BBA 75753

SOLUTE AND SOLVENT FLOW ACROSS MAMMALIAN RED CELL MEMBRANE

HOW TO TEST FOR ONSAGER RECIPROCAL RELATION

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(Received April 26th, 1971)

SUMMARY

Equations are derived for the flow of solvent and an uncharged solute across the red cell membrane. The differential equation which describes the time course of cell volume change is solved using perturbation analysis. A method to test the Onsager reciprocal relation is presented. Previously determined experimental values were substituted in the final equation to show how this analysis can be used to test for the Onsager reciprocal relation.

INTRODUCTION

When mammalian red cells which are placed in a medium containing an isomolal concentration of an impermeant solute are rapidly mixed with a solution containing a suitable concentration of a permeant solute, the cell volume initially shrinks and then returns to its initial volume. Cell volume reaches a well defined minimum which is achieved when the volume of the solute moving into the cell is exactly balanced by the volume of water moving outward in response to the remaining osmotic pressure gradient. The details of the experimental technique and the rapid-stop flow apparatus used for rapid mixing are discussed elsewhere[1-4].

A great deal of information about the passive properties of the cell membrane can be obtained from the knowledge of the theoretical equation that describes the time course of cell volume. For example, in the shrinking phase, water and the permeant solute are moving in the opposite direction, and thus there is a solvent drag effect opposing solute diffusion. On the other hand, during the swelling phase there is a solvent drag helping solute diffusion. Lack of theoretical guidance has limited the use of the experimentally measured time course of cell volume. Only the minimum point on the curve has been used so far to calculate the diffusional permeability coefficient, ω, of a permeating molecule[4].

ANALYSIS

When solvent and an uncharged solute are moving across a membrane, KEDEM AND KATCHALSKY[5] and KATCHALSKY AND CURRAN[6], give the following relations to express the volume flow and solute flux,

$$J_v = -L_p\Delta\pi_i + L_{pd}\Delta\pi_s \quad (1)$$

$$J_s = (1 + L_{dp}/L_p)\bar{c}_s J_v + \omega\Delta\pi_s \quad (2)$$

in which J_v is the volume flow per unit area in cm/sec, and flow into the cell is considered to be in the positive direction. The osmotic pressure due to the impermeant solute (NaCl) is denoted by $\Delta\pi_i$, which is defined as $\Delta\pi_i = RT(c^\circ_i - c_i^{\Delta x})$; $\Delta\pi_i$ has units of dynes/cm^2. The solution bathing the external surface of the cell membrane is denoted by the superscript $^\circ$ and that bathing the internal surface by Δx. L_p, L_{pd} and L_{dp} are phenomemelogical coefficients in cm^3/dyne per sec ($\sigma \equiv -L_{pd}/L_p$ and $\sigma' \equiv -L_{dp}/L_p$). $\Delta\pi_s$ is the osmotic pressure of the permeable solute and is defined as $\Delta\pi_s = RT(c^\circ_s - c_s^{\Delta x})$. The c's denote concentrations in osmoles/cm^3. J_s is solute flux per unit area in moles/sec. $\bar{c}_s$ is the average concentration and is defined as $\bar{c}_s = (c^\circ_s - c_s^{\Delta x})/\ln c^\circ_s/c_s^{\Delta x}$. The subscripts i and s denote impermeant and permeable solutes, respectively. The diffusional permeability coefficient, ω, is expressed in moles/dyne per sec.

Rewriting Eqs. 1 and 2 in terms of non-dimensional variables as was done by JOHNSON AND WILSON[7], one obtains

$$v(dv/d\tau) = -(1 + a)v + (1 + as) \quad (3)$$

$$(ds/d\tau) = (1 - \sigma')(\bar{c}_s/c^\circ_s)(dv/d\tau) + r(v - s)/v \quad (4)$$

where $v = V'/V'_0$, $\tau = A'l_pRTc^\circ_i t/V'_0$, $a = \sigma c_s{}^\circ/c_i{}^\circ$, $s = n_s/c_s{}^\circ V'_0$, $r = \omega/L_p c_i{}^\circ$ in which A' is the red cell surface area, considered to remain constant[1,8]; V' and V'_0 are, respectively, the volume of cell water at $t = 0$, and at any given time; and n_s is the amount of permeant solute in the cell water.

Solving for s from Eqn. 3 and equating $ds/d\tau$ with Eqn. 4 one obtains the following differential equation:

$$(v^2)(d^2v/d\tau^2) + (v)(dv/d\tau)^2 + (1 + a + r)(v)(dv/d\tau) -$$
$$(a)(v)(1 - \sigma')(\bar{c}_s/c^\circ_s)(dv/d\tau) + r(v - 1) = 0 \quad (5)$$

The concentrations c^0_s and $c_i{}^0$ remain constant during the time course of cell volume changes since the volume occupied by red cells is only 1% of the total volume of the suspension[1].

Presence of the time dependent term, $\bar{c}_s$, and of $(dv/d\tau)^2$ contributes to the difficulty in integrating this equation. SHA'AFI *et al.*[4,9] have derived a similar equation and made use of it in the region of minimum volume, *i.e.* when $dv/d\tau = 0$. They have used this condition to determine the permeability coefficient, ω, for various solutes across the membranes of human and dog red cells. However, the use of this condition is limited to the determination of ω and no other information can be obtained about the other parameters, in particular σ'.

All the three phenomenological coefficients L_p, σ and ω have been determined experimentally across mammalian red cell membranes and many other biological

membranes[4,9–13]. At the present, there is no available method to determine σ', and it has always been assumed to be equal to σ. The difficulty in measuring σ' is not experimental but rather theoretical in nature.

In terms of the new variables, $\bar{c}_s$ can be written as:

$$\bar{c}_s = (c^\circ_s)\,(x)/\ln\,(1 + x) \tag{6}$$

where

$$x = \{(v)\,(dv/d\tau) + (v - 1)\}\,\{av\}^{-1} = (s - v)/(v) \tag{7}$$

The logarithm in Eqn. 6 may be expanded by means of the series:

$$\ln\,(1 + x) = 2(x)\,(x + 2)^{-1} + \sum_{n=1}^{\infty} \{(2)\,(2n + 1)^{-1}(x)^{2n+1}(x + 2)^{-(2n+1)}\} \tag{8}$$

By inspection, $0 \leqslant s/v \leqslant 1.0$ and $|x| \leqslant 1.0$; the equality sign for x holds only at $t = 0$, when $s = 0$. In practice, the higher order terms in Eqn. 8 can be neglected. The maximum error is less than 4%.

Substituting for $\bar{c}_s$ in Eqn. 5 and collecting terms one obtains the following differential equation:

$$(2v^2)\,(d^2v/d\tau^2) + (v)\,(dv/d\tau)^2\,(1 + \sigma') + (v)\,(dv/d\tau)\,(1 + 2r + 2a\sigma' + \sigma') + (1 - \sigma')\,(dv/d\tau) + 2r(v - 1) = 0 \tag{9}$$

At the minimum point, $\tau = \tau_m$, $v = v_m$, $s = s_m$, and $(dv/d\tau) = 0$. Solving for v_m from Eqn. 3 one obtains the following relation:

$$1 - v_m < a \tag{10}$$

For small a, the change in cell volume is small, and a solution to Eqn. 9 can be obtained by a perturbation analysis where v is expanded as a power series in a, as given by Eqn. 11.

$$v = 1 + av_1 + a^2v_2 \ldots \tag{11}$$

The value of $a = \sigma c^\circ_s/c_1^\circ$ can be controlled experimentally. Furthermore, when a is small, ω can be considered constant independent of J_v. SHA'AFI *et al.*[4] have found that ω depends slightly on J_v.

Substituting Eqn. 11 in Eqn. 9, collecting terms of the same power of a, and neglecting a^3 and higher order terms result in series of equations as follows:

$$d^2v_1/d\tau^2 + (1 + r)\,(dv_1/d\tau) + rv_1 = 0 \tag{12}$$

$$2d^2v_2/d\tau^2 + 2(1 + r)\,(dv_2/d\tau) + 2rv_2 = -4v_1(d^2v_1/d\tau^2) - (1 + \sigma')\,(dv_1/d\tau)^2 - (c_1v_1 + 2\sigma')\,(dv_1/d\tau) \tag{13}$$

where $c_1 = (1 + 2r + \sigma')$.

The boundary conditions which must be satisfied are as follows: $v_j\,(0) = 0$ and $v_j\,(\infty) = 0$, for $j = 1, 2, \ldots$ The solutions of Eqns. 12 and 13 which satisfy the boundary conditions are given by Eqns. 14 and 15.

$$v_1 = A\{\exp\,(-r\tau) - \exp\,(-\tau)\} \tag{14}$$

$$v_2 = \{A^2\}\,\{(B - \sigma' r\tau)\,\exp\,(-r\tau)\} + \{A^2\}\,\{(B + \sigma'\tau)\,\exp\,(-\tau)\} + \{A^2\}\,\{(A_{11})\,(2r^2 - r)^{-1}\exp\,(-2r\tau) + (A_{22})\,(4 - 2r)^{-1}\exp\,(-2\tau) +$$

$$(A_{12}/r) \exp(-r\tau - \tau)\} \tag{15}$$

where

$$A = 1/(r-1),\ A_{11} = \{(r/2)\}\{(1+\sigma')(1-r) - 2r\}$$

$$A_{22} = (r-2),\quad A_{12} = \{1/2\}\{(r-1)(r+\sigma') + r^2 + 3\}$$

and

$$B = -\{1/2\}\{(A_{11})(2r^2-r)^{-1} + (A_{22})(4-2r)^{-1} + (A_{12}/r)\}$$

Within the order $(1-v_m)^3$, the cell volume time course is given by Eqn. 16

$$v = 1 + \{aA\}\{1 + aA(B-\sigma' r\tau)\}\{(\exp(-r\tau)\} - \{aA\}\{1 - aA(B+\sigma'\tau)\}$$

$$\{\exp(-\tau)\} + \{aA\}^2\{A_{11}(2r^2-r)^{-1}\exp(-2r\tau) +$$

$$(A_{22})(4-2r)^{-1}\exp(-2\tau) + (A_{12}/r)\exp(-\tau r - \tau)\} \tag{16}$$

Similar analysis can be used to derive a similar equation if, at $t = 0$, the permeating solute is present in the cell at a given concentration.

APPLICATION

In the case where the Onsager reciprocal relation holds, *i.e.* $\sigma = \sigma'$, Eqn. 16 can be used to determine the three coefficients, L_p, ω and σ, which characterize the cell membrane. The experimentally determined time course of cell volume can be fitted by Eqn. 16. The three phenomenological coefficients can then be easily calculated from the parameters which give the best fit. This kind of analysis is not limited to mammalian red cells, but can be used for sea urchin eggs, plant cells, squid giant axon, blood brain barrier, Ehrlich ascites tumor cells and many other systems. In some cases the surface area cannot be assumed constant, and its variation with time must then be taken into consideration.

The value of this analysis is not in calculating the phenomenological coefficients since other methods can be used equally well, if not better[1,2,4,9,10]. Its value lies in the fact that for the first time, at least in biological systems, it is possible, with the aid of this analysis, to calculate σ' and thus test for the Onsager reciprocal relation. For this analysis to be of use it must satisfy two conditions: (a) the theoretically generated time course of cell volume changes must be sensitive to variations in σ'; (b) the analysis must be relatively simple to use.

Let us consider the following experiment in order to see how this analysis can be used. A suspension of red blood cells, at a low hematocrit (about 1%) and in an isoosmotic NaCl solution (0.3 osmole/l), is rapidly mixed with an equal volume of solution containing 0.3 osmole/l NaCl and 0.188 osmole/l of permeating solute. Assume that we are dealing with dog red cells and the permeating solute is propionamide. The details of experimental techniques are thoroughly discussed elsewhere[1,2,4,9,10]. It is worth noting that the small perturbation technique used by FARMER AND MACEY[3,10] is probably better suited for this analysis than that used by SHA'AFI *et al.*[4]. Under the present experimental conditions $L_p = 1.55 \cdot 10^{-11}$ cm^3/dyne per sec, $A' = 1.24 \cdot 10^{-6}$ cm^2, $V_0' = 0.49 \cdot 10^{-10}$ cm^3 (cell water is 72%), $\sigma = 0.56$, and $\omega = 7 \cdot 10^{-15}$ moles/dyne per sec. These values are taken from SHA'AFI *et al.*[9,14,15]. Using these parameters, $a = 0.35$, $r = 1.5$ and $A = 2.0$. The theoretical time course of cell volume changes,

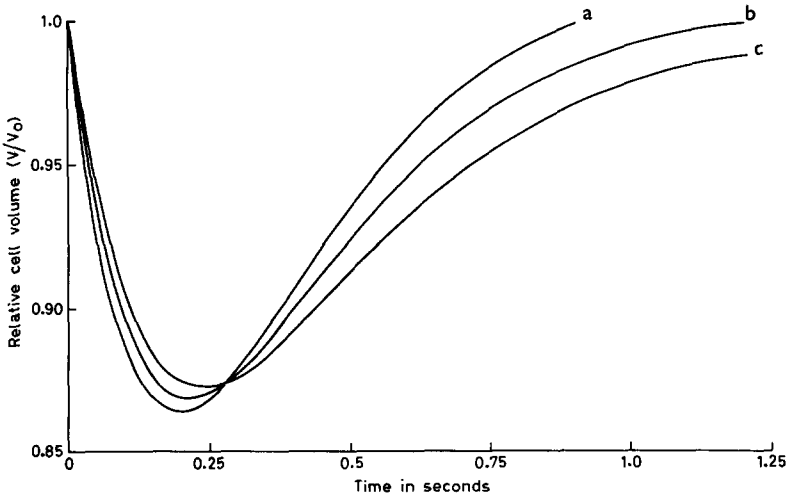

Fig. 1. Computer generated curves for the variation of relative cell volume with time, for three values of σ'. *a* is for $\sigma' = 0.9$, *b* is for $\sigma' = 0.56$, and *c* is for $\sigma' = 0.2$.

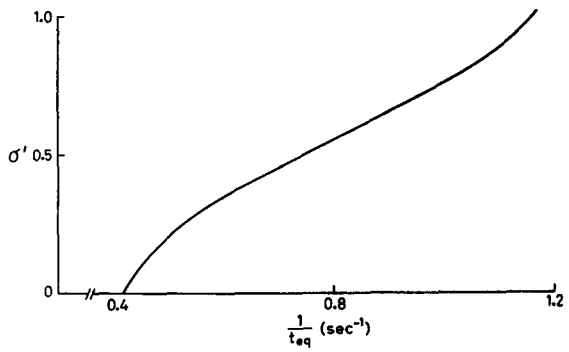

Fig. 2. Variation of σ' with the inverse of equilibration time.

TABLE I

VARIATION OF EQUILIBRATION TIME WITH σ'

σ'	t_{eq} (*sec*)
0	2.40
0.1	2.25
0.2	2.00
0.3	1.80
0.4	1.50
0.5	1.35
0.6	1.15
0.7	1.05
0.8	0.95
0.9	0.92
1.0	0.85

calculated from Eqn. 16 for three values of σ', is shown in Fig. 1. The sensitivity of cell volume to variation in σ' is quite good. Other solutes were also tested in both dog and human red cells. There are three important points which can be easily determined along the curve describing the time course of cell volume changes. These points are the time required to reach the minimum volume, t_m, the volume of the cell at the minimum, V_m, and the time required to reach equilibrium, t_{eq}. The first two points are not very sensitive to changes in σ', as evident from Fig. 1, whereas t_{eq} is extremely sensitive to changes in σ'.

Table I summarizes the values of t_{eq} for different values of σ', and Fig. 2 shows the variation of σ' with the inverse of t_{eq} for the solute propionamide in dog red cells. There is a large variation in t_{eq}, from the experimental viewpoint, since changes in cell volume can be followed experimentally every 0.005 sec[4]. With the help of Fig. 2 it becomes extremely a simple matter to determine σ'. The authors are well aware of the importance of coupling this theoretical analysis with experimentation. The lack of experimental setup prevents us from doing so.

ACKNOWLEDGEMENT

We should like to express our thanks to Professor A. K. Solomon for his stimulating discussions about this problem. A great deal of thought and formulation of this problem have been carried out by one of us (R. I. Sha'afi) during his stay at the Biophysical Laboratory, Harvard Medical School, Boston, Mass., U.S.A. The assistance of Mr. G. Boyadjian of the University Computer Center is greatly appreciated. This study has been supported in part by the unversity medical sciences research program.

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