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COMPUTER-SIMULATED UREA REFLECTION COEFFICIENTS IN HUMAN RED CELLS

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Summary

Relative volume curves for human red cells were generated with a computer program to simulate the experimental results obtained by rapidly mixing red cells and urea. Known values for the permeability variables were used in the Kedem and Katchalsky (Biochim. Biophys. Acta (1958) 27, 229—246) solute and solvent flux equations. The different values used for the reflection coefficient, σ, were 0.62, 0.75 and 1.0. The theoretical computer curves with $\sigma = 0.75$ compared closely with the experimental stopped-flow curves.

Reflection coefficients, σ, are necessary to formulate mathematical models for nonelectrolyte permeability across red cell membranes, as shown by Macey and Wadzinski [1]. The purpose of this paper is to compare computer-simulated results for the rapid penetrant urea with the recent experimental σ data reported by Owen and Eyring [2].

To ascertain the amount of error, if any, present in the recently determined σ, it is necessary to compare experimental and theoretical red cell relative volume curves. Fig. 1A illustrates the effect of rapidly mixing human red cells with urea in a stopped-flow instrument (for more data, see Fig. 3 in ref. 2). Fig. 1B represents a plot of the negative tangents of the "initial" slopes of the data in Fig. 1A. Fig. 1C shows computer-simulated curves at three different σ values ($\sigma = 0.62$, 0.75 and 1.0). The concentrations of the permeant molecule urea, C_s^0, are listed as post-mixing values, which is a factor of 8/9 of the pre-mixing experimental values previously reported [2]. The theoretical curves in Fig. 1C were obtained by using a fourth-order Runge-Kutta routine to integrate numerically the Kedem and Katchalsky [3] equations for volume J_v and solute J_s flux across the cell membrane.

$$J_v = -L_p(RT\Delta C_m + \sigma RT\Delta C_s) \tag{1}$$

$$J_s = (1-\sigma)\bar{C}_s J_v + P_s\Delta C_s \tag{2}$$

After the "non-solvent" and "apparent osmotic" volumes were considered, the final forms of Eqns. 1 and 2 which were used to obtain the curves in Fig. 1B are shown in Eqns. 3 and 4.

$$\frac{dV}{dt} = \frac{L_p ART}{V_i} \left[\frac{1-\frac{b_m}{V_i} \quad C_m^i}{V-\frac{b_m}{V_i}} - C_m^o - \sigma(C_s^o - C_s^i) \right] \tag{3}$$

$$\frac{dC_s^i}{dt} = \left[((1-\sigma)\bar{C}_s - C_s^i)\frac{dV}{dt} + \frac{\omega ART}{V_i}(C_s^o - C_s^i) \right] \Big/ \; V - \frac{b_s}{V_i} \tag{4}$$

where V is the relative cell volume (volume/V_i), V_i is the isotonic cell volume, b is the fractional portion of the cell which is apparently osmotically unavailable. L_p and ω are the osmotic and solute permeability coefficients, C is con-

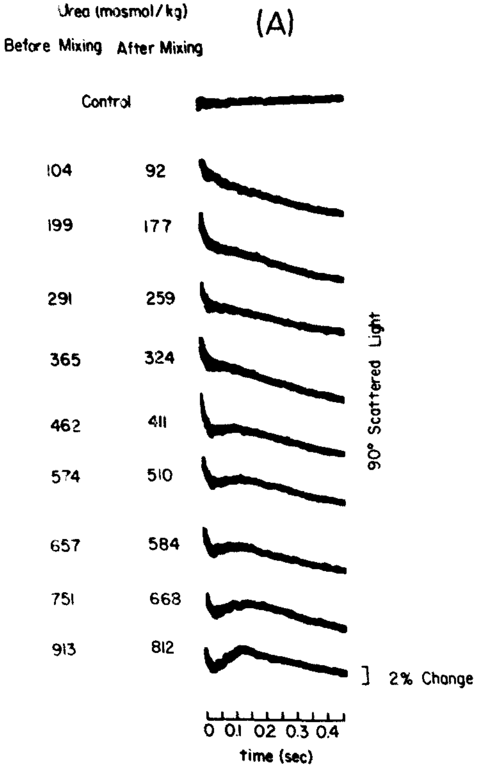

Fig. 1A. For legend see following page.

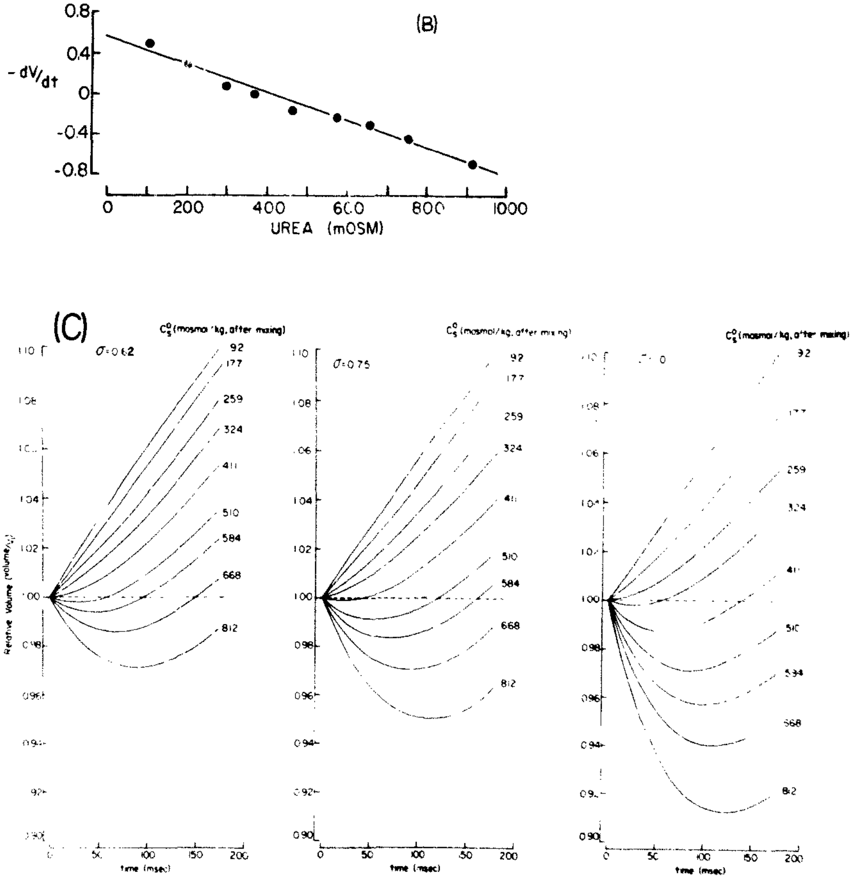

Fig. 1. (A) The effect of different urea concentrations on the rate of change in intensity of 90° scattered light from a human red cell suspension, dV/dt, where V is the response of the photomultiplier tube in volts. Since red cell volume is indirectly proportional to the intensity of 90°-scattered light, the photomultiplier voltage was an index of relative cell volume, i.e. a negative voltage change corresponds to swelling and a positive voltage change denotes shrinking. The control trace represents the rapid mixing of ≈ 8 vol. of isotonic buffer with 1 vol. of an isotonic red cell suspension. This data is from ref. 2. (B) Tangents were drawn to the curves in A between approx. 20 and 130 ms, and the negative of the slopes of these tangents was plotted as a function of urea concentration. The urea concentration obtained by interpolation from (—dV/dt = 0) was 404 mosmol/kg, which with the isotonic buffer concentration of 305 mosmol/kg, gave a σ of 0.75. The urea concentrations used here were pre-mixing concentrations to conform with the original procedure of Goldstein and Solomon [5]. This data is from ref. 2 and the ordinate was previously incorrectly labeled twice its actual value, but this does not affect the value of σ. (C) Computer-simulated, relative-volume curves for red cells mixed with varying outside concentrations of urea, C_s^0, at given σ values. The values used in Eqns. 3 and 4 to generate these curves were: $A = 1.42 \cdot 10^{-6}$ cm^2 [6,7], $T = 298$ [2], $L_p = 7.7 \cdot 10^{-12}$ cm^3/dyne-s [8], $\omega = 11 \cdot 10^{-15}$ mol/dyne-s [9], $V_i = 8.7 \cdot 10^{-11}$ cm^3 [10], $C_m^i = 0.300 \cdot 10^{-3}$ osmol/cm^3 [2], $C_m^0 = 0.034 \cdot 10^{-3}$ osmol/cm^3 [2] $((1 - (b_m/V_i))_{\text{theory}} = ((1 - (b_m/V_i))_{\text{experimental}} \times$ apparent osmotic value $(0.717)(0.8) = 0.53$ [11], $(b_s/V_i) = 1 - 0.717 = 0.283$ [11] and $C_s = (C_s^0 - C_s^i)/\ln(C_s^0/C_s^i)$ [3].

TABLE I

COMPARISON OF UREA σ VALUES IN HUMAN RED CELLS

$\sigma_{average}$	Investigators	Ref.	Procedure
0.62	Goldstein and Solomon (1960)	4	Rapid-flow
0.55	Sha'afi, Rich, Mickulecky and Solomon (1970)	7	Stopped-flow
>1	Macey and Wadzinski (1974)	1	Computer-simulated fit of red cell volume data in ref. 8.
0.79	Owen and Eyring (1975)	2	Stopped-flow
0.71	Solomon, Milgram and Kirkwood (1975)	12	Computer-simulation re-evaluation of the σ in ref. 4 (?)
0.79	Owen (this work)	—	Computer-simulated fit of red-cell, light-scattering data in ref. 2.

centration, $\bar{C}_s$ is the mean concentration of the permeant species within the membrane, A is the urea and R and T have their usual meanings. The subscripts in Eqns. 3 and 4, i, m and s denote isotonic, impermeant and permeant species, while the superscripts, i and o represent concentrations inside and outside the cell.

At least four similarities can be shown with the recent experimental red-cell, light-scattering data Fig. 1A and B and the theoretical cell volume curves at $\sigma = 0.75$ in Fig. 1C:

(1) *C_s^o values which initially gave swelling, no volume change and shrinking.* In Fig. 1A, the experimental "null" C_s^o, or the interpolated permeant molecule concentrations which caused a zero time slope of zero, was 404 mosmol/kg (pre-mixing). This corresponds to a post-mixing null C_s^o of 360 mosmol/kg. The theoretical curves in Fig. 1C indicate a null C_s^o of 260 mosmol/kg for $\sigma = 0.62$, 360 mosmol/kg for $\sigma = 0.75$ and 460 mosmol/kg for $\sigma = 1.0$. In Fig. 1A, the experimental data indicate that at C_s^o 365 mosmol/kg (pre-mixing) or 324 mosmol/kg (post-mixing) the cells were swelling and at C_s^o 462 mosmol/kg (pre-mixing) or 411 mosmol/kg (post-mixing) the cells were shrinking, so qualitatively the experimental data near and at the null C_s^o agrees with the theoretical curves at $\sigma = 0.75$. When comparing the experimental light-scattering data with the theoretical relative volume curves in Fig. 1C the changes occur in opposite directions, i.e. light-scattering increases as cell volume decreases [4].

(2) *The position of the minimum volume point.* The theoretical minimum point for the curve at C_s^o 812 mosmol/kg (post-mixing) or 913 mosmol/kg (pre-mixing) in Fig. 1C for $\sigma = 0.75$ is at about 110 ms, but the experimental minimum, shown in Fig. 1A, is at approx. 130 ms. If Eqns. 3 and 4 are considered correct, then the experimental points must be shifted approx. 20 ms. This apparent shift may be due to an initial mixing phenomonon which is unrelated to the osmotic properties of the cells, since it manifests itself in all the C_s^o tested. Therefore, the procedure used previously [2] of ignoring the initial 20 ms fortuitously gave slope measurements starting at "zero time".

(3) *The ratio of the slopes of the C_s^o curves.* During the initial 20 ms the theoretical curves in Fig. 1C show considerable differences at the same C_s^o but different σ, e.g. at C_s^o 812 mosmol/kg the slopes become increasingly steeper from $\sigma = 0.62$ to 1, while at C_s^o 92 mosmol/kg the slopes decrease slightly as σ increases. The ratios of the theoretical slopes at ((812 mosmol/kg)/

(92 mosmol/kg)) in Fig. 1C are approximately: −1 at $\sigma = 0.62$, −1.5 at $\sigma = 0.75$ and −3 at $\sigma = 1$. The experimental slope ratio from Fig. 1A at the comparable premixing C_s^0 values, ((913 mosmol/kg)/(104 mosmol/kg)), is about −1.4, which is in good agreement with computer simulated data at $\sigma = 0.75$.

(4) *The time at which the (relative volume versus time) slope is measured.* The calculated value at σ, using the "zero-time" method [5], decreases from the σ value given to the computer as the apparent initial observation time increases. This can be demonstrated with the theoretical curves at a given σ of 0.75 in Fig. 1C. Interpolation at 5 ms after mixing with the 324 and 411 mosmol/kg C_s^0 curves yielded a calculated σ of 0.74. A plot of the zero-time slopes, obtained from tangents drawn to the curves in Fig. 1 during the initial 20 ms, as a function of each C_s^0 for a given σ of 0.75 gave a calculated σ of 0.71. Increasingly lower σ values were calculated from slopes taken at longer apparent initial observation times, e.g. a σ of 0.53 was calculated from slopes at 55 ms and a σ of 0.46 was found at 75 ms.

Calculating σ from the theoretical curves (comparison 4) indicates that the slopes must be determined close to zero time to obtain excellent agreement between the given σ and the calculated σ values. Consequently, since the experimental data and theoretical curves at $\sigma = 0.75$ are qualitatively similar (comparisons 1, 2, 3), the experimental σ of 0.75 [2] must have been determined from slopes close to zero-time. Therefore, this suggests that this representative example where $\sigma = 0.75$ and the average σ of 0.79 is probably accurate.

Table I shows the comparison of our average value of σ of 0.79 [2] with several other reported σ values. The range is indeed wide between various σ values and illustrates the necessity for continued interest in this area of red cell nonelectrolyte permeability research.

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References

1 Macey, R.I. and Wadzinski, L.T. (1974) Fed. Proc. 33, 2323–2326.
2 Owen, J.D. and Eyring, E.M. (1975) J. Gen. Physiol. 66, 251–265.
3 Kedem, O. and Katchalsky, A. (1958) Biochim. Biophys. Acta 27, 229–246.
4 Owen, J.D., Steggall, M. and Eyring, E.M. (1974) J. Membrane Biol. 19, 79–92.
5 Goldstein, D.A. and Solomon, A.K. (1960) J. Gen. Physiol. 44, 1–17.
6 Westerman, M.P., Pierce, L.E. and Jensen, W.N. (1961) J. Lab. Clin. Med. 57, 819–824.
7 Wieth, J.O., Funder, J., Gunn, R.B. and Brahm, J. (1974) in Comparative Biochemistry and Physiology of Transport. (Bolis, L., Bloch, K., Luria, S.E. and Lynen, F., eds.), North-Holland Publishing Company, Amsterdam.
8 Sha'afi, R.I., Rich, G.T., Mikulecky, D.C. and Solomon, A.K. (1970) J. Gen. Physiol. 52, 941–954.
9 Galey, W.R., Owen, J.D. and Solomon, A.K. (1973) J. Gen. Physiol. 61, 727–746.
10 Rich, G.T., Sha'afi, R.I., Romualdez, A. and Solomon, A.K. (1968) J. Gen. Physiol. 52, 941–954.
11 Savitz, D., Sidel. V.W. and Solomon, A.K. (1964) J. Gen. Physiol. 48, 79–94.
12 Solomon, A.K., Milgram, J. and Kirkwood, D.H (1975) Biochim. Biophys. Acta 406, 157–160.