Measurement of Mean Red-Cell Thickness by the Use of a Probability Function

When attempting to find the mean thickness of any kind of circular and biconcave red cell, one has either to be content with the mean corpuscular thickness, obtained by treating the cell as a cylinder and dividing the mean cell volume by πr^2, or one has to measure the dimensions of red cells on edge[1], thus obtaining the greatest thickness, the least thickness, and so on. The former method does not take the shape of the cell into consideration, and the latter method is laborious. This communication describes a new method, intermediate in accuracy but sufficiently reliable for many purposes. It depends on the fact that the number of red cells seen on edge, in a preparation in which the cells are randomly oriented, depends on the mean dimensions of the cells, including the diameter. Virtually no cell will be seen exactly on edge; but a number will present themselves in the direction of observation in such a way that their observed thickness will be less than a selected value w—where w is a little larger than t, the true mean thickness. The probability f of such cells being seen in the preparation is

$$f = \frac{w - t}{d - t} \qquad (1)$$

an expression for which we are indebted to Dr. R. T. Cox (the proof of this expression is a little too long to be included here; but we shall be glad to communicate it to anyone who is interested), and which treats the cross-section of the red cell as a rectangle with a semicircle at each end.

A preparation of red cells in plasma is made between slide and coverslip, and is examined under conditions of reasonable high resolution ($\times$ 20 objective, N.A. 0·5, $\times$ 5 eyepiece, and Köhler illumination). The preparation is stirred up by pressing on the coverslip so that the red cells move in the currents thus produced, and several fields are photographed (Leica 111 f camera with Adox *KB*-17 film) at an exposure of 0·001 sec., provided by a Strobonar IV electronic flash. After development, the fields are projected at a magnification of about $\times$ 800, and a cell is considered to be on edge if its image falls within two lines separated by a distance w and drawn on a glass plate; it is convenient to choose two values for w, one about 1·5 times and the other about twice the estimated cell thickness. The values chosen for w are not particularly important, although they must be known exactly in μ. The number of red cells on edge and the total number of cells in the fields examined give f, from which t, the mean cell thickness, can be calculated for each of the two values of w. Virtually the same value of t is usually obtained from the two values of w.

This method has given excellent results. For the red cells of man $t = 2{\cdot}07 \pm 0{\cdot}1\mu$, which is in good agreement with the value found by photography of human red cells on edge[1]. For rabbit red cells, $t = 2{\cdot}1 \pm 0{\cdot}1\mu$, which again agrees with direct observations if one allows for the limitations which the latter suffer because of diffraction phenomena at the cell edge. For the mouse red cell, $t = 2{\cdot}05 \pm 0{\cdot}05\mu$.

A corollary to expression (1) is that if no red cell or ghost presents itself on edge, the cells or ghosts are necessarily spherical. Their volume is then $4\pi r^3/3$, where r is their radius, and their surface area is $4\pi r^2$.

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[1] Ponder, E., *Quart. J. Exp. Physiol.*, **20**, 29 (1930).

Electrical Properties of the Plasma Membrane of Erythrocytes at Low Frequencies

Earlier investigations of the dielectric properties of biological interfaces dealt with the plasma membrane of erythrocytes and obtained from its capacitance a value of its thickness near $10\varepsilon_M$A., where ε_M is dielectric constant of membrane[1]. Other contributions considered the possibility of a frequency dependence of cellular membrane impedance which can be expressed by a power law[2]. Both frequency dependence of membrane and complexity arising from variability in cell size and shape may affect similarly dielectric properties of cellular suspensions in the R_F-range.

Thus, measurements of biological interfaces at lower frequencies than previously investigated are indicated to differentiate power law behaviour of interface from form and size variation effects.

This study was undertaken to obtain data of resistivity and capacitance of erythrocytes at low frequencies. Accurate impedance measurements on red cell suspensions were obtained in a frequency-range of 10–200,000 c./s. Heparinized fresh beef blood was washed with Ringer tyrode, pH 7·6, and centrifuged to 80–90 per cent volume concentration of erythrocytes. The packing was done to reduce artefacts due to electrode polarization[3]. This concentrated suspension was placed in a cylindrical electrolytic cell, the impedance of which was measured in a precision bridge[4]. Temperature was stabilized to 25° C. $\pm$ 0·01° C. Effects of distributed capacitances and leakage conductances were eliminated by a substitution technique. The measurements of impedance as function of frequency (f) were made with high accuracy, resistance to $\pm$ 0·0005 per cent except at 10 c./s. and capacity to $(0{\cdot}5/f_{\text{kc.}} \pm 0{\cdot}5)$ per cent. Changes due to small temperature variations and sedimentation were eliminated by alternating experimental measurements with control readings at 1 kc./s. The small drift of the control readings with time was found independent of frequency and used for determining corrections for the experimental data at other frequencies.

Electrode polarization impedance, which appears in series with the sample impedance, was nearly independent of sample volume. Hence, the difference between measurements at two electrode spacings could be taken to be proportional to the impedance of the differential blood volume[3,5]. Accuracy of these difference measurements was kept high by minimizing

Table 1

kc./s.	$\frac{\varepsilon}{\varepsilon(1\text{ kc./s.})}$	kc./s.	$\frac{\varepsilon}{\varepsilon(1\text{ kc./s.})}$
0·05	1·0	5	0·991
0·1	1·0	10	0·987
0·2	1·00	20	0·983
0·5	1·00	50	0·978
1	1·00	100	0·973
2	0·996	200	0·961