J. Membrane Biol. 10, 367–371 (1972)

Independent Pathways for Water and Solute Movement across the Cell Membrane

Richard M. Hays

Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461

Received 1 November 1972

Summary. In published studies of the relationship between movement of nonelectrolytes across cell membranes and the lipid solubility of these test molecules, it is generally found that a number of the smaller, more water-soluble molecules deviate significantly from the general pattern relating permeability (or reflection coefficient) to lipid solubility. This is often true of the amides, for example, whose reflection coefficients are considerably lower than expected on the basis of lipid solubility. While this has been interpretep in terms of the movement of these solutes through aqueous channels in the membrane, it now appears that many of these "deviant" molecules may cross the membrane by means of carrier-mediated diffusion, independent of osmotic water flow. This has important implications for studies in which equivalent pore radius has been estimated from the reflection coefficients of small hydrophilic molecules, and for our present concepts of membrane structure.

Our understanding of cell membrane structure is based to an important extent on studies of membrane permeability. The early work of Overton (1899) on the movement of nonelectrolytes across cell membranes has had a particularly strong influence on biologists up to the present time. Overton proposed that the rate of penetration of cell membranes by nonelectrolytes was correlated with their lipid/water partition coefficients; thus, highly lipid-soluble molecules would be expected to have higher permeability coefficients across predominantly lipid membranes than more polar, water-soluble species.

The general validity of Overton's suggestion has been supported by studies of a variety of biological membranes, including *Nitella* (Collander, 1954), rabbit gallbladder (Wright & Diamond, 1969), frog choroid plexus (Wright & Prather, 1970) and red cell (Sha'afi, Gary-Bobo & Solomon, 1971). There has been a generally good correlation between the permeability coefficient (or reflection coefficient) of uncharged molecules and the

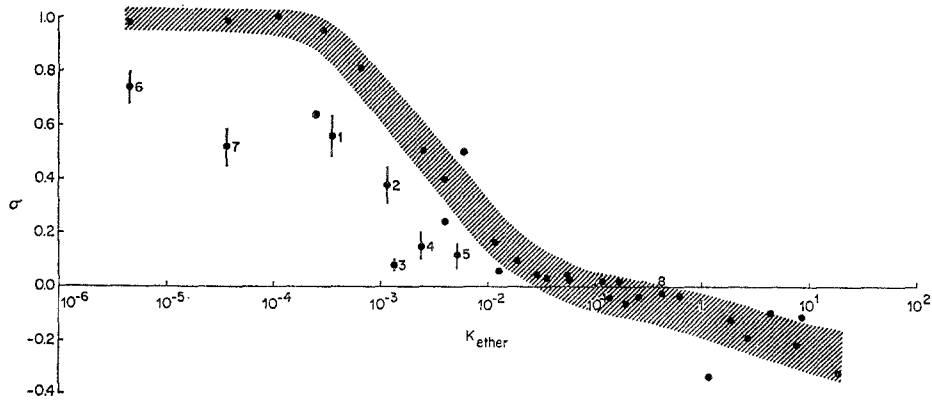

Fig. 1. The relation between σ and the ether/water partition coefficient. The ordinate shows the average σ for nonelectrolytes in the frog choroid plexus; the abscissa shows the ether/water partition coefficient. The numbered points are, 1 urea, 2 methyl urea, 3 formamide, 4 acetamide, 5 ethylene glycol, 6 D-glucose, 7 L-arabinose, and 8 pinacol; the standard deviations are represented by the bars on each point. The shaded band indicates the general pattern of the other points, and, apart from the fact that the width of the band indicates the standard deviation of the results, the band has no theoretical significance. (From Wright and Prather, 1970)

water/lipid partition coefficient. Exceptions, however, are usually encountered: for example, certain small amides proved to have much lower reflection coefficients than a large group of nonamides with comparable ether/water partition coefficients, in studies by Wright and Diamond of rabbit gallbladder (1969). A similar situation exists in frog choroid plexus (Fig. 1); here, urea, methylurea, formamide, and acetamide, as well as ethylene glycol, D-glucose and L-arabinose have unexpectedly low reflection coefficients.

How can we account for the deviation of this group of compounds from the general pattern of nonelectrolytes? It is of some importance to do so, since these water-soluble molecules have been among those whose reflection coefficients have been used in the determination of equivalent pore radii of cell membranes (Goldstein & Solomon, 1960; Fenstermacher & Johnson, 1966; Hays, Harkness & Franki, 1970). It has been assumed that the more water-soluble compounds would be those which penetrate the membrane via aqueous channels, and that the equivalent radius of such channels could be estimated from the reflection coefficients of a series of such compounds. Fig. 2 shows an estimate by us of the equivalent pore radius of the luminal membrane of the toad bladder in the presence and absence of vasopressin. A series of small, water-soluble compounds were placed in the luminal bathing medium of the toad bladder, and their ability to retard osmotic

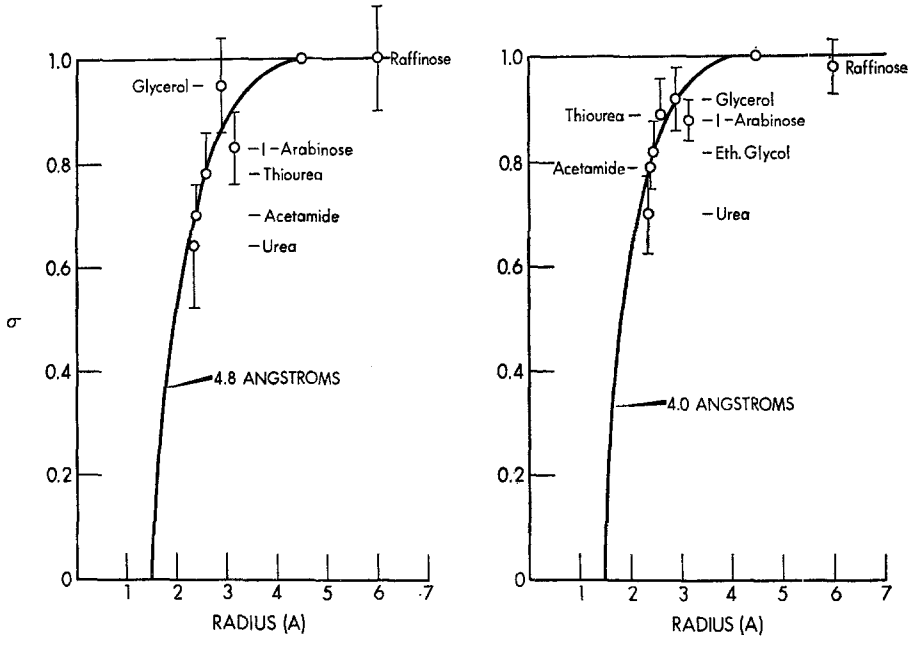

Fig. 2. Reflection coefficients of small molecules in control bladders (*left*) and vasopressin-treated bladders (*right*). Vertical bars, ± 1 standard error. Sucrose, the reference molecule, is shown as an open circle at 4.5 Å. (From Hays, Harkness and Franki, 1970)

water flow across the bladder was compared to that of sucrose, which was assigned a σ of 1. Thus, the "osmotic efficiency" of a series of molecules could be tested, and correlated, in this study, with their mean radius. It can be seen from Fig. 2 that a reasonably good fit of the data was obtained to theoretical curves for equivalent pore radii of 4.8 Å in the absence of vasopressin, and 4.0 Å in the presence of vasopressin. Our conclusion at the time was that the barrier limiting diffusion of these test molecules contained aqueous channels with the radii listed above, and that vasopressin did not increase the dimensions of these channels.

However, it should be noted that the test molecules in this study included urea, acetamide and ethylene glycol, some of the "deviant" molecules shown in Fig. 1. This raises an important question: in view of the atypical behavior of these solutes can we be certain that they are simply moving across the membrane passively in aqueous channels? And, further, if this is not the case, can we employ these test molecules in estimates of equivalent pore radius? In their consideration of the anomalous behavior of small polar solutes, Diamond and Wright (1969) and Wright and Prather (1970) suggested that they might permeate the membrane via the lipid phase rather

than via aqueous pores, and therefore be of little value in estimating pore radius.

The studies of Macey and Farmer (1970) on water and solute permeability across the red cell have thrown new light on this problem. These workers found that phloretin (the aglucone of phlorizen) markedly inhibited the movement of urea, methylurea and glycerol across the red cell membrane, but had no effect on osmotic water flow. There was, then, a clear dissociation between the movement of water and the movement of certain solutes, and it was concluded that these solutes might be transported via a phloretin-inhibited, facilitated diffusion system. We have obtained comparable results in studies of water and solute movement across the toad bladder (Franki, Levine & Hays, 1972): phloretin markedly inhibited the movement of urea and all other amides tested, but had no effect on osmotic water flow, or the movement of ethanol and ethylene glycol. It seems entirely possible at this point that the penetration of cell membranes by certain nonelectrolytes (in addition to the sugars, whose transport has long been recognized as carrier-mediated), takes place in some polar or nonpolar region of the membrane, independant of the pathway for water. We know too little about this pathway to provide any detailed description of a carrier mechanism, but the findings indicate that there is a component of the membrane that has an affinity for certain small solutes. Selectivity undoubtedly varies with the particular tissue under consideration, but for each tissue there is probably a group of small nonelectrolytes whose movement is carrier-mediated. This does not mean that small polar solutes cannot be used to estimate pore radius, but the list of solutes that meet the requirement of moving passively through the membrane in aqueous channels has been reduced by the experiments cited above. Only further investigation will clarify this problem.

In their important work entitled "A Physical Interpretation of the Phenomenological Coefficients of Membrane Permeability" (1961), Kedem and Katchalsky expressed the relationship between the reflection coefficient σ and the permeability coefficient for a given solute ω as follows:

$$\sigma = 1 - \frac{\omega \bar{V}_s}{L_p} - \frac{K f_{sw}}{\varphi_w (f_{sw} + f_{sm})} \tag{1}$$

where $\bar{V}_s$ is the partial molar volume of the solute, L_p the mechanical filtration coefficient for water, K a distribution coefficient, f_{sw} and f_{sm} the frictional coefficients between solute and water, or solute and membrane, respectively, and φ_w the volume fraction of water in the membrane. For the case in which solute and solvent penetrate the membrane through different pathways, there is no frictional interaction between solute and solvent, $f_{sw} = 0$,

and the equation becomes

$$\sigma = 1 - \frac{\omega \overline{V}_s}{L_p}. \tag{2}$$

This expression describes the independence of solute and water movement which, unexpectedly, has assumed new importance in our approach to the problem of membrane structure. Aharon Katchalsky recognized how much can be gained from unexpected findings, and would have considered new possibilities with the boundless enthusiasm that marked his whole life.

References

Collander, R. 1954. The permeability of *Nitella* cells to non-electrolytes. *Physiol. Pl.* **7**:420.

Diamond, J. M., Wright, E. M. 1969. Biological membranes: The physical basis of ion and non-electrolyte selectivity. *Annu. Rev. Physiol.* **31**:581.

Fenstermacher, J. D., Johnson, J. A. 1966. Filtration and reflection coefficients of the rabbit blood-brain barrier. *Amer. J. Physiol.* **211**:341.

Franki, N., Levine, S., Hays, R. M. 1972. Evidence that vasopressin opens independent pathways for water and urea in the cell membrane. *Proc. 5th Int. Congr. Nephrology*, p. 78 (*Abstr.*).

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.

Hays, R., Harkness, S. H., Franki, N. 1970. The movement of urea and other small molecules across the toad bladder. *In:* Urea and the Kidney. B. Schmidt-Nielsen, editor. p. 149. *Excerpta Med., Amst.*

Kedem, O., Katchalsky, A. 1961. A physical interpretation of the phenomenological coefficients of membrane permeability. *J. Gen. Physiol.* **45**:143.

Macey, R. I., Farmer, R. E. L. 1970. Inhibition of water and solute permeability in human red cells. *Biochim. Biophys. Acta* **211**:104.

Overton, E. 1899. Über die allgemeinen osmotischen Eigenschaften der Zelle, ihre vermutlichen Ursachen und ihre Bedeutung für die Physiologie. *Vjschr. Naturforsch. Ges. Zürich* **44**:88.

Sha'afi, R. I., Gary-Bobo, C. M., Solomon, A. K. 1971. Permeability of red cell membranes to small hydrophilic and lipophilic solutes. *J. Gen. Physiol.* **58**:238.

Wright, E. M., Diamond, J. M. 1969. Patterns of non-electrolyte permeability. *Proc. Roy. Soc. (London) B* **172**:227.

Wright, E. M., Prather, J. W. 1970. The permeability of the frog choroid plexus to nonelectrolytes. *J. Membrane Biol.* **2**:127.