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EFFECTS OF MEDIUM ACETATE ON CELLULAR VOLUME IN RABBIT RENAL CORTICAL SLICES

BY KENNETH R. COOKE AND ANTHONY D. C. MACKNIGHT
From the Department of Physiology, University of Otago Medical School, P.O. Box 913, Dunedin, New Zealand

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SUMMARY

1. Slices of rabbit renal cortex were incubated at 25 °C in media in which acetate replaced chloride.

2. There was gross cellular swelling in isosmotic 132 mM-acetate medium, and this swelling was unique in that, with a normal medium potassium concentration, it was accompanied by a substantial increase in cellular potassium content.

3. This accumulation of potassium, but not the cellular swelling, was dependent upon metabolism and inhibited by ouabain.

4. This accumulation of potassium was not dependent upon the cellular swelling. It also occurred in a hyperosmotic acetate medium in which swelling was minimized.

5. In isosmotic media, the cellular swelling was proportional to medium acetate concentration and was also affected markedly by medium pH, being greatest at an initial medium pH of 7·1 and least at pH 7·7.

6. The swelling was reversed and cellular composition restored when tissue was re-incubated in NaCl medium. Ouabain (10^{-3} M) largely prevented this recovery in volume.

7. The results are consistent with plasma-membrane-based theories, on the assumption that membranes are much more permeable to undissociated acetic acid than they are to the acetate ion. They are inconsistent with the expectations of an alternative hypothesis (the association–induction hypothesis) which ascribes the maintenance of cellular composition to properties of cellular proteins and cellular water rather than to those of the plasma membrane.

8. The results do not favour the suggestion that cellular swelling itself results in irreversible cellular damage.

9. The results are consistent with the hypothesis that the ouabain-inhibitable Na–K-ATPase plays a major role in the regulation of cellular volume. No alternative metabolically dependent volume regulating mechanism need be postulated to explain them.

INTRODUCTION

Cellular volume remains constant in a variety of tissues incubated *in vitro* in oxygenated media, the composition of which resembles that of the interstitial fluid (Macknight & Leaf, 1977). Swelling of cells can be induced by incubation in

hyposmotic media, by inhibition of metabolism, by agents which increase plasma membrane permeability to sodium, or by incubation in isosmotic media in which caesium, rubidium or potassium replace sodium mole for mole as the predominant cation (Macknight & Leaf, 1977). With the obvious exception of the swelling induced by a high medium potassium concentration, such swelling has been associated invariably either with no change in cellular potassium content or, more commonly, with loss of cellular potassium.

Although effects on cellular volume of substitutions of medium cations have been studied extensively, less information is available about the role of anions in determining cellular volume. The reason for this is that the predominant medium anion, chloride, is thought to be distributed between medium and cellular water as a consequence of the metabolically dependent movements of cations (sodium and potassium). Occasionally, however, in a variety of *in vitro* experiments medium chloride is replaced by an anion to which the plasma membrane is thought to be impermeable (usually sulphate). In such media cellular swelling can be minimized, if not prevented, under conditions which would normally result in an increased cellular water content.

In contrast to the use of a chloride substitute to prevent cellular swelling, we report here experiments using renal cortical slices in which the replacement of chloride by acetate in an isosmotic medium has been used to produce gross, but reversible, cellular swelling which is unique in that, with normal medium sodium and potassium concentrations, potassium content is increased markedly in association with the swelling.

The results of these experiments provide important information about three aspects of cellular function which remain controversial. First, they are readily explained by the widely accepted 'pump-leak' or 'double-Donnan' hypothesis (Tosteson & Hoffman, 1960; Leaf, 1956) which describes the normal regulation of cellular volume, and cation contents, in terms of specific properties of the plasma membrane. They are not predicted, however, by the association–induction hypothesis of Ling and his associates (Ling, 1962; Ling & Peterson, 1977; Ling, Ochsenfeld, Walton & Bersinger, 1980; Negendank, 1982) which attributes the maintenance of cellular composition to organization of cellular macromolecules and consequent polarization of cellular water, rather than to the properties of the plasma membranes. Secondly, they reveal that even swelling sufficient to approximately double cellular volume can be completely reversed with the restoration of a normal cellular ionic composition. They therefore argue against the possibility (Leaf, 1973) that cellular swelling *per se* is a major contributor to the cellular damage with which it is so often associated. Thirdly, the results are consistent with the hypothesis that the ouabain-inhibitable Na–K-ATPase plays the major role in the regulation of cellular volume. They do not require the existence of any alternative metabolically dependent volume regulating mechanism as has been postulated (Kleinzeller & Knotková, 1964; Macknight, 1968*a*; Whittembury & Proverbio, 1970).

METHODS

Media. The sodium chloride medium used for the incubation of slices had the following composition (mM): Na^+, 148; K^+, 5; Ca^{2+}, 2; Mg^{2+}, 1; Cl^-, 132; SO_4^{2-}, 1; acetate, 10; buffered with phosphate, 8; at a pH approximating 7·3. The measured osmolality of this medium was 280 mosmol/kg H_2O.

In the sodium acetate medium, 122 mM-acetate replaced 122 mM-chloride giving a final concentration of 132 mM-acetate and of 10 mM-chloride. The pH of this medium approximated 7·4, and its measured osmolality was 285 mosmol/kg H_2O. In one experiment slices were exposed to graded concentrations of acetate in isosmotic media. These media were prepared by mixing the appropriate volumes of the two isosmotic media. In other experiments, slices were incubated in hyperosmotic media. These had the same composition as the isosmotic media except for the addition of 150 mmol/l sodium chloride to the sodium chloride medium and 150 mmol/l of sodium acetate to the sodium acetate medium. The measured osmolalities of these media were 547 and 552 mosmol/kg H_2O respectively. In one experiment the effects of medium pH were investigated. Sodium chloride and sodium acetate media were modified in that 8 mM-HEPES buffer was added to the media, and the concentration of phosphate buffer reduced from 8 to 2 mM. These alterations were made to overcome the possible precipitation of medium calcium phosphate with changes in medium pH. The final pHs of the media before incubation were 7·10, 7·40 and 7·70, the pHs being adjusted by adding the appropriate amounts of sodium hydroxide. The resulting changes in medium sodium concentration, at the greatest, were no more than 5 mM.

In one set of experiments, extracellular spaces were estimated using [*carboxyl*-^{14}C]inulin obtained from New England Nuclear Corporation. The final concentrations of radioactivity in the media were between 0·1 and 0·2 μCi/ml. Where indicated, ouabain octahydrate and iodoacetamide (Sigma Chemical Co., U.S.A.) were dissolved in media immediately before use.

Procedure. Adult male rabbits were stunned by a blow on the head. Kidneys were removed and placed in ice-cold isosmotic sodium chloride medium where they remained till sliced by Cohen's (1945) modification of the method of Deutsch (1936). Slices 200–300 μm thick were used for all experiments. About twenty-four slices could be obtained from each kidney. Slices with any macroscopically detectable medulla were rejected, but no effort was made to distinguish between slices from different cortical regions. Slices were immediately transferred to oxygenated isosmotic sodium chloride medium at 25 °C where they remained stirring vigorously until all slices were cut. They were then placed in fresh oxygenated isosmotic sodium chloride medium at 25 °C for 15 min. These slices are referred to in the text and Tables as equilibrated slices. Slices were then incubated in fresh media under the conditions shown in the Tables. Slices to be transferred to acetate media were washed rapidly with a large volume of acetate medium before incubation in the acetate medium. This minimized the transfer of chloride to the new incubation medium.

Analyses. Tissue water content was determined by loss of weight after drying for at least 2 h in a hot-air oven at 105 °C (Little, 1964). Ions and extracellular markers were extracted from the tissue overnight in 10 ml 0·1 M-nitric acid (Little, 1964; McIver & Macknight, 1974). Sodium and potassium in these acid extracts were measured with an EEL flame photometer and chloride determined by the method of Cotlove, Trantham & Bowman (1958) using an Aminco chloride titrator. Radioactivity in medium and slice extracts was determined by liquid scintillation counting in a three-channel Packard TriCarb Liquid Scintillation counter using 2 ml sample in 17 ml Triton C-100-toluene fluor. Medium osmolalities were determined by freezing point depression on an Advanced Instrument Co. osmometer, model 3 W, or by a vapour point technique using a Wescor model 5100 C osmometer, and pHs were measured with a Radiometer model 26 pH meter.

Presentation of results. Tissue water contents are expressed in kg H_2O/kg tissue dry wt., and ion contents are presented in mmol/kg tissue dry wt. The cellular water and ions have been calculated from the [^{14}C]inulin distribution in slices (McIver & Macknight, 1974). Cellular acetate contents have been estimated in two ways: by summing algebraically the respective gains of cation (sodium plus potassium) and losses of chloride, and by multiplying the water gained by total medium anion concentration and adding to this the loss of cell chloride. These two methods yielded very similar results, and the mean of the two has been used as the best available estimate of cellular acetate content. This divided by the appropriate water content provides an estimate of cellular acetate concentration.

The values quoted in the text and Tables are the means $\pm$ S.E. of mean of the number of

observations shown in parentheses. The statistical significance of differences between groups has been determined using Student's t test.

RESULTS

Incubation of rabbit renal cortical slices in oxygenated medium in which acetate replaced chloride as the dominant anion resulted in marked tissue swelling (Table 1), which occurred within 60 min and was sustained over a further 60 min incubation.

TABLE 1. The effects of incubation in acetate medium on the composition of rabbit renal cortical slices

	Cellular contents			
Incubated in	Water (kg/kg dry wt.)	Sodium (mmol/kg dry wt.)	Potassium (mmol/kg dry wt.)	Chloride (mmol/kg dry wt.)
Sodium chloride medium 60 min (23)	2·99±0·03	221±4	352±4	227±9
Sodium acetate medium 60 min (23)	5·08±0·07	398±9	504±13	33±3
Δ	2·09	177	152	194
P	<0·001	<0·001	<0·001	<0·001
Sodium chloride medium 120 min (22)	3·05±0·03	232±5	337±3	224±5
Sodium acetate medium 120 min (23)	4·82±0·12	357±11	474±15	33±7
Δ	1·77	125	137	191
P	<0·001	<0·001	<0·001	<0·001

All slices were equilibrated in oxygenated sodium chloride medium at 25 °C. They were then randomly distributed to either oxygenated sodium chloride medium or oxygenated sodium acetate medium where they remained for up to 120 min at 25 °C. Data are pooled from kidney slices obtained from three rabbits.

This swelling was accompanied by large increases in both sodium and potassium, and by a loss of most of the tissue chloride content. Fig. 1 illustrates the time course of the cellular swelling and estimated acetate accumulation from a separate series of experiments. A new steady state seemed to be reached by about 30 min. However, there was a tendency for water and ion contents to decrease between 60 and 120 min (Table 1).

To analyse these changes in tissue composition in terms of changes in cellular water and electrolytes, inulin space, which provides an appropriate estimate of extracellular water in this tissue (McIver & Macknight, 1974), was determined (Table 2). As a percentage of tissue water, the extracellular space in sodium chloride medium was similar to that previously reported (McIver & Macknight, 1974). However, the extracellular space expressed in this way decreased as tissue water increased, and increased as tissue water decreased (Table 2). In contrast, the calculated extracellular water content (kg H_2O/kg dry wt.) remained remarkably constant at about

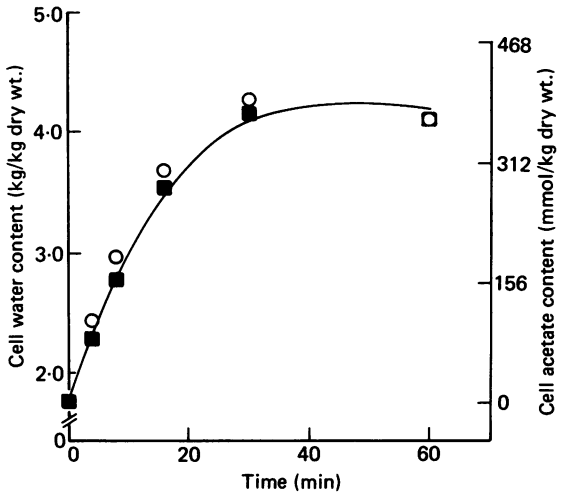

Fig. 1. The time course of the cellular swelling (■) and uptake of acetate (○) when rabbit renal cortical slices were transferred from sodium chloride to sodium acetate medium. Values shown are the means of eight observations in each group.

TABLE 2. The extracellular space (percentage tissue water) of rabbit renal cortical slices incubated in sodium chloride or sodium acetate media

Incubated in	Extracellular space (% tissue water)	Tissue water (kg/kg dry wt.)	Extracellular water (kg/kg dry wt.)
Sodium chloride medium 60 min (8)	30·4 ± 1·0	2·91 ± 0·05	0·89 ± 0·04
Sodium chloride medium 120 min (8)	32·6 ± 0·8	3·01 ± 0·02	0·98 ± 0·02
Δ	2·2	0·10	0·09
P	> 0·10	> 0·05	> 0·05
Sodium acetate medium 60 min (8)	20·2 ± 0·8	4·99 ± 0·10	1·01 ± 0·05
Sodium acetate medium 120 min (8)	20·8 ± 1·0	4·76 ± 0·12	0·99 ± 0·03
Δ	0·6	0·23	0·02
P	> 0·60	> 0·10	> 0·60
Sodium chloride medium + 150 mmol/l sodium chloride (8)	42·5 ± 0·9	2·16 ± 0·04	0·92 ± 0·03
Sodium acetate medium + 150 mmol/l sodium acetate (8)	31·4 ± 1·3	3·60 ± 0·07	1·13 ± 0·04
Δ	11·1	1·44	0·21
P	< 0·001	< 0·001	< 0·001

All slices were equilibrated in oxygenated sodium chloride medium at 25 °C. They were then randomly distributed to either oxygenated sodium chloride medium or oxygenated sodium acetate medium with [^{14}C]inulin. After 60 min, some slices were transferred from either sodium chloride or sodium acetate medium to the corresponding oxygenated hyperosmotic medium containing [^{14}C]inulin at the same specific activity, and others taken for analysis. After a further 60 min all remaining slices were taken for analysis.

1·0 kg H_2O/kg dry wt. (Table 2). The mean of all values was 0·99 ± 0·003 kg H_2O/kg dry wt.).

These measured extracellular spaces were used to correct the appropriate tissue contents for extracellular water and ions, and the cellular contents derived from the tissue analysis presented in Table 1 are shown in Table 3. Incubation in sodium acetate medium resulted in a doubling of cellular water content. Accompanying this, both cellular sodium and potassium contents rose substantially and cellular chloride

TABLE 3. Cellular composition of rabbit renal cortical slices incubated in either sodium chloride or sodium acetate medium

	Cellular contents			
Incubated in	Water (kg/kg dry wt.)	Sodium (mmol/kg dry wt.)	Potassium (mmol/kg dry wt.)	Chloride (mmol/kg dry wt.)
Sodium chloride medium 60 min	2·08	86	347	108
Sodium acetate medium 60 min	4·05	244	499	23
Δ	1·97	158	152	−85
Sodium chloride medium 120 min	2·06	85	333	94
Sodium acetate medium 120 min	3·81	208	469	22
Δ	1·75	123	136	−72

Values calculated from tissue compositions given in Table 1 using the mean extracellular spaces given in Table 2.

content fell markedly. These gains in cation content in acetate medium were appropriate to the gains in cellular water. With an extracellular cation content of 156 mmol/l H_2O, each kg of water gained by the cells would require the uptake of 156 mmol of cation to maintain isosmolality between cells and medium. After 60 min incubation cells had gained 1·97 kg H_2O/kg dry wt. and 310 mmol cation ($Na^+ + K^+$), and after 120 min incubation, a gain of 1·75 H_2O/kg dry wt. was associated with an uptake of 259 mmol cation ($Na^+ + K^+$). These values give ratios of cation uptake : water uptake of 157 and 148 respectively, in excellent agreement with the theoretical value of 156 mmol/kg H_2O. In contrast to the gain of additional cation, which is associated with an isosmotic uptake of water, the sum of ($Na^+ + K^+$) in the control tissues incubated in sodium chloride medium is appreciably greater than might be anticipated for an isosmotic intracellular fluid (ratio of cation : water of 208, Table 3). In part, this will reflect the presence within the cells of polyvalent anions. In addition, in many epithelia it seems that chemical analysis yields an over-estimate of cellular sodium (and chloride) content (Macknight, 1980). Nevertheless, though the absolute values for cellular sodium may be too high, the gains of cation which accompany the swelling of the cells appear to be quantitatively correct.

The increased water and cation contents in acetate medium must be associated with uptake of acetate, the predominant extracellular anion. Cellular chloride content fell and the uptake of acetate by the cells after 60 min should be equal to the net gain

of cation (310 mmol) plus the loss of cellular chloride (85 mmol), a net uptake of 405 mmol of acetate. After 120 min, the acetate gain calculated in this way is 331 mmol/kg dry wt. Using the alternative method of calculation outlined in Methods, acetate uptakes of 392 (60 min) and 345 mmol (120 min) are estimated. It is this uptake of acetate by the cells which we believe is responsible for the cellular swelling (see Discussion).

TABLE 4. Effects of ouabain on composition of rabbit renal cortical slices incubated 60 min in either sodium chloride or sodium acetate medium

	Tissue contents			
Incubated in	Water (kg/kg dry wt.)	Sodium (mmol/kg dry wt.)	Potassium (mmol/kg dry wt.)	Chloride (mmol/kg dry wt.)
Sodium chloride medium (8)	3·07±0·05	234±5	369±6	229±10
Sodium acetate medium (8)	5·34±0·15	412±16	539±14	40±8
Δ	2·27	178	170	189
P	<0·001	<0·001	<0·001	<0·001
Sodium chloride medium +10^{-3} M-ouabain (8)	3·02±0·08	437±5	121±3	254±8
Sodium acetate medium +10^{-3} M-ouabain (8)	4·83±0·07	689±14	119±3	51±6
Δ	1·81	252	2	203
P	<0·001	<0·001	>0·60	<0·001
		Cellular contents		
Sodium chloride medium	2·07	86	364	97
Sodium acetate medium	4·34	264	534	30
Δ	2·27	178	170	67
Sodium chloride medium +10^{-3} M-ouabain	2·02	289	116	122
Sodium acetate medium +10^{-3} M-ouabain	3·83	541	114	41
Δ	1·81	252	2	81

All slices were equilibrated in oxygenated sodium chloride medium at 25 °C. They were then randomly distributed to either oxygenated sodium chloride medium, oxygenated sodium acetate medium or to these media containing 10^{-3} M-ouabain where they remained for 60 min. The cellular contents in this and subsequent Tables and graphs were derived using an extracellular water content of 1·0 kg/kg dry wt. (Table 2).

If it is assumed that all non-inulin space ions are within cells and in free solution, the cellular concentrations of these ions can be derived. Though cellular sodium concentration rose (from 41 in sodium chloride medium to 60 mmol/kg cell H_2O in sodium acetate medium after 60 min) and cellular potassium concentration fell (from 167 to 123 mmol/kg cell H_2O after 60 min), and similar changes can be calculated after 120 min incubation, these changes were relatively small compared with the changes in water and cation contents. That is, the cells continued to maintain cation distributions typical of metabolizing tissue despite gross cellular swelling.

Normally, cellular accumulation of potassium is inhibited by cardiac glycosides such as ouabain. Similarly, in these cells swollen in acetate medium, ouabain also

inhibited cellular uptake of potassium (Table 4). In both sodium chloride and sodium acetate medium, ouabain resulted in a decrease of cellular potassium content to similar levels, and cellular swelling in the ouabain-treated tissues incubated in acetate medium was now associated exclusively with a net gain of sodium, appropriate to the uptake of water (ratio of sodium gained: H_2O gained/kg dry wt. = 140 mmol/kg H_2O).

That the uptake of potassium in sodium acetate medium was dependent upon cellular metabolism was established by incubating slices for 120 min in sodium acetate medium containing 10^{-3} M-iodoacetamide, bubbled with nitrogen (Table 5). The swelling itself was clearly not dependent upon metabolism for it was comparable to that seen in oxygenated sodium acetate medium. However, it was now associated exclusively with uptake of sodium and the net gain of cation remained appropriate to the uptake of water.

To determine whether the extent of the cellular swelling and changes in electrolyte contents were related to the concentration of acetate in the medium, an experiment was performed in which slices were incubated for 120 min in isosmotic media in which different ratios of acetate to chloride were used (Fig. 2). It is clear that as the medium acetate concentration increased relative to the chloride concentration, cellular water, sodium and potassium contents rose progressively and cellular chloride content fell. Thus the degree of cellular swelling and the magnitude of the change in ionic contents is dependent upon the medium acetate concentration. Note, however, that the swelling increases disproportionately as medium concentration is increased. In all of these media, cellular potassium concentrations remained physiological.

The extent of the cellular swelling in acetate medium was also dependent upon medium pH within a pathophysiological range (initial pHs 7·10, 7·40 and 7·70). Control slices incubated at these pHs in sodium chloride medium maintained their normal compositions (Table 6). In contrast, in the acetate media, there was a marked dependency of cellular volume on medium pH, with cellular water contents ranging from 5·70 at the most acid pH (a threefold increase in water content) to 3·58 at pH 7·70. However, the increases in cellular potassium content were similar at all three medium pHs, and it was cellular sodium content which varied with water content. There was also a tendency for cellular chloride content to fall as medium pH increased.

Finally, two studies were performed to examine relationships between cellular swelling and potassium content. In the first, slices were incubated for 60 min in either isosmotic sodium chloride or sodium acetate media and were then transferred to media of about twice normal osmolality (Table 7). The increased osmolality minimized the cellular swelling normally seen in sodium acetate medium and resulted in a cellular water content closer to that observed in slices incubated in sodium chloride medium (2·63 kg H_2O/kg dry wt. compared with 2·08 kg H_2O/kg dry wt. in sodium chloride medium, Table 3). However, cellular potassium content was, if anything, higher in the hyperosmotic acetate medium (552 mmol/kg dry wt.) than it was in isosmotic acetate medium (499 mmol/kg dry wt., Table 3). Thus the increase in cellular potassium content in acetate medium was not dependent upon cellular swelling. Again, ouabain prevented this net uptake of potassium. Instead sodium was the cation accumulated (Table 7).

TABLE 5. Effects of metabolic inhibition on the composition of rabbit renal cortical slices incubated in sodium acetate medium

Incubated in	Water (kg/kg dry wt.)	Sodium (mmol/kg dry wt.)	Potassium (mmol/kg dry wt.)	Chloride (mmol/kg dry wt.)
	Tissue contents			
Sodium acetate medium 120 min (8)	4·76±0·12	396±18	421±9	14±2
Sodium acetate medium $+10^{-3}$ M-iodoacetamide 120 min (8)	5·08±0·06	773±11	72±2	46±3
Δ	0·32	377	349	32
P	<0·05	<0·001	<0·001	<0·001
		Cellular contents		
Sodium acetate medium	3·76	248	416	4
Sodium acetate medium $+10^{-3}$ M-iodoacetamide	4·08	625	67	36
Δ	0·32	377	349	32

All slices were equilibrated in oxygenated sodium chloride medium and then transferred either to oxygenated sodium acetate medium or nitrogenated sodium acetate medium containing 10^{-3} M-iodoacetamide, where they remained for 120 min.

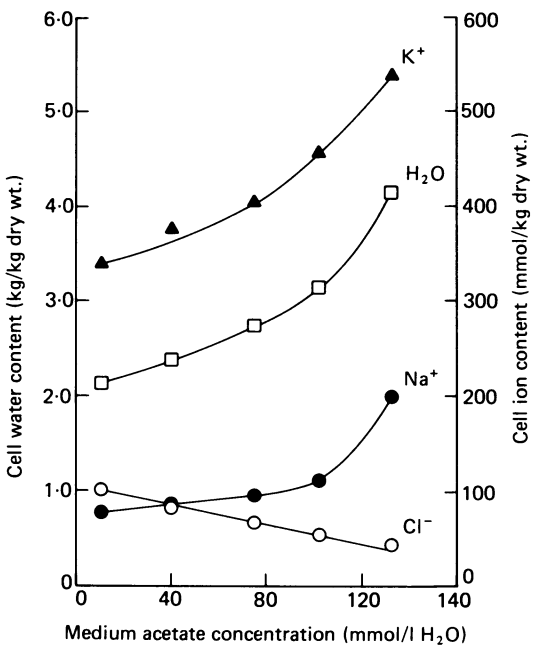

Fig. 2. The relationship between medium acetate concentration and cellular composition. Slices were equilibrated in oxygenated sodium chloride medium (10 mM-acetate). They were then transferred to oxygenated media in which acetate replaced chloride mole for mole to provide media with acetate concentrations of 10, 41, 75, 102 or 132 mM respectively, and incubated for 120 min. Values shown are the means of seven or eight observations in each group.

TABLE 6. Effects of medium pH on tissue composition

		Medium pH		
	Equilibrated			
Initial pH	7·40	7·10	7·40	7·70
Final pH				
Sodium chloride medium		7·17	7·48	7·75
Sodium acetate medium		7·20	7·52	7·79
	Tissue contents			
Medium	Water (kg/kg dry wt.)			
Sodium chloride medium (8)	2·87 ± 0·05	2·85 ± 0·03	2·90 ± 0·02	2·91 ± 0·03
Sodium acetate medium (8)		6·70 ± 0·05	5·40 ± 0·06	4·58 ± 0·08
Δ		3·85	2·50	1·67
P		<0·001	<0·001	<0·001
	Sodium (mmol/kg dry wt.)			
Sodium chloride medium (8)	214 ± 4	195 ± 3	197 ± 2	190 ± 4
Sodium acetate medium (8)		566 ± 8	369 ± 8	279 ± 7
Δ		371	172	90
P		<0·001	<0·001	<0·001
	Potassium (mmol/kg dry wt.)			
Sodium chloride medium (8)	295 ± 3	335 ± 7	331 ± 3	332 ± 3
Sodium acetate medium (8)		438 ± 13	468 ± 7	465 ± 14
Δ		103	137	133
P		<0·001	<0·001	<0·001
	Chloride (mmol/kg dry wt.)			
Sodium chloride medium (8)	198 ± 3	198 ± 2	192 ± 2	198 ± 2
Sodium acetate medium (8)		69 ± 2	46 ± 3	34 ± 2
Δ		129	146	164
P		<0·001	<0·001	<0·001
	Cellular contents			
	Water (kg/kg dry wt.)			
Sodium chloride medium	1·87	1·85	1·90	1·91
Sodium acetate medium		5·70	4·40	3·58
Δ		3·85	2·50	1·67
	Sodium (mmol/kg dry wt.)			
Sodium chloride medium	66	47	49	42
Sodium acetate medium		418	221	131
Δ		371	172	89
	Potassium (mmol/kg dry wt.)			
Sodium chloride medium	290	330	326	327
Sodium acetate medium		433	463	460
Δ		103	137	133
	Chloride (mmol/kg dry wt.)			
Sodium chloride medium	66	66	60	66
Sodium acetate medium		59	36	22
Δ		7	24	44

All slices were equilibrated in oxygenated sodium chloride medium, pH 7·40, at 25 °C. They were then randomly distributed to either oxygenated sodium chloride medium or oxygenated sodium acetate medium of the initial pHs shown, where they remained for 60 min at 25 °C.

It was of interest that cells compensated for the increased medium osmolality in both sodium chloride and sodium acetate medium in part by a loss of cellular water and in part by an increase in cellular ionic contents. In both cases cellular water decreased to approximately 60% of its value in isosmotic media and cellular cation content increased by approximately 10%. Furthermore, in both the isosmotic and

TABLE 7. Effects of hyperosmotic incubation media on the composition of rabbit renal cortical slices

	Tissue contents			
Incubated in	Water (kg/kg dry wt.)	Sodium (mmol/kg dry wt.)	Potassium (mmol/kg dry wt.)	Chloride (mmol/kg dry wt.)
Sodium chloride medium +150 mM-sodium chloride (15)	2·25±0·04	394±14	390±3	447±16
Sodium acetate medium +150 mM-sodium acetate (15)	3·63±0·05	575±16	556±12	15±2
Δ	1·39	181	167	432
P	<0·001	<0·001	<0·001	<0·001
Sodium acetate medium +150 mM-sodium acetate +10^{-3} M-ouabain (7)	3·25±0·09	863±33	194±8	30±2
*Δ	0·38	288	389	15
P	<0·001	<0·001	<0·001	<0·001
	Cellular contents			
Sodium chloride medium +150 mM-sodium chloride	1·25	96	385	165
Sodium acetate medium +150 mM-sodium acetate	2·63	277	552	5
Δ	1·38	181	167	160
Sodium acetate medium +150 mM-sodium acetate +10^{-3} M-ouabain	2·25	565	189	20
*Δ	0·27	288	363	15

All slices were equilibrated in oxygenated sodium chloride medium, and then transferred for 60 min to either oxygenated sodium chloride medium or oxygenated sodium acetate medium. Slices in sodium chloride medium were then transferred to oxygenated sodium chloride medium containing an additional 150 mM-NaCl and slices in sodium acetate medium were transferred either to sodium acetate medium containing an additional 150 mM-sodium acetate or, in one experiment, to this medium which also contained 10^{-3} M-ouabain. Slices were incubated 60 min in these hyperosmotic media.

*Δ, differences produced by ouabain in hyperosmotic acetate media.

hyperosmotic media, acetate resulted in the same fractional increase in cellular water content, the ratios cell water content in acetate medium : cell water content in sodium chloride medium being respectively 1·94 and 1·92.

Secondly, the extent to which a normal cellular composition could be restored when slices incubated in isosmotic acetate medium were returned to sodium chloride medium was explored (Table 8). Clearly, extensive recovery occurred with loss of an

TABLE 8. Effects of re-incubation in sodium chloride medium after incubation in sodium acetate medium

Incubated in	Tissue contents: Water (kg/kg dry wt.)	Sodium (mmol/kg dry wt.)	Potassium (mmol/kg dry wt.)	Chloride (mmol/kg dry wt.)
Sodium chloride medium 60 min (8)	3·05±0·04	270±8	318±2	233±5
Sodium acetate medium 60 min (7)	4·58±0·09	374±7	420±4	32±3
Sodium acetate medium +ouabain 60 min (8)	5·11±0·06	806±22	97±1	55±2
Sodium chloride medium 120 min (8)	2·98±0·04	255±7	316±3	226±5
Sodium chloride medium 60 min following the acetate medium (8)	3·09±0·04	293±7	297±2	255±5
Sodium chloride medium +ouabain 60 min following sodium acetate medium +ouabain 60 min (8)	4·51±0·05	698±23	57±6	469±13
	Cellular contents			
Sodium chloride medium 60 min	2·05	122	313	101
Sodium acetate medium 60 min	3·58	226	415	22
Sodium acetate medium $+10^{-3}$ M ouabain 60 min	4·11	658	92	45
Sodium chloride medium 120 min	1·98	107	311	94
Sodium chloride medium 60 min after sodium acetate	2·09	145	292	123
Sodium chloride medium $+10^{-3}$ M ouabain 60 min after sodium acetate +ouabain	3·51	550	52	337

All slices were equilibrated in oxygenated sodium chloride medium at 25 °C. They were then randomly distributed to either the oxygenated sodium chloride medium or oxygenated sodium acetate medium with or without ouabain, 10^{-3} M. After 60 min some slices were taken for analysis, the remainder either remained in sodium chloride medium or were returned to sodium chloride medium, the slices exposed to ouabain in the acetate medium being incubated in sodium chloride medium with ouabain.

isosmotic fluid, and after 60 min tissue composition was comparable to that of slices maintained throughout in sodium chloride medium. Thus, even gross cellular swelling, of itself, appeared not to affect the subsequent ability of cells to maintain a normal volume with a high cellular potassium content. Interestingly, however, the restoration of water and cation contents, but not of chloride content, was impeded markedly by ouabain. Indeed, the chloride contents became much greater with ouabain.

Finally, on the assumption that the distribution of acetate is determined by non-ionic diffusion (see Discussion), cellular pH has been estimated (Table 9). Too much significance should not be attached to these estimates given the imprecision in estimating cellular acetate concentrations, and possible variations in medium pHs during incubation which were not assessed routinely. Furthermore, the equation used in the estimation assumes that the plasma membrane is virtually impermeable to the

TABLE 9. Estimates of cell pH

Medium acetate concentration (mmol/l)	Medium pH	Cell pH
Table 3		
132, 60 min	7·40	7·27
132, 120 min	7·40	7·23
Table 4		
132, 60 min	7·40	7·26
132 + ouabain, 10^{-3} M, 60 min	7·40	7·25
Table 5		
132, 120 min	7·40	7·24
132 + iodoacetamide, 10^{-3} M, 120 min	7·40	7·22
Table 6		
41, 60 min	7·33	7·18
75, 60 min	7·37	7·17
120, 60 min	7·39	7·20
132, 60 min	7·41	7·25
Table 7		
132, 60 min	7·20	7·06
132, 60 min	7·52	7·34
132, 60 min	7·79	7·58
Table 8		
282, 60 min	7·40	7·26
282 + ouabain, 10^{-3} M, 60 min	7·40	7·24
Table 9		
132, 60 min	7·40	7·20
132 + ouabain, 10^{-3} M, 60 min	7·40	7·24

Cell pHs were estimated from the distribution of acetate with cellular acetate concentration calculated as described in the Methods section, using a pK_a of 4·7, according to the equation (Roos & Boron, 1981):

$$pH_c = pK_c + \log\left[\frac{TA_c}{TA_m}(10^{pH_m - pK_m} + 1) - 1\right],$$

where subscripts c and m refer to cell and medium respectively, and TA = total content of dissociated and undissociated species. In fact the concentration of undissociated acid is so low that TA approximates acetate concentrations very closely in both medium and cell.

acetate ion whose distribution ratio is dependent entirely upon the difference between medium and cell pHs and is uninfluenced by plasma membrane potential. In the absence of independent assessments of cell pHs or of plasma membrane potentials, the application of more sophisticated equations (e.g. equation 10 of Roos, 1975) is unwarranted. However, it seems that cellular pH approximates 7·25 when medium pH is about 7·40, and that cellular pH increases progressively when medium pH is elevated, though this increase is less than that of the medium.

DISCUSSION

Rabbit renal cortical epithelial cells in slices incubated at 25 °C in isosmotic oxygenated medium of normal potassium concentration (5 mM) became grossly swollen within 30 min (Fig. 1) when chloride was replaced by acetate as the predominant medium anion. This swelling is unique in that it is accompanied by a large increase in cellular potassium content. In all other situations in which swelling has been described in renal cortical tissue incubated in medium of normal potassium concentration, cells have either retained a normal potassium content (hyposmotic media, Hughes & Macknight, 1976), or, more commonly, have lost potassium (metabolic inhibition, Mudge, 1951; non-diuretic mercurials, Macknight, 1968*b*; substitution of caesium or rubidium for medium sodium, Hughes & Macknight, 1977). Furthermore, the swelling here was not dependent upon metabolism, though the associated accumulation of potassium was (Table 5), and the changes in composition were largely reversed when tissues were re-incubated in sodium chloride medium (Table 8). In addition, the accumulation of potassium was inhibited by ouabain (Tables 4 and 8), and was not dependent upon the cellular swelling, for it occurred equally when swelling was inhibited in a hyperosmotic acetate medium (Table 7).

The mechanism of the cellular swelling

Three sets of experiments bear directly on this question; the cellular swelling was not dependent upon metabolism (Table 5), it increased with increasing medium acetate concentration (Fig. 2), and it was sensitive to medium pH (Table 6). All these observations are consistent with the following hypothesis, illustrated in Fig. 3. The plasma membrane, while relatively permeable to the undissociated weak acid, acetic acid (HAc), is much less permeable to the acetate ion (Ac^-). Thus, acetic acid will enter cellular water and, under steady-state conditions, will approach the same concentration as acetic acid in the medium. From the Henderson–Hasselbalch equation, and using a pK of 4·7 for the buffer under these experimental conditions (Documenta Geigy 7th edition, p. 279) medium acetic acid concentration (and, therefore, cell acetic acid concentration) will approximate 0·26 mM with medium pH 7·4 and a medium acetate concentration of 132 mM; but in the cell acetic acid will dissociate to hydrogen ions and acetate ions. With the membrane permeability to acetate ions sufficiently low, the concentration of acetate ions at equilibrium will be determined largely by the cellular pH, and not by the plasma membrane potential. (The steady-state distribution of acetate under such conditions provides a measure of cellular hydrogen ion concentration if it can be assumed that pK_a is the same in both medium and cell water, as in Table 9.)

The cell will now contain an excess of osmotically active solute, and water will enter the cell to maintain isosmolality between medium and cell. Thus the cells gain water and swell. (Such cellular swelling when tissues are incubated in solutions containing weak acids was indeed described by Overton in 1902 (cited by Roos & Boron, 1981) who used the osmotic swelling as an index of acid entry. More recently, swelling of muscle fibres has been described when they are incubated in a medium containing high concentrations of lactate (Roos, 1975). However, to our knowledge, there are no reports of net potassium accumulation associated with such swelling.)

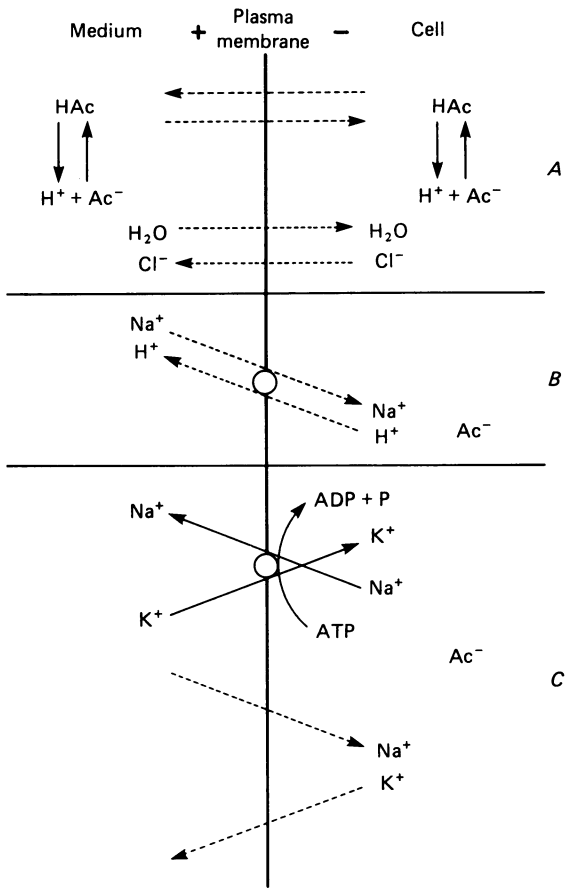

Fig. 3. The mechanism of swelling in acetate media. *A*, acetic acid (HAc) equilibrates across the plasma membrane which is relatively impermeable to the acetate ion (Ac^-). The resulting cellular accumulation of solute causes a net entry of water to the cells with swelling. There is also a loss of cellular chloride ions to the medium in which the chloride ion concentration has been reduced. There are two further processes which will accompany the changes in *A*. In *B*, the cellular hydrogen ions are exchanged for extracellular sodium ions. The mechanism illustrated involves sodium–hydrogen ion counter-transport but there is no direct experimental evidence for this from the present results. In *C*, the sodium ions gained are actively extruded from the cells by the Na–K-ATPase. This step can be inhibited by ouabain or by metabolic inhibition. The final cellular composition reflects the extent of acetate accumulation and the relative activities of the Na–K pump compared with the rates of passive diffusion of these ions across the membrane.

The accumulation of acetate must be accompanied by a transient increase in cellular hydrogen ions. Though some of these hydrogen ions might be buffered intracellularly, the increase is so great that cellular pH would drop if they were not expelled from the cell. (Since cellular acetate content is estimated at about 360 mmol/kg dry wt. in these experiments, cells must also have gained transiently some 360 mmol H^+/kg dry wt.) Estimates of the pH of mammalian cell water vary from tissue to tissue, but it is now generally accepted that cellular pH is higher than

would be predicted were hydrogen ions in electrochemical equilibrium across the plasma membrane (Roos & Boron, 1981). The mechanism or mechanisms involved in maintaining the lower than predicted cellular hydrogen ion concentration remain to be established, but one attractive possibility in renal tissue is that a sodium–hydrogen ion counter-transport system driven by the energy inherent in the sodium gradient allows extrusion of hydrogen ions from the cells (Boron & Boulpaep, 1980; Kinsella & Aronson, 1981). This is illustrated in Fig. 3*B* and would explain the loss

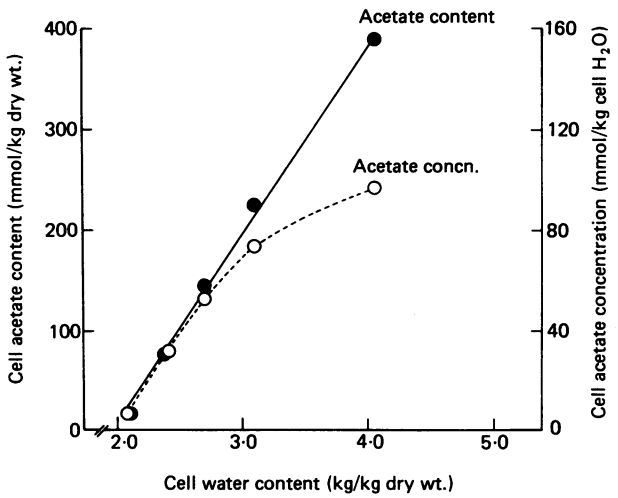

Fig. 4. The relationship between cell acetate content (●——●) and cell acetate concentration (○---○) with increasing cell water content. Data obtained from the experiments illustrated in Fig. 2.

of hydrogen ions from the cell both in metabolizing and non-metabolizing tissue, for such loss is not dependent directly on cellular metabolism and would continue after metabolic inhibition until the sodium gradient was dissipated. (Note, however, that the present results, though consistent with sodium–hydrogen ion counter-transport, do not establish this as the mechanism either wholly or partly involved, and in an experiment in which acetate medium contained 2 mM-amiloride, a specific inhibitor of this counter-transport process (Kinsella & Aronson, 1981), cellular swelling was not affected significantly. This result is, however, difficult to interpret since at medium sodium concentrations of 148 mM, even 2 mM-amiloride is only partially effective in blocking the counter-transport mechanism (Kinsella & Aronson, 1981). Further work is, therefore, required to explore the mechanisms involved in hydrogen ion loss from the cells under these experimental conditions.)

Whatever the mechanism, sodium is gained by the cells with acetate, but the extent of this gain will be determined by the activity of the Na–K-ATPase (Fig. 3*C*), and by the relative membrane permeabilities to sodium and to potassium. Thus, in metabolizing cells, a large fraction of the sodium gained will be replaced by potassium, but, with metabolic inhibition or with ouabain, a specific inhibitor of the enzyme, sodium is the only cation accumulated.

The present results support the first part of the hypothesis in that the extent of the cellular swelling with different medium acetate concentrations and with different

medium pHs can be explained readily. The degree of swelling however, is not a linear function of medium acetate concentration (Fig. 2). Rather, the swelling is greater the greater the concentration. The reason for this is that, though acetate content increases linearly with water content, the rate of increase of cell acetate concentration falls progressively as cell acetate and water contents increase (Fig. 4). Thus increasing quantities of acetate and water must be gained by the cells to achieve the required cellular acetate concentration as medium acetate concentration increases.

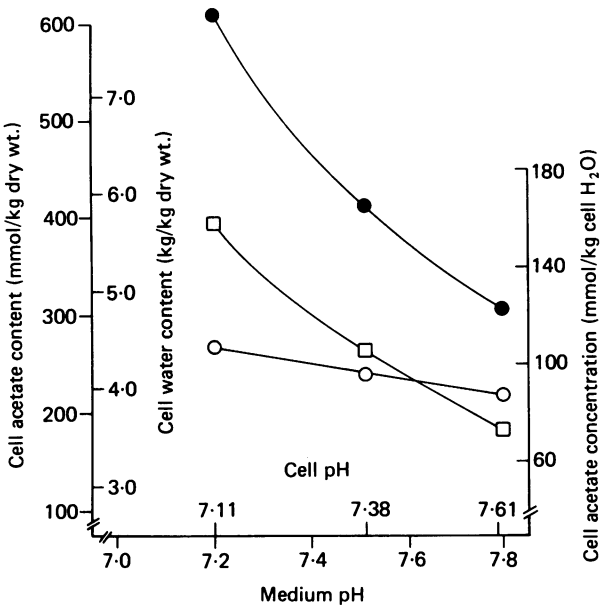

Fig. 5. Relationships between cell water content (□), cell acetate content (●) and acetate concentration (○), and medium (and cell) pH.

It is not possible to predict the effects which alterations in medium pH might have on cellular volume because of possible changes in cellular pH, which could influence the cellular acetate accumulation considerably. For example, with a medium acetate concentration of 132 mM, were cellular pH to remain constant, with a value of 7·2, cell acetate concentration would range from 132 mM at a medium pH of 7·2, to 32 mM at a medium pH of 7·8, with, of necessity, a large variation in cellular water content. Conversely, if cellular pH always adjusted to a fixed interval below the medium pH, cellular acetate concentration would remain approximately constant, as would cellular water content. In fact, experimentally, something between these two extremes occurred. Though estimates (Table 9) reveal that cell pH did increase as medium pH increased, the increase was less, so that the cell interior became relatively more acid as the medium became more alkaline. The consequence of this was that both the cellular water content and calculated cellular acetate contents decreased markedly as the medium became more alkaline (Fig. 5). Again, note the large increase in water and acetate contents required for a modest increase in cellular acetate concentration.

From these considerations, therefore, the behaviour of the tissue under both these

sets of experimental conditions is explicable entirely in terms of membrane permeability to the undissociated weak acid and relative impermeability to the acetate ion.

In addition, the estimates of cell pH made from the distribution of acetate between medium and cell (Table 9) support this conclusion for they agree well with estimates made in experiments with renal proximal tubular cells designed specifically to assess cellular pH (summarized by Boron & Sackin, 1983).

In summary, the hypothesis illustrated in Fig. 3 describes the behaviour of the renal cortical cells incubated in acetate medium in terms of specific properties of the plasma membrane: relative permeability to undissociated acetic acid, relative impermeability to the acetate ion, an exchange process allowing extracellular sodium to displace hydrogen ions from the cells thus preventing any appreciable alteration in cellular pH, and an energy-dependent active transport process, the Na–K-ATPase, which limits sodium accumulation and results in a marked increase in cellular potassium content. This hypothesis, based on a central role of the plasma membrane, accounts adequately for all of the experimental observations.

The relevance of the results to an alternative theory of cellular organization

Whilst most workers agree as to the importance of the properties of the plasma membrane in determining cellular volume and composition, an alternative, the association–induction hypothesis, which relies upon properties attributed to cellular constituents, has been promoted vigorously by Ling and his associates (for example; Ling, 1962; Ling & Peterson, 1977; Ling *et al.* 1980; Negendank, 1982). In this hypothesis, metabolism provides ATP which is adsorbed to specific binding sites on cellular protein molecules thereby controlling their electronic and steric configurations. The protein–water–ion system now exists in its living state, and the bulk of cellular potassium is adsorbed on certain anionic protein sites which have adsorption energies more favourable for potassium than for sodium. In contrast to potassium, little cellular sodium is adsorbed, the rest remains in the cell water but at a lower concentration than in the medium because cellular water, existing in a state of multilayers polarized by a matrix of extended polypeptide chains, has a diminished ability to dissolve it and all other medium solutes (Ling, 1962). This hypothesis, therefore, ascribes the existence of a unique cellular environment to properties of the cellular constituents and not to special characteristics of the plasma membrane.

Two aspects of the present results seem not to be accounted for readily by the association–induction hypothesis. First, why do the cells swell in an acetate medium? Secondly, why should cellular potassium content actually increase with such cellular swelling?

In the association–induction hypothesis, two major factors determine cellular water content: one, the multilayer adsorption of cell water, which is affected by ATP (Ling & Peterson, 1977; Negendank, 1982) and the other, salt-linkages formed between adjacent charged or fixed groups on the same or on neighbouring protein molecules (Ling & Peterson, 1977). Cellular swelling will be favoured as these salt-linkages are broken, and the 'swelling power' of a univalent salt will, therefore, depend both on the affinity of the fixed anions for the cation and upon the affinity of the fixed cations for the anion of the univalent salt. If both affinities are low, little

if any swelling would be predicted, as few of the linkages between protein charged groups will be broken. In the case of sodium acetate, neither the sodium nor the acetate ion is strongly preferred and little swelling should occur. (In Ling, 1962, p. 172, acetate lies with the sulphate anion at the bottom of the Table which ranks the relative adsorption energies of anions, and the relative ineffectiveness of potassium sulphate in causing cell swelling when compared with potassium chloride is attributed by Ling & Peterson (1977) to the fact that, though the potassium ion is preferred by the fixed anions, the sulphate anion is not strongly preferred by the fixed cations. From the theory, one would anticipate even less tendency for swelling when neither cation nor anion is preferred by the fixed charges.)

The additional accumulation of potassium by cells is equally puzzling in terms of the association–induction hypothesis. Since cellular volume has increased, it might be argued that salt-linkages have necessarily been disrupted and that, since the fixed anions forming such linkages favour potassium over sodium, potassium must be accumulated. Aside from begging the question of the cause of the initial disruption, which of itself could modify the selectivity of the fixed anionic sites (for example, a local change in pH might be suggested with hydrogen ions released from acetic acid breaking the salt-linkages, but the sites would then be occupied by hydrogen ions and unavailable to potassium ions), this argument ignores the observation that potassium is also accumulated to a somewhat greater extent (Table 7) when swelling is largely prevented by a hyperosmotic acetate medium. It also fails to offer a ready explanation for the fact that, in acetate media, sodium is accumulated instead of potassium, which is actually lost from the cells after ouabain (Tables 4 and 7) and after metabolic inhibition (Table 5). Indeed the similarity of the swelling in acetate medium whether or not cells are metabolizing is, of itself, an argument against the association–induction hypothesis which would ascribe the swelling in non-metabolizing tissue not to a single process, as does the membrane-based hypothesis (the accumulation of acetate in cell water) but to two processes – metabolic inhibition which disrupts ATP adsorption to specific protein binding sites, thereby altering their properties and depolarizing the cellular water, and salt-linkage disruption due to the medium ions themselves. From the association–induction hypothesis, therefore, there seems to be no *a priori* reason why metabolizing and non-metabolizing cells should swell to the same extent in acetate media.

The present results, therefore, are inconsistent with the behaviour predicted by the association–induction hypothesis.

The restoration of cellular composition upon re-incubation in sodium chloride medium

It has been argued (Leaf, 1973) that cellular swelling, of itself, could contribute to the associated impairment of cellular function and the eventual failure of metabolically inhibited cells to recover even when optimum physiological conditions are restored. In this view, as cells swell the inevitable separation of cellular membrane-bound enzymes disrupts the orderly flow of essential sequential reactions. If the swelling is sufficiently great, this disruption may result in irreversible cellular injury.

In the present experiments it has been possible for the first time to produce cellular swelling in metabolizing cells in a medium of normal extracellular osmolality and

potassium concentration and to separate the effects of swelling itself from the effects of metabolic inhibition. Indeed, the swelling associated with acetate in renal cortical slices over 60 min is considerably greater than that produced by metabolic inhibition in a sodium chloride medium over the same time (for example, Hughes & Macknight, 1977, Table 2). Despite this gross swelling, cellular potassium content increased markedly, and estimated potassium concentration, though lower, was well sustained (167 mM in sodium chloride, 123 mM in acetate medium, from Table 3). Similarly, the estimated cellular concentration of sodium remained substantially lower than the medium concentration (41 mM in sodium chloride medium, 60 mM in acetate medium, from Table 3). The maintenance of these typical cellular cation concentrations was dependent on metabolism (Table 5) and, specifically, on the continued activity of the ouabain-sensitive Na–K-ATPase (Table 4). Therefore, as judged by the ability to maintain cellular ionic composition, gross swelling has not interfered substantially with a central metabolically dependent process.

Furthermore, cellular composition can be restored essentially to normal when swollen slices are transferred from acetate medium back to sodium chloride medium (Table 8). Thus even gross swelling, to an extent much greater than that associated with metabolic inhibition over 60–120 min, does not irreversibly damage cellular function as assessed from the ability to maintain a normal volume and cationic composition; processes which are both very sensitive to impairment of cellular metabolism.

We conclude, therefore, that even gross swelling of metabolizing cells does not, of itself, result in irreversible cellular damage.

The role of Na–K-ATPase in volume regulation

In a variety of tissues, recovery of cellular water content when metabolically inhibited cells have their metabolism restored appears not to be inhibited by cardiac glycosides (Macknight & Leaf, 1977). This observation, together with the lack of swelling of metabolizing cells incubated with ouabain (for example, Table 4, sodium chloride media), has led to the hypothesis that some mechanism other than the ouabain-inhibitable Na–K-ATPase is involved in volume regulation (Kleinzeller & Knotková, 1964; Macknight, 1968*a*; Whittembury & Proverbio, 1970). The recovery in volume when slices swollen in acetate medium are restored to sodium chloride medium, and the effects of ouabain on this recovery (Table 8) are, therefore, of importance.

The loss of cellular water which accompanies the return to sodium chloride medium can be presumed to result from the reversal of the diffusion gradient for undissociated acetic acid. As acetic acid diffuses from the cell, its cellular concentration will fall, and acetate concentration will also fall as new acetic acid is formed from cell acetate. The decrease in cellular acetate which results must be accompanied either by a loss of cellular cations or by a gain of extracellular chloride to maintain electroneutrality. With the Na–K-ATPase inhibited by a high concentration of ouabain (10^{-3} M), relatively little volume recovery occurred and tissue water content remained about 70% greater than in the slices incubated without ouabain. Though some cation was lost, there was also an appreciable uptake of chloride by the cells. This presumably replaced cell acetate. In contrast, in the absence of ouabain, volume and ionic

composition were restored to normal values. In particular, cellular chloride did not increase substantially above normal. This implies that Na–K-ATPase actively extruded sodium from the cells and played a key role in the process of recovery. (The loss of cellular potassium presumably reflects the tendency for potassium concentration to rise as cellular water decreases, with the increased concentration promoting net passive diffusion of potassium from the cells.)

The present results, therefore, favour the hypothesis that the Na–K-ATPase plays a major part in cellular volume regulation as well as in maintaining the normal cellular concentrations of potassium and sodium. They are, therefore, in agreement with the recent demonstration that, in species sensitive to cardiac glycosides, ouabain largely prevents the recovery in volume following cellular swelling induced by inhibition of metabolism (Cooke, 1981). The relatively small recovery in volume in ouabain-inhibited tissue cannot be taken as providing evidence for a contribution from an energy-dependent ouabain-insensitive mechanism but could simply reflect the relative rates at which acetate is lost from the cells (as acetic acid) and chloride is gained. These passive processes do not involve any energy-dependent extrusion of sodium.

In summary, the present results provide a description of a unique situation, gross cellular swelling in an isosmotic medium of normal potassium concentration, which is accompanied by a metabolically dependent, ouabain-inhibitable, accumulation of potassium. These results are readily interpretable in terms of plasma-membrane-based theories, assuming that membrane permeability to undissociated acetic acid is much greater than it is to the acetate ion. They are not, however, consistent with the association–induction hypothesis which seeks to explain cellular composition and volume in terms of specific properties of cellular proteins and their interrelationships with cell water. The results also argue against the suggestion that cellular swelling is, of itself, detrimental to cellular function and results in irreversible cellular damage, and they favour a predominant role of the ouabain-inhibitable Na–K-ATPase in volume regulation.

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