FACTORS AFFECTING THE SWELLING OF KIDNEY CORTEX SLICES AT 0°

A. KLEINZELLER

Laboratory for Cellular Metabolism, Biological Institute, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)

(Received January 4th, 1960)

SUMMARY

Factors affecting swelling of slices of rabbit kidney cortex at 0° in isotonic salines containing Na^+ as the main cation and Cl^- as the bulk anion were examined.

1. Tissue hydration and chlorides increase with increasing concentration of K^+ in the leaching medium. A similar swelling effect was shown for Rb^+, but not for Li^+.

2. A linear relationship was shown to exist between the apparent membrane potential, calculated from the Donnan ratio of potassium, and the logarithm of the external potassium concentration.

3. An inverse relationship was demonstrated between tissue water and the apparent Donnan ratios of chloride and potassium: as compared with controls leached at 0° in standard saline $\left(\frac{[Cl^-]_o}{[Cl^-]_i} = 1.495 \pm 0.037; \frac{[K^+]_i}{[K^+]_o} = 8.86 \pm 0.35\right)$ factors increasing tissue swelling, *e.g.*, 0.2–0.6 *mM* $HgCl_2$, 0.7–1.4 *mM* Esidrone, 2–149 mEqu K^+, 67 and 134 mEqu Rb^+, decreased $\frac{[Cl^-]_o}{[Cl^-]_i}$ and $\frac{[K^+]_i}{[K^+]_o}$, whilst 1–6 m*M* Ca^{2+}, Ba^{2+}, Sr^{2+} and Mn^{2+} decreased tissue swelling and also increased these ratios.

4. The relationships found between tissue water and the apparent Donnan ratios of chloride and potassium correspond to a derived relationship of the osmotic gradient and the ratio $\frac{[Cl^-]_o}{[Cl^-]_i}$ for a double Donnan system.

INTRODUCTION

It is now well established that animal tissues swell on immersion at 0° in isotonic sodium chloride or in isotonic salines of the KREBS-RINGER type (*e.g.*, ROBINSON[1], MUDGE[2], AEBI[3], WHITTAM AND DAVIES[4]) and reach within about 2 h an apparent equilibrium between the tissue water and electrolytes and the medium; the term "balanced state" of the tissue has been suggested by CONWAY[5] to denote this apparent equilibrium. The mechanism of the swelling process has been the subject of a number of investigations. Evidence has been presented suggesting that the swelling of *e.g.* kidney cortex slices at 0° may be accounted for by a double DONNAN system (LEAF[6]), although osmotic changes due to the breakdown of intracellular macromolecules into smaller moieties may also contribute to the swelling process (CONWAY, GEOGHEGAN

AND MCCORMACK[7]); in both these investigations it was assumed that the active transport of solutes (and water) at 0° was negligible and that the balanced state of the tissue represented an equilibrium, as opposed to a steady state. However, a basically different view was advanced by MAIZELS AND REMINGTON[8]. These authors pointed out that the water and electrolyte content of the tissue after leaching at 0° could hardly represent an equilibrium state since, due to the presence of an indiffusible anion in a Donnan system, there would be a tendency of the water and electrolytes to continuously enter the cell with the eventual result that the cells would burst. The view was therefore put forward that at 0–2° active transport of sodium (and water) counteracted the influx of sodium and water due to Donnan forces. The apparent equilibrium between the tissue solutes and the medium after leaching at 0° was thus considered to be the result of a new steady state of the cellular components.

It has been shown elsewhere (KLEINZELLER AND CORT[9,10]) that 0.2–1.4 *mM* mercurial preparations, both inorganic and organic, and also 0.6 m*M* Cu^{2+} and 1.2 *mM* Ag^{+}, considerably increase the swelling of kidney cortex slices during leaching for 2.5 h at 0° in isotonic sodium chloride, increase the fluxes of sodium and potassium and of urea at the balanced state of the tissue components, and decrease the apparent Donnan ratios of potassium and chloride ions. On the other hand, 1–6 *mM* Ca^{2+}, and also ions of some other alkaline earths were found to have the opposite effect, *i.e.* decreasing the swelling of the tissue and the fluxes of the cations and of urea at the balanced state, and also increasing the apparent Donnan ratios of potassium and chloride ions (KLEINZELLER AND CORT[11]). A detailed investigation of the relationship between the tissue electrolytes and the water after leaching kidney cortex slices at 0°, and the effect of various agents thereon, appeared therefore desirable, especially since it was known from the now classical work of BOYLE AND CONWAY[12] that potassium greatly affected the swelling of some tissues, *e.g.* muscle.

It will be shown here that potassium and rubidium ions increase the swelling of kidney cortex slices at 0° in a similar way to that in muscle. Furthermore, evidence will be presented for a relationship between the apparent Donnan ratios of potassium and chloride, and the water content of the tissue. The simplest explanation of this observation appears to be a Donnan system with some agents, which affect tissue swelling, directly influencing the charge of the cellular membrane.

A preliminary account of these results has been presented by KLEINZELLER[13].

EXPERIMENTAL

The experimental methods used here have been described in detail elsewhere (KLEINZELLER AND CORT[11]) and are only summarized here. Unless otherwise stated, kidney cortex slices of healthy rabbits were leached 2.5 h in a saline solution of the following composition (in mEqu/l redistilled water): 142.5 Na^{+}; 5.1 K^{+}; 7.6 Li^{+}; 148 Cl^{-}; 7.2 HPO_4^{2-}; pH 7.4, or in salines with various cations replacing Li. The salines were cooled with crushed ice, the cut slices then transfered into these, and the salines with the slices maintained at 0° in an ice-chest, with occasional shaking. The following tissue components were estimated: Tissue water, Na and K (by flame photometry) and Cl^{-} potentiometrically by the method of SANDERSON[14]. Results, representing the mean of at least two analyses, are expressed in ml H_2O and mEqu of ions per

100 g dry tissue solids (DS). For the calculation of the apparent intracellular concentrations of potassium and chloride, the value of the inulin extracellular space of 0.20 ml/g tissue was taken for slices leached in standard saline or in the presence of mercurials, whilst for slices leached in the presence of alkaline earths the slightly higher value of 0.23 ml/g was used (KLEINZELLER AND CORT[10,11]). Molal concentrations (mEqu/kg H_2O) of ions are denoted by square brackets, indices *i* and *o* refering to the intracellular and extracellular spaces, respectively.

As an organic mercurial, Esidrone (*i.e.*, theophyllino–mercuri–monooxypropylamido–sodiumquinolate, Ciba, Basel, Switzerland) was used.

RESULTS

The effect of some metabolic inhibitors on the swelling of kidney cortex slices at 0°

The effect of some metabolic inhibitors on the swelling of kidney cortex slices at 0° was examined in order to test whether any active transport could be demonstrated at this temperature, as suggested by MAIZELS AND REMINGTON[8]; if this were so, substances such as dinitrophenol (DNP) or iodoacetate might be expected to inhibit such a transport process and bring about an increased swelling of the slices.

It may be sufficient to relate that these experiments were clearly negative; DNP at concentrations up to 0.5 *mM* did not affect the swelling; it has been shown elsewhere (KLEINZELLER AND CORT[10]) that 1–2 *mM* iodoacetate and also a number of other agents known to block thiol groups were similarly found to be without effect on the swelling. Unless evidence can be provided that DNP and other inhibitors do not penetrate into the cells at 0°, these negative results are suggestive of the view that no active transport of sodium (and water) takes place at 0° in kidney cortex slices.

The effect of alkaline cations on the swelling of kidney cortex slices at 0°

AEBI[3] has shown that equivalent replacement of sodium by potassium in the leaching saline or incubating medium at 37° produced an increased swelling of kidney cortex slices; no comments were offered as to the mechanism of this swelling effects of potassium.

The findings of AEBI were confirmed and extended also for tissue chloride. The results of a representative experiment, presented in Fig. 1, show that increasing concentrations of K^+ in an isotonic saline bring about an increase of tissue water, potassium and chloride, and a decrease of tissue sodium. These results are in agreement with those obtained by BOYLE AND CONWAY[12] for muscle, KLEINZELLER AND RYBOVÁ[15] for brain cortex slices, and by KLEINZELLER, KOLÍNSKÁ AND FOLBERGROVÁ[16] for pigeon pancreas slices. It thus may be assumed that the swelling effect of potassium in these tissues is brought about by the same mechanism, *i.e.* by a Donnan system as described by BOYLE AND CONWAY.

If from these results the apparent $[K^+]_i$ is calculated and the apparent membrane potential φ $\left(\varphi = 54 \log \frac{[K^+]_i}{[K^+]_o}\right)$ then plotted against log $[K^+]_o$, a straight line is obtained, as shown in Fig. 2. Such a result is in agreement with the view that the cellular membrane of kidney cortex cells is freely permeable to potassium. The apparent Donnan ratio of chloride decreased in this experiment from the value of 1.53 (no potassium in the medium) to 1.19 (149 mEqu K^+/kg medium), corresponding to a change of apparent membrane potential from 10 to 4.17 mV.

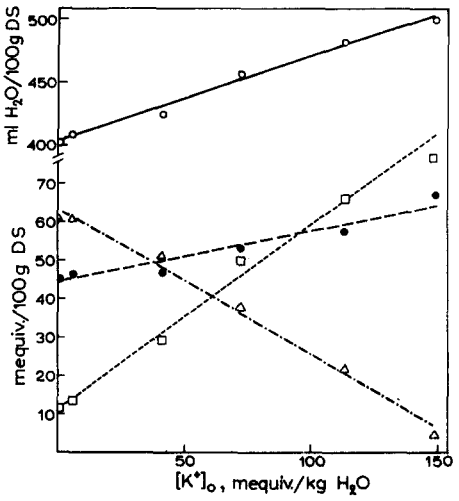

Fig. 1. Effect of varying concentrations of potassium in the leaching medium on tissue water and electrolytes: kidney cortex slices leached 2.5 h at 0° in 0.154 *M* NaCl, buffered with 0.05 vol. 0.1 *M* Na-phosphate buffer, pH 7.4, or in salines with 0.154 *M* KCl equivalently replacing NaCl. ○—○ H_2O (ml/100 g DS); △-·-·-△ Na^+, □----□ K^+, ●—● Cl^-, all in mEqu/100 g DS.

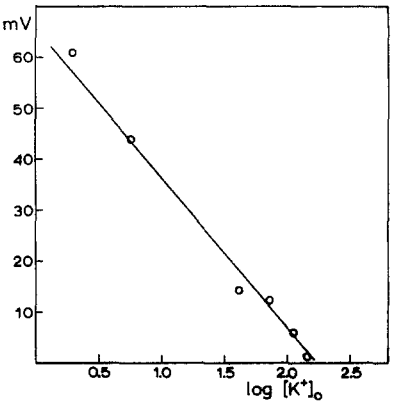

Fig. 2. Relationship of apparent membrane potential as a function of varying concentrations of K^+ in the leaching medium. For conditions of experiment, see Fig. 1. The apparent membrane potential

$$\varphi = 54 \log \frac{[K+]_i}{[K+]_o}, \text{ in mV.}$$

The effect of potassium is not specific for this ion, the same effect being shown by Rb^+, but not by Li^+ (Table I). This result appears to be in agreement with the view of CONWAY[5] that the difference in the behaviour of these two cations is a reflection of the diameter of their hydrated form, hydrated Rb^+ being considerably smaller than Li^+ and therefore freely diffusible through the cellular membrane.

TABLE I

EFFECT OF VARIOUS ALKALINE CATIONS ON TISSUE WATER AND ELECTROLYTE DISTRIBUTION IN KIDNEY CORTEX SLICES LEACHED AT 0°

Slices leached 2.5 h at 0° in standard saline (see Experimental section) or in salines in which NaCl was equivalently replaced by 0.154 *M* solutions of other alkaline chlorides.

Exp. No.	*Cation (mEqu/l)*	*H_2O (ml/100 g DS)*	*Na^+*	*K^+*	*Cl^-*
			(mEqu/100 g DS)		
1	142.5 Na^+	406	53.6	16.2	45.5
	72.4 K^+	484	36.6	48.2	53.2
	144.8 K^+	510	14.0	76.1	54.5
	60.0 Rb^+	437	36.9	—	51.1
	120.0 Rb^+	491	13.9	—	56.6
	78.0 Li^+	426	39.4	18.1	42.1
	145.0 Li^+	433	9.0	12.9	42.8
2	142.5 Na^+	409	61.3	13.4	46.9
	149.0 K^+	499	5.1	76.5	66.0
	149.0 Rb^+	440	5.8	—	54.5
	149.0 Li^+	409	5.1	8.7	46.5

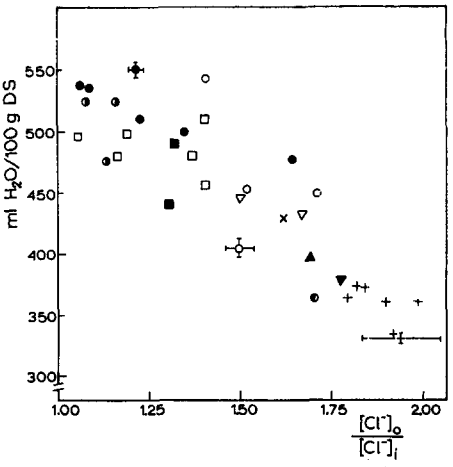

Fig. 3. Relationship of tissue water as a function of apparent Donnan ratio of chloride: tissue leached 2.5 h at 0° in standard saline (see EXPERIMENTAL) and modified salines with following additions: ○, control; ●, 0.2–0.6 *mM* $HgCl_2$; ◑, 0.7–1.4 *mM* Esidrone; □, 6–149 mEqu KCl replacing NaCl in the saline; ■, 67 and 134 mEqu RbCl replacing NaCl in the saline; ▽, 134 mEqu LiCl replacing NaCl; +, 1–6 m*M* $CaCl_2$; ▼, 6 *mM* $SrCl_2$; ▲, 6 *mM* $BaCl_2$; ◐, 6 *mM* $MnCl_2$; ×, 6 *mM* $MgCl_2$. Lines represent S.E. for both tissue water and apparent chloride ratio ($n = 10$) for following conditions: ⊢⊙⊣, standard saline; ⊢●⊣, 0.6 *mM* $HgCl_2$; ⊢⊣, 6 *mM* $CaCl_2$.

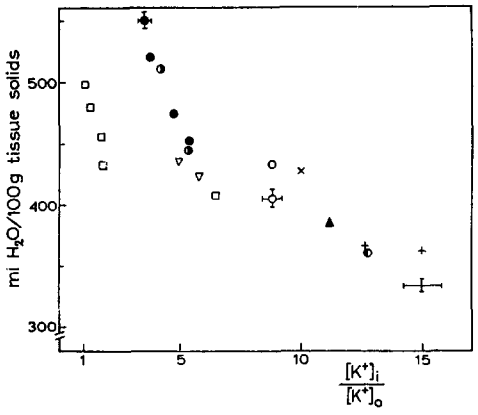

Fig. 4. Relationship of tissue water as a function of apparent Donnan ratio of potassium: for conditions of experiment and symbols see Fig. 3.

The relationship between tissue water and the apparent Donnan ratios of chloride and potassium

Reference has been made to the findings that whilst mercurial preparations increase the swelling of kidney cortex slices at 0° and decrease the apparent Donnan ratios of chloride and potassium, Ca^{2+} and some other alkaline earths have the opposite effect (KLEINZELLER AND CORT[10,11]). It was considered of interest to relate the apparent Donnan ratio of chloride and potassium to tissue water after leaching the slices in the presence of various agents. The results are shown in Figs. 3 and 4. It will be seen that, compared with controls leached in the standard saline, an increased apparent Donnan ratio of chloride, brought about by 1–6 *mM* Ca^{2+} (or other alkaline earths), corresponds to a decreased swelling of tissue; on the other hand, agents producing increased swelling of the slices, *i.e.* varying concentrations of K^+, Rb^+, 0.2–0.8 *mM* $HgCl_2$ and 0.7–1.4 m*M* Esidrone, also showed a decreased apparent Donnan ratio of chloride. A similar relationship holds for the apparent Donnan ratio of potassium and tissue water.

Since it is known that the Donnan ratio of freely diffusible ions is a determinant of the membrane potential, the results presented above suggest a relationship between the membrane potential of kidney cortex cells at 0° and the degree of cellular swelling.

DISCUSSION

Two aspects of the swelling of kidney cortex slices will be considered here, *i.e.* factors determining the apparent equilibrium of tissue components with the surrounding medium at 0° (the balanced state of the tissue) and the physico-chemical aspects

of the relationship found between the Donnan ratios of chloride and potassium (and/or the apparent membrane potential), and the cell water.

It was pointed out above that a negative result with dinitrophenol and thiol-group inhibitors other than mercurials on tissue swelling at 0° suggest the view that no active transport of sodium (and water) takes place at this temperature. The balanced state of the tissue may thus be taken to represent an apparent equilibrium. It should be noted that no positive evidence is available for the opposite view, *i.e.* an active transport of sodium (and water) at 0°, most of the authors in the field agreeing that at this temperature no other transport processes take place but those due to diffusion (*e.g.*, GLYNN,[17], SNELL AND LEEMAN[18], CORT AND KLEINZELLER[19]).

If it is acceptable that the balanced state of the tissue at 0° represents an apparent equilibrium, physical rather than chemical factors counteracting the continuous influx of water and solutes from the leaching medium into the cells, due to Donnan forces, have to be considered. It is suggested, in accord with LEAF[6] that the distensibility (or elasticity) of the cellular membrane and intracellular structures (or possibly connective tissue surrounding the cell) might represent such a factor. As far as the author is aware there now exists some direct experimental evidence in favour of such a view: thus ERNST AND TIGYI[20] found that swelling of muscle in isotonic NaCl at 0° is significantly lowered by slight stretching, this of necessity changing the mechanical properties of the cellular membrane and intra-cellular structures (or connective tissue surrounding the muscle fibres).

There is now considerable evidence available that the cellular membrane of kidney cortex cells is freely permeable to K^+ (CORT AND KLEINZELLER[21]) and results presented above), Cl^- (WHITTAM[22], KLEINZELLER AND CORT[9]) and at 0° also to Na^+ (CORT AND KLEINZELLER[19]). Since these ions represent the bulk of those present in the leaching medium used in the experiments described above, and also the bulk of intracellular freely diffusible ions, it may be feasible to consider only their distribution. The following treatment, although necessarily a simplification, follows and extends arguments of LEAF[6] and BOYLE AND CONWAY[12].

A two-compartment system, separated by a semipermeable membrane, and at equilibrium, is assumed. Furthermore, the following simplifying assumptions are made: (a) none of the freely permeable ions are bound by components of the inner compartment (this assumption may not be fully valid for the cell); (b) activity coefficients equal 1.0; (c) ions in the inner compartment are evenly distributed (this assumption also may not be fully valid for the cell).

Let $[Na^+]_o$, $[K^+]_o$, $[Cl^-]_o$ denote molal concentrations of these ions in the outer compartment. Then

$$[Na^+]_o + [K^+]_o = [Cl^-]_o \tag{1}$$

For the inner compartment, sodium and potassium are the main cations, whilst Cl^- and non-diffusible anion A^{n-} represent the bulk of anions. Thus

$$[Na^+]_i + [K^+]_i = [Cl^-]_i + n[A^{n-}]_i \tag{2}$$

For a Donnan distribution the following relationship will hold:

$$\frac{[Na^+]_i}{[Na^+]_o} = \frac{[K^+]_i}{[K^+]_o} = \frac{[Cl^-]_o}{[Cl^-]_i} \tag{3}$$

and the membrane potential p will be:

$$\varphi = -\frac{RT}{F} \ln \frac{[Na^+]_i}{[Na^+]_o} = -\frac{RT}{F} \ln \frac{[K^+]_i}{[K^+]_o} = -\frac{RT}{F} \ln \frac{[Cl^-]_o}{[Cl^-]_i} \quad (4)$$

When applying these terms to cells, it ought to be noted that for reasons stated above the values of intracellular ion concentrations, Donnan ratios and membrane potentials, calculated from analyses of tissue water and electrolytes, may only approximate actual values; they are therefore designated as apparent values.

Denoting the osmotic pressure in the outer compartment π_o, that of the inner compartment π_i, it is known (see LEAF[6]) that the difference between the osmotic pressures of both compartments, $\Delta\pi = \pi_i - \pi_o$, can be expressed as follows:

$$\Delta\pi = RT\{([Na^+]_i + [K^+]_i + [A^{n-}]_i + [Cl^-]_i) - ([Na^+]_o + [K^+]_o + [Cl^-]_o)\} \quad (5)$$

We are now concerned with the question of the relationship of the Donnan ratio of chloride to $\Delta\pi$. By replacing in equation (5) the value of $([Na^+]_o + [K^+]_o)$ according to eq. (1), and the value of $([Na^+]_i + [K^+]_i)$ from eq. (2), and re-arranging, it will be seen that

$$\frac{\Delta\pi}{RT} = 2\,[Cl^-]_i + (n+1)\,[A^{n-}]_i - 2\,[Cl^-]_o \quad (6)$$

It thus follows that by maintaining experimental conditions, and consequently also $[Cl^-]_o$ constant, the osmotic gradient between the inner and outer compartments should linearly increase with increasing $[Cl^-]_i$. Since $\Delta\pi$ determines the influx of water into the inner compartment, an increased swelling of the inner compartment with increased $[Cl^-_i]$ would follow, as demonstrated experimentally in Fig. 1 above, and by BOYLE AND CONWAY[12] for muscle.

In order to relate the ratio $\frac{[Cl^-]_o}{[Cl^-]_i}$ to $\Delta\pi$, a knowledge of the approximate value of $(n+1)[A^{n-}]_i$ in eq. (6) is desirable. Assuming now that the number of charges per molecule of the non-diffusible anion is considerably greater than 1, we may write, using equation (2):

$$(n+1)\,[A^{n-}]_i \doteq n[A^{n-}]_i = [Na^+]_i + [K^+]_i - [Cl^-]_i \quad (7)$$

Thus by estimating the apparent values of $[Na^+]_i$, $[K^+]_i$ and $[Cl^-]_i$, the approximate value of $(n+1)[A^{n-}]_i$ can be obtained. In Table II the appropriate values are compiled as obtained by the author for rabbit kidney cortex, leached 2.5 h at 0° in the standard saline described above (KLEINZELLER AND CORT[11]), and as calculated from the data of WHITTAM (*cf.* LEAF[6]) for guinea-pig kidney cortex, leached for 2 h at 0° in a saline of similar composition. For the calculation of the data for rabbit kidney cortex the value of the inulin space was 0.20 ml per g tissue. The data of WHITTAM were calculated for an inulin space of 0.25 ml/g tissue (WHITTAM[22]); if the somewhat lower value of 0.20 ml/g, as found by CONWAY AND GEOGHEGAN[23] is taken, the agreement between both sets of values will be still closer.

It follows from Table II that there is satisfactory agreement of the apparent intracellular concentrations of the various components and also of the apparent Donnan ratios of chloride and potassium for both species. The relevant apparent value of $(n+1)[A^{n-}]_i$ may be taken to approximate 90 mEqu/kg intracellular water.

TABLE II

APPARENT INTRACELLULAR ION CONCENTRATIONS IN KIDNEY CORTEX OF RABBIT AND GUINEA-PIG AFTER LEACHING AT 0°

The data for guinea-pig kidney cortex calculated from values of tissue water and electrolytes, found by WHITTAM, as quoted by LEAF[6]. Values for rabbit ± S.E. (n = 10).

Apparent ion conc. (mEqu/kg intracellular water)	*Kidney coertx*	
	Rabbit	*Guinea-pig*
$[Na^+]_i$	136.6 ± 2.2	132
$[K^+]_i$	45.2 ± 1.8	50.5
$[Cl^-]_i$	95.4 ± 2.4	82
$n[A^{n-}]_i = [Na^+]_i + [K^+]_i + [Cl^-]_i$	87.0 ± 3.4	100.5
$\frac{[K^+]_i}{[K^+]_o}$	8.86 ± 0.35	8.7
$\frac{[Cl^-]_o}{[Cl^-]_i}$	1.495 ± 0.037	1.64

On plotting the ratio $\frac{[Cl^-]_o}{[Cl^-]_i}$ against $\frac{\Delta\pi}{RT}$ for the above set of conditions, the hyperbolic curve shown in Fig. 5 is obtained. It will be seen that this curve corresponds to the trend of the relationship of tissue water as a function of the apparent $\frac{[Cl^-]_o}{[Cl^-]_i}$, as shown in Fig. 3.

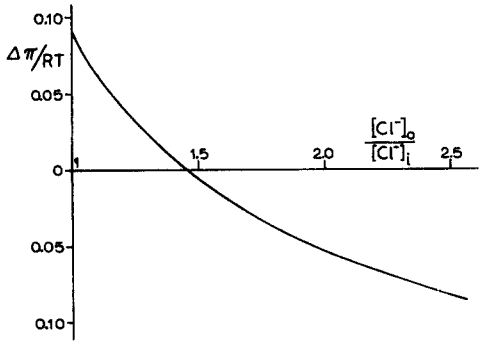

Fig. 5. Relationship of osmotic gradient as a function of the Donnan ratio of chloride: curve computed according to equation (6), for $(n + 1)[A^{n-}]_i$ = 90 mEqu/kg intracellular water. For details see Text.

A similar relationship of $\Delta\pi$ as a function of $\frac{[K^+]_i}{[K^+]_o}$ might be derived, taking into account the Donnan distribution of freely diffusible ions (eq. 3). However, this relationship could not be verified experimentally for kidney cortex slices leached at 0°, the average apparent value of $\frac{[K^+]_i}{[K^+]_o}$ being 8.86 ± 0.35, that of $\frac{[Cl^-]_o}{[Cl^-]_i}$ 1.495 ± 0.037 and that of $\frac{[Na^+]_i}{[Na^+]_o}$ 1.1. It should be mentioned that it has been found in a number of tissues that apparent $\frac{[K^+]_i}{[K^+]_o} > \frac{[Cl^-]_o}{[Cl^-]_i}$, the directly measured membrane potentials corresponding to intermediate values (SHANES[24]). It may be assumed that the activity coefficient of intracellular K^+, as opposed to Cl^-, is lower than 1.0,

possibly due to partial binding of potassium by intracellular components; such a view would explain the observation that K^+ cannot be completely removed from kidney cortex cells even by prolonged leaching in isotonic sodium chloride, while all tissue chloride can be readily removed by leaching the slices *e.g.* in isotonic sulphate (KLEINZELLER AND CORT[9]) or nitrate (unpublished results).

A simple Donnan system could not, however, explain the opposite effects of mercurials and alkaline earths. The increased swelling of kidney cortex slices in the presence of mercurial preparations cannot be explained by a Donnan distribution, taking into account their low effective concentrations (0.2–1.4 *mM*) and also their firm binding by tissue components. A Donnan distribution of Ca^{2+} would be expected to lead to an increased swelling of the tissue, since the relationship should hold:

$$\frac{[Cl^-]_o}{[Cl^-]_i} = \frac{\sqrt{[Ca^{2+}]_i}}{\sqrt{[Ca^{2+}]_o}}$$

However, the finding that both these groups of compounds have the opposite effect on the apparent Donnan ratios of chloride and potassium and on the membrane properties of kidney cortex cells, may point to the following tentative interpretation: if it is assumed that these compounds alter directly the ionic charge of the membrane by reacting with its components, then an inspection of eq. (4) will show that a corresponding redistribution of freely permeable ions would have to take place, with consequent changes of cellular hydration. Thus, *e.g.*, a direct increase of the membrane potential by Ca^{2+} would secondarily lead to an increase of the Donnan ratios of freely diffusible ions. Evidence for the localization of calcium at the cellular membrane of various cells has been summarized elsewhere (KLEINZELLER AND CORT[11], SHANES[24]) as well as evidence for calcium affecting the membrane potential of muscle and nerve tissue.

Finally, mention should be made of findings that also in some other cells opposite effects of mercurial preparations and calcium on cellular hydration were observed. Thus in brain cortex slices the tissue swelling in isotonic NaCl at o° is increased by mercurial preparations (KLEINZELLER AND CORT[9]) and by potassium (KLEINZELLER AND RYBOVA[15]), while Ca^{2+} decreases swelling (RYBOVÁ[25]); also in yeast cells $HgCl_2$ in milimolar concentrations increases the cell volume at o°, while Ca^{2+} decreases the volume (Dr A. KOTYK, personal communication). It would appear, therefore, that a common mechanism is involved in these changes.

ACKNOWLEDGEMENT

The author is greatly indebted to Mrs. J. FRIEDMANNOVÁ for invaluable technical assistance.

REFERENCES

1 J. R. ROBINSON, *Proc. Royal Soc., Ser. B*, 137 (1950) 378.
2 G. H. MUDGE, *Am. J. Physiol.*, 165 (1951) 113.
3 H. AEBI, *Helv. Physiol. et Pharmacol. Acta*, 11 (1953) 96.
4 R. WHITTAM AND R. E. DAVIES, *Biochem. J.*, 55 (1953) 880.
5 E. J. CONWAY, *Physiol. Revs.*, 37 (1957) 84.
6 A. LEAF, *Biochem. J.*, 62 (1956) 241.
7 E. J. CONWAY, H. GEOGHEGAN AND J. I. MCCORMACK, *J. Physiol.* (London), 130 (1955) 427.

[8] M. MAIZELS AND M. REMINGTON, *J. Physiol.* (London), 143 (1958) 283.
[9] A. KLEINZELLER AND J. H. CORT, *Biochem. J.*, 67 (1957) 15.
[10] A. KLEINZELLER AND J. H. CORT, *Abstr. Commns. IVth Intern. Congr. Biochemistry*, Vienna, 1958, p. 78.
[11] A. KLEINZELLER AND J. H. CORT, *Physiol. Bohemoslov.*, 9 (1960) 106.
[12] P. J. BOYLE AND E. J. CONWAY, *J. Physiol.* (London), 100 (1941) 1.
[13] A. KLEINZELLER, *Čs. Physiologie*, 9 (1960) 21.
[14] P. H. SANDERSON, *Biochem. J.*, 52 (1952) 502.
[15] A. KLEINZELLER AND R. RYBOVÁ, *J. Neurochem.*, 2 (1957) 45.
[16] A. KLEINZELLER, J. KOLÍNSKÁ AND J. FOLBERGROVÁ, *Biokhimiya*, 24 (1959) 1401.
[17] I. M. GLYNN, *Progr. Biophys.*, 8 (1957) 241.
[18] F. M. SNELL AND C. P. LEEMAN, *Biochim. Biophys. Acta*, 25 (1957) 311.
[19] J. H. CORT AND A. KLEINZELLER, *J. Physiol.* (London), 142 (1958) 208.
[20] E. ERNST, J. TIGYI AND J. NAGY, *Acta Physiol. Acad. Sci. Hung.*, 6 (1954) 135.
[21] J. H. CORT AND A. KLEINZELLER, *Biochim. Biophys. Acta*, 23 (1957) 321.
[22] R. WHITTAM, *J. Physiol.* (London), 131 (1956) 542.
[23] E. J. CONWAY AND H. GEOGHEGAN, *J. Physiol.* (London), 130 (1955) 438.
[24] A. M. SHANES, *Pharmacol. Revs.*, 10 (1958) 59.
[25] R. RYBOVÁ, *Physiol. Bohemoslov.*, 9 (1960) 116.

Biochim. Biophys. Acta, 43 (1960) 41–50

EFFECTS OF INSULIN ON MONOSACCHARIDE TRANSPORT AND INCORPORATION OF AMINO ACIDS INTO PROTEIN IN DIAPHRAGM DIFFERENTIATED WITH PHLORIZIN

F. C. BATTAGLIA*, K. L. MANCHESTER** AND P. J. RANDLE
Department of Biochemistry, University of Cambridge, Cambridge (Great Britain)

(Received February 8th, 1960)

SUMMARY

The effects of phlorizin on monosaccharide transport, amino acid transport and amino acid incorporation into protein have been compared in isolated rat diaphragm. Conditions have been found under which phlorizin inhibits the uptake of glucose and membrane transport of D-xylose and D-galactose and the effect of insulin on these processes without affecting incorporation of [^{14}C]glycine into protein and the stimulation of this process by insulin. The results provide further evidence for the view that effects of insulin on amino acid incorporation are not dependent upon an effect of the hormone on carbohydrate metabolism.

INTRODUCTION

Insulin *in vitro* increases both uptake of glucose and incorporation of [^{14}C]amino acids into protein by isolated rat diaphragm muscle. There are points of evidence to

* Present address: Department of Physiology, Yale University, New Haven, Connecticut, U.S.A.
** Mr and Mrs John Jaffé Donation Student, Royal Society.