BBA 75026

EFFECT OF SALINE OSMOLARITY ON THE STEADY-STATE LEVEL OF WATER AND ELECTROLYTES IN KIDNEY CORTEX CELLS

ARNOŠT KLEINZELLER, JÁRA NEDVÍDKOVÁ AND ANNA KNOTKOVÁ

Laboratory for Cell Metabolism, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)

(Received August 29th, 1966)

SUMMARY

Variation of saline osmolarity (220–420 mosM) with mannitol or sucrose at constant $[Na^+]_o$ and $[K^+]_o$ produced the following changes in the aerobic steady-state levels of solutes in slices of rabbit kidney incubated at 25°.

(1) The extracellular inulin space increased from 0.209 to 0.277 kg water/kg tissue when the external osmolarity was increased from 220–420 mosM.

(2) With increasing osmolarity, tissue water decreased, the cells behaving as imperfect osmometers; tissue Na^+, K^+ and Cl^- (in mequiv/kg tissue dry wt.) remained constant. When active transport was virtually stopped at 0°, the osmotically produced changes in tissue water were considerably greater and were accompanied by a simultaneous movement of Na^+ and Cl^-, cellular K^+ behaving as an osmotically inactive component.

(3) The membrane potential E in isotonic saline was 39.6 ± 1.3 mV. Saline osmolarity reversibly produced significant changes in E. *i.e.*, an increase of 11.5 mV at 220 mosM and a drop of 12.3 mV at 420 mosM.

(4) The dependence of the steady-state membrane potential on external osmolarity was compared with changes in the diffusion potentials of electrolytes.

(a) Observed values of E closely coincided with $E_{^{36}Cl}$ while E_{Cl} was somewhat lower;

(b) the electrochemical gradient of Na^+ decreased with increasing osmolarity;

(c) the electrochemical gradient of K^+ increased with increasing osmolarity.

(5) Efflux kinetics of propanediol at various external osmolarities did not indicate a marked change in the membrane leak for this substance.

(6) The results are discussed. It is suggested that with increasing external osmolarities the activity of the Na^+ pump is decreased while the activity of a K^+ pump may be enhanced.

Abbreviation: DW, tissue dry weight.

INTRODUCTION

Evidence has been summarized previously[1] in favour of the view that, in addition to the function of the leak-and-pump system, the volume of some animal cells is also determined by mechanical forces due to the elasticity of the cell membrane and/or intracellular structures. The present investigation was initiated to further test this view. External pressure produced by saline osmolarity might be expected to affect not only the cell volume but also the ionic distribution. Additional interest in the problem was raised by the observation of USSING[2] that a variation in external osmolarity produced marked changes in the function of the Na^+ pump in epithelial cells of frog skin.

The present report is concerned with the effect of external osmolarity on the steady-state levels in kidney cortex slices of water and electrolytes and of the cell membrane potential; the saline osmolarity was varied with 'non-permeable' substances, *i.e.* mannitol or sucrose*.

MATERIALS AND METHODS

Slices of rabbit kidney cortex were used throughout. Where required, tissue from several animals was pooled in order to obtain material for a sufficient number of analyses.

Incubation media. A prerequisite for the study was a rigid maintenance of the external concentrations of bulk ions (Na^+, K^+ and Cl^-), the saline osmolarity being varied only by a metabolically inert component.

Phosphate-salines of the Krebs–Ringer type (similar to Medium II A of KREBS[3], containing a physiological concentration of Ca^{2+}) were used; the osmolarity was varied by addition of mannitol, or, in some instances, of sucrose. To facilitate a study of the effect of hypotonic salines on the electrolyte distribution, the concentration of Na^+ in the media had to be reduced. For experiments at 25°, the saline contained the following components (mM): Na^+, 104.7; K^+, 6.3; Ca^{2+}, 2.7; Mg^{2+}, 1.25; Cl^-, 87.4; HCO_3^-, 3.4; SO_4^{2-}, 1.25; phosphate, 10.2; α-oxoglutarate (as substrate), 4.1. The pH was 7.4. For experiments at 0°, substrate (α-oxoglutarate) and Ca^{2+} were omitted from the above saline; it has been shown previously[4] that Ca^{2+} decreases tissue swelling. Some further modifications of the salines used at 0° will be mentioned in the text.

Experimental procedure. Groups of slices (about 600 mg) were incubated aerobically (gaseous phase O_2) for 60 min, in 100-ml conical flasks containing 15 ml saline, by shaking in a Dubnoff metabolic incubator. At the end of the incubation, the slices were removed from the flasks, blotted and used for analyses of tissue water and electrolytes. This incubation procedure was sufficient to ensure a steady-state distribution of tissue components; identical values were obtained when the reverse procedure was employed, *i.e.* the cells were first allowed to swell by soaking the tissue at 0° in an isotonic Ca^{2+}-free saline at 0° and then the slices were aerobically incubated at 25° for 60 min in the appropriate saline.

For experiments at 0°, groups of slices were soaked for 2.5 h in the appro-

* An account of these results has been presented at the 2nd International Biophysics Congress, Vienna, September 1966.

priate ice-cold salines in dishes immersed in crushed ice. The media were repeatedly replaced in order to obtain a balanced state of tissue components.

After incubation, tissue water and electrolytes were determined as described in detail elsewhere[5]. For the estimation of the extracellular space the tissue was incubated and treated as stated above, the medium containing 0.4% (w/v) inulin. The amount of tissue permitted at least 6 analyses. The concentration of electrolytes in the salines and the osmolarity were also checked. From the results obtained the apparent intracellular ionic concentrations and Donnan ratios were computed, the intracellular and external concentrations being denoted by square brackets and the indices i and o, respectively. Two corrections had to be applied for the calculation of the apparent intracellular ionic concentrations as follows:

(a) The extracellular space was taken to be identical with the inulin space (see ref. 6) and was assumed to be constant at various degrees of tissue swelling[7]. However, this last assumption was found to be only an approximation, as shown in Fig. 1. It will be seen that the inulin space significantly increased with increasing saline osmolarity.

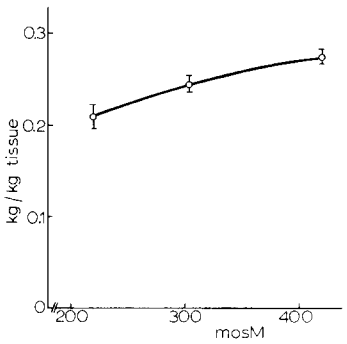

Fig. 1. Effect of saline osmolarity on the inulin space of kidney cortex slices. Slices were incubated aerobically (O_2) for 60 min at 25° in salines contained 0.4% (w/v) inulin; the saline osmolarity was varied by using mannitol. The inulin space is expressed in kg tissue water/kg tissue ± S.E., $n = 16$.

(b) Especially at higher concentrations of mannitol or sucrose in the media, these non-electrolytes in the tissue significantly contribute to the tissue weight, thus affecting the calculated values of cell water and ionic concentrations. Therefore, the observed wet and dry weights had to be corrected for the amount of mannitol or sucrose in the tissue on the assumption that these substances only enter the inulin extracellular space, *i.e.* 0.25 kg/kg tissue (*cf.* refs. 6, 7). This assumption is not quite correct since it has been shown[8,9] that these saccharides also enter a cellular compartment (the mannitol space in kidney cortex slices was about 0.4 kg/kg tissue, see ref. 9).

The errors due to the above assumptions not being quite correct are relatively small (not exceeding 3% of the values), and do not materially affect the reported findings.

The results are expressed as follows: tissue water, kg/kg tissue dry wt. (DW);

tissue electrolytes, mequiv/kg DW; apparent intracellular ionic concentrations, mM. Values ± S.E. are presented.

Analytical methods. Tissue water was determined gravimetrically from the difference between wet and dried tissue (95° overnight), Na and K by flame photometry (E.E.L. instrument); Cl^- by potentiometric titration[10]; inulin by the method of COLE (see ref. 11); saline osmolarity from the depression of the freezing point (Osmometer of the Advanced Instruments Comp., Newton Highlands, Mass., U.S.A.).

In some experiments, the incubation salines were labelled with ^{36}Cl, and the apparent Donnan ratios $[^{36}Cl^-]_o/[^{36}Cl^-]_i$ were determined. ^{36}Cl in the tissue extract and in the salines was measured using a Tracerlab scintillation liquid counter.

Measurement of the membrane potential of kidney cortex cells. The technique described by WHITTEMBURY[12] was employed. Selected microelectrodes filled with 3 M KCl were used (tip diameter less than 1 μ, resistance about 10 MΩ, tip potential less than 6 mV); checks were performed to ensure that the tip potential was not affected by changes in saline osmolarity within 220–420 mosM. Slices were placed between nylon netting in a plexiglass chamber filled with the appropriate saline, and the tissue was brought to an aerobic steady state as to its mineral constituents by aeration and gentle flow of the saline at 25° through the chamber. After about 30 min the cells were impaled, and the potential was read on a Radiometer (Copenhagen, Denmark) S.E. 26 instrument. A calomel electrode immersed in the saline in the lower part of the chamber served as reference. Up to 5 successful impalements could be achieved with each slice. In this way, results for 3 different saline osmolarities were obtained, values ± S.E. being given.

The effect of saline osmolarity on the membrane potential, E, was alsoistudied as follows. Slices were first aerobically incubated at 25° for 45 min in isoton c saline and then transferred to the chamber; a steady-state ionic distribution was assured by maintaining the slices in the chamber for a further 20 min. Then a cell was impaled, and the potential was found to be steady for more than 30 min. With the microelectrode inserted in the cell the saline was exchanged at a given time interval, a medium of different osmolarity being permitted flow through the chamber, and the time course of E was followed until a new steady state was established. As a control, the original saline was reintroduced into the chamber, and the potential changes were again recorded. It will be seen from values given in RESULTS that the changes in E produced by variations in saline osmolarity were reversible. Results are expressed in mV ± S.E.

Measurement of efflux kinetics of propanediol. The technique described earlier[13] was used. Tissue was first loaded with [1-^{14}C]propanediol-1,3 by aerobic incubation for 60 min at 25° in salines of different osmolarities also containing 3 mM of the label (0.5 μC/ml). Subsequently, the washing-out of the label into non-labelled media was followed.

Materials. [1-^{14}C]propanediol-1,3 was prepared by Mr. I. BENEŠ. Commercial mannitol was purified by repeated recrystallization. All other reagents were commercial preparations.

RESULTS

Effect of varying external osmolarity on the steady-state distribution of water and electrolytes at 0°

An increase in saline osmolarity from 220 to 420 mosM with mannitol or sucrose, the ionic concentrations being constant, produced a decrease in tissue water while the ion content in the tissue (mequiv/kg DW) remained unchanged. The result of such an experiment is shown in Fig. 2. Here the tissue was first allowed to reach a balanced state of cell constituents by soaking for 2.5 h in an isotonic saline at 0°, the osmolarity of the medium being adjusted to 310 mosM by sucrose; under these conditions a swelling of the cells accompanied by an entry of Na^+ and Cl^- and a loss of tissue K^+ takes place. Subsequently, the tissue was aerobically incubated for 60 min at 25° in salines of the appropriate osmolarity, thus assuring a steady-state distribution of cell components, the tissue extruding water, Na^+ and Cl^- and reaccumulating K^+ (see refs. 14 and 15). In order to obtain a linear plot for the Van 't Hoff relationship between external osmolarity, π_{exp}, and the equilibrium volume of space surrounded by a semipermeable membrane, the reciprocal of the relative saline osmolarity (π_{iso}/π_{exp}) was plotted on the abscissa; the subscripts *iso* and *exp* denote isotonic and experimental conditions.

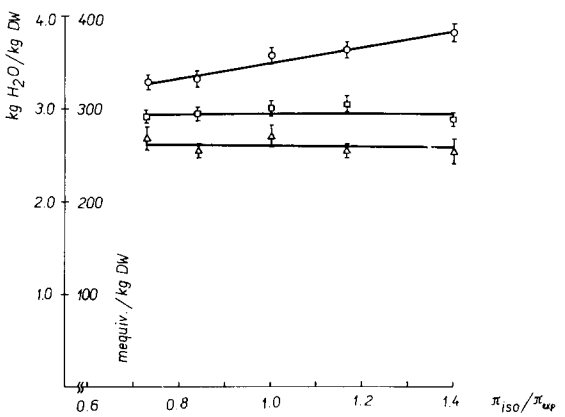

Fig. 2. Effect of saline osmolarity on the steady-state levels of tissue water and cations in kidney cortex slices at 25°. For conditions of the experiment see text. ○, kg water/kg DW; □, mequiv K^+/kg DW; △, mequiv Na^+/kg DW. Values ± S.E., $n = 6$. Abscissa, reciprocal of the relative osmolarity, π, the subscripts *iso* and *exp* denoting isotonic and experimental conditions, respectively.

Evidence that the above result corresponded to a steady-state distribution of tissue water and electrolytes was obtained by two further procedures: (a) the slices were directly aerobically incubated for 60 min at 25° in salines of different osmolarities; (b) the tissue was first aerobically incubated at 25° for 60 min in isotonic saline and subsequently transferred for 30 min into salines of appropriate osmolarity. In both experiments mannitol was used to vary the tonicity of the medium. The values for tissue water and electrolytes were identical with those found under conditions described for Fig. 2.

It will be noted that on the plot a linear relationship between tissue water and the reciprocal of saline osmolarity was obtained. However, the cells obviously behaved as rather imperfect osmometers, the tissue water decreasing only 15% when the osmolarity doubled. To some extent, such a result might be due to the fact that the cells are not impermeable to not-actively transported sugars and polyols (see refs. 8 and 9). However, this could only be a partial explanation since under steady-state conditions the space for sucrose or mannitol is considerably less than 100% in various tissues[8,9] and this was also confirmed in our laboratory by using [^{14}C]sucrose.

The second interesting aspect of the results shown in Fig. 2 concerns the practically constant values of tissue cations at varying saline osmolarities. In separate experiments it was found that the level of tissue Cl^- (mequiv/kg DW) did not change either. The cell volume decreasing with increasing π_{exp}, the apparent intracellular ionic concentrations $[Na^+]_i$, $[K^+]_i$ and $[Cl^-]_i$ of necessity increased, thus producing considerable changes in the concentration gradients of these ions.

Effect of saline osmolarity on the distribution of water and electrolytes at 0°

In view of the above results it was of interest to examine the extent to which metabolic activity of the cells contributed to the relevant features. Therefore, the effect of external osmolarity on the distribution of tissue water and electrolytes after prolonged soaking at 0° was studied. It has been shown by BURG AND ORLOFF[16] and ROBINSON[17] that, contrary to the view held in our laboratory (see, *e.g.*, ref. 18), some active ion transport takes place even at 0°. However, this represents only a minor fraction of that occurring at 25°: according to ROBINSON[17] the respiration of kidney cortex slices at 0° is about 7% of that at 25°. The results obtained at 0° by the present procedure may thus approach values corresponding to a virtual exclusion of active transport.

The saline osmolarity, when varied with mannitol, produced changes in the distribution of tissue water and electrolytes as shown in Fig. 3. When the values

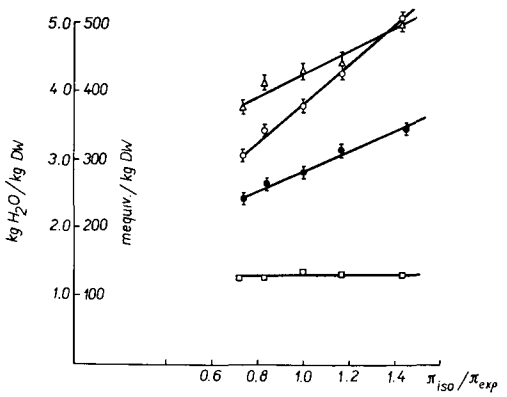

Fig. 3. Effect of saline osmolarity on the levels of tissue water and electrolytes in kidney cortex slices at 0°. Slices exhaustively washed in salines of various osmolarities for 2.5 h at 0°; the Ca^+-free salines were of the Krebs–Ringer phosphate type, $[Na^+]_0$ = 106 mM, mannitol being used to vary osmolarity. ○ tissue water kg/kg DW; △, Na^+; □, K^+; ●, Cl^-; all electrolytes in mequiv/kg DW. Values ± S.E., n = 9.

were plotted against the reciprocal of the relative saline osmolarity, linear relationships were obtained for all components studied. However, while tissue water, Na^+ and Cl^- decreased with increasing π_{exp}, tissue K^+ remained constant.

A comparison of the results given in Figs. 2 and 3 makes several aspects noteworthy. The cells behaved at 0° as much better, though still imperfect, osmometers than under conditions in which cell metabolism was fully operating at 25°. Furthermore, the decrease in cell water due to external osmotic pressure at 0° accompanied was by a loss of Na^+ and Cl^-. The loss of a NaCl solution from the cells, brought about by external osmotic pressure due to a non-permeable substance, can be readily understood for a system in which both these ions can move freely through leaks in the membrane.

In contrast to the behaviour of Na^+ and Cl^-, it will be seen that no net changes in tissue K^+ occurred at a wide range of external osmolarities. Such result provides evidence for the view that most if not all cell K^+ at 0° does not participate in osmotic phenomena of kidney cortex cells. Such a conclusion is consonant with the observation that a considerable portion of tissue K^+ remains in the cells after prolonged washing with isotonic K^+-free salines at 0°, and this potassium is not exchangeable with $^{42}K^+$ (*cf.* refs. 19–21). Admittedly, some residual active accumulation of K^+ may take place at 0° (see refs. 16 and 17); however, even in the presence of metabolic inhibitors the amounts of tissue K^+ could never be reduced below some 60 mequiv/kg DW (see WIGGINS[22]). Thus, the conclusion reached here appears to be in satisfactory agreement with data on the behaviour of tissue K^+ at 0° obtained by a variety of experimental approaches.

The cellular water being a function not only of external osmolarity but also of the intracellular ionic concentrations, the effect of varying external osmolarities on tissue water at different $[Na^+]_o$ was studied while $[K^+]_o$ remained constant. The results are shown in Fig. 4.

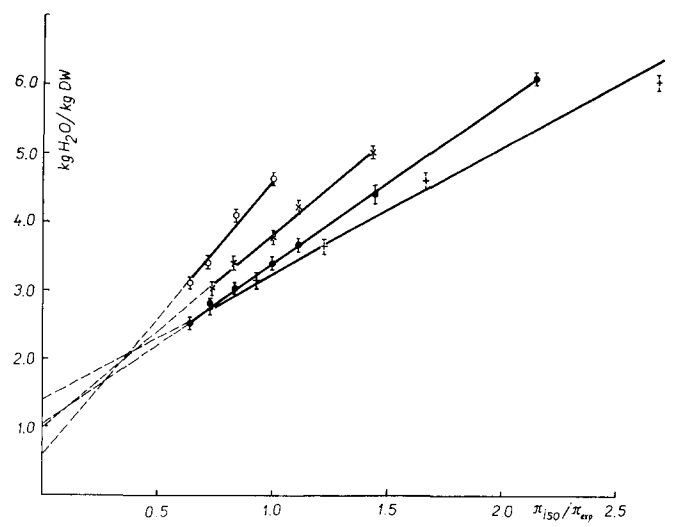

Fig. 4. Effect of saline osmolarity on tissue water in kidney cortex slices at 0°. The experimental procedure was as described in the legend to Fig. 3. Salines: Ca^+-free media, $[Na^+]_o$ also being varied; mannitol was used to adjust osmolarity. $[Na^+]_o$, mM: ○, 150; ×, 106; ●, 57.6; +, 28.5. Values ± S.E., $n = 6$–8.

It will be seen that, with increasing $[Na^+]_o$, tissue water increased at all osmolarities used. The intersect of the linear plots with the ordinate indicates an apparent non-compressible space of kidney cortex cells of the order of 1 kg tissue water/kg DW. A non-compressible space of tissues may be due to structural components of cells.

In these experiments tissue electrolytes were affected by external osmolarities in the same manner as shown for one particular $[Na^+]_o$ on Fig. 3. Details are therefore not given. The concentration of NaCl in the aqueous solution lost from the cells when the external osmolarity was increased was somewhat lower than that in the outer medium.

The effect of saline osmolarity on the membrane potential of kidney cortex cells

The steady-state membrane potential of renal cells bathed at 25° in isotonic saline (including 90 mM mannitol) was 39.6 ± 1.3 mV (range 14–77 mV, 94 impalements). Fig. 5 shows that a change in π_{exp} markedly affected the membrane potential E: on transition from isotonic to hypotonic saline, the membrane potential systematically increased by an average of 11.5 ± 2.9 mV ($n = 8$), this difference being highly significant ($P < 0.01$); hypertonicity consistently brought about a drop in E of 12.3 ± 2.6 mV ($n = 6$, $P < 0.01$).

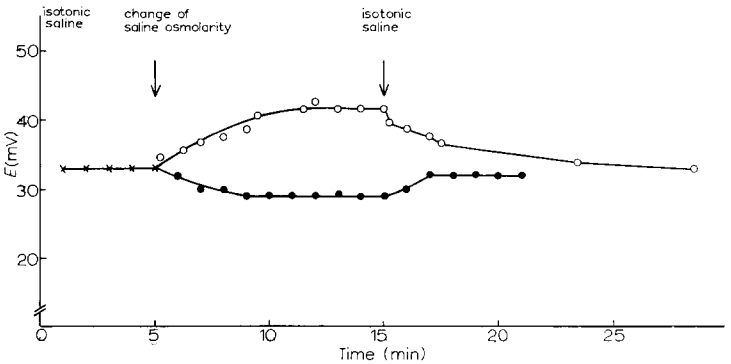

Fig. 5. Effect of saline osmolarity on the membrane potential of kidney cortex cells. The slices were first brought to a steady state of tissue components by aerobic incubation at 25° in isotonic saline, then transferred to a plexiglass chamber, and a cell was impaled. Arrows indicate the time when the saline tonicity was changed. The results of two such experiments are given, the potentials in isotonic saline coinciding. ×, isotonic saline (308 mosM); ○, hypotonic saline (208 mosM); ●, hypertonic medium (420 mosM).

The changes in membrane potential produced by varying π with mannitol at constant $[Na^+]_o$ and $[K^+]_o$ were completely reversible, as shown by the time-course of the experiments. The results obtained from this type of experiment thus provided evidence that the membrane potential decreased with increasing saline osmolarity.

Comparison of the effect of saline osmolarity on the membrane potential and the Nernst diffusion potentials of electrolytes

It is now universally accepted that the membrane potential is produced by an uneven distribution of ions between the cells and their external environment, due essentially to active transport (*cf.* ref. 23). A comparison of the observed membrane

potential E with the Nernst diffusion potentials computed from the apparent ionic distribution was therefore of interest. In Fig. 6, the observed values of E and the calculated Nernst potentials are plotted against the reciprocal of the relative external osmolarity. The scales on the ordinate were chosen to show that a Donnan ratio of 10 corresponds to a Nernst diffusion potential of 58 mV. The Nernst potentials are denoted by E with a subscript indicating the ion concerned. From the difference between the membrane potential and the Nernst diffusion potential the electrochemical gradient of each ion can be evaluated.

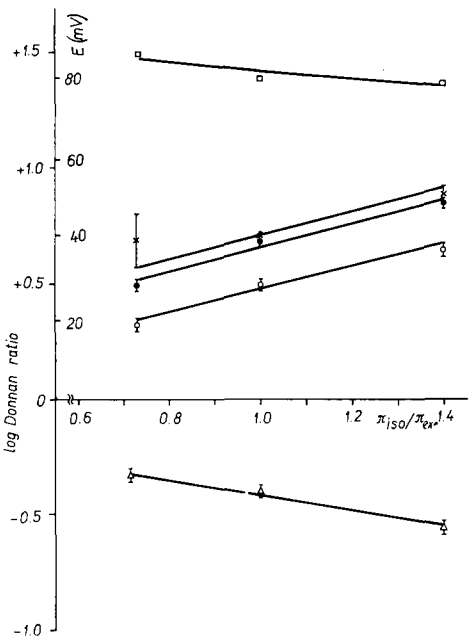

Fig. 6. Effect of saline osmolarity on the steady-state membrane potential and Nernst diffusion potentials of electrolytes in kidney cortex cells. Slices were incubated aerobically for 60 min at 25° in salines of varying osmolarity. Symbols: ×, membrane potential E; ○, E_{Cl}; ●, $E_{^{36}Cl}$; □, E_K; △, E_{Na}. Mean values ± S.E., n not less than 6.

The following observations arise from the results represented in Fig. 6.

(a) The membrane potential decreased with increasing π_{exp}. In view of the results reported above (Fig. 5) it appeared reasonable to consider that the relationship between E and the external osmolarity approached linearity although the mean value of E in hypertonic saline differed only little from that at π_{iso}: this is undoubtedly due to the fact that while the greater number of impalements was carried out with cells at a steady state in the isotonic saline and a wide range of E was observed, only 8 such measurements were carried out under hypertonic conditions.

(b) The logarithm of the apparent Donnan ratios of Na^+, K^+ and Cl^- showed linear dependence on π_{exp}. At all osmolarities tested E_{Cl} ran parallel to E whereas $E_{^{36}Cl}$ practically coincided with E. It has been shown elsewhere that about $^1/_3$ of the intracellular Cl^- is very slowly if at all exchangeable with $^{36}Cl^-$, and this fraction of

cell chloride can hardly participate in the osmotic and electrochemical phenomena; consequently, the Donnan ratio $[^{36}Cl^-]_o/[^{36}Cl^-]_i$ would be markedly higher than that for the chemically determined chloride. The fact that the electrochemical gradient of $^{36}Cl^-$ appears to be zero provides evidence in favour of a passive distribution of chloride between cells and saline at all osmolarities studied.

E_{Na} proved to be a mirror image of E. This is fully consonant with the operation of a Na^+ pump. However, with increasing π_{exp} the difference between E and E_{Na} decreased, suggesting that the driving mechanism for Na^+ extrusion decreased with increasing osmolarity.

The dependence of E_K on saline osmolarity was unexpected. The positive difference between E and E_K might possibly be due to a portion of cellular K^+ not participating in osmotic and electrochemical phenomena of renal cells; such a view cannot readily be excluded since a considerable fraction of cellular K^+ is only slowly exchangeable with external label at 25° (*cf.* ref. 24). If this were the only explanation of the results shown in Fig. 6, the true Donnan ratio of K^+ would be lower than that computed on the basis of chemical determinations, and E_K should change in the same direction as E for a system behaving as a potassium electrode; since in the present experiments the total cellular K^+ per unit DW remained constant during changes in external osmolarity, E_K should run parallel to E. Fig. 6 shows, however, that the positive difference between E_K and E actually increased with increasing osmolarity. This result cannot be readily reconciled with a purely passive distribution of cellular K^+. Thus, the possibility of an active transport of K^+ independent of the Na^+ pump should be considered (note the opposite changes in the respective electrochemical gradients with π_{exp}); the activity of such a K^+ pump would then be enhanced by increasing saline osmolarity.

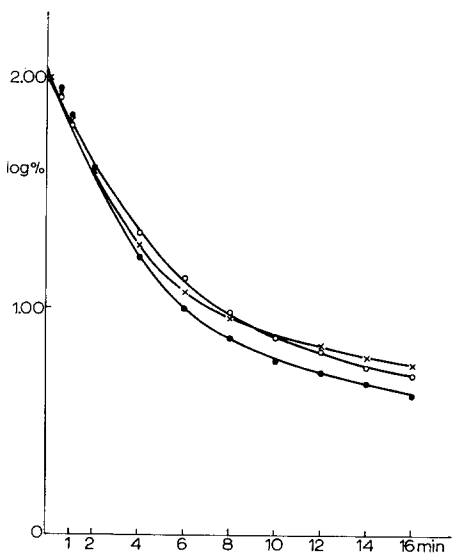

Fig. 7. Effect of saline osmolarity on the kinetics of propanediol efflux. Slices were first loaded with 3 mM [1,3-^{14}C]propanediol by aerobic preincubation for 60 min at 25° in salines of different osmolarities; then the washing-out of the label into non-labelled media was followed at 25°. Salines: ○, 220 mosM; ×, 310 mosM; ●, 440 mosM.

Effect of saline osmolarity on the efflux of propanediol

It has been suggested[25] that external osmotic pressure affects the membrane, altering its permeability to water. Such an effect might be demonstrable by following the efflux from the cells of a water-soluble, non-metabolizable substance which moves through the membrane in a purely passive manner. Propanediol satisfies this requirement, its steady-state distribution in tissue water being 81.5 ± 5.0% (see ref. 6). It will be seen from Fig. 7 that the efflux kinetics did not markedly differ when the saline osmolarity during the loading and washing-out procedure varied from 220 to 420 mosM.

To interpret this result correctly, it must be remembered that the rate constant is not directly related to the permeability constant unless the volume of the compartment from which the efflux takes place is constant too. This follows from the well-known equation for a unidirectional flux

$$\frac{dc_i}{dt} = -kc_i = -M_{out}\frac{A}{V}c_i$$

where c_i denotes the intracellular concentration of the solute studied, k the rate constant, M_{out} the flux, A the surface of the cell and V its volume. Of these values, the cell volume V certainly varies when the saline osmolarity alters (see above). Thus, a correction of the rate constants derived from efflux kinetics has to be made for the volume changes if results are to be compared for varying osmolarities. However, even then no systematic effect of saline osmolarity on the efflux of propanediol could be found. Thus the above experiments do not provide evidence in favour of a change in the passive permeability of the renal cell membrane for propanediol.

DISCUSSION

The results reported here clearly demonstrate that changes in external osmolarity brought about by mannitol or sucrose (*i.e.* substances penetrating through the cell membrane only to a limited extent) produce marked effects on cell water and apparent intracellular ionic concentrations. The osmotic effects of mannitol or sucrose differ in essential details from those brought about by readily permeable substances, *i.e.* LiCl, Tris–chloride, choline–chloride or urea (*cf.* ref. 1 for a preliminary statement).

In this report an effect of external osmotic pressure on the extracellular space has been described, the inulin space decreasing with increasing cellular swelling. This observation is in agreement with some data reported earlier[26] and also with the qualitatively similar findings on the frog ventricle (Dr. W. McD. ARMSTRONG, personal communication), but contrasts with the data of ROBINSON[7] for kidney cortex slices. A decrease in the extracellular space with increased swelling of the cells appears to be reasonable: such swelling would be expected to occur in all three dimensions.

The effect of external osmotic pressure on the steady-state volume of renal cells and tissue electrolytes at 25°, shown in Fig. 2, has to be analyzed in the light of results obtained from the study of electrochemical ionic gradients.

From the results presented in Fig. 6 it appears that the operation of the Na^+ pump is reduced with increasing external osmotic pressure. Such result is fully

consonant with the conclusion of USSING[2], based on the effect of external osmolarity produced by urea on the short-circuit Na^+ current in frog skin. It has also been shown[27] that during mannitol diuresis the net Na^+ reabsorption in dog kidney tubules is significantly reduced. An analysis of the apparent intracellular concentration and electrochemical gradient of Na^+ thus fully bears out the conclusions obtained from a study of the net Na^+ movement across the epithelial cell membrane.

The observation of an enhancing effect of external osmolarity on the electrochemical gradient of K^+ was rather surprising. For the frog muscle ADRIAN[28] showed that at constant $[K^+]_o$ the resting membrane potential actually increased when the saline osmolarity was raised with sucrose, and this increase in E corresponded to a rise of $[K^+]_i$. Thus, the muscle obeys the Nernst equation for a potassium electrode. The kidney cortex cells were found to behave in a different manner, *i.e.* with rising π_{exp}, $[K^+]_i$ increased whereas E fell. This result can hardly be explained in terms of either a purely electrogenic Na^+ pump (*i.e.* K^+ behaving passively) or a linked Na^+–K^+ exchange pump (*cf.* USSING[23]); for either mechanism one might expect changes in E_K to follow E. The observation shown on Fig. 6 thus suggests that a K^+ pump independent of that for Na^+ operates in kidney cortex cells* and that the activity of this pump is enhanced by increased external osmotic pressure. In this context it ought to be mentioned that some unexpected transport phenomena have recently also been observed in the physiologically related frog skin: in hypertonic solutions an active transport of sucrose was found although under isotonic conditions not sugar was transported[30,31].

The behaviour of renal cells described does not appear to be specific for this tissue. Preliminary experiments carried out by Dr. K. SIGLER in this laboratory showed that within a limited range of saline osmolarities at constant ionic concentrations, the volume of frog oocytes decreased with increasing π_{exp}; simultaneously, E decreased, the apparent electrochemical potentical of Na^+ fell whereas that of K^+ actually increased.

No suggestion can at present be offered concerning the detailed mechanism by which external osmotic pressure affects the active transport. The experiment represented in Fig. 7 did not indicate any marked effect of saline osmolarity on the leak for propanediol. Further studies will be required to elucidate whether the passive leaks for Na^+ and K^+ of the renal cell membrane might be affected by osmotic pressure.

The described effects of saline osmolarity on the active transport of Na^+ and K^+ suggest an explanation for the observed relationships between cell volume and tissue electrolytes presented in Fig. 2. With increasing external osmolarity, Na^+ would be extruded more slowly from the cells while K^+ would accumulate more rapidly; consequently, because of the increased intracellular concentrations of both these ions, shrinkage of the cells due to external osmotic pressure would be much less under these conditions than one would expect when the function of the ionic pumps is greatly reduced by low temperature (Fig. 3).

At 0° the renal cortex cells did not behave as perfect osmometers. Several factors may contribute to this. Firstly, a residual active transport of ions still appears to operate at 0°. In agreement with the view of BURG AND ORLOFF[16] and of ROBIN-

* Since this paper was sent to press, GIEBISCH, CLOSE AND MALNIC[29] reported evidence for a K^+ pump in renal tubular cells on the basis of experiments *in vivo*.

SON[17], unpublished experiments have shown that the apparent E_{Na} was lower by about 5 mV than the observed E (7.5 ± 0.5 mV, $n = 22$). Secondly, the non-compressible space of the order of 1 kg tissue water/kg DW (see Fig. 4.) may represent another factor. This non-compressible space can hardly be identified with 'bound' water; as mentioned above, the space for the water-soluble, non-metabolizable propanediol in kidney cortex slices at steady-state conditions at 25° was 81.5% of tissue water, *i.e.* about 0.5 kg water/kg DW did not serve as solvent for this substance. It is thus reasonable to assume that the non-compressible space is to a considerable extent due to intracellular structures.

For extreme values of saline osmolarities at 0°, the tissue water differed by a factor of 2–3 (Fig. 4), whereas the amount of tissue K^+ remained constant. Consequently, an increase in π_{exp} at constant $[K^+]_o$ raised several-fold the apparent $[K^+]_i$. This can hardly be due to a residual active transport of K^+ at 0°. Evidence has been summarized above (*cf.* refs. 19–22) that at this temperature most tissue K^+ cannot be washed out by K^+-free salines and is not exchangeable with $^{42}K^+$. The result reported here can thus be taken as evidence that most of the intracellular K^+ at 0° does not participate in the osmotic properties of renal cells.

Finally, the observed relationships between the external osmolarity, cell volume and ionic distribution in kidney cortex cells may serve to support the contention[1] that in addition to the system of pump(s) and leak(s), hydrostatic pressure, intracellular and extracellular, also participates in the maintenance of the cell volume.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Dr. K. JANÁČEK for valuable discussions and to Mr. I. BENEŠ for the preparation of [1,3-^{14}C]propanediol. The courtesy of Dr. G. WHITTEMBURY, I.V.I.C., Caracas, Venezuela, in providing information on the construction of the chamber for potential measurements prior to publication is gratefully acknowledged.

REFERENCES

1 A. KLEINZELLER, *Arch. Biol. Liège*, 76 (1965) 217.
2 H. H. USSING, *Acta Physiol. Scand.*, 63 (1965) 41.
3 H. A. KREBS, *Biochim. Biophys. Acta*, 4 (1950) 249.
4 A. KLEINZELLER AND J. H. CORT, *Physiol. Bohemoslov.*, 9 (1960) 106.
5 A. KLEINZELLER, A. A. LEV, P. G. KOSTYUK AND A. KOTYK, in the press.
6 A. KLEINZELLER AND A. KNOTKOVÁ, *Biochim. Biophys. Acta*, 126 (1966) 604.
7 J. R. ROBINSON, *Proc. Roy. Soc. Ser.B*, 137 (1950) 378.
8 E. BOZLER, *Am. J. Physiol.*, 200 (1961) 651.
9 L. E. ROSENBERG, S. J. DOWNING AND S. SEGAL, *Am. J. Physiol.*, 202 (1962) 800.
10 J. A. RAMSAY, R. H. J. BROWN AND P. C. CROGHAN, *J. Exptl. Biol.*, 32 (1955) 822.
11 J. S. D. BACON AND D. BELL, *Biochem. J.*, 42 (1948) 397.
12 G. WHITTEMBURY, *J. Gen. Physiol.*, 48 (1965) 699.
13 A. KLEINZELLER, K. JANÁČEK AND A. KNOTKOVÁ, *Biochim. Biophys. Acta*, 59 (1962) 239.
14 G. H. MUDGE, *Am. J. Physiol.*, 165 (1951) 113.
15 A. LEAF, *Biochem. J.*, 62 (1956) 241.
16 M. B. BURG AND J. ORLOFF, *Am. J. Physiol.*, 207 (1964) 983.
17 J. R. ROBINSON, *J. Physiol., London*, 177 (1965) 112.
18 A. KLEINZELLER, *Biochim. Biophys. Acta*, 43 (1960) 41.
19 G. H. MUDGE, *Am. J. Physiol.*, 173 (1953) 511.

20 A. KLEINZELLER, *Mosbach Colloquium Ges. physiol. Chem., 1961*, Springer, Heidelberg, 1961, p. 45.
21 E. C. FOULKES, *Am. J. Physiol.*, 203 (1962) 655.
22 P. M. WIGGINS, *Biochim. Biophys. Acta*, 88 (1964) 503.
23 H. H. USSING, in H. H. USSING, P. KRUHØFFER, J. HESS THAYSEN AND N. A. THORN, *The Alkali Metal Ions in Biology*, Springer, Heidelberg, 1960, p. 57.
24 A. KLEINZELLER AND A. KNOTKOVÁ, *Physiol. Bohemoslov.*, in the press.
25 J. M. DIAMOND, *J. Physiol., London.* 183 (1966) 58.
26 A. KLEINZELLER AND J. H. CORT, *Physiol. Bohemoslov.*, 10 (1961) 349.
27 F. G. KNOX, J. S. FLEMING AND D. W. RENNIE, *Am. J. Physiol.*, 210 (1966) 751.
28 R. H. ADRIAN, *J. Physiol., London.* 133 (1956) 631.
29 G. GIEBISCH, R. M. CLOSE AND G. MALNIC, *2nd International Biophysics Congress, Vienna, 1966*, Abstr. 308.
30 T. J. FRANZ AND J. T. VAN BRUGGEN, *Proc. 10th Meeting Biophysical Society, Boston, 1966*, p. 139.
31 H. H. USSING, *Proc. N.Y. Acad. Sci.*, 137 (1966) 513.