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A PROPOSED TEST FOR THE PORE HYPOTHESIS

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SUMMARY

A theoretical analysis is presented for the calculation of tritiated water (^{3}HHO) permeability coefficient in red blood cells during osmotic water flow. This method can also be used to calculate the permeability coefficients of small hydrophilic solutes under similar experimental conditions. With the aid of this analysis it becomes feasible experimentally to test the pore model hypothesis for the red blood cell membrane. Previously determined experimental values were substituted for parameters in the final equation to show how this hypothesis can be tested.

The concept of the pore model for biological membranes and in particular for the human red blood cell membrane has not been totally accepted by the leading investigators in this field in spite of a large body of evidence[1]. Even those who are strong advocates of this view concede that the experimental data at hand do not rule out other interpretations but are merely consistent with the pore model hypothesis. Even though some of the outstanding arguments put forth by DAINTY[2] against this concept have been experimentally ruled out by SHA'AFI *et al.*[3] and RICH *et al.*[4], there is, however, one important point as yet unresolved. This has to do with experimental verification of the effect of osmotic water flow on the diffusion of tritiated water (^{3}HHO) and tracer diffusion of small hydrophilic solutes such as urea. Measurements on both the stop-flow and continuous-flow apparatus can be combined to provide an excellent opportunity for the investigation of this question. This can be done by measuring ^{3}HHO diffusion in red blood cells under conditions in which the cell volume is continuously changing during the course of the experiment. ^{3}HHO movement can be measured while osmotic flow of water is taking place in either the same or the opposite direction of ^{3}HHO movement. If the concept of the pore model is valid, one would expect an increase in ^{3}HHO movement when the osmotic water flow is in the same direction as the ^{3}HHO; a decrease would be expected when it is in the opposite direction. Similar behavior would be expected for the radioactive movement of small hydrophilic solutes which are assumed to permeate the cell membrane *via* the aqueous pores. SOLOMON[1] is well aware of the importance of this kind of approach. Such findings would be hard to interpret without thinking in terms of a porous-like structure for the red blood cell membrane. Lack of theoretical guidance was the main reason for not carrying out such investigation. The needed theoretical analysis is presented in this short communication.

The basic equations which govern volume flow and tracer diffusion in red blood cells are as follows[3,5]:

$$dV_q/dt = -AL_pRT(c_{i0}-c_{ii}) \quad (1)$$

$$dP/dt = -Kp + Kq \quad (2)$$

in which V_q is the volume of cell water, L_p is the hydraulic water conductivity which has been shown to be independent of time but depends on medium osmolality or direction of water flow[6,7]. R and T have their usual meanings, c_{i0} and c_{ii} are the concentrations of the impermeative solute (NaCl) in the medium and the cell, respectively, t is time and A is the cell surface area which is assumed to be constant in the course of the experiment[4]. K is a constant and is the same for the inward and outward movement of the tracer, since the membrane is assumed to be symmetrical[5], p and q are the specific radioactivities in the medium and the cell, respectively, and P is the amount of radioactivity in the medium $= V_p p$. c_{i0} varies with time and cannot be taken as constant as is usually done[3].

But

$$c_{i0} = c^0_{i0}V^0_p/V_p,\ c_{ii} = c^0_{ii}V^0_q/V_q \text{ and } V_q + V_p = V^0_q + V^0_p = V^0_q\left(1 + \frac{1}{H^0}\right)$$

where V_p is the volume of the medium, H is defined as V_q/V_p, and the superscript ^{0} refers to $t = 0$. Eqn. 1 can be rewritten as follows:

$$-(a+c)^{-1}x\,dx + ab(a+c)^{-2}\,dx + b^2ac(a+c)^{-2}(cx+ax-bc)^{-1}\,dx = -dt \quad (3)$$

in which $a = AL_pRTc^0_{i0}/V^0_q$, $b = 1 + H^0$, $c = AL_pRTc^0_{ii}/V^0_q$ and $x = V_q/V^0_q$. Integrating Eqn. 3 for the boundary condition $x = 1$ when $t = 0$ one gets:

$$(1-x^2)(2a+2c)^{-1} + ab(a+c)^{-2}(x-1) + ab^2c(a+c)^{-3}\ln\frac{cx+ax-bc}{c+a-bc} = -t \quad (4)$$

By inspection, $0 < (ax+cx-bc)/(a+c-bc) \leq 2$ since in an actual experiment the cell volume does not change by more than 20 %, *i.e.* $0.8 \leq x \leq 1.2$ (ref. 6). The natural logarithm can be expanded within these limits in terms of a power series as follows:

$$\ln\left(\frac{ax+cx-bc}{a+c-bc}\right) = \frac{ax+cx-bc}{a+c-bc} - 1 - \tfrac{1}{2}\left(\frac{ax+cx-bc}{a+c-bc} - 1\right)^2 + \ldots$$

For an actual experiment the higher order terms are less than 2 %. The value of x calculated from Eqn. 4 is as follows:

$$x = B/2n \pm (B^2 + 4nE + 4nt)^{\frac{1}{2}}/2n \quad (5)$$

Upon using actual experimental values for these parameters, it turns out that the positive root is for the case when the cells are swelling, *i.e.* $1 \leq x \leq 1.2$ and the negative root is for the case in which the cells are shrinking, *i.e.* $0.8 \leq x \leq 1.0$; where

$$m = a + c - bc$$

$$n = (m^2 + ab^2c)(2am^2 + 2cm^2)^{-1}$$

$$B = ab/m^2$$

$$E = -ab(a+c)^{-2} + (2a+2c)^{-1} - 2ab^2c(3m^2 + 4bcm + b^2c^2)(2m)^{-2}(a+c)^{-3}$$

The parameters m, n, B, and E all are constants and can be easily calculated from experimental conditions.

Assuming that the back flow of radioactive tracers is negligible during the course of the experiment, Eqn. 2 can be rewritten as follows:

$$dp/p + dV_p/V_p = -K dt/V_p \tag{6}$$

But

$$V_p = V_q^0(b/H^0 - x) = (V_q^0/2n)(\alpha \pm \lambda)$$

where

$$\lambda^2 = B^2 + 4nE + 4nt,\ \alpha = 2nb/H^0 - B$$

The above assumption is certainly valid in the case of small hydrophilic nonelectrolytes such as urea since the maximum amount that enters the cells during the course of the experiment is about 25 % of the total medium radioactivity. A straightforward analysis yields the following solution to Eqn. 6:

$$\ln\left[\frac{p(\alpha+\lambda)}{p^0(\alpha+\lambda^0)}\right] = -\frac{K}{V_q^0}\left[\lambda - \lambda^0 + \alpha \ln\frac{\alpha+\lambda^0}{\alpha+\lambda}\right] \text{ for } 0 < x \leq 1.0 \tag{7a}$$

and

$$\ln\left[\frac{p(\alpha-\lambda)}{p^0(\alpha-\lambda^0)}\right] = -\frac{K}{V_q^0}\left[\lambda^0 - \lambda + \alpha \ln\frac{\alpha-\lambda^0}{\alpha-\lambda}\right] \text{ for } 1.0 \leq x \leq 2 \tag{7b}$$

In order to see how p/p^0, which is an experimentally determined quantity, varies with time, let us consider the following experiment: A suspension of red blood cells at a given hematocrit and in iso-osmotic solution (286 mosM) is rapidly mixed with an equal volume of a hyperosmotic solution (338 mosM) containing tritiated water (^{3}HHO). After mixing, the time-course of the disappearance of ^{3}HHO from the medium is followed. This experimental setup is identical with those of PAGANELLI AND SOLOMON[5], BARTON AND BROWN[8], RICH *et al.*[6] and VIEIRA *et al.*[9], except that the solution which contains ^{3}HHO is hyperosmotic instead of iso-osmotic. The details of the experimental procedure are discussed elsewhere[5,8]. Under the present experimental conditions $L_p = 1 \cdot 10^{-11}$ cm^3/dyne·sec, $A = 1.67 \cdot 10^{-6}$ cm^2, $V_q^0 = 4.9 \cdot 10^{-11}$ cm^3, $RTc_{ii}^0 = 6.98 \cdot 10^6$ dynes/cm^2, $RTc_{io}^0 = 7.62 \cdot 10^6$ dynes/cm^2, and $H^0 = 0.3$. These values are taken from ref. 3. Using these parameters, λ^0 and α can be easily calculated as: $\lambda^0 = 0.159$ sec, $\alpha = 2.48$ sec. Table I gives the values of p/p^0 calculated from

TABLE I

TIME-COURSE OF MEDIUM SPECIFIC ACTIVITY FOR TWO VALUES OF ^{3}HHO RATE CONSTANT

Time (*sec*)	*Medium specific activity*, p/p^0	
	$K/V_q^0 = 50$ *sec*$^{-1}$	$K/V_q^0 = 80$ *sec*$^{-1}$
0	1.0	1.0
0.005	0.92	0.88
0.010	0.85	0.78
0.015	0.78	0.69
0.020	0.72	0.61

Eqn. 7a using two different values of K/V_q^0. These values of K/V_q^0 and the range in time interval represent actual experimental values[8,9]. For such experimental conditions the higher order terms in the series expansion contribute less than 1 % to the total value of the function.

To test the validity of the pore hypothesis, one first determines the value of K/V_q^0 under conditions when no volume flow of water is taking place, *i.e.* the solution which contains ^{3}HHO is iso-osmotic. This can be done using any of the procedures discussed in refs. 5, 6, 8 and 9. Then one determines the value of K/V_q^0 by plotting the left-hand side of Eqn. 7a or 7b against the right-hand side as shown in Fig. 1

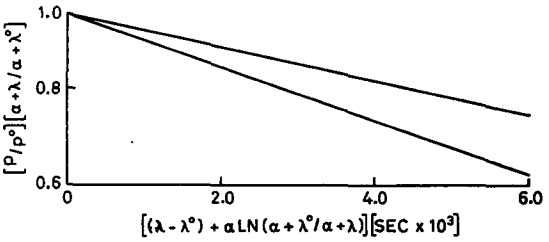

Fig. 1. The right-hand side of Eqn. 7a is plotted against the left-hand side for two values of K/V_q^0. The upper line is for $K/V_q^0 = 50$ sec^{-1} and the lower line is for $K/V_q^0 = 80$ sec^{-1}.

for the data in Table I under two different experimental conditions: first, when the solution containing ^{3}HHO is hypo-osmotic and second, when the solution is hyperosmotic. If the pore hypothesis is not valid, one should expect the value of K/V_q^0 to be the same for the two conditions and equal to the value determined when no volume flow of water is taking place. On the other hand, if the pore hypothesis is valid, one should expect that the value of K/V_q^0 determined under the hypo-osmotic condition to be greater than that determined under hyperosmotic case. Moreover, the value of K/V_q^0 determined when no osmotic water flow is taking place should lie between the hypo-osmotic case and the hyperosmotic case. The value of L_p to be used must correspond to the experimental condition[6,7]. The same analysis can be used when dealing with the radioactive movement of small hydrophilic nonelectrolytes which are assumed to permeate the cell *via* the aqueous pores. Further evidence for or against the pore hypothesis can also be obtained using the same procedure with red blood cells of other mammalian species, whose membranes are characterized by different pore sizes[6].

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