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MATHEMATICAL DESCRIPTION OF ADHESION OF LYMPHOCYTES FLOWING THROUGH A GLASS BEAD COLUMN

Investigation of phenomena associated with the retention of cells in model structures simulating certain traits of the microcirculation network during the cell flow through the channels of this system is important for two reasons. On one hand, processes playing an important role in the life cycle of cells circulating in the blood and lymph, particularly as regards various functions of organs and tissues such as lungs, spleen lymphatic nodes and others, are involved. On the other hand, model studies may contribute to a better knowledge of the physical and physico-chemical phenomena which are basic for processes of cell adhesion, e.g., passing of cells through narrow vessels, interaction between cells.

In this paper we attempt to describe the retention of cells (lymphocytes) in the glass bead column from the mathematical point of view. Other aspects of the problem have been discussed in [1], [2], [4], [8].

A cell suspension of constant density and constant velocity flows through a glass tube which is filled with small glass beads. The diameter of the glass tube is about 12 mm and the experiments were carried out with 5 lengths of the column: 1.2, 1.6, 2.0, 2.4, and 2.8 cm. The diameters of the beads vary from 150 to 300 μm. A single lymphocyte may be considered as a sphere of diameter of about 8 μm. During perfusion some cells are stopped on the surface of glass beads. We make the following assumptions about this phenomenon:

a. On the surface of the glass beads there are hypothetical active centres which retain the cells flowing through the bed. The number of these sites in the column is finite.

b. In the process of retaining the cells on the surface of the beads each cell occupies one site which further takes no part in the process. The maximal number of cells which may be retained in the bead bed corresponds to the number of sites (active centres) in the column.

c. During perfusion, the adhesion properties of cells, the properties of the bead surface and the properties of the medium do not change.

d. The number of cells retained on the bead bed per time unit depends thus on the concentration of the inflowing cells, the number of active centres and on the adhesion properties of the cells.

e. The packing of the beads in the column is homogeneous. Thus the maximal number of cells stopped on each cross-section of the column by a plane perpendicular to the axis of the tube is constant.

The mathematical problem is to find a differential equation describing the velocity of retention of cells in every layer perpendicular to the axis of the tube.

The most general description of the phenomenon in the layer is given by the differential equation

$$\frac{\partial A}{\partial t} = f(g, A_M - A, x, t),$$

where $g = g(x, t)$ is the suspension concentration in the layer of coordinate x (i.e. the distance of the layer from the entry of the column is x) at the moment t, A_M denotes the maximal capacity of the layer (it is assumed that A_M is independent of x), $Adx = A(x, t)dx$ is the number of cells captured in the layer at the level x up to the moment t, and the quantity $A(x, t)$ is the density of captured cells up to the moment t at the level x.

The mathematical description presented above should be supplemented by some quantitative postulates concerning the adhesion process. Some similarities to physico-chemical aspects of the process are suggested by the equation

$$\frac{\partial A}{\partial t} = an(A_M - A), \qquad n = vgS, \tag{1}$$

where a is a constant number, independent of x and t, v is the mean linear velocity of the flow of cellular suspension into the packing and S is the mean area of the cross-section of the bed. If v is constant and if $S(x)$ is the area of the cross-section at the level x, then

$$S = \frac{1}{a}\int_0^a S(x)dx,$$

where a is the length of the column.

The second equation describing the process is the equation of transport through the medium:

$$\frac{1}{S}\frac{\partial A}{\partial t} + \frac{\partial g}{\partial t} + v\frac{\partial g}{\partial x} = 0. \tag{2}$$

The foregoing equations are considered in the domain $\{x \geqslant 0, t \geqslant x/t\}$ with the boundary conditions $g(0, t) = g_0 = \text{const}$ and $A(x, x/v) = 0$.

For the column of the length x the experimental results are expressed by

$$W(x, t) = 1 - \frac{Svg(x, t)}{Svg_0},$$

which gives the rate of cell retention W at the moment t. The function $W(x, t)$ derived from (1) and (2) with the boundary condition specified above takes the form

$$(3) \qquad W(x, t) = \frac{(\exp\{aA_M x\} - 1)\exp\{anx/v\}\exp\{-ant\}}{1 + \exp\{aA_M x\}\exp\{anx/v\}\exp\{-ant\}}.$$

On the other hand, we have

$$(3') \qquad W(x, t) = \frac{c(x)e^{-bt}}{1 + c(x)e^{-bt}},$$

where $c(x) = (\exp\{aA_M xS\} - 1)\exp\{g_0 xS\}$ and $b = g_0 vS$.

It has been shown that the curves of the form (3) do not fit to the experimental data [3]. Namely, for every x (column length) there exist two numbers a and A_M such that the curve (3) approximates well the experimental curve, but do not exist universal numbers a and A_M good for all considered x. Then, to find a function which describes the empirical data with good approximation, we generalize the form (3') so that the coefficient b changes in dependence on the variable x (bed length). Therefore, we consider the approximation of experimental curves of the form

$$(4) \qquad W(x, t) = \frac{c(x)e^{-b(x)t}}{1 + c(x)e^{-b(x)t}}.$$

If the curves represented by (4) approximate the experimental ones, then the parameter b must essentially depend on x.

For every x we found two parameters, b and c, such that the mean-square error between the experimental curve and that of the form (4) is minimal. We used the procedure described by Kreczmar [6] and the calculations were performed on a GIER computer in the Computer Centre of the University of Warsaw.

Fig. 1 shows the experimental and theoretical curves. In Figs. 2 and 3 the dependence of parameters b and c on x (theoretical and experimental) is presented. With a good approximation we can assume that

$$(5) \qquad b(x) = 0.31e^{-0.28x} \quad \text{and} \quad c(x) = 3.1(e^x - 1)e^{-0.28x}.$$

Starting from equations (2) and (4) the differential equation of the thin layer may be found.

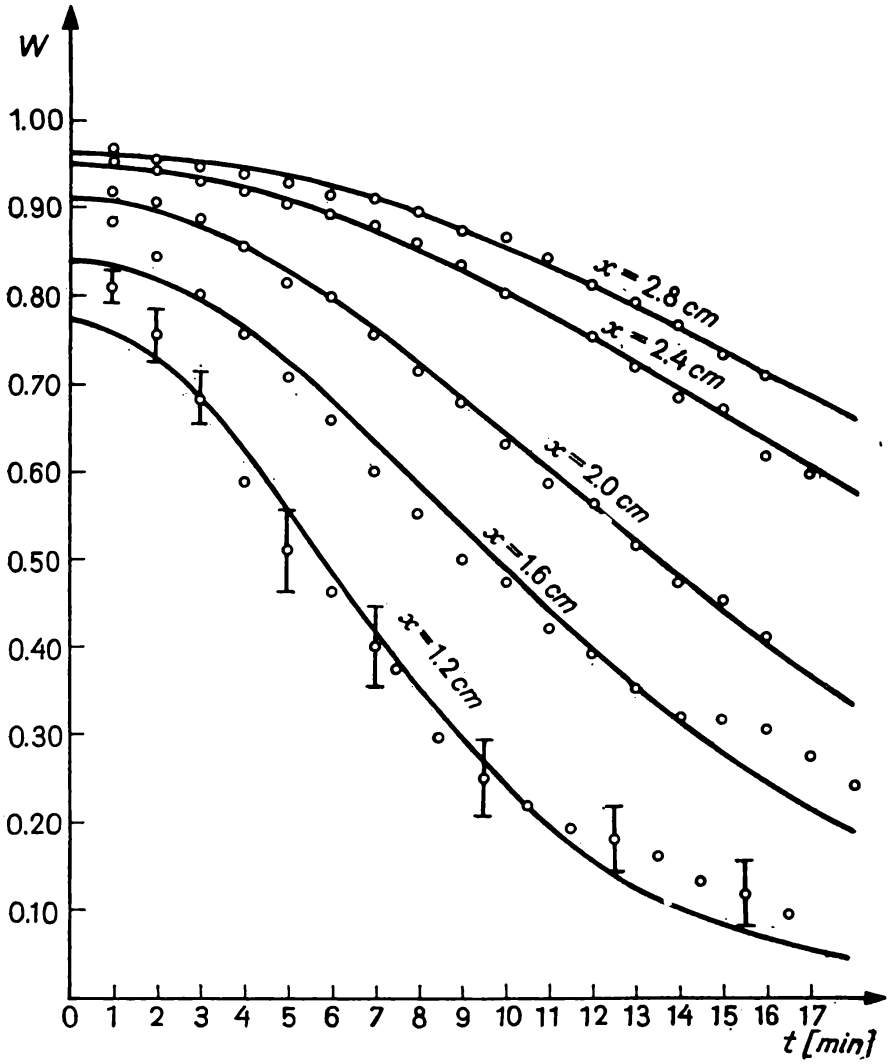

Fig. 1. Comparison of experimental (circles) and theoretical (continuous lines) curves for studied column lengths x; theoretical curves are implied by the equation of cell uptake (4). Vertical bars denote 95% confidence intervals

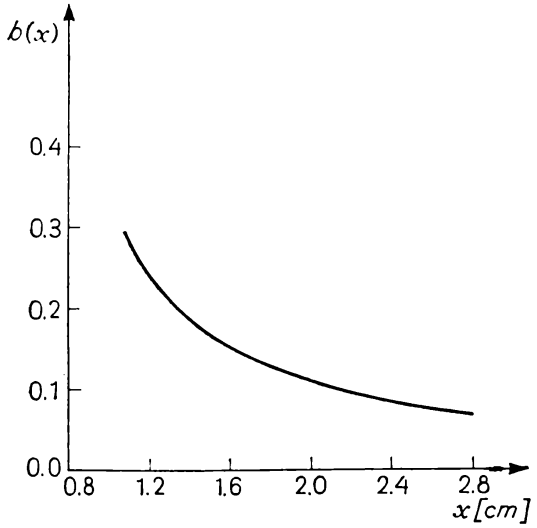

Fig. 2. Dependence of the function $b(x)$ on the column length x

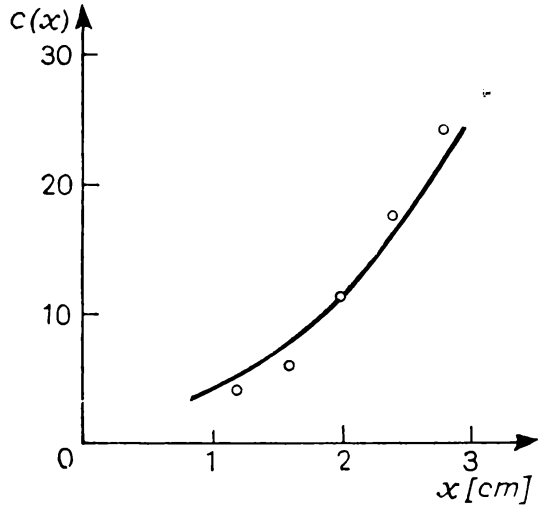

Fig. 3. Dependence of the function $c(x)$ on the column length x

Since $1-W(x, t) = g(x, t)/g_0$, we have

$$g = g(x, t) = \frac{g_0}{1+c(x)e^{-b(x)t}}. \tag{6}$$

Hence, computing the partial derivatives $\partial g/\partial t$ and $\partial g/\partial x$, and putting them in (2) we get

$$\frac{\partial A}{\partial t} = g_0 \frac{c(x)e^{-b(x)t}}{[1+c(x)e^{-b(x)t}]^2}\left[v\frac{c'(x)}{c(x)} - tvb'(x) - b(x)\right]. \tag{7}$$

Dividing (7) by $g(x, t)$ (i.e. using (6)) we obtain

$$\frac{1}{g}\frac{\partial A}{\partial t} = v\frac{c'(x)e^{-b(x)t}}{1+c(x)e^{-b(x)t}} - vb'(x)t\frac{c(x)e^{-b(x)t}}{1+c(x)e^{-b(x)t}} - b(x)\frac{c(x)e^{-b(x)t}}{1+c(x)e^{-b(x)t}}. \tag{8}$$

Integrating (7) with respect to t and taking into account

$$\lim_{t\to\infty} A(x, t) = A_M,$$

we get

$$\frac{b(x)}{g_0}(A_M - A) + \frac{b(x)}{g_0}(g_0 - g) = vc'(x)\frac{e^{-b(x)t}}{1+c(x)e^{-b(x)t}} - vb'(x)t\frac{c(x)e^{-b(x)t}}{1+c(x)e^{-b(x)t}} - v\frac{b'(x)}{b(x)}\log\left(1+c(x)e^{-b(x)t}\right). \tag{9}$$

Subtracting (9) from (8) and taking into account (6) we get the differential equation of the thin layer:

$$\frac{\partial A}{\partial t} = \frac{g}{g_0}b(x)(A_M - A) + gv\frac{b'(x)}{b(x)}\log\frac{g_0}{g}. \tag{10}$$

The boundary conditions are the same as for the linear equation (2). Since we did not use the form (5) of the functions $b(x)$ and $c(x)$, equation (10) is valid for any functions $b(x)$ and $c(x)$. In the case where $b(x) = \text{const}$, equation (10) takes the form (1). The function on the right-hand side of (10) depends explicitly on x.

It can be proved that the right-hand side of equation (10) depends essentially on x, i.e. there exists no equivalent form of (10) such that its right-hand side does not depend explicitly on x.

THEOREM. *The value $\partial A/\partial t$ is not defined only by the variables g and $A_M - A$, i.e. there exists no function $H(g, A_M - A)$ of two variables such that*

$$\frac{\partial A}{\partial t} = H(g, A_M - A).$$

Proof. We assume $b(x)$ and $c(x)$ are of the form

$$b(x) = \lambda e^{-\beta x} \quad \text{and} \quad c(x) = \alpha(e^{x}-1)e^{-\beta x},$$

where λ, β, α are some constants.

Multiplying equation (9) by g_0 and using (6) we get

$$b(x)(A_M-A)+b(x)(g_0-g) = vg_0\frac{c'(x)}{c(x)}\left(1-\frac{g}{g_0}\right)-vg_0b'(x)t\left(1-\frac{g}{g_0}\right)-vg_0\frac{b'(x)}{b(x)}\log\frac{g}{g_0}.$$

Dividing the last equation by $b(x)$ and taking into account that $b'(x)/b(x) = -\beta$ we obtain

$$\frac{1}{v}[(A_M-A)-(g_0-g)] = \frac{c'(x)}{b(x)c(x)}(g_0-g)+\beta t(g_0-g)+\frac{\beta g_0}{\lambda}e^{\beta x}\log\frac{g_0}{g}.$$

Let

$$G(x,t,g) = \frac{1}{v}(A_M-A).$$

Then

$$G(x,t,g) = \frac{c'(x)}{b(x)c(x)}(g_0-g)+\beta t(g_0-g)-\frac{1}{v}(g_0-g)+g_0\frac{\beta}{\lambda}e^{\beta x}\log\frac{g_0}{g}.$$

Formula (6) can be reversed with respect to t. Then $t = t(x,g)$ and

$$g(x,t(x,g)) = g \quad \text{for all } x \text{ and } g. \tag{11}$$

Differentiating equation (11) with respect to x we obtain

$$\frac{\partial g}{\partial x}+\frac{\partial g}{\partial t}\frac{\partial t}{\partial x} = 0. \tag{12}$$

Set

$$F(x) = G(x,t(x,g),g).$$

Differentiating the last equality with respect to x and using (12) we get

$$\frac{dF}{dx} = \frac{\partial G}{\partial x}+\frac{\partial G}{\partial t}\frac{dt}{dx} = \frac{\partial G}{\partial x}+\frac{\partial G}{\partial t}\left(-\frac{\partial g/\partial x}{\partial g/\partial t}\right)$$

$$= \left(\frac{\partial g}{\partial t}\right)^{-1}\det\begin{pmatrix}\frac{\partial G}{\partial x} & \frac{\partial G}{\partial t}\\ \frac{\partial g}{\partial x} & \frac{\partial g}{\partial t}\end{pmatrix} = \det\begin{pmatrix}\frac{\partial G}{\partial x} & \frac{\partial G}{\partial t}\\ \frac{\partial g}{\partial x}\left(\frac{\partial g}{\partial t}\right)^{-1} & 1\end{pmatrix}$$

$$= (g_0-g)\det\begin{pmatrix}\left(\frac{c'}{bc}\right)'+g_0\frac{\beta^2}{\lambda}e^{\beta x}\frac{\log g_0-\log g}{g_0-g} & \beta\\ -\frac{c'}{bc}-\beta t & 1\end{pmatrix}$$

$$= (g_0-g)\left[\frac{c'}{bc}+g_0\frac{\beta^2}{\lambda}e^{\beta x}\frac{\log g_0-\log g}{g_0-g}+\beta\frac{c'}{bc}+\beta^2 t\right],$$

i.e.

$$\frac{1}{g_0-g}\frac{dF}{dx}=\frac{e^{(\beta+1)x}}{\lambda}-\frac{(1+\beta)(e^x-1)-1}{(e^x-1)^2}-\frac{\beta+\beta^2}{\lambda}e^{\beta x}+$$

$$+g_0\frac{\beta^2}{\lambda}e^{\beta x}\frac{\log g_0-\log g}{g_0-g}+\beta\frac{e^{(\beta+1)x}}{\lambda(e^x-1)}-\frac{\beta}{\lambda}e^{\beta x}+\beta^2 t.$$

For g close enough to g_0 the term

$$g_0\frac{\log g_0-\log g}{g_0-g}$$

is close to 1. We recall that $t(x, g)$ is the time at which the concentration at the level x is equal to g. Evidently, if $g \to g_0$, then $t = t(x, g) \to +\infty$ for any $x > 0$. Consequently,

$$dF/dx \to +\infty \quad \text{as } g \to g_0$$

for every $x > 0$. Therefore,

$$\frac{dF}{dx}(x) > 0 \quad \text{for every } x > 0$$

if g is close enough to g_0. On the other hand, it is easy to see that

$$\lim_{x\to 0}\frac{dF}{dx}(x) = -\infty.$$

Therefore, there exists an x_0 such that

$$\frac{dF}{dx}(x_0) = 0$$

and dF/dx changes its sign at x_0. It means that for some values $\bar{g}$ and $\bar{F}$ there exist at least two solutions (x, t) of equations $\bar{g} = g(x, t)$ and $\bar{F} = G(x, t, \bar{g})$. Since the function $b(x)$ is one-to-one, it follows from equation (10) that the derivative $\partial A/\partial t$ is not defined by the number of free places $G = v^{-1}(A_M - A)$ and the density g. This completes the proof.

We do not claim that equation (10) is the only one which describes the real process. Since the curves of the form (4) with the suitable functions $b(x)$ and $c(x)$ approximate very well the experimental curves (together with the first derivatives), practically we can consider the solution of equation (10) as a very good approximation of the real process. Of course, if we took another good approximation of experimental data, we would obtain an equation different from (10). Perhaps we would have some difficulties with finding a so simple final form.

On the right-hand side of (10) there are two terms. For every considered length x there is a moment such that the contribution of every term is not less than 1/3 of the whole value of the right-hand side. So no one term can be omitted.

The Theorem gives us an interesting conclusion concerning the investigated process. Since the right-hand side of (10) depends explicitly on x, in the real equation the same phenomenon must occur. It means that the layers in the column do not act independently, i.e. the adhesion process is not a direct sum of the processes in each layer. We observe a more complex picture, namely the action of every layer depends on its place in the column. This dependence distinguishes the adhesion process of cells in the glass bead column from the adhesion process occurring in geology (so-called colmatage process). In the colmatage process the column acts as a direct sum of layers, i.e. the differential equation describing the process does not depend explicitly on x ([6], [7]).

The present state of knowledge of adhesion does not allow us to draw any further conclusions. Perhaps the obtained equations could suggest some directions of experiments, but this will be the matter of future.

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MATEMATYCZNY OPIS ADHEZJI LIMFOCYTÓW, PRZEPŁYWAJĄCYCH PRZEZ KOLUMNĘ KULEK SZKLANYCH

STRESZCZENIE

Celem pracy jest znalezienie równania różniczkowego, opisującego proces adhezji limfocytów do szkła w zawiesinie przepływającej przez kolumnę kulek szklanych. Punktem wyjścia są eksperymentalnie znalezione zależności stężenia zawiesiny od czasu, mierzone po wyjściu zawiesiny z kolumny. Znalezione równanie pozwala na wyciągnięcie wniosku, że proces adhezji w dowolnej warstwie kolumny i w dowolnej chwili nie jest tylko funkcją stężenia zawiesiny i stanu nasycenia warstwy, lecz zależy explicite od położenia tej warstwy w kolumnie.
