J. Physiol. (1971), **212**, *pp.* 641–653
With 6 *text-figures*
Printed in Great Britain

THE EFFECTS OF HIGH CONCENTRATIONS OF AN ELECTROLYTE ON THE SWELLING OF NON-METABOLIZING TISSUE SLICES

BY K. J. DAVEY AND D. C. G. SKEGG
From the Department of Physiology, University of Otago Medical School, Dunedin, New Zealand

(*Received 1 June 1970*)

SUMMARY

1. Rat kidney cortical slices, with metabolism suppressed by iodoacetate, were incubated anaerobically at 26° C in hyperosmotic saline media. Changes in tissue composition with time, up to 12 hr, were studied. Despite initial shrinkage, gross swelling occurred, even in saline media of more than twice the concentration of normal extracellular fluid.

2. The composition of non-metabolizing kidney slices incubated at 26° C in a balanced saline medium containing 7 g polyethylene glycol 6000 (PEG 6000)/100 ml. was determined at intervals up to 12 hr. The tissue water, sodium and chloride contents had reached constant levels by 6 hr. Potassium continued to leak slowly from the tissue, but there was no significant further loss between 10 and 12 hr.

3. Non-metabolizing kidney slices were incubated in PEG medium containing additional quantities of either a non-electrolyte (400 m-osmole glucose/kg H_2O) or an electrolyte (400 m-osmole NaCl/kg H_2O), both of which penetrated the tissue to attain approximately uniform concentrations in cells and media. Whereas the slices in the glucose medium attained the same equilibrium water content as those incubated in PEG medium alone, the final water content of slices in the medium containing additional NaCl was significantly lower. This difference might have resulted from depression of intracellular colloid osmotic pressure by the high salt concentration.

INTRODUCTION

Owing to the colloid osmotic pressure of their contents, cells tend to gain water and solutes from their surroundings. It is commonly held that this tendency is balanced by the active expulsion of certain ions (Wilson, 1954). When the metabolism of a tissue slice incubated in isotonic saline is suppressed, the slice gains sodium ions, chloride ions, and water (Mudge, 1951*a*, *b*; Leaf, 1956).

Opie (1949), Robinson (1950) and Aebi (1953) reported that the swelling of isolated, non-metabolizing tissues could be prevented by immersing them in saline solutions approximately twice as concentrated as normal extracellular fluids. It has long been known that the colloid osmotic pressure of protein solutions is depressed by high salt concentrations (Lillie, 1907), because of a reduction in that component of colloid osmotic pressure which results from the Donnan excess of diffusible ions (Hitchcock, 1924). Leaf (1956, 1959) cited Lillie's work and suggested that high concentrations of salts prevented swelling by depressing the colloid osmotic pressure of the intracellular contents. Robinson (1965) suggested that the effects of changes in ionic strength on colloid osmotic pressure might play a part in the regulation of cellular volume *in vivo*.

There has, in fact, been no unequivocal demonstration of these effects in animal tissues. Furthermore, it is not obvious how high concentrations of crystalloid substances, able to penetrate the cellular membrane, could prevent the swelling of non-metabolizing slices completely. Although salts should reduce the Donnan excess of diffusible ions, the oncotic effect of the intracellular proteins should not be entirely abolished. In the experiments of Opie, Robinson and Aebi, the tissues were incubated over relatively short periods and it is conceivable that swelling was not observed because insufficient time was available for all changes to occur.

The present investigation includes kinetic studies of the behaviour of non-metabolizing rat kidney slices in various hyperosmotic saline media. In some experiments, swelling was prevented by balancing the colloid osmotic pressure of the intracellular contents with a non-penetrating solute, polyethylene glycol 6000 (PEG 6000), added to the external medium (Wiggins, 1964, 1965). Finally, non-metabolizing kidney slices were incubated in a medium containing sufficient PEG to prevent gross swelling, to which had been added either a large amount of an electrolyte (sodium chloride) or an osmotically equivalent amount of a non-electrolyte (glucose) which was not expected to affect colloid osmotic pressure.

METHODS

Materials. All reagents were commercial products of analytical grade where possible. Polyethylene glycol of average molecular weight 6,000 (PEG 6000) was obtained from Union Carbide Chemicals Company, U.S.A. Glucose oxidase and peroxidase were obtained from C. F. Boehringer and Sons, Germany.

Media. 'Ordinary medium' contained ions in the following concentrations (m-equiv/kg H_2O): Na^+, 144; K^+, 5; Ca^{2+}, 5; Mg^{2+}, 2; Cl^-, 132; SO_4^{2-}, 2; either acetate, 9 (equilibration medium) or iodoacetate, 9 (incubation medium). The medium was buffered with 8 mM phosphate at pH 7·33.

Media containing PEG 6000, glucose or additional NaCl were prepared by dissolving weighed amounts of solute in ordinary medium containing iodoacetate.

(i) Hyperosmotic saline media were prepared to give measured osmolalities of approximately 200, 300 and 400 m-osmole/kg H_2O above that of ordinary medium (Table 1).

(ii) A medium containing 7 g PEG/100 ml. of ordinary medium was prepared. In the final experiments, glucose or additional NaCl was added to this PEG medium to raise its osmolality by approximately 400 m-osmole/kg H_2O (Table 2). Media containing glucose were prepared fresh before each experiment.

Preparation of slices. Adult male white rats from an inbred strain, weighing 250–450 g, were allowed water and stock pellet food *ad libitum* until the time of the experiment. They were stunned by a blow on the head and bled from the carotid arteries. The kidneys were rapidly removed, decapsulated, and halved in the frontal plane. Slices of cortex, about 0·3 mm thick, were cut with a razor blade following

TABLE 1. Details of hyperosmotic saline media

	Osmolality (m-osmole/ kg H_2O)	Na^+ (m-equiv/kg H_2O)	Cl^- (m-equiv/kg H_2O)
Ordinary medium	285	144	132
Medium A	485	265	253
Medium B	581	325	313
Medium C	673	386	373

TABLE 2. Details of media containing polyethylene glycol 6000

	Osmolality (m-osmole/ kg H_2O	Na^+ (m-equiv/kg H_2O)	Cl^- (m-equiv/kg H_2O)	Glucose (m-mole/ kg H_2O)
PEG medium	340	152	139	—
PEG medium + NaCl	770	374	362	—
PEG medium + glucose	770	152	138	385

Cohen's (1959) development of the method of Deutsch (1936). No more than four slices were cut from each half kidney. The outermost slices were not discarded since Macknight (1967) showed that under experimental conditions like those employed here, these slices behaved similarly to inner cortical slices.

Slices, as cut, were immediately placed in a beaker of stirred oxygenated ordinary medium, containing acetate, at room temperature. Once the last slice had been cut, all slices remained in this equilibration medium for a further 15 min. Equilibration to remove urine, blood cells, and debris from the slices enabled a uniform composition to be attained. Control slices were then taken for analysis and the remaining slices were transferred to the incubation vessels.

Incubation. Groups of five to seven slices were incubated in small bottles, containing 20 ml. of medium. Each bottle was fitted with a rubber stopper pierced by two tubes for the passage of nitrogen, which was bubbled through the medium for 5 min. The vessels were sealed, so that each contained an atmosphere of nitrogen, and were then placed in a Gallenkamp shaking incubator which thermostatically controlled the temperature at 26° C. In most experiments, slices were removed for analysis 15 min, 30 min, 1 hr, 2, 4, 8 and 12 hr after the commencement of incubation. In all cases, a few slices were taken randomly from each bottle. Nitrogen was again bubbled through the media and the bottles were resealed so that incubation of the remaining slices continued under anaerobic conditions.

Analytical methods. Slices were blotted to remove excess moisture and weighed on a Mettler single-pan semimicro balance. They were dried in an air oven at 105° C and extracted with 0·1 N-HNO_3, as described by Little (1964). Water content was taken as the loss of weight during drying.

Sodium and potassium in the acid extracts were determined with an EEL flame photometer using external standards made up in 0·1 N-HNO_3. PEG and glucose at the concentrations encountered did not interfere with these measurements. Chloride was determined by the method of Cotlove, Trantham & Bowman (1958).

Preliminary experiments showed that drying in an air oven reduced the amount of glucose recoverable by acid extraction. Therefore when tissue glucose was to be estimated, twice as many slices were incubated in glucose medium as in other media. When taken for analysis, these were separated randomly into two groups. One group was dried in the normal way and extracted for ion analyses. The other group was not dried but was extracted wet with 0·1 N-HNO_3 for glucose analysis. It was assumed that the two groups of slices had attained the same composition. Glucose in the acid extracts was determined enzymatically, with glucose oxidase and peroxidase, using the method of Huggett & Nixon (1957) as desciibed by Bergmeyer & Bernt (1965).

Measurement of osmolality. The osmolality of each medium was determined with a Fiske osmometer, calibrated using standards supplied by Fiske Associates Inc., U.S.A.

Expression of results. Tissue water, ion and glucose contents have been expressed in terms of unit mass of dry matter.

In the final experiments, where slices were incubated in media containing high concentrations of sodium chloride or glucose, the extra weight of solutes entering the slices was subtracted from the measured dry weights to allow comparison of slices from different media. Corrections were also made for the weight of PEG remaining in the slices, on the assumption that PEG was confined to an extracellular phase corresponding to 26 % of the weight of the moist tissue (Macknight, 1968), in which it had attained the same concentration as in the medium (Wiggins, 1965).

The significance of differences between groups of observations was assessed by Student's t test.

RESULTS

Incubation of non-metabolizing slices in media containing high concentrations of sodium chloride

Changes in composition of slices incubated in media made hyperosmotic with sodium chloride are illustrated in Figs. 1 and 2. For clarity, values for the 15 min period and all values for medium B are omitted. The 15 min results lay between the control and 30 min results for their respective media, while results for medium B always lay between corresponding values for media A and C.

Water contents. Whereas slices in ordinary medium swelled from the onset of incubation, those in the hyperosmotic media shrank initially, regained their volume, and then continued to swell. The initial shrinkage and recovery of volume of the slices in hyperosmotic media were complete within the first hour. The subsequent swelling of slices in all media was most rapid during the first 4 hr of incubation. Thereafter the rate of

swelling decreased, but the differences between water contents at 8 and 12 hr were statistically significant ($P < 0{\cdot}05$) in three of the four media. Hence it may be concluded that most slices were still swelling at the end of incubation. After 12 hr incubation, those in ordinary medium had more than doubled their water content: from 2·64 to 5·56 kg H_2O/kg dry matter.

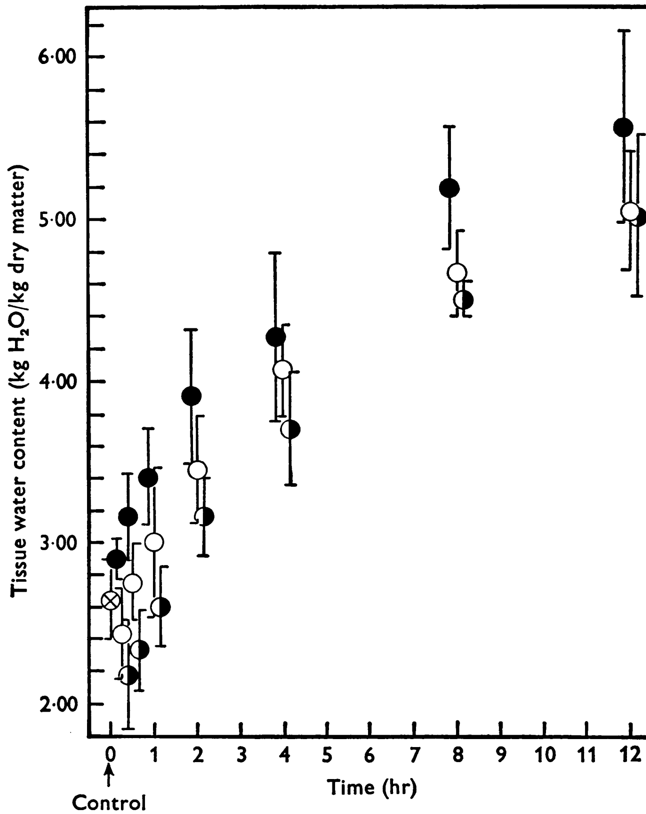

Fig. 1. Water contents of non-metabolizing slices incubated at 26° C for up to 12 hr in ordinary medium, and in media containing additional quantities of NaCl. Each value represents the mean ± S.D. of six to sixteen separate observations. ⊗ control; ● ordinary medium; ○ medium A (ordinary medium + 200 m-osmole NaCl/kg H_2O); ◑ medium C (ordinary medium + 400 m-osmole NaCl/kg H_2O).

The water contents of slices from the other three media had by this time reached approximately 5·0 kg H_2O/kg dry matter.

The degree to which slices shrank initially depended upon the concentration of the medium; those in the strongest media lost the most water. Comparison of slices from any two media before 12 hr shows that,

at any particular time, those from the more concentrated medium had a lower water content.

The amounts of swelling shown by slices in all three hyperosmotic media, between 1 and 4 hr, were almost identical: 1·07 kg H_2O/kg dry matter (medium A), 1·07 kg/kg (medium B), 1·09 kg/kg (medium C). The slices in ordinary medium were swelling somewhat less rapidly at this stage;

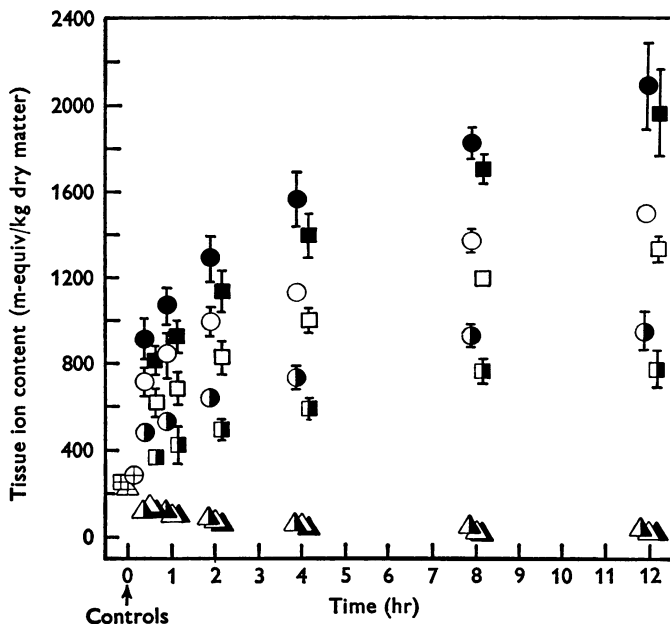

Fig. 2. Ion contents of non-metabolizing slices incubated at 26° C for up to 12 hr in ordinary medium, and in media containing additional quantities of NaCl. Each value represents the mean ± S.D. of six to ten separate observations. Control values are indicated by crossed symbols (eighteen observations). Ordinary medium, ◑ Na^+, ◨ Cl^-, ▲ K^+; ordinary medium + 200 m-osmole NaCl/kg H_2O, ○ Na^+, □ Cl^-, △ K^+; ordinary medium + 400 m-osmole NaCl/kg H_2O, ● Na^+, ■ Cl^-, ▲ K^+.

their water content increased by 0·87 kg H_2O/kg dry matter during the same interval. These slices had shown no initial shrinkage, and had undergone their most rapid swelling in the first 2 hr.

Ion contents. The tissue sodium and chloride contents of all slices rose throughout the experiment, while potassium contents fell quite rapidly during the first hour and thereafter at a slower rate. These changes are illustrated in Fig. 2.

The most rapid influx of sodium and chloride occurred during the early part of incubation, especially in medium C, where the tissue sodium content increased from 282 to 760 m-equiv/kg dry matter in the first 15 min.

After 12 hr the tissue sodium content in medium C had risen to 2090 m-equiv/kg dry matter. The increase in chloride content was of similar magnitude. Slices in less concentrated media had gained smaller quantities of these ions during this time. At any stage of incubation, the amounts of sodium and chloride were greater in slices from the more concentrated media.

Incubation of non-metabolizing slices in ordinary medium containing PEG 6000

The results are illustrated in Fig. 3. Slices in ordinary medium containing 7 g PEG/100 ml. shrank from 2·74 to 2·33 kg H_2O/kg dry matter within 10 min, but had returned to the control water content within 2 hr. A steady water content, slightly above that of the control, was maintained from 4 to 12 hr: there was no significant difference between the values at these times ($P > 0{\cdot}3$).

Sodium and chloride contents increased for the first 6 hr, but there was no significant further increase between 6 and 12 hr (for Na^+, $P > 0{\cdot}05$; for Cl^-, $P > 0{\cdot}2$). Four fifths of the tissue potassium were lost during the first 4 hr. Subsequent loss was small and slow: the results at 10 and 12 hr were not significantly different ($P > 0{\cdot}05$).

Experiments lasting up to 48 hr at an incubation temperature of 0·5° C showed similar, though slower, changes.

Incubation of non-metabolizing slices in PEG media with high concentrations of sodium chloride or glucose

In each experiment, three media were used: ordinary medium containing 7 g PEG/100 ml., and this PEG medium containing additional quantities of either sodium chloride (the 'NaCl medium') or glucose (the 'glucose medium') (Table 2). The results are illustrated in Figs. 4, 5 and 6.

Water contents. Slices in all three media shrank at first. After 30 min the water content of slices in the PEG medium had decreased from the control value of 2·60 kg H_2O/kg dry matter to 2·45 kg/kg, but the water content of those in the other two media had fallen much further: to 1·93 kg/kg (glucose medium) and 1·79 kg/kg (NaCl medium).

After the initial shrinkage, slices in all three media recovered their volume and reached a steady water content which was maintained without significant increase between 8 and 12 hr ($P > 0{\cdot}4$ for glucose medium; $P > 0{\cdot}2$ for NaCl medium). But whereas the addition of glucose to the PEG medium did not affect the equilibrium water content attained (no significant difference between slices from the PEG medium and glucose medium after 6 hr), the addition of NaCl significantly reduced the equilibrium water content of the slices ($P < 0{\cdot}05$ for values in PEG medium

and NaCl medium at 12 hr). The difference was 0·31 kg H_2O/kg dry matter which is 11 % of the control water content. Fig. 4 also shows that the slices in the NaCl medium swelled much more slowly than those in the glucose medium, especially during the first 2 hr after their initial shrinkage.

Ion and glucose contents. The sodium and chloride contents of slices in the PEG and glucose media were almost identical and were the same as

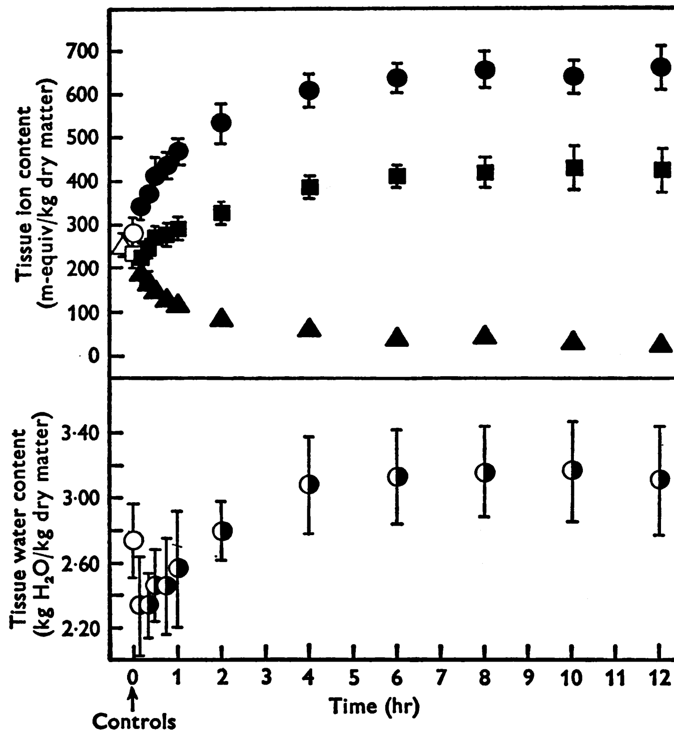

Fig. 3. Composition of non-metabolizing slices incubated at 26° C for up to 12 hr in ordinary medium containing 7 g PEG 6000/100 ml. Each value represents the mean ± S.D. of fourteen to twenty-four separate observations. Open symbols indicate controls. ◑ H_2O, ● Na^+, ■ Cl^- and ▲ K^+.

described for the previous series. The influx of sodium and chloride into slices in the NaCl medium ran parallel to that observed in the other two groups, but at a much higher level. The sodium content after 12 hr was 1242 m-equiv/kg dry matter compared with the control value of 262 m-equiv/kg dry matter. The content of these ions appeared to be still rising slightly between 8 and 12 hr ($P < 0·02$, for Na^+; $P < 0·05$, for Cl^-). The potassium contents of slices in all media showed the same trend as described

for the previous series, falling quite rapidly at first, but very slowly during the later stages of incubation. These results are illustrated in Fig. 5.

The glucose contents of slices incubated in the glucose medium are plotted in Fig. 6. They rose rapidly from a control value of zero to 1175 m-mole/kg dry matter after 6 hr. However, even after 8 hr, these slices still seemed to be gaining glucose slowly ($P < 0{\cdot}05$ for values at 8 and 12 hr).

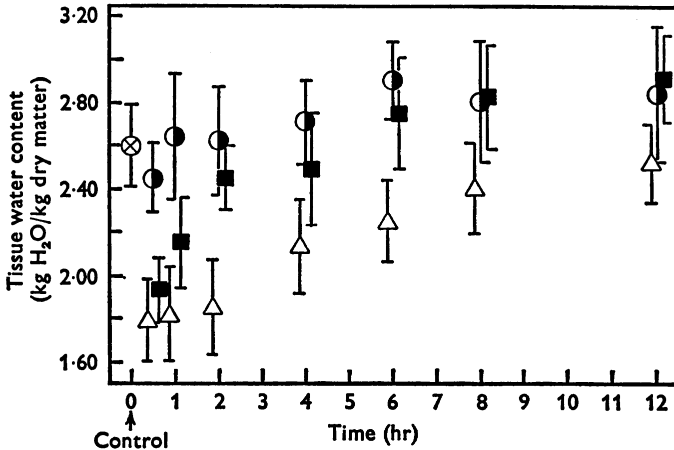

Fig. 4. Water contents of non-metabolizing slices incubated at 26° C for up to 12 hr in ordinary medium containing 7 g PEG/100 ml., and in PEG medium containing either glucose or additional NaCl. Each value represents the mean ± S.D. of ten to twelve separate observations. ⊗ control; ◑ PEG medium; ■ PEG medium + 400 m-osmole glucose/kg H_2O; △ PEG medium + 400 m-osmole NaCl/kg H_2O.

DISCUSSION

The results showed that swelling of non-metabolizing kidney slices was not prevented by incubation in hyperosmotic saline media, even when these were more than twice the concentration of extracellular fluid. Although the slices in concentrated media did initially shrink, this was an osmotic transient and was reversed within an hour. The temporary loss of water was presumably a consequence of the greater permeability of the cellular membrane to water than to solutes such as sodium and chloride ions. However, the slower entry of ions, accompanied by water, eventually resulted in swelling.

Although, at any time, slices in the more concentrated media contained less water, this was not necessarily due to depression of colloid osmotic pressure. Heckmann & Parsons (1959) discussed the swelling of incubated rat liver slices, in a chilled isosmotic saline solution, in two stages. The first phase of redistribution of ions and water, after the cessation of active

transport, was followed by colloid osmotic swelling in which the unbalanced colloid osmotic pressure of the intracellular contents caused a net uptake of water, accompanied by solutes. To provide evidence for the depression of colloid osmotic pressure, it would seem necessary to show that the slices in our more concentrated media swelled at a slower rate,

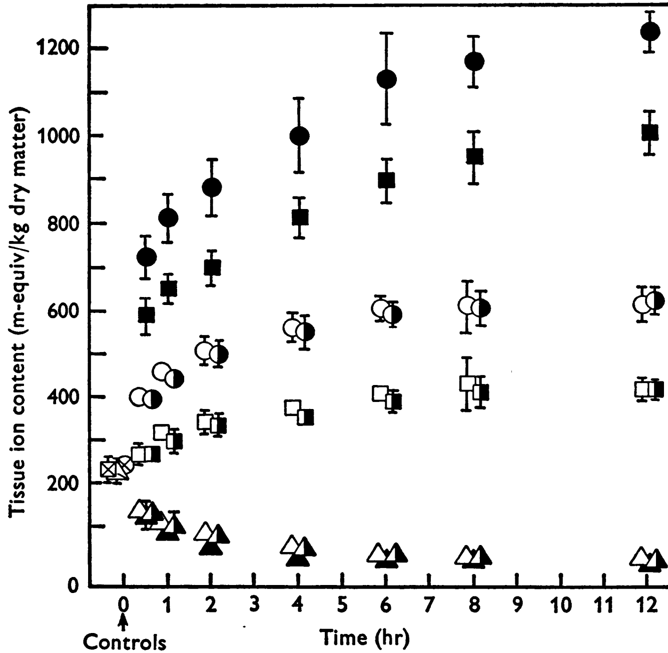

Fig. 5. Ion contents of non-metabolizing slices incubated at 26° C for up to 12 hr in ordinary medium containing 7 g PEG/100 ml., and in PEG medium containing either glucose or additional NaCl. Each value represents the mean ± S.D. of ten to twelve separate observations. Control values are indicated by crossed symbols (sixteen observations). PEG medium, ○ Na^+, □ Cl^-, △ K^+; PEG medium + 400 m-osmole NaCl/kg H_2O, ● Na^+, ■ Cl^-, ▲ K^+; PEG medium + 400 m-osmole glucose/kg H_2O, ◑ Na^+, ◨ Cl^-, ◭ K^+.

at least during the second phase of swelling. In fact, slices in three of the four media were swelling at the same average rate between 1 and 4 hr. Deductions from rates of swelling, however, require the uncertain assumption that the permeabilities of slices in the different media were the same.

In the subsequent experiments with media containing PEG, it was hoped that equilibrium states would be established, so that comparisons between slices in different media could be made readily. By eliminating gross swelling, the uncertain consequences of alteration of the spatial arrangement within the cells should have been avoided.

The transient shrinkage of slices incubated in media containing 7 g PEG/100 ml. (Fig. 3) was due to the greater initial activity of water in the slices than in the medium. But as PEG diffused into the extracellular space, and as sodium and chloride entered the cells, this initial shrinkage was reversed. Subsequent attainment of a steady water content indicates that the activities of water in the slices and medium became equal. The osmotic effect of the non-penetrating PEG molecules, together with other

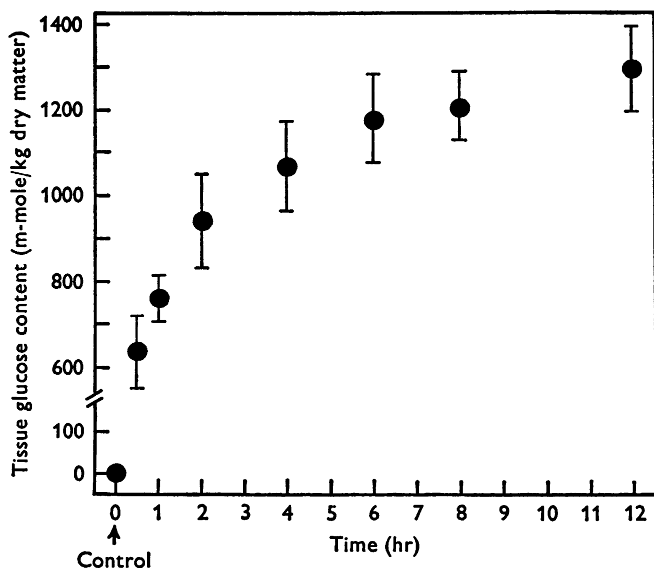

Fig. 6. Glucose content of non-metabolizing slices incubated at 26° C for up to 12 hr in ordinary medium containing 7 g PEG/100 ml. with added glucose. Each value represents the mean ± S.D. of ten to twelve separate observations.

forces opposing swelling (e.g. elastic tension within the tissue), balanced the effect of the colloid osmotic pressure of the cellular contents. That these changes occurred more slowly at 0·5° C than at 26° C may be attributed to the slower diffusion at low temperatures.

During the latter part of incubation, the water, sodium, potassium, and chloride contents of slices in the PEG medium were all unchanging, both at 0·5 °C and at 26° C. This constant composition indicates that an equilibrium state was established. The slices in media containing glucose or extra NaCl also attained water contents which were constant for at least 4 hr, and it has been assumed that equilibrium was established in these slices, although the tissue contents of glucose or sodium and chloride seemed to be still rising slightly after 8 hr. These very slow changes may have been the result of autolytic or other deterioration of the tissue during

the long period of incubation. Nevertheless, it seems unlikely that autolysis importantly affected the quantities being measured because slices in all three media maintained virtually constant composition over periods of several hours, even though autolysis, which is a progressive phenomenon, was presumably continuing. Interpretation of the results also depends on the assumption that metabolism was completely suppressed by iodoacetate and nitrogen.

The addition of a penetrating solute to the PEG medium would be expected to lead to a greater initial shrinkage of slices, as an osmotic transient, which should be reversed as the added solute became evenly distributed between the cells and medium. Only if the penetrating solute affected the colloid osmotic pressure of the cellular contents (or the elastic properties of the tissue) should the final equilibrium volume be altered. As predicted, addition of glucose (a penetrating non-electrolyte) to the medium did result in greater initial shrinkage and did not significantly alter the equilibrium water content (Fig. 4). But addition to the PEG medium of an osmotically equivalent amount of sodium chloride produced a different result. The initial shrinkage was greater but the slices then swelled more slowly, attaining a significantly lower equilibrium water content than slices in either the PEG medium or PEG medium containing glucose. This difference could readily be explained by a reduction of colloid osmotic pressure as the sodium and chloride ions penetrated the cells and increased the ionic strength of the fluid within them.

This work was carried out in partial fulfilment of requirements for the degree of B.Med.Sc. of the University of Otago during the tenure by both authors of Junior Research Scholarships from the Medical Research Council of New Zealand. We are indebted to Professor J. R. Robinson for his continual guidance and encouragement, and to Dr P. M. Wiggins and Dr A. D. C. Macknight for helpful discussions.

REFERENCES

AEBI, H. (1953). Elektrolyt-Akkumulierung und Osmoregulation in Gewebsschnitten. *Helv. physiol. pharmac. Acta* **11**, 96–121.

BERGMEYER, H-U. & BERNT, E. (1965). Determination with glucose oxidase and peroxidase. In *Methods of Enzymatic Analysis*, ed. BERGMEYER, H-U. New York: Academic Press.

COHEN, P. P. (1959). Methods for preparation and study of tissues. In *Manometric Techniques*, ed. UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. Minneapolis: Burgess.

COTLOVE, E., TRANTHAM, H. V. & BOWMAN, R. L. (1958). An instrument and method for automatic, rapid, accurate, and sensitive titration of chloride in biological samples. *J. Lab. clin. Med.* **51**, 461–468.

DEUTSCH, W. (1936). An improvement of Warburg's method for cutting tissue slices for respiratory experiments. *J. Physiol.* **87**, 56–57*P*.

HECKMANN, K. D. & PARSONS, D. S. (1959). Changes in the water and electrolyte content of rat-liver slices *in vitro*. *Biochim. biophys. Acta* **36**, 203–213.

HITCHCOCK, D. I. (1924). Proteins and the Donnan equilibrium. *Physiol. Rev.* **4**, 505–531.

HUGGETT, A. ST G. & NIXON, D. A. (1957). Enzymic determination of blood glucose. *Biochem. J.* **66**, 12P.

LEAF, A. (1956). On the mechanism of fluid exchange of tissues *in vitro*. *Biochem. J.* **62**, 241–248.

LEAF, A. (1959). Maintenance of concentration gradients and regulation of cell volume. *Ann. N.Y. Acad. Sci.* **72**, 396–404.

LILLIE, R. S. (1907). The influence of electrolytes and of certain other conditions on the osmotic pressure of colloidal solutions. *Am. J. Physiol.* **20**, 127–169.

LITTLE, J. R. (1964). Determination of water and electrolytes in tissue slices. *Analyt. Biochem.* **7**, 87–95.

MACKNIGHT, A. D. C. (1967). Differences in composition between outer and inner cortical slices from rat kidney. *Proc. Univ. Otago med. Sch.* **45**, 38–39.

MACKNIGHT, A. D. C. (1968). The extracellular space in rat renal cortical slices incubated at 0·5° and 25°. *Biochim. biophys. Acta* **163**, 85–92.

MUDGE, G. H. (1951*a*). Studies on potassium accumulation by rabbit kidney slices: effect of metabolic activity. *Am. J. Physiol.* **165**, 113–127.

MUDGE, G. H. (1951*b*). Electrolyte and water metabolism of rabbit kidney slices: effect of metabolic inhibitors. *Am. J. Physiol.* **167**, 206–223.

OPIE, E. L. (1949). Movement of water in tissues removed from the body and its relation to movements of water during life. *J. exp. Med.* **89**, 185–208.

ROBINSON, J. R. (1950). Osmoregulation in surviving slices from the kidneys of adult rats. *Proc. R. Soc.* B **137**, 378–402.

ROBINSON, J. R. (1965). Water regulation in mammalian cells. *Symp. Soc. exp. Biol.* **19**, 237–258.

WIGGINS, P. M. (1964). Selective accumulation of potassium ion by gel and kidney slices. *Biochim. biophys. Acta* **88**, 593–605.

WIGGINS, P. M. (1965). A kinetic study of the state of potassium in kidney tissue. *Biochim. biophys. Acta* **109**, 454–466.

WILSON, T. H. (1954). Ionic permeability and osmotic swelling of cells. *Science, N.Y.* **120**, 104–105.