ANALYTICAL DETERMINATION OF THE SURFACE AREA OF ERYTHROCYTES

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The diffusion, osmosis, and metabolism of the erythrocyte take place through its surface, the area of which determines the intensity of adsorption of various substances by the erythrocyte, notably autologous and foreign plasma proteins. The surface area of the erythrocytes is therefore an important hematologic index. Naturally, methods of determining this index have attracted the attention of workers in different branches of biology.

Experimental determination of the surface area of the erythrocyte (S) is difficult because of its complex shape, and various approximate formulas have therefore been suggested on the basis of simplified models of the erythrocyte [3-7]. The most accurate method of determining S is that proposed by A. L. Chizhevskii [1].

Regarding the erythrocyte as the figure formed by rotation of the erythrocyte cross section, Chizhevskii determined the area of this cross section by using Goulden's theorem, according to which

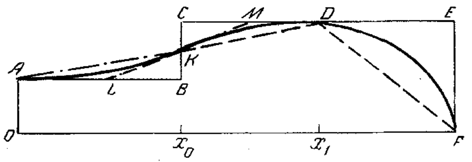
$$S = 2\pi l X_0,$$

where l is the length of the curve and X_0 the distance of the axis of rotation from the center of gravity of the curve. From the results of his calculations Chizhevskii concluded that for a normocyte with diameter $d = 7.5\ \mu$, height of torus $H = 1.8\ \mu$, and height along the central axis $h = 0.9\ \mu$, $S = 107\ \mu^2$.

At the present time Goulden's theorem provides the most accurate method of determining S, but Chizhevskii's method has certain disadvantages. The most important of these is that the equation which he deduced is a special case for fixed parameters of the erythrocyte. However, these parameters vary in the erythrocyte, so that for each set of parameters (H, h, d) the equation for the surface area of the cell must be obtained and approximately integrated. Further, the equation does not enable the error arising during calculation of the surface to be determined.

The authors propose a method of determining the surface area of the erythrocyte which is free from these disadvantages. It can be used to determine S for different sets of parameters without laborious calculations, and also to determine the possible error on the basis of a theorem enunciated by the authors.

Let us start from the well known fact that the erythrocyte has a biscuit-shaped cross section, symmetrical relative to a given straight line, and its surface can be regarded as the surface formed by rotation of this cross section around an axis perpendicular to the given straight line.



Cross section of erythrocyte with enveloping and enveloped broken lines. ABK and KCEF) segments of the enveloping broken line; FDK and AK) segments of the enveloped broken line; K) point of inflection.

One quarter of this cross section is shown in the figure. Let us note certain characteristic points on this section, having the axis ox along the line of symmetry. Let us place the origin in the center of the cross section. $A(o, \frac{h}{2})$ lies on the axis of rotation, $K(X_0, Y_0)$ is the point of inflection, $D(x_1, \frac{H}{2})$ is the maximum of the erythrocyte section, anf $F(\frac{d}{2}, o)$ is the point farthest from the axis of rotation. In this case $h < H$. There are two lemmas. Lemma 1. During rotation of two convex broken lines, with common end points, about a given axis, areas are formed of which the larger corresponds to the enveloping broken line. Proof of this is elementary. Lemma 2.

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TABLE 1. S for $H = \frac{d}{4}$ and $h = \frac{d}{8}$ and Calculated by Formula (4) and by A. L. Chizhevskii

d (in μ)	S (in μ^2)	Δ	After Chizhevskii (in μ^2)
6,0	71,24	0,34	70,37
6,5	83,50	0,42	84,37
7,0	96,82	0,47	95,70
7,5	111,60	0,53	109,46
8,0	126,46	0,61	123,05

TABLE 2. S_{min} and S_{max} of Erythrocytes with Certain Diameters

d (in μ)	S_{min} (in μ^2)	Δ	S_{max} (in μ^2)	Δ
6,0	70,70	0,12	85,03	0,39
6,5	82,96	0,14	99,80	0,47
7,0	95,21	0,16	115,74	0,54
7,5	110,45	0,18	132,86	0,62
8,0	126,64	0,21	156,20	0,71

During rotation of a convex curve and the broken line enveloping it, having common end points, about a given axis areas are formed, of which the larger is that corresponding to the broken line. Proof rests on lemma 1. A broken line is inscribed inside a convex curve, and the proof is completed by passage to the limit.

If enveloping and enveloped broken lines are now constructed around the erythrocyte profile (the broken line ABCEF in the figure is enveloping, and the line AKDF is enveloped), the theorem can be formulated.

The surface of the erythrocyte is contained between two surfaces formed by rotation of the enveloping and enveloped broken lines.

As a first, rough approximation, to obtain the half-surface the broken lines l_1 = ABCEF and l_2 = AKDF may be taken, such that

$$S_{l_1} < S < S_{l_2}. \quad (1)$$

For a more accurate calculation, the number of broken lines in individual parts of the curve must be increased, thereby approximating the left and right bounds of the inequality (1). The arithmetical mean of these bounds must be taken as the approximate value of the surface area, in which case the upper limit of the absolute error will be equal to the half-difference between these bounds.

To simplify the calculations and obtain an acceptable result, let us assume that arc DF is the outline of an ellipse with semiaxes $(\frac{d}{2} = x_1;\ \frac{H}{2})$. In that case the upper limit of the half-surface of the erythrocyte will be the surface formed by rotation of the broken line ALKMD and the arc DF, while the lower limit will be the surface formed by rotation of the broken line AKD and the arc DF.

We can write:

$$\pi\left\{X_0^2 + X_0X_1 + \frac{X_1^2}{2} + 3X_0\sqrt{a^2 + \left(\frac{X_0}{2}\right)^2} + (3X_0 + X_1)\sqrt{b^2 + \left(\frac{X_1 - X_0}{2}\right)^2}\right\} + S_e < S < \pi\left[2X_0\sqrt{X_0^2 + a^2} + 2(X_0 + X_1)\sqrt{b^2 + (X_1 - X_0)^2}\right] + S_e, \quad (2)$$

where $a = y_0 - \frac{h}{2}$, $b = \frac{H}{2} - y_0$,

$$S_e = 4\pi\int_0^{\frac{H}{2}} (x_1 + x)\sqrt{1 + (x^1y)^2}\,dy.$$

The value of x is determined from the equation of an ellipse

$$\frac{4x^2}{(d - 2x_1)^2} + \frac{4y^2}{H^2} = 1. \quad (3)$$

In the overwhelming majority of cases $H \geq d = 2X_1$ and the integral included in formula (3) can be calculated accurately.

When $H > d = 2X_1$

$$S_e = 2\pi x_1 HE\left[1-\left(\frac{d-2x_1}{H}\right)^2\right]+\frac{\pi^2(d-2x_1)}{4\sqrt{H^2-(d-2x_1)^2}},$$

where $E(\alpha^2)=\int\limits_0^{\frac{\pi}{2}}\sqrt{1-\alpha^2\operatorname{Sin}^2 t}\,dt$ the elliptic integral [2].

When $H = d = 2X_1$

$$S_e = \pi^2 X_1 + \pi(d-2X_1).$$

If $H < d = 2X_1$, the value of S_e can be calculated approximately.

In the special case examined by A. L. Chizhevskii, when $H = 2h = \frac{d}{4}$, we find the coordinates of the point of inflection $K - X_0 = 0.2d$; $y_0 = \frac{H+h}{4}$, and the abscissa of the maximum $X_1 = \frac{d}{3}$. Then, according to formula (2),

$$\pi d\left[\frac{\sqrt{16d^2+25(H-h)^2}}{50}+\frac{4\sqrt{64d^2+225(H-h)^2}}{225}+\frac{d}{18}+\frac{2}{9}dE(\varepsilon^2)+\frac{H^2}{4d}\ln\frac{1+\varepsilon}{1-\varepsilon}\right]<S<$$
$$\pi d\left[\frac{3\sqrt{4d^2+25(H-h)^2}}{100}+\frac{7\sqrt{16d^2+225(H-h)^2}}{450}+\frac{7d}{45}+\frac{2}{9}dE(\varepsilon^2)+\frac{H^2}{4d}\ln\frac{1+\varepsilon}{1-\varepsilon}\right], \quad (4)$$

where $\varepsilon=\frac{\sqrt{d^2-9H^2}}{d}$ represents the eccentricity of an ellipse with axes $(\frac{d}{3}, H)$. Calculations show that in this case the relative error does not exceed 0.6%. Data obtained by formula (4) and by A. L. Chizhevskii [1] are given in Table 1.

Not only the diameter of erythrocytes may vary, but also the values of H and h. The values of S given in Table 1 were calculated for the minimal values: $H_{min} = 1.87\ \mu$ and $h_{min} = 0.9\ \mu$ when $d = 7.5\ \mu$. The variability of S in normal conditions is an interesting question. $S = S_{max}$ at H_{max} and h_{min}, $S = S_{min}$ at H_{min} and h_{max}, and S_{max} were determined for values $H = \frac{d}{3}$ and $h = \frac{3d}{25}$. In this case $\varepsilon = 0$ and $E(\varepsilon^2) = 1$. Substituting these values in formula (4) we obtain: $0.742\pi d^2 < S_{max} < 0.762\pi d^2$; $S_{max} = 0.752\pi d^2 = 2.36d^2$, and $\Delta < 0.011\pi d^2$. S_{min} was determined at $H = \frac{d}{4}$ and $h = \frac{d}{6}$. In this case $E^2 = 0.641$ and $E(\varepsilon^2) = 1.3957$. Substituting these values in formula (4) we obtain $0.624\pi d^2 < S_{min} < 0.625\pi d^2$; $S_{min} = 0.625\pi d^2 = 1.96d^2$, and $\Delta < 0.001\pi d^2$.

Values of S_{max} and S_{min} for certain diameters of erythrocytes are given in Table 2. The results of the calculations show that the surface area of erythrocytes in normal conditions forms a scatter amounting ±10% about the arithmetical mean value. The surface area of erythrocytes as determined by A. L. Chizhevskii is close to S_{min} and approximately 10% below the mean.

From the inequality (2) deduced above the surface area of erythrocytes can also be calculated if they are of pathological size.

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