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A NEW THEORY OF TRANSPORT FOR CELL MEMBRANE PORES

I. GENERAL THEORY AND APPLICATION TO RED CELL

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SUMMARY

Previous theories for the transport of solutes through cell membrane pores have not considered the fact that the pore may be so small that the water and solute molecules cannot pass by each other. A theory has been developed in this paper for such a pore. Transfer of solute through the pore requires movement of the whole pore "plug". Relations are derived between the macroscopic permeability constants and the pore structure which are much more general and rigorous than any previous result. For example, the average number of water molecules per pore can be determined just from the values of the hydraulic and diffusive permeability of the whole membrane to water. This derivation does not require any assumptions about the pore shape or about the interactions of the water molecules with each other or with the pore walls. The theory is applied to the experimental data for the red blood cell. Although there is general agreement between the theory and experiment, there are some important discrepancies which are discussed. According to arguments given in detail in an appendix, it appears that there is a serious methodological error in the measurements made by Goldstein and Solomon (1960) (J. Gen. Physiol. 44, 1–17) of the red cell reflection coefficients and these values were not used when the theoretical and experimental results were compared.

INTRODUCTION

The existence of aqueous pores in cell membranes is currently an unsettled question. One of the difficulties in answering this question is the lack of a satisfactory quantitative theory of the kinetics of transport in pores of the size postulated (≈ 3 Å radius). The only theory which has so far been able to make quantitative predictions about pore structure is based on the assumption that the water can be treated as a continuum. For example, in calculating the resistance to diffusion of the solute in the pore it is assumed that the equations which describe the fall of a marble through a very long cylindrical tube filled with water are applicable to the membrane pore [1]. The problem with this assumptions is clearly illustrated by the following example. Consider the diffusion of urea through a 3.5-Å radius pore. The sum of the diameter

of the urea molecule (> 4 Å) plus the water molecule (≈ 3 Å) is greater than the pore diameter so that the urea molecule effectively plugs the pore and the continuum idea that the water flows around the urea molecule is completely wrong. In this paper a quantitative theory of the kinetics of transport in pores which are effectively plugged by the solute (as in the above example) will be presented. It turns out that this assumption leads to a remarkable simplification of the problem and allows for a rigorous derivation of the kinetics. Predictions from this theory will then be compared with experimental values for red cells. In the second paper of this series [2] it will be shown by the use of computer simulation (molecular dynamics) that the basic assumptions of this theory are correct for one particular type of solvent.

The derivation will be given first for the important special case where the solute is a tracer of water and then for the general case of an arbitrary solute. The special case for water is discussed separately because it provides a simple illustration of the basic idea of the theory and because the results for this case should have important experimental applications.

NOTATION

N	Avogadro's number
β	frictional coefficient
ω	diffusive permeability constant
σ_s, σ_v	reflection coefficient for solute flux and volume flux equations, respectively
L_p, L_p^e, L_p^s	hydraulic permeability of the entire membrane; of a single pore that contains only water; or of a pore that contains one solute molecule, respectively
$c(x)$, $\bar{c}$, Δc	concentration, integrated average concentration, and concentration difference in molar units
N_p, N_p^e, N_p^s	total number of pores; number of pores that contain no solute; and number that contain one solute molecule, respectively
n_w	average number of water molecules per pore
l	pore length
V_p, V_p^s	volume occupied in bulk solution by contents of a pore that contains only water or one solute molecule, respectively
ΔP_a, ΔP_F, ΔP_0	applied pressure difference; pressure difference equivalent to drag force; pressure difference produced by force on solute in bulk solution (osmotic)
F	force
$U(x)$	potential energy of force
κ_s	relative partition constant
J_s	solute flux (molar)
J_v, J_v^e, J_v^f	volume flux through total membrane, through pores that contain only water; and through pores that contain one solute molecule
$\bar{v}$	drift velocity
$\overline{V}_w$, $\overline{V}_s$	molar volume of water; solute, respectively

SPECIAL CASE: SOLUTE IS A TRACER OF WATER

In this section it will be assumed that the pore radius is so small that two water molecules cannot get by each other. It will be shown that for this special case the following general relationship among the average number of water molecules per pore (n_w), the molar volume of water ($\overline{V}_w$), the diffusive (ω), and hydraulic permeability (L_p) of the membrane to water can be derived:

$$n_w = \frac{L_p}{\omega \overline{V}_w} \tag{1}$$

Eqn 1 is remarkable because it allows one to obtain information about a single pore (n_w) from two macroscopic measurements. For example, if the experimental values of $1.35 \cdot 10^{-13}$ moles/dyne per s for ω and $1.2 \cdot 10^{-11}$ cm^3/dyne per s for L_p for the human red cell [3] are substituted in Eqn 1, a value of about 5 water molecules per pore is found. As will be shown, this result requires only the assumption that (1) the pore is narrow enough that the water molecules cannot pass each other and (2) that the water traverses the cell membrane only via the pores.

The derivation of Eqn 1 is based on a generalization of the Einstein derivation [4] of the relationship between the coefficient of diffusion (D) and the frictional drag (β):

$$D = \frac{kT}{\beta} \tag{2}$$

Consider the following hypothetical experiment. One has a beaker of water which is in a force field F (Fig. 1A). The beaker contains a small concentration of a tracer of water which is identical to water except that each tracer molecule feels a force F and the water does not feel the force. (For example, the tracer could be slightly denser than water and then the force would be gravitational.) The tracer will distribute itself in the beaker according to the Boltzmann distribution:

$$\begin{aligned} c(x) &= c(0) \cdot e^{-U(x)/kT} \\ &= c(0) \cdot e^{Fx/kT} \end{aligned} \tag{3}$$

where $c(x)$ is the tracer concentration, $U(x)$ is the potential of the force and for a constant force (F) : $U(x) = -Fx$. The basic idea of Einstein was that the equilibrium

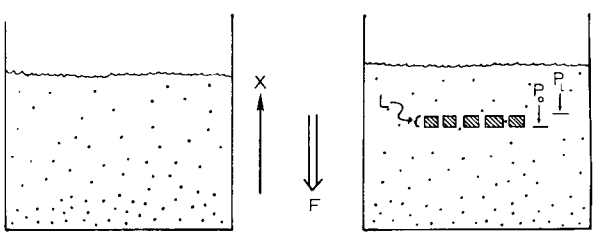

Fig. 1. The "hypothetical" experiment that is used in the derivation. An equilibrium concentration gradient of the solute(dots) is established by the external force (F). After the gradient has been established (A) the membrane is placed in the beaker (B).

concentration distribution (Eqn 3) could be thought of as resulting from a balance between a diffusive flux (J_D) and a drift flux (J_F):

$$J_D = -D\frac{dc}{dx} = -J_F = -c\bar{v} = -c\frac{F}{\beta} \tag{4}$$

where $\bar{v}$ is the drift velocity and, by definition, $F = \beta\bar{v}$. Substituting the Boltzmann relationship (Eqn 3) into Eqn 4 yields Einstein's fundamental relationship (Eqn 2).

This same procedure can be applied to the membrane problem. Imagine that a membrane is placed in the beaker (in which the tracer has the equilibrium distribution of Eqn 3) with the pores aligned parallel to the force (Fig. 1B). Since the membrane clearly does not disturb the equilibrium state, the bulk tracer concentration is still given by Eqn 3. Thus, there is a concentration difference (Δc) across the membrane:

$$\Delta c = c(0) - c(l) = c(0)\cdot(1 - e^{Fl/kT}) \tag{5}$$

where l is the membrane thickness and x = 0 is arbitrarily placed at the lower edge of the membrane. Since the system is at complete equilibrium, there cannot be any net flux in the pores. Extending Einstein's basic hypothesis, I will assume that this equilibrium of tracer flux in the membrane can be thought of as the result of a balance between a diffusive tracer flux (J_D) and a drift tracer flux (J_F). The diffusive flux is defined by:

$$J_D = \omega RT\Delta c = \omega RTc(0)\cdot(1 - e^{Fl/kT}) \tag{6}$$

Equating this flux to the drift flux one can obtain a relation between ω and the frictional coefficient (β). So far this analysis is completely general and can be applied to any membrane. In general, in order to use this relationship, β must be determined from the solution for some particular model of the transport process (for example, continuum flow in a cylindrical pore). However, for the special case of pores in which the water molecules cannot pass each other, the drift flux can be directly related to the macroscopic hydraulic permeability as is shown by the following derivation. Consider such a pore containing a tracer molecule drifting under the force F. For the tracer molecules to move along the pore in a given direction, all the molecules ahead of it must also move so that this drift can be stopped by applying some pressure difference ΔP_F to the bulk solutions across the pore. Since this pressure difference produces exactly the same flux through the pore as the force, the drift flux can be expressed in terms of ΔP_F instead of F. Thus, if L_p^e is the hydraulic permeability per pore, and N_p^w is the number of pores that contain a tracer of water ("full") and it is assumed that there is no more than one tracer per pore (see below) then the volume flux through the "full" pores (J_v^f) due to the applied force F is:

$$J_v^f = N_p^w L_p^e \Delta P_F \tag{7}$$

Since the molecules cannot pass each other, every tracer that crosses the membrane must carry a volume v_p equal to the volume the pore contents occupy in the bulk solution:

$$J_v^f = Nv_p J_F \quad \text{or} \quad J_F = \frac{N_p^w L_p^e \Delta P_F}{Nv_p} \tag{8}$$

where N is Avogadro's number and J_F is the molar flux. The relationship between ΔP_F and F is obtained from the condition that the work done by an infinitesimal displacement (dx) by the tracer must be just balanced by the work done by ΔP_F:

$$F\mathrm{dx} = \Delta P_F \mathrm{d}V$$

where $\mathrm{d}V$ is the volume displacement in the bulk solutions. From the definition of V_p it can be seen that:

$$\mathrm{d}V = \frac{\mathrm{dx}}{l} V_p \quad \text{or} \quad \Delta P_F = \frac{Fl}{V_p} \tag{9}$$

With at most one tracer per pore, N_p^w is equal to the number of tracer molecules in the membrane which is simply equal to the total (bulk) volume of water molecules in the membrane ($N_p V_p$) times the average tracer concentration (where N_p is the total number of pores):

$$N_p^w = N \bar{c}_w N_p V_p \tag{10}$$

The average tracer concentration is (using Eqn 3):

$$\bar{c}_w = \frac{1}{l}\int_0^l c(\mathrm{x})\mathrm{dx} = \frac{c(0)}{l}\frac{kT}{F}(\mathrm{e}^{Fl/kT} - 1) = -\frac{kT}{Fl}\Delta c \tag{11}$$

Substituting Eqns 9–11 into Eqn 8, the following expression for J_F is obtained:

$$J_F = \frac{kT N_p L_p^e \Delta c}{V_p} \tag{12}$$

Equating the drift flux (Eqn 12) to the diffusive flux (Eqn 6):

$$N\omega = \frac{N_p L_p^e}{V_p} \tag{13}$$

Finally, since $N_p L_p^e$ is equal to L_p (the membrane hydraulic permeability when there are no permeable solutes) and n_w is equal $NV_p/\bar{V}_w$, Eqn 1 is obtained:

$$n_w = \frac{L_p}{\omega \bar{V}_w} \tag{1}$$

This derivation is very general in the sense that no assumptions about interactions between the water and pore wall or about the partition of the water between the membrane and bulk solution are made. The derivation uses only quantities which are defined in terms of bulk fluid measurements. In particular, it is not necessary to use undefined quantities such as the concentration or pressure in the pore. In addition to the assumption that the water molecules cannot get past each other, two new ideas are used in this derivation. First, it is assumed that one can extend to the membrane Einstein's basic concept that an equilibrium can be regarded as the balance of two opposing non-equilibrium fluxes. Secondly, it is assumed that the drag force F can be replaced by an equivalent pressure difference (Eqn 9). Both these assumptions are verified by computer simulation in the second paper of this series [2] for the case where the solvent consists of hard smooth spheres.

GENERAL CASE: ARBITRARY SOLUTE AND PRESSURE DIFFERENCE

Most theoretical and experimental investigations of membrane permeability have used the following two phenomenological equations, derived from irreversible thermodynamics, to describe the kinetics of transport of non-electrolytes [5]:

$$J_s = \omega RT\Delta c + (1-\sigma_s)\bar{c}J_v \tag{14}$$

$$J_v = L_p(\Delta P_a - \sigma_v RT\Delta c) \tag{15}$$

where J_s and J_v are the solute and volume flux, σ_s and σ_v are the reflections coefficients for the two equations (and may be different), $\bar{c}$ is some average of the concentration on the two sides, and ΔP_a is the applied pressure difference. In this section, the kinetics of a pore in which the water and solute cannot pass each other will be derived in the form of Eqns 14 and 15 and the expressions for the parameters ω, σ_s, σ_v and L_p will be obtained.

The extension of the preceding derivation given for water to an arbitrary solute requires three modifications: (1) the hydraulic permeability of a pore which contains a solute (L_p^s) may not be the same as for a pore that contains only water (L_p^e). (2) The volume the pore contents occupy in the bulk solution will depend on whether the pore contains a solute (V_p^s) or only the solvent water (V_p). (3) A concentration difference can produce a volume flux (osmotic) when the solute is not identical to water.

Volume flux

The derivation will first be carried out for the case in which there is no applied pressure difference ($\Delta P_a = 0$). The derivation is based again on the thought experiment shown in Fig. 1B in which the membrane is placed in a beaker containing the solute. It is assumed that the solution is dilute enough for there to be at most one solute per pore. It is again assumed that only the solute feels the force F so that at equilibrium the solute will be distributed according to the Boltzmann relation (Eqn 3). Accordingly, the concentration difference across the membrane is again given by Eqn 5. It is again assumed that the equilibrium condition in the pores is the result of a balancing of two equal and opposite fluxes: (1) the solute and volume flux (described by Eqns 14 and 15) produced by the concentration difference and (2) the solute and volume flux produced by the applied force. Thus, in the following derivation, the fluxes produced by the force will be determined and they will then be set equal to the fluxes that would be produced by the concentration difference in the absence of the force.

In addition to the concentration difference across the membrane there is also a pressure difference ΔP_0 (see Fig. 1B) due to the force on the solute in the bulk solution parallel to the membrane. This pressure is equal to the total force on the solute molecules in a slab of water of thickness l and unit area:

$$\Delta P_0 = P(0) - P(l) = NF\int_0^l c_s(x)dx = -NFl\bar{c}_s \tag{16}$$

where $\bar{c}_s$ (the average concentration in the bulk fluid) is again given by Eqn 11 so that Eqn 16 can be rewritten as:

$$\Delta P_0 = RT\Delta c \tag{17}$$

Now the volume flux across the membrane results from two factors: the pressure difference ΔP_0 which is exerted across all the pores and the force F which acts only on the pores which contain a solute. As in the case when the solute was a tracer of water, the effect of F can be replaced by an equivalent pressure difference (ΔP_F) which is given by Eqn 9:

$$\Delta P_F = \frac{Fl}{V_p^s} \tag{18}$$

In this equation $V_p{}^s$ (the volume occupied in the bulk solution by a pore that contains a solute molecule) is used in place of V_p. Now one can immediately write down the volume flux produced by the force. For the empty pores, the volume flux is due only to the pressure difference ΔP_0:

$$J_v^e = N_p^e L_p^e \Delta P_0 \tag{19}$$

where $N_p{}^e$ and $L_p{}^e$ are the number and hydraulic permeability of the pores that contain no solute ("empty"). For the pores that contain a solute, the volume flux is the result of both ΔP_0 and ΔP_F:

$$J_v^f = N_p^s L_p^s(\Delta P_0 + \Delta P_F) \tag{20}$$

Then the volume flux produced by the concentration difference must be equal and opposite to the sum of these two volume fluxes. Also, if there is an applied pressure difference (ΔP_a) it will be felt simply as an additive pressure term in Eqns 19 and 20 so that the total volume flux produced by the concentration and applied pressure differences is given by:

$$J_v = N_p L_p^e \left[\frac{N_p^e}{N_p}(\Delta P_a - \Delta P_0) + \frac{N_p^s L_p^s}{N_p L_p^e}(\Delta P_a - \Delta P_0 - \Delta P_F)\right] \tag{21}$$

with $N_p{}^e = N_p - N_p{}^s$, this equation can be rewritten as:

$$J_v = \alpha L_p \left(\Delta P_a - \Delta P_0 - \frac{N_p^s L_p^s}{\alpha N_p L_p^e}\Delta P_F\right) \tag{22}$$

where:

$$\alpha = 1 - \frac{N_p^s}{N_p}\left(1 - \frac{L_p^s}{L_p^e}\right) \quad \text{and} \quad L_p = N_p L_p^e$$

The assumption that there is no more than one solute molecule per pore is equivalent to the assumption that:

$$\frac{N_p^s}{N_p} \ll 1 \quad \text{or} \quad \alpha \approx 1 \tag{23}$$

Thus, the deviation of α from 1 represents a second-order concentration-dependent correction to the kinetic equations. This correction is usually small and in the rest of

this paper α will be assumed to be 1. It will be shown below that this assumption (Eqn 23) is satisfied for the red cell.

Eqn 22 can be simplified if a constant κ_s (defined by Eqn 24) is introduced (using Eqn 11):

$$N_p^s = \kappa_s N N_p V_p \bar{c}_s = \kappa_s N N_p V_p \frac{kT}{Fl} \Delta c \tag{24}$$

Taking the ratio of Eqns 10 and 24:

$$\kappa_s = \frac{N_p^s \bar{c}_w}{N_p^w \bar{c}_s} \tag{25}$$

Thus κ_s is the solute partition coefficient between the membrane and bulk solution, relative to the partition of water. Substituting Eqns 17, 18 and 24 into Eqn 21 (with $\alpha = 1$) yields an expression for J_v in the form of Eqn 15:

$$J_v = L_p(\Delta P_a - \sigma_v RT\Delta c)$$

with

$$\sigma_v = 1 - \kappa_s \frac{L_p^s}{L_p^e} \frac{V_p}{V_p^s} \tag{26}$$

Solute flux

Eqn 8 relates the volume flux through the pores that contain a solute (J_v^f) to the solute flux (J_F) through the same pores due to the force F:

$$J_v^f = N V_p^s J_F \tag{27}$$

In this equation the volume occupied in the bulk solution by the contents of a pore that contain a solute molecule (V_p^s) has been used. The solute flux that results from the concentration difference must be equal and opposite to this flux where J_v^f is given by Eqn 20 so that the total solute flux (including the effect of ΔP_a) is given by:

$$J_s = \frac{N_p^s L_p^s}{N V_p^s} (\Delta P_a - \Delta P_0 - \Delta P_F) \tag{28}$$

This equation can be put into the same form as Eqn 14 by the following manipulations. Adding and subtracting the same term and using Eqns 17–24:

$$\begin{aligned} J_s &= \frac{N_p^s L_p^s}{N V_p^s} (\Delta P_a - \Delta P_0 - \Delta P_F) + \frac{N_p^s (L_p^s)^2 \kappa_s \Delta P_0 V_p}{N (V_p^s)^2 L_p^e} - \frac{N_p^s (L_p^s)^2 \kappa_s \Delta P_0 V_p}{N_p (V_p^s)^2 L_p^e} \\ &= \frac{L_p^s \kappa_s V_p \bar{c}}{V_p^s L_p^e} J_v + \frac{N_p \kappa_s L_p^s V_p}{N (V_p^s)^2} \Delta P_0 \left(1 - \frac{N_p^s L_p^s}{N_p L_p^e}\right) \end{aligned} \tag{29}$$

The second term in the parenthesis is negligible (Eqn 23) and can be dropped. Eqn 29 is now in the same form as Eqn 14 (since $\Delta P_0 = RT\Delta c$) and the parameters ω and σ_s can be identified:

$$\omega = \frac{N_p \kappa_s L_p^s V_p}{N(V_p^s)^2} \quad \text{and} \quad \sigma_s = 1 - \kappa_s \frac{L_p^s}{L_p^e} \frac{V_p}{V_p^s} \tag{30}$$

Comparing Eqns 26 and 30 it can be seen that σ_s and σ_v are identical. This equality can be obtained from the irreversible thermodynamic derivation of Eqns 14 and 15 if one assumes that Onsager's reciprocity relationship is valid. In order to simplify the argument, the ΔP_0 term was not included in the derivation for the special case where the solute was a tracer of water. This omission is justified since it is easy to show that $\Delta P_F \gg \Delta P_0$ (see Eqns 9, 10, 16 and 23). For this special case ($V_p = V_p^s$, $L_p^s = L_p^e$, and $\kappa_s = 1$) the general expression for ω (Eqn 30) reduces to Eqn 13 and $\sigma = 0$.

Substituting the expression for ω, σ can be rewritten as:

$$\sigma = 1 - \frac{N\omega V_p^s}{L_p} \tag{31}$$

Eqn 31 is very similar to the expression for the reflection coefficient (σ'_v) for a membrane in which the solute moves by dissolution and diffusion through the lipid film [6]:

$$\sigma'_v = 1 - \frac{\omega \bar{V}_s}{L_p} \tag{32}$$

The only difference between Eqns 31 and 32 is that the pore volume replaces the solute volume. If the pore is so narrow that two water molecules cannot pass by each other then Eqn 13 is valid and Eqn 31 can be rewritten as:

$$\sigma = 1 - \frac{\omega_s V_p^s}{\omega_w V_p} \tag{33}$$

In the next section these theoretical results will be compared with the experimental results for the red blood cell. At this point it may be useful to summarize the general assumptions behind these results. All of the above results apply only to the kinetics of transport through homogeneous pores. The derived equations will not be valid for the membrane if a significant fraction of the water or solute crosses the membrane by dissolving in the lipid or if the pores are heterogeneous. The basic distinguishing assumption of the theory presented in this paper is that the solute molecule plugs the pore. It is important to further distinguish between two different types of plugging: (1) the pore is so small that two water molecules cannot pass each other; and (2) the pore is small enough that the water molecule cannot get by the solute molecule, but two water molecules are able to pass each other. Eqn 30 for ω and Eqns 26, 30 or 31 for σ require only assumption (2). If the more restrictive assumption (1) is valid, then Eqn 1 can be derived (and the number of water molecules per pore can be determined) and σ can be written in the form of Eqn 33.

COMPARISON OF THEORY WITH RED BLOOD CELL EXPERIMENTS

The red cell is the best available model for studying the permeability properties of a cellular membrane. Its small size and simple structure eliminates the problem

of unstirred layers or intracellular compartments. Despite this apparent simplicity, recent studies have demonstrated that the permeability characteristics are complicated. For example, there is some evidence that the reflection coefficient and the hydraulic and diffusive permeability may be functions of the external osmolarity or may depend on the direction and magnitude of the water flow [7–10]. In this section it will be assumed that, to a first approximation, this complexity can be neglected in a comparison of the theory with the experimental results. Obviously, this comparison can be only qualitative until these complexities are explained.

Non-electrolyte permeability

The experimental data for the human red blood cell that will be used to test the theory are shown in Table I. All of the data in this table (except for the ω of erythritol) are from the publications of A. K. Solomon or his colleagues and should be directly comparable*. Only solutes with a low lipid solubility (ether/water partition coefficients less than that of water) and which are not supposed to have a carrier mechanism were included.

TABLE I

COMPARISON BETWEEN THEORETICAL PREDICTIONS AND EXPERIMENTAL MEASUREMENTS OF NON-ELECTROLYTE RED CELL PERMEABILITY (ω)

The molecular radius is determined from molar volume as described in the text.

Solute	Radius (Å)	ω ($\times 10^{-15}$ moles/dyne per s)	Experimental (ω/ω_{for})	Theoretical (ω/ω_{for})
Water	1.5	136 [3]		
Formamide	2.07	20 (18–22) [8, 27]	(1)	(1)
Urea	2.11	15 (14–16) [8]	0.75	0.71
Ethylene glycol	2.26	6 [28]	0.3	0.34
Acetamide	2.3	5 [8]	0.25	0.26
Methyl urea	2.34	2 [8]	0.1	0.2
1,3-Dimethyl urea	2.52	1.1 [8]	0.05	0.02
Erythritol	2.6	$1.2 \cdot 10^{-3}$ [28]	$< 10^{-4}$	0

The theoretical expression for ω is given by Eqn 30. The four parameters κ_s, L_p^s, V_p, V_p^s are not known and cannot be easily measured so that it is not possible to directly test Eqn 30. However, L_p^s (the hydraulic resistance of a pore that is plugged by a solute molecule) and V_p^s should be determined primarily by the water in the pore and should be relatively independent of the solute. Thus, to a first approximation, the ratio of the permeabilities of two solutes (Eqn 30) should be equal to just the ratio of the equilibrium partition constant of the solutes:

$$\frac{\omega_1}{\omega_2} = \frac{\kappa_1}{\kappa_2}$$

* Permeability data (ω) for some additional solutes have been recently published [11]. These data have not been used in Table I because the authors state that a method is used which tends to underestimate the permeability and that these results are not directly comparable with previous results.

Since κ_s is a function of the change in the free energy of the solute as it moves from the aqueous solution to the inside of the pore, it will depend in part on the chemical nature of the solute and the pore walls. However, it is of interest to see how much of the variation in ω can be explained just from simple steric effects. If the solute behaved like a hard sphere of radius a and the pore consisted of hard walls of radius r, then:

$$\frac{\omega_1}{\omega_2} = \frac{\kappa_1}{\kappa_2} \approx \frac{(r-a_1)^2}{(r-a_2)^2} \tag{34}$$

The application of Eqn 34 introduces the possibility of an important "fudge factor" because of the large range of acceptable values for the solute radius which are available for the investigator to choose from. I have chosen to define the solute radius on the basis of the molar volume of the pure solute [12]. I assumed that the molecules are packed as cubes with sides of length d in the pure substance so that

$$d^3 N\rho = M$$

where ρ is the solid density and M is the molecular weight. The radius of interaction is assumed to be equal to $d/2$. These values are listed in Table I.

The permeability of erythritol (Table I) is about 10^{-4} times that of urea (and part of this may be due to a carrier) which implies that the pore radius should be roughly equal to or less than the radius of erythritol (2.6 Å) if Eqn 34 is correct. Taking 2.6 Å as a rough estimate of r in Eqn 34 the solute permeabilities relative to formamide can be calculated and are shown in Table I ("Theoretical"). It can be seen that the agreement between experiment and theory is good.

In the theoretical derivation it was assumed that only a small fraction of the pores contained a solute molecule (Eqn 23) so that the concentration-dependent terms could be neglected (i.e. $\alpha \simeq 1$). If only steric factors are considered then the fraction of pores that contain a solute (Eqn 24) can be approximated by:

$$\frac{N_p^s}{N_p} = \kappa_s N V_p \bar{c}_s \approx N\pi(r-a)^2 l \bar{c}_s \tag{35}$$

where l is the pore length. Since l and r are probably less than 50 Å and 3.5 Å, respectively, N_p^s/N_p for urea is less than 0.18 c (molar). For a concentration of 0.3 molar, less than 6 % of the pores contain a solute molecule and Eqn 23 is easily satisfied.

Reflection coefficient

The bulk of the available experimental data for the reflection coefficient of the red cell comes from the paper of Goldstein and Solomon [13]. However, as is discussed in detail in Appendix, there appears to be a serious methodological error in this paper, and these data will not be used here. Only the more recent (and probably more reliable, see Appendix) data for the reflection coefficients that are listed in Table II will be relied on in this section.

The volume (V_p^s) occupied in the bulk solution by the contents of a pore that contains a solute molecule can be determined from Eqn 31 if the values of ω, σ, and L_p are known. This equation is based on the assumption that the pore is so narrow that the water and solute molecule cannot get by each other. If, in addition, the pore

TABLE II

EXPERIMENTAL VALUES OF RED CELL REFLECTION COEFFICIENTS (σ) WHICH ARE USED TO TEST THE THEORY

Solute	Species	ω ($\cdot 10^{-15}$ moles/dyne per s)	σ
Urea [8]	Human	15	0.55
Urea [24]	Beef	5.2	0.73
Ethylene glycol [10]	Beef	0.15	0.82
Glycerol [10]	Beef	0.0078	0.96
Glycerol [25, 26]	Human	0.7*	1.0*
Glycerol [25, 26]	Pig	0.027*	1.0*

* These values are for 37 °C, all the rest are for room temperature.

is so narrow that two water molecules cannot get by each other, then V_p (the bulk volume of a pore that contains only water) is also determined by Eqn 13, and Eqn 31 can be written in the form of Eqn 33. Substituting the values of σ and ω for the human red cell for urea (Table II) and an L_p of $1.2 \cdot 10^{-11}$ cm^3/dyne per s into Eqn 31 yields a value of V_p^{urea} of $6 \cdot 10^{-22}$ cm^3 while a value of $1.5 \cdot 10^{-22}$ cm^3 for V_p is found from Eqn 13. Since it is unlikely that just the presence of a single urea molecule in the pore could lead to this 4-fold increase in volume, other explanations for this discrepancy should be sought (assuming that the experimental values are correct). One possibility is that the pore may be of such a size that the water molecules can get around each other but cannot get by the solute so that Eqn 13 is not valid. Another possibility is that a large fraction of the water permeability may be due to permeation through the membrane lipid rather than through the pores. For example, if the value of ω_w for the pore itself was only one-fourth the experimental value, then the two values for the pore volume would agree. In either case, the value of $6 \cdot 10^{-22}$ cm^3 would be the more accurate one. If the molecules were packed into the pore with the same density as in the bulk solution, this value would correspond to a pore 28 Å long, assuming the radius of 2.6 Å inferred from the erythritol impermeability.

The ratio of the permeabilities of two solutes can be obtained from Eqn 31:

$$\frac{1-\sigma_1}{1-\sigma_2} = \frac{\omega_1 V_p^1}{\omega_2 V_p^2} \approx \frac{\omega_1}{\omega_2} \tag{36}$$

where it has been assumed in the last term of this equation that the pore "volume" should be relatively independent of the solute. This is only a rough approximation and it is possible that different solutes could be arranged in a pore in such a way that the pore contents have markedly different volumes in the bulk solution. Applying Eqn 36 to the data in Table II for the beef erythrocyte, it is easy to show that if the value of σ for urea (0.73) is correct, then both glycerol and ethylene glycol should have a σ close to 1. Although this prediction is satisfactory for glycerol, it clearly disagrees with the experimental value for ethylene glycol (0.82). One explanation for this discrepancy (other than that the approximation in Eqn 36 or the experimental values or both are incorrect) is that the actual value of ω of urea for the pore is less than the experimental value as would be the case were there a carrier mechanism for urea.

"Carrier" behavior of the red cell pore

As was mentioned above, the relative partition coefficient (κ_s) is determined by the change in free energy that occurs when the solute molecule exchanges the water molecules with which it is interacting in the aqueous solution for the chemical components of the pore wall. Obviously, chemical interactions will play an important part in this process and it is an oversimplification to assume that the value of κ_s is determined primarily by steric factors as in Eqn 34. This chemical interaction can lead to kinetic behavior that is more commonly interpreted in terms of carriers. For example, it has been recently observed that in human erythrocytes phloretin greatly reduces the permeability of urea while the permeability of water is not significantly affected [14, 15]. This observation is difficult to explain in terms of the classical pore concept where the solute molecule simply exchanges one aqueous environment (bulk phase) for another (pore); and Macey and Farmer [14] regard this observation as evidence against the existence of pores. However, for the pore model considered in this paper in which the solute interacts directly with the pore wall, it does not seem unlikely that phloretin could reduce the κ_s for urea while not significantly affecting the permeability to water.

DISCUSSION

The idea that the pore may be so narrow that two molecules cannot get past each other was first suggested by Hodgkin and Keynes [16] to explain the observed flux ratios of K^+ in nerve. In their theoretical analysis they assumed that the pore consisted of a series of sites that were filled with K^+ and that when K^+ was added at one end of the pore it caused the release of another K^+ at the other end. This is a special case of a more general theory which has been considered in detail by Heckmann [17]. This theory is based on a very idealized model in which it is assumed that the pore consists of a series of sites over which the solute hops. The solute–solute interactions are defined by a set of rules such as "a molecule can jump to its neighboring site only if that site is empty" and the solvent–solute interactions are usually neglected. Barrer [18] has developed a similar theory to describe the movement of solute in zeolites. These theories are so idealized that they have not proven very useful in interpreting experimental permeability data in terms of the physical structure of the pore.

The approach of irreversible thermodynamics can be used to relate the three membrane parameters ω, σ and L_p to three more fundamental "frictional" constants, but, because of its generality, it yields little information about the pore structure. At the other extreme, the membrane parameters can be related to an "equivalent pore radius" by the use of the continuum theory. The continuum theory makes the assumption that the water is a continuum (i.e. consists of point particles), that the solute behaves like a macroscopic body, and that the pore is cylindrical. Although this theory has been shown to be qualitatively correct in larger pores [19, 20], its validity certainly must decrease as the dimensions of the pore approach the dimension of the water molecule and in the limit where the pore is so narrow that the water and solute cannot pass around each other, it breaks down completely. The theory developed in this paper depends primarily on the single assumption that the pore is so narrow that the water and solute cannot get past each other. (In addition, it is assumed either that all the membrane permeability is due to transport through the homogeneous pores,

or that one knows the value of ω, σ and L_p for the pores. This assumption is required for all pore theories.) In some respects the present theory is more general than that of irreversible thermodynamics since, for example, it is not necessary to assume linearity, Onsager's relation, or the existence of frictional constants. Unlike the continuum theory, no assumptions are made about the behavior of the water and solute in the pore, about the type of interactions with the pore wall, or about the pore shape (except for the basic condition that it is narrow enough). It is remarkable that, despite this generality, the theory still provides a way to obtain information about the structure of a single pore from measurements of ω, σ and L_p. For the special case where the pore is so narrow that two water molecules cannot get by each other, n_w (the number of water molecules per pore) can be determined just from a measurement of the diffusive and hydraulic permeability of water (Eqn 1). For the case where the water can get past another water molecule but cannot get by a solute molecule, V_p^s (the volume occupied in the bulk solution by the contents of a pore that is filled with water and one solute molecule) can be determined from the measurements of ω, σ and L_p (Eqn 31). Although it is not possible to determine the pore dimensions just from a knowledge of n_w or V_p^s, they are important experimental parameters. If, in addition, it is assumed that (1) the relative partition coefficient (κ_s) is determined primarily by the steric factor given in Eqn 34 and (2) that there is little variation in the values of L_p^s and V_p^s for the different solutes, then the "equivalent pore radius" can be obtained from Eqn 34. These assumptions (especially the first) would appear to be rather questionable and are, at best, only rough approximations. A knowledge of the "equivalent" radius and the value of n_w or V_p^s describes the pore geometry in as much detail as can be expected from this kind of experiment.

As was shown above, the data for the red cell membrane are in rough agreement with this theory although there are some important discrepancies. This analysis of the red cell data was used to illustrate how the experimental results can be analyzed in terms of this theory and the important implications that arise from a disagreement between theory and experiment. Because there is so little reliable experimental data available (especially for the reflection coefficient), no definite conclusions can be drawn from this analysis.

APPENDIX

Evaluation of the "zero time method" of measuring the reflection coefficient

Goldstein and Solomon [13] made the first measurements of the reflection coefficient of the red cell membrane in 1960. These data still represent the main body of the available experimental values and they have played an important role in the evaluation of various membrane theories. For example, they have been used repeatedly to calculate an equivalent pore radius [21] and as a test for the existence of pores [6]. In examining these data in order to apply them to the theory developed in this paper, I was surprised to find a methodological error that raises serious questions about the validity of the experimental values.

The experimental procedure of Goldstein and Solomon is based on a measurement of the red cell volume change when the external solution is suddenly replaced by a permeant. The reflection coefficient is determined by the use of Eqn 15 (with $\Delta P_a = 0$):

$$J_v = RTL_p(\sigma \Delta c_s - \Delta c_i) \tag{1A}$$

where Δc_s and Δc_i are the concentration difference of the permeant and impermeant, respectively. At time zero:

$$J_v^0 = RTL_p(\sigma \Delta c_s^0 - \Delta c_i^0) \tag{2A}$$

where $\Delta c_s{}^0$ and $\Delta c_i{}^0$ are the initial concentration differences and are known. In the "zero time method" $\Delta c_s{}^0$ is varied until $J_v{}^0$ is zero and then σ_{exp} is determined from:

$$\sigma_{exp} = \frac{\Delta c_i^0}{\Delta c_s^0} \tag{3A}$$

All this is completely straightforward. The difficulty is in determining the value of $J_v{}^0$. The experimental apparatus of Goldstein and Solomon [13] allowed them to measure J_v at approx. 45, 90, 140 and 190 ms after mixing. These values were then extrapolated to obtain $J_v{}^0$. It will be shown below that these times are not early enough to allow one to make an accurate extrapolation.

A simple way to illustrate the error in the method is to determine the theoretical curve for J_v for a cell with a known "true" σ and then to compare this value of σ with the experimental value (σ_{exp}) determined by the method of Goldstein and Solomon [13]. Eqns 14 and 15 provide an exact set of differential equations for the change in the red cell volume which can be solved by approximation or numerical techniques [22, 23]. The results for acetamide are shown in Fig. 2.

The conditions have been chosen to match as closely as possible the experimental conditions of Goldstein and Solomon [13]. At $t = 0$, the normal external medium

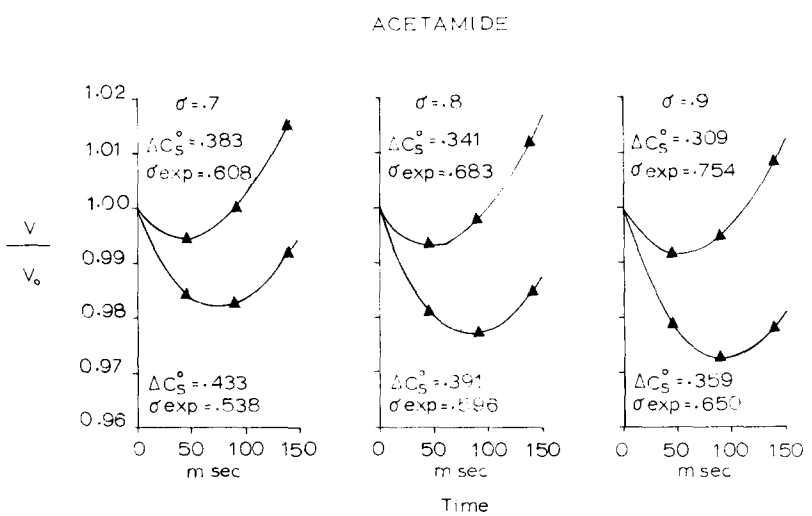

Fig. 2. Theoretical change in red cell volume (V/V_0) as a function of time (t) when the cell is suddenly placed in a solution of acetamide at the indicated concentration ($\Delta c_s{}^0$). The values of $\Delta c_s{}^0$ and the corresponding σ_{exp} at the top and bottom of the figure are for the upper and lower curves, respectively. The three panels correspond to the volume curves for three different values of the true reflection coefficient (σ) of the cell. The "Δ" marks the points in time at which Goldstein and Solomon [13] could make measurements. See Appendix for details.

(0.3 molar impermeant) is replaced by a solution containing a variable concentration of permeant and 0.067 molar impermeant. An L_p of $1.2 \cdot 10^{-11}$ cm^3/dyne per s and the ω listed in Table I were used in Eqns 14 and 15 which were then integrated numerically by a Runge–Kutta method. Since the red cells occupy less than 3 % of the total volume, it could be assumed that the concentrations in the external medium were constant.

The three panels in Fig. 2 show the theoretical curves for three different values of the (true) reflection coefficient. The curves are labeled with the external concentration of the permeant. The points in time at which experimental measurements were made are indicated by "Δ". The procedure of Goldstein and Solomon [13] was to determine what value of Δc_s^0 made the value of J_v extrapolated back to $t = 0$ equal to zero and then calculate σ_{exp} from Eqn 3A. It is not clear from their paper exactly how the extrapolation was performed. They state that "the curves through the points were drawn by eye and the tangent at zero time was also drawn by eye." The only example they show is for the case when glycerol (which has a very small permeability) was the permeant and the volume changes are so slow that the extrapolation is relatively straightforward. In Fig. 2, curves are shown for two different concentrations which (depending on how the extrapolation was performed) should have bracketed the concentration that Goldstein and Solomon would have interpreted as producing a zero J_v^0. It can be seen from Fig. 2, that this extrapolation can lead to a large error because the volume curve goes through a minimum at about 75 ms. For example, in the first panel in Fig. 2, a Δc_s^0 of between 0.433 and 0.383 corresponding to a σ_{exp} of between 0.538 and 0.608 (from Eqn 3A with $\Delta c_i^0 = 0.233$) would have been calculated from the apparent extrapolation of J_v when the true σ was 0.7.

For the solutes with higher permeabilities, such as urea or formamide, the error is much greater. Fig. 3 shows the results for urea. It can be seen that the largest value of σ that can be obtained by the procedure of Goldstein and Solomon (when the true σ is 1) is only 0.538. It is difficult to understand how the experimental value of 0.62 could have been obtained by Goldstein and Solomon.

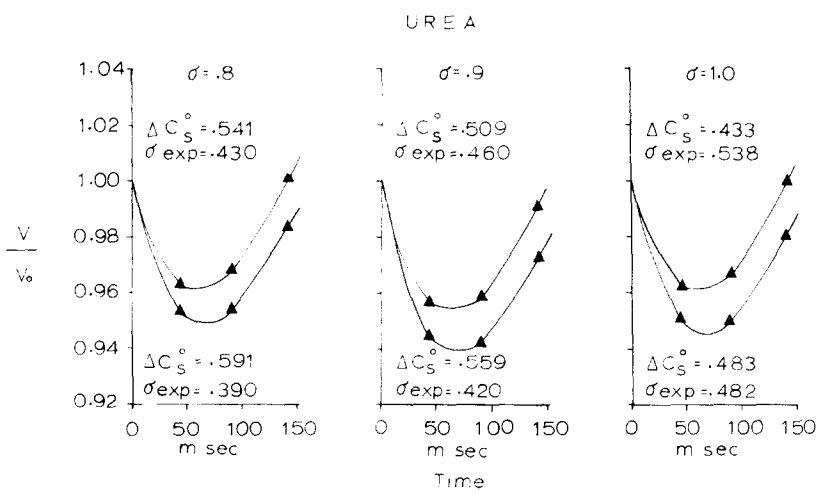

Fig. 3. Same as Fig. 2 except that urea is the solute.

As can be seen in Figs 2 and 3, the experimental points do not extrapolate back to the initial volume. Goldstein and Solomon observed this same effect and assumed that it was the result of the different refractive indices of the different concentrations of permeant that were used.

These results clearly indicate that the experimental procedure of Goldstein and Solomon can lead to large errors in the evaluation of σ and that these values cannot be trusted. Since 1960, several other measurements of the red cell reflection coefficients which should be more reliable have been made. These results are summarized in Table II. Using a stop flow apparatus which can make continuous measurements of volume changes within about 10 ms of mixing, Sha'afi et al. [8] found a σ for urea of 0.55 by the "zero time method". Farmer and Macey [10, 24] have used a similar apparatus to determine σ by a procedure that utilizes the whole time course of the volume change. Wessels and coworkers [25, 26] found a σ of 1 for glycerol in the human and beef erythrocyte by the use of a new method of analysis of the hemolysis times.

REFERENCES

1 Solomon, A. K. (1968) Gen. Physiol. 51 (5, Pt. 2), 3353
2 Levitt, D. G. and Subramanian, G. (1974) Biochim. Biophys. Acta 373, 132–140
3 Vieira, F. L., Sha'afi, R. I. and Solomon, A. K. (1970) J. Gen. Physiol. 55, 451–466
4 Einstein, A. (1956) Investigations on the Theory of the Brownian Movement (Furth, R., ed.), Dover Publications, Inc.
5 Katchalsky, A. and Curran, P. F (1965) Nonequilibrium Thermodynamics in Biophysics, Harvard University Press, Cambridge, Mass.
6 Stein, W. D. (1967) The Movement of Molecules across the Cell Membrane, Academic Press, New York
7 Rich, G. T., Sha'afi, R. I., Romualdez, A. and Solomon, A. K. (1968) J. Gen. Physiol. 52, 941
8 Sha'afi, R. I., Rich, G. T., Mikulecky, D. C. and Solomon, A. K. (1970) J. Gen. Physiol. 55, 427–450
9 Farmer, R. E. L. and Macey, R. I. (1970) Biochim. Biophys. Acta 196, 53
10 Farmer, R. E. L. and Macey, R. I. (1972) Biochim. Biophys. Acta 255, 502–516
11 Naccache, P. and Sha'afi, R. I. (1973) J. Gen. Physiol. 62, 714
12 Sha'afi, R. I., Gary-Bobo, C. M. and Solomon, A. K. (1971) J. Gen. Physiol. 58, 238–258
13 Goldstein, D. A. and Solomon, A. K. (1960) J. Gen. Physiol. 44, 1–17
14 Macey, R. I. and Farmer, R. E. L. (1970) Biochim. Biophys. Acta 211, 104–106
15 Owen, J. D. and Solomon, A. K. (1972) Biochim. Biophys. Acta 290, 414–418
16 Hodgkin, A. L. and Keynes, R. D. (1955) J. Physiol. 128, 61
17 Heckmann, K. (1972) Passive Permeability of Cell Membranes (Kreuzer, F. and Slegers, J. F. G., eds), Biomembranes, Vol. 3, pp. 127–153, Plenum Press, London
18 Barrer, R. M. (1971) Molecular Sieve Zeolites-II (Advances in Chemistry Series, 102), pp. 1–34, American Chemical Society, Washington, D.C.
19 Beck, R. E. and Schultz, J. S. (1972) Biochim. Biophys. Acta 255, 273
20 Levitt, D. G. (1973) Biophys. J. 13, 186–206
21 Sha'afi, R. I. and Gary-Bobo, C. M. (1973) in Progress in Biophysics and Molecular Biology (Butler, J. P. V. and Noble, D., eds), Vol. 26, pp. 103–146, Pergamon Press, Oxford
22 Johnson, J. A. and Wilson, T. A. (1967) J. Theor. Biol. 17, 304–311
23 Hempling, H. G. (1967) J. Cell Physiol. 70, 237–256
24 Farmer, R. E. L. and Macey, R. I. (1972) Biochim. Biophys. Acta 290, 290–299
25 Wessels, J. M. C., Pals, D. T. F. and Veerkamp, J. H. (1973) Biochim. Biophys. Acta 291, 169–177
26 Wessels, J. M. C. and Veerkamp, J. H. (1973) Biochim. Biophys. Acta 291, 190–196
27 Savitz, D. and Solomon, A. K. (1971) J. Gen. Physiol. 58, 259–266
28 Weith, J. O. (1971) J. Physiol. 213, 435–453