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CONTROL OF WATER CONTENT OF RESPIRING KIDNEY SLICES BY SODIUM CHLORIDE AND POLYETHYLENE GLYCOL

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SUMMARY

1. Cortical slices from kidneys of adult rats were incubated for 1 and 2 hr at 25 °C in oxygenated solutions containing 5 mM-K, 2·5 mM-Ca and Mg, 0·007 M-phosphate buffer (pH 7·4) and 5 mM-glucose, together with 1, 2, 4 and 6 % polyethylene glycol (PEG 6000) and 46, 92, 123, 154 and 231 mM-NaCl.

2. There were no significant differences in water content between slices incubated for 1 and for 2 hr.

3. The slices contained more water when the concentration either of NaCl or of PEG 6000 was lower.

4. Larger increases in the contribution of NaCl to total osmolarity were required to prevent increases in water content accompanying relatively small reductions in the contribution of PEG 6000. At a physiological salt concentration around 300 m-osmole/l., it took 10 m-osmole/l. of additional NaCl to compensate for the loss of 1 m-osmole/l. PEG 6000 and keep the volume of cell water normal.

5. The much greater contribution, osmole for osmole, which PEG 6000 made to tonicity suggests that metabolic activity failed to make the cell membranes effectively semipermeable; they were still substantially permeable to NaCl.

6. Comparison with earlier results suggested that ionic strength affected colloid osmolarity similarly in respiring and in non-metabolizing cells; and that respiration at 25 °C somehow kept the concentration of diffusible solutes in the cells sufficiently lower than outside to compensate for a swelling effect of intracellular colloid osmolarity which, in the absence of metabolism, could be balanced by 20 m-osmole/l. PEG 6000 added to the medium.

INTRODUCTION

When their metabolism is inhibited, mammalian cells swell grossly in solutions resembling normal extracellular fluids (Robinson, 1960). Renal cortical cells approximately double their volume (Robinson, 1950) by taking up a solution resembling the medium (Leaf, 1959). Such swelling has been called 'non-osmotic' to emphasize that it does not depend upon dilution of the medium, and 'colloid-osmotic' to indicate that the driving force arises from the higher concentration of colloidal solutes, principally proteins, in the cells (Willbrandt, 1942). The volume attained presumably reflects a balance between diminishing colloid osmolarity as intracellular macromolecules are diluted and elastic forces set up as the tissue is stretched. This

equilibrium volume can be reduced in three ways: (1) by low concentrations of non-penetrating solutes added to the medium; (2) by high concentrations of penetrating electrolytes which depress the Donnan component of colloid osmolarity; and (3) by restoring metabolism, without additions to the medium.

In the absence of metabolic activity a non-penetrating polyethylene glycol, PEG 6000, of molecular weight about 6000, was about 40 times as effective as the penetrating salt, NaCl, in restoring the normal water content of cells in rat kidney slices (Robinson, 1971). The interaction of all three factors has been investigated by comparing the effects of NaCl and of PEG 6000 on the volume of respiring cells in rat kidney slices. If metabolic pumping caused the cell membranes to behave as ideally semipermeable, neither solute should penetrate, and they should both be equally effective, osmole for osmole, in strong contrast with their very different effects on non-metabolizing cells. In fact PEG 6000 was still far more effective than NaCl when the cells were respiring.

METHODS

Solutions. The following stock solutions were prepared.

A, NaCl: 462, 308, 246, 184 and 92 mM.

B, polyethylene glycol, PEG 6000 (Union Carbide Chemical Company, U.S.A.): 120, 80, 40 and 20 g $l.^{-1}$ in a solution containing 10 mM-KCl, 5 mM-$CaCl_2$ and 5 mM-$MgSO_4$.

C, Na phosphate buffer, M/7·5, adjusted to pH 7·4, with 20 g $l.^{-1}$ of glucose added just before use.

Equal volumes (2 ml.) of appropriate pairs of solutions A and B were placed in twenty 25 ml. conical flasks to give a series of solutions containing 231, 154, 123, 92 and 46 mM-NaCl with 60, 40, 20 and 10 g $l.^{-1}$ PEG 6000 together with 5 mM-KCl, 2·5 mM-$CaCl_2$ and 2·5 mM-$MgSO_4$; 0·2 ml. of the buffer, C, was finally added to each flask.

Procedure. Cortical slices prepared by Cohen's (1945) modification of the method of Deutsch (1936) from kidneys of adult male, black and white hooded rats of a Lister strain maintained by the Animal Breeding Department of the Otago University Medical School were equilibrated for 15 min at room temperature in well stirred and aerated 'ordinary medium' (Robinson, 1956) to remove erythrocytes and cellular debris, and were then briefly drained on glass and distributed among the twenty flasks. The flasks, each containing six to eight slices, were filled with oxygen, stoppered, and incubated in a shaking water-bath at 25 °C. Half the slices from each flask were harvested after 1 hr, and the flasks refilled with oxygen and replaced for a second hour, after which the rest of the slices were removed. All slices were lightly blotted on removal and then placed in small weighed tubes for determination of the water content of the tissue by the loss of weight after drying overnight in an air oven at 105 °C. Tissue water content was calculated as kg per kg of PEG-free dry matter by subtracting from the weight of each dried sample as much PEG 6000 as would have been contained in a volume of medium corresponding to one quarter of the weight of the moist sample.

To facilitate interpretation of the results, a representative volume of cell water, V_{cw}, was calculated and expressed as a percentage, V'_{cw}, of the cell water per kg of dry matter, $V°_{cw}$, in a 'normal' respiring slice with a water content of 0·76 kg kg^{-1}. Measurements using inulin as a marker suggested that, regardless of swelling, about one quarter of the moist mass of a renal cortical slice was extracellular fluid (Robinson, 1950). Although this may be an underestimate (McIver & Macknight, 1974), when the present results were recalculated taking 0·35 instead of 0·25 of a moist slice by weight as extracellular fluid, the values obtained for V'_{cw}, referred to $V°_{cw}$ calculated on the same basis, were identical in ten instances, differed by 1% in eight and by 2% twice.

If weights of moist tissue, dried tissue and cell water are mw, dw and cw, and if extracellular fluid provides one quarter of the moist weight, cw = 0·75 mw − dw, and V_{cw} (in kg kg^{-1} dw) =

0·75 mw/dw − 1. The water content of the tissue, W_T, in kg kg^{-1} dw, is (mw − dw)/dw, = mw/dw − 1. Hence 0·75 W_T = 0·75 mw/dw − 0·75, and therefore V_{cw} = 0·75 W_T − 0·25.

For 'normal' respiring tissue with 0·76 kg kg^{-1} of water, W_T = 0·76/0·24 = 3·17 kg kg^{-1} dw. The 'normal' representative cell water content, $V°_{cw}$, was therefore taken to be 3·17 × 0·75 − 0·25 = 2·38 − 0·25 = 2·13 kg kg^{-1} dw.

The contributions of PEG 6000 to the osmolarities of the solutions were obtained from a continuous curve made by plotting values reported by Heyer, Cass & Mauro (1970) who measured osmotic pressure of solutions of a polyethylene glycol of similar weight with a membrane osmometer. They appear in the left-hand columns of Tables 1 and 2. The values used previously (Robinson, 1971) were obtained in the same way.

The contributions of the other solutes to the osmolarities of the 4·2 ml. medium in which each batch of slices was incubated were calculated by taking the osmotic coefficient to be 0·9 for Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, $H_2PO_4^-$ and SO_4^{2-}; and 1·0 for glucose. These values appear as 'non-PEG m-osmole/l.' at the heads of the columns in Tables 1 and 2. The principal solutes contributing to the Non-PEG osmolarities were always Na^+ and Cl^- ions. For example, in the medium with 305 m-osmole/l. of non-PEG solute, 283 m-osmole came from the 154 mM-NaCl and 22 m-osmole from other solutes.

RESULTS

Results for slices incubated for 2 hr were not distinguishable from those for slices incubated for 1 hr. The results were therefore pooled, and Table 1 shows data (mean ± S.D.) for W_T from six experiments in each of the twenty solutions. An earlier series of pilot experiments at 37 °C gave similar results; but their scatter was greater, and they have not been included. The concentrations of NaCl and of PEG 6000 shown at the heads of the columns and at the ends of the rows in Table 1 are halves of the concentrations of these solutes in the solutions A and B which were mixed in equal volumes to make the media in which the slices were incubated. The contributions of PEG 6000 and of the other solutes to the osmolarities of the media are also indicated.

TABLE 1. Water contents W_T (means ± S.D.), expressed in kg kg^{-1} PEG-free dry matter, of slices incubated in media with different concentrations of PEG 6000 and NaCl, but with constant concentrations of other solutes (see Methods). Each group = six experiments

NaCl (mM) ...	46	92	123	154	231
Non-PEG ...	120 m-osmole/l.	200 m-osmole/l.	252 m-osmole/l.	305 m-osmole/l.	438 m-osmole/l.
PEG 6000					
60 g l.$^{-1}$ 17·5 m-osmole/l.	3·78 ± 0·18	3·16 ± 0·06	2·86 ± 0·13	2·74 ± 0·04	2·55 ± 0·11
40 g l.$^{-1}$ 9·0 m-osmole/l.	3·77 ± 0·13	3·35 ± 0·06	3·11 ± 0·07	2·95 ± 0·05	2·71 ± 0·06
20 g l.$^{-1}$ 3·7 m-osmole/l.	3·90 ± 0·10	3·43 ± 0·06	3·22 ± 0·04	3·05 ± 0·02	2·97 ± 0·11
10 g l.$^{-1}$ 1·5 m-osmole/l.	3·95 ± 0·12	3·46 ± 0·12	3·29 ± 0·07	3·13 ± 0·09	2·93 ± 004

Inspection of Table 1 shows that the water content of the tissue was greater when the concentration either of PEG 6000 or of electrolyte in the medium was smaller. Moreover, a relatively large increase in non-PEG osmolarity was required to prevent swelling associated with a small decrease in the contribution of PEG 6000 to total osmolarity. This is qualitatively what was found for non-metabolizing slices (Robin-

son, 1971). Estimates of cell water volume (V_{cw}) were made as explained under Methods, and were also expressed as percentages (V'_{cw}) of the cell water volume of 'normal' respiring slices in order to facilitate more detailed comparisons of the effects of PEG 6000 and of non-PEG osmolarity on the water content of respiring and of non-metabolizing cells. Reciprocal figures, which proved more convenient to plot because the graphs were linear, were calculated as $10{,}000/V'_{cw}$, so that a 'normal' content of cell water is indicated by the same figure, 100, on the reciprocal scale. Values of V'_{cw} and of the reciprocal $10^4/V'_{cw}$ are set out in Table 2.

TABLE 2. Representative cell water volumes, V'_{cw} (as % of 'normal' value; see Methods) and reciprocals, $10^4/V'_{cw}$ (means ± S.D.) for slices respiring in media with different concentrations of PEG 6000 and NaCl, but with constant concentrations of other solutes (see Methods). Each group = six experiments

NaCl (mM) ...		46	92	123	154	231
Non-PEG ...		120 m-osmole/l.	200 m-osmole/l.	252 m-osmole/l.	305 m-osmole/l.	438 m-osmole/l.
PEG 6000						
60 g l.$^{-1}$	V'_{cw}	123 ± 5·9	100 ± 1·9	90 ± 4·1	86 ± 1·3	78 ± 3·4
17·5 m-osmole/l.	$10^4/V'_{cw}$	82 ± 3·9	100 ± 1·9	111 ± 5·2	116 ± 1·7	128 ± 5·5
40 g l.$^{-1}$	V'_{cw}	123 ± 4·2	107 ± 1·9	98 ± 2·2	93 ± 1·6	84 ± 1·9
9 m-osmole/l.	$10^4/V'_{cw}$	82 ± 2·8	94 ± 1·7	102 ± 2·3	107 ± 1·8	119 ± 2·6
20 g l.$^{-1}$	V'_{cw}	127 ± 3·2	110 ± 1·9	103 ± 1·3	96 ± 0·6	94 ± 3·5
3·7 m-osmole/l.	$10^4/V'_{cw}$	79 ± 2·0	91 ± 1·6	97 ± 1·2	104 ± 0·7	106 ± 3·9
10 g l.$^{-1}$	V'_{cw}	129 ± 3·9	111 ± 3·8	105 ± 3·2	99 ± 2·9	92 ± 1·3
1·5 m-osmole/l.	$10^4/V'_{cw}$	78 ± 2·4	90 ± 3·1	95 ± 2·0	101 ± 2·9	109 ± 1·5

Fig. 1 shows values of $10^4/V'_{cw}$ for slices respiring at 25 °C plotted against the contributions of PEG 6000 to the osmolarities of the media; the concentrations of NaCl and the total contributions of Non-PEG solutes are indicated in the caption. Corresponding results for non-metabolizing slices in a medium to which non-PEG solutes contributed 305 m-osmole/l. (Robinson, 1971) are plotted on the same axes for comparison. The steeper slope of the graph for non-metabolizing slices suggests that the volume of respiring cells was less affected than the volume of non-metabolizing cells by changes in the concentration of PEG 6000 in the medium. Corresponding graphs (not reproduced) plotted against non-PEG osmolarity suggested that respiring cells were more sensitive than non-metabolizing cells to changes in the concentration of non-PEG solutes, principally NaCl. Earlier experiments had suggested that respiring cells were relatively resistant to changes in external concentrations of salts, although below 200 m-osmole/l., when respiration was impaired, the curves for respiring slices became steeper than those for chilled slices (Robinson, 1950, Fig. 10). The conditions differed in that in the more recent experiments all media contained PEG 6000, the respiring slices were at 25 °C instead of 38·5 °C, and metabolism was depressed with cyanide and iodoacetate as well as by chilling. However, the respiring cells should have been relatively more affected by NaCl compared with PEG 6000 than non-metabolizing cells if metabolic activity made the membranes of the respiring cells effectively less permeable to Na^+ and Cl^- ions.

Extrapolation of the graph for non-metabolizing slices in Fig. 1 confirms that

the volume of cell water should be approximately doubled by suppressing metabolism in a 305 m-osmolar saline medium. The addition of about PEG 6000 20 m-osmole/l. prevented this swelling and made the medium isotonic for non-metabolizing slices, whereas no added PEG 6000 was required to make the 305 m-osmolar medium isotonic for respiring slices. Intersections of the lines drawn through the several sets of points for respiring slices with the horizontal line at $10^4/V'_{cw} = 100$ indicate the contributions required from PEG 6000 to make the volumes of cell water normal in media with different concentrations of non-PEG solutes, i.e. to make

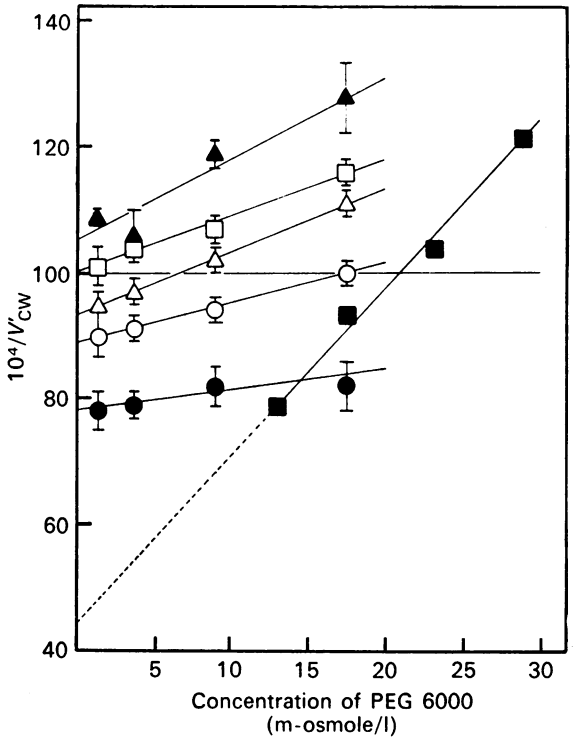

Fig. 1. Reciprocals, $10^4/V'_{cw}$, of representative cell water content (see text) for kidney slices incubated in saline media with added PEG 6000. Standard deviations and means. ●, 46 mM-NaCl; 120 m-osmole/l. non-PEG solute; respiring. ○, 92 mM-NaCl; 200 m-osmole/l. non-PEG solute; respiring. △, 123 mM-NaCl; 252 m-osmole/l. non-PEG solute; respiring. □, 154 mM-NaCl; 305 m-osmole/l. non-PEG solute; respiring. ▲, 231 mM-NaCl; 438 m-osmole/l. non-PEG solute; respiring. ■, 154 mM-NaCl; 305 m-osmole/l. non-PEG solute; non-metabolizing. The interrupted line indicates extrapolation.

these media isotonic. Values of $10^4/V'_{cw}$ were similarly plotted against total osmolarities for all the media, with their different concentrations of PEG 6000. The intersections of these graphs with horizontal lines at $10^4/V'_{cw} = 100$ indicated the concentrations of non-PEG solutes required to keep cell volumes normal in the presence of different concentrations of PEG 6000. The resulting estimates of the contributions from PEG 6000 that should be required to make solutions with different concentrations of non-PEG solutes isotonic for respiring and for non-respiring slices are plotted against the concentrations of non-PEG solutes in Fig. 2.

Fig. 2 confirms that whereas a saline solution of about 300 m-osmole/l. was isotonic for respiring slices, the addition of about 20 m-osmole/l. of PEG 6000 was necessary to make the same solution isotonic when metabolism was suppressed. The slopes of the two curves permit estimates of the relative effectiveness of PEG 6000 and of the other solutes in the media in controlling cellular volume. The most physiologically relevant comparison is at a non-PEG concentration of 300 m-osmole/l., which is about the osmolarity of mammalian extracellular fluids. For non-metabolizing slices the tangent at non-PEG solute 300 m-osmole/l. had a slope of $-1/30$.

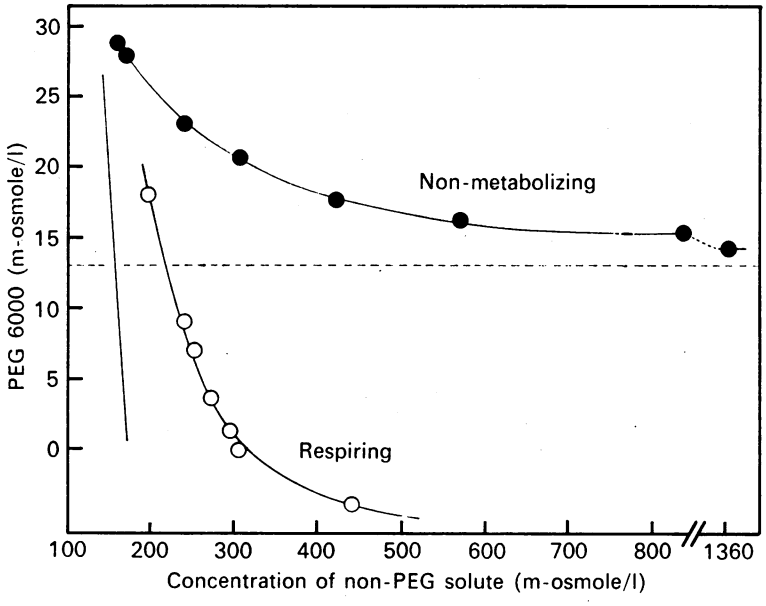

Fig. 2. Concentrations (m-osmole/l.) of PEG 6000 required to maintain a normal representative volume of cell water ($V'_{cw} = 100$) in presence of different concentrations of non-PEG solute. ●, Non-metabolizing slices. ○, Slices respiring at 25 °C. Note the break in the x-axis made to accommodate the point plotted at 1360 m-osmole/l. non-PEG solute. The straight line (upper left) shows the slope of -1 which would indicate that PEG 6000 and non-PEG solutes (principally NaCl) were equally effective, osmole for osmole, in controlling cellular volume. The horizontal interrupted line indicates a non-PEG solute concentration of 13 m-osmole/l. (see Discussion).

This implies that an increase of 30 m-osmole/l. in the concentration of non-PEG solutes, principally NaCl, could compensate for the removal of PEG 6000 1 m-osmole/l. The slope of the tangent at 300 m-osmole/l. to the curve for respiring slices is $-1/10$, implying that PEG 6000 1 m-osmole/l. could replace 10 m-osmole/l. of non-PEG solute in keeping the medium isotonic. Thus, osmole for osmole, PEG 6000 was about 30 times as effective as non-PEG solute in controlling the volume of non-metabolizing cells and about 10 times as effective as non-PEG solute in controlling the volume of cells respiring at 25 °C. This supports the suggestion from Fig. 1 that, compared with non-metabolizing cells, respiring cells were relatively more sensitive to alterations in the concentration of electrolytes and less sensitive to alterations in the concentration of PEG 6000 in the medium.

DISCUSSION

If metabolic activity kept sodium out of respiring cells so effectively that they behaved as though their membranes were semipermeable, two consequences should follow. First, the cells should swell in diluted media; and secondly, NaCl and PEG 6000 added to the medium should be equally effective, osmole for osmole, in opposing swelling, for both solutes should be excluded from the cells and should act in the same way, by increasing osmolarity. The slope of -1 which would indicate that PEG 6000 and other solutes were equally effective is indicated by a straight line at the left in Fig. 2.

The fact that PEG 6000 was so much more effective than NaCl must mean that metabolic activity left the cell membranes still substantially permeable to Na and Cl ions, which were the principal solutes in the media. A high permeability to these ions might indeed be expected because proximal renal tubular cells *in vivo* reabsorb each minute about half their own volume of a glomerular filtrate similar in composition to the 305 m-osmolar medium. Na is believed to enter the epithelial cells passively from the lumen and to be extruded actively across the basal or peritubular membranes. These membranes are the most probable site of exchanges measured in experiments with cortical slices, which consist for the most part of proximal tubules with collapsed lumina (Bojesen & Leyssac, 1965). The behaviour of slices plunged suddenly into diluted saline media provides direct evidence of permeability to sodium and chloride. Respiring slices placed for 2 min in an oxygenated medium diluted with an equal volume of water lost no K and gained no water, but they lost, per kg of dry matter, 210 m-mole Na and 200 m-mole Cl, about half their initial content (Robinson, 1977).

Membranes through which Na and Cl ions pass so rapidly cannot be regarded as semipermeable. Alterations in the concentration of Na and Cl ions should therefore have no more than a brief transient effect upon the volumes of cells with such membranes as a result solely of the ions' non-specific contribution to osmolarity. These ions could, however, act in their own right as electrolytes to increase ionic strength and so depress the Donnan component of the colloid osmolarity of non-diffusible polyelectrolytes (mainly proteins) in the cells. This was probably the chief action by which increasing concentrations of NaCl reduced the volume of non-metabolizing cells (Robinson, 1971). The fact that the curves for respiring and for non-metabolizing slices in Fig. 2 became roughly parallel and PEG 6000 20 m-osmole/l. apart above a non-PEG concentration of about 300 m-osmole/l. suggests that respiration exerted a rather constant effect while the concentration of electrolyte affected colloid osmolarity similarly in the respiring and in the non-metabolizing cells. The convergence of the two curves at lower concentrations of non-PEG solutes may be attributed to impairment of respiration as the saline medium was diluted (Robinson, 1950); this would tend to alter the behaviour of the respiring slices towards that of non-metabolizing ones. Respiration appears to retain some effect even at very low concentrations of Na and Cl. Allison (1976) reported that kidney slices incubated in media with less than 4 mM-Na and Cl could retain a normal water content if the medium contained sufficient PEG 6000; the concen-

trations required were 14·6 g/100 ml when incubation was anaerobic and 13·9 g/100 ml. when it was aerobic at 25 °C.

It remains to consider the equilibrium of cells under different conditions. Non-metabolizing slices had a normal water content when the medium contained PEG 6000 20 m-osmole/l. as well as non-PEG solutes 305 m-osmole/l. (Fig. 1). The swelling effect of intracellular macromolecules was then balanced by non-penetrating PEG 6000 20 m-osmole/l., presumably assisted by elastic forces of unknown magnitude within the tissue. Fig. 2 shows how the concentration of PEG 6000 needed to keep the water content normal increased when the concentration of NaCl in the medium was made less. Since Fig. 2 refers to conditions in which cellular volumes were constant, it may be assumed that the elastic forces resisting swelling did not vary. If these forces were also small, the concentrations of PEG 6000 needed to make the media isotonic provide estimates of the colloid osmolarity in the cells and of its variation as the concentration of electrolyte was altered. The asymptotic approach of the curve for non-metabolizing slices to a value of about 13 m-osmole/l. at high concentrations of non-PEG solute suggests that the colloid osmolarity of the intracellular macromolecules would not become less than about 13 m-osmole/l. even if the Donnan component were to be reduced to zero (i.e. if the macromolecules had no charge or the ionic strength became infinitely large). At a physiological concentration of salt, around 300 m-osmole/l., the Donnan component of 7 m-osmole/l. was, as in the plasma about one third of the total osmolarity. This, however, was 20 m-osmole/l., of the same order as Tosteson's (1964) suggestion of 25–30 m-osmole/l. for erythrocytes, and much greater than the colloid osmolarity of the plasma. In terms of actual pressure required for equilibrium across a membrane impermeable only to the macromolecules, 20 m-osmole/l. corresponds to 388 mmHg at body temperature or 372 mmHg at the experimental temperature of 25 °C.

Respiring cells maintained a normal volume in the 305 m-osmolar saline medium without any added PEG 6000. The transition from non-metabolizing slices in 305 m-osmolar saline plus PEG 6000 20 m-osmole/l. to slices with the same water content respiring in the same saline without PEG 6000 can be considered to occur in two steps. The extrapolated line in Fig. 1 shows the swelling of non-metabolizing slices on removal of PEG 6000 from the medium. With the concentration of PEG 6000 reduced to zero, there was no non-penetrating solute, elastic forces in the swollen tissue had presumably increased, and the cells had doubled their water content. The effect of restoring respiration is represented by moving up the y-axis until a normal water content is attained without any PEG 6000 in the medium.

For the activity of water to be the same in the cells and in the surrounding media, osmolarity must have been lower in the respiring than in the non-metabolizing cells. If the intracellular colloids were still present, together with their counter-ions, colloid osmolarity should have been undiminished, and processes supported by respiration must either have developed contractile forces sufficient to increase pressure in the cells by 372 mmHg, or else they must have expelled enough non-colloidal solute to reduce the intracellular osmolarity by 20 m-osmole/l., which is between 6 and 7 %. On Leaf's (1959) view, the essential action supported by respiration is the extrusion of Na, which, together with Cl, becomes an external,

non-penetrating solute and replaces the PEG 6000 20 m-osmole/l. which could maintain equilibrium with the same water content in the absence of metabolism.

This implies that in order to compensate for the contribution of macromolecules to intracellular osmolarity, the total concentration of diffusible ions inside respiring cells must be kept about 20 m-osmole/l. less than the total concentration of diffusible ions outside, rather than being greater as would be predicted by the conditions of the Gibbs–Donnan membrane distribution for a system in equilibrium. The difference might appear large enough to detect by the analysis of tissues, but there are uncertainties about the concentrations and valencies of organic anions in cells, and also about the possibility that cations and even water are bound in ways which preclude their expected contributions to osmolarity (Robinson, 1960).

The osmometric behaviour of cells *in vivo* still requires some explanation. Alterations in the osmolarity of extracellular fluids are normally associated with closely parallel alterations in the concentration of NaCl and in ionic strength. If cell membranes are permeable and volume equilibrium is in part dynamic, an increase in the external concentration of Na should lead to a more rapid passive influx which, unless it were matched at once by an accelerated active efflux through the pumps, would tend to increase cell solute content and reduce the shrinkage that might be expected to result from the increased osmolarity. At the same time the increasing ionic strength should diminish the colloidal component of intracellular osmolarity, and this should favour a reduction in cellular volume. It is hoped to elucidate the roles of these several factors by measuring transient volume changes after the external concentration of NaCl is suddenly changed without altering the osmolarity of the medium.

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