Characterization of Biological Membranes by Equivalent Pores

A. K. SOLOMON

From the Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts 02115

The description of the permeability properties of biological membranes in terms of parallel pathways for lipid-insoluble and lipid-soluble molecules has its roots in the studies of Overton (1) and Collander and Barlund (2) and has been presented in detail in several books, such as those of Davson and Danielli (3) and Höber (4). The concept of the equivalent pore as a description of the path taken by lipid-insoluble molecules grew from the treatments of Koefoed-Johnsen and Ussing (5) and Pappenheimer, Renkin, and Borrero (6) and has been used by Solomon and coworkers (7–11) to characterize the behavior of single cell membranes, particularly those of red cells. More powerful mathematical treatments based on irreversible thermodynamics have been applied to biological systems, and new experimental evidence bearing on the equivalent pore concept has been obtained both from biological systems and from organic membranes. It appears desirable, therefore, to reexamine both the theoretical basis for the equivalent pore concept and the evidence bearing on its validity when applied to single biological membranes.

Since a great deal of the discussion will be concerned with diffusion, it is important to understand the nature of this process, which was perhaps best described by Einstein (12): "the molecular theory of heat affords a . . . point of view from which the process of diffusion can be considered. The process of irregular motion which we have considered as the heat . . . content of a substance will operate in such a manner that the single molecules of a liquid will alter their positions in the most irregular manner thinkable. This wandering about of the molecules of the solute—fortuitous to a certain extent—will have as a result that the original non-uniform distribution of concentration of the solute will gradually give place to a uniform one."

Diffusion may be contrasted with viscous or bulk flow, in which a number of molecules in a liquid move together in response to a physical force, often a pressure gradient. Account is taken of the attractive forces between neighboring portions of the fluid. As Prandtl and Tietjens (13) point out, "in homogeneous fluids the behavior of individual fluid particles is not of particular interest; one only wants to know the state of motion and its alteration with time at every point." The matter was put very succinctly by Onsager (14), who stated: "viscous flow is a relative motion of adjacent portions of a liquid. Diffusion is a relative motion of its constituents."

When solvent passage through a membrane can take place only by dissolution of the molecules in the membrane, solvent transport takes place by diffusion alone. When a pressure gradient is imposed across a pipe whose diameter is greater by orders

of magnitude than that of the solvent and solute molecules, viscous flow alone is of primary importance. We are interested in the intermediate range between these two extremes, and shall try to show what equations, still largely empirical, may be applied to fluxes through channels with dimensions comparable to those of solute and solvent. We shall start by an examination of the relationship of channel dimensions to frictional coefficients, including the Staverman reflection coefficient, and then examine diffusion coefficients as an index of channel radius. Next we shall discuss the further information that can be obtained from the relation of the diffusion coefficient of water through a membrane to its hydraulic conductivity under a pressure gradient. Finally, these several methods will be applied to a description of channels in red cell membranes.

HYDRODYNAMIC RELATIONS BETWEEN PORE DIMENSIONS AND FRICTION

Several equations have been put forward to describe the quantitative relationship between pore dimensions and frictions. In the case of free diffusion in one dimension in a solution, Fick's law can be stated as

$$J_s = -D_s(\delta c/\delta x). \tag{1}$$

J_s is the solute flux in moles per unit time and area, c is concentration in moles/cm^3, and x is distance. D_s is the diffusion coefficient in free solution (cm^2/sec), equal to RT/f^o, in which R and T have their usual meanings and f^o is the friction of 1 mole of solute with water. In order to describe membrane permeability, Pappenheimer et al. (6) used an equation similar to the following:

$$J_s = D_s A_s(\Delta c/\Delta x). \tag{2}$$

The partial differential has been replaced by a difference taken across the membrane (taken in the opposite direction to produce the change in sign); Δx is considered to represent the total path length through the membrane, probably greater than the membrane thickness because of tortuosity. The additional restrictions to diffusion introduced by the membrane are included in the factor A_s, which includes two terms. First are the geometrical restrictions introduced by the fact that the diffusion area is usually limited to a portion of the total membrane area. Since we shall be concerned primarily with transport through apertures that behave as pores traversing the membrane, the first term may be expressed as A_p/A, the ratio of the total pore area to the total membrane area. The additional restrictions due to friction experienced by the solute in diffusing through the membrane are included in the second term, A_{sd}/A_p. A_{sd} is the total apparent area for solute diffusion; A_{sd}/A_p has a limiting value of 1.0 when the pores are so large that solute-membrane friction may be neglected. Thus $A_s = (A_p/A)(A_{sd}/A_p)$. In diffusion through biological membranes it is not possible to separate A_s and Δx, so the parameter to be determined is $A_s/\Delta x$. In the case of artificial membranes there are techniques for determining A_p/A and also methods by which Δx may be approximated, though with varying degrees of success.

In order to describe the friction of a particle within the membrane pore, Pappen-

heimer et al. (6) used the following equation which Ladenburg (15) had derived on hydrodynamic grounds to correct Stokes' law for viscous drag between particles and the walls of a cylinder in which the measurement was made:

$$g'/g^{o\prime} = 1 + 2.4\alpha \tag{3}$$

in which g' is the friction exerted on the solute molecule because of interactions within the pore, and $g^{o\prime}$ that in free solution; $\alpha = a/r$, the ratio of the radius of the solute molecule, a, to the pore radius, r; the primed symbols are used to denote the Ladenburg equation. Pappenheimer et al. also pointed out that an additional factor had to be considered to take account of the probability that a particle will actually enter the pore. Assuming that a molecule could only enter the pore by diffusion if it did not strike the rim of the pore, Pappenheimer et al. (6) used the following:

$$A_{sd}/A_p = (1 - \alpha)^2/(1 + 2.4\alpha) = (1 - \alpha)^2 g^{o\prime}/g'. \tag{4}$$

Renkin (16) pointed out that the following equation, derived by Faxen (17) on theoretical grounds, is to be preferred to the Ladenburg equation:

$$g^o/g = 1 - 2.104\alpha + 2.09\alpha^3 - 0.95\alpha^5. \tag{5}$$

Faxen's equation was derived to describe the friction of spheres in tubes and depends upon the implicit hydrodynamic requirement that the fluid be considered a continuum. In addition, he gave several important conditions to be observed in the application of his equation. The most restrictive appears to be that molecules constituting the fluid in the tube be small compared with the sphere. As will be discussed, this equation has been applied to situations in which this condition is not fulfilled. The other conditions appear to be more generally satisfied in studies of membrane permeability.

In a detailed study Bacon (18) had shown that the viscosity of falling spheres could be well described by the Faxen equation. Bacon computed viscosities from the terminal velocity of falling balls of 0.1–0.8 cm diameter in tubes of 2.5–7.9 cm diameter and found the Faxen equation to be valid for values of α as great as 0.32, though the Ladenburg equation gave errors as great as 40% at this value of α. Calculated viscosities were within 1% of the absolute viscosities over a range of 7.5–3600 poises. Renkin (16) combined the Faxen equation with the equation for steric hindrance at the pore entrance to give the following equation, in which the restriction to diffusion is expressed in terms of the ratio of the apparent area for diffusion, A_{sd}, to the total pore area, A_p:

$$A_{sd}/A_p = (1 - \alpha)^2(g^o/g). \tag{6}$$

Renkin also derived a similar equation for bulk flow through the pores, and used this in a discussion of experiments on ultrafiltration through cellophane membranes. Renkin assumed that laminar flow takes place through the membrane and that the velocity of the flow in each laminar shell depends upon the distance of the shell from the axis of the tube. This gives a parabolic velocity profile as described by the Poiseuille

equation. Using a steric hindrance factor derived by Ferry (19), Renkin gave the following equation for the frictional effects in bulk flow (A_{sf} is the apparent area for filtration):

$$A_{sf}/A_p = [2(1 - \alpha)^2 - (1 - \alpha)^4](g^o/g). \quad (7)$$

In this instance, the term in the square brackets has been adjusted to take account of the velocity profile, whereas the g^o/g term remains as for diffusion. Fig. 1, taken from Renkin's paper, shows the relation between the apparent pore areas calculated by equation 6 and equation 7. In general, it is not possible to measure A_p in biological membranes, so water flow is measured also and the ratio A_{sf}/A_{wf} is used in

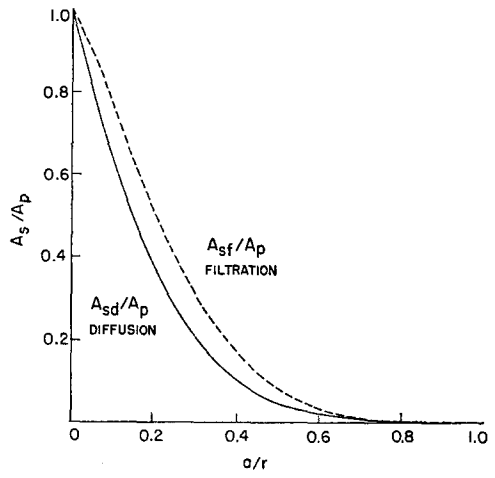

FIGURE 1. A_s/A_p, the ratio of the apparent area for solute *diffusion* to the total pore area (A_{sd}/A_p), and the ratio of the apparent area for solute *filtration*, to total pore area (A_{sf}/A_p), are expressed as a function of the ratio of the radius, a, of the solute molecule to the radius, r, of the equivalent pore. The full line (diffusion) is drawn according to equation 6, and the dashed line (filtration) is drawn according to equation 7. The figure has been redrawn, with permission, from Renkin (16).

the computation. This is given by

$$\frac{A_{sf}}{A_{wf}} = \frac{[2(1 - \alpha_s)^2 - (1 - \alpha_s)^4](g_s^o/g_s)}{[2(1 - \alpha_w)^2 - (1 - \alpha_w)^4](g_w^o/g_w)} \quad (8)$$

in which the subscript s refers to use of the solute radius, and w refers to the water radius.

The assumptions on which both equations 6 and 7 have been developed, and particularly their extension to equivalent pores so narrow that the molecular mechanism of diffusion and of bulk flow is not understood, mean that both equations are to be considered as empirical descriptions of the relation between frictions and pore dimensions. Their applicability rests upon the ability of these two equations to provide a self-consistent description of the membrane pore. Thus it is necessary that the parameters derived from equations 6 and 7 be consistent with data derived from all other measurements of membrane permeability if they are to be considered applicable to pores of dimensions comparable with those of solute and solvent molecule.

RELATION BETWEEN REFLECTION COEFFICIENT AND FRICTIONAL COEFFICIENTS

In 1951, Staverman (20) introduced the reflection coefficient, σ, to describe the osmotic properties of leaky semipermeable membranes which permit restricted passage of solute. The osmotic pressure developed in such a system is given by $\pi_{obs} = \sigma\pi_{theor}$, in which π_{obs} is the measured osmotic pressure and π_{theor} is the van't Hoff theoretical osmotic pressure. $(1 - \sigma)$ was to be obtained in an idealized ultrafiltration experiment as the ratio of the solute concentration after it had flowed through the membrane to the solute concentration before, after correction for the diffusion of the solute down its concentration gradient. In 1956, Durbin, Frank, and Solomon (21) first applied these considerations to a biological membrane. The passage of lipid-insoluble molecules was considered to be restricted to pores, and the membrane was treated operationally as an array of equivalent pores. They devised the equation

$$1 - \sigma = A_{sf}/A_{wf} \tag{9}$$

to relate the reflection coefficient to the hydrodynamic frictions. A_{sf} and A_{wf} are the apparent areas for filtration of solute and water as given in equation 8. Subsequently, a consistent theoretical treatment of coupled flows in membranes, with the methods of irreversible thermodynamics, was introduced by Kedem and Katchalsky in 1958 (22), and a deeper insight into the relations between forces and flows in such systems was obtained by the use of phenomenological coefficients which obeyed the Onsager reciprocal relations. In the same year, Spiegler (23) derived a relationship between the phenomenological coefficients and frictional coefficients which he introduced to describe the multiple interactions between solute, solvent, and membrane in Newtonian terms. In the systems to be discussed, three frictional coefficients are important: f_{sw}, the friction between 1 mole of solute and the water; f_{sm}, the friction between 1 mole of solute and the membrane; and f_{wm}, the friction between 1 mole of water and the membrane.

In 1961, Dainty (24) derived a relationship which related σ to the Spiegler frictional coefficients f_{sw} and f_{sm}; subsequently Dainty and Ginzburg (25) derived the following relation to apply when solute passage was restricted to the pores:

$$1 - \sigma - \frac{\omega_s \bar{V}_s}{L_p} = \frac{K_s^c f_{sw}}{f_{sw} + f_{sm}} = \frac{A_{sf}}{A_{wf}} \tag{10}$$

in which K_s^c is the partition coefficient for the solute between the water in the pores and the external solutions. ω_s is the permeability coefficient for the solute, $\bar{V}_s$ the solute partial molar volume, and L_p the hydraulic conductivity of the membrane. The term $\omega_s\bar{V}_s/L_p$ enters because σ is defined under the condition of zero volume flow, whereas the relation with the frictional coefficients is derived at zero water flow. Equation 10 may also be given as

$$1 - \sigma' = A_{sf}/A_{wf},$$

so that

$$\sigma' = \sigma + \omega_s \bar{V}_s / L_p .$$

At zero volume flow, the solute flow must be balanced by solvent flow, and the correction term $\omega_s \bar{V}_s / L_p$ is the contribution of that solute flow. When the solute flows only through pores that offer considerable restriction to its passage, the correction term is usually small. However, in cases in which solute can move preferentially to solvent, as by dissolution in the membrane, the $\omega_s \bar{V}_s / L_p$ term can become important and lead to the production of negative anomalous osmosis. Kedem and Katchalsky (26) come to a slightly different conclusion about the relationship between the frictional coefficients and the apparent pore areas. They use the ratio of the apparent pore areas for diffusion, A_{sd}/A_{wd}, rather than the ratio for filtration, A_{sf}/A_{wf}, given by Dainty and Ginzburg. Kedem and Katchalsky give the following equation (their equation 4-11):

$$1 - \sigma - \frac{\omega_s \bar{V}_s}{L_p} = \frac{A_{sd}}{A_{wd}} . \tag{11}$$

Since the difference between A_{sf}/A_{wf} and A_{sd}/A_{wd} is appreciable, it is necessary to choose between these two theoretical derivations. The difference lies in the use of the tortuosity factor, Θ, originally introduced by Mackie and Meares (27) and used by Kedem and Katchalsky in their derivation of equation 11. $\Delta x/\Theta$, is used by them to represent "in an overall manner the water path in the membrane." However, the tortuosity factor was introduced by Mackie and Meares to apply to polymeric membranes in which the membrane exerts no mechanical sieve action on the movement of the solute. Indeed, sieve actions are important even in the theory originally derived by Mackie and Meares, as pointed out by Lagos and Kitchener (28), who criticized the whole concept of tortuosity as lacking in rigor and taking no account of the size of the moving particle. Furthermore, Ginzburg and Katchalsky (29) have shown experimentally that the tortuosity in Visking dialysis tubing is dependent on the size of the solute molecule. When the tortuosity is not introduced into the derivation of equation 11, it can be shown that the equality to A_{sd}/A_{wd} does not hold, and we may conclude that the treatment of Dainty and Ginzburg is correct.

EXPERIMENTAL STUDY OF RELATION BETWEEN $(1 - \sigma)$ AND A_{fs}/A_{wf} IN VISCOUS FLOW

Two experimental investigations bearing on this relation in artificial membranes have been made, one by Renkin (16) in 1955, and the other by Durbin (30) in 1960. In the light of more recent knowledge the results of both investigations need further evaluation.[1] For example, Renkin's equations for ultrafiltration do not include any

[1] Lakshminarayanaiah (58) has criticized the measurements of Renkin (16) and Durbin (30) on the gound that their membranes were supported when filtration measurements were made, and not when diffusion measurements were made. Both authors point out that filter paper was interposed between the membrane and its support in order to permit filtration over the entire membrane area. Lakshminarayanaiah also followed this procedure, but found a significant increase in the hydraulic

explicit mention of the reflection coefficient, and Durbin's measurements do not consider the effect of the $\omega_s \bar{V}_s / L_p$ term; neither author has been concerned with the effect of the unstirred layer. In both cases, the molecular dimensions used for the various solutes are not necessarily correct. This is particularly important in the case of H_2O, for which the radius was considered to be 1.9–2.0 A, much larger than the currently accepted value of 1.5 A.

The artificial membranes are not very thick, and the pores in these membranes may be only of the order of 1 mm long. It is desirable to see whether the usual velocities of flow are sufficient to establish laminar flow in these pores by the usual hydrodynamic criteria; otherwise the entire pore might represent a transition region. The Reynolds numbers are so small (of the order of 10^{-9} at usual velocities) that the length required to reach laminar flow is negligible, even for Sylvania wet gel with an equivalent pore radius of the order of 100 A, the largest in the group.

As Dainty (31) and Ginzburg and Katchalsky (29) have pointed out, the presence of the unstirred layer leads to great difficulties in the interpretation of studies of diffusion and bulk flow across artificial membranes. The effect, as Dainty (31) has shown, is of much less significance in bulk flow than in diffusion. In Renkin's ultrafiltration experiments the stirring bar rested directly on the membrane on the filtrand side, so the stirring must have been very efficient. Both Renkin and Durbin characterized their membranes by measuring the diffusion of tritiated water, THO, as will be discussed in a later section. From the results of these measurements it is possible to compute the unstirred layer effect for Visking dialysis tubing in both studies, based on the assumption of a layer 25 μ thick on the side to which the tracer was added, and no unstirred layer on the pure solvent side of the membrane. In both cases, the effect on the membrane diffusion coefficient amounts to a few per cent, so that no correction need be applied in the case of bulk flow.

The equation that Renkin used in computing the results of his ultrafiltration experiments can be shown to be almost equivalent to the usual expression derived by irreversible thermodynamics. Renkin's equation is

$$c_2 = \left(\frac{A_{sf}}{A_{wf}}\right) c_1 + \frac{dn/dt}{J_v} \tag{12}$$

where c_1 is the concentration of the filtrand and c_2 is the concentration of the filtrate. J_v is the volume flow (volume per unit time and area). dn/dt is the rate of solute diffusion caused by the concentration difference, that is,

$$(dn/dt) = (J_s)_{J_v=0} = \omega_s \Delta\pi_s$$

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conductivity without the support. It is not clear how much of this may be ascribed to pressure-induced stretching of the membrane. Lakshminarayanaiah obtained a similar effect in his diffusion measurements, but the contribution of the unstirred layer could not be measured exactly. Since the validity of the criticism depends upon exact details of membrane stretching and the importance of the unstirred layer in Lakshminarayanaiah's measurements, it is very difficult to assess the importance of this objection to the experiments of Renkin and Durbin.

in which J_s is solute flow in moles per unit time and area and $\Delta\pi_s = RT(c_1 - c_2)$. Renkin's experimental measurements seem to have been made under conditions close to the steady state, since the ratio c_1/c_2 did not vary appreciably during the course of the experiment. Multiplying through by J_v, and remembering that $A_{sf}/A_{wf} \approx (1 - \sigma)$, we obtain:

$$J_v c_2 \approx J_s \approx (1 - \sigma)c_1 J_v + \omega_s \Delta\pi_s . \tag{13}$$

In the steady state, $J_v c_2$ is approximately equal to J_s. Since c_1/c_2 varies between 0.6 and 1.0, c_1 is not very far from $\bar{c}_s$ ($\bar{c}_s$ approximates the mean concentration and is

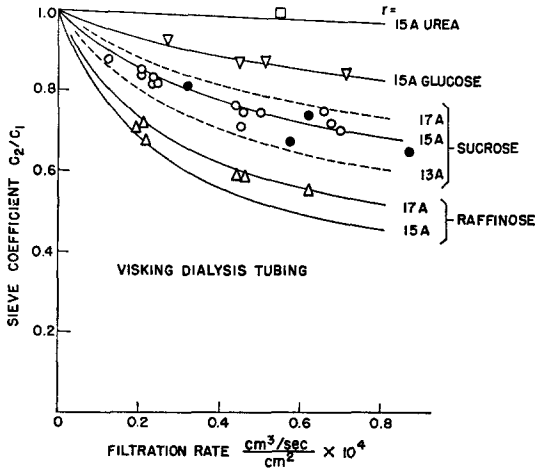

FIGURE 2. The sieving coefficient of Visking dialysis tubing plotted by Renkin (16) following equation 8 and 12. r, equivalent pore radius. The figure has been redrawn with permission.

defined by $\bar{c}_s = (c_2 - c_1)/\ln(c_2/c_1)$). Equation 13 is not very different from the usual equation for solute flux,

$$J_s = (1 - \sigma)\,\bar{c}_s J_v + \omega_s \Delta\pi_s . \tag{14}$$

One reason equation 13 is approximate is that no allowance has been made for the $\omega_s \bar{V}_s/L_p$ term in the substitution of $(1 - \sigma)$ for A_{sf}/A_{wf}. However, as will be shown in the discussion of Durbin's experiments, corrections due to this term are very small for filtration through porous membranes when the solute and solvent traverse the same channels. Fig. 2 shows a comparison of Renkin's experimental data with theoretical curves drawn according to equations 8 and 12; the data are consonant with a 15 A equivalent pore radius. The agreement is only qualitative, since no correction has been made by the introduction of more recent values for the molecular radii of H_2O and the solutes. Nonetheless Fig. 2 offers support for the treatment of hydrodynamic friction in filtration by equations dependent on the relative dimensions of the filtered molecule and the equivalent pore.

The data presented by Durbin (30) for bulk flow through Visking dialysis tubing can be recomputed so that equation 10 may be tested directly. σ for D_2O was 0.002 in this membrane, and the $\omega_s\bar{V}_s/L_p$ term amounts to 0.01, so that σ' for D_2O is effectively 0.01. The effect of the $\omega_s\bar{V}_s/L_p$ term on the other solutes is unimportant. As will be discussed later, Soll (32) has shown that the most appropriate parameter for the effective molecular dimension in bulk flow may be obtained by treating the permeating molecule as a cylinder and obtaining its radius from molecular models. In general this leads to smaller radii than those used by Durbin. Since the inulin used by Durbin had a mean molecular weight of 3100, about half the value of 5600 which

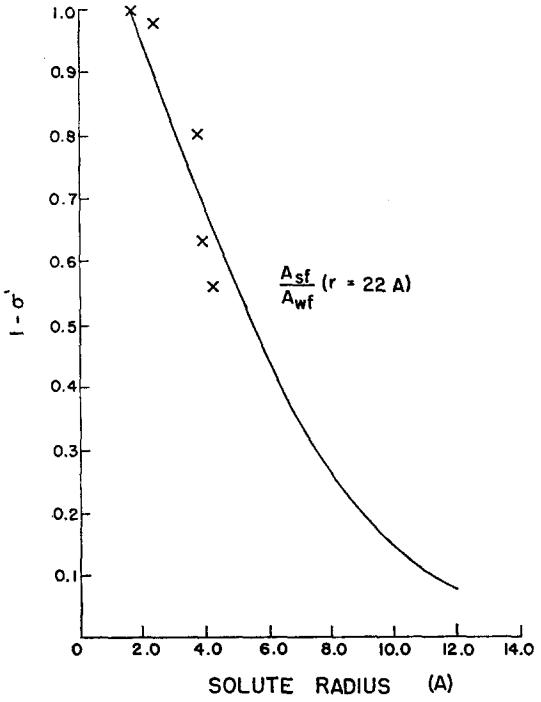

FIGURE 3. $(1 - \sigma')$, recomputed from Durbin's data (30), plotted as a function of solute radius. The point for albumin, to which Durbin has assigned a radius of 37 A which is beyond the limit of the figure is at $1 - \sigma' = 0$ as expected. r, equivalent pore radius.

characterizes the most homogeneous preparations (33), these data have not been included. As expected, $\sigma = 1.0$ for bovine serum albumin, to which Durbin assigns a radius of 37 A. Fig. 3 shows the recomputed data for the equivalent pore radius as determined by least squares on a computer. The value comes to 22 A, essentially unchanged from the 23 A figure used by Durbin, based on his original assumptions.

DIFFUSION COEFFICIENTS AS AN INDEX OF EQUIVALENT PORE DIMENSIONS

As early as 1930, Friedman and Kraemer (34) used diffusion in the study of the structure of gelatin gels. They estimated the equivalent pore radius in three different gels by a study of the diffusion coefficients of sucrose, glycerol, and urea. Essentially, their method consisted in the application of the Ladenburg correction (equation 3) term to

compute an equivalent pore radius from the restriction offered by the gel to free diffusion. The molecular radii were determined from the Stokes-Einstein relationships, and corrections were made for mechanical blocking by the gelatin and the viscosity

TABLE I

CALCULATION OF PORE SIZE FROM DIFFUSION IN GELATIN*

Substance diffusing	Size of pores		
	5% gel	10% gel	15% gel
	A	*A*	*A*
Urea	47	15	5
Glycerol	57	17	10
Sucrose	55	14	8

* Data from Friedman and Kraemer (34).

of the solution in which the experiments were performed. The results they obtained are given in Table I, and it can be seen that the agreement is very good for pores with equivalent radius in the range of 10–50 A. Friedman (35) obtained similar results on

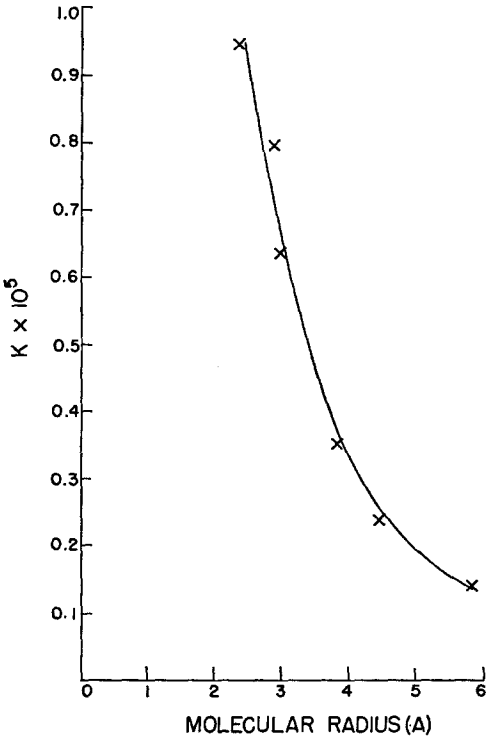

FIGURE 4. Diffusion coefficients, K, in a 5% gelatin gel from the data of Friedman (35) for methanol, ethanol, urea, glycerol, glucose, and lactose. The radii have been computed from equivalent spheres based on dimensions taken from molecular models. The curve has been drawn by eye and is not based on theory.

agar gels, though the agreement among pore radii was not as good. Friedman also made an extensive study of the diffusion of a number of nonelectrolytes into a 5% gelatin gel. Fig. 4 shows the relation between his diffusion coefficient, denoted by K,

and the equivalent sphere radius of the diffusing solute, computed from molecular models. The figure illustrates the increasing restriction on diffusion in such a gel with increasing size of the diffusing molecule. The quantitative interpretation of these data must be accepted with some reserve, however, since Friedman and Shearer (36) have subsequently shown that nonelectrolytes exert an appreciable and concentration-dependent effect upon the diffusion of urea in gelatin gels. Although Friedman made a correction for this effect in the original calculation, it is not entirely clear that all the factors were taken into account. Furthermore, Gary-Bobo (personal communication; see also reference 37) has studied diffusion in 10% gelatin gels and found that molecules such as inulin and hemoglobin can permeate such gels, a result which would not be expected from the Friedman and Kraemer model. However, gelatin varies from batch to batch and the experimental methods and conditions differed greatly, so that the two sets of data are not directly comparable.

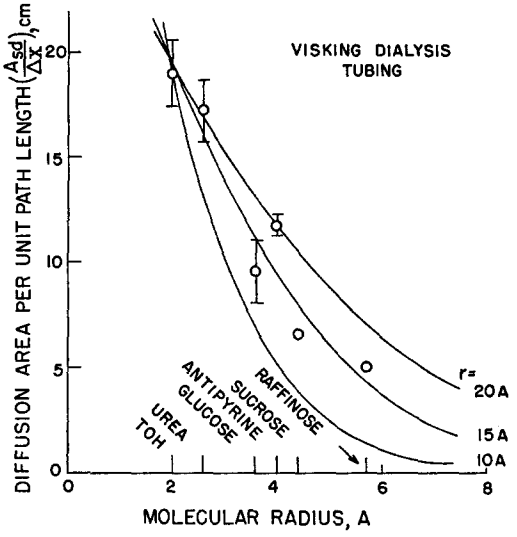

FIGURE 5. Apparent diffusion area per unit path length in Visking dialysis tubing as measured by Renkin, and reproduced with permission, (16). The molecular radii are those given by the authors The length of the bars give. standard errors of the means *r*, equivalent pore radius.

Pappenheimer et al. (6) in 1951 first used diffusion coefficients to characterize a biological system in studies on capillary permeability. These authors found that the restricted diffusion area across the capillary wall could be described with reasonable accuracy by equation 4 which combines the Ladenburg restriction within the pore with a correction for steric hindrance at the entrance to the equivalent pore. These results will not be discussed in detail, since it is not clear that diffusion took place across a single homogeneous barrier in this complex system.

Subsequently, Renkin (16) made a study of restricted diffusion through porous cellophane membranes. He made use of the Faxen treatment and fitted his data to equation 6. Fig. 5 shows that this equation provided a reasonable fit to the data for an equivalent pore radius of 15 A for Visking dialysis tubing. These measurements bring out the consistency in the application of these equations to pores of relatively small dimensions. As has been discussed in the previous section, 15 A was obtained as the

equivalent pore radius for this membrane in Renkin's measurements of the restriction offered to filtration.

A number of other studies have been made with synthetic membranes, particularly ion-exchange resins. There are two examples of studies in which the apparent porosity of the membranes could be controlled by physical means. In neither case was any abrupt change in membrane parameters observed as the porosity went from the

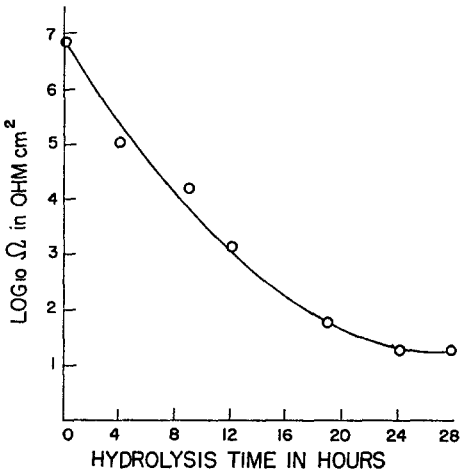

FIGURE 6. Resistance of polyvinyl-butyral membranes hydrolyzed at 60°C in 4 N sulfuric acid, redrawn, with permission, from Gregor and Kantner (38). The abscissa gives hydrolysis time in hours.

lowest to the highest value. This is an important observation, since it indicates no discontinuity which might be ascribed to sudden changes in the nature of the fluid in pores of apparent dimensions of 20–100 A. Gregor and Kantner (38) prepared membranes of polyvinyl butyral film whose porosity was controlled by the time of hydrolysis in 4 N sulfuric acid. As shown in Fig. 6, the ohmic resistance in the membrane

TABLE II

DIFFUSION OF NONELECTROLYTES IN RESIN*

Relative humidity	Grams H_2O per 100 g dry resin	$D \times 10^6$		
		Glycerol	Glucose	Sucrose
			cm²/sec	
0.980	48.8	1.50	0.94	0.15
0.902	30.4	0.35	0.23	0.02
0.807	19.3	0.06	0.05	0.01

* Data, with permission, from Lagos and Kitchener (28).

varied smoothly with hydrolysis time. In the tightest membrane the permeability coefficient for urea was 0.049 as compared to 0.019 for sucrose, which would roughly correspond to a membrane with an equivalent pore radius in the 15–30 A range.

Lagos and Kitchener (28) studied the diffusion of three nonelectrolytes in strips of polystyrenesulfonic acid ion-exchange resins whose permeability could be closely controlled by the relative humidity in the experimental chamber. As Table II shows,

these membranes could discriminate between glycerol, glucose, and sucrose, and no marked discontinuities of behavior appear as the hydration is changed by a factor of 3.

Peterson and Gregor (39) report that the effective pore diameter of the cation-permeable membrane Nalfilm 1 is 6.0 A, as measured by the diffusion of unhydrated quaternary ammonium ions of different size The restriction to diffusion was calculated by equation 5, the Faxen equation. This 6.0 A value was in good agreement with an average distance of separation of fixed sites of 8.7 A calculated from volume measurements. The equivalent pore diameter was also used to calculate the hindered diffusion for the co-ion, Cl. The calculated value for the ratio of diffusion coefficient in the membrane to the free diffusion coefficient was 0.016 as compared with an experimental value of 0.014. Less good agreement was found for the counterion, K; this has been ascribed to electrostatic binding to the fixed charges.

Subsequently Kawabe et al. (40) measured the pore radii of Nalfilm 1 and several other polyethylene-styrene graft copolymer resins. They found that more consistent results could be obtained on the basis of an equivalent slit model than on that of a cylindrical equivalent pore model. These authors used equations similar to equation 6, replacing the steric hindrance term with the one appropriate for a slit, taken from Ferry (19) and using the slit form of the Faxen equation (41). They computed the steric hindrance term and the Faxen term separately and have emphasized the importance of each separate term in the computation. In the usual Faxen treatment, terms higher than the fifth power of α are neglected; Kawabe et al. point out that the seventh-power term should also be included. Equivalent slit radii of about 5 A were given for Nalfilm 1, and similar values for the other resins studied. Consistent results were obtained from the diffusion of both alkali cations and tetraalkylammonium ions.

RELATION OF WATER DIFFUSION TO OSMOTIC FLOW AS AN INDEX OF EQUIVALENT PORE DIMENSIONS—THEORY

Koefoed-Johnsen and Ussing (5) and Pappenheimer et al. (6) have independently pointed out that the ratio of the hydraulic conductivity, measured under either an osmotic or a hydraulic pressure gradient, to the transmembrane water-diffusion coefficient, as measured by tracers, provides information which can be used to calculate an equivalent pore radius for the channels in the membrane. The tracer measurement provides a value for $A_s/\Delta x$ (equation 2) which is $A_w/\Delta x$ when the substance traced is water. If the same pore dimensions determine both hydraulic conductivity and diffusion, and if Poiseuille's law may be applied to flow through these pores, and if the only substance flowing through the pores is water,

$$J_v = (A_w/\Delta x)(r^2/8\eta_w)\Delta P \qquad (15)$$

in which η_w is the viscosity of water and ΔP is the pressure difference. Thus r^2 may be obtained in principle from these two separate measurements. These measurements were first applied by Pappenheimer et al. to the determination of the equivalent pore radius in capillary membranes. They obtained a value of 30 A, the same figure which

they obtained from the restricted diffusion through capillary membranes. This consistency would seem to provide good evidence for the general applicability of this treatment, but the evidence is clouded by the fact that these authors did not take account of the influence of the reflection coefficient on osmotic pressure, as has been discussed by Kedem and Katchalsky (22). Furthermore, as already pointed out, it is unlikely that only a single barrier is traversed between capillary lumen and interstitial fluid; the presence of series barriers would introduce further complexities.

Durbin et al. (21) showed that the mathematical derivations given by Koefoed-Johnsen and Ussing and by Pappenheimer et al. were equivalent when the solutions were dilute and the osmotic volume flow was small. In a review article, Solomon (9) gave the following equations for the computation of the equivalent pore radius, r, by this method:

$$\lambda = (8\eta_w D_w/k')(P_f/P_d - 1). \tag{16}$$

λ is related to r by equation 17 below. D_w is the diffusion coefficient for water, and k' is a conversion factor $(RT/\bar{V}_w)$ having the value of 1.35×10^9 dynes/cm^2 at 23°C. At this temperature, $(8\eta_w D_w/k') = 14.3 \times 10^{-16}$ cm^2. P_f and P_d are permeability coefficients for filtration and diffusion respectively, and must be expressed in identical units. For small equivalent pores, it is necessary to take account of the difference between the steric hindrance in diffusion and bulk flow, that is, the difference between equations 6 and 7. This may be done by computing the equivalent pore radius by the following equation:

$$r = -a_w + \sqrt{2a_w^2 + \lambda} \tag{17}$$

in which a_w is the radius of the water molecule.

When Paganelli and Solomon (7) first extended this treatment to apertures in single biological membranes with radii as small as 4 A, they emphasized the conjectural nature of the extrapolation to these dimensions. They pointed out that though there was experimental evidence to support the use of equations 6 and 7 in the description of flow through cellophane membranes of 15 A pore radius, there was none below that radius. As has been discussed, equation 5 has subsequently been shown by Peterson and Gregor (39) to give consistent results for radii of 6 A. Paganelli and Solomon also pointed out the difficulties inherent in the assumption of bulk values for the diffusion coefficient and viscosity of water when it is contained within the membrane. They introduced the term *equivalent* pore radius to describe the operational nature of this description: a radius equivalent to the pore radius of an ideal membrane containing uniform, circular pores in which diffusion and bulk flow may be described by equations of Fick and Poiseuille. The assumption is *nowhere* made that all the pores are uniform and circular, that they all have the same radius, or that they remain fixed in position or in time. Indeed, the equivalent pore radius need not be the actual pore radius. Paganelli and Solomon pointed out that the equivalent pore radius for the human red cell "may be regarded for the moment as an attempt to describe in operational terms a physical property of a complex biological membrane."

In a subsequent review, Solomon (9) stressed the predictive use of the concept to

express the passive permeability properties of biological membranes to hydrophilic molecules by a single parameter which describes the steric and frictional properties of the pore. The ultimate test of the validity of the concept of the equivalent pore radius is whether this parameter does indeed provide a consistent descriptive account of the passive qualities of simple biological membranes, independent of the method of measurement. In a subsequent section, it will be shown that this seems to be the case for the red cell membranes of man, dog, and beef.

Mikulecky (42) has recently discussed the theoretical relation between diffusion and viscous flow in an article which is prefaced by a quotation from Onsager (14). Onsager's first paragraph, which has already been used in our introduction, is followed by: "Strictly speaking, the two [viscous flow and diffusion] are inseparable; for the 'hydrodynamic' velocity in a diffusion mixture is merely an average determined by some arbitrary convention." Mikulecky derives the equation for water flow through the membrane and emphasizes the arbitrary nature of the assumptions necessary for its solution. One set of assumptions comprises the ones that have been used in determining the equivalent pore by equation 16. Mikulecky points out the need for experimental verification of these relations and stresses the absolute requirement that consistent results be obtained when the model is subjected to a variety of experimental tests.

Is it reasonable to assign bulk viscosity and diffusion coefficients to the fluid within the equivalent pores? Fortunately, as Blank (Personal communication) has pointed out the diffusion coefficient and the viscosity appear in equation 16 as the product, $D_w\eta_w$. In classical terms, the Stokes-Einstein equation gives $D_w = RT/6\pi\eta_w a_w$, so that the product $D_w\eta_w$ is independent of the viscosity within the equivalent pore and is a function only of universal constants and the radius of the water molecule. Even though Stokes' law does not strictly apply to such small molecules, it is a reasonable first approximation, and it seems clear that the product $D_w\eta_w$ is much less dependent on the properties of the fluid within the equivalent pore than either the diffusion coefficient alone or the viscosity alone.

A similar conclusion can be reached from a consideration of the ratio in frictional terms. The apparent area for filtration is given by equation 7:

$$A_{sf}/A_p = [2(1 - \alpha)^2 - (1 - \alpha)^4](g^o/g) \tag{7}$$

and the apparent area for diffusion is given by equation 6:

$$A_{sd}/A_p = (1 - \alpha)^2(g^o/g). \tag{6}$$

The ratio of the two is

$$A_{sf}/A_{sd} = 2 - (1 - \alpha)^2. \tag{18}$$

Within the pore all the frictional terms in the Faxen equation for g^o/g (equation 5) cancel out, and one is left with only the ratio of the steric hindrance terms, which has already been introduced into the computation as equation 17. Thus, from this viewpoint also, the ratio of the two permeability coefficients is far less sensitive to the friction within the pore than is either coefficient alone.

Longuet-Higgins and Austin (43) have considered the theoretical problems concerned with the application of Poiseuille's law to pores in which the dimensions are so small that one solvent molecule cannot "overtake" another. On theoretical grounds an equation is derived in which the coefficient for hydrodynamic flow depends on the self-diffusion coefficient for the solute rather than on the fourth power of the radius as used in Poiseuille's law. Longuet-Higgins and Austin conclude that for equivalent pore radii less than 4.5 A, a diffusional mechanism will be operative; above this value, the mechanism of transport will be hydrodynamic, by Poiseuille flow. As will be shown in a later section, the coefficients for hydrodynamic flux are experimentally greater than those for diffusion in biological membranes characterized by a 4.5 A equivalent pore radius. Thus experimental evidence indicates that the equivalent pore radius of a channel in which diffusion alone is operative is appreciably smaller than that proposed by Longuet-Higgins and Austin.

When the channels are so narrow that water transport can take place only by diffusion through the channel, the flux is proportional to the channel area, that is, to r^2. When the channels are so large that the diffusional component may be neglected in comparison with viscous flow, Poiseuille's law obtains and flux is proportional to r^4. In the transitional region the flux must be proportional to some power of r greater than 2 and less than 4. The question at issue for the present purposes is the relationship of this power to the radius of the equivalent pore. As Mikulecky has pointed out, the theoretical value depends upon the model chosen. In practice it would seem likely that for pores of 4–6 A radius, the experimental power would lie between 3 and 4. Since, as will be shown later, agreement between the several methods of determining equivalent pore radii is quite good, it would appear that the sensitivity of the method to the power of the radius may be relatively small in the range of 4–6 A. An adequate theoretical treatment that covers the transitional region will probably be based on statistical considerations; it is of the highest importance that such a treatment be beveloped soon.

EXPERIMENTAL STUDIES ON VISCOSITY AND FLOW IN SMALL CHANNELS

The experimental evidence bearing on the viscosity of fluid in very small pores depends on the system used. Fedyakin (44) studied the thermal expansion of water in glass capillaries of radius from 100 to 1000 A. He found that the coefficient of volume expansion had a different temperature coefficient from that in bulk solution, and also depended on the pore radius. He concluded that the liquid structure in the capillary differed from that in bulk solution. In a later paper Fedyakin (45) found the viscosity of water to be linearly dependent on capillary tube radius in the range from 200 to 1000 A. In a review of these and other studies, Derjaguin (46) concluded that the viscosity of solutions in boundary layers changed jumpwise at a small distance from the wall. Derjaguin also pointed out that sliding along the walls of microcapillaries probably plays a major role in the flow process. He also interpreted the thermal expansion studies of Fedyakin as indicating that the structure of water in microcapillaries is more compact than in bulk solution, and that no breakup and rearrangement of structure occurs when the temperature is raised, in contrast to the behavior in bulk

solution. All this evidence has been obtained in glass and mica systems and is entirely different from what has been found in cellophane membranes and resins.

For example, the studies of Madras et al. (47) lead to a different conclusion. Water was driven through a swollen cellophane membrane by a hydrostatic pressure difference, and the flow was expressed as $J_v = K\Delta P/\eta\Delta x$. Their results on the temperature dependence of $K/\eta\Delta x$ have been plotted in Fig. 7 and show, as expected, that the

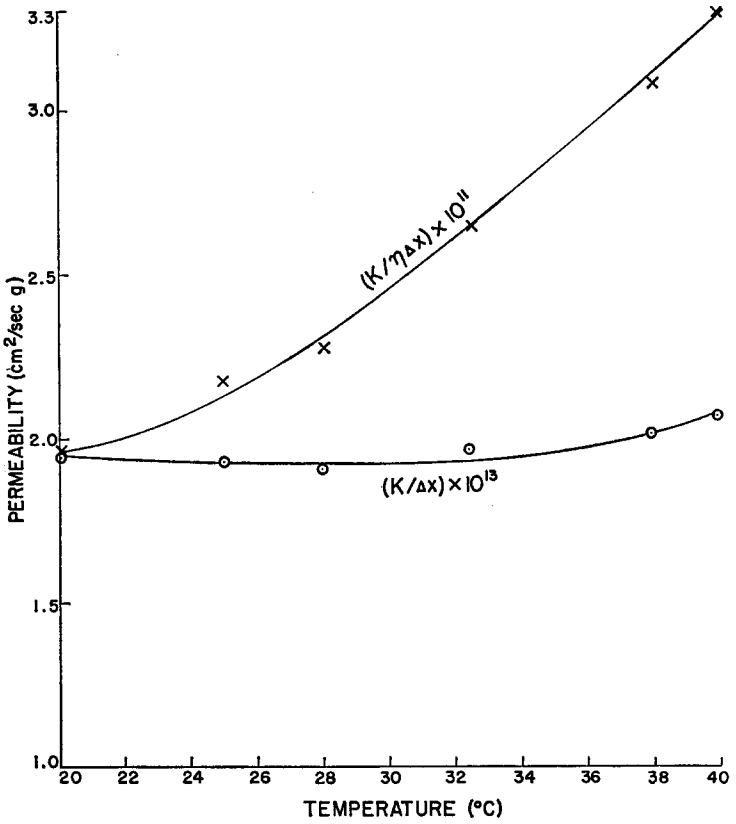

FIGURE 7. Permeability coefficients for bulk water flow under a hydrostatic pressure head, as measured by Madras et al. (47). The upper curve represents the change in the coefficient, $K/\eta\Delta x$, as a function of temperature. The lower curve has been corrected by multiplying by the bulk viscosity of water, η.

coefficient, $K/\eta\Delta x$, is a function of temperature. However, when this coefficient is multiplied by η, the bulk viscosity of water, to produce $K/\Delta x$, it is apparent that the permeability coefficient, K, is virtually independent of temperature. This cellophane membrane has been characterized by a comparison of the water content with the hydraulic conductivity, and found to have an equivalent pore radius of 15 A. This value may well be low, as indicated by the studies of Renkin (16), who showed this method to give a consistently lower value of equivalent pore radius than that obtained by the other methods we have discussed. In all cases, J_v was found to be proportional

to ΔP, which Madras et al. have interpreted as evidence of viscous flow. These results are in conflict with the evidence of Fedyakin and the conclusions of Derjaguin. It must be emphasized that the systems are different and that flow in narrow glass microcapillaries may indeed be different from that in cellophane membranes. Behavior in membranes of cellophane and collodion and in ion-exchange resins appears to be self-consistent.

Another example of flow through small apertures that is different from that in cellophane membranes is to be found in the Debye and Cleland (48) study of flow of *n*-decane through porous Vycor tubing under a hydrostatic pressure head. In this system, they compute that the capillary radius would be about 26 A if the porous tube were considered to be an array of uniform parallel capillaries. Debye and Cleland varied the temperature and measured the permeability coefficient, K, which should be independent of the viscosity. As the viscosity of the *n*-decane changed from about 2×10^{-3} poise to 13×10^{-3} poise, the permeability coefficient, instead of remaining constant as expected, decreased from about 4.5×10^{-16} cm^2 to 2.6×10^{-16} cm^2. Debye and Cleland suggest that their results may be accounted for by a bimodal model. An external sheath is considered free to slide along the wall of the equivalent pore. Thus the layer at the surface of the wall is not static as is assumed in the derivation of Poiseuille's law. Within the moving sheath, there is a region in which the velocity profile follows Poiseuille's law. In this treatment, there are two adjustable constants, the thickness of the sliding layer, and the friction between the layer and the wall. A sliding layer has also been proposed by Derjaguin, who suggests that such an effect in microcapillaries might result in flow proportional to the third rather than the fourth power of the radius.

The characteristics of flow of pure liquids in small capillaries depend upon two frictional coefficients, the solvent-solvent friction and the solvent-membrane friction. Thus there is no experimental contradiction between the results of Debye and Cleland and those obtained with cellophane membranes, since both the solvent and the membrane are different. The situation is somewhat different when the results of Fedyakin and those described by Derjaguin are compared with those on cellophane membranes. Here the membrane is different and the solvent is the same. The results obtained by Fedyakin and his colleagues are apparently characteristic of water layers as thick as 1000 A, in which the water-water friction ought to predominate. It is very hard to reconcile these findings with those of Madras et al., who showed that flow through cellophane membranes was governed by the bulk water viscosity coefficient, which is a measure of water-water friction. The results of many other studies both in artificial membranes and in ion-exchange resins are consistent with those of Madras et al., and we may be justified in extrapolating them to apertures as small as the equivalent pores in biological membranes. However, as the equivalent pore radius becomes smaller, the relative importance of the solvent-membrane friction increases, and in this respect cellophane membranes and ion-exchange resins are hardly satisfactory models for biological systems.

Evidence for an ordered flow in biological membranes of 4–6 A equivalent radius comes from the studies of Soll (32). Soll concluded that the small lipid-insoluble solute molecules usually used in permeability experiments were not truly spherical, as was ordinarily assumed. He treated them as cylinders and used molecular models to

obtain the diameter, d, and length, h. Subsequently Soll studied the validity of the cylindrical model by making a statistical analysis of correlations with both d and h. He analyzed the reflection coefficients determined on red cell membranes of man (11) and dog (49), as will be described later. There was a significant correlation between the solute diameter and the reflection coefficient. Correlation with the cylinder length was of less significance and could be ascribed to the interdependence of cylinder diameter and length.

The diffusion coefficient in free solution (data from Longsworth, reference 50) was then examined and found to be significantly correlated with the length of the equivalent cylinder rather than the diameter. Table III compares the correlation coefficients in the two cases and indicates that there is a substantial and measurable difference between hydraulic flow through pores of 4–6 A equivalent radius and diffusion in free solution. Soll concludes that the dynamics associated with bulk solvent flow tend to align the long axis of the cylindrical molecule parallel to the direction of flow.

TABLE III

CORRELATION COEFFICIENTS BETWEEN MOLECULAR DIMENSIONS, REFLECTION COEFFICIENTS IN RED CELL MEMBRANES AND FREE DIFFUSION

	Correlation coefficients	
	With log (solute diameter, d)	With log (solute length, h)
Log* $(1 - \sigma)$	0.89	0.38
Log‡ diffusion coefficient	0.30	0.88

* For nine solutes measured on human red cell membranes by Goldstein and Solomon (11).

‡ For the seven of those solutes whose diffusion coefficients were measured in free solution by Longsworth (50).

EXPERIMENTAL CHARACTERIZATION OF ARTIFICIAL MEMBRANES BY COMPARISON OF HYDRAULIC CONDUCTIVITY AND TRACER WATER DIFFUSION

Though the process of osmosis is well understood thermodynamically, there is no truly satisfactory kinetic theory of the process (see Dainty, reference 51 for a discussion). Early experimental evidence that the kinetics of bulk flow differed from tracer diffusion was given for biological membranes by Pappenheimer et al. (6), Pappenheimer (52), and Durbin et al. (21). Later, Mauro (53) studied both processes in a cellophane membrane and found, for the same gradient, that bulk flow was 730 times greater than tracer diffusion flow. Robbins and Mauro (54) studied these two permeability processes in a series of three collodion membranes of graded porosity. They calculated equivalent pore radii by equation 16 and obtained consistent results for their tightest membrane, whose equivalent pore radius was calculated as 21 A. This figure was consistent with the permeability of inulin, for which σ was calculated to be 0.62. Less consistent results were obtained with the membranes of larger pore radius,

for reasons which are not clear. Meschia and Setnikar (55) measured osmotic flow in a collodion membrane with pores whose radius was about 90 A, as calculated by equation 16. This estimate would seem to be in qualitative agreement with their determination that $\sigma = 0.02$ for raffinose, which in cylindrical conformation has a 4.1 A radius and 19 A length. Meschia and Setnikar demonstrated experimentally the importance of σ in determining the direction and velocity of solvent flow in osmotic measurements.

Thau et al. (56) have characterized a series of artificial membranes by the ratio P_f/P_d, which they denote as g. Though no account has been taken of the unstirred layer, Ginzburg and Katchalsky (29) have pointed out that it plays a negligible role in membranes of low permeability, and all but one of the membranes studied by Thau et al. had ω's of 10^{-15} mole/dyne sec. The series ranged from paper coated with polyethyl acrylate as an example of a liquid membrane to cellophane as an example of a membrane that was truly porous. The ratio P_f/P_d varied from 1.1 for polyethyl acrylate–coated paper to 80 for cellophane. When $P_f/P_d = 1$, there can be no viscous flow and the only passage through the membrane is by dissolution of single molecules of water in the membrane and subsequent independent diffusion of water molecules across the membrane. When the P_f/P_d ratio is very much greater than 1, there is a large component of bulk flow, and this process, in which water-water friction is of great importance, predominates. As already discussed, viscous flow is expected to be dominant through pores of large equivalent radius, because this flow depends upon the fourth power of the radius, whereas diffusion depends only upon the square. Classically, any P_f/P_d value greater than unity has been interpreted as an indication of viscous flow and thus as an implication of the presence of a porous structure. However, in the special membranes which were made by coating paper with liquid or filling the apertures in polyvinyl chloride membranes with liquid, Thau, Bloch, and Kedem found P_f/P_d ratios up to 2.1. They consider these membranes to behave as liquids rather than as porous structures, so that their results indicate that water-water friction within the membrane can be of the same order as the water-membrane friction in some instances; hence in specialized structures there appear to be ways to transport water molecules in small clusters other than by bulk flow.

In other membranes such as the acrylamide polymer gel studied by White (57), the P_f/P_d ratio seems to provide a good index of the pore dimensions. White varied the polymer concentration from 5 to 35%, and the permeability coefficient varied almost logarithmically with polymer concentration, as Fig. 8 shows. P_f/P_d also varied smoothly with polymer concentration, as shown in Fig. 9. White estimated his equivalent pore radius from the ratio of the hydraulic conductivity to the membrane water content, which, as has been discussed by Renkin (16), probably gives too low an estimate. At the highest polymer concentration, White calculated the equivalent pore radius to be 5 A. Under these conditions P_f/P_d was 1.75, indicating that there still remained a substantial viscous flow contribution. The variation of P_f/P_d is smooth and uniform, as is the change in the properties of the polymer. Bulk flow surely predominates in the polymers with the largest pores, and there seems to be no reason to ascribe the 1.75 ratio in the tightest membranes to any cause other than the continued participation of viscous flow in the transport of water across the membrane.

Lakshminarayanaiah (58) used the P_f/P_d ratio to measure the equivalent pore radius of two cation-exchange membranes, using equations similar to 16 and 17. One of these membranes, denoted as AMF C-103, had previously been characterized by Kawabe et al. (40). These authors had studied the diffusion of alkyl cations and tetraalkylammonium ions, and had found that their data were best fitted by a slit model with an equivalent pore radius of about 5 A for AMF C-103; this result is similar to their findings with Nalfilm 1, already discussed. From his data on the diffusion of tritiated water and viscous water flow, Lakshminarayanaiah concluded that AMF C-103 was characterized by an equivalent pore radius of 7.6 A, by using a cylindrical pore model. This must be considered very good agreement and illustrates again the consistency of the results obtained when small apertures are characterized by these several methods.

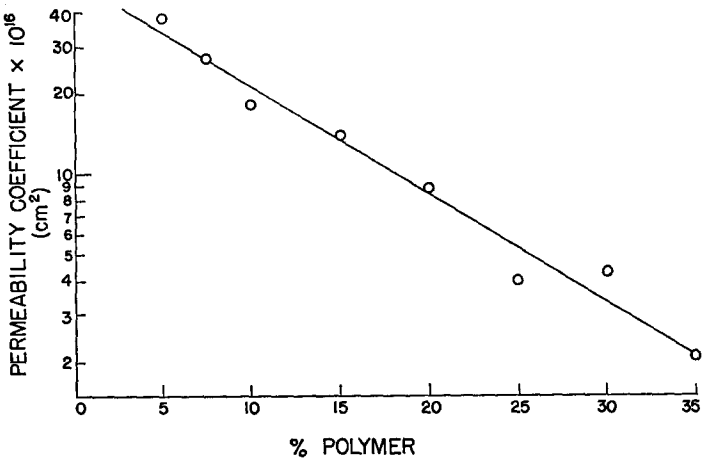

FIGURE 8. The permeability coefficient, K, of an acrylamide polymer as a function of the percentage of polymer in the gel, redrawn, with permission, from White (57). K is similar to the permeability coefficient in Fig. 7 and to that used by Debye and Cleland (48).

The membranes probably most similar to biological ones are the bimolecular lipid membranes originally described by Mueller et al. (59), which are probably less than 100 A thick. They have been prepared from brain lipids and are presumed to be similar to the lipid bilayers found between the protein sheets in biological membranes. In this instance there is no evidence supporting the presence of any structure with porelike characteristics. Although initial reports suggested that the P_f/P_d ratio might be greater than 1 for such membranes, these observations have subsequently been attributed to the effect of the unstirred layer. Cass and Finkelstein (60) have now shown that $P_f = P_d$ in such a membrane, exactly in accord with expectations based on the classical treatment. In general, $P_f > P_d$ for single biological membranes such as the red cell membrane, as will be discussed below. The difference between this inequality and the equality found by Cass and Finkelstein for lipid bilayers strongly

supports the view that red cell membranes are characterized by structures that permit viscous flow.

EQUIVALENT PORE DIMENSIONS IN RED CELL MEMBRANES

No modern coherent theory has been developed to describe the motions of solute molecules in small pores. Equations from several sources have been used to provide partial solutions to different aspects of the problem. As we have stressed, most of these do not rest on a firm theoretical ground, and they are to be considered, at best, as

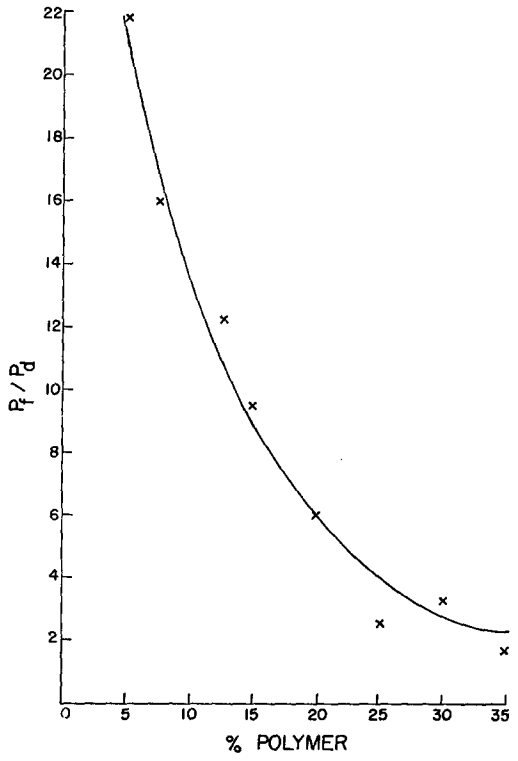

FIGURE 9. P_f/P_d as a function of the polymer concentration in the gel whose hydraulic conductivity has been illustrated in Fig. 8. The data have been taken from White (57).

semiempirical. Since the equivalent pore model is based on a semiempirical theory, the best test of the efficacy of the model is the consistency of the results obtained. Fortunately, in the case of cellophane and ion-exchange membranes there is a good deal of evidence in support of the main features.

The same assumptions are involved in the measurement of the equivalent pore radius of biological tissues, and the same requirement exists that consistent results be obtained when the passive permeability of the membrane to lipid-insoluble molecules is investigated by all the means available. Since any barrier in series with the membrane causes a modification of the permeability properties, we shall limit our discussion to mammalian red cells, in which a single membrane can be studied in free suspension. The methods available are the diffusion coefficients of selected lipid-insoluble

solutes, the reflection coefficients of similar solutes, and the P_f/P_d ratio for water. Detailed investigations in which two or more of these parameters have been measured have been made for three species: man, beef, and dog.

Diffusion Measurements in Red Cells

In man, the diffusion of small lipid-insoluble molecules into the red cells is very rapid, and no direct measurements of permeability coefficients for such molecules ($D_wA_w/\Delta x$ in equation 2) have yet been published. However, the time required for red cells to hemolyze in solutions of permeating substances is an effective index of solute diffusion (see Davson and Danielli, reference 3). Table IV presents a comparison of hemolysis times in human red cells for two homologous series of compounds. The data in the upper part of the table for the lipophilic monocarboxylic acids indicate that progression from a 3-carbon to a 5-carbon acid is accompanied by about a 2-fold decrease

TABLE IV

PERMEABILITY PROPERTIES OF HUMAN RED CELL MEMBRANES

Lipophilic monocarboxylic acids	Time for 50% penetration(61)
	sec
Propionic acid (3-carbon)	0.412
Butyric acid (4-carbon)	0.379
Valeric acid (5-carbon)	0.218
Hydrophilic alcohols	**Relative time of hemolysis (62)**
	sec
Ethylene glycol (2-carbon)	1.7
Glycerol (3-carbon)	60.0
Erythritol (4-carbon)	10,750.0

in hemolysis time, a relatively small increase in permeability. However, the relative hemolysis times for the three hydrophilic alcohols in the lower part of the table change by almost four orders of magnitude. These data provide strong support for the existence of separate routes of entrance for the two classes of substances. Table IV shows that there is a particularly steep increase in hemolysis time between glycerol and erythritol. Since the viscometric radii (63) of these two molecules are 3.1 and 3.5 A respectively, these data would be consonant with an equivalent pore radius greater than 3.5 A, since the cells do indeed hemolyze in erythritol.

Giebel and Passow (64) have studied the diffusion permeability of beef red cells to homologous series of both mono- and dicarboxylic acids by an ingenious method based on the exchange of intracellular Cl. The lipophilic monocarboxylic acids penetrate very much faster than the hydrophilic dicarboxylic acids; Giebel and Passow ascribe this difference to dissolution of the lipophilic molecules in the membrane fabric. In the case of the dicarboxylic acids there is a sharp decrease in permeability between molecules 7–7.5 A long and those 9 A long. Giebel and Passow consider

this decrease to represent steric hindrance and conclude that the equivalent pore radius lies between 3.8 and 4.5 A. Such an equivalent pore radius is in good agreement with the observations of Laris (65), who used a chemical method to measure the permeability of beef red cells to glucose (viscometric radius, 4.2 A). He found no measurable entrance of this molecule into the cells even after 19–24 hr of incubation; this would suggest that the upper limit for the equivalent pore radius is less than 4.2 A.

Wilbrandt (66) studied the entrance of a series of hexoses and pentoses into dog red cells by measuring volume changes resulting from solute permeation. No entrance of glucose could be detected by this method. It will be shown below that in this species σ for glucose is very close to 1, so that no detectable volume changes would necessarily be expected. Galactose, whose viscometric radius is also 4.2 A, entered slowly. The pentose arabinose (viscometric radius 3.8 A) permeated somewhat more rapidly. Laris (65) used chemical analyses to detect a slow entrance of glucose into dog red cells over a 6 hr period. His results are thus consistent with Wilbrandt's and point to an equivalent pore radius somewhat greater than 4.2 A.

Reflection Coefficient Measurements in Red Cells

Goldstein and Solomon (11) measured the reflection coefficients of a series of lipid-insoluble nonelectrolytes in human red cells. In order to avoid correction for penetration by the permeating solute, they used a rapid-reaction, continuous-flow method and extrapolated their data to zero time. They measured the volume flow, J_v, and found by interpolation the concentration of external permeant which would reduce J_v to zero at zero time. Since σ is defined, at $J_v = 0$, as the ratio of the theoretical osmotic pressure difference of the impermeant species to that of the permeant species, the computation is not difficult. The use of the flow method was subsequently criticized by Dainty (31), who suggested that the presence of an unstirred layer might affect the accuracy of the results. Subsequently, Sha'afi et al. (67) measured the thickness of the unstirred layer in an improved rapid-reaction, stop-flow apparatus of a similar type and found it to be about 5.5 μ. This is so small that it does not affect either the measurements of the reflection coefficient or those of osmotic or diffusional permeability by the flow method.

Rich et al. (49) also measured the reflection coefficients of a number of hydrophilic nonelectrolytes in the dog red cell, by using the improved rapid-reaction stop-flow apparatus. σ is related to the equivalent pore radius by equation 10 [$(1 - \sigma') = A_{sf}/A_{wf}$], in which $\sigma' = \sigma + \omega_s \bar{V}_s/L_p$, and equation 8 which relates A_{sf}/A_{wf} to the equivalent pore dimensions. Rich et al. also showed that the difference between σ and σ' was relatively unimportant for urea in dog red cells. It is also unimportant for the other molecules studied, since ω_s decreases very much more rapidly than $\bar{V}_s$ increases. They computed a value of 0.95 for σ_{glucose} and found that volume flow due to this difference could not be detected by their apparatus, in agreement with Wilbrandt's (66) observations. Fig. 10 shows the results of the reflection coefficient studies in both man and dog, fitted by least squares to equivalent pore radii determined by equations 8 and 10. The values of 4.3 A for man and 6.0 A for dog obtained by this method agree well with the results of the solute diffusion measurements.

P_f/P_d Measurements in Red Cells

Water diffuses into human red cells very rapidly with a half-time of about 7 msec. The diffusion permeability coefficient (P_d) of tritiated water in human red cells was measured by Paganelli and Solomon (7) in a rapid-reaction, continuous-flow apparatus. At the same time the hydraulic conductivity (P_f) was measured by Sidel and

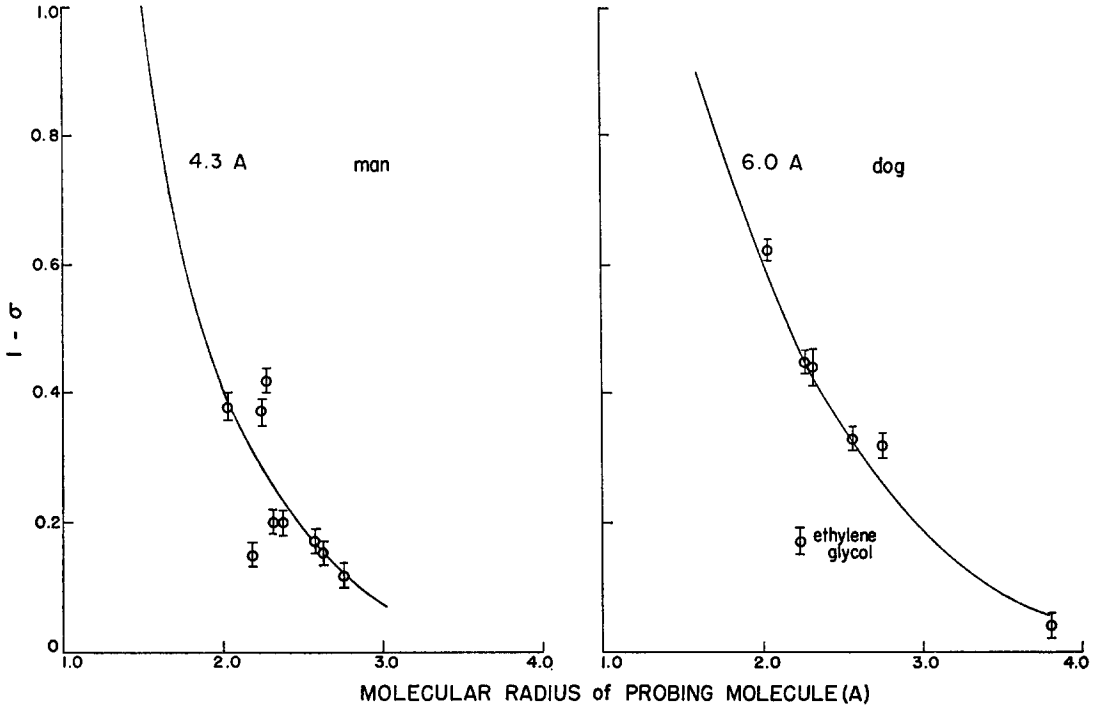

FIGURE 10. The relationship of $(1 - \sigma)$ to the molecular radius of the probing molecule, from the data of Goldstein and Solomon (11) and of Rich et al. (49). The curves are drawn according to equations 8 and 10. The molecular radii are viscometric radii from Schultz and Solomon (3) and the curves have been fitted by least squares on a computer. The length of the bars gives standard errors of the mean. The aberrant point for dog is ethylene glycol.

Solomon (68) in a different type of flow apparatus. These values of P_f and P_d were then combined to compute an equivalent pore radius according to equations 16 and 17. More recently, Barton and Brown (69) have made tracer diffusion measurements in an improved continuous-flow apparatus which enabled them to make observations over a longer time period. They found the rate constant for THO diffusion in human red cells to be 0.091 $msec^{-1}$ rather than 0.119 $msec^{-1}$ as given by Paganelli and Solomon. Sha'afi et al. (67) have also made a new measurement of P_f and obtained a value in very good agreement with that found earlier by Sidel and Solomon. Using

the new data (67, 69), the equivalent pore radius for human red cells becomes 4.5 A, in good agreement with the other results.

Villegas et al. (8) measured the P_f/P_d ratio in beef and dog. Subsequent measurements of P_f by Rich et al. (49) agreed well with the data on beef; the equivalent pore radius is calculated to be 4.1 A, in good agreement with the solute diffusion studies. In the case of the dog, the recent measurement by Rich et al. (49) was not in agreement with the earlier one given by Villegas, Barton, and Solomon, so a new measurement (49) was also made of P_d, using the improved equipment of Barton and Brown (69). The equivalent pore radius calculated from these results by equations 16 and 17 is 5.9 A, in good agreement with the values found by the other methods.

Table V shows a comparison of the results obtained by all the methods of investigation in all three species. For each species the results are in very close agreement and entirely consistent. This provides strong support for the application of the equivalent pore concept to the measurement of channel dimensions in single biologi-

TABLE V
EQUIVALENT PORE RADIUS IN RED CELLS OF DIFFERENT SPECIES

Species	Equivalent pore radius		
	Diffusion method	Reflection coefficient method	P_f/P_d method
	A	*A*	*A*
Man	>3.5	4.3	4.5
Dog	>4.2	6.2	5.9
Beef	3.8–4.2	—	4.1

cal membranes. Some reservations still remain, the most important being that no account has been taken of the influence of the medium osmolarity and the red cell volume on the parameters that have been measured. As Ginzburg and Katchalsky (29) have shown, such effects take place in cellophane membranes, and preliminary studies in our laboratory indicate that they also affect the hydraulic conductivity of red cell membranes.

The ability to separate steric restrictions from other interactions with the membrane opens the way to a number of important experiments. One intriguing question concerns the nature of the water-membrane friction within the equivalent pores. A comparison of the apparent activation energy for water diffusion in human red cells, whose equivalent pore radius is 4.4 A, and in dog red cells, whose equivalent pore radius is 6.1 A, should provide important data and may lead to a partial experimental separation of solvent-solvent and solvent-membrane friction in small pores. Once the normal steric hindrance and friction are established, the behavior of aberrant lipid-insoluble molecules can be interpreted in terms of reactions taking place within the pore. In this connection studies of temperature coefficients will be most important. Several molecules are already known to affect the permeability of ions, other solutes,

or solvent. They should be studied to determine whether their effect may be ascribed to alterations in the equivalent pore. Segregation of the permeability properties that are attributable to the equivalent pore makes it possible to study the permeability of lipid-soluble molecules, and thus to approach directly the measurement of chemical and physical forces within the lipid layer of the membrane.

A great deal more has been learned since the equivalent pore theory was first applied to the very small apertures that characterize single biological membranes. It is gratifying to know that the use of frictional coefficients and the treatment of coupled flows by irreversible thermodynamic methods leads to theoretical equations in accord with those initially made. Recent studies of ion exchange resins have given consistent results for pores of 6–8 A equivalent radius. The ratio of the osmotic permeability coefficient to the diffusion permeability coefficient of lipid bilayers is unity, in accord with our expectations for a nonporous structure. In cellophane membranes water appears to retain its bulk viscosity as the apertures are shrunken from 25 A down to about 5 A. All of this evidence from several disparate sources lend support to the use of the equivalent pore theory in the characterization of biological membranes.

The author would like to express his thanks to Drs. S. R. Caplan, C. Gary-Bobo, and R. Sha'afi for stimulating discussion and criticism.

REFERENCES

1. OVERTON, E. 1895. Über die osmotischen Eigenschaften der lebenden Pflanzen- und Tierzelle. *Vierteljahrschr. Naturforsch. Ges. Zürich.* **40**:159.
2. COLLANDER, R., and H. BARLUND. 1933. Permeabilitätsstudien an *Chara ceratophylla*. II. Die Permeabilität für Nichtelektrolyte. *Acta Botan. Fennica.* **11**:1.
3. DAVSON, H., and J. F. DANIELLI. 1952. The Permeability of Natural Membranes. Cambridge University Press, London.
4. HÖBER, R. 1945. Section 4: The surface of the protoplast, its properties and its architecture. *In* Physical Chemistry of Cells and Tissues. The Blakiston Co., Philadelphia. 227.
5. KOEFOED-JOHNSEN, V., and H. H. USSING. 1953. The contributions of diffusion and flow to the passage of D_2O through living membranes. *Acta Physiol. Scand.* **28**:60.
6. PAPPENHEIMER, J. R., E. M. RENKIN, and L. M. BORRERO. 1951. Filtration, diffusion and molecular sieving through peripheral capillary membranes. *Am. J. Physiol.* **167**:13.
7. PAGANELLI, C. V., and A. K. SOLOMON. 1957. The rate of exchange of tritiated water across the human red cell membrane. *J. Gen. Physiol.* **41**:259.
8. VILLEGAS, R., T. C. BARTON, and A. K. SOLOMON. 1958. The entrance of water into beef and dog red cells. *J. Gen. Physiol.* **42**:355.
9. SOLOMON, A. K. 1959. Equivalent pore dimensions in cellular membranes. *Proc. Natl. Biophys. Conf. 1st, Columbus, Ohio, 1957.* 314.
10. SOLOMON, A. K. 1961. Measurements of the equivalent pore radius in cell membranes. *In* Symposia Čsav. Membrane Transport and Metabolism. A. Kleinzeller and A. Kotyk, editors. Publishing House of ths Czechoslovak Academy of Sciences, Prague. 94.

11. GOLDSTEIN, D. A., and A. K. SOLOMON. 1960. Determination of equivalent pore radius for human red cells by osmotic pressure measurement. *J. Gen. Physiol.* **44**:1.
12. EINSTEIN, A. 1908. The elementary theory of the Brownian motion. *Z. Elektrochemi.* **14**:235. R. Fürth, editor. 1956. Investigations of the Theory of Brownian Movement. Dover Publications, Inc., New York.) 76.
13. PRANDTL, L., and O. G. TIETJENS. 1934. Fundamentals of Hydro- and Aeromechanics. Dover Publications, Inc., New York. 71.
14. ONSAGER, L. 1945. Theories and problems of liquid diffusion. *Ann. N. Y. Acad. Sci.* **46**:241.
15. LADENBURG, R. 1907. Über die innere Reibung zäher Flüssigkeiten und ihr Abhängigkeit vom Druck. *Ann. Physik.* **22**:287.
16. RENKIN, E. M. 1955. Filtration, diffusion, and molecular sieving through porous cellulose membranes. *J. Gen. Physiol.* **38**:225.
17. FAXEN, H. 1922. Die Bewegung einer starren Kugel längs der Achse eines mit zäher Flüssigkeit gefüllten Rohres. *Arkiv Mat., Astron. Fysik.* **17**(27).
18. BACON, L. R. 1935. Measurement of absolute viscosity by the falling sphere method. *J. Franklin Inst.* **221**:1322.
19. FERRY, J. D. 1936. Statistical evaluation of sieve constants in ultrafiltration. *J. Gen. Physiol.* **20**:95.
20. STAVERMAN, A. J. 1951. The theory of measurement of osmotic pressure. *Rec. Trav. Chim.* **70**:344.
21. DURBIN, R. P., H. FRANK, and A. K. SOLOMON. 1956. Water flow through frog gastric mucosa. *J. Gen. Physiol.* **39**:535.
22. KEDEM, O., and A. KATCHALSKY. 1958. Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. *Biochim. Biophys. Acta.* **27**: 229.
23. SPIEGLER, K. S. 1958. Transport processes in ionic membranes. *Trans. Faraday Soc.* **54**:1409.
24. DAINTY, J. 1961. Discussion. *In* Symposia Čsav. Membrane Transport and Metabolism. A. Kleinzeller and A. Kotyk, editors. Publishing House of the Czechoslovak Academy of Sciences, Prague. 109.
25. DAINTY, J., and B. Z. GINZBURG. 1963. Irreversible thermodynamics and frictional models of membrane processes, with particular reference to the cell membrane. *J. Theoret. Biol.* **5**:256.
26. KEDEM, O., and A. KATCHALSKY. 1961. A physical interpretation of the phenomenological coefficients of membrane permeability. *J. Gen. Physiol.* **45**:143.
27. MACKIE, J. S., and P. MEARES. 1955. The sorption of electrolytes by a cation-exchange resin membrane. *Proc. Roy. Soc. (London), Ser. A.* **232**:485.
28. LAGOS, A. E., and J. A. KITCHENER. 1960. Diffusion in polystyrenesulphonic acid ion-exchange resins. *Trans. Faraday Soc.* **56**:1245.
29. GINZBURG, A., and A. KATCHALSKY. 1963. The frictional coefficients of the flows of non-electrolytes through artificial membranes. *J. Gen. Physiol.* **47**:403.
30. DURBIN, R. P. 1960. Osmotic flow of water across permeable cellulose membranes. *J. Gen. Physiol.* **44**:315.
31. DAINTY, J. 1963. Water relations of plant cells. *Advan. Botan. Res.* **1**:179.

32. SOLL, A. 1967. A new approach to molecular configuration applied to aqueous pore transport. *J. Gen. Physiol.* **50:** 2565.
33. PHELPS, C. F. 1965. The physical properties of inulin solutions. *Biochem. J.* **95:** 41.
34. FRIEDMAN, L., and E. O. KRAEMER. 1930. The structure of gelatin gels from studies of diffusion. *J. Am. Chem. Soc.* **52:**1295.
35. FRIEDMAN, L. 1930. Structure of agar gels from studies of diffusion. *J. Am. Chem. Soc.* **52:**1311.
36. FRIEDMAN, L., and W. N. SHEARER. 1934. The effect of non-electrolytes upon diffusion in gelatin gels. *J. Am. Chem. Soc.* **56:**1323.
37. GARY-BOBO, C. 1965. L'effet d'exclusion moléculaire dans le gel de gélatine isoélectrique à 0, 23 et 38°C. *J. Chim. Phys.* **58:**923.
38. GREGOR, H. P., and E. KANTNER. 1957. Preparation and characterization of polymeric membranes of graded porosity. *J. Phys. Chem.* **61:**1169.
39. PETERSON, M. A., and H. P. GREGOR. 1959. Diffusion-exchange of exchange ions and nonexchange electrolyte in ion-exchange membrane systems. *J. Electrochem. Soc.* **106:**1051.
40. KAWABE, H., H. JACOBSON, I. F. MILLER, and H. P. GREGOR. 1966. Functional properties of cation-exchange membranes as related to their structures. *J. Colloid Sci.* **21:**79.
41. FAXEN, H. 1922. Der Widerstand gegen die Bewegung einer starren Kugel in einer zähen Flüssigkeit die zwischen zwei parallelen ebenen Wänden eingeschlossen ist. *Ann. Physik.* **68:**89.
42. MIKULECKY, D. C. 1967. On the relative contribution of viscous flow vs. diffusional (frictional) flow to the stationary state flow of water through a "tight" membrane. *Biophys. J.* **7:**527.
43. LONGUET-HIGGINS, H. C., and G. AUSTIN. 1966. The kinetics of osmotic transport through pores of molecular dimensions. *Biophys. J.* **6:**217.
44. FEDYAKIN, N. N. 1961. The thermal expansion of water in microcapillaries. *Dokl. Akad. Nauk. SSSR.* **138:**1389.
45. FEDYAKIN, N. N. 1962. The motion of liquid in microcapillaries. *Russ. J. Phys. Chem. (English Transl.).* **36:**1450.
46. DERJAGUIN, B. V. 1965. Recent research into the properties of water in thin films and in microcapillaries. *Symp. Soc. Exptl. Biol.* **19:**55.
47. MADRAS, S., R. L. MCINTOSH, and S. G. MASON. 1949. A preliminary study of the permeability of cellophane to liquids. *Can. J. Res. B.* **27:**764.
48. DEBYE, P., and R. L. CLELAND. 1959. Flow of liquid hydrocarbons in porous Vycor. *J. Appl. Phys.* **30:**843.
49. RICH, G. T., R. I. SHA'AFI, T. C. BARTON, and A. K. SOLOMON. 1967. Permeability studies on red cell membranes of dog, cat, and beef. *J. Gen. Physiol.* **50:** 2391.
50. LONGSWORTH, L. G. 1963. Diffusion in the water-methanol system and the Walden product. *J. Phys. Chem.* **67:**689.
51. DAINTY, J. 1965. Osmotic flow. *Symp. Soc. Exptl. Biol.* **19:**75.
52. PAPPENHEIMER, J. R. 1953. Passage of molecules through capillary walls. *Physiol. Rev.* **33:**387.

53. MAURO, A. 1957. Nature of solvent transfer in osmosis. *Science.* **126**:252.
54. ROBBINS, E., and A. MAURO. 1960. Experimental study of the independence of diffusion and hydrodynamic permeability coefficients in collodion membranes. *J. Gen. Physiol.* **43**:523.
55. MESCHIA, G., and I. SETNIKAR. 1958. Experimental study of osmosis through a collodion membrane. *J. Gen. Physiol.* **42**:429.
56. THAU, G., R. BLOCH, and O. KEDEM. 1966. Water transport in porous and nonporous membranes. *Desalination.* **1**:129.
57. WHITE, M. L. 1960. The permeability of an acrylamide polymer gel. *J. Phys. Chem.* **64**:1563.
58. LAKSHMINARAYANAIAH, N. 1967. Permeation of water through cation exchange membranes. *Biophys. J.* **7**:511.
59. MUELLER, P., D. O. RUDIN, H. TI TIEN, and W. C. WESCOTT. 1962. Reconstitution of cell membrane structure *in vitro* and its transformation into an excitable system. *Nature.* **194**:979.
60. CASS, A., and A. FINKELSTEIN. 1967. Water permeability of thin lipid membranes. *J. Gen. Physiol.* **50**:1765.
61. GREEN, J. W. 1949. The relative rate of penetration of the lower saturated monocarboxylic acids into mammalian erythrocytes. *J. Cellular Comp. Physiol.* **33**:247.
62. HÖBER, R., and S. L. ØRSKOV. 1933. Untersuchungen über die Permeergeschwindigkeit von Anelektrolyten bei den roten Blutkörperchen verschiedener Tierarten. *Pflügers Arch. Ges. Physiol.* **231**:599.
63. SCHULTZ, S. G., and A. K. SOLOMON. 1961. Determination of the effective hydrodynamic radii of small molecules by viscometry. *J. Gen. Physiol.* **44**:1189.
64. GIEBEL, O., and H. PASSOW. 1960. Die Permeabilität der Erythrocytenmembran für organische Anionen. *Pflügers Arch. Ges. Physiol.* **271**:378.
65. LARIS, P. C. 1958. Permeability and utilization of glucose in mammalian erythrocytes. *J. Cellular Comp. Physiol.* **51**:273.
66. WILBRANDT, W. 1938–39. Die Permeabilität der roten Blutkörperchen für einfache Zucker. *Pflügers Arch. Ges. Physiol.* **241**:302.
67. SHA'AFI, R. I., G. T. RICH, V. W. SIDEL, W. BOSSERT, and A. K. SOLOMON. 1967. The effect of the unstirred layer on human red cell water permeability. *J. Gen. Physiol.* **50**:1377.
68. SIDEL, V. W., and A. K. SOLOMON. 1957. Entrance of water into human red cells under an osmotic pressure gradient. *J. Gen. Physiol.* **41**:243.
69. BARTON, T. C., and D. A. J. BROWN. 1964. Water permeability of the fetal erythrocyte *J. Gen. Physiol.* **47**:839.