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On the Equivalent Pore Radius

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Introduction

Galey and Brahm (1985) have recently proposed that hydrodynamic analysis and, in particular, the equivalent pore theory have failed to define pore size in the human red cell membrane. On the basis of this conclusion they suggest that single-file diffusion is the mechanism by which water crosses the red cell membrane. The present article shows that Galey and Brahm's equations are based on a misunderstanding of the hydrodynamic principles applicable to transport through small pores and hence that their conclusion is not valid. The possible role of single-file diffusion is examined in detail, and it is shown that the physical chemical and irreversible thermodynamic behavior of the system are entirely consistent with conventional, rather than single-file, diffusion.

Discussion

When Paganelli and Solomon proposed (1957) the equivalent pore theory to describe the properties of the aqueous channel in the membrane of the red cell, we derived the equations from Renkin's (1954) careful study of the hydrodynamic restrictions covering the passage of small molecules through cylindrical channels. The basic premise, which was put forward initially by Pappenheimer, Renkin and Borrero (1951) and independently by Koefoed-Johnsen and Ussing (1953), arises from the fact that, in macroscopic systems, osmotic flow through a small cylindrical channel is proportional to the fourth power of the radius of the tube (Poiseuille's law) while diffusion is proportional to the second power (Fick's law). To express these relations quantitatively, it is necessary to take explicit account of steric hindrance which Renkin (1954) did, using the equations of Faxen (1922) to describe steric effects within the channel and those of Ferry (1936) to describe steric hindrance at the entrance. Our equations, as stated in further detail in a subsequent review (Solomon, 1968), give the equivalent pore radius, r, as a function of the ratio of the osmotic permeability, P_f, to the diffusional permeability, P_d, as follows:

$$r = -a_w + (2a_w^2 + \lambda)^{1/2} \tag{1}$$

$$\lambda = K(P_f/P_d - 1) \tag{2}$$

in which a_w is the radius of the water molecule, taken as 1.5 Å. $K = 8\eta_w D_w \bar{V}_w/RT$ in which the subscript w refers to water; η, viscosity; D, diffusion coefficient; $\bar{V}$, the partial molar volume; R and T have their usual meanings. At 20°C, $K = 14.5 \times 10^{-16}$ cm^2. P_f and P_d must be expressed in identical units.

Galey and Brahm (1985) have evaluated the equivalent pore theory using predictions made on the basis of their equation, for which no derivation is given

$$r = ((P_f/P_d)K')^{-2} \tag{3}$$

and have concluded that the theory is not valid. This is an error in logic. When it is found that a theory based on one set of equations [Eqs. (1) and (2)] does not conform to predictions from another equation [Eq. (3)], the conclusion can only refer to the applicability of the various equations and has no bearing on the validity of the theory, unless the original equations can be shown to be in error. Galey and Brahm have advanced no evidence to show that our equations are wrong, given the limitations which have been carefully spelled out by Paganelli and Solomon (1957; Solomon, 1968), or that there is

Key Words red cell membrane · equivalent pore · water transport · diffusion · osmosis · aqueous channel

any theoretical argument why their equation should be preferred to ours. It appears that Galey and Brahm's conclusion arises primarily from the use of an equation that neglects several essential factors which we have included explicitly, and we conclude, therefore, that their observations have little bearing on the validity of the equivalent pore theory.

As Galey and Brahm have pointed out, recent values of P_f differ from that originally given by Sidel and Solomon (1957) and recent values of P_d appear to differ from the initial one (Paganelli & Solomon, 1957), so that it is desirable to make a new computation of the human red cell equivalent pore radius. Such comparisons require explicit accounting for the physical chemical conditions under which the measurements to be compared are made, a consideration which was not applied by Galey and Brahm. Since $P_d = k(V'/A)$, in which k is the rate constant, the parameter which is measured, and V' and A are the cell water volume (= 0.72 times the cell volume, V) and cell surface area, it is necessary to refer all measurements to the same V/A ratio as well as the same temperature. Indeed, the changes in P_d to which Galey and Brahm call attention arise from more accurate recent estimates of red cell area and volume and not from changes in measurements of the water flux. These two geometric parameters have changed from the cell area of 1.63×10^{-6} cm^2 and a volume of 87×10^{-12} cm^3 used by Paganelli and Solomon to the present values (Jay, 1975; Canham & Burton, 1968) of $A = 1.35 \times 10^{-6}$ cm^2 and $V = 100 \times 10^{-12}$ cm^3. When Villegas, Barton and Solomon (1958) made their determination of P_d for dog and beef red cells, they pointed out that Paganelli and Solomon had made an error in their determination by including a zero time point obtained from hematocrit measurements in a time course in which subsequent time points were obtained from isotope measurements.[1] Subsequently, Barton and Brown (1964) made a new determination of human red cell P_d in which the zero time point was omitted. Their value, after corrections for A, V and T, agrees well with subsequent determinations, which are summarized by Dix and Solomon (1984) in their Table I. Dix and Solomon conclude that the best value for the diffusional permeability is $4.22 \pm 0.49 \times 10^{-13}$ cm sec^{-1} at 20°C, based on the values of A and V given above and a cell water content of 0.72. This value is an average of 10 determinations by nine groups of investigators, which include measurements of ^{3}HHO by two different methods and seven NMR measurements by various T_1 and T_2 methods. It does not include Brahm's (1982) value of 2.4×10^{-13} cm sec^{-1} at 25°C, which is outside of the range of the values given by the other investigators.

The recent study of P_f by Terwilliger and Solomon (1981) was made with very small gradients of osmotic pressure that minimizes nonlinear errors. Their values of $P_f = 1.8 \pm 0.1 \times 10^{-11}$ cm^3 dyne^{-1} sec^{-1} was obtained at 25–26°C with $A = 1.37 \times 10^{-6}$ cm^2 and $V = 1.04 \times 10^{-12}$ cm^3 and agrees very well with that of Galey (1978) of $1.84 \pm 0.05 \times 10^{-11}$ cm^3 dyne^{-1} sec^{-1} (after conversion to Terwilliger and Solomon's A/V) and that of Rich et al. (1968) of 1.8×10^{-11} cm^3 dyne^{-1} sec^{-1} (after conversion) obtained previously in this laboratory. These recent values are higher than the original value of Sidel and Solomon (1957), and it is this difference which has caused an increase in the equivalent pore radius. After conversion[2] to 20°C and the A/V values of Dix and Solomon, $P_f = 1.6 \times 10^{-11}$ cm^3 dyne^{-1} sec^{-1} and $P_f/P_d = 5.1$.

When these values are substituted in Eqs. (1) and (2), the equivalent pore radius at 20°C is calculated to be 6.5 ± 0.6 Å, much smaller than the 9-Å radius computed by Galey and Brahm (1985). This difference may be ascribed to Galey and Brahm's neglect of the diffusional component of osmotic flow [*compare* Eq. (2) with Eq. (3)] as well as the steric factors described by Ferry (1936) which hinder entrance to the aqueous channel [included explicitly in Eq. (1)]. Galey and Brahm next increase the radius to 12.5 Å by subtracting the putative contribution of water conductivity through the red cell lipids. Macey, Karan and Farmer (1972) had suggested that the 46% of diffusional flux across the red cell membrane that is not inhibited by the mercurial sulfhydryl reagent, pCMBS (*p*-chloromercuribenzene sulfonate) crosses by diffusion through the lipids. However, Dix and Solomon (1984) pointed out that the permeability coefficient of cholesterol containing lipids was two to ten times too small to ac-

[1] From a 1985 vantage, it may seem odd that a single time point might have a major effect on a time course. But thirty years ago, in 1954–56 when these experiments were done, determination of ^{3}H radioactivity was very difficult, the radiation being so soft that it could not penetrate even the thinnest counter windows, and scintillation counters were not yet available. For each determination, the water samples, which were very small, were introduced into an evacuated gas handling apparatus and the ^{3}HHO was converted to ^{3}HCH$_3$ by a Grignard reaction in the apparatus. This was used as the filling gas in a proportional counter, to which it was transferred by physical chemical gas handling methods, using apparatus which had been developed and constructed by Robinson (1951) in our laboratory. Each single determination required 20 individual steps. As a result, there were only four time points for each 10 msec time course, not counting the zero time point, computed from the hematocrit. As we subsequently realized, hematocrit values depend upon the degree of packing, a function of centrifuge speed, and thus contain assumptions that are absent in determinations made by isotope dilution.

[2] Temperature conversions were made using the activation energy for the hydraulic conductivity of the human red cell of 3.3 kcal M^{-1} given by Vieira et al. (1970).

count for this flux. Furthermore, Dix and Solomon found that perturbing red cell membranes with the lipophilic anesthetic, halothane, had no effect on red cell water permeability, whereas the same membrane concentration of halothane caused a significant increase in the water permeability of liposomes prepared from total red cell lipids. These experiments indicate that there is little water flux through red cell membrane lipids *in situ*. Carruthers and Melchior (1983) obtained a similar result in their studies of the osmotic water permeability of red cells. They found that the osmotic permeability coefficient for whole red cells was more than 500 times greater than that for vesicles prepared from a total red cell lipid extract.

The salient fact about the sieving properties of the red cell equivalent pore in man is that the pore is essentially impermeable to glucose (except for facilitated diffusion) though it readily passes three and four carbon nonelectrolytes. Measurements of a CPK model of glucose give orthogonal radii of 4.8 × 4.0 × 2.4 Å. Glucose is a highly hydrogen bonded molecule so that it is reasonable to assign it at least a single shell of hydration, which would increase each dimension by 3 Å, twice the radius of the water molecule. The hydrated glucose molecule is too big to pass through a uniform cylindrical pore of 6.5 Å radius without the intervention of some mechanism to promote exchange of components of the hydration shell with hydrogen bonding areas on the inside of the pore.

Diffusion in Bulk Solution *vs.* Single-file Diffusion

Moura et al. (1984) and Galey and Brahm (1985) have suggested that single file diffusion through a pore whose radius is less than 3 Å could provide an alternative explanation for the observation that the P_f/P_d ratio is greater than unity in human red cells. There is a profound physical difference between the fundamental mechanism of diffusion in bulk solution and single file diffusion. The self-diffusion coefficient for water in free solution, D_w, is given (in classical terms) by the Stokes-Einstein equation, $D_w = RT/(6\pi\eta_w N_{Av} a_w)$. The temperature dependence is entirely contained in the term, $F(T) = D_w\eta_w/T$. Wang (1951), who determined the activation energy for self-diffusion in bulk water to be 4.8 kcal M^{-1}, reported that $F(T)$ was independent of temperature over the range from 5–25°C, as shown in the Table. Wang interpreted these data as evidence that the self diffusion of water consists of movement of individual molecules of water, notwithstanding the structural properties of bulk water.

In 1970, we (Vieira, Sha'afi & Solomon, 1970) determined the activation energy for diffusional and osmotic flow of water across the human and dog red cell. All the temperature-dependent parameters for diffusion across the red cell membrane (Solomon, 1968) can be placed in a term, analogous to $F(T)$, $F'(T) = P_d\eta_w/T$. The Table shows that $F'(T)$ is also independent of temperature (Solomon, 1972). Following Wang, we interpret these data as evidence that water diffusion across the dog red cell membrane is dominated by diffusion of individual molecules (i.e., water-water interactions) and that water membrane interactions are of considerably less importance. This conclusion is strongly supported by the observation that the apparent activation energy for water diffusion across dog red cells is 4.9 ± 0.3 kcal M^{-1}, in excellent agreement with the 4.8 kcal M^{-1} for free diffusion in bulk solution.

Villegas et al. (1958) determined P_f/P_d for dog red cells to be 6.3. They pointed out that this figure was much larger than the value of 2.4 for man, determined at about the same time and with the same apparatus, techniques and assumptions. This indicates that the equivalent pore radius of the dog red cell is larger than that of man, in conformity with the classical observation that dog red cells are readily permeable to glucose. As a result, water-membrane interactions would be expected to be more important in human red cells. The activation energy for red cell membrane diffusion[3] in man is 6.0 ± 0.2 kcal M^{-1}, consistent with some water-membrane interaction, presumably as a result of hydrogen bond formation. Gary-Bobo and Solomon (1971) showed that increased hydrogen bonding capacity increased

Table.

Self diffusion of H_2O (°C)	D_w (cm² sec⁻¹ × 10⁵)	$D_w\eta_w/T$ (dyne deg⁻¹ × 10¹⁰)
5.00	1.426 ± 0.018	7.77
10.00	1.675 ± 0.025	7.73
15.00	1.97 ± 0.020	7.79
25.00	2.57 ± 0.022	7.70
H_2O diffusion across dog red cell membranes (°C)	**P_d (cm sec⁻¹ × 10³)**	**$P_d\eta_w/T$ (dyne cm⁻¹ deg⁻¹ × 10⁷)**
7	3.1 ± 0.3	1.58
22	5.0 ± 0.3	1.63
37	7.2 ± 0.5	1.61

The self-diffusion data are taken from Wang (1951) and the dog data from Vieira et al. (1970).

[3] Our value for the activation energy for diffusion is somewhat greater than, but in reasonable agreement with, subsequent determinations of 5.3 ± 0.1 kcal M^{-1} by Conlon and Outhred (1978), 5.0 ± 0.5 kcal M^{-1} by Brahm (1982) and 4.8 kcal M^{-1} by Macey et al. (1972).

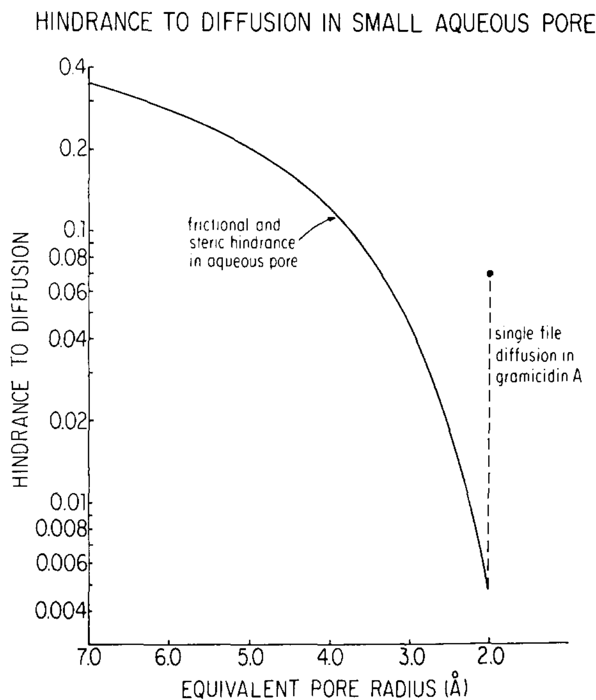

Fig. The curve for the steric and frictional restriction for conventional diffusion through pores is computed from the restricted diffusion equation of Renkin

$$A_{sd}/A_p = (1 - \alpha)^2 (1 - 2.104\alpha + 2.09\alpha^3 - 0.95\alpha^5)$$

in which A_{sd}/A_p is the restricted diffusion factor and α is a/r in which a is the radius of the water molecule, taken as 1.5 Å, and r is the pore radius in Å. This equation, in slightly modified form, provides a very good fit (Solomon et al., 1983) to the data of Holz and Finkelstein (1970) on nonelectrolyte diffusion through amphotericin B ($P_f/P_d = 3.0$) and nystatin pores ($P_f/P_d = 3.3$), with equivalent radii of about 4 Å. For gramicidin A, Dani and Levitt (1981) report that the single-file diffusion coefficient is about 7% of the free diffusion coefficient of bulk water. Since we do not know how this point is connected to the conventional restricted diffusion curve, we have arbitrarily drawn the dashed vertical line.

the activation energy from 5.5 kcal M^{-1} to 7.7 kcal M^{-1} for water diffusion through pores in cellophane membranes with $P_f/P_d = 1.65$-1.71.

For single-file diffusion, Levitt (1984) gives the equation

$$D = D_0 N \tag{4}$$

to relate D, the effective diffusion coefficient of the entire row of water molecules in the channel, to D_0, the diffusion coefficient the water molecule would have if it were all alone in the channel, which is governed by the water-membrane interaction. N is the number of water molecules in the channel. The physical difference between single-file diffusion and conventional diffusion lies in the coupling between the water molecules. Molecules crossing large water filled pores by free diffusion follow an irregular path and may wander in many directions before they emerge on the other side. In contrast, in single-file diffusion, the molecules are constrained to move in one direction only, and the motion of each molecule is closely coupled to that of its neighbors. This has a somewhat unexpected consequence. If a large pore is gradually shrunken in diameter, the diffusional flux will fall smoothly, initially slowly and then increasingly more rapidly until the pore approaches the dimensions of single-file diffusion. At this point the curve will change its direction, perhaps suddenly, and the flux will rise to the value consonant with single-file diffusion, as shown in the Figure. This confers a distinct biological advantage on single file pores which should be able to transport water much more rapidly than would be possible by diffusion through pores of significantly larger diameter.

Rosenberg and Finkelstein (1978) have pointed out a related aspect of these relationships. In ordinary diffusion through water-filled channels, P_f/P_d decreases as the channels become narrower until $P_f = P_d$ when the channels close. In contrast, for single-file diffusion, P_f/P_d is larger than it would be for conventional diffusion. Rosenberg and Finkelstein give a value of 5 for this ratio in the 2-Å gramicidin A channels and Dani and Levitt (1981) find 9. Either of these figures is larger than the value of $P_f/P_d = 3$, determined by Holz and Finkelstein (1970) for the much larger 4-Å amphotericin B and nystatin channels. The physical explanation arises from another aspect of the coupling between water molecules. In conventional flow through a pipe, driven by hydrostatic or osmotic pressure differences, cylindrical shells of water move past one another at a rate governed by the frictional coefficient, the viscosity η_w. This results in the parabolic velocity profile that follows from Poiseuille's law. In single-file osmotic flow, there is no slippage since each molecule propels directly the one in front of it and consequently the net flux is greater.

This difference between conventional and single-file osmotic and diffusional flow provides a method of reasoning which, in principle, would enable us to discriminate between single-file and conventional diffusion. If we accept the argument that dog red cells have larger pores than human ones, based on the passive permeability of dog red cells to glucose and the agreement of the activation energy data with that for bulk diffusion, we can use the P_f/P_d ratio to examine the question of single-file diffusion. If a significant fraction of water crossed the human red cell by single-file diffusion, the P_f/P_d ratio would be larger than that for dog, rather than

smaller as experimentally observed. We may conclude that the movement of water across the pores in the red cell membranes of dog and man is entirely consistent with the physical chemistry of conventional diffusion and there is no singularity which suggests single-file diffusion.

Osmotic Flow through Pores

The classical relationship between applied pressure gradients and viscosity observed in macroscopic systems was shown to extend to pores with an equivalent pore radius of 15 Å by Madras, McIntosh and Mason (1949), who showed that the product of hydraulic conductivity, L_p, and η_w was essentially independent of temperature between 20 and 40°C in cellophane membranes. In small pores, as discussed above, it is necessary to correct the observed L_p by subtracting the diffusional component, given by $P_d\bar{V}_w/RT$ (in which $\bar{V}_w$ is the partial molar volume of water). After making this correction for cellophane membranes with an equivalent pore radius of 4.5 Å ($P_f/P_d = 3.2$), Gary-Bobo and Solomon (1971) found that the product of the bulk water flow and water viscosity was independent of temperature over the range from 2 to 30°C. This observation shows that the viscous properties of water in the cellophane membrane are close to those in bulk solution. In the dog red cell membrane, as Sha'afi and Gary-Bobo (1973) pointed out, the product of bulk water flow and bulk water viscosity is independent of temperature over the range from 5 to 37°C, and in the human red cell this product depends only slightly on temperature, rising approximately 12% over the same temperature range. The activation energies for osmotic flow in dog and human red cells are 3.7 ± 0.7 and 3.3 ± 0.4 kcal M^{-1}, probably not significantly different from the activation energy of 4.2 kcal M^{-1} for the viscosity of bulk water. Thus, viscous flow across these red cell membranes is entirely consistent with expectations based on the physical chemistry of conventional viscous flow in bulk solution and there are no striking differences between red cells of dog and man which might suggest single-file diffusion.

Coupling between Urea and Water Flow

The single-file channel in gramicidin A excludes urea, whereas urea rapidly permeates the human red cell membrane. Since the exclusion of urea is characteristic of single-file diffusion, Galey and Brahm argue that urea utilizes an independent transport system to enter the human red cell. The question of whether urea and water are transported in separate channels can be answered by examining the coupling between these fluxes. The irreversible thermodynamic parameter, σ, the reflection coefficient (which varies between limits of 0 and 1) measures the interaction between solute and solvent in an aqueous channel. Theoretically[4], if $\sigma = 1$ (or approximately 1), there is no interaction between solute and solvent as the solute permeates the cell membrane. If σ is significantly <1, there is an interaction between solute and solvent in an aqueous channel so that these two components of the solution do not cross the membrane independent of one another. Recently, Chasan and Solomon (1985) have made a new measurement of σ_{urea} in the human red cell and found it to equal 0.70 ± 0.02, which is significantly less than 0.95, the theoretical value for independent flow of urea and water across the human red cell membrane. This value is higher than those of 0.62 ± 0.02 and 0.55 ± 0.02 previously obtained in this laboratory by Goldstein and Solomon (1960) and Sha'afi et al. (1970) and somewhat lower than the figure of 0.79 ± 0.02 obtained by Owen and Eyring (1975). Levitt and Mlekoday (1983), who have measured σ_{urea} in the human red cell by a method in which all three permeability coefficients are measured from analysis of a single shrink-swell curve, report that their best value is 1.0 but that 0.75 also gives a satisfactory fit[5] to their data. Thus, Levitt and Mlekoday's evidence is consistent with σ_{urea} being significantly less than unity and all the other measurements show this to be the case. These measurements show that there is a significant interaction of urea with water, as solute and solvent cross the membrane, so a single-file channel which excludes urea is inconsistent with the experimental data.

The stubborn experimental observation on which all parties agree is that the P_f/P_d ratio is very much greater than unity. As we have pointed out (Solomon, 1968), this observation is characteristic of aqueous channels in a wide variety of porous

[4] The exact criterion for interaction between solute and solvent in a membrane aqueous channel is (Katchalsky & Curran, 1965):

$$\sigma < 1 - \omega\bar{V}_s/P_f$$

The second term on the right is ordinarily very small for nonlipophilic solutes in biological membranes. For urea in the human red cell membrane, the correction term is 0.05, based on our values for the urea permeability coefficient ω, and for $\bar{V}_s$ and P_f given in Chasan and Solomon (1985).

[5] Smith, Myslik and Dix (1986) have conducted a sensitivity analysis of this method of determining the permeability coefficients and have shown that σ cannot be determined accurately without independent determination of the other two parameters, which may explain the spread in Levitt and Mlekoday's data.

structures, including collodion membranes and cation exchange membranes, as well as biological ones. Since neither the physical chemical evidence, nor the irreversible thermodynamic evidence, provide a compelling argument for single-file diffusion, we conclude that water crosses the red cell membrane through an equivalent pore whose radius of 6.5 ± 0.6 Å is large enough to permit the passage of small nonelectrolytes.

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References

Barton, T.C., Brown, D.A.J. 1964. Water permeability of the fetal erythrocyte. *J. Gen. Physiol.* **47**:839–849

Brahm, J. 1982. Diffusional water permeability of human erythrocytes and their ghosts. *J. Gen. Physiol.* **79**:791–819

Canham, P.B., Burton, A.C. 1968. Distribution of size and shape in populations of normal human red cells. *Circ. Res.* **22**:405–422

Carruthers, A., Melchior, D.L. 1983. Studies of the relationship between bilayer water permeability and bilayer physical state. *Biochemistry* **22**:5797–5807

Chasan, B., Solomon, A.K. 1985. Urea reflection coefficient for the red cell membrane. *Biochim. Biophys. Acta* **821**:56–62

Conlon, T., Outhred, R. 1978. The temperature dependence of erythrocyte water diffusion permeability. *Biochim. Biophys. Acta* **511**:408–418

Dani, J.A., Levitt, D.G. 1981. Water transport and ion-water interaction in the gramicidin channel. *Biophys. J.* **35**:501–508

Dix, J.A., Solomon, A.K. 1984. Role of membrane proteins and lipids in water diffusion across red cell membranes. *Biochim. Biophys. Acta* **773**:219–230

Faxen, H. 1922. Die Bewegung einer starren Kugel Längs der Achse eines mit zäher Flüssigkeit gefüllten Rohres. *Arkiv. Mat., Astron. Fysik.* **17**:27

Ferry, J.D. 1936. Statistical evaluation of sieve constants in ultrafiltration. *J. Gen. Physiol.* **20**:95–104

Galey, W.R. 1978. Determination of human erythrocyte membrane hydraulic conductivity. *J. Membr. Sci.* **4**:41–49

Galey, W.R., Brahm, J. 1985. The failure of hydrodynamic analysis to define pore size in cell membranes. *Biochim. Biophys. Acta* **818**:425–428

Gary-Bobo, C.M., Solomon, A.K. 1971. Effect of geometrical and chemical constraints on water flux across artificial membranes. *J. Gen. Physiol.* **57**:610–622

Goldstein, D.A., Solomon, A.K. 1960. Determination of equivalent pore radius for human red cells by osmotic pressure measurement. *J. Gen. Physiol.* **44**:1–17

Holz, R., Finkelstein, A. 1970. The water and nonelectrolyte permeability induced in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *J. Gen. Physiol.* **56**:125–145

Jay, A.W.L. 1975. Geometry of the human erythrocyte. *Biophys. J.* **15**:205–222

Katchalsky, A., Curran, P.F. 1965. Nonequilibrium Thermodynamics in Biophysics. p. 131. Harvard University Press, Cambridge

Koefoed-Johnsen, V., Ussing, H.H. 1953. The contributions of diffusion and flow to the passage of D_2O through living membranes. *Acta Physiol. Scand.* **28**:60–76

Levitt, D.G. 1984. Kinetics of movement in narrow channels. *Curr. Topics Membr. Transp.* **21**:181–197

Levitt, D.G., Mlekoday, H.J. 1983. Reflection coefficient and permeability of urea and ethylene glycol in the human red cell membrane. *J. Gen. Physiol.* **81**:239–253

Macey, R.I., Karan, D.M., Farmer, R.E.L. 1972. Properties of water channels in human red cells. *In*: Biomembranes. Vol. 3, pp. 331–340. F. Kreuzer and J.F.G. Slegers, editors. Plenum, New York

Madras, S., McIntosh, R.L., Mason, S.G. 1949. A preliminary study of the permeability of cellophane to liquids. *Can. J. Res. B* **27**:764–779

Moura, T.F., Macey, R.I., Chien, D.Y., Karan, D., Santos, H. 1984. Thermodynamics of all-or-none water channel closure in red cells. *J. Membrane Biol.* **81**:105–111

Owen, J.D., Eyring, E.M. 1975. Reflection coefficients of permeant molecules in human red cell suspensions. *J. Gen. Physiol.* **66**:251–265

Paganelli, C.V., Solomon, A.K. 1957. The rate of exchange of tritiated water across the human red cell membrane. *J. Gen. Physiol.* **41**:259–277

Pappenheimer, J.R., Renkin, E.M., Borrero, L.M. 1951. Filtration, diffusion and molecular sieving through peripheral capillary membranes. *Am. J. Physiol.* **167**:13–46

Renkin, E.M. 1954. Filtration, diffusion and molecular sieving through porous cellulose membranes. *J. Gen. Physiol.* **38**:225–243

Rich, G.T., Sha'afi, R.I., Romualdez, A., Solomon, A.K. 1968. Effect of osmolality on the hydraulic permeability coefficient of red cells. *J. Gen. Physiol.* **52**:941–954

Robinson, C.V. 1951. A methane, proportional counting method for the assay of tritium. *Rev. Sci. Instrum.* **22**:353–355

Rosenberg, P.A., Finkelstein, A. 1978. Water permeability of gramicidin A-treated lipid bilayer membranes. *J. Gen. Physiol.* **72**:341–350

Sha'afi, R.I., Rich, G.T., Mikulecky, D.C., Solomon, A.K. 1970. Determination of urea permeability in red cells by minimum method. *J. Gen. Physiol.* **55**:427–450

Sha'afi, R.I., Gary-Bobo, C.M. 1973. Water and nonelectrolytes permeability in mammalian red cell membranes. *Prog. Biophys. Molec. Biol.* **26**:103–146

Sidel, V.W., Solomon, A.K. 1957. Entrance of water into human red cells under an osmotic pressure gradient. *J. Gen. Physiol.* **41**:243–257

Smith, K.R., Myslik, J., Dix, J.A. 1986. Determination of reflection coefficient from scattered light intensity in osmotic shock experiments. *Biophys. J.* **49**:273*a*

Solomon, A.K. 1968. Characterization of biological membranes by equivalent pores. *J. Gen. Physiol.* **51**:335*s*–364*s*

Solomon, A.K. 1972. Propeties of water in red cell and synthetic membranes. *In:* Biomembranes. Vol. 3, pp. 299–300. F. Kreuzer and J.F.G. Slegers, editors. Plenum, New York

Solomon, A.K., Chasan, B., Dix, J.A., Lukacovic, M.F., Toon, M.R., Verkman, A.S. 1983. The aqueous channel in the red cell membrane: Band 3 as a channel for anions, cations, nonelectrolytes, and water. *Ann. N.Y. Acad. Sci.* **414**:97–124

Terwilliger, T.C., Solomon, A.K. 1981. Osmotic water permeability of human red cells. *J. Gen. Physiol.* **77**:549–570

Vieira, F.L., Sha'afi, R.I., Solomon, A.K. 1970. The state of water in human and dog red cell membranes. *J. Gen. Physiol.* **55**:451–466

Villegas, R., Barton, T.C., Solomon, A.K. 1958. The entrance of water into beef and dog red cells. *J. Gen. Physiol.* **42**:355–369

Wang, J.H. 1951. Self-diffusion and structure of liquid water. *J. Am. Chem. Soc.* **73**:510–513

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