazol

INTRODUCTION

In the thirty years of their existence, the filtration methods based on passing erythrocyte suspensions through membranes with calibrated pores comparable in diameter with the cell size have become a powerful tool in studying a vast scope of problems concerning the mechanical properties of erythrocytes [1–3]. First, filtration methods are used to biophysically assess changes in the mechanical properties of erythrocytes [4]. Second, they have proved helpful in disease diagnosis and monitoring of therapy. Third, they may be a good supplement to the procedures used in quality control of banked donor blood (e.g., at the stage of development of preserving solutions and storage conditions), assessment of the effects of drugs, etc. [5–8].

The testing techniques usually include incubation of cells with the drug to be tested. To facilitate the detection of the potential effect of the drug, the so-called stress models are often used, in which cells are tested in the presence of some damaging agent [3]. Stress factors most often used in such models include oxygen in damaging concentrations, pH and osmolarity changes [9, 10], and chemicals affecting the cell membrane (e.g., glutaraldehyde, ionophore A23187, chlorpromazine, acetylphenyl hydrazine, pentoxifylline, etc.) [11, 12].

The usual reasoning behind the use of such stress tests is that stressful incubation conditions may augment the response of cells to the drug under study and thereby make more obvious the difference between the control and the experimental erythrocytes. Pre-exposure of erythrocytes to stress appears useful in diagnostic screening of these cells by filtration methods, especially in the cases when erythrocytes undergo increased sequestration by phagocytosis within the organism [12]. When such kinetic versions of the method are used, it may be of importance how the filtration parameters depend on the incubation time, the level of the stress factor, etc. In this study, we examined the effect of Ephazol [N-methyl-(1-oxy-1-phenylisopropyl)ammonium tetrachloropalladate] on the filtration properties of erythrocytes. As Ephazol-containing preparations produce a wide variety of biological effects, immunomodulation included [13, 14], we suggested that the palladium complex

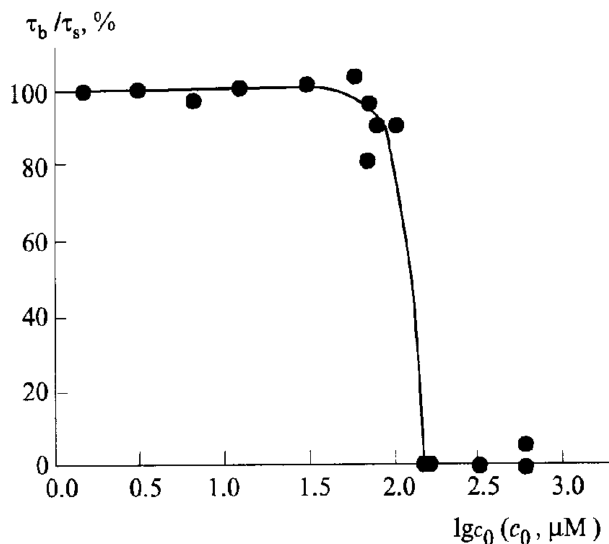

Fig. 1. Concentration dependence of the filterability index (τ_b/τ_s, percent) of an erythrocyte suspension incubated with Ephazol for 60 min.

Ephazol might be membranotropic, and considered it to be a stress agent.

EXPERIMENTAL

Preparation of erythrocyte suspensions. Erythrocyte suspensions were prepared from donor blood collected in the glucose–citrate preserving solution Glugicir. Erythrocytes were sedimented by centrifugation at 2000 *g* for 10 min and washed twice with physiological saline and then once with 10 mM HEPES (pH 7.4) containing 140 mM NaCl. Every time, the supernatant was removed together with the top layer of the sedimented cells. The osmolarity of solutions was determined with an OMKA 1Ts-01 cryoscopic osmometer (Russia). The erythrocytes were incubated with Ephazol at a suspension hematocrit of 15–25%. Ephazol was prepared in the same buffer that was used to resuspend the erythrocytes.

Filterability measurements. Erythrocyte filterability was determined with a modified Hanss hemorheometer [15] at a constant hydrostatic pressure of 6 cm H_2O. Nuclear membrane filters were made of a 7-μm-thick polyethylene terephthalate film with pores 3 μm (average) in diameter. The time that a fixed volume (250 μl) of erythrocyte suspension took to pass through a filter was recorded to an accuracy of 0.1 s. The τ_b/τ_s ratio, with τ_b and τ_s being the buffer and suspension passage times for a given filter, was used as the quantitative measure of erythrocyte filterability. The worse the filterability, the lower the filterability index.

Filterability measurements were performed with 1% erythrocyte suspensions. The same buffer was used for measuring τ_b, resuspending the erythrocytes, and diluting the suspensions to 1% hematocrit. The working solutions were filtered through 0.22-μm filters to remove mechanical debris.

RESULTS AND DISCUSSION

Ephazol significantly decreased the erythrocyte filterability. The τ_b/τ_s ratio depended on both the incubation time and the Ephazol concentration. Figure 1 shows the concentration dependence of the filterability index, τ_b/τ_s, for erythrocytes incubated with Ephazol for 60 min. One can see that, with a fixed incubation time, the filterability depended on the Ephazol concentration in a clearly threshold manner. The characteristic feature of the curve is the steep slope in the threshold region: although we varied the Ephazol concentrations from 1 to 1000 μM, the major changes took place in a narrow range from 60 to 80 μM (threshold).

The dependence of the type observed might be explained by the kinetics of changes in the properties of erythrocytes during incubation with Ephazol. This is clearly evident from the time course of filterability loss observed in the erythrocyte suspension after addition of Ephazol at nearly threshold concentrations (Fig. 2). In all cases, the rate of filterability loss increased with increasing the time of incubation with Ephazol.

As the mechanisms whereby Ephazol affects the erythrocyte filterability are unknown at present, we cannot construct a kinetic description of the process. In order to consider the simplest hypothesis about the effect of Ephazol on erythrocytes and to analyze the conditions that might lead to the curves typical of autoaccelerated processes, we constructed a model based on the following assumptions.

(i) Any suspension consists of the major fraction characterized by a certain passage time through pores

(τ_{bas}), and the minor fraction (comparable in size with the number of filter pores) that does not pass through the filter.

(ii) Incubation of erythrocytes in the presence of Ephazol increases the fraction of nonfilterable cells.

The filtration rate of a buffer without cells may be written as

$$\frac{dV}{d\tau} = wn_0, \tag{1}$$

where V is the buffer volume passed through the filter, τ is the current filtration time, w denotes the specific filtration rate of the buffer (i.e., the volume passing through a pore per unit time), and n_0 is the total number of pores in the filter.

Therefore,

$$\tau_b = \frac{V_0}{wn_0}, \tag{2}$$

where V_0 is the volume of the aliquot applied to the filter.

Further, the filtration rate for a cell suspension will be described by the set of equations (3)–(5). This set of equations was proposed in [16] to describe the filtration of a dilute erythrocyte suspension containing a small fraction of nonfilterable leukocytes. Equation (5) is written under the assumption that, for dilute suspensions, the kinetics of passage through a filter is accurately described by the kinetics of the passage of the suspending medium through empty pores (pores that are not occupied by cells).

$$\frac{dn_1}{d\tau} = k_1(n_0 - n_1 - n_2) - k_2 n_1, \tag{3}$$

$$\frac{dn_2}{d\tau} = k_3(n_0 - n_1 - n_2), \tag{4}$$

$$\frac{dV}{d\tau} = w(n_0 - n_1 - n_2). \tag{5}$$

Here, n_0, n_1, and n_2 denote the total number of filter pores, the number of pores occupied by cells from the major subpopulation, and the number of pores clogged by nonfilterable cells; k_1 and k_3 are the fractions of pores that become occupied per unit time by cells from the major (filterable) and minor

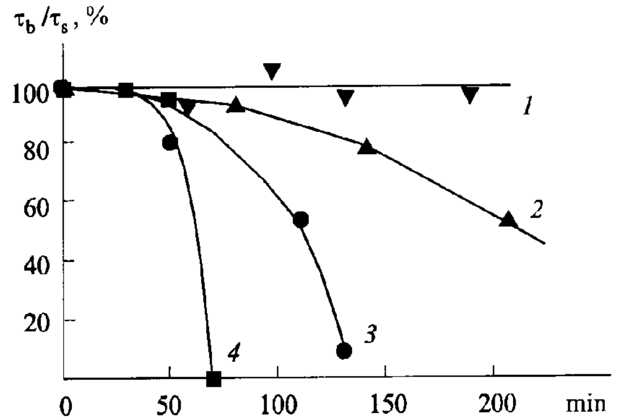

Fig. 2. Filterability index (τ_b/τ_s, percent) as a function of the incubation time in the presence of (*1*) 0.03 (*2*) 0.06, (*3*) 0.07, and (*4*) 0.08 mM Ephazol.

(nonfilterable) subpopulations, respectively, (i.e., the specific pore-blocking rates for the major and minor subpopulations), and k_2 is the fraction of pores emptied per unit time (i.e., the specific rate of cell passage through pores; $k_2 = 1/\tau_{bas}$).

The set may be easily solved analytically by using a steady-state approximation for n_1. By setting $dn_1/d\tau = 0$, we obtain

$$n_1 = (n_0 - n_2)\frac{k_1}{k_1 + k_2}.$$

Substituting this value of n_1 into equation (4) and performing simple calculations, we arrive at

$$\frac{dn_2}{d\tau} = rk_3(n_0 - n_2).$$

Here, $r = k_2/(k_1 + k_2)$.

If $n_2 = 0$ at $\tau = 0$, integration leads to

$$n_2 = n_0[1 - \exp(-rk_3\tau)].$$

With these estimates of n_1, and n_2 substituted into (5), calculations give

$$\frac{dV}{d\tau} = wrn_0[1 - \exp(-rk_3\tau)].$$

Integrating the latter expression for the boundary conditions $V = 0$ at $\tau = 0$ and $V = V_0$ at $\tau = \tau_s$, we find the filtration time for the suspension:

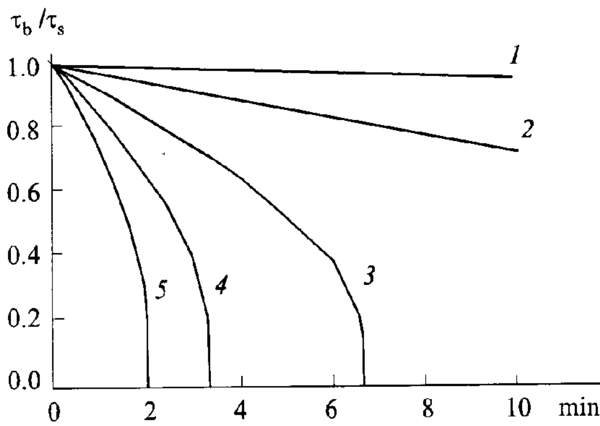

Fig. 3. Dependence of the filterability index τ_b/τ_s on the incubation time calculated according to equation (6) for $\beta = \alpha C_0 t$, r = 1, and the following $\alpha hQt_b/C_0$ values: (*1*) 0.01, (*2*) 0.05, (*3*) 0.15, (*4*) 0.30, and (*5*) 0.50.

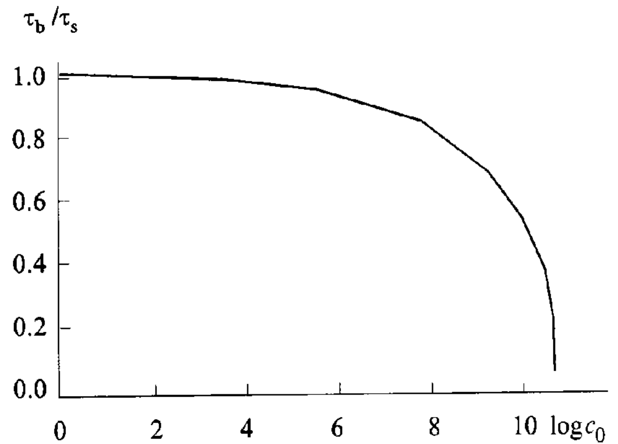

Fig. 4. Dependence of the filterability index τ_b/τ_s on the $\lg C_0$ calculated according to equation (6) for $\beta = \alpha C_0 t$, r = 1, and $\alpha hQt_b t$ = 1.

$$\tau_s = \frac{\ln(wn_0/(wn_0 - k_3 V_0))}{rk_3}.$$

This expression may be rewritten in the form convenient for comparison with the experiment by using relation (2):

$$\tau_b/\tau_s = \frac{rk_3\tau_b}{\ln(1/(1 - k_3\tau_b))}. \quad (6)$$

For analysis of how the τ_b/τ_s ratio depends on the experimental parameters (the duration of incubation with Ephazol and its concentration), k_3 must be expressed in these terms. Its physical meaning in (4) allows us to write it in the form:

$$k_3 = Qh\beta,$$

Here, Q is the coefficient characterizing the filter, h is the hematocrit value (in fractions), and $\beta = f_{(\alpha, C, t)}$ is a function reflecting the changes in the concentration of pore-clogging cells induced by incubation with Ephazol at concentration C for time t. Coefficient α is the specific efficacy of Ephazol (or sensitivity of the cells under study to the drug).

Let us analyze the dependence of filterability on the Ephazol concentration, C, and the incubation time, t, for several most practically significant types of the function β.

(a) $\beta = \alpha C_0$. In this case, the filterability index does not depend on the incubation time:

$$\tau_b/\tau_s = \frac{rQh\alpha c_0\tau_b}{\ln(1/(1 - Qh\alpha c_0\tau_b))}.$$

We will not examine this situation in more detail,* as the effect of Ephazol is clearly time-dependent. Therefore, we have to hypothesize about the law whereby the filterability of erythrocytes changes in the presence of Ephazol. Let us consider the simplest possibilities.

(b) $\beta = \alpha C_0 t$. This corresponds to a rather common case of a dose-proportional effect. As follows from (5), the dependence of the filterability index τ_b/τ_s on any of the multiplicands in the product $\alpha QhC_0 t$ will be of the same form. To make more illustrative the comparison of the theoretical curves with the experimental data, the dependence of the filterability index τ_b/τ_s on the incubation time t calculated for several values of C_0 is presented in Fig. 3, and the concentration dependence (τ_b/τ_s vs. $\ln C_0$) calculated for the fixed t is shown in Fig. 4. Evidently, the theoretical curves correspond well to the experimental dependences shown in Figs. 1 and 2.

* The τ_b/τ_s vs. t dependences can be analyzed using the relationship for the case below, taking t = 1.

(c) $\beta = C_0(1 - e^{-\alpha t})$. This is also a common situation: when acting, the drug is spent proportionally to its current concentration. If the time is fixed, the term $(1 - e^{-\alpha t})$ is constant; therefore, the dependence of the filterability index τ_b/τ_s on the $\ln C_0$ will have the same shape as that shown in Fig. 4. However, in this case, the invariance with respect to the product $C_0 t$ is not observed.

The form of the temporal dependence of the filterability index τ_b/τ_s is determined by the value of the multiplicand QhC_0t_b. If C_0 is such that $QhC_0t_b < 1$, the curve will be concave (relative the time axis) and have a limit depending on the C_0 value. If C_0 is such that $QhC_0t_b > 1$, the curve will have an inflection at a position depending on the C_0 value. The greater the C_0, the higher the position of the inflection. The curve begins to resemble the curve for the dependence described under (a). Figure 5 shows the curves of both types.

As follows from the aforesaid, all the kinetics considered give rise to such dependences of the filterability index τ_b/τ_s on the initial Ephazol concentration as those shown in Figs. 2 and 4. Therewith, the shape of the filterability–incubation time plot depends on whether the drug acts following the zero-order or the first-order kinetics. In the latter case, the shape of the curve also depends on the initial drug concentration (more exactly, on whether the product QhC_0t_b is less or more than unity.

Note that the constant α in the expression for β may be interpreted as a measure of the effect produced by the drug on the cells and, as such, may be useful in comparing the drugs in potency, or in quantitatively assessing the erythrocytes from different donors, or cells from the same donor differently processed or stored.

In real erythrocyte suspensions, cells are somehow distributed with respect to their specific rates of passage through pores, rather than constitute two subpopulations, as in the model.

Numerical analysis shows that the dependence of the filterability index τ_b/τ_s on the time of incubation with the drug or on its concentration does not change qualitatively when such cell distribution is taken into consideration. However, in this case, the shape of the curve depends on the distribution shape and width and on how these distribution parameters change during incubation with Ephazol. Both the experimental data and the results of modeling indicate that the effects observed (the accelerated time- and dose-dependent filterability loss in the presence of Ephazol) is a manifestation of the nonlinear dependence of the filtration rate on the parameters of the cell distribution with respect to the rate of passage of an individual cell through a pore. This "amplifying" property of the method may prove to be useful in many situations, especially in assessing the effects of weakly but continuously or repeatedly acting membranotropic drugs, in diagnostic measurements, etc. This property must be taken into account if some drugs are compared in the membranotropic activity. On the other hand, as small and sometimes uncontrollable variations in experimental conditions may bring the system below or above the response threshold, a possibility exists of misinterpreting the data.

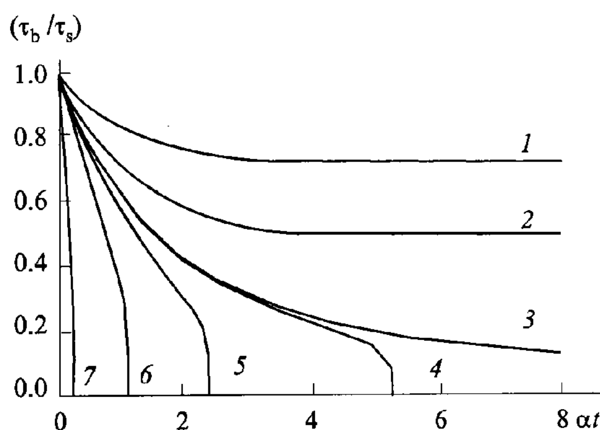

Fig. 5. Dependence of the filterability index τ_b/τ_s on the incubation time according to equation (6) for $\beta = C_0(1 - e^{-\alpha t})$, $r = 1$ and the following values of the product $h\,Q \cdot t_b \cdot C_0$: (*1*) 0.50, (*2*) 0.80, (*3*) 1.00, (*4*) 1.05, (*5*) 1.10, (*6*) 1.50, and (*7*) 5.00.

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