J. Physiol. (1972), **225**, *pp.* 555–564
With 3 text-figures
Printed in Great Britain

THE INFLUENCE OF ELECTROLYTES ON THE VOLUME OF NON-METABOLIZING RENAL CORTICAL CELLS

By D. J. L. McIVER and A. E. G. RAINE
From the Department of Physiology, University of Otago Medical School, Dunedin, New Zealand

(*Received 21 February 1972*)

SUMMARY

1. The metabolism of rat renal cortex slices was inhibited by iodoacetate and anoxia, and swelling was prevented by the presence in the medium of 7·2 g polyethylene glycol 6000/100 ml. (referred to as PEG medium).

2. Slices were incubated for up to 12 hr in PEG medium, and in PEG media containing 440 m-osmole/kg H_2O of an electrolyte (LiCl, NaCl, KCl or RbCl), or a non-electrolyte (glucose).

3. It was concluded that the slices in all media were at equilibrium with the medium after incubation for 8 hr.

4. Slices in the medium containing glucose reached the same equilibrium water content as those in the PEG medium, but slices in all the electrolyte media had significantly lower equilibrium water contents, although these did not differ significantly from each other.

5. It is suggested that the results demonstrate a non-specific effect of electrolytes on the swelling of non-metabolizing cells.

INTRODUCTION

Despite the fact that their colloids depress the activity of the intracellular fluids, metabolizing cells maintain stable volumes. Leaf (1956, 1959) accounted for this stability in terms of a 'double Donnan' system, in which Na, actively held in an obligatorily extracellular position, balanced the osmotic pressure of the cell colloids, and prevented swelling.

Many workers have shown that swelling does occur when the metabolism of isolated tissues is interfered with, thereby inactivating the mechanisms normally responsible for Na exclusion. However, Opie (1949), Robinson (1950) and others found that this swelling was prevented by immersion of the tissue in highly concentrated solutions of NaCl. Deyrup (1953) suggested that these observations could be explained on the basis of a greater permeability of the cell membrane to water than to solutes; if

the time of observation was sufficiently short and the external solute concentration sufficiently high, the cells should initially shrink as they lost water before the extracellular solute had time to penetrate the intracellular phase. In contrast, Leaf (1956) advanced another explanation for these observations. From the experiments of Lillie (1907) and the theory developed by Donnan (1911), it was known that high concentrations of electrolytes reduced the osmotic pressure of charged colloids. Leaf suggested that this phenomenon might explain why the slices in Robinson's experiments failed to swell.

Davey & Skegg (1971) confirmed Deyrup's explanation by showing, in a kinetic study, that non-metabolizing kidney slices, incubated in media containing high concentrations of NaCl, did actually swell after an initial shrinkage. However, they also obtained evidence to support Leaf's suggestion that high concentrations of electrolytes could reduce cellular colloid osmotic pressure. When non-metabolizing slices were prevented from swelling by the addition of the impermeant solute polyethylene glycol of average mol. wt. 6000 (PEG 6000) to the medium, slices which were incubated with a high concentration of an electrolyte (NaCl) attained a significantly lower final water content than slices incubated in a medium containing an equal osmotic activity of a non-electrolyte, glucose. Since analysis showed that both NaCl and glucose had freely penetrated the slice, Davey & Skegg concluded that their results were consistent with the hypothesis that NaCl reduced the cellular colloid osmotic pressure.

If this interpretation is correct, then from Donnan's theory the depressing effect of electrolytes should depend only on their activity and valence, and not on their chemical nature. In the present experiments, the effects of several electrolytes were compared; univalent cations only were chosen because of uncertainties regarding the intracellular activities of di- or polyvalent ions. Non-metabolizing kidney slices were incubated in media containing PEG 6000, and a high concentration of either an electrolyte (LiCl, NaCl, KCl or RbCl), or a non-electrolyte (glucose). The results confirmed the earlier finding that the presence of a permeant electrolyte reduced the equilibrium water content of non-metabolizing tissue slices, while the presence of a penetrating non-electrolyte had no effect on water content. In addition, it was found that this effect of the electrolyte was independent of the nature of the univalent cation used. A preliminary account of some of this work has been published (McIver & Raine, 1971).

METHODS

Media. The balanced saline medium, called 'ordinary medium' and similar to that of Robinson (1949), contained ions in the following concentrations m-mole/kg H_2O: Na^+ 145, K^+ 5, Ca^{2+} 2·5, Mg^{2+} 1, Cl^- 133, SO_4^{2-} 1. (All media were buffered at pH

7·35 with 8 mM phosphate buffer.) Media in which slices were equilibrated before incubation contained 9 mM Na acetate as substrate, and incubation media contained 9 mM Na iodoacetate.

Media containing polyethylene glycol 6000 (Union Carbide Chemical Company, U.S.A.) and additional solutes were prepared by dissolving weighed amounts of solute in ordinary iodoacetate medium. A medium containing 7·2 g PEG/100 ml. solution was prepared, since preliminary experiments had showed that slices incubated in this medium achieved the same water content as control slices which were incubated in oxygenated ordinary medium. This ensured that the suppression of metabolism by iodoacetate and nitrogen did not of itself alter the final water content of the slices. Hyperosmotic media were prepared from the PEG medium by adding sufficient solute (LiCl, NaCl, KCl, RbCl or glucose) to raise the osmolality of the medium by approximately 440 m-osmole/kg H_2O. The composition of all the media is shown in Table 1.

Preparation of slices. Kidneys of adult male rats from an inbred black and white strain were sliced using Cohen's (1959) modification of the method of Deutsch (1936). No more than four cortical slices were taken from each half kidney. Slices were immediately equilibrated at room temperature for at least 15 min in a large volume of oxygenated ordinary medium containing acetate. Slices thus equilibrated attain a uniform control state, to which incubated slices can be referred, rather than to the variable composition of slices cut fresh from the kidney (Robinson, 1961).

Incubation. After removal of control slices for analysis, the equilibrated slices were transferred to the incubation vessels. About five slices were incubated with 20 ml. medium in glass bottles through which nitrogen was bubbled for 15 min before the bottles were sealed for the remainder of the experiment. Incubation was carried out in a Gallenkamp shaking incubator at 25° C. Slices were removed after incubation for 30 min, 1 hr, 2 hr, 4 hr, 8 hr and 12 hr.

Analytical techniques. Water and electrolyte contents were determined by the method described by Little (1964). Incubated slices were blotted, weighed in borosilicate glass bottles, and dried in an air oven at 105° C. After cooling in a desiccator they were reweighed, and the weight of water was taken as the difference between the wet and dry weights. Each dried slice was extracted with 0·1 M-HNO_3, and the electrolytes were determined in this acid extract. Li^+, Na^+ and K^+ were determined with an EEL flame photometer, using external standards in 0·1 M-HNO_3. In the concentrations used PEG and glucose did not interfere with the measurement nor was there any evidence that the presence of any of these three electrolytes interfered with the measurement of any other. Rb^+ was measured by emission at 780 mμ with a Beckman DU flame spectrophotometer. At the highest concentrations of Rb^+ encountered there was evidence of slight interference by Na^+, K^+ and Ca^{2+}; as Sutter, Platman & Fieve (1970) found, with atomic absorption spectrophotometry, all of these ions enhanced the measurement of Rb^+. On the other hand, there was no interference from PEG or any other substances present in the extract. Rb^+ standards were made up containing appropriate amounts of Na^+, K^+ and Ca^{2+} to overcome the interference. However, Rb^+ interfered so strongly with K^+ that it was impossible to measure K^+ on the EEL photometer when Rb^+ was present, and K^+ measurements have not been made for slices incubated in Rb^+ media. Chloride was measured by the method of Cotlove, Trantham & Bowman (1958).

Glucose was determined on wet slices with glucose oxidase and peroxidase (C. F. Boehringer and Sons, Germany), using the method of Huggett & Nixon (1957), described by Bergmeyer & Bernt (1965). Davey & Skegg (1971) showed that drying reduced the amount of glucose detectable in slice extracts. Therefore, in every experiment in which glucose contents were determined, some wet slices were analysed, and the glucose contents of the dry slices were calculated by extrapolation from these values.

The osmolalities of the media were determined from their freezing point depressions using a Fiske thermo-electric osmometer, and standards supplied by Fiske Associates Ltd, U.S.A. Preliminary experiments had showed that freezing point depression measurements over-estimated the osmolality of PEG solutions at higher temperatures. Presumably this was mainly because of the marked solvent-ordering capacity of PEG. Thus, the osmolalities shown in Table 1 give only relative, not absolute, estimates of the water activities in the different media at 25° C.

Expression of results. Entry of solute from hyperosmotic media may cause a significant increase in the final dry weight of a slice; hence, for valid comparison of slices incubated in different media, the dry weights of slices were corrected for weights

TABLE 1. Composition of media

	Osmolality (m-osmole/kg H_2O)	Na^+	K^+	Li^+	Rb^+	Cl^-	Glucose	PEG (g/100 ml.)
		m-mole/kg H_2O						
Ordinary medium	282	145	5	—	—	133	—	—
PEG medium	342	145	5	—	—	133	—	7·2
PEG + LiCl	779	145	5	224	—	357	—	7·2
PEG + NaCl	780	377	5	—	—	365	—	7·2
PEG + KCl	782	145	242	—	—	370	—	7·2
PEG + RbCl	780	145	5	—	232	365	—	7·2
PEG + glucose	785	145	5	—	—	133	389	7·2

of additional solutes contained in them. PEG was corrected for on the basis that it occupied the same space as inulin (Macknight, 1968). Electrolytes were corrected for by expressing Cl^- (present in excess of that found in slices incubated in PEG medium under the same conditions) as a weight of LiCl, NaCl, KCl or RbCl. Glucose was corrected for by assuming that dried slices had contained the same glucose concentration as wet slices incubated under the same conditions.

Ion, water and glucose contents have been expressed in terms of unit weight of dry matter. Concentrations of a solute in the tissue water have been calculated from the division of the tissue solute content by the tissue water content. The resulting ratio is not intended to imply that all of the solute was in free solution, nor that all of slice water was solvent. Individual groups of observations have been expressed as the average of the observations, ± S.E. of the mean. Student's *t* test was used to assess the significance of differences between groups of observations.

RESULTS

In six separate experiments, slices were incubated simultaneously either in PEG medium or in one of the five hyperosmotic media.

Water contents. These are illustrated in Fig. 1. Control slices analysed at the end of equilibration had a mean water content of 2·74 kg H_2O/kg dry matter (S.E. of mean ± 0·04 from fourteen determinations). Slices in all media showed a pronounced decrease in water content at the onset of incubation. This loss of water can be readily explained as an osmotic

transient; water moved down its activity gradient out of the tissue, into the hyperosmotic medium, before the solute penetrated the cells.

This initial water loss was least for the slices incubated in the PEG medium, which had an osmolality approximately 440 m-osmole/kg H_2O less than that of the other media. The slices in the PEG medium had a water content of $2{\cdot}21 \pm 0{\cdot}03$ kg H_2O/kg dry matter after 30 min, but had returned

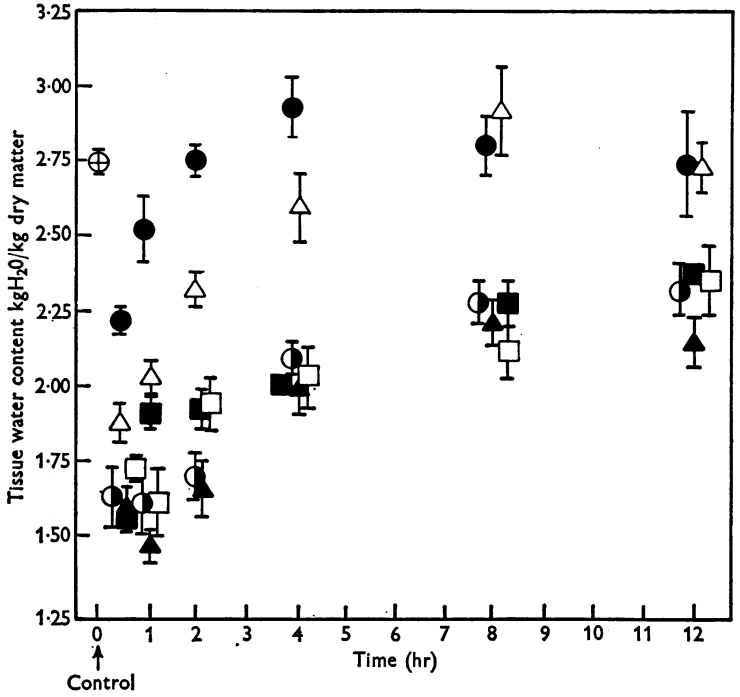

Fig. 1. Water contents of non-metabolizing slices incubated at 25° C for up to 12 hr in PEG medium (●), and PEG media containing added amounts of glucose (△), LiCl (▲), NaCl (◑), KCl (□) or RbCl (■). Each value represents the mean ± S.E. of mean of six to fifteen separate observations. ⊕ indicates control (fourteen observations).

to the control water content within 2 hr, and showed no significant change between 2 and 12 hr ($P > 0{\cdot}6$). The 12 hr water content of these slices was almost identical to that of the controls: $2{\cdot}73 \pm 0{\cdot}17$ kg H_2O/kg dry wt.

Slices incubated in the medium containing added glucose shrank to a water content of $1{\cdot}88 \pm 0{\cdot}07$ kg H_2O/kg dry matter at 30 min, but regained water and maintained a steady content between 8 and 12 hr ($P > 0{\cdot}2$). The final water content for these slices was the same as that for slices incubated in PEG medium ($2{\cdot}73 \pm 0{\cdot}08$ kg H_2O/kg dry matter).

During the first hour of incubation, the lowest water content values were

reached by slices incubated in the media containing added electrolytes. As with slices incubated in PEG medium, or in PEG medium with added glucose, slices in these media subsequently increased in water content, reaching values at 12 hr of $2{\cdot}15 \pm 0{\cdot}10$ kg H_2O/kg dry matter (PEG medium + LiCl), $2{\cdot}32 \pm 0{\cdot}10$ kg H_2O/kg dry matter (PEG medium + NaCl), $2{\cdot}36 \pm 0{\cdot}12$ kg H_2O/kg dry matter (PEG medium + KCl), and $2{\cdot}39 \pm 0{\cdot}03$ kg H_2O/kg dry matter (PEG medium + RbCl). Between 8 and 12 hr the water contents of slices in each of these media were constant: $P > 0{\cdot}6$ for LiCl medium; $P > 0{\cdot}7$ for NaCl medium; $P > 0{\cdot}1$ for KCl medium; $P > 0{\cdot}1$

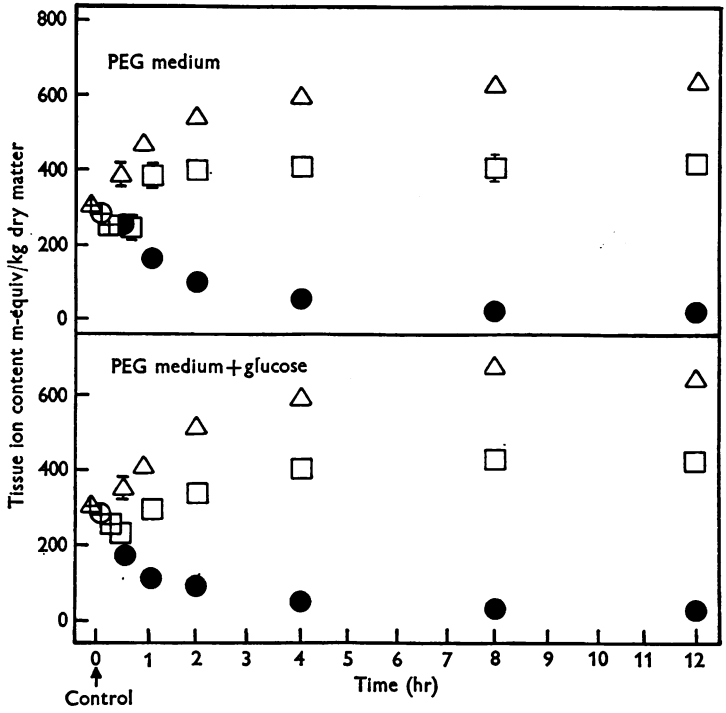

Fig. 2. Ion contents (△ Na^+, ● K^+, □ Cl^-) of non-metabolizing slices incubated at 25° C for up to 12 hr in PEG medium, and in PEG medium containing an added amount of glucose. Each value represents the mean ± S.E. of mean of six to fifteen separate observations. ⊕ indicates controls (fourteen observations).

for RbCl medium. Also, at any time from 4 hr onwards the water contents of slices in these four media were not affected by differences in the cation present in the medium. By contrast, however, the final water contents of slices incubated in media containing added electrolytes were all significantly lower than those of slices incubated in either the PEG or the PEG + glucose media; this was true at both 8 and 12 hr ($P < 0{\cdot}05$, in all cases).

Ion and glucose contents. The ion contents of slices incubated in PEG medium and PEG medium with added glucose are illustrated in Fig. 2. The distribution of ions was very similar in both media. The Na^+ and Cl^- which entered the slice maintained a steady level between 4 and 12 hr ($P > 0{\cdot}1$ for Na^+ in each medium; $P > 0{\cdot}7$ for Cl^-). The K^+ contents of

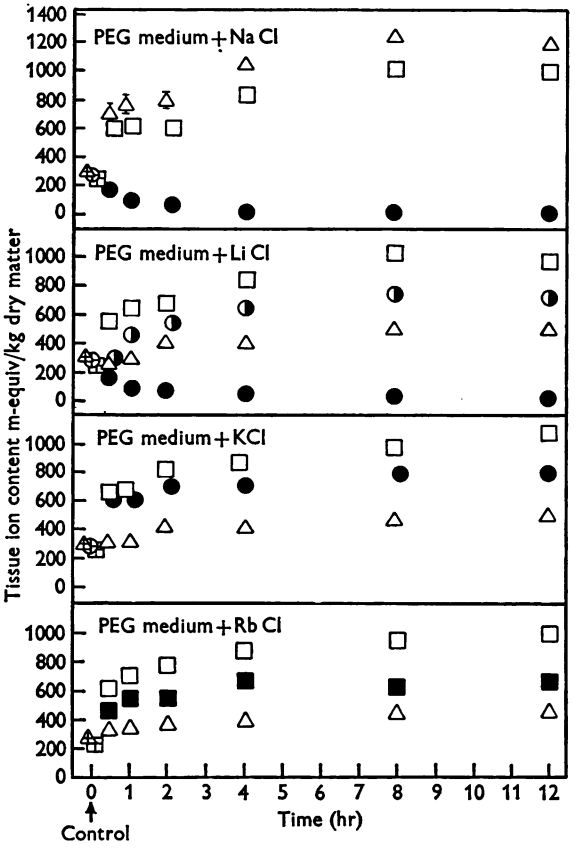

Fig. 3. Ion contents (△ Na^+, ● K^+, □ Cl^-, ◑ Li^+, ■ Rb^+) of non-metabolizing slices incubated at 25° C for up to 12 hr in PEG media containing added amounts of LiCl, NaCl, KCl or RbCl. Each value represents mean ± S.E. of mean of six to fourteen separate observations. ⊕ indicates controls (fourteen observations).

slices fell continuously with time. For slices incubated in media containing added glucose, the glucose content rose to 1150 mM glucose/kg dry matter by the end of 8 hr, and it did not change during the remainder of the experiment ($P > 0{\cdot}6$).

The ion contents of slices incubated in media containing added electrolytes are shown in Fig. 3. Except in the medium containing added KCl the

K content of slices fell throughout the experiment. All other ions entered the tissue from the medium, and reached constant levels before 8 hr (P values for comparison of 8 and 12 hr tissue ion content ranged between $P > 0{\cdot}2$ and $P > 0{\cdot}9$, except for Cl in the PEG medium + KCl, where $P > 0{\cdot}05$).

The concentrations of solutes in the tissue water were also calculated. By 8 hr these concentrations were somewhat higher than the medium concentrations shown in Table 1. For example, the 8 hr Na concentration for slices incubated in PEG medium was 228 ± 7 m-mole/kg H_2O, while the Cl concentration was 148 ± 8 m-mole/kg H_2O, and the glucose concentration in the glucose medium was 396 ± 14 m-mole/kg H_2O. All solutes entering the tissue reached steady concentrations before 4 hr ($P > 0{\cdot}5$, in all cases). Where K efflux was shown to occur, the K concentration fell throughout the experiment.

DISCUSSION

In the present study, four penetrating electrolytes reduced the final volumes of non-metabolizing cells, while a penetrating non-electrolyte did not, even though the water activity in all media was the same. To interpret these effects of electrolytes on slice water content, it is necessary to decide if the water and permeant solutes in the slices reached a true equilibrium with the medium. At equilibrium it is possible to ignore the uncertain effects on the membrane of iodoacetate and the different medium compositions, since then the distribution of solvent and permeant solutes is independent of membrane permeability.

For all slices, the water and solute contents were unchanging over the latter part of incubation. This unchanging composition is evidence that equilibrium had been reached, as Davey & Skegg (1971) concluded in their experiments. It is possible, though, that PEG slowly penetrates the cells under these conditions. Macknight (1968) considered that inulin, of similar molecular size to PEG 6000, probably penetrated the cells of non-metabolizing kidney slices incubated for considerably shorter periods than those in the present experiments. If PEG does enter the cells to any marked extent, not only is the correction of slice weights for PEG invalid, but also it cannot be assumed that a true equilibrium was reached. However, experiments using [^{14}C]PEG 4000 and [^{3}H] inulin simultaneously (McIver & Macknight, 1972) have shown both that the PEG space closely approximated the inulin space, and that it remained constant for up to 12 hr of suppressed metabolism. Thus, it seems very likely that in these experiments an equilibrium distribution of water and permeant solutes was reached.

At equilibrium, there is no difference in water activity across the cell

membranes and the water content of the slice should depend on the balance of those forces tending to cause the cell to shrink, and those tending to cause it to swell. Extracellular PEG provides the major shrinking force, while the cell colloids cause swelling. Some of the cell colloids are probably in free solution, while others are aggregated to form weakly cross-linked gels (Katchalsky, 1954). Dissolved colloids cause swelling by the absorption of fluid down its activity gradient, while those colloidal polyelectrolytes which are organized in a gel-like form tend to swell until the mutually repulsive forces exerted by their charged sites are balanced by the 'mechanical' effects of distorting their matrix (Helfferich, 1962).

Permeant electrolytes should reduce the osmotic pressure of dissolved colloids by reducing the 'Donnan excess' of diffusible ions which accompanies charged macromolecules (Hitchcock, 1924). They should also reduce the swelling of charged gels by reducing the repulsive forces between adjacent charged sites (Katchalsky, 1964). Further, for dissolved colloids and weakly cross-linked gels (such as are probably found in the cell), the reduction in swelling should depend only on the activity and valence of the permeant electrolyte, and not on its chemical nature. Permeant non-electrolytes, on the other hand, should have no effect on swelling, since their concentration in the medium and in the cells should be the same at equilibrium, and they should not affect the properties of the intracellular macromolecules.

The present experiments suggest that the electrolytes reduced the equilibrium volume of the cells by either or both of the mechanisms outlined above. This effect of electrolytes on non-metabolizing cells is dependent on the ionic strength of the medium. Robinson (1971) has demonstrated an inverse relation between medium NaCl concentration and the amount of PEG necessary to prevent swelling in non-metabolizing slices. Thus, it is clear that in the absence of metabolically dependent volume regulation at least, the electrolyte concentration of the medium can influence cellular volume. However, a model of cell volume regulation in metabolizing cells, incorporating both the conventional 'leak and pump' model, and the effects of electrolytes on cell colloids, has yet to be developed.

This work was supported by the Medical Research Council of New Zealand. We are grateful to Professor J. R. Robinson, and to members of the staff of the Department of Physiology for continual help and advice.

REFERENCES

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.

DAVEY, K. J. & SKEGG, D. C. G. (1971). The effects of high concentrations of an electrolyte on the swelling of non-metabolizing tissue slices. *J. Physiol.* **212**, 641–654.

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

DEYRUP, I. (1953). A study of fluid uptake of rat kidney slices *in vitro*. *J. gen. Physiol.* **36**, 739–749.

DONNAN, F. G. (1911). *Z. Elektrochem.* **17**, 572. Cited by HELFFERICH, 1962.

HELFFERICH, F. (1962). *Ion Exchange*. New York: McGraw-Hill.

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**, 12*P*.

KATCHALSKY, A. (1954). Polyelectrolyte gels. In *Progress in Biophysics*, vol. 4, ed. BUTLER, J. A. V. & RANDALL, J. T. New York: Academic Press. London: Pergamon Press.

KATCHALSKY, A. (1964). Polyelectrolytes and their biological interactions. *Biophys. J.* **4**, 1, 2, 9–41.

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.

McIVER, D. J. L. & MACKNIGHT, A. D. C. (1972). The distribution of ^{14}C-polyethylene glycol 4000 in non-metabolizing rat renal cortical slices. *Proc. Univ. Otago med. Sch.* **50**, 13–15.

McIVER, D. J. L. & RAINE, A. E. G. (1971). The influence of ionic strength and pH on the volume of non-metabolizing cells. *Proc. Univ. Otago med. Sch.* **49**, 34–35.

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.

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. (1949). Some effects of glucose and calcium upon the metabolism of kidney slices from adult and newborn rats. *Biochem. J.* **45**, 68–74.

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

ROBINSON, J. R. (1961). Exchanges of water and ions by kidney slices determined by a balance method. *J. Physiol.* **158**, 449–460.

ROBINSON, J. R. (1971). Control of water content of non-metabolizing kidney slices by sodium chloride and polyethylene glycol (PEG 6000). *J. Physiol.* **213**, 227–234.

SUTTER, E., PLATMAN, S. R. & FIEVE, R. R. (1970). Atomic absorption spectrophotometry of rubidium in biological fluids. *Clin. Chem.* **16**, 602–605.