BBA 76137

PERTURBATION OF RED CELL VOLUME

DETERMINATION OF MEMBRANE TRANSPORT PARAMETERS FOR RAPID PENETRANTS

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(Received June 30th, 1972)

SUMMARY

In the presence of a rapid non-electrolyte penetrant an osmotic perturbation of red cell volume results in a complex re-equilibration of solute and water. The kinetics can be described by the sum of two real exponentials whose time constants are functions of experimental conditions and transport parameters. It is shown that determination of the time constants is sufficient for calculation of the permeability coefficient ω and the reflection coefficient σ when the osmotic filtration coefficient L_p is known. To facilitate measurement of the time constants an experimental approach is described in which alternately one of the two exponentials is greatly reduced in magnitude. Thus two types of single-exponential experiments are suggested: one type to measure the faster time constant, another to measure the slower time constant. This single-exponential approach was used to obtain values of σ and ω for urea crossing the membrane of the beef erythrocyte.

INTRODUCTION

The use of small concentration gradients and "volume perturbation" in the measurement of membrane transport parameters of intact cells has been previously discussed[1,2]. While this technique is of considerable interest for routine determination of parameters, perhaps its principal advantage is that it permits a detailed description of the concentration dependence of the membrane parameters which control the entry and exit of small molecules. For example, the use of small gradients led to the discovery that in beef and human red cells the osmotic filtration coefficient L_p is independent of salt concentration but depends upon the direction of osmotic flow[1]. Further, for certain slowly permeable non-electrolytes in beef red cells the reflection coefficient σ and the permeability coefficient ω were also independent of salt concentration with little dependence upon direction of flow[2]. In addition, all three parameters were independent of penetrant concentration over a wide range.

Since the previous work has dealt with simplified systems, *i.e.* the presence of no solute or the presence of a slowly penetrating non-electrolyte, it is important

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to treat the more general problem which arises when a rapid non-electrolyte penetrant is present. A rapid penetrant may cross the membrane almost as fast as water. Thus, the second-order system of equations describing the perturbation kinetics cannot be reduced to lower order. Separation of the two exponentials which describe a volume perturbation of this kind is much more difficult, and the analytical relationships between time constants and transport parameters are more complex.

To facilitate data analysis an experimental approach is described (single-exponential approach) which eliminates the necessity of extracting time constants from a composite of two, similar, overlapping exponentials. Initial conditions are chosen so that the magnitude of either the faster or the slower exponential is small. The remaining exponential then predominates, and its time constant can be determined more accurately. Two experiments, one for each of the two exponentials, are then necessary for each set of conditions. This procedure leads to increased accuracy in determining values of the parameters, but may require several trials to find the proper initial conditions (equivalent to having a good initial guess for the parameters)

The single-exponential approach has been used to obtain the parameters of urea transport in beef red cells. In mammalian erythrocytes urea is an extraordinarily rapid penetrant[3], and determination of its transport properties has always been hampered by experimental and analytical difficulties[4,5]. The single-exponential approach leads directly to values for σ and ω.

CALCULATIONS

In terms of cell volume (V), penetrant content (S), and external osmotic pressures (π_m for impermeable solutes, π_s for permeable solutes) a general system of equations can be written[2] which describes the kinetic change in state (V, S) of the cell following a sudden step change in solution composition from $\pi_m - \Delta\pi_m$, $\pi_s - \Delta\pi_s$ to π_m, π_s at $t = 0$

$$\frac{dV}{dt} = k_1\left[\frac{1-b}{V-b} + \frac{\sigma S}{V-a} - (\pi_m + \sigma\pi_s)\right] \tag{1}$$

$$\frac{dS}{dt} = (1-\sigma)\bar{\pi}_s\frac{dV}{dt} + k_2\left(\pi_s - \frac{S}{V-a}\right) \tag{2}$$

where t is time (s) and σ is the penetrant reflection coefficient (unitless). $\bar{\pi}_s$ is a mean value of penetrant osmotic pressure defined by $(\pi_s + S/(V-a))/2$ for small changes. a and b are constants which are related to the non-water volume of the cell.

Let V_{iso} represent the cell volume at isotonicity (cm^3) and C_{iso}, the isotonic concentration of impermeable solutes (osmoles$\cdot cm^{-3}$). RT is the universal gas constant times absolute temperature (dyne$\cdot$cm$\cdot$mole^{-1}). V is in units of V_{iso} ($V = 1$ at isotonicity), π_m and π_s are in units of RTC_{iso} ($\pi_m = 1$ at isotonicity), and S is in units of $V_{iso}C_{iso}$ ($S = 1$ when the amount of internal penetrant equals the equivalent number of osmoles of non-penetrant in a unit volume V_{iso} of isotonic solution). The coefficients k_1 and k_2 (each in units of s^{-1}) are then

$$k_1 = RTL_pAC_{iso}/V_{iso} \tag{3}$$

$$k_2 = RT\omega A/V_{iso} \tag{4}$$

where L_p is the osmotic filtration coefficient ($cm^3 \cdot dyne^{-1} \cdot s^{-1}$), ω is the permeability coefficient of the penetrant (moles$\cdot$dyne$^{-1}\cdot$s^{-1}) and A is the area of the cell membrane (cm^2).

For very rapid penetrants (with respect to water) the term $(1-\sigma)$ in Eqn 2 should be replaced by $(1-\sigma-\omega\bar{V}_s/L_p)$ where $\bar{V}_s$ is the partial molal volume of the penetrant. This is evident from the Kedem–Katchalsky[6] equations under conditions of zero hydrostatic pressure gradient*.

At equilibrium Eqns 1 and 2 give the final state of the cell

$$V_\infty = b + (1-b)/\pi_m \tag{5}$$

$$S_\infty = (V_\infty - a)\pi_s \tag{6}$$

The initial state is

$$V_0 = V_\infty - \Delta V = b + (1-b)/(\pi_m - \Delta\pi_m) \tag{7}$$

$$S_0 = S_\infty - \Delta S = (V_\infty - \Delta V - a)\,(\pi_s - \Delta\pi_s) \tag{8}$$

where ΔV and ΔS represent the total increase in V and S (final *minus* initial). $\Delta\pi_m$ and $\Delta\pi_s$ represent the perturbing osmotic pressures. From the initial and final states we get (for small changes)

$$\Delta V = -(1-b)\pi_m^{-2}\Delta\pi_m \tag{9}$$

$$\Delta S = \pi_s\Delta V + (1-b)\gamma^{-1}\pi_m^{-1}\Delta\pi_s \tag{10}$$

where γ is defined by

$$\gamma = \frac{V_\infty - b}{V_\infty - a} = \left(1 + \frac{b-a}{1-b}\pi_m\right)^{-1} \tag{11}$$

For small perturbations the nonlinear system (Eqns 1 and 2) can be linearized by expanding the right-hand side in a Taylor's series and neglecting the higher-degree terms. Using Eqns 5 and 6 the linearized system becomes.

$$\frac{dV}{dt} = -k_1\left[\frac{\pi_m}{V_\infty - b} + \frac{\sigma\pi_s}{V_\infty - a}\right](V - V_\infty) + \frac{k_1\sigma}{V_\infty - a}(S - S_\infty) \tag{12}$$

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* Using the original notation of Kedem and Katchalsky[6] the equations describing volume flow and solute flow are

$$J_v = L_p(-\Delta\pi_i - \sigma RT\Delta c_s)$$

$$\dot{n}_s = J_v(1-\sigma)c_s + \omega RT\Delta c_s + \omega\phi\Delta\pi_i$$

for $\phi = c_s\bar{v}_s \ll 1$ and $\Delta P \simeq 0$ The term $\omega\phi\Delta\pi_i$, which for convenience is usually neglected, should be retained for very rapid penetrants (*i e.* when ω is large) If the volume flow equation is solved for $\Delta\pi_i$ and the resulting expression substituted into the equation for solute flow, we have ($\phi \ll 1$)

$$\dot{n}_s = J_v(1 - \sigma - \omega\bar{v}_s/L_p)c_s + \omega RT\Delta c_s$$

which explains the correction for very rapid penetrants

$$\frac{dS}{dt} = (1-\sigma)\pi_s \frac{dV}{dt} + \frac{k_2\pi_s}{V_\infty - a}(V - V_\infty) - \frac{k_2}{V_\infty - a}(S - S_\infty) \tag{13}$$

Defining $t' = t/\tau_w$ where[1]

$$\tau_w = (1-b)k_1^{-1}\pi_m^{-2} \tag{14}$$

and using Eqn 11 gives

$$\frac{dV}{dt'} = \alpha(V - V_\infty) + \beta(S - S_\infty) \tag{15}$$

$$\frac{dS}{dt'} = \left[(1-\sigma)\pi_s\alpha + \frac{k_2}{k_1}\frac{\pi_s}{\pi_m}\gamma\right](V - V_\infty) + \left[(1-\sigma)\pi_s\beta - \frac{k_2}{k_1}\frac{\gamma}{\pi_m}\right](S - S_\infty) \tag{16}$$

where

$$\alpha = -(1 + \sigma\gamma\pi_s/\pi_m) \tag{17}$$

$$\beta = \sigma\gamma/\pi_m \tag{18}$$

The characteristic equation for this linearized system is

$$\lambda^2 + \left(1 + \sigma^2\gamma\frac{\pi_s}{\pi_m} + \frac{k_2}{k_1}\frac{\gamma}{\pi_m}\right)\lambda + \frac{k_2}{k_1}\frac{\gamma}{\pi_m} = 0 \tag{19}$$

The eigenvalues are the solution to this equation

$$\lambda_\pm = -\frac{1}{2}\left(1 + \sigma^2\gamma\frac{\pi_s}{\pi_m} + \frac{k_2}{k_1}\frac{\gamma}{\pi_m}\right) \pm \frac{1}{2}\sqrt{\left(1 + \sigma^2\gamma\frac{\pi_s}{\pi_m} + \frac{k_2}{k_1}\frac{\gamma}{\pi_m}\right)^2 - 4\frac{k_2}{k_1}\frac{\gamma}{\pi_m}} \tag{20}$$

and they are both real and negative. For very rapid penetrants σ^2 should be replaced by $\sigma(\sigma + \omega\bar{V}_s/L_p)$.

Using the eigenvalues the following general solution to the linearized system (Eqns 12 and 13) can be verified by substituion.

$$V - V_\infty = \frac{(\alpha - \lambda_+)\Delta V + \beta\Delta S}{(\lambda_+ - \lambda_-)} e^{\lambda_- t'} - \frac{(\alpha - \lambda_-)\Delta V + \beta\Delta S}{(\lambda_+ - \lambda_-)} e^{\lambda_+ t'} \tag{21}$$

$$S - S_\infty = \frac{(\alpha - \lambda_+)\,[(\alpha - \lambda_-)\Delta V + \beta\Delta S]}{\beta(\lambda_+ - \lambda_-)} e^{\lambda_+ t'} - \frac{(\alpha - \lambda_-)\,[(\alpha - \lambda_+)\Delta V + \beta\Delta S]}{\beta(\lambda_+ - \lambda_-)} e^{\lambda_- t'} \tag{22}$$

From Eqns 9, 10, 17 and 18, however

$$\alpha\Delta V + \beta\Delta S = (1-b)\pi_m^{-2}(\Delta\pi_m + \sigma\Delta\pi_s) \tag{23}$$

So the volume perturbation part (Eqn 21) can be written

$$V - V_\infty = (1-b)\pi_m^{-2}[E_- e^{-t/\tau_-} + E_+ e^{-t/\tau_+}] \tag{24}$$

where

$$E_- = \frac{(1 + \lambda_+)\Delta\pi_m + \sigma\Delta\pi_s}{(\lambda_+ - \lambda_-)} \tag{25}$$

$$E_+ = \frac{(1 + \lambda_-)\Delta\pi_m + \sigma\Delta\pi_s}{(\lambda_- - \lambda_+)} \tag{26}$$

$$\tau_- = -\tau_w/\lambda_- \tag{27}$$

$$\tau_+ = -\tau_w/\lambda_+ \tag{28}$$

Thus the volume change is described by the sum of two real exponentials with time constants τ_- and τ_+. For small k_2 Eqns 25, 26, 27 and 28 reduce to expressions identical with those previously reported for slow penetrants[2].

We are particularly interested in the conditions which lead to a disappearance of one of the two exponentials (*i.e.* $E_- = 0$ or $E_+ = 0$). When initial conditions are selected so that the volume change is a single exponential, problems of initial mixing are alleviated, and larger volume changes can be utilized. σ and ω can be calculated from two or more separate single-exponential experiments.

From Eqn 19 we solve for the relative permeability

$$\frac{k_2}{k_1} = x + \sigma^2 y \tag{29}$$

where

$$x = -\lambda\frac{\pi_m}{\gamma} \tag{30}$$

$$y = -\frac{\lambda}{\lambda + 1}\pi_s \tag{31}$$

so for two different experimental conditions (i, j) and assuming constant parameters in this range we have two simultaneous algebraic equations

$$\frac{k_2}{k_1} = x_i + \sigma^2 y_i \tag{32}$$

$$\frac{k_2}{k_1} = x_j + \sigma^2 y_j \tag{33}$$

These can be solved to give

$$\sigma^2 = \frac{x_i - x_j}{y_j - y_i} \tag{34}$$

and

$$\frac{k_2}{k_1} = \frac{y_j x_i - y_i x_j}{y_j - y_i} \tag{35}$$

where x_i, x_j, y_i, y_j are given by Eqns 30 and 31 under the proper conditions. Thus σ and ω can becomputed (for known L_p) from the time constants of a slow exponential experiment ($E_- = 0$) and a rapid ($E_+ = 0$), or they can be computed from two slow exponential experiments ($E_- = 0$). In the latter case the two experiments must have different final states. We have used the first method to obtain σ and ω for urea in beef red cells.

EXPERIMENTAL PROCEDURE

Beef blood was obtained and defibrinated by slow stirring at a local slaughterhouse. It was filtered through cheesecloth and stored at about 2 °C until used. The resulting hematocrit (International Model MB Micro-capillary Centrifuge), osmolality (Fiske Mark III Osmometer) and cell counts of the blood were determined. Red cell volume was calculated using hematocrit and cell count with no correction for trapped plasma. Cell area was estimated using a right circular cylinder approximation[7]. The quantity b was determined from a plot of hematocrit *vs* inverse tonicity. The value for a was taken to be 0.24 for these cells[2].

The isotonic salt solution used as a standard for preparing cell suspensions consisted of (all Cl^- salts) 133 mM Na^+, 5.4 mM K^+, 2.6 mM Ca^{2+}, 1.8 mM Mg^{2+}, 32 mM Tris. Its pH was about 7.4, and its osmolality was near 300 mosmoles/kg. Hypertonic and hypotonic solutions contained the same relative proportion of salts. Urea was added to make the desired concentrations.

The photometric apparatus has been previously described[1]. It measures the change in turbidity of a cell suspension following a disturbance of solution composition.

5 ml of a 1.0% suspension of beef cells were pipetted into the cuvette of the apparatus. After a short baseline, 0.5–0.6 ml of an appropriate solution was injected by solenoid into the cuvette, and the resulting perturbation of cell volume was recorded (Grass Model 5D Polygraph) as a change in turbidity. The suspension was withdrawn from the cuvette and its final osmolality measured. Time constants of cell shrinking were obtained from the recorded data.

RESULTS

In Table I are the experimental conditions and results of our red cell shrinking experiments. There were several experiments over a two-day interval all using blood from the same animal. The cells were somewhat swollen since they were initially suspended in a solution containing 236 mosmoles/l salt (a tonicity of 0.82), but this had the advantage of yielding slower time constants. Time constants for the same experiment done on two consecutive days (Nos. 1 and 3) gave good agreement.

The data of Table I were used to calculate the parameters σ and ω using Eqns 34 and 35 Three calculations were made using rapid and slow exponential experiments (Nos. 1 and 2, Nos. 3 and 4, Nos. 3 and 5). The values of σ and ω were then averaged. Calculation of these parameters using slow exponentials only (*i.e.* Nos. 2 and 4, Nos. 2 and 5) was considered inaccurate because of the close proximity of the final states. Also, such a calculation was found to be very sensitive to the difference $b-a$. Table II shows the results of the calculations when $b-a$ was assumed to be zero and when $b-a$ was 0.18. For $a = b$, the average values were $\sigma = 0.64$ and

TABLE I

DETAILS OF UREA SHRINKING EXPERIMENTS WITH BEEF ERYTHROCYTES (B 1/30 LM)

The cells were equilibrated at an initial state defined by the solution composition "Before injection" (c_m = salt concentration, c_s = non-electrolyte concentration) Upon injection of the "Injection solution" the cells approached a final state defined by the "After injection" solution composition The first three experiments were of the single-exponential type, *i e* one of the two exponentials was very small (see Fig 1) The last two experiments were double-exponential only the well-defined slower exponential was used in these cases Time constants τ were obtained from averages of several runs (number in parentheses) In Expts No 1 and 2 the blood had been stored for one day. In the other experiments, for two days Plasma isotonicity was 288 mosmoles/l, and the temperature was 25 °C τ_w was 0 276 s at isotonicity

Expt No	*Exponential type*	*Before injection* *		*Injection solution* *		*After injection* *		π_m	π_s/π_m	τ (*s*)
		c_m	c_s	c_m	c_s	c_m	c_s			
1	Rapid −	236	153	470	460	261	186	0 906	0 713	0 192 (5)
2	Slow +	236	153	576	0	272	137	0 944	0 504	0 504 (5)
3	Rapid −	236	153	470	460	261	186	0 906	0 713	0 198 (7)
4	Slow +	236	153	236	0	236	137	0 820	0 580	0 627 (5)
5	Slow +	236	153	303	0	243	137	0 844	0 564	0 586 (3)

* All concentrations given in mosmoles/l

TABLE II

CALCULATION OF PARAMETERS ASSUMING $b-a = 0$ AND $b-a = 0\ 18$

The reflection coefficient σ and the permeability coefficient $RT\omega$ for urea were calculated from pairs of experiments The averages show a significant dependence upon the difference $b-a$ (V_{iso}/A was $0\ 55 \cdot 10^{-4}$ cm)

Expt No	$b-a = 0$		$b-a = 0\ 18$	
	σ	$RT\omega \cdot 10^4$ (*cm/s*)	σ	$RT\omega \cdot 10^4$ (*cm/s*)
1–2	0 67	1 06	0 75	1 36
3–4	0 64	1 04	0 72	1 33
3–5	0 62	1 07	0 71	1 36
Average	0 64	1 06	0 73	1 35

$RT\omega = 1.06 \cdot 10^{-4}$ cm/s For $a = b-0.18$, the average values were $\sigma = 0.73$ and $RT\omega = 1.35 \cdot 10^{-4}$ cm/s. The latter values are preferred because they correspond to reasonable values for a and b ($a = 0.24$, $b = 0.42$)[2].

DISCUSSION

Use of time constants

We have seen that a perturbation of red cell volume in the presence of a rapid non-electrolyte penetrant such as urea results in a complex re-equilibration of solute and water which can be described by the sum of two real exponentials (Eqn 24). The time constants of these exponentials are complicated functions of experimental conditions (π_m and π_s/π_m) and of the transport parameters (σ^2, ω/L_p and L_p) Given

L_p or τ_w from previous measurement and assuming constant parameters over the range of experimental conditions, two time constants are sufficient information to compute the parameters σ and ω using Eqns 34 and 35.

In certain cases it is possible to simplify the relationships. Thus, when both rapid and slow exponentials have the same final state, Eqns 34 and 35 then become (using Eqns 3 and 4)

$$\omega/L_p = C_{iso}(\pi_m/\gamma)\tau_w^2(\tau_-\tau_+)^{-1} \tag{36}$$

and

$$\sigma^2 = (\pi_m/\gamma)(1 - \tau_w/\tau_+)(\tau_w/\tau_- - 1)/\pi_s \tag{37}$$

These important equations are particularly useful for the special case of very small perturbations where both exponentials are present and measurable.

It will be noted that expressions involving the time constants generally contain the quantity γ. Gamma (γ), defined by Eqn 11 is, in general, less than unity because the difference $b - a$ is normally greater than zero[8]. In many analyses of cell volume changes it has implicitly been assumed that $b = a$. The consequences of such an assumption are shown in Table II where calculated values of σ are shown to be 13 % too low and values of ω are too low by 22 %.

Single-exponential conditions

To simplify experimental measurement and analysis of data we have suggested choosing initial conditions so that alternately one of the two exponentials is small. (Single-exponential conditions are obtained by setting the numerators of Eqns 25 and 26 to zero). Advantages of reducing the size of one of the exponentials are (1) a higher signal-to-noise ratio because of the increase in magnitude of the remaining exponential and (2) easier and more accurate separation of exponentials because of the smaller contribution by the attenuated exponential.

When measuring the rapid exponential the conditions for $E_+ = 0$ should be observed carefully. If the slow exponential has an appreciable magnitude then the rapid exponential will tend to approach a pseudo-equilibrium state which may be significantly different from the true final equilibrium state. This problem can be the source of error in the calculated results. Some degree of flexibility is allowed, thus Fig. 1 shows good results even though the $E_+ = 0$ condition was not precisely satisfied (Table I).

We mentioned that an alternate procedure for determination of constant parameters was to obtain time constants for two or more slow exponential experiments whose final states are widely different. This approach eliminates the problem of measuring the time constant of the rapid exponential. A disadvantage, however, is an increased dependence of the calculated results upon the value of $b-a$. The difference $b-a$ may not be known accurately, although for the non-electrolytes studied so far it seems to be near 0.17–0.19. On the other hand, experiments of this type may be particularly useful for studying the functional properties of a and b.

With regard to calculation of the parameters σ and ω, it was implicitly assumed that these parameters were independent of cell size and solute level over the range of final states used in this study. Such constancy was found with the non-electrolytes, glycerol and ethylene glycol[2] However, a dependence upon the direction of water

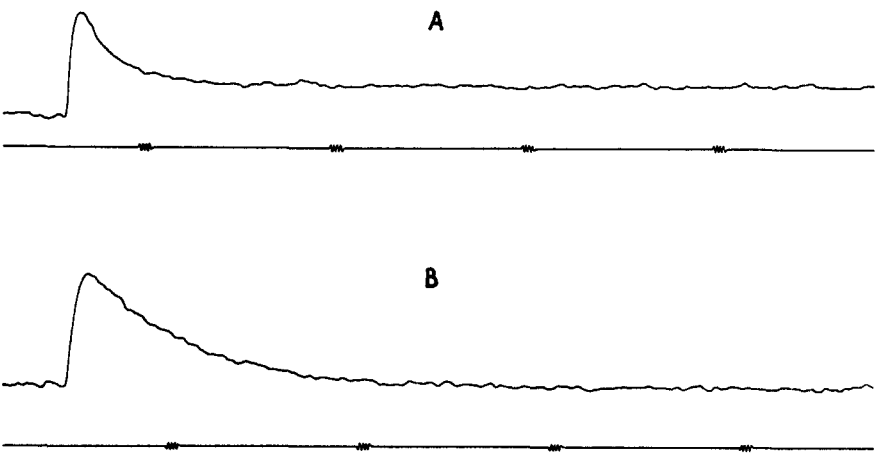

Fig. 1 Polygraph traces from individual shrinking experiments investigating urea transport in beef erythrocytes (B 1/30 LM) by the single-exponential approach Trace A shows the rapid (−) exponential Here, experimental conditions have been selected so that the magnitude of the slow exponential is small (E_+ approx 0). This experiment corresponds to No 1 of Table I. Trace B shows the slow (+) exponential where the rapid exponential has been made small (E_- approx 0). This experiment corresponds to No. 2 of Table I The time interval is 1 s between marks

flow is possible and has not been investigated for the case of a rapid penetrant like urea. Here, the problem was minimized by utilizing only shrinking experiments. Thus the value of τ_w used in these calculations was obtained under conditions of cell shrinking. For swelling experiments, one should probably use a value of τ_w obtained from osmotic swelling experiments. The correct τ_w to use with the slower exponential experiments is particularly open to question at this time and is undoubtedly related to the mechanism of rectification of water flow[1].

Comparison of results

Our current values for urea transport in the beef erythrocyte are $\sigma = 0.73$ and $RT\omega = 1.35 \cdot 10^{-4}$ cm/s at 25 °C. We could find no independently measured values of σ for beef red cells. However, Jacobs[9] reported a permeability (obtained by the hemolysis time method) of $RT\omega = 1.8 \cdot 10^{-4}$ cm/s at 20 °C for beef red cells. This value is higher than ours. The difference may in part be explained by the fact that Jacob's calculations did not take into account the non-unity σ of urea.

The parameters have been reported for other species of red cells. Values for σ of 0.38[10] and 0.55–0.62[11,5] for dog and human, respectively, are somewhat smaller than our value of 0.73 for beef. This is consistent with the suggestion that beef red cells may have a significantly smaller "equivalent pore radius"[2] In qualitative agreement with a pore model is the higher permeability reported for urea in the human erythrocyte where $RT\omega = 3.2 \cdot 10^{-4}$ cm/s at 21 °C[12]. However, it is clear that a simple pore model is not sufficient to explain the mechanism of urea transport because of increasing evidence that urea may react chemically with the red cell membrane[13,14].

ACKNOWLEDGEMENTS

The authors are grateful for the technical assistance of Mrs Frances Johnson. This work was supported in part by a grant from the National Science Foundation

(GB11981) and in part by a post-doctoral research fellowship from the Bay Area Heart Research Committee.

REFERENCES

1 R E L Farmer and R I Macey, *Biochim Biophys. Acta*, 196 (1970) 53
2 R E L Farmer and R I Macey, *Biochim Biophys Acta*, 255 (1972) 502.
3 M H Jacobs, H N. Glassman and A. K. Parpart, *J. Exp Zool*, 113 (1950) 277.
4 W Wilbrandt, *Pflugers Arch Ges Physiol*, 245 (1941) 1
5 R I Sha'afi, G T Rich, D C Mikulecky and A K Solomon, *J Gen Physiol*, 55 (1970) 427.
6 O Kedem and A Katchalsky, *Biochim Biophys Acta*, 27 (1958) 229
7 W F Emmons, *J Physiol*, 64 (1927) 215
8 C M Gary-Bobo and A K Solomon, *J Gen Physiol*, 52 (1968) 825
9 M H Jacobs, in E S Guzman Barron, *Modern Trends in Physiology and Biochemistry*, Academic Press, New York, 1952, p 149
10 G T Rich, R I Sha'afi, T. C. Barton and A K Solomon, *J Gen Physiol*, 50 (1967) 2391
11 D A Goldstein and A. K Solomon, *J Gen Physiol*, 44 (1960) 1
12 D Savitz and A K Solomon, *J Gen Physiol*, 58 (1971) 259
13 R I Sha'afi, C M Gary-Bobo and A K Solomon, *J Gen Physiol.*, 58 (1971) 238
14 R I. Macey and R E L Farmer, *Biochim Biophys Acta*, 211 (1970) 104