OSMOTIC CORRECTION TO ELASTIC AREA COMPRESSIBILITY MEASUREMENTS ON RED CELL MEMBRANE

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ABSTRACT In a recent article (*Biophys. J.* **16**:585, 1976), we reported measurements of the elastic area compressibility modulus of red cell membranes using micropipette aspiration on osmotically preswollen red cells. Subsequently, we have analyzed the effects of osmotic and hydrostatic pressure driving forces across the cell membrane in conjunction with the mass conservation equation; we find that the change in cell volume due to the reversible movement of water out of the cell can produce one-third of the movement of the cell projection in the pipette tip. Since the actual volume change is too small to measure directly (about 1% of the total cell volume), we have used an indirect experimental method to provide critical evaluation of the analysis of cell volume change versus applied pressure; this is based on the model that the change in cell volume is inversely proportional to the cellular osmotic strength. We have increased the cellular cation concentration with a drug, nystatin, and measured the elastic area compressibility modulus corrected for osmotic volume changes as a function of cellular osmotic strength. We find that the corrected elastic area compressibility modulus is independent of cellular osmotic strength, which supports the model and calculated correction for the osmotic effect. The elastic area compressibility modulus is 450 dyn/cm at 25°C instead of 300 dyn/cm, determined previously.

INTRODUCTION

Rand (1964) originally applied micropipette aspiration to osmotically swollen red blood cells to produce lysis; no deformation of the cell was observed when the suction pressure was applied. Recently, we used the same technique (Evans et al. 1976) on osmotically swollen red cells. With a combined video-microscope system, we were able to detect small reversible displacements of the cell projection in the pipette in response to the applied pressure (see Fig. 1). This reversible movement of the cell projection in the pipette is the result of a change in cell area plus a change in cell volume.[1] Because of the small applied pressures, 0.1 atmosphere, the original analysis assumed that the change in cell volume was negligible and that the movement of the edge of the cell in the pipette was the result of only changes in membrane area. Subsequently, we have

[1]The reversible movements of the cell projection associated with volume and surface area changes occur in less than a second; after the pressure is applied, the cell volume and surface area are constant for periods of time of the order of 1 min or longer. Periods of time longer than 1 min produce irreversible volume changes shown to depend on how well the tonicities inside and outside the pipette are matched.

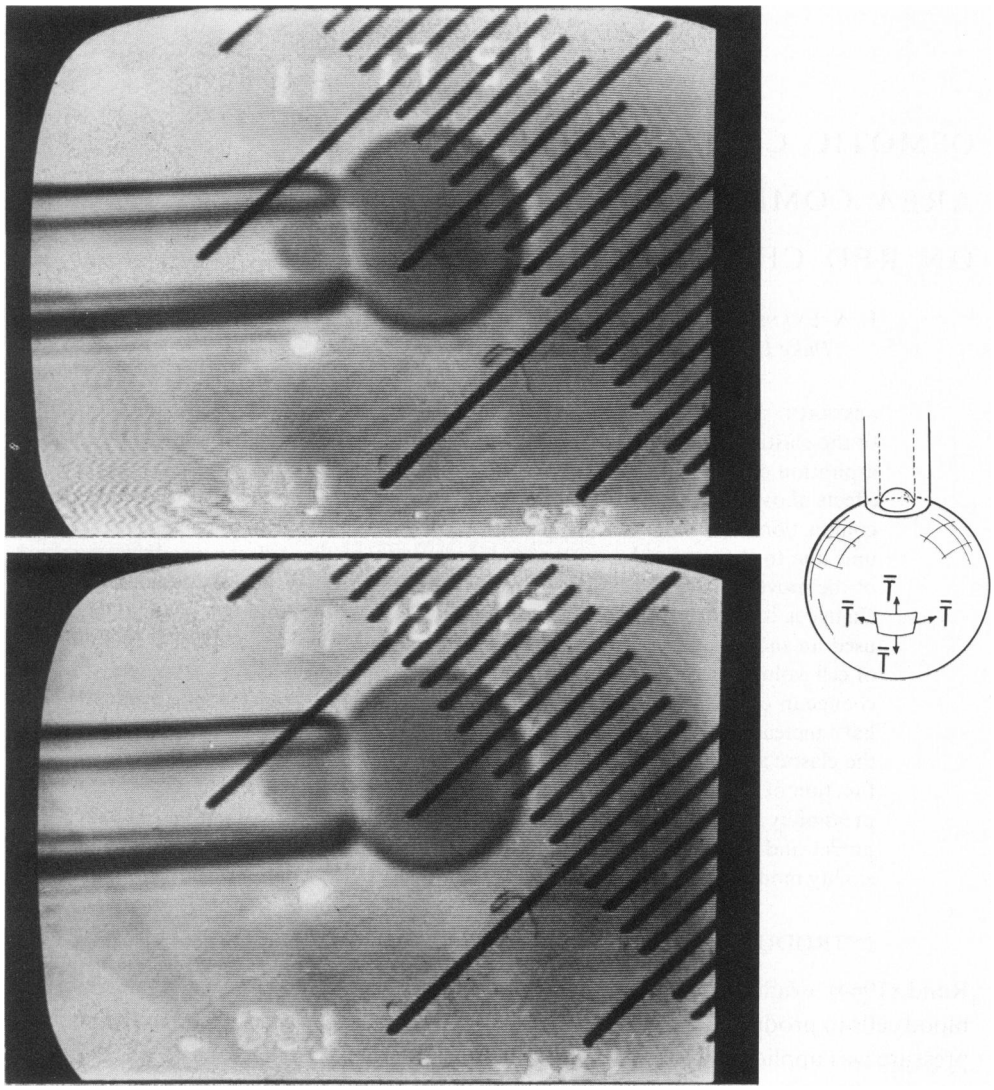

FIGURE 1 The figure on the right is a schematic of the micropipette aspiration of an osmotically swollen red blood cell; the isotropic membrane tension is illustrated for an element of the membrane. On the left, the video record is shown of a red cell, subjected to a suction pressure of about 4 mm Hg in the upper photograph, followed by a pressure of about 60 mm Hg in the lower photograph (the micrometer scale in the picture is 10 div = 9 μm); the response of the cell projection to applied pressure can be seen by comparing the two photographs.

analyzed the effects of osmotic and hydrostatic pressure driving forces across the cell membrane. In conjunction with the mass conservation equation, we found that the change in cell volume due to the reversible movement of water out of the cell could represent about one-third of the movement of the cell projection in the pipette tip. Because the magnitude of the calculated volume effect is large and since the actual vol-

ume change is too small to measure directly (about 1% of the total cell volume), we have developed an experimental method that critically evaluates the analysis of cell volume change versus applied pressure. Therefore, the reversible change in red cell membrane surface area can be determined from the movement of the cell projection in the pipette, exclusive of cell volume change.

THEORETICAL ANALYSIS

Details of the micropipette experiment are documented in Evans et al. (1976). The procedure is illustrated in Fig. 1. The micropipette suction of osmotically preswollen red cells produces isotropic tension in the membrane surface. The relationship between membrane isotropic tension and the pipette suction pressure is given by Rand (1964) and Evans et al. (1976). The reversible change in cell projection length, ΔL, is observed to be linearly related to the pipette suction pressure, ΔP, and to the calculated membrane isotropic tension, $\overline{T}$. Since the cell deformation and the associated area change produced by the tension are reversible, an elastic constitutive relation can be written between the isotropic tension and the fractional change in area, $\Delta\alpha = \Delta A/A_0$.

$$\overline{T} = K \cdot \Delta\alpha, \tag{1}$$

where ΔA and A_0 are the change in cell membrane area and total membrane area, respectively. The elastic modulus for area compressibility, K, is the material property to be determined from the aspiration experiment. The change in membrane area, ΔA, can be derived from the movement of the cell projection in the pipette if the change in cell volume, ΔV, is known. Since the changes are small, the area change can be approximated by the first-order term in a Taylor series expansion,

$$\Delta A \cong \pi[D_p \cdot \Delta L \cdot (1 - D_p/D_{c_o}) + 4\Delta V/\pi D_{c_o}], \tag{2}$$

where D_p is the pipette diameter and D_{c_o} is the initial diameter of the spherical portion of the cell outside the pipette. If the volume is assumed to stay constant, then the isotropic tension can be plotted directly against the fractional change in area as shown in the *lower* curve in Fig. 2 (this data is from a single red cell experiment where the cell is preswollen at a tonicity of 155 mosmol); the area compressibility modulus is calculated from the slope, $\Delta\overline{T}/\Delta\alpha$. On the other hand, if the volume changes with applied suction pressure, then the area change must be corrected for the change in volume as in Eq. 2. Since the volume change is too small to measure directly, we will model the osmotic equilibrium for the cell using mass transport equations to calculate the volume change. The critical assumptions in the analysis will be: (*a*) the permeability of water is uniform and constant over the cell surface; (*b*) negligible transport of water occurs where the membrane is adjacent to the pipette wall (i.e. the cylindrical section of the cell projection in the pipette); (*c*) the flux of water out of the cell is given by the linear transport equation,

$$\dot{m}_w = k(\Delta P_w + \Delta\Pi_w), \tag{3}$$

where k is the filtration coefficient for water flow per unit area, ΔP_w is the hydrostatic pressure difference across the membrane (inside minus outside), and $\Delta \Pi_w$ is the osmotic pressure difference (outside minus inside); (*d*) the osmotic pressure is given by the van't Hoff equation,

$$\Delta \Pi_w = \beta(C_o - C_i), \tag{4}$$

where β is the gas constant times absolute temperature, C_o and C_i are the molar concentrations of solute species outside and inside the cell, respectively; (*e*) concentration gradients within the cell are assumed negligible. Because the pressure difference across the membrane hemispherical cap inside the pipette is greater than the pressure difference across the membrane of the outside cell portion, there must be filtration of water out of the cell projection into the pipette and an equal absorption of water into the cell from the outside medium if the cell volume is to remain constant. The constant volume equilibrium is determined by

$$0 = k(\Delta P_w + \Delta \Pi_w)_p \cdot A_p + k(\Delta P_w + \Delta \Pi_w)_c \cdot A_c, \tag{5}$$

where the first term is the filtration of water out of the cell projection into the pipette and the second term is the absorption of water from the outside medium into the cell. A_p and A_c are the area of the hemispherical cap in pipette and the area of the spherical outside portion, respectively. If we assume that the ionic strengths of the media inside and outside the pipette are equal, then the osmotic pressure difference will be the same in both terms of Eq. 5; therefore, using the relationship between hydrostatic pressure difference and the isotropic membrane tension, we obtain

$$\Delta \Pi_w = -4\bar{T} \cdot \left(\frac{A_p/D_p + A_c/D_c}{A_p + A_c} \right). \tag{6}$$

The resulting change in solute strength inside the cell is given by the van't Hoff relation Eq. 4. The fractional change in cell volume is ideally related to the fractional change in solute strength inside the cell by,

$$\Delta V/V_0 = -R_w \cdot (\Delta C/C_i). \tag{7}$$

The coefficient, R_w, is the fraction of total cell volume that is water. Ponder (1971) has used R_w to represent the water that is osmotically "active," as determined from experiment. For osmotically preswollen cells, the volume percent water is about 80–85%; however, the measured R_w coefficient is about 0.6 instead of 0.8 (Evans and Fung, 1971).

By combining Eqs. 4, 6, and 7, the fractional change in volume can be written as a function of membrane isotropic tension,

$$\frac{\Delta V}{V_0} = -\frac{4R_w\bar{T}}{\beta C_i} \left(\frac{A_p/D_p + A_c/D_c}{A_p + A_c} \right) \tag{8}$$

The isotropic tension is measured in units of dynes per centimeter; all dimensions

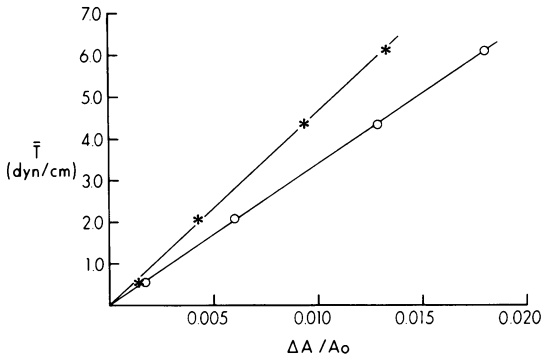

FIGURE 2

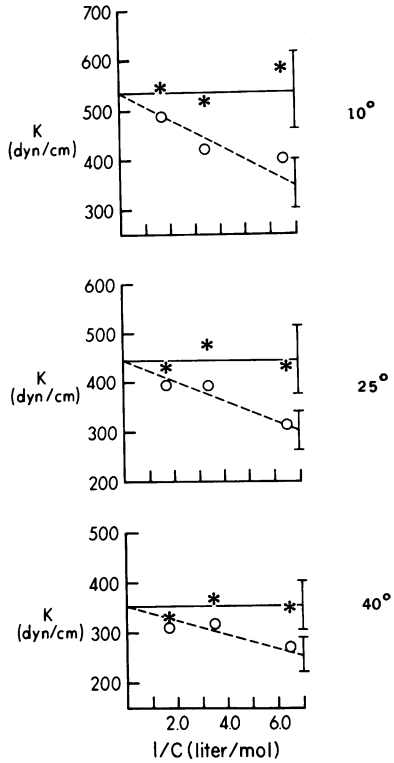

FIGURE 3

FIGURE 2 The isotropic tension is plotted against the fractional change in area for a single red cell experiment; the isotropic tension is determined by the pipette suction pressure; the fractional change in area is calculated using Eq. 2 for both $\Delta V \equiv 0$ (the uncorrected lower curve) and the osmotic volume correction of Eq. 8, which gives the corrected upper curve. This red cell was preswollen at a tonicity of 155 mosmol.

FIGURE 3 The elastic area compressibility moduli of nystatin-treated cells are plotted versus one over the osmolarity in which the cells were swollen (in liters per mole); the data points are the average moduli for about 20 cells, calculated with and without the osmotic volume correction. Experiments are shown for three different temperatures. The solid line and dashed line in each plot represent the predicted behavior of the area compressibility modulus with and without the osmotic volume correction, respectively; these lines are calculated using the corrected and uncorrected average moduli obtained from measurements of several hundred untreated red cells.

are in centimeters; the concentration of solutes is in moles per liter; and the coefficient, β, ranges between 2.27–2.60 $\times$ 10^7 dyn-liter/mol $\cdot$ cm^2 for a temperature range of 0°–40°C. By using Eq. 8 in Eq. 2 for the change in area, the isotropic tension is plotted versus the fractional change in area exclusive of osmotic volume change artifact in the *upper* curve in Fig. 2. The magnitude of the osmotic volume correction is significant; consequently, we must pose the question: how can we experimentally verify the model used for the osmotic volume change? *Or* how can we avoid the problem of water movement in the micropipette experiment?

EXPERIMENT

It is apparent from Eq. 8 that the volume change can be reduced by increasing the osmotic concentration, C_i, at which the cell is swollen. Increasing the solute concentration not only reduces the volume correction, but it also provides an experimental test of the model used to calculate the volume correction. Because the membrane has such low permeability to cations, it is not simple to increase the cellular cation concentration. Fortunately, there is a drug, nystatin, shown to increase the membrane permeability to cations significantly (Cass and Dalmark, 1973). Equally important, the membrane regains its low permeability to cations after the nystatin is washed out from the red cell suspension. Consequently, it is possible to swell red cells osmotically at tonicities much greater than 150 mosmol to obtain spheroidal cells. For instance, we have produced swollen, nearly spherical red cells at 580 mosmol; here, the volume correction is only about 25% of the value at 150 tonicity. If our model for the osmotic correction is valid, then the volume correction should approach zero as one over the concentration approaches zero; likewise, the slope of the isotropic tension versus the uncorrected fractional change in area should approach the corrected value. The slope is the elastic area compressibility modulus.

Method for Increasing Ionic Strength Inside Red Cells

Treatment of the cells with nystatin essentially followed the procedure established by Cass and Dalmark (1973). Cells were drawn in heparin and washed three times in 294 mosmol phosphate-buffered saline (30 mM phosphate) with pH 7.37–7.43. In advance, three incubating solutions had been prepared: the first contained 132.6 mM KCl, 19.4 mM NaCl, 0.47 mM KH_2PO_4, and 2.37 mM Na_2HPO_4; the second contained approximately double this concentration, and the third approximately quadruple the first concentration. Sucrose (27.0 mM) was added to each solution, and the pH was adjusted to the range 7.2–7.3. The cells were divided into three groups labeled $N \times 1$, $N \times 2$ and $N \times 4$, and suspended at 7.0% hematocrit in the appropriate incubating solutions. Nystatin (Mycostatin, E. R. Squibb & Sons, Princeton, N.J.), 20 mg/ml in dimethyl sulfoxide had been added at a concentration of 30 μg/ml. The cells were then incubated at 0°C for about 60 min. Cells were washed five times at 32–38°C: twice in nystatin-free incubating solution, and three times in the same solution containing 0.5 g/100 ml bovine serum albumin (BSA).

For the micropipette experiments the cells were swelled by resuspending them at very low hematocrit in phosphate-buffered saline of the appropriate osmolarity: 155 mosmol, 294 mosmol, or 580 mosmol. These solutions contained 0.5 g/100 ml BSA and had pH 7.37–7.43. The osmolarities were measured by freezing point depression and matched the osmolarity of the micropipette solutions within ±1.0 mosmol. All solutions contained penicillin (100 U/ml) and streptomycin (100 μg/ml) to retard bacterial growth.

RESULTS AND DISCUSSION

Fig. 3 contains the plots of elastic area compressibility modulus of nystatin-treated cells as a function of the osmolarity in which the cells were swollen and aspirated with the micropipette; each set of data is for a specific temperature. These figures show the area compressibility moduli calculated with and without the osmotic volume correction. Each data point is the average of the moduli calculated for about 20 individual cells; the representative standard deviation is illustrated in the figure. The solid line and dashed line represent the predicted behavior of the area compressibility modulus as a function of one over the osmolarity; these lines are obtained using the corrected and uncorrected average moduli obtained from measurements of several hundred un-

treated red cells. The area compressibility modulus corrected for osmotic volume changes is observed to be independent of the cellular osmotic concentration; also, the average value obtained for the nystatin-treated cells is the same as for the untreated cells with the volume correction. This evidence supports the model and assumptions proposed for the osmotic effect. In addition, it appears that the nystatin procedure does not measurably affect the membrane elastic area compressibility. The average value of the area compressibility modulus is temperature dependent; however, the correction for volume change appears to be valid at each temperature. The temperature dependence of the average area compressibility modulus will be presented in a forthcoming paper. The most important observation from these experiments is that the red cell membrane is even more resistant to area dilation than our first estimate, e.g. about 450 dyn/cm at 25°C, in contrast to the original value of 300 dyn/cm.

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