## PRELIMINARY NOTE

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### Inhibition of water and solute permeability in human red cells

The passive permeability properties of cell membranes are often interpreted in terms of a mosaic membrane model which consists largely of a lipid matrix interspersed with small polar pores. The lipid matrix accounts for the strong correlation between solute permeabilities and their oil solubilities. Pores (water-filled channels) are added because (1) they offer some explanation for the empirical discrepancy between diffusional water permeability and osmotic water permeability and (2) they offer a means to account for the size-dependent permeability properties of small polar solutes. The existence of pores implies that the transfer of water and the penetration of small hydrophilic solutes should be strongly related. In this report we deal primarily with this interdependence of water and solute transport. We first show that the sulfhydryl reagents PCMB (*p*-chloromercuribenzoate) and PCMBS (*p*-chloromercuribenzene sulfonate) can produce a dramatic decrease in osmotic water permeability. Next we correlate this decrease with simultaneous changes in solute permeabilities. Finally, by use of phloretin, we alter the permeabilities of certain solutes and measure corresponding changes in water permeability. The results show that it is possible to dissociate the major fractions of solute penetration and water transfer.

Permeabilities were estimated by a modification of the ØRSKOV[1] photometric method. Human red cell suspensions (1.4 %) were subjected to a sudden step change in osmotic pressure (and in solute concentration). The subsequent cell volume change was followed by measurements of light transmission. Details of the technique have been described in an earlier publication[2]. Concentration perturbations were kept small so that volume changes were restricted to $\pm$ 10 %. Under these conditions the kinetic volume curves are exponential functions of time, and analysis of the Kedem–Katchalsky equations shows that the relevant permeability coefficients are inversely proportional to the exponential time constants[2,3].

When a suspension of cells is preincubated with either PCMB or PCMBS, the osmotic water permeability ($L_p$) is drastically reduced. The effect is reversible; if excess cysteine is added, the depressed level of $L_p$ returns promptly to control values. Fig. 1 shows the dependence of $L_p$ on concentrations of PCMB and PCMBS. The control value for $L_p$ corresponds to a permeability coefficient of about 200 $\mu$/sec. The maximal effect of these reagents is to reduce $L_p$ by a factor of 10, *i.e.* to a permeability of about 20 $\mu$/sec. This latter figure is similar to the diffusional permeabilities obtained with lecithin-cholesterol bilayers[4]. This suggests that the action of PCMB and PCMBS may be to inhibit water flow through aqueous channels (pores) leaving the lipid portions of the membrane as the only alternative for water transport.

In correlating changes in water permeability with changes in solute permeability, we first note that, in contrast to its effect on water transport, PCMBS increases cation leakage by an order of magnitude[5]. Since PCMBS has no effect on $SO_4^{2-}$ permeability[6], its action on cation transport should not be ascribed to a simple alteration of membrane fixed charge. Further, the inhibition of water transport by

Abbreviations: PCMB, *p*-chloromercuribenzoate; PCMBS, *p*-chloromercuribenzene sulfonate.

PCMBS cannot be attributed to a change in cation reflection coefficient, because the amplitude of the osmotically induced volume change is not reduced. It seems simplest to conclude that most if not all of the water transport occurs through channels that are inaccessible to both cations and anions.

Turning to small polar nonelectrolytes, our results with a series of glycols and ureas are illustrated in Fig. 2A. The permeabilities of water, urea, and methylurea are substantially reduced by PCMBS. Glycerol permeability is reduced to a lesser extent, and the other solutes show no significant change. The significance of correlating changes in the permeabilities of glycerol and water is questionable since glycerol is presumably transported *via* a facilitated diffusion system which is sensitive to sulfhydryl reagents[7]. This leaves urea and methylurea as candidates for penetration through the major water channels.

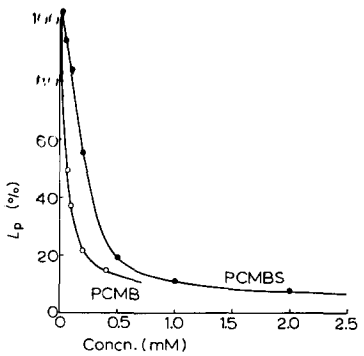


Fig. 1. Dependence of osmotic water permeability ($L_p$) on concentration of PCMB and PCMBS. Cells were preincubated with the indicated concentrations of PCMB or PCMBS for about 75 min at 22°. $L_p$ was calculated from time constants of cell swelling (25°) following a step change in medium osmolality from 320 to 286 mosM. Values of $L_p$ have been normalized to their control value (100%).

If we turn to the converse aspect, *i.e.* alter solute permeability and correlate this with changes in water permeability, we find further complications even with urea and methylurea. Fig. 2B shows our results using the glucose inhibitor, phloretin. Results with phloretin and PCMBS are similar with the important exception that phloretin does not change water permeability. Nevertheless, urea and methylurea transport are inhibited by large factors. By increasing the phloretin dosage it is possible to inhibit urea permeability by a factor of 50 with negligible influence on water permeability.

Combining the PCMBS and phloretin results, we see that it is possible to dissociate water and solute transport for each solute examined. It would appear that water channels transport water and very little else. The data could be accounted for by assuming that PCMBS somehow imposes a new type of selectivity on a set of homogenous pores, or by postulating the simultaneous existence of several types of selective pores. Although these interpretations cannot be refuted, the pore model now loses its basic appeal, simplicity.

Alternatively, the explanation proposed for the dissociation of water and small solute permeabilities in toad bladder may also be applicable to red cells[8–10]. It assumes the existence of two permeability barriers in series. One barrier provides the principal resistance for small solutes, while the other impedes water flow. The two barriers

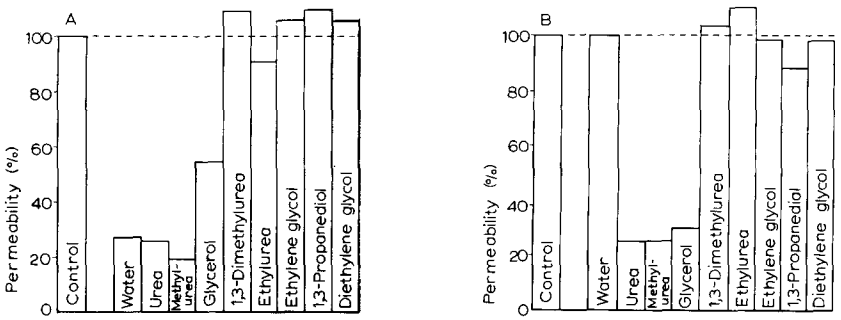


Fig. 2. Effects of PCMBS and phloretin on the permeabilities of water and several solutes. Water permeability was measured as described in Fig. 1. Solute permeabilities were estimated from time constants of cell swelling (25°) following a step change in solute concentration from 0 to 60 mM in an otherwise isotonic medium. Permeabilities have been normalized to the control values (100 %). Cells were preincubated for about 75 min at 22° with: (A) 0.4 mM PCMBS and (B) 0.5 mM phloretin (*plus* 0.5 % ethanol).

respond differently to specific reagents such as vasopressin and amphotericin B. An arrangement of series barriers in *Chara ceratophylla* has also been suggested on other grounds by LIEB AND STEIN[11]. The series-barrier hypothesis has the additional attraction that it predicts rectification of osmotic water flow[2,12].

A more likely explanation is that urea and methylurea, like glycerol and glucose, are transported *via* a phloretin–PCMBS-inhibited, facilitated diffusion system. A similar proposition has been advanced by HUNTER *et al.*[13] to account for the effects of tannic acid on urea transport. The major pathway for the other solutes would then be through the lipid matrix[14]. Occasionally these solutes might enter a water channel. This would not contribute substantially to the total solute transfer, yet it could account for reflection coefficients which differ from unity[15].

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*Department of Physiology-Anatomy,*
*University of California,*
*Berkeley, Calif. 94720 (U.S.A.)*

ROBERT I. MACEY
ROBERT E. L. FARMER

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