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COMPUTER SIMULATION OF UREA PERMEABILITY IN HUMAN RED CELLS

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There has been considerable interest recently in comparing computer simulated red cell nonelectrolyte permeability information with previously reported experimental data [1—4]. Macey and Wadzinski [2] reported that they could not fit a computer simulated curve to the experimental urea permeability data of Sha'afi et al. [5] when they used Sha'afi et al.'s values [5] for the various parameters in the Kedem and Katchalsky volume and solute flux equations [6]. In order to obtain a comparable simulated curve they had to use a lower permeability coefficient, ω, and a higher reflection coefficient, σ, in their computer program. Since this appeared to be an apparent paradox, we report here the effect of utilizing different parameters in the Kedem and Katchalsky equations to fit Sha'afi et al.'s data.

It is necessary to briefly describe the procedure Sha'afi et al. [5] used to fit the Kedem and Katchalsky equations

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

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

can be rearranged [5] so that at the red cells' minimum volume, where $J_v = 0$,

$$\omega = V'_{\min}\,(d^2V'/\mathrm{d}t^2)_{\min}/A^2L_pRT(\pi_i^o-\pi_i^{\Delta x}) \tag{3}$$

where V' is the cell volume minus the portion which is osmotically unavailable, A is the cell area, L_p is the hydraulic permeability coefficient, π_i is the osmotic pressure due to the impermeant solute outside and inside (superscript o and Δx, respectively) the cell membrane, and R and T have their usual meanings. Using Eqn. 3 to solve for ω is routinely referred to as Solomon's "minimum method". A value for σ is not needed in order to solve Eqn.

3 for ω and Sha'afi et al. [5] reported a value for σ obtained in a set of independent experiments.

To generate computer simulated red cell volume vs. time curves with a fourth-order Runge-Kutta routine to numerically integrate Eqns. 1 and 2 is straightforward [7] and the fact that Macey and Wadzinski's results do not agree with Sha'afi et al.'s suggests the possibility that some of the assumptions used by Sha'afi et al. [5] to obtain their minimum method Eqn. 3 were incorrect. Perhaps using the information at the minimum volume point is not complete, as compared to fitting the entire curve. Since several parameters are in Eqn. 1 and 2 it is possible to obtain the same generated curve by, for example, increasing one parameter and decreasing another. Table I shows the values for the various parameters used by Sha'afi et al. [5], Macey and Wadzinski [2] and this work to fit the experimental data of Sha'afi et al. as shown in Fig. 1. Macey and Wadzinski used Sha'afi et al.'s values for L_p, A and V, but in order to fit the experimental data they had to use a lower ω and a higher σ value than had been previously reported. The fit we obtained in this work was equally good, but we used different values than either Macey and Wadzinski or Sha'afi for each of the five parameters (see Table I). Since the report of Sha'afi et al., considerable improvements and more accurate equipment has been used to independently measure these parameters [8–12].

TABLE I

HUMAN RED CELL PERMEABILITY INFORMATION UTILIZED TO SOLVE THE KEDEM AND KATCHALSKY VOLUME AND SOLUTE FLUX EQUATIONS AS SHOWN IN FIG. 1.

	ω (10^{-15}mol/ dyne per s)	σ	L_p (10^{-12} cm^2/ dyne per s)	A (10^{-6} cm^2)	V_i (10^{-11} cm^3)
Sha'afi et al.	14.1	0.55	7.67	1.67	8.7
Macey and Wadzinski	9.28	1.0	7.67	1.67 (?)	8.7 (?)
This work	11	0.8	11.7	1.35	9.4

The computer generated curve in Fig. 1 from this work was obtained by using Eqns. 1 and 2 and after the "non-solvent" and "apparent osmotic" values were considered, as well as rewriting the volume flux to cell volume change and solute flux was rewritten to express changes in internal permeant solute concentration, Eqns. 1 and 2 yield:

$$\frac{\mathrm{d}V}{\mathrm{d}t} = \frac{L_p ART}{V_i} \left[\frac{\left(1-\frac{b_m}{V_i}\right) C_m^i}{\left(V-\frac{b_m}{V_i}\right)} - C_m^o - \sigma \left(C_s^o - C_s^i\right) \right] \tag{4}$$

$$\frac{\mathrm{d}C_s^i}{\mathrm{d}t} = \left[\left((1-\sigma)\,\bar{C}_s - C_s^i\right) \frac{\mathrm{d}V}{\mathrm{d}t} + \frac{\omega ART}{V_i} \left(C_s^o - C_s^i\right) \right] \Big/ \left(V - \frac{b_s}{V_i}\right) \tag{5}$$

Where V represents the relative cell volume or ((the volume at time = t)/(isotonic volume, V_i)), b is the fractional portion of the cell which is appar-

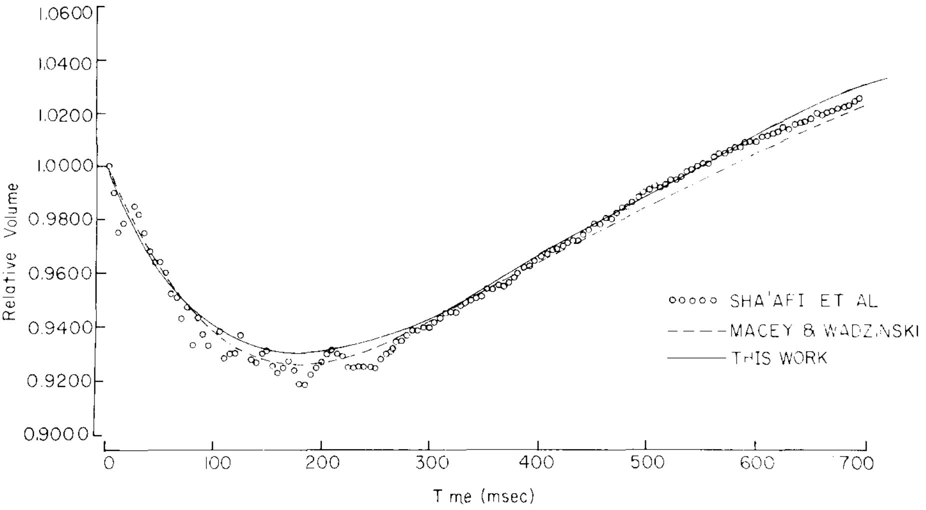

Fig. 1. The comparison of computer simulated curves with experimental data. The theoretical curves were generated using Eqns. 4 and 5 in the text with the values for ω, σ, L_p and A as given in Table I. Also, $T = 298^\circ$ K, $C_m^i = 0.270 \cdot 10^{-3}$ osmol/cm^3 [5], $C_m^o = 0.185 \cdot 10^{-3}$ osmol/cm^3 [5], $((1-(b_m/V_i))$ theory $= ((1-(b_m/V_i)_{\text{experimental}} \times$ apparent osmotic value $= (0.717)(0.8) = 0.57$ [15], $(b_s/V_i) = 1-0.717 = 0.283$ [15] and $C_s^o = 0.375 \cdot 10^{-3}$ osmol/cm^3 [5].

ently osmotically unavailable, L_p and ω are the osmotic and solute permeability coefficients, C is the concentration with the superscripts i and o denoting inside and outside the cell and the subscripts m and s representing impermeant and permeant solute, $\bar{C}_s$ is the mean concentration of the permeant species within the membrane or $(C_s^o - C_s^i)/\ln(C_s^o/C_s^i)$ [6], A is the area and R and T have their usual meanings.

In conclusion, these results support*: (1) the experimental observations of Sha'afi et al. [5], (2) the temperature controlled stopped-flow ω of $11 \cdot 10^{-5}$ mol/dyne per s urea results of Galey et al. [13] (albeit obtained via the minimum method), as well as the rapid-flow result of $13 \cdot 10^{-15}$** mols/dyne per s obtained recently by Weith et al. [8], (3) the urea σ results of 0.79 as reported by Owen and Eyring [9] and Owen [4], (4) the Farmer and Macey L_p result of $11.7 \cdot 10^{-12}$ cm^3/dyne per s [10] as well as the Blum and Forster result of $12.1 \cdot 10^{-12}$ cm^3/dyne per s [11] and (5) the red cell A of $1.35 \cdot 10^{-6}$ cm^2 and the red cell isotonic volume of $9.4 \cdot 10^{-11}$ cm^3 as reported by Evans and Fung [12].

The computer curves shift accordingly when the parameters are individually varied: (1) decreasing ω shifts all the points downwards, especially at the longer times; (2) increasing σ shifts all the points downwards; (3) increasing L_p makes the curve steeper and shifts them downwards in the initial and middle portions of the curve, while it shifts the latter portion of the

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* It should be pointed out that Owen [4] used the older L_p, A and V_i data of Sha'afi et al. [5] but the newer ω [13] data to find that there was a qualitative similarity between the theoretical and experimental σ work for $\sigma = 0.79$. The qualitative observations of Owen would not be greatly affected by the observations in this study.

**Calculated from their rate constant value of 6.6 s^{-1} by assuming a cell volume of 94 μm^3, surface area of 13 μm^2 [12] and a cell water volume fraction of 0.72.

curve upwards; (4) increasing A shifts the curve upwards; (5) increasing V_i shifts the curve upwards; (6) using Levitt's [14] value for $\bar{C} = 0.5\,(C_s^o + C_s^i)$ instead of that of Kedem and Katchalsky [6] $\bar{C} = (C_s^o - C_s^i)/\ln\,(C_s^o/C_s^i)$ shifts the longer time points slightly upwards; (7) increasing C_m^o shifts the curve downwards; (8) using Levitt's [1] form of the Kedem and Katchalsky equations shifts the curve upwards.

In summary, it should be noted that the purpose of this report is not to prove that the parameters used in this work are correct since they fit the experimental data. Instead, the intent here is to demonstrate that it is possible to obtain a good fit with recently reported parameter values, although different combinations of slightly varied parameter values in Table I would also yield a similar fit.

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