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Emission of Microvesicles and Erythrocytic Sphericitation

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Abstract. We have examined mathematically the "cause-effect" relationship between the phenomenon of emission of microvesicles and that of erythrocytic sphericitation, especially when the age of the cell is the main factor involved.

Key words: Microvesicle — Senescence — Sphericitation — Erythrocyte — Sphericity index

Introduction

The ageing human erythrocyte (RC) has a tendency to lose membrane material together with peripheral intracellular contents in the form of spherical microvesicles having an average diameter of 10^2 nanometers. During senescence, the sphericitation of the erythrocyte is a marked tendency, and it manifests itself with an intensity directly proportional to the erythrocyte tendency to "spherulize" (i.e. to emit microvesicles as mentioned above) (Bocci et al. 1979; Pessina et al. 1978).

The "spherulization" process can thus be interpreted as a concause or stimulus to the sphericitation. We have not been in this case interested in examining the complex mechanism underlying the start and stabilization of the "spherulization" phenomenon. Rather, our objective has been that of studying in analytico-numerical terms the way in which "spherulization" bears upon "sphericitation" of the erythrocyte. The latter process is directly associated with "spherulization" but is not explainable only in those terms, but rather by a multiplicity of factors (mechanical, thermic and biochemical) the list of which is, in this case, useless to compile. The emission of microvesicles can be conceived as a temporal sequence of elementary events (production of a single spherule or microvesicle) governed by a statistical law on the nature of which, at the present moment, plausible hypotheses do not seem possible.

The diameter of a single spherule produced is a dependent variable, nonetheless subject to a precise physical tie (as we will demonstrate later on) which is dependent on the RC's geometry previous to emission. This tie is determinable with a sufficient accuracy if, at each time, the volume (V) and surface area (S) of the RC which the spherule originates from can be measured and controlled.

Analytical Procedure

With regard to the length unit, we decided to use micrometers ($\mu m = 10^{-6}$ m) bringing literature's metric data back to this unit when they were expressed differently (nanometers $\longrightarrow$ micrometers). Therefore:

$$V = \dots \mu m^3; \qquad S = \dots \mu m^2; \quad \text{etc.}$$

The radius (r) of a generic emitted spherule is given by $x (r \equiv x)$. As the estimator of the adaptation to the spherical morphology we have used the dimensionless quantity called "normalized sphericity index (J)".

$$J \equiv \mathrm{N} \frac{V^{2/3}}{S}$$

where N = "normalizing factor" $= \sqrt[3]{36\pi}$

Because of the presence of N:
$0 < J \leqslant 1$ for every geometrical figure with $V > 0$
that is
$0 \leqslant J \leqslant 1$ for every geometrical figure without any sort of limitation,
and
$J = 1$ only for perfectly spherical bodies.

Let us denote $J(x)$ or $y(x)$ the normalized sphericity index of an RC after emission of a microvesicle (presumed to be approximately spherical) with a radius, $x (x \geqslant 0)$.

$$J(x = 0) \equiv J(O) \equiv J_0$$

is, obviously, the normalized sphericity index of the RC previous to its spherulization. By analogy, V_0 indicates the erythrocytic volume when $x = 0$, and S_0 indicates the surface area of the RC when $x = 0$. V_0 and S_0 are known or assumed as such.

Since the emission of a single microvesicle probably proceeds with a mechanism involving evagination of the erythrocytic membrane (which is extraordinarily flexible "in toto" and "in loco" but, to all intents and purposes, an inextendable thin shell), not only does the relationship:

$$V(x) = V_0 - \frac{4}{3}\pi x^3$$

assume physical significance, but also (even if less evidently) the following:

$$S(x) = S_0 - 4\pi x^2$$

Therefore, on the basis of this, we can define $J(x)$:

$$J(x) \equiv y(x) = \mathrm{N}\,\frac{\left(V_0 - \frac{4}{3}\pi x^3\right)^{2/3}}{S_0 - 4\pi x^2} \qquad (\mathrm{N} = \sqrt[3]{36\pi})$$

We know, "a priori", that $x \geqslant 0$. For the moment, let us ignore such a physical limitation (which will not be the only one, as we will see further on) for a complete study, on the sole mathematical level, of the curve corresponding to $y(x)$ on the whole OXY cartesian plane. Having calculated the first, second and third derivatives of $y(x)$ with respect to x, we find the following general pattern (schematic representation, Fig. 1)

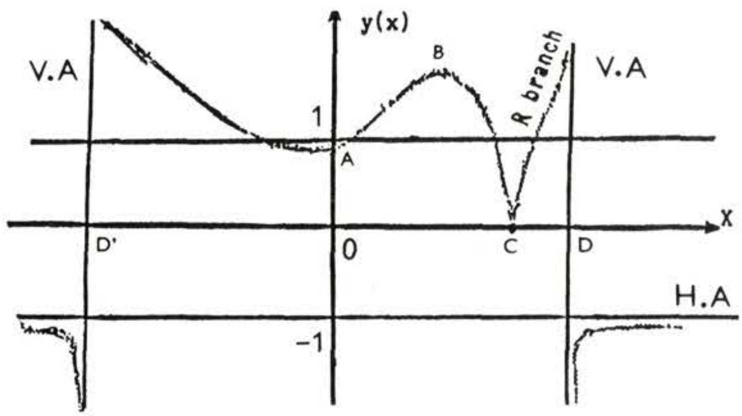

Fig. 1. Graph of the function $J(x)$. H.A. — horizontal asymptote, V.A. — vertical asymptote

Notable points on the curve are those labeled A, B, C, D, and D′. The respective coordinates are:

$\mathrm{A} \equiv \mathrm{A}(x = 0,\ y = J_0)$: sole intersection of the curve with the y-axis
: point of relative minimum

$\mathrm{B} \equiv \mathrm{B}\left(x = 3\frac{V_0}{S_0},\ y = \frac{J_0}{\sqrt[3]{1 - J_0^3}}\right)$: point of relative maximum

$\mathrm{C} \equiv \mathrm{C}\left(x = \sqrt[3]{\frac{3}{4}\frac{V_0}{\pi}},\ y = 0\right)$: sole intersection of the curve with the x-axis
: sharp-cornered point with coincident vertical tangents

$\mathrm{D} \equiv \mathrm{D}\left(x = \sqrt[2]{\frac{1}{4}\frac{S_0}{\pi}},\ y = 0\right)$

: positioning of the two vertical asymptotes

$$D' \equiv D\left(x = -\sqrt[2]{\frac{1}{4}\frac{S_0}{\pi}},\ y = 0\right)$$

There is an interesting relationship between J_0 and the ordinate of the maximum-point, which we will call y_B. In fact:

$$\left[J_0 > J_{crit} = \sqrt[3]{\frac{1}{2}}\right] \quad \text{involves } [y_B > 1]$$

Since $J_{crit} \cong 0.7937$, it is evident that in Fig. 1 (general pattern of the curve corresponding to $y(x)$) we considered a $J_0 > 0.7937\ldots$ Finally, let us note that a following implication exists:

$$[J_0(<1) \quad \rightarrow \quad 1] \Rightarrow \left[\overline{CD} \quad \rightarrow \quad 0 \qquad \begin{array}{c}\text{i.e. obsolescence of the}\\ \text{"R" branch}\end{array}\right]$$

Up to now our analysis has been of a purely mathematical nature. Let us then introduce the physical limitations, pertaining to the phenomenon under examination. These are essentially:

$$\begin{cases} x \geqslant 0 \\ V_0 - \frac{4}{3}\pi x^3 > 0 \\ S_0 - 4\pi x^2 > 0 \end{cases}$$

The physical domain of $y(x)$ is therefore defined as

$$\begin{cases} 1^{st}\text{ case: } (0 < J_0 \leqslant J_{crit}) \text{ implies } [0, x_B] \\ 2^{nd}\text{ case: } (J_{crit} < J_0 \leqslant 1) \text{ implies } [0, x_S] \end{cases}$$

x_S is that value of x for which, simultaneously:

$$\begin{array}{ll} (^*) & \begin{cases} x_S < x_B \\ y(x = x_S) = 1 \end{cases} \\ (^{**}) & \end{array}$$

The condition (**) is met by determining x_S. The subscript "S" indicates that "x" refers to the superior extreme of the physical definition range of $y(x)$. The determination of x_S was not obtained by way of a mathematical analysis but rather by using a graphical method. We adopted the graphical method since the mathematical one involved an algebrical equation of the fourth degree, making it rather impracticable. Iterative techniques were also used.

Similar considerations are also valid for investigation of inflexion points (or rather of the only inflexion point in the physical domain). Since and "old" RC is closer to the spherical shape than a "young" or "mature" one, we can accept for it the following starting data:

"old" RC : Volume $\cong 116\ \mu m^3$ (against $V = 94\,\mu m^3$ for a "mature" RC)

: Surface area $\cong 135\ \mu m^2$

Therefore,

$$J_0 = \sqrt[3]{36\pi}\frac{\sqrt[3]{116^2}}{135} \cong 0.85203\ldots > J_{crit} \cong 0.7937$$

Thus, only the 2[nd] case will have both a biological and a physical meaning. With simple calculations we obtain:

$$x_B = 3\frac{V_0}{S_0} = 2.5(7)\ \mu m$$

$$(x_C = 3.0254487\ \mu m, \qquad x_D \cong 3.277645\ \mu m)$$

$$Y_B = \frac{J_0}{\sqrt[3]{1 - J_0^3}} \cong 1.1748$$

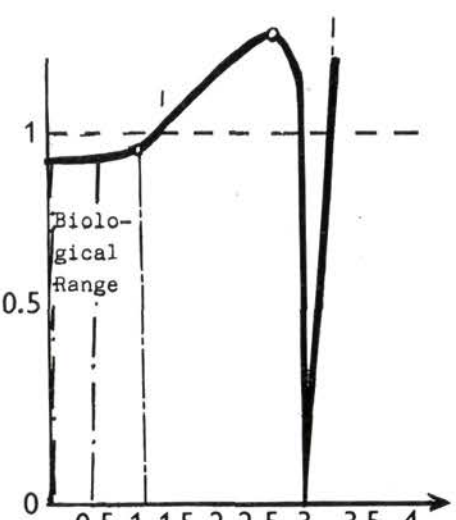

Fig. 2. Magnification of the graph of $J(x)$ function with regard to a portion of its domain.

$$y(x=1)=\sqrt[3]{36\pi}\,\frac{\sqrt[3]{\left(116-\frac{4}{3}\pi\right)^2}}{(135-4\pi)}\cong(4.83597586)\frac{23.209025}{122.433629}\cong 0.9167$$

Discussion and Conclusion

It is evident that, whatever the value of x in the range $[0, x_S]$ may be, the normalized sphericity index that pertains to the RC after the emission of a microvesicle cannot but be greater than the previous one and will approach the unit value proportionally to x getting closer to x_S.

The sphericitation of RC becomes a forced final stage, reached after a period of time (Δt) which is in relation to the law of distribution of the random variable x. Little is known about this law except that it depends on x_S. The value of the initial X_S is imposed by the original (V_0, S_0) couple.

Since $\lim\limits_{J\to 1} x_S=0$, not only is the inexorable evolution of the process toward the complete sphericitation of the residual RC evident, but also the impossibility for the RC [with normalized sphericity index between $J_0(>J_{crit})$ and 1] to emit a spherule with a radius greater than the corresponding value of x_S which, in the sequence of spherulations, unavoidably tends to zero. In other terms, a RC with $J<1$ and x_S 1.3 μm cannot, for example, generate a microvesicle with a volume of 9.5 μm^3, that is a spherule with a volume of 9% ($\div$ 12%) that of the "*ab origine*" erythrocytic volume. The final passage of the residual RC to the completely spherical shape halts the spherulation process (high inextensibility and decreased flexibility of the membrane).

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