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THE VOLUME DEPENDENCE OF THE ERYTHROCYTE WATER DIFFUSION PERMEABILITY

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SUMMARY

The product of the water diffusion permeability and the membrane area of a human erythrocyte has been found to be nearly independent of the cell volume. The product was measured by an NMR technique. This result conflicts with previous flow tube determinations but is in accord with recent measurements of the hydraulic permeability and various solute permeabilities. The results are consistent with the major part of the water fllux traversing the membrane through a fixed number of pores. There may also be a minor non-pore flux. It appears to be practicable to follow volume changes in the red blood cell by an NMR technique.

INTRODUCTION

The red cell membrane is commonly thought to be non-uniform: water and many other polar substances apparently permeate through localised pathways or pores. One of a variety of techniques by which the properties of these aqueous pathways can be elucidated, consists in studying the effects of cell volume variations on water and solute permeabilities. Such studies should help to clarify for example whether the same pores are accessible to all permeants and whether their numbers or properties depend on cell volume, membrane area or the tonicity of the external solution.

There is another reason for studying the relationship between water permeability and cell volume. The water exchange time can now be monitored in small blood samples using a nuclear magnetic resonance (NMR) technique[1]. If a precise relationship between exchange time and cell volume could be established, the NMR method for monitoring the exchange time would provide an alternative to light scattering as a way of following volume changes during solute permeability determinations.

The effect of cell volume on the water diffusion permeability was first investigated by Villegas *et al.*[2] who reported an unexpected negative correlation between the volume and the permeability of beef erythrocytes. Solomon[3] concluded that a similar relationship was valid for water diffusion into human red blood cells, although

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the K^+ permeability appeared to be independent of volume. He inferred that water and potassium enter *via* different routes and he reconciled the strange behaviour of the water permeability with the pore concept by invoking the rather awkward hypothesis that the pores shrink as the cells swell.

The hydraulic water permeability is apparently independent of cell volume[4–7], as are the permeabilities of two neutral hydrophilic solutes, glycerol and ethylene glycol[7,8].

The present paper reports some further measurements of the water diffusion permeability at various cell volumes.

METHODS

The water exchange time was measured by an NMR technique which has been described previously[1]. Some of the present work was carried out at a higher resonance frequency (30 MHz) than was used previously (9 MHz). The higher resonance frequency provides a gain in sensitivity, permitting smaller samples to be used, but does not otherwise affect the experimental conditions.

Samples were prepared by adding to one part of human blood one part of a solution containing 50 mM $MnCl_2$ and various amounts of NaCl (Series A) or 1.1 parts of a solution containing 45 mM $MnCl_2$, 12 mM NaCl and various amounts of sucrose (Series B). As explained elsewhere[1] Mn^{2+} is essential to the measurement procedure.

The exchange times were determined 5 min after preparing the samples. The temperature was then 37 °C. The haematocrits were assessed after centrifugation in microhaematocrit tubes for 5 min at $12000 \times g$. The haematocrit of the whole blood was also measured. From these data, the relative volumes of the cells in the various samples were calculated.

Each series of measurements was performed on blood from a single specimen collected from an adult male by venipuncture. Different donors supplied the blood used in the two series. The blood was stored in heparinised collection tubes at 4 °C for not more than 24 h. No significant changes in the haematocrit were observed during storage.

RESULTS AND DISCUSSION

In the present experiments, the water diffusion exchange time T_e was determined as a function of cell volume V. The diffusion permeability P is related to these quantities by the equations

$$P = V_w/(AT_e) \tag{1}$$

and

$$V_w = V - V_s \tag{2}$$

A is the area of the cell membrane. V_w and V_s are the volumes within the cell occupied by water and other substances, respectively. V_w is approximately equal to $0.72V$ in a human red cell under physiological conditions[9].

The membrane area A was not monitored, so the results give direct information only about the product PA where

$$PA = V_w/T_e \tag{1a}$$

If V_w/T_e is constant, PA must be independent of cell volume. Inspection of Fig. 1 reveals that this was very nearly true under the present experimental conditions. A 1.7:1 change in cell volume caused a variation of less than 10% in the product PA.

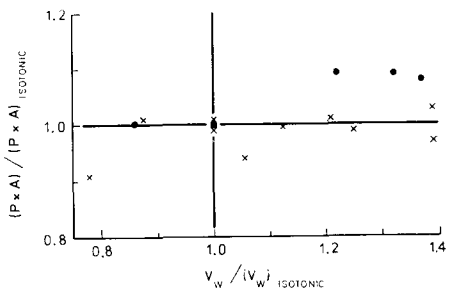

Fig. 1. The product of the water diffusion permeability and membrane area of human erythrocytes plotted against cell water volume. ●, Series A, external NaCl varied; ×, Series B, external sucrose varied.

In the past it has commonly been assumed that the membrane area is independent of cell volume[2,5,7,8]. The justification for this comes originally from the work of Ponder[10]. If the constant-area postulate is accepted, the present results suggest that the water diffusion permeability is nearly independent of cell volume, increasing very slightly at large volumes. However, the present experiments spanned quite a wide volume range and it is not inconceivable that A did vary. If so, changes in A were apparently compensated by inverse changes in P. An inverse relationship between P and A would be expected if water penetrated the membrane solely through a fixed number of pores. The small increase in PA that was observed could be attributed on such a model to either a small non-pore diffusion flux which increased with membrane area or to a slight dependence of the pore properties on cell volume or area. Alternatively it might be an artifact resulting from an incorrect assignment of the non-water volume V_s.

Equivalent results were obtained in both series of measurements. This suggests that the external electrolyte concentration has no influence on the volume dependence of the water permeability, since in Series A the external tonicity was controlled by varying NaCl, in Series B by varying sucrose.

The present results do not support the conclusions drawn from flow tube tracer studies[2,3]. Solomon[3] summarised the flow tube data in his Fig. 7, where a strong negative relationship can be discerned between a quantity termed "relative water diffusion" and the cell volume. Solomon[3] does not explicitly define "relative water diffusion" but reference to the earlier paper by Villegas *et al.*[2], some of whose data are incorporated in Solomon's diagram, reveals it to be T_e^{-1} in the terminology of the present paper. T_e^{-1} and cell volume will be negatively related even if PA is constant (*cf.* Eqns 1 and 2). The data from Solomon's Fig. 7 have therefore been replotted in

Fig. 2 according to the format used for the present data in Fig. 1. The product PA, determined by the flow tube method, does in fact appear to decrease with volume. However, the scatter is large and the correlation in the case of the human cells considered alone is not statistically significant (2 tailed test, 10% level). There may be a discrepancy between the flow tube and NMR results but further work with the flow tube apparatus would be required to establish this conclusively.

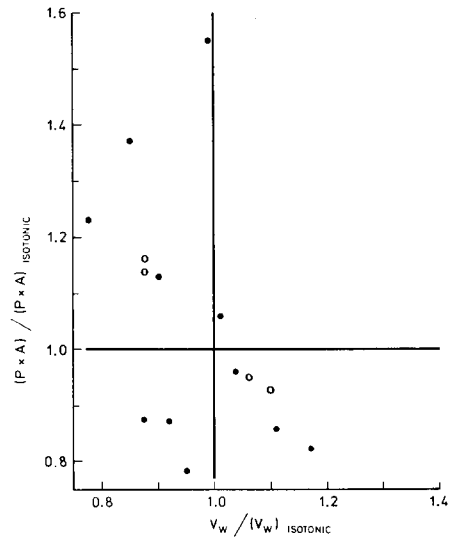

Fig. 2. Flow tube water diffusion data graphed according to the format of Fig. 1. ●, Human erythrocytes[3]; ○, Beef erythrocytes[2].

At present, we cannot really say whether either type of measurement is systematically in error. In the present work, the plasma Mn^{2+} concentration was of necessity[1] quite high. Possibly Mn^{2+} altered the membrane in such a way that the permeability at physiological cell volume was not changed but the variation of permeability with volume was suppressed. This is not a very plausible hypothesis but hypotheses which might account for a systematic bias in the flow tube data tend to be equally unrealistic.

There is now considerable evidence[4–7] that the hydraulic permeability·area product does not depend on cell volume. There is, however, some controversy concerning whether the hydraulic permeability (a) is independent of the external osmolality[6,7] or (b) increases with decreasing external osmolality[4]. In both the present experiments and the radio-tracer work of Solomon *et al.*[2,3] an increment in volume was associated with a decrement in the external osmolality. The present data (Fig. 1) therefore tend, if anything, to favour hypothesis (a), while the trend displayed by the radio-tracer data (Fig. 2) would not be anticipated on either of the above hypotheses.

Regardless of the possibility of systematic error, there appears to exist a reproducible, almost linear, relationship between cell volume and the water exchange time determined by the NMR method. The NMR method therefore offers a new way of monitoring cell volume which may sometimes be useful when determining solute per-

meabilities by measuring cell swelling. The water exchange time may be altered by large hydraulic fluxes[11]. Thus the method would be best applied to solutes whose permeabilities are small compared with that of water.

CONCLUSIONS

(1) The product of the area of human erythrocytes and their water diffusion permeability measured by a NMR technique has been found to be almost independent of cell volume. (2) This independence is not in agreement with earlier work on the water diffusion permeability but is consistent with recent work which suggests that the hydraulic water permeability and various solute permeabilities are independent of cell volume. (3) The results are consistent with the hypothesis that the major part of the water diffusion flux traverses the membrane through a fixed number of pores. There may also be a minor non-pore flux. (4) The NMR technique may be used to monitor changes in the volumes of red blood cells.

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