Determination of Equivalent Pore Radius for Human Red Cells by Osmotic Pressure Measurement

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ABSTRACT A new method has been developed to measure the equivalent pore radius in cellular membranes, and has been applied to human red cells. When red cells are suddenly introduced into a non-isosmolar concentration of non-lipid–soluble non-electrolyte molecules, water will enter or leave the cell. The rate of cell swelling or shrinking is determined and extrapolated to zero time to give the initial rate of volume change. By suitable adjustment of the concentration of the external solution the initial rate may be brought to zero. The transient equilibrium concentration, determined by interpolation from experimental data, gives a measure of Staverman's reflection coefficient, σ. The zero time method has enabled us to determine σ for nine permeant molecules. σ is directly related to the equivalent pore radius; the experimental data lead to a value of 4.2 Å for the equivalent pore radius in man, in good agreement with the previous figure of 3.5 Å given by Paganelli and Solomon. The zero time method offers a number of advantages over previous methods for determination of this parameter. It requires no measurement of the rate of water entrance into the cell, and is essentially independent of the kinetics of cell swelling. It may be applied to a variety of living cells so that many additional membranes may now be characterized in terms of their equivalent pore radius.

The present experiments have been undertaken to provide an independent estimate of the equivalent pore radius in the membrane of the human red blood cell. The previous figure of 3.5 Å has been determined by Paganelli and Solomon (1) from their measurements of the rate of water diffusion into the red cell, coupled with measurements of water entrance under an osmotic pressure gradient, as given by Sidel and Solomon (2). In view of the numerous assumptions involved in Paganelli and Solomon's estimate, it is desirable to approach the problem by an independent method, which in the present in-

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stance involves a study of the rate of entrance of a number of lipid-insoluble molecules of graded size into the red cell. It has been customary to make such measurements by observing the time course of cell swelling subsequent to exposure of the red cell to solutions containing permeant molecules. The permeability coefficient has usually been determined by the use of equations given by Jacobs (3), which are based on the application of van't Hoff's equation, $\pi = RTC$ (π is osmotic pressure, C is concentration, and R and T have their usual meaning). However, Staverman (4) has pointed out that an important correction is required in cases in which van't Hoff's equation is applied to molecules which can penetrate the membrane. Thus Jacobs' equations cannot be used in precisely those cases which are of most interest to us, those in which the probing molecules enter the red cell almost as readily as water. In these cases it is first necessary to make a determination of Staverman's reflection coefficient, the correction factor σ, which is defined as the ratio of the operative osmotic pressure to the theoretical van't Hoff osmotic pressure.

The method we have adopted to measure σ is the "zero time method" which depends upon a determination of the concentration of permeant molecules required to cause the initial rate of water entrance into the cell to become zero. When the external concentration rises above this transient osmotic equilibrium value, water leaves the cell at zero time; when it falls below, water enters the cell at zero time. By interpolation it is possible to find the transient equilibrium concentration, and thus determine σ. In the discussion, it will be shown that σ provides a measure of the equivalent pore radius. Thus the determination of the permeability coefficients for the probing molecules is not necessary to our purpose, which is primarily the determination of the equivalent pore radius of the red cell in man by a new and independent method.

EXPERIMENTAL METHOD

Equipment

The rapid flow method of measuring reaction velocity was used to determine the rate of water entrance into human red cells. The equipment is shown schematically in Fig. 1. Whole blood diluted with standard buffer was delivered into a mixing chamber and combined with either a control or test solution, the proportion of blood to solution being 1:2.5. The resulting mixture was forced through an adjustable length of tubing and into a light-scattering chamber in which the red cell volume was measured photometrically. By changing the length of tubing between the mixing and scattering chambers, it was possible to obtain a measure of cell volume at approximately 45, 90, 140, and 190 milliseconds after mixing. The equipment has been described in detail by Sidel and Solomon (2) in their report on the osmotically in-

duced flow of water into human red cells. The only modification made in the present experiments was the substitution of 1 liter glass bottles for the original 100 ml. burettes.

For the photometric volume measurement a collimated beam of white light was directed into the scattering chamber at right angles to the direction of fluid flow. A photomultiplier, placed at right angles, both to the direction of flow and to the incident light beam, measured the light scattered by the cells. This signal had been shown (2) to bear a linear relationship to the average size of the red cells in the chamber. The reaction times were determined by measuring the volume rate of fluid flow and utilizing the known system geometry.

The pressure which forced the fluid through the system was provided by a mixture of CO_2 and air (5 and 95 per cent, respectively) under a pressure of about 8 pounds

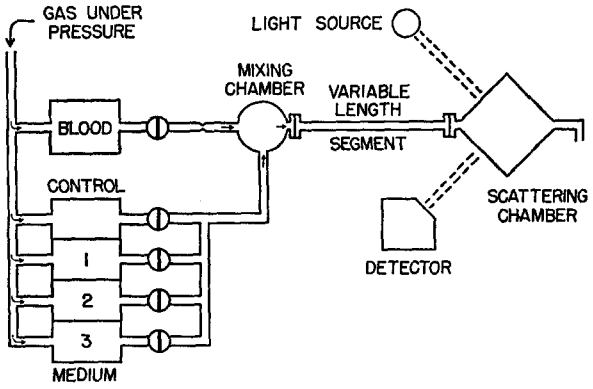

FIGURE 1. Schematic representation of the apparatus.

per square inch. The velocity of flow was always greater than 185 cm./sec., and usually greater than 190 cm./sec. In this velocity range the pressure *vs.* flow rate relationship is characteristic of turbulent flow, as previously shown (2) for this equipment.

Procedure

Blood of young normal human male volunteers was collected through siliconized needles into closed plastic bags (Fenwal). Each bag contained 2000 units of heparin as anticoagulant for 500 ml. of blood. All blood was used within 6 hours of venesection.

Twenty to 30 minutes before an experiment 1 volume of blood was diluted with 2.3 volumes of a standard buffer having the following composition: NaCl, 117 mM; $MgCl_2$, 0.5 mM; $CaCl_2$, 1.2 mM; Na_2HPO_4, 1.7 mM; NaH_2PO_4, 4.2 mM; KCl, 4 mM; Na_2CO_3, 13.5 mM. The osmolarity of this suspension medium was 290 milliosmoles per liter as measured by freezing point depression with an Aminco-Bowman apparatus. Just before the beginning of an experiment the suspension was shaken gently, aerated to pH 7.4 with 5 per cent CO_2–95 per cent air, and placed on a magnetic stirrer.

In the previous experiments of Sidel and Solomon (2), the photometric measure-

ments of cell volume were made by comparison with an isosmotic control solution of standard buffer. This procedure afforded a certain measure of protection against artifacts caused by the mixing process. In the present set of experiments, this problem is considerably magnified because the test substances are no longer electrolytes. As soon as the blood suspension is mixed with a test solution, the ionic strength of the medium and, in particular, the Cl concentration are instantaneously altered. As Jacobs (5) and Jacobs and Stewart (6) have pointed out, this results in an immediate pH shift, which is followed by a shift in cell volume. In order to compensate for these environmental changes, the control solution in the present series of experiments contains glucose at an isosmotic concentration (as defined by freezing point measurements). Glucose, which does not significantly penetrate the red cell membrane within 200 milliseconds, exerts its full osmotic effect throughout the experiment.

TABLE I

OLIVE OIL-WATER PARTITION COEFFICIENTS OF PERMEANT MOLECULES (8)

Compound	Olive oil-water partition coefficient ($\times 10^3$)
Ethylene glycol	0.49
Propylene glycol	5.7
Glycerol	0.07
Urea	0.15
Methylurea	0.44
Thiourea	1.37
Acetamide	0.83
Propionamide	3.6
Malonamide	0.08

The results of Höber and Ørskov (7) indicate that both the molecular volume and the chemical nature of the molecule are parameters of importance when the rate of penetration is determined by the osmotic hemolysis method. For this reason our probing molecules were chosen from three homologous series of non-lipid–soluble non-electrolytes. The following compounds were used: urea, methylurea, thiourea; ethylene glycol, propylene glycol, glycerol; acetamide, propionamide, and malonamide. The glycerol was Merck, "reagent grade." All other compounds were obtained from the Eastman Kodak Co. and were of "white label" purity except for thiourea which was "practical grade." The lipid solubility of these compounds, as measured in terms of olive oil–water partition coefficients by Collander and Bärlund (8), is given in Table I.

The control solution bottle was filled with 0.30 M glucose. A mixture of this solution with standard buffer (in the proportion 2.5:1) had the same freezing point depression as the standard buffer by itself within the limits of experimental error which are $\pm$ 2.5 milliosmoles per liter. The three test solution bottles were each filled with a separate concentration of probing molecule solution (made up of distilled water with no added salts). The concentrations were chosen so that at least one of them was high enough to cause initial shrinking of the cells when test solution and cell suspen-

sion were mixed. The concentration sets were generally 0.2, 0.3, 0.4 M or 0.3, 0.4, 0.5 M, the latter set being used for compounds to which the membrane was found to be especially permeable. Letting A_1, A_2, A_3 represent the three different concentrations of substance A, the sequence of mixing with blood followed the pattern: control, A_1, A_2, A_3, control. This sequence was repeated a total of three times at each of four reaction times. The difference between the signal recorded by mixing with A_1, A_2, or A_3 and that obtained by mixing with the control was used as a measure of cell volume.

Since a small but progressive volume change occurred when the cells were exposed to the isosmotic glucose, it was important to determine the glucose control reading at each reaction time. This phenomenon was interpreted as the summation of all effects which occur when the ionic medium surrounding the cells is diluted with 2.5 times its own volume of non-electrolyte. The temperature was essentially constant during an experiment, the total range for all experiments being 24 ± 2°C.

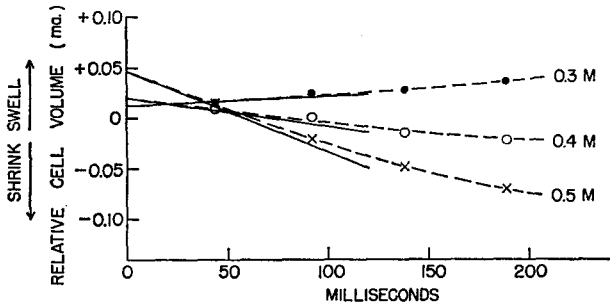

FIGURE 2. Time course of relative volume changes for an experiment in which glycerol was the probing molecule. The true cell volume bears a linear relation to the relative volume which is measured by the current passing through a recorder. The zero time slopes are shown for the three concentrations of glycerol used.

RESULTS AND DISCUSSION

Each experiment provided data from which the relative cell volume could be calculated at four reaction times for three separate concentrations of probing molecule solutions, as shown in Fig. 2. The curves through the points were drawn by eye and the tangent at zero time was also drawn by eye. These curves provided the data from which the zero time rate of cell swelling could be determined as a function of extracellular concentration of the permeant molecule. It will be noted that the initial volumes do not share a common origin. We are indebted to Dr. Stanley G. Schultz for pointing out that these differences may be ascribed to the different refractive indices of the three solutions. Fig. 3 shows the data obtained in an experiment in which the probing molecule was glycerol. The three points fall on a straight line which crosses the zero slope axis at a concentration slightly greater than isosmolar. When NaCl was used as probing molecule the same straight line relation-

ship was observed, and the line cut the zero slope axis exactly at the isosmolar concentration as expected, since there is no net flux of NaCl into the cell from the buffer solution. The isosmolar concentrations determined for the nine permeant molecules in experiments of this type are given in Table II. In accord with the considerations of Staverman (4), they are larger than would be expected from van't Hoff's classical equation.

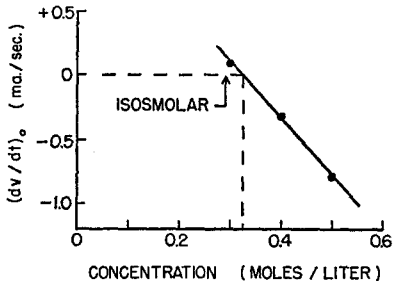

FIGURE 3. Zero time slope as a function of the concentration of probing molecule. The broken lines show the method of obtaining the isosmolar concentration for glycerol.

TABLE II

ISOSMOLAR CONCENTRATIONS OF PERMEANT MOLECULES

Compound	No. of experiments	Mean isosmolar concentration	Standard error of mean	Relative hemolysis times*
		M	M	t'
Ethylene glycol	6	0.46	0.05	1.7
Propylene glycol	3	0.34	0.03	
Glycerol	5	0.33	0.02	60
Urea	5	0.47	0.04	0.3
Methylurea	5	0.36	0.02	2.3
Thiourea	5	0.34	0.02	57
Acetamide	4	0.50	0.05	0.9
Propionamide	4	0.36	0.03	1.2
Malonamide	4	0.35	0.03	1025

* The relative hemolysis times t' are obtained from Höber and Ørskov (7). These authors define $t' = (t_2 - t_1)/t_1$ in which t_1 = time of hemolysis in 0.02 M NaCl and t_2 = time of hemolysis in 0.5 M non-electrolyte +0.02 M NaCl.

The experiments actually measure the concentration of the permeant molecule solution which the cell sees as equivalent to an equal volume of standard buffer when 2.5 volumes of water solution are added to 1 volume of standard buffer. For this reason the units of the isosmolar concentration are given in M. Since, as shown in Table III, the freezing point depression of the probing molecules is very close to ideality in our concentration range, molar concentration units are very close to units of osmols per liter.

Interpretation of the Physical Phenomenon

The observation that the isosmolar concentration for the compounds in Table II is greater than 0.29 M is equivalent to the statement that the cell membrane

is permeable to these compounds. In the last column of the table the isosmolar concentrations are compared with relative hemolysis times obtained by Höber and Ørskov (7); it can be seen that the more readily the molecule penetrates the membrane, the greater the mean isosmotic concentration. The expected osmotic pressure for a test molecule which exerts its full osmotic effect is $\pi_{theor} = RTC$. As previously stated, the relationship of the observed osmotic pressure, π_{obs}, to π_{theor} has been generally investigated by Staverman (4), who defines the reflection coefficient, σ, to equal the ratio π_{obs}/π_{theor}. A given concentration of permeant molecules never exerts its full osmotic effect, even at $t = 0$ when no molecules have penetrated the membrane. The reflection coefficient, σ, provides a quantitative measure of the extent to which the membrane can distinguish solvent and solute molecules.

TABLE III

FREEZING POINT DEPRESSION (9)

Molal concentration	0.04	0.07	0.10	0.20	0.30	0.40	0.50	1.0
	Molal freezing point depression [°C./(mols/1000 gm. H_2O)]							
Compound								
Urea	1.850						1.863	
Thiourea				1.82		1.72		
Glycerol				1.87			1.89	
Acetamide			1.83	1.82				1.82
NaCl		3.498	3.478		3.40			

σ may be formally related to the concentrations of solute on the two sides of the membrane, as has been done by Kedem and Katchalsky (10). Following their approach, we obtain an equation which is valid at $t = 0$ under conditions of transient osmotic equilibrium.

$$\sigma_{t_0} = (\sum_j c_j^i - \sum_j c_j^o)/(c_s^o)_{isos} \qquad (1)$$

in which

σ_{t_0} = value of σ at t = 0

$\sum_j c_j^i$ = the sum of the concentrations of all the non-permeant *intracellular* species

$\sum_j c_j^o$ = the sum of the concentrations of all the non-permeant *extracellular* species

$(c_s^o)_{isos}$ = the initial extracellular concentration of the permeant species when $(dv/dt)_0 = 0$

Details of the derivation are given in the Appendix.

The Correlation of σ with the Radius of the Test Molecules

In Table IV the experimental values for σ are compared with the radii of the permeant molecules. The various physical and theoretical methods used to

estimate molecular radii give widely differing values for small non-electrolyte molecules. For this reason we have chosen to make scale models of the nine permeant molecules (using theoretical bond angles and atomic radii) and to measure the dimensions of the models directly. Measurements were made along three perpendicular axes while the molecules were in their largest and smallest steric configurations. The largest and smallest radii, as given in Table IV, were then determined from

$$r = 0.5\ (d_1 d_2 d_3)^{1/3} \tag{2}$$

where d_1, d_2, d_3 are the diameters measured along the three perpendicular axes. Unfortunately, neither this set of radii, which does not include any

TABLE IV

EXPERIMENTAL VALUES OF σ COMPARED WITH MOLECULAR RADII*

Compound	σ	Standard error of mean	Largest radius	Smallest radius	Mean radius
			Å	Å	Å
Glycerol	0.88	0.02	2.73	2.75	2.74
Propylene glycol	0.85	0.04	2.70	2.52	2.61
Thiourea	0.85	0.02	2.19	2.17	2.18
Malonamide	0.83	0.04	2.48	2.65	2.57
Methylurea	0.80	0.02	2.38	2.36	2.37
Propionamide	0.80	0.03	2.39	2.23	2.31
Ethylene glycol	0.63	0.03	2.34	2.13	2.24
Urea	0.62	0.02	1.95	2.10	2.03
Acetamide	0.58	0.03	2.29	2.24	2.27

* Measured on Catalin models.

allowance for hydration, nor any other set presently available, can be considered to give a completely satisfactory estimate of effective molecular size in solution. Nonetheless, Table IV indicates that increases in σ may well be correlated with increases in the molecular radius. In the succeeding section, these molecular model radii will be compared with the radius of the water molecule. We have adopted a figure of 1.5 Å for the radius of the water molecule, as determined in x-ray diffraction studies by Morgan and Warren (11). No water of hydration is included in any of the radii, and the figures, though arbitrary, are consistent.

Paganelli and Solomon (1) have studied the ratio of the osmotic to the diffusion flow of water into human red blood cells. They interpreted the observed ratio as a measure of an equivalent pore radius for an ideal red cell membrane pierced by uniform cylindrical pores. If Poiseuille's law is assumed to hold in such an ideal membrane, the mean equivalent pore radius is 3.5 Å. The relationship between an estimate of the equivalent pore radius

and σ may be obtained by the use of an equation given by Durbin, Frank, and Solomon (12). These authors related σ to the apparent pore areas for filtration of water and solute as follows:—

$$1 - \sigma = A_{sf}/A_{wf} \tag{3}$$

in which A_{sf} and A_{wf} are the apparent areas for filtration of solute and water respectively. These apparent pore areas for filtration are a measure of the resistance offered by the membrane to the movement of water and solute under an applied pressure gradient, as defined by Renkin (13).

Since σ for each of the test molecules has been determined, the values of A_{sf}/A_{wf} are known. Renkin (13) gives the following equations which provide a basis for expressing the ratio, A_{sf}/A_{wf}, in terms of the molecular radius, a, and the equivalent pore radius, r:

$$A_{sf} = A_o[2(1 - a/r)^2 - (1 - a/r)^4][1 - 2.104a/r + 2.09(a/r)^3 - 0.95(a/r)^5] \tag{4}$$

$$A_{wf} = A_o[2(1 - a_w/r)^2 - (1 - a_w/r)^4][1 - 2.104a_w/r + 2.09(a_w/r)^3 - 0.95(a_w/r)^5] \tag{5}$$

in which

A_o = the geometrical pore area in the membrane
A_w = the radius of the water molecule

Therefore:

$$1 - \sigma = \frac{[2(1 - a/r)^2 - (1 - a/r)^4][1 - 2.104a/r + 2.09(a/r)^3 - 0.95(a/r)^5]}{[2(1 - a_w/r)^2 - (1 - a_w/r)^4][1 - 2.104a_w/r + 2.09(a_w/r)^3 - 0.95(a_w/r)^5]} \tag{6}$$

Durbin (14, 15) has shown that this equation provides a satisfactory fit to the data he obtained in cellulose membranes with pore radii of 20 to 80 Å. Using Equation 6 and the crystallographic radius of 1.5 Å for water, it is possible to derive the family of curves of $(1 - \sigma)$ as a function of the permeant molecular radius. The parameter of these curves, shown in Fig. 4, is the equivalent pore radius. This family of curves makes it possible to find the equivalent pore radius which best fits the experimental values of σ. The resultant equivalent pore radius, 4.2 Å, shown in Fig. 5, is in very good agreement with the previous value (1) of 3.5 Å.

Since the two methods of determination are independent, the agreement is particularly significant. In the present method, σ provides a direct measure

of A_{sf}/A_{wf}, whose relationship to the equivalent pore radius is given by Equation 6. Given values for the molecular radii, a and a_w, the remaining parameter is the variable of interest, the equivalent pore radius. Thus the

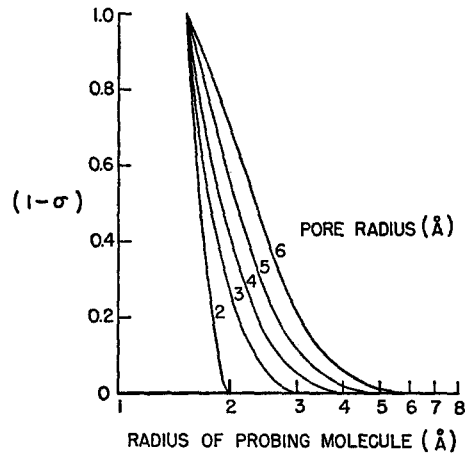

FIGURE 4. $(1 - \sigma)$ as a function of the radius of the probing molecule. Equation 6 generates a theoretical family of curves which has the radius of the pore as the parameter.

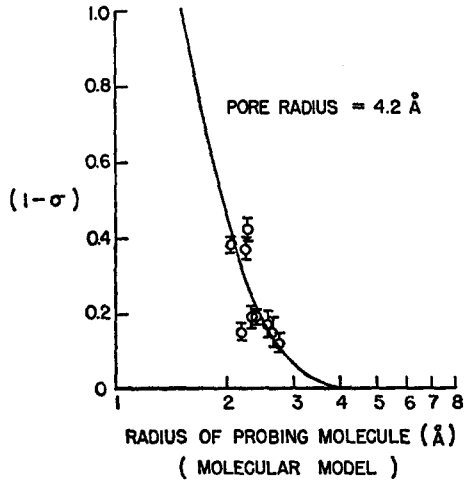

FIGURE 5. The experimental values of $(1 - \sigma)$ for the nine permeant molecules are shown as a function of the radius of the probing molecule. The theoretical curve which provides the best fit is also shown. It is drawn for a pore of radius 4.2 Å.

measurements do not depend in any way on the previous determinations of the rate of water flux into the cell, either under an osmotic pressure gradient or a concentration gradient. A major source of error appears to lie in the estimation of the molecular radius of the permeant molecules, particularly when these molecules are assumed to be anhydrous, as is necessary when the

radius is computed from molecular models. In solution, these molecules certainly move as though they were associated with some water, either because of hydrodynamic drag or loose chemical association. Thus, for example, Robinson and Stokes (16) suggest that each glycerol molecule in solution moves as though accompanied by 1 molecule of water. Considerations such as these would suggest that the present 4.2 Å figure is a lower estimate, though they would only introduce an uncertainty of the order of 0.5 Å. A further uncertainty arises from the extension of Equation 3 to pores of such small equivalent radius, since Durbin's (15) studies have established its validity only to a lower limit of 20 Å equivalent pore radius. A similar remark applies to the use of Renkin's equations in this domain.

Nonetheless, the assignment of an equivalent pore radius in the 3.5 to 4.5 Å range is supported by qualitative evidence from the experiments in which hemolysis time was used as an index of cell permeability. From Table II, the relative hemolysis times as determined by Höber and Ørskov (7) for the permeant molecules ethylene glycol and glycerol are 1.7 and 60. When the size of the permeant molecule is increased by the introduction of one additional CHOH group, as for erythritol, the relative hemolysis time rises steeply to 10,750. Table IV gives the molecular radius of ethylene glycol as 2.24 Å and of glycerol as 2.74 Å. The additional group in erythritol leads to a molecular radius of 3.06 Å for this compound. The increase in hemolysis time as the permeant molecule radius approaches 3 Å is in qualitative agreement with the assignment of an equivalent pore radius of 3.5 to 4.2 Å, which would offer considerable resistance to the passage of erythritol.

In the case of lipid-soluble molecules, no such sharp demarcation is evident. Green (17) has studied the passage of the lipid-soluble aliphatic straight chain acids across the cellular membrane. Expressed in his measure of permeability, the 3-carbon propionic acid requires 0.41 second for 50 per cent penetration. This figure may be compared to values of 0.38 second for butyric (4C) acid and 0.22 second for valeric (5C) acid. In the lipid-soluble molecules there is no sharp difference in permeability as the number of carbon atoms climbs above three. This observation is consistent with the existence of two separate routes of entrance into the cell: one through the fabric of the membrane, according to Overton's rule (18) and the other through the pores for the lipid-insoluble non-electrolytes.

The method of determining equivalent pore radius by zero time measurement of osmotic pressure offers a number of advantages. It does not require any determination of the permeability of the cell to water, either under an osmotic pressure gradient or a concentration gradient, and hence is independent of the influence of the cell contents on measurement of water permeability. Secondly, it is essentially independent of the kinetics of entrance of either water or permeant molecule into the cell. This is a characteristic ad-

vantage enjoyed by null methods. It is only necessary that the restoring forces be symmetrical around the normal volume of the cell; that is, that the elastic and other restoring forces change continuously as the cell moves from a volume somewhat lower than normal to a volume somewhat greater than normal. This is a particularly important consideration since the kinetics of cell swelling are not yet completely resolved. Thirdly, the method minimizes the effect of parallel routes for transport across the cellular membrane because the rate of zero time water entrance is essentially determined in the first 150 milliseconds after the reaction begins. Parallel processes will only interfere when their rates are of the same order of magnitude. This is probably the reason that results obtained with glycerol are compatible with those obtained with other permeant molecules, even though, as Stein and Danielli (19) have suggested, glycerol may penetrate the human red cell by interaction with membrane components. It would also appear that a limited lipid solubility might be permitted to the permeant molecules, as long as entrance by this route were neither immediate, nor overwhelming. However, the method is directly sensitive to steric hindrance arising from the interaction of charges within the pore with permeant ions or dipoles. Consequently the interaction between the pore charges and the non-electrolyte molecules we have used must be weak. The permeabilities of all the permeant molecules in the present study apparently form a homogeneous series when examined by the criterion of the equivalent pore radius. This behavior may be contrasted with that observed by Höber and Ørskov (7) who had earlier found it necessary to group these molecules according to their chemical nature in order to account for the relative hemolysis time data.

Furthermore, the present method is also applicable to a wide variety of other cells, including those whose shape changes are so complex that no kinetic theory of cell swelling has been developed. In the present experiments, we have used the flow method to measure the time course of volume changes; in other cells, in which the rate of water entrance is slower, as is generally the case, less complex methods may be used. For example, the rate of water entrance into *Sepia* axon (20) is so slow that the volume changes may be measured from microscopic observations of cell diameter. It is not even necessary to translate such microscopic observations into terms of water entrance since cell diameter or any other parameter that is a single-valued function of cell volume may be used. The rate of change of all such variables goes to zero when the cell volume is constant; as long as they exhibit no discontinuities around this point, they serve as valid indices of cell volume. Consequently it should now prove possible to characterize a wide variety of cellular membranes according to their equivalent pore radius.

APPENDIX

Derivation of Equation 1

Following the approach of Kedem and Katchalsky (10), we begin by considering a two component system at constant temperature. This system is composed of two solutions of solute s in solvent w, separated by a membrane of zero thickness. The treatment of Kedem and Katchalsky may not be applied directly because their equations are expressed in terms of a complicated function of a mean concentration of permeant molecule. Their mean concentration, c_s, as defined[1] in their Equation 14, is not defined under our experimental conditions in which the internal concentration of permeant molecules is zero, at $t = 0$, the time to which our calculations refer.

The general expression for the dissipation function, Φ, will be Kedem and Katchalsky's equation (12)

$$\Phi = (\mu_w^o - \mu_w^i)\dot{n}_w + (\mu_s^o - \mu_s^i)\dot{n}_s \tag{7}$$

in which

$(\mu^o - \mu^i)$ = the difference in the chemical potential of a component taken across the membrane; the superscripts denote the outer and inner compartments, respectively

$\dot{n}_s$ or $\dot{n}_w$ = the number of moles of solute or solvent entering compartment i per second per unit area

Assuming that we are dealing with ideal solutions:

$$\mu^o - \mu^i = \bar{v}\Delta p + RT[\ln \gamma^o - \ln \gamma^i] \tag{8}$$

where

$\bar{v}$ = the partial molal volume of the component

Δp = hydrostatic pressure difference across the membrane

γ = the mole fraction of the component

R and T have their usual meanings

Evaluating (8) for the solvent, we obtain

$$\begin{aligned}\mu_w^o - \mu_w^i &= \bar{v}_w \Delta p + RT\left[\ln\left(\frac{c_w^o}{c_w^o + c_s^o}\right) - \ln\left(\frac{c_w^i}{c_w^i + c_s^i}\right)\right] \\ &= \bar{v}_w \Delta p - RT\left[\ln\left(1 + \frac{c_s^o}{c_w^o}\right) - \ln\left(1 + \frac{c_s^i}{c_w^i}\right)\right] \\ &= \bar{v}_w \Delta p - RT\left[\frac{c_s^o}{c_w^o} - \frac{c_s^i}{c_w^i}\right]\end{aligned} \tag{9}$$

since c_s is ordinarily very much smaller than c_w.

[1] Kedem and Katchalsky (10) define c_s by the equation, $\Delta \ln c_s \equiv (\Delta c_s/c_s)$, in which $\Delta c_s = c_s^o - c_s^i$ and $\Delta \ln c_s = \ln c_s^o/c_s^i$.

Now if we set

$$c_s^o + c_s^i = 2\,\bar{c}_s \qquad c_s^o - c_s^i = \Delta c_s$$
$$c_w^o + c_w^i = 2\,\bar{c}_w \qquad c_w^o - c_w^i = \Delta c_w$$

then we can obtain

$$\mu^o - \mu^i = \bar{v}_w \Delta p - \frac{RT}{\bar{c}_w}\left[\Delta c_s - \Delta c_w \left(\frac{\bar{c}_s}{\bar{c}_w}\right)\right] \tag{10}$$

where we have assumed $\left(\frac{\Delta c_w}{\bar{c}_w}\right)^2 \ll 1$

This can be rewritten

$$\mu_w^o - \mu_w^i = \bar{v}_w \Delta p - (1 - \alpha) RT \frac{\Delta c_s}{\bar{c}_w} \tag{11}$$

where $\alpha \equiv \left(\frac{\bar{c}_s}{\bar{c}_w}\right)\left(\frac{\Delta c_w}{\Delta c_s}\right)$

For the solute we can obtain the equation

$$\begin{aligned} \mu_s^o - \mu_s^i &= \bar{v}_s \Delta p + RT\left[\ln \frac{c_s^o}{c_w^o} - \ln \frac{c_s^i}{c_w^i}\right] \\ &= \bar{v}_s \Delta p + RT\left[\ln\left(1 - \frac{\Delta c_w}{c_w^o}\right) - \ln\left(1 - \frac{\Delta c_s}{c_s^o}\right)\right] \end{aligned} \tag{12}$$

where we have set $\frac{c_s}{c_s + c_w} = \frac{c_s}{c_w}$

We may simplify equation (12) as follows:—

$$\ln\left(1 - \frac{\Delta c_w}{c_w^o}\right) \cong -\frac{\Delta c_w}{c_w^o}, \quad \text{since } \Delta c_w / c_w^o \text{ is small}$$

$$\ln\left(1 - \frac{\Delta c_s}{c_s^o}\right) = \left(-\frac{\Delta c_s}{c_s^o}\right) - \frac{1}{2}\left(-\frac{\Delta c_s}{c_s^o}\right)^2 + \frac{1}{3}\left(-\frac{\Delta c_s}{c_s^o}\right)^3 - \cdots = -\sum_{k=1}^{\infty} \frac{1}{k}\left(\frac{\Delta c_s}{c_s^o}\right)^k$$

Therefore we obtain for the solute

$$\mu_s^o - \mu_s^i = \bar{v}_s \Delta p + RT(\Sigma - \beta)\frac{\Delta c_s}{c_s^o} \tag{13}$$

where

$$\Sigma \equiv \sum_{k=1}^{\infty} \frac{1}{k}\left(\frac{\Delta c_s}{c_s^o}\right)^{k-1}$$

$$\beta \equiv \left(\frac{c_s^o}{c_w^o}\right)\left(\frac{\Delta c_w}{\Delta c_s}\right)$$

Using (11) and (13) in equation (7), we obtain

$$\Phi = [\dot{n}_w \bar{v}_w + \dot{n}_s \bar{v}_s]\Delta p + \left[\frac{\dot{n}_s}{c_s^o}(\Sigma - \beta) - \frac{\dot{n}_w}{\bar{c}_w}(1 - \alpha)\right] RT\Delta c_s \tag{14}$$

We can extend the equation to cover the situation where there are concentrations of impermeant molecules on both sides of the membrane. If all the concentrations of these impermeants, c_j, are small compared to c_w, then $\mu_s^o - \mu_s^i$ is still given by equation (13). The expression for $(\mu_w^o - \mu_w^i)$ becomes

$$\mu_w^o - \mu_w^i = \bar{v}_w \Delta p - (1 - \alpha)RT\frac{\Delta c_s}{\bar{c}_w} - (1 - \alpha_j)RT\left[\frac{\sum_j c_j^o - \sum_j c_j^i}{\bar{c}_w}\right] \tag{15}$$

where

$$\alpha_j \equiv \left[\frac{\sum_j (c_j^o + c_j^i)}{2\bar{c}_w}\right]\left[\frac{\Delta c_w}{\sum_j c_j^o - \sum_j c_j^i}\right]$$

We can therefore form Φ for this system by using equations (13) and (15) in equation (7). We may equate this expression for Φ to an expression of the form

$$\Phi = [\dot{n}_w \bar{v}_w + \dot{n}_s \bar{v}_s]X_v + \left[\frac{\dot{n}_s}{c_s^o}(\Sigma - \beta) - \frac{\dot{n}_w}{\bar{c}_w}(1 - \alpha)\right] X_D \tag{16}$$

where X_v and X_D represent the pressures conjugate to our arbitrarily selected flows. Collecting the coefficients for $\dot{n}_w$ and $\dot{n}_s$ and solving for X_v and X_D, one obtains

$$X_D = RT\Delta c_s + \left\{\frac{c_s^o \bar{v}_s(1 - \alpha_j)}{\bar{c}_w \bar{v}_w(\Sigma - \beta) + c_s^o \bar{v}_s(1 - \alpha)}\right\}\Delta\pi_j \tag{17}$$

$$X_v = \Delta p - \left\{\frac{(\Sigma - \beta)(1 - \alpha_j)}{\bar{c}_w \bar{v}_w(\Sigma - \beta) + c_s^o \bar{v}_s(1 - \alpha)}\right\}\Delta\pi_j \tag{18}$$

in which $\Delta\pi_j = RT(\sum_j c_j^o - \sum_j c_j^i)$

We use Kedem and Katchalsky's equations (19, 21, and 29) to write

$$J_v = L_p X_v + L_{pD} X_D = L_p[X_v - \sigma X_D] \quad \text{since} \quad \sigma = -\frac{L_{pD}}{L_p} \tag{19}$$

Then

$$\begin{aligned} J_v = L_p\left[\Delta p - \frac{(\Sigma - \beta)(1 - \alpha_j)}{\bar{c}_w \bar{v}_w(\Sigma - \beta) + c_s^o \bar{v}_s(1 - \alpha)}\Delta\pi_j\right] \\ - \sigma L_p\left[RT\Delta c_s + \frac{c_s^o \bar{v}_s(1 - \alpha_j)}{\bar{c}_w \bar{v}_w(\Sigma - \beta) + c_s^o \bar{v}_s(1 - \alpha)}\Delta\pi_j\right] \end{aligned} \tag{20}$$

During our experiments $J_v = 0$, and $\Delta p = 0$. Therefore

$$\sigma = \frac{-(\Sigma - \beta)(1 - \alpha_j)\Delta\pi_j}{RT\Delta c_s[\bar{c}_w \bar{v}_w(\Sigma - \beta) + c_s^o \bar{v}_s(1 - \alpha)] + c_s^o \bar{v}_s(1 - \alpha_j)\Delta\pi_j}$$

$$= \frac{-\Delta\pi_j}{\left[\bar{c}_w \bar{v}_w + c_s^o \bar{v}_s \frac{(1 - \alpha)}{(\Sigma - \beta)}\right] \frac{RT\Delta c_s}{1 - \alpha_j} + \frac{c_s^o \bar{v}_s}{(\Sigma - \beta)} \Delta\pi_j} \tag{21}$$

At the time of measurement, $t = 0$, $\Delta c_s = c_s^o$, and $\Sigma = \sum_{k=1}^{\infty} \frac{1}{k} \left(\frac{\Delta c_s}{c_s^o}\right)^{k-1}$ becomes very large. Therefore

$$\sigma_{t_0} = -\frac{\Delta\pi_j}{\frac{\bar{c}_w \bar{v}_w}{1 - \alpha_j} RT\Delta c_s} = \frac{\sum_j c_j^i - \sum_j c_j^o}{\frac{\bar{c}_w \bar{v}_w}{1 - \alpha_j} c_s^o} \tag{22}$$

Equation 22 may now be evaluated at $t = 0$, using the definition of α_j given in Equation 15

$$\sum_j c_j^i = 0.290 \text{ osmole/liter}$$

Because we mix 1 volume of buffered blood ($\sim$15 per cent cells) with 2.5 volumes of test solution,

$$\sum_j c_j^o = 0.290 \frac{0.85}{3.35} = 0.074 \text{ osmole/liter (the activity coefficient change is negligible)}$$

Δc_w may be estimated as 0.5 mole/liter
$2\bar{c}_w \cong 111$ moles/liter

Therefore

$$\alpha_j \cong 0.01$$

and

$$\frac{\bar{c}_w \bar{v}_w}{1 - \alpha_j} \cong 1$$

The final expression therefore reduces to Equation 1

$$\sigma_{t_0} = \left(\sum_j c_j^i - \sum_j c_j^o\right)/(c_s^o)_{\text{isos}} = 0.216/(c_s^o)_{\text{isos}}$$

where $(c_s^o)_{\text{isos}}$ = (2.5/3.35) [mean isosmolar concentration of permeant molecule]

Note that $\sigma_{t_0} = \frac{0.290\ [1 - (0.85/3.35)]}{[\text{mean isosmolar concentration}]\ (2.5/3.35)}$

$$= \frac{0.290}{[\text{mean isosmolar concentration}]}$$

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