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CONTROL OF WATER CONTENT OF NON-METABOLIZING KIDNEY SLICES BY SODIUM CHLORIDE AND POLYETHYLENE GLYCOL (PEG 6000)

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SUMMARY

1. Cortical slices from kidneys of adult rats were equilibrated for 24 and 48 hr in the refrigerator in solutions containing 5 mM-K, 2·5 mM-Ca and Mg, M/15 phosphate buffer (pH 7·4), 5 mM cyanide and iodoacetate with 5, 6, 7 and 8 % polyethylene glycol (PEG 6000) and 77, 154, 308, 462 and 770 mM-NaCl.

2. There were no consistent differences in water content between slices after 24 and after 48 hr.

3. Slices contained more water when either the concentration of NaCl or that of PEG was reduced. The increase in water content induced by lowering the concentration of either was prevented by a higher concentration of the other.

4. Water content was held constant at 3·25 $kg.kg^{-1}$ dry PEG-free tissue despite a fall in [NaCl] from 770 to 77 mM (more than 1200 m-osmole.kg^{-1}) by increasing PEG from 5·1 to 7·7 % corresponding to an increase of the order of 15 m-osmole.kg^{-1}.

5. It is suggested that PEG 6000 was so much more effective than NaCl, osmole for osmole, because PEG 6000 acted as a non-penetrating solute, whereas NaCl penetrated the slices and reduced the 'Donnan excess' component of the intracellular colloid osmotic pressure so that this could be balanced by a smaller concentration of PEG.

INTRODUCTION

That cells swell when external solutions are diluted and shrink when these solutions become more concentrated has been explained by supposing that they behave like osmometers, adjusting their volume and maintaining osmotic equilibrium by gaining or losing water through functionally semi-permeable membranes (Peters, 1944, 1950). When their metabolism is

impaired, cells also swell greatly even when the external medium is not diluted; but then they take up a fluid which is not very different from physiological saline (Leaf, 1959). This kind of swelling is somewhat analogous to the uptake of fluid into capillaries when the net hydrostatic pressure tending to cause ultrafiltration is less than the colloid osmotic pressure of the plasma proteins, and its driving force should be the unbalanced colloid osmotic pressure of intracellular proteins. Tosteson (1964) pointed out that the swelling of erythrocytes in hypotonic saline media also resembles colloid osmotic swelling in that it can be prevented by concentrations of albumin which are far too small to compensate for the diminished osmolality of the diluted saline (Lowenstein, 1960). It is also possible that external NaCl controls the volume of cells through the influence of ionic strength upon colloid osmotic pressure as well as by a non-specific contribution to external osmolality (Robinson, 1965). This hypothesis has been tested by comparing the efficacies of NaCl and of a non-penetrating solute (polyethylene glycol) in controlling the water content of kidney slices under conditions set up to suppress metabolism. Polyethylene glycol of molecular weight about 6000 turned out to be from 10 to 100 times more effective than NaCl, osmole for osmole.

METHODS

Solutions. The following stock solutions were prepared:

A. NaCl, 1540, 924, 616, 308 and 154 m-mole.l^{-1}

B. Polyethylene glycol, PEG 6000 (Union Carbide Chemical Co., U.S.A.), 16, 14, 12 and 10 % in a solution containing 10 mM-KCl, 5 mM-$CaCl_2$ and 5 mM-$MgSO_4$.

C. Sodium phosphate buffer, M/7·5, pH 7·4, containing 100 mM sodium iodoacetate and 100 mM sodium cyanide.

Mixing equal volumes of pairs of solutions *A* and *B* gave a series of twenty mixtures all containing 5 mM-K^+, 2·5 mM-Ca^{2+} and 2·5 mM-Mg^{2+} with 770, 462, 308, 154 and 77 mM added NaCl and 8, 7, 6 or 5 % PEG. To each mixture was added 1/20 of its volume of the buffer *C* to supply the usual working concentration, M/15, of phosphate buffer and 5 m-mole.$l.^{-1}$ of each inhibitor.

Procedure. Portions (2 ml.) of these solutions were placed in twenty short wide, roundbottomed, stoppered tubes. Kidneys from rats of the black and white hooded Lister strain bred at the Animal Department of the Otago University Medical School were sliced by Cohen's (1945) modification of the method of Deutsch (1936) and equilibrated with magnetic stirring in the 'ordinary medium' (Robinson, 1956) at room temperature for 15 min to remove erythrocytes and debris. Cortical slices were distributed into drying tubes to determine their initial water content and into the twenty prepared tubes, at least two slices, a total of 50–70 mg of moist tissue, in each tube. The tubes were then stoppered and placed in the refrigerator, where the slices were allowed to equilibrate with the various solutions. The tubes were inverted and shaken at intervals to ensure mixing, and the slices were harvested in two batches, after 24 and after 48 hr, blotted lightly on filter paper, and transferred to weighed drying tubes which were weighed before and again after drying at 105° C to constant weight. Since previous work in the Department (Little, 1965) had shown

that PEG 6000 was largely confined to the extracellular phase of kidney slices, the weight of PEG contained in a volume of solution corresponding to 25 % of the moist weight (Robinson, 1950) was subtracted from the dry weight of each slice, and the water contents expressed as kg.kg^{-1} dry, PEG-free tissue.

RESULTS

The slices as used had an average initial water content of 3·45 kg.kg^{-1} dry matter (S.D. ± 0·26 from fifteen determinations). There were no significant differences between the water content of slices after 24 and after 48

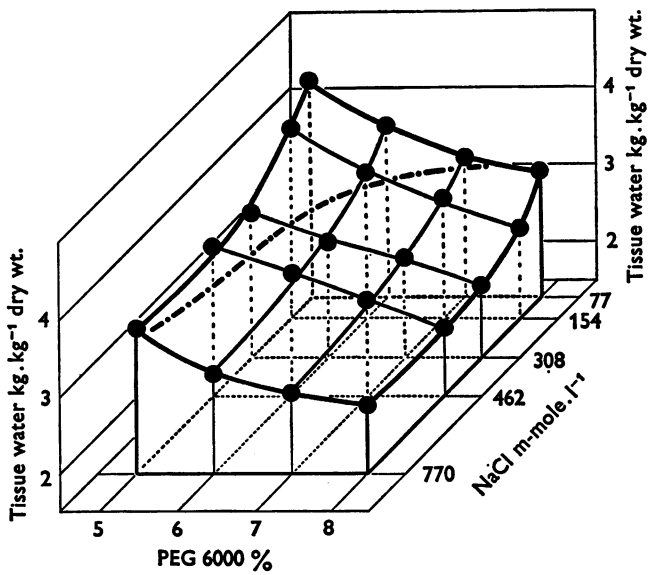

Fig. 1. Water contents (kg.kg^{-1} dry, PEG-free tissue) of non-respiring kidney slices equilibrated for 24–48 hr in different concentrations of NaCl and PEG 6000. Data from Table 1. -·-·-·- represents the contour line for constant water content of 3·25 kg.kg^{-1} dry, PEG-free tissue.

hr, so that equilibration may be presumed to have been complete. The experiment was repeated three times with remarkably similar results. These are shown in Table 1 and illustrated in Fig. 1 in which each plotted point gives the average from six measurements, three after 24 hr, and three after 48 hr equilibration in each solution. Fig. 1 shows in a single diagram the effect of varying the concentration of PEG 6000 at different concentrations of NaCl and that of varying the concentration of NaCl at different concentrations of PEG 6000. Swelling produced by lowering the concentration either of NaCl or of PEG could be prevented by raising the concentration of the other. The dot-dash curve is a contour line showing the concentrations of PEG 6000 and of NaCl required to maintain a con-

stant and approximately 'normal' water content of 3·25 kg.kg^{-1} dry tissue, corresponding to 76·5% tissue water by weight. The most striking observation is the small increase in the concentration of PEG that sufficed to compensate for quite large reductions in concentrations of NaCl. Following the dot-dash contour line from left to right, the effect of reducing [NaCl] from 770 to 77 m-mole.l.$^{-1}$ was countered by increasing PEG from 5·1 to 7·7 %. Thus an increase of PEG from 5·1 to 7·7 % compensated for a tenfold reduction of NaCl from 5× 'isotonic' to ½ 'isotonic'.

TABLE 1. Tissue water (kg.kg^{-1} dry PEG-free tissue) of non-respiring kidney slices equilibrated for 24–48 hr in NaCl-PEG 6000 mixtures

	NaCl concn. (m-mole.l.$^{-1}$)				
	77	154	308	462	770
8% PEG	3·16	2·65	2·42	2·36	2·35
7% PEG	3·31	3·05	2·78	2·75	2·53
6% PEG	3·69	3·37	2·99	3·05	2·75
5% PEG	4·33	3·92	3·32	3·45	3·32

Precisely what contribution PEG made to osmolality could not be determined because of difficulties in freezing the PEG solutions in the Fiske osmometer. An estimate was obtained from measurements which Heyer, Cass & Mauro (1970) made with a membrane osmometer, although these were not on the same batch of PEG 6000. A curve drawn from their results suggests that the contributions of 8, 7, 6 and 5 % PEG to osmolality were of the order of 29, 23, 17·5 and 13 m-osmole.kg^{-1} respectively. Hence the contribution to osmolality of the increase in concentration of PEG that sufficed to compensate for a reduction of (NaCl) by 700 mM (more than 1200 m-osmole.kg^{-1}) was of the order of 15 m-osmole.kg^{-1}, which suggests that PEG and NaCl were not acting to control cellular water content in the same manner.

DISCUSSION

The slices took up water when either the external concentration of NaCl or that of PEG was reduced, but nothing can be deduced with certainty from the relation between water content and external osmolality because the factors that determine the volume of the cells at equilibrium are likely to include elastic forces in the tissue and in gel structures in the cells. These rather ill-defined forces should oppose swelling, and they should increase as the water content of the tissue increases and so affect the equilibrium volume. They cannot be estimated, but if they remained constant when water content, and presumably cell volumes, were held constant, their contribution could be ignored, and solutions which maintained the same equilibrium water content could be regarded as osmotically equivalent.

Two hypotheses, either of which might account for the observation that the swelling which occurred when the external concentration of NaCl was reduced could be prevented, and the cells' volume presumably held constant, by increasing the external concentration of PEG are:

1. That the cells behaved as classical osmometers. The increase in concentration of PEG that was needed to restore the original water content should then have increased the external osmolarity precisely as much as it had been reduced by lowering the concentration of NaCl.

2. That the cells behaved as colloid osmometers. The increase in concentration of PEG that was needed to restore the original water content should then have increased the external effective osmolarity as much as the intracellular colloid osmotic pressure had been increased by the reduction in ionic strength when the external NaCl was diluted.

On the former hypothesis the reduction in concentration of NaCl and the compensating increase in concentration of PEG should produce equal and opposite contributions to osmolality. On the latter hypothesis PEG should be far more effective than NaCl in osmotically equivalent concentrations. Furthermore, if the increase in the external concentration of PEG required to prevent swelling with any given reduction in the concentration of NaCl corresponds to an increase in colloid osmotic pressure in the cells, then the curve obtained by plotting the concentration of PEG required to maintain a constant water content against the concentration of NaCl should resemble curves relating colloid osmotic pressure to the concentration of penetrating electrolyte.

Fig. 2 shows such a comparison. The circles represent the osmolal contributions of PEG (according to the data of Heyer, Cass & Mauro, 1970) plotted against the concentrations of NaCl in which they gave a water content of 3·25 $kg.kg^{-1}$. The open circles show 10 × the values given by Höber (1945) for the colloid osmotic pressure of a 0·001 molar solution of a 17-valent colloidal polyelectrolyte in different concentrations of NaCl. They illustrate the depression of colloid osmotic pressure as the 'Donnan excess' component is reduced by increasing the ionic strength with a penetrating electrolyte. The similarity in shape of the two curves is striking. The slope of the tangent at a physiological salt concentration of 150 m-$mole.l.^{-1}$ suggests that an increase in [NaCl] of 200 m-$mole.l.^{-1}$ should change the intracellular colloid osmotic pressure by 10 m-$osmole.kg^{-1}$, in remarkably close agreement with the comparison of NaCl and albumin for erythrocytes by Lowenstein (1960). The actual value of about 20 m-$osmole.kg^{-1}$ for the intracellular colloid osmotic pressure in physiological saline is, moreover, of the same order as Tosteson (1964) suggested for erythrocytes.

The present results offer an explanation for the observation of Davey

& Skegg (1971) that the addition of NaCl (400 m-osmole.kg^{-1}) to the 'isotonic' medium in which non-respiring kidney slices were maintained in nitrogen at 26° C without gross swelling by 7 % PEG 6000 reduced the water content after 12 hr equilibration from 2·90 to 2·50 kg. kg^{-1} dry matter, whereas the addition of glucose (400 m-osmole.kg^{-1}) produced a similar initial transient shrinkage but left the water content after equilibration unaltered. Analysis showed that both the NaCl and the glucose had

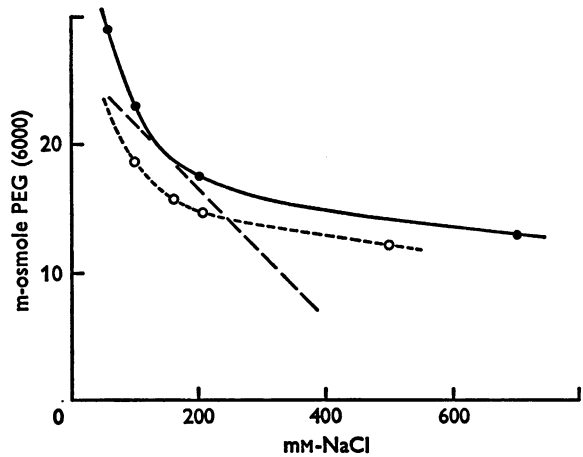

Fig. 2. ● Contributions to osmolality (according to Heyer *et al.* 1970) of concentrations of PEG 6000 required to maintain constant water content of 3·25 kg.kg^{-1} dry, PEG-free tissue in non-respiring kidney slices equilibrated at different concentrations of NaCl. ○ 10 × colloid osmotic pressure of 0·001 M-$Na_{17}R$ in different concentrations of NaCl (Höber, 1945). The straight line, tangent to the experimental curve at a physiological concentration of NaCl (150 mM) shows a slope of − 1 m-osmole per 20 mM-NaCl.

penetrated freely into the slices, and Davey & Skegg concluded that their observations might be explained if the NaCl exerted a depressing effect upon colloid osmotic pressure which the penetrating non-electrolyte did not share.

It seems reasonable to conclude that PEG, which was so much more effective than NaCl, osmole for osmole, in resisting the swelling of non-respiring kidney slices, was excluded from the cells and functioned as a non-penetrating solute balancing the colloid osmotic pressure of intracellular protein, whereas NaCl functioned as a penetrating electrolyte which reduced the colloid osmotic effect of intracellular protein. This does not necessarily contradict the traditional view that sodium chloride functions as an external osmotically active solute when metabolic conditions are normal, for then active extrusion confers a functional semi-permeability upon the cell membranes and keeps sodium outside as a non-

penetrating ion. Under normal conditions an increase in the concentration of external NaCl should lead to a corresponding increase in the concentration of potassium inside the cells, whereas under the conditions of the experiments increasing the concentration of NaCl in the medium should rather have increased the concentration of sodium in the cells. The effect on colloid osmotic pressure should, however, hardly depend upon whether the ionic strength was augmented by sodium or by potassium, so that a decrease in the Donnan excess component of colloid osmotic pressure associated with increasing ionic strength may be a factor contributing to the control of cell volume under normal metabolic conditions when sodium is largely excluded. The present experiments have shown that sodium chloride can still control cellular volume when metabolism is suppressed and sodium is not excluded; they also suggest strongly that under these conditions the mechanism is the effect of NaCl as an electrolyte upon colloid osmotic pressure rather than a non-specific contribution of NaCl to total osmolality. If colloid osmotic pressure is indeed a substantial factor in controlling the volume of cells under normal as well as under the experimental conditions, its importance may be that it should make the volume of cells relatively insensitive to exchanges of intracellular potassium for sodium. It would have the further advantage of making cellular volume relatively independent of external osmolality as such. Indeed it would enable cells to retain stability and control of their volume whilst possessing membranes sufficiently permeable to permit exchanges of solutes, including the principal bulk cations of the intracellular and extracellular fluids.

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