J. Physiol. (1977), **267**, *pp.* 113–136
Printed in Great Britain

EFFECTS OF REPLACING MEDIUM SODIUM BY CHOLINE, CAESIUM, OR RUBIDIUM, ON WATER AND ION CONTENTS OF RENAL CORTICAL SLICES

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

(*Received 16 July 1976*)

SUMMARY

1. Renal cortical slices from rat, rabbit, and guinea-pig were incubated in media in which choline, caesium or rubidium replaced sodium.

2. Slices of rabbit and guinea-pig renal cortex incubated in oxygenated choline Ringer decreased in volume initially and did not swell over 3 hr at 25° C. There was a steady loss of potassium. Inhibition of metabolism (N_2 + 1 mM iodoacetamide) caused some swelling. Ouabain, 10 mM, in choline Ringer affected neither loss of potassium nor tissue water content.

3. Slices of rat renal cortex similarly incubated in choline Ringer swelled over 3 hr at 25° C whether or not metabolism was inhibited; ouabain (15 mM) affected neither tissue potassium loss nor tissue water content.

4. Incubation in choline Ringer containing either 0·2 mM *p*-chloromercuribenzoic acid, or 1 mM ethacrynic acid increased the tissue water content of guinea-pig renal cortical slices.

5. Depletion of cellular potassium (by preliminary incubation in oxygenated potassium-free sodium Ringer with 10 mM ouabain at 30° C) resulted in increased tissue water content when rabbit renal cortical slices were subsequently incubated in oxygenated choline Ringer at 25° C for 3 hr.

6. There was no evidence of energy-dependent extrusion of water or ions from either equilibrated rat or rabbit renal cortical slices leached at 0·5° C and then reincubated at 25° C in choline Ringer.

7. Rat and guinea-pig renal cortical slices leached at 0·5° C and reincubated at 25° C swelled in rubidium Ringer and in caesium Ringer. There was no evidence of energy-dependent water or ion extrusion when metabolism was restored after leaching in either of these media. Metabolizing rat slices but not guinea-pig slices swelled faster than slices whose metabolism was inhibited.

8. These results lend no support to the mechano-chemical hypothesis which ascribes cellular volume regulation to a contractile mechanism squeezing isotonic extracellular fluid from the cells. Instead it is suggested that cellular water content in these experiments reflects the balance between the rate of loss of potassium (and chloride) from the cells and the rate of uptake of extracellular cation (and chloride) into the cells – these rates reflecting both the electrochemical potential gradients of the ions and membrane permeability to them. The implications in relation to the hypothesis of ouabain-insensitive cellular volume regulation are discussed.

INTRODUCTION

It is widely held that cellular water content remains constant despite the colloid osmotic effect of cellular macromolecules, this swelling force being offset by the exclusion of sodium from the cells (Leaf, 1956). This exclusion of sodium has been attributed to the activity of the sodium pump (Tosteson, 1964; Woodbury, 1965) which, using energy from metabolism provided by the hydrolysis of ATP through (Na–K)-activated, Mg-dependent ATPase (Skou, 1965) balances passive influx of sodium by active extrusion.

Over the past decade, since the work of Kleinzeller & Knotková (1964*a*, *b*), a number of workers using different preparations *in vitro* have challenged this hypothesis largely because cardiac glycosides, known inhibitors of Na–K ATPase, do not necessarily cause cellular swelling (kidney cortex cells, Kleinzeller & Knotková, 1964*a*; Macknight, 1968*a*; Maude, 1969; Whittembury, 1968; liver cells, Macknight, Pilgrim & Robinson, 1974; skeletal muscle, Kleinzeller & Knotková, 1964*b*; smooth muscle, Daniel & Robinson, 1971). Alternative hypotheses to explain the regulation of cellular volume have included a second cardiac glycoside-insensitive, ethacrynic acid-inhibited, sodium pump (Whittembury, 1968; Whittembury & Proverbio, 1970) and a mechano-chemical hypothesis (Kleinzeller & Knotková, 1964*a*, *b*; Kleinzeller, 1972) which attributes the regulation of cellular volume to some type of cellular contractile mechanism. Support for this latter hypothesis is claimed for some experimental observations in which sodium in the incubation medium has been replaced by other cations (Tris, choline, lithium; Kleinzeller, 1972). Under such conditions it is suggested that cellular volume continues to be regulated normally, showing that active sodium transport may not be necessary for volume regulation.

This paper reports the results of experiments in which slices from rat, rabbit and guinea-pig renal cortex have been incubated under a variety of conditions in which sodium has been replaced by choline, caesium or rudibium. Cooke (1975, 1976*a*, *b*) has recently provided preliminary reports of the effects of incubation of mammalian renal cortical slices in

media in which lithium or potassium replaced sodium. His observations are complementary to those presented here.

METHODS

Media. The sodium Ringer had the following composition (mM) Na^+, 145; K^+, 5; Ca^{2+}, 2·5; Mg^{2+}, 1; Cl^-, 133; SO_4^{2-}, 1; acetate, 10; buffered with phosphate 8; at pH 7·25. The osmolarity of this medium was 280 m-osmole. Choline, caesium, rubidium and potassium Ringer were prepared by substituting the appropriate cation for Na in the medium. Ouabain octahydrate (Sigma Chemical Co; U.S.A.), iodoacetamide (Koch–Light Laboratories Ltd, U.K.), *p*-chloromercuribenzoic acid (PCMB, Nutritional Biochemical Corp., U.S.A.) and ethacrynic acid (gift of Merck, Sharp and Dohme (NZ) Ltd) were dissolved in media immediately before use. Because of calcium precipitation, media containing 0·2 mM-PCMB had only 1·0 mM calcium. Control media in these experiments also contained only 1·0 mM calcium.

Procedure. Adult male rats, rabbits or guinea-pigs were stunned by a blow on the head. Kidneys were removed and placed in ice-cold sodium Ringer 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. Slices were immediately transferred to oxygenated sodium Ringer at 25° C where they remained, vigorously stirred, until all slices were cut. They were then placed in fresh oxygenated sodium Ringer at 25° C for 15 min. These slices are referred to in the text and Tables as equilibrated slices. They were then transferred to fresh media and incubated under the conditions shown in the Tables.

Whenever slices were transferred from sodium Ringer to sodium-free media they were first washed in a large volume (220 ml.) of the appropriate oxygenated sodium-free Ringer for 2 min before being incubated in the new medium. Incubation solutions were changed after 10, 30, and 60 min of incubation to ensure that the sodium concentration of the medium did not increase importantly. Initially, forty slices were incubated in 110 ml. medium. As slices were removed for analysis the number decreased but the volume remained at 110 ml. The sodium concentrations of the sodium-free media were checked by flame photometry at the end of each experiment and were never more than 0·22 mM. Sodium-free media to which 1 mM sodium iodoacetamide had been added contained appropriately more sodium.

Initially, the same experiments were performed with rat, rabbit and guinea-pig renal cortical slices. However, since rabbit and guinea-pig slices behaved very similarly but rat slices differed somewhat in their behaviour, subsequent experiments were usually performed with slices from rat and either guinea-pig or rabbit.

Analyses. Tissue water content was determined by loss of weight after drying for at least 2 hr in a hot air oven at 105° C (Little, 1964). Ions were extracted from the tissue overnight in 0·1 M-nitric acid (Little, 1964). Sodium and potassium in these acid extracts were measured with an EEL flame photometer, or, in experiments with caesium and rubidium media, cations were determined by atomic absorption spectroscopy. Technical difficulties prevented the accurate measurement of tissue rubidium contents. Chloride was measured by the method of Cotlove, Trantham & Bowman (1958) using an Aminco Chloride titrator.

Presentation of results. Tissue water contents are expressed in kg water/kg tissue dry wt., and ion contents are presented in m-mole/kg tissue dry wt. Cellular water and ion contents presented in the text for slices transferred from sodium to choline Ringer were calculated assuming that the extracellular space comprises 34% tissue water (McIver & Macknight, 1974). Small changes in tissue water in renal

cortical slices do not appreciably alter extracellular space expressed as a % tissue water. Tissue choline, caesium and rubidium contents were not measured in these experiments. However, the other main cations, sodium and potassium, together with tissue water and chloride contents, were always measured, and since the difference between the tissue (sodium and potassium) and the tissue chloride must reflect tissue choline content it is possible to quantify tissue choline content, at least approximately.

The values quoted in the text and tables are the mean ± S.E. of mean of the number of observations shown. The statistical significance of differences between groups has been determined using Student's t test.

RESULTS

Incubation of renal cortical slices in choline Ringer

Slices of rat (Table 1), rabbit (Table 2) and guinea-pig (Table 3) were incubated under aerobic conditions for up to 3 hr in either sodium Ringer, sodium Ringer containing ouabain, choline Ringer or choline Ringer containing ouabain. In the same experiments some slices were incubated anaerobically with 1 mM iodoacetamide to suppress metabolism.

In all three species tissue water and chloride contents remained constant in sodium Ringer under control aerobic conditions and also with ouabain; though ouabain caused a substantial gain in sodium and loss of potassium. In rabbit and guinea-pig slices these losses of potassium were comparable to those produced by metabolic inhibition with iodoacetamide. As well as causing potassium loss, metabolic inhibition resulted in marked tissue swelling with net uptake of water, sodium and chloride. These results, similar to those obtained previously in this laboratory and by others, served as control observations. The effects of ouabain were consistent with the hypothesis that cellular volume regulation involves a ouabain-insensitive mechanism.

There were important differences in behaviour between the slices from the three species incubated in choline Ringer. Slices of rat renal cortex did not maintain their volume but swelled over 3 hr incubation, gaining water and chloride, the rate of swelling being similar in oxygenated choline Ringer, oxygenated choline Ringer with ouabain, and under anaerobic conditions with iodoacetamide. Cells lost potassium throughout, the rate of loss being unaffected by ouabain, suggesting that the absence of medium sodium had effectively inhibited the ouabain-sensitive sodium–potassium pump. Though tissue choline content was not measured in these experiments, the large rapid loss of sodium in the first minutes of incubation must have been accompanied by a corresponding gain in choline, for neither cellular chloride nor water changed by amounts equivalent to the loss of sodium which occurred (in the first 10 min, sum of sodium lost + potassium lost = 141 m-mole/kg dry wt.; neither chloride, which decreased by

TABLE 1. The behaviour of rat renal cortical slices incubated for up to 3 hr in sodium or in choline Ringer (n = 8 for each group)

	Equilibrated (sodium Ringer)	Incubated (min)				
		10	30	60	120	180
		Water content (kg/kg dry wt.)				
Sodium Ringer	2·64 ± 0·04	2·68 ± 0·03	2·77 ± 0·07	2·70 ± 0·03	2·66 ± 0·06	2·61 ± 0·04
Sodium Ringer + 15 mM ouabain		2·62 ± 0·05	2·62 ± 0·02	2·46 ± 0·05	2·59 ± 0·04	2·72 ± 0·04
Sodium Ringer + 1 mM iodoacetamide		2·88 ± 0·07	3·19 ± 0·05	3·78 ± 0·03	4·48 ± 0·07	4·69 ± 0·05
		Sodium content (m-mole/kg dry wt.)				
Sodium Ringer	220 ± 6	194 ± 3	218 ± 12	196 ± 5	232 ± 7	231 ± 7
Sodium Ringer + 15 mM ouabain		258 ± 11	277 ± 7	281 ± 10	335 ± 20	360 ± 11
Sodium Ringer + 1 mM iodoacetamide		298 ± 5	390 ± 4	553 ± 36	669 ± 16	704 ± 17
		Potassium content (m-mole/kg dry wt.)				
Sodium Ringer	269 ± 5	287 ± 3	275 ± 10	293 ± 3	272 ± 9	280 ± 7
Sodium Ringer + 15 mM ouabain		221 ± 4	205 ± 5	200 ± 6	178 ± 4	158 ± 5
Sodium Ringer + 1 mM iodoacetamide		227 ± 5	180 ± 5	135 ± 7	105 ± 3	78 ± 3
		Chloride content (m-mole/kg dry wt.)				
Sodium Ringer	264 ± 8	240 ± 3	259 ± 11	245 ± 4	254 ± 7	263 ± 10
Sodium Ringer + 15 mM ouabain		236 ± 8	242 ± 6	232 ± 6	254 ± 7	285 ± 8
Sodium Ringer + 1 mM iodoacetamide		276 ± 6	330 ± 5	444 ± 8	575 ± 11	647 ± 10
		Water content (kg/kg dry wt.)				
Choline Ringer		2·71 ± 0·08	2·91 ± 0·07	3·08 ± 0·09	3·31 ± 0·12	3·62 ± 0·10
Choline Ringer + 15 mM ouabain		2·70 ± 0·04	2·74 ± 0·05	2·95 ± 0·11	3·20 ± 0·08	3·43 ± 0·08
Choline Ringer + 1 mM iodoacetamide		2·77 ± 0·03	2·88 ± 0·03	3·11 ± 0·06	3·43 ± 0·10	3·69 ± 0·07
		Sodium content (m-mole/kg dry wt.)				
Choline Ringer		9 ± 1	9 ± 2	9 ± 2	10 ± 3	5 ± 2
Choline Ringer + 15 mM ouabain		9 ± 2	13 ± 2	9 ± 1	10 ± 4	6 ± 1
Choline Ringer + 1 mM iodoacetamide		9 ± 2	14 ± 3	16 ± 4	16 ± 3	19 ± 5

TABLE 1 (*cont.*)

	Equilibrated (sodium Ringer)	Incubated (min) 10	30	60	120	180
		Potassium content (m-mole/kg dry wt.)				
Choline Ringer		220 ± 5	187 ± 4	160 ± 5	131 ± 6	101 ± 4
Choline Ringer + 15 mM ouabain		214 ± 3	186 ± 2	170 ± 3	130 ± 4	116 ± 4
Choline Ringer + 1 mM iodoacetamide		209 ± 6	180 ± 3	140 ± 6	103 ± 3	84 ± 5
		Chloride content (m-mole/kg dry wt.)				
Choline Ringer		234 ± 9	274 ± 18	309 ± 23	356 ± 21	425 ± 29
Choline Ringer + 15 mM ouabain		237 ± 6	242 ± 10	276 ± 18	334 ± 19	382 ± 16
Choline Ringer + 1 mM iodoacetamide		256 ± 5	295 ± 4	311 ± 11	403 ± 20	476 ± 17

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly distributed to the media shown. The media were continuously gassed by either oxygen, or, when iodoacetamide was present, nitrogen.

TABLE 2. The behaviour of rabbit renal cortical slices incubated for up to 3 hr in sodium or in choline Ringer (n = 6–8 for each group)

	Equilibrated (sodium Ringer)	Incubated (min) 10	30	60	120	180
		Water content (kg/kg dry wt.)				
Sodium Ringer	2·84 ± 0·09	2·72 ± 0·04	2·85 ± 0·11	2·84 ± 0·06	2·84 ± 0·08	2·78 ± 0·06
Sodium Ringer + 10 mM ouabain		2·61 ± 0·08	2·82 ± 0·06	2·94 ± 0·12	2·70 ± 0·05	2·77 ± 0·07
Sodium Ringer + 1 mM iodoacetamide		2·79 ± 0·09	2·89 ± 0·08	3·20 ± 0·14	4·63 ± 0·13	4·69 ± 0·10
		Sodium content (m-mole/kg dry wt.)				
Sodium Ringer	248 ± 10	255 ± 8	267 ± 7	265 ± 7	290 ± 8	298 ± 15
Sodium Ringer + 10 mM ouabain		344 ± 11	391 ± 12	432 ± 14	431 ± 11	436 ± 18
Sodium Ringer + 1 mM iodoacetamide		306 ± 8	396 ± 12	517 ± 17	794 ± 29	820 ± 15
		Potassium content (m-mole/kg dry wt.)				
Sodium Ringer	283 ± 2	311 ± 10	323 ± 9	323 ± 7	319 ± 10	316 ± 15
Sodium Ringer + 10 mM ouabain		209 ± 10	166 ± 6	146 ± 5	121 ± 7	110 ± 4
Sodium Ringer + 1 mM iodoacetamide		260 ± 7	191 ± 4	119 ± 7	98 ± 5	82 ± 6

TABLE 2 (*cont.*)

	Equilibrated (sodium Ringer)	Incubated (min) 10	30	60	120	180
		Chloride content (m-mole/kg dry wt.)				
Sodium Ringer	262 ± 12	276 ± 7	301 ± 9	306 ± 9	320 ± 16	321 ± 5
Sodium Ringer + 10 mM ouabain		286 ± 8	307 ± 6	328 ± 12	316 ± 12	320 ± 13
Sodium Ringer + 1 mM iodoacetamide		309 ± 9	303 ± 8	375 ± 15	700 ± 36	730 ± 15
		Water content (kg/kg dry wt.)				
Choline Ringer		2·50 ± 0·08	2·50 ± 0·08	2·67 ± 0·07	2·42 ± 0·07	2·60 ± 0·07
Choline Ringer + 10 mM ouabain		2·63 ± 0·06	2·44 ± 0·05	2·54 ± 0·04	2·57 ± 0·08	2·62 ± 0·09
Choline Ringer + 1 mM iodoacetamide		2·87 ± 0·16	2·65 ± 0·10	2·79 ± 0·09	3·27 ± 0·14	3·97 ± 0·14
		Sodium content (m-mole/kg dry wt.)				
Choline Ringer		29 ± 5	20 ± 2	15 ± 2	18 ± 3	16 ± 4
Choline Ringer + 10 mM ouabain		39 ± 6	19 ± 2	22 ± 2	18 ± 3	19 ± 5
Choline Ringer + 1 mM iodoacetamide		46 ± 4	29 ± 5	27 ± 5	21 ± 5	28 ± 8
		Potassium content (m-mole/kg dry wt.)				
Choline Ringer		246 ± 5	240 ± 4	217 ± 5	197 ± 4	162 ± 10
Choline Ringer + 10 mM ouabain		249 ± 4	224 ± 5	215 ± 4	185 ± 4	165 ± 2
Choline Ringer + 1 mM iodoacetamide		249 ± 3	199 ± 6	136 ± 2	115 ± 2	93 ± 3
		Chloride content (m-mole/kg dry wt.)				
Choline Ringer		215 ± 9	230 ± 10	228 ± 4	237 ± 11	247 ± 8
Choline Ringer + 10 mM ouabain		225 ± 7	205 ± 10	219 ± 3	222 ± 8	239 ± 12
Choline Ringer + 1 mM iodoacetamide		259 ± 14	240 ± 10	271 ± 14	392 ± 17	499 ± 22

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly distributed to the media shown. The media were continuously gassed by either oxygen, or, when iodoacetamide was present, nitrogen.

TABLE 3. The behaviour of guinea-pig renal cortical slices incubated for up to 3 hr in sodium or in choline Ringer (n = 8 for each group)

	Equilibrated (sodium Ringer)	Incubated (min) 10	30	60	120	180
		Water content (kg/kg dry wt.)				
Sodium Ringer	2·69 ± 12	2·69 ± 0·09	2·50 ± 0·06	2·69 ± 0·09	2·46 ± 0·09	2·47 ± 0·09
Sodium Ringer + 10 mM ouabain		2·58 ± 0·08	2·83 ± 0·08	2·52 ± 0·04	2·51 ± 0·04	2·47 ± 0·09
Sodium Ringer + 1 mM iodoacetamide		3·00 ± 0·12	3·16 ± 0·11	3·06 ± 0·06	3·63 ± 0·08	4·42 ± 0·07
		Sodium content (m-mole/kg dry wt.)				
Sodium Ringer	207 ± 12	203 ± 13	207 ± 8	219 ± 9	230 ± 10	250 ± 13
Sodium Ringer + 10 mM ouabain		290 ± 7	352 ± 9	343 ± 7	371 ± 9	396 ± 15
Sodium Ringer + 1 mM iodoacetamide		263 ± 10	319 ± 13	414 ± 5	537 ± 31	635 ± 41
		Potassium content (m-mole/kg dry wt.)				
Sodium Ringer	279 ± 20	283 ± 11	255 ± 9	284 ± 13	258 ± 16	263 ± 20
Sodium Ringer + 10 mM ouabain		204 ± 16	169 ± 12	114 ± 4	116 ± 5	100 ± 8
Sodium Ringer + 1 mM iodoacetamide		283 ± 14	239 ± 17	146 ± 9	114 ± 8	104 ± 5
		Chloride content (m-mole/kg dry wt.)				
Sodium Ringer	203 ± 9	209 ± 8	195 ± 6	214 ± 6	216 ± 6	224 ± 9
Sodium Ringer + 10 mM ouabain		215 ± 7	236 ± 9	230 ± 4	241 ± 9	265 ± 15
Sodium Ringer + 1 mM iodoacetamide		238 ± 12	263 ± 9	288 ± 6	416 ± 15	590 ± 25
		Water content (kg/kg dry wt.)				
Choline Ringer		2·35 ± 0·11	2·26 ± 0·07	2·25 ± 0·11	2·77 ± 0·05	2·69 ± 0·07
Choline Ringer + 10 mM ouabain		2·32 ± 0·08	2·34 ± 0·11	2·36 ± 0·05	2·38 ± 0·05	2·30 ± 0·07
Choline Ringer + 1 mM iodoacetamide		2·50 ± 0·06	2·48 ± 0·09	2·64 ± 0·08	2·99 ± 0·11	3·24 ± 0·0
		Sodium content (m-mole/kg dry wt.)				
Choline Ringer		24 ± 2	23 ± 3	16 ± 2	16 ± 3	14 ± 2
Choline Ringer + 10 mM ouabain		27 ± 1	20 ± 1	16 ± 2	14 ± 2	12 ± 2
Choline Ringer + 1 mM iodoacetamide		24 ± 2	20 ± 2	17 ± 2	15 ± 2	12 ± 2

TABLE 3 (*cont.*)

	Equilibrated (sodium Ringer)	Incubated (min)				
		10	30	60	120	180
		Potassium content (m-mole/kg dry wt.)				
Choline Ringer		241 ± 15	200 ± 10	200 ± 14	189 ± 8	169 ± 25
Choline Ringer + 10 mM ouabain		246 ± 20	223 ± 12	205 ± 15	191 ± 19	148 ± 21
Choline Ringer + 1 mM iodoacetamide		242 ± 16	197 ± 11	161 ± 9	143 ± 18	84 ± 9
		Chloride content (m-mole/kg dry wt.)				
Choline Ringer		172 ± 10	170 ± 4	168 ± 8	147 ± 3	159 ± 6
Choline Ringer + 10 mM ouabain		165 ± 4	162 ± 7	163 ± 4	193 ± 7	187 ± 4
Choline Ringer + 1 mM iodoacetamide		182 ± 4	196 ± 6	220 ± 6	322 ± 30	402 ± 27

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly distributed to the media shown. The media were continuously gassed by either oxygen, or, when iodoacetamide was present, nitrogen.

5 m-mole/kg dry wt., nor cellular water content, which increased by 0·02 kg/kg dry wt., changed significantly). Maizels & Remington (1958) have shown that choline enters cells in rat renal cortical slices.

Since slices whose metabolism was inhibited swelled more rapidly in sodium Ringer than in choline Ringer, the cells under these conditions must have been more permeable to sodium than to choline (chloride being the major extracellular anion in both media, this difference in rate of swelling could not reflect variation in anion permeability). However, cellular potassium loss was virtually identical in the two media under conditions of metabolic inhibition. This suggests that loss of cellular potassium was limited not by the rate of entry of cation to the cells (sodium or choline) but by the rate at which potassium could leave the cells.

In contrast to the tissue swelling observed in rat slices, neither rabbit nor guinea-pig slices swelled over three hours in oxygenated choline Ringer, with or without ouabain. Indeed in both species in the first 10 min there were initially significant losses of water ($P < 0·05$) (rabbit lost 0·23 kg/kg dry wt., guinea-pig 0·22 kg/kg dry wt.) and of chloride (32 and 15 m-mole/kg dry wt. respectively) so that some of the cellular cation lost (sodium and potassium) was accompanied by loss of chloride and water rather than exchanged for choline. As in the rat, potassium loss in choline Ringer was unaffected by ouabain, an observation which is consistent with

the hypothesis that removal of medium sodium is as effective an inhibitor of the Na–K ATPase as is ouabain under these conditions. Whittam & Willis (1963) reported a similar result in rabbit renal cortical slices incubated in choline Ringer at 38° C with and without ouabain.

In contrast to the initial shrinkage and lack of swelling in oxygenated slices, metabolic inhibition in choline Ringer caused some swelling in both rabbit and guinea-pig slices though such swelling, like that in the rat slices, was markedly less than was observed in slices whose metabolism was inhibited in sodium Ringer.

TABLE 4. The behaviour of equilibrated rat renal cortical slices leached and reincubated in sodium or in choline Ringer (n = 7–8)

	Water content (kg/kg dry wt.)	Sodium content	Potassium content	Chloride content
		(m-mole/kg dry wt.)		
Equilibrated	2·64 ± 0·04	246 ± 8	249 ± 7	240 ± 8
Leached and reincubated in sodium Ringer				
Leached 150 min	3·02 ± 0·08	360 ± 13	183 ± 4	295 ± 11
Reincubated				
2 min	2·70 ± 0·05	281 ± 5	227 ± 3	259 ± 6
10 min	2·73 ± 0·07	269 ± 12	244 ± 7	251 ± 8
30 min	2·67 ± 0·05	233 ± 8	271 ± 6	247 ± 10
60 min	2·97 ± 0·09	258 ± 8	288 ± 5	303 ± 19
Leached and reincubated in choline Ringer				
Leached 150 min				
Reincubated	2·83 ± 0·05	13 ± 2	184 ± 2	238 ± 9
2 min	2·77 ± 0·03	12 ± 3	174 ± 3	237 ± 5
10 min	2·72 ± 0·06	10 ± 2	158 ± 2	239 ± 11
30 min	2·90 ± 0·05	11 ± 4	141 ± 4	282 ± 8
60 min	2·98 ± 0·06	5 ± 1	125 ± 5	301 ± 13

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly transferred either to sodium or to choline Ringer at 0·5° C gassed with nitrogen where they remained 150 min. They were then reincubated in oxygenated medium, of the same composition in which they had been leached, for up to 60 min at 25° C.

Considered together these results suggested that the cell membranes of renal cortical cells from different species differed somewhat in their permeability to choline, those in the rat being more permeable than those in rabbit or guinea-pig. It seemed possible that metabolic inhibition in rabbit and guinea-pig increased membrane permeability to choline, the resultant swelling thus reflecting increased diffusional entry of choline into cells rather than inhibition of a metabolically dependent mechanism excluding choline as it would other cations and thus regulating cellular volume.

To examine this problem further, experiments were performed to investigate the changes in tissue water content when slices from rat or rabbit equilibrated at 25° C in oxygenated sodium Ringer were incubated at 0·5° C under anaerobic conditions (leaching) in either sodium or choline Ringer, and then reincubated at 25° C in oxygenated medium of the same composition in which they had been leached (since rabbit and guinea-pig renal cortical slices have behaved so similarly in the preceding series of experiments only rabbit slices were compared with rat slices in this group of experiments). Table 4 shows the effects on rat renal cortical slices of leaching for 2·5 hr and reincubation for up to 60 min. Table 5 contains the

TABLE 5. The behaviour of equilibrated rabbit renal cortical slices leached and reincubated in sodium or in choline Ringer (n = 8)

	Water content (kg/kg dry wt.)	Sodium content	Potassium content	Chloride content
		(m-mole/kg dry wt.)		
Equilibrated	3·12 ± 0·07	240 ± 8	314 ± 4	265 ± 6
Leached and reincubated in sodium Ringer				
Leached 300 min	3·72 ± 0·03	325 ± 7	271 ± 6	353 ± 10
Reincubated				
2 min	3·22 ± 0·07	299 ± 5	276 ± 4	275 ± 5
10 min	3·04 ± 0·05	269 ± 10	286 ± 5	260 ± 6
30 min	3·00 ± 0·09	301 ± 16	279 ± 5	274 ± 11
60 min	3·12 ± 0·06	299 ± 10	271 ± 5	278 ± 7
Leached and reincubated in choline Ringer				
Leached 300 min	2·98 ± 0·08	25 ± 3	188 ± 6	287 ± 1
Reincubated				
2 min	3·11 ± 0·05	32 ± 3	175 ± 6	313 ± 8
10 min	3·30 ± 0·6	23 ± 1	153 ± 7	334 ± 12
30 min	3·14 ± 0·11	25 ± 3	150 ± 3	320 ± 11
60 min	3·12 ± 0·11	18 ± 3	125 ± 7	320 ± 15

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly transferred either to sodium or to choline Ringer at 0·5° C, gassed with nitrogen, where they remained 300 min. They were then reincubated in oxygenated medium, of the same composition in which they had been leached, for up to 60 min at 25° C.

results for rabbit slices. Since it has been found in this laboratory that rabbit slices equilibrated at 25° C in oxygenated medium before leaching hardly swell after 2·5 hr at 0·5° C (K. R. Cooke, unpublished observations) the rabbit slices were leached 5 hr before being reincubated for up to 60 min. Slices from both species leached in sodium Ringer showed the expected tissue swelling with uptake of water, sodium and chloride and loss of potassium, changes which were rapidly reversed by restoration of

metabolism in oxygenated sodium Ringer at 25° C. In contrast, however, rat slices leached in choline Ringer did not swell as much though they lost both sodium and potassium in exchange for choline (since tissue chloride did not alter significantly). During reincubation there was no detectable extrusion of water or chloride. Slices continued to lose potassium and gained some water and chloride over the 60 min. Slices of rabbit renal cortex did not swell over 5 hr of leaching in choline Ringer and again there was no evidence of net extrusion of water or chloride when metabolism was restored.

TABLE 6. The behaviour of guinea-pig renal cortical slices incubated for up to 3 hr in media with or without PCMB, 0·2 mM (n = 8 for each group)

	Water content (kg/kg dry wt.)	Sodium content	Potassium content (m-mole/kg dry wt.)	Chloride content
Equilibrated	3·24 ± 0·06	245 ± 5	327 ± 9	224 ± 2
Incubated in sodium Ringer				
60 min	2·92 ± 0·06	235 ± 7	310 ± 7	230 ± 11
180 min	2·73 ± 0·04	220 ± 3	306 ± 4	208 ± 3
Incubated in sodium Ringer + PCMB				
10 min	3·03 ± 0·05	248 ± 5	315 ± 4	234 ± 11
30 min	3·12 ± 0·07	291 ± 16	289 ± 12	241 ± 10
60 min	3·02 ± 0·05	277 ± 7	288 ± 6	229 ± 6
120 min	3·13 ± 0·12	353 ± 21	258 ± 6	272 ± 17
180 min	3·30 ± 0·08	396 ± 13	246 ± 6	318 ± 8
Incubated in choline Ringer				
60 min	2·67 ± 0·05	20 ± 3	245 ± 2	174 ± 6
180 min	2·64 ± 0·04	19 ± 2	190 ± 6	178 ± 4
Incubated in choline Ringer + PCMB				
10 min	2·85 ± 0·10	35 ± 3	283 ± 10	187 ± 6
30 min	2·61 ± 0·03	34 ± 3	238 ± 8	175 ± 5
60 min	2·65 ± 0·04	28 ± 2	212 ± 4	182 ± 6
120 min	2·91 ± 0·05	23 ± 3	179 ± 9	227 ± 11
180 min	3·06 ± 0·06	19 ± 3	153 ± 5	268 ± 8

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly transferred to the oxygenated media shown where they remained up to 180 min. Since the full time course of behaviour in sodium and choline Ringer had already been investigated (Table 3), only 60 and 180 min analyses were made of controls in media without PCMB.

These results thus provided no evidence for metabolically dependent volume regulation producing extrusion of choline and thereby chloride and water from the cells. Instead, they reinforced the belief that the behaviour of slices incubated in choline Ringer reflected the permeability of the cellular membranes to choline.

One test of such an hypothesis is to alter membrane permeability and examine the behaviour of slices incubated in choline Ringer. Slices of guinea-pig renal cortex were equilibrated in oxygenated sodium Ringer at 25° C and then transferred to either sodium Ringer, sodium Ringer containing 0·2 mM-PCMB, choline Ringer or choline Ringer containing 0·2 mM-PCMB. Table 6 shows the results of these experiments. PCMB, a non-diuretic mercurial, increases cellular membrane permeability causing cellular swelling but seems not to block the mechanisms involved in energy-dependent volume regulation (Macknight, 1968*b*). All slices in choline Ringer showed initial shrinkage. However, in contrast to slices incubated in choline Ringer alone, the presence of PCMB resulted in eventual tissue swelling with gain of water and chloride and some increased loss of potassium. A similar pattern was seen in the slices incubated in sodium Ringer with PCMB. These results were therefore consistent with the hypothesis that the water and ion contents of slices incubated in choline Ringer reflected membrane permeability to choline rather than exclusion of choline from the cells by an energy-dependent mechanism.

It has been suggested from work with guinea-pig slices (Whittembury & Proverbio, 1970) that cellular volume regulation in renal cortical tissue involves a ouabain-insensitive second sodium pump specifically inhibited by ethacrynic acid. Alternatively, the effects of ethacrynic acid on renal slices have been ascribed to an inhibition of cellular metabolism together with an increased membrane permeability (Macknight, 1969; Poat, Poat & Munday, 1970; Epstein, 1972). It was therefore of interest to examine the effects of ethacrynic acid on guinea-pig cortical slices incubated in choline Ringer (Table 7), for in the absence of medium sodium the inhibition by ethacrynic acid of a specific sodium pump should be without effect on cellular volume. It is obvious however that ethacrynic acid resulted in substantial cellular swelling in choline Ringer. Even greater cellular swelling was observed in slices incubated in sodium Ringer with ethacrynic acid. The effects of ethacrynic acid in choline Ringer are therefore consistent with an important effect of this diuretic on membrane permeability either directly or secondary to inhibition of cellular metabolism.

Because cellular potassium concentration might be important in determining the rate of cellular swelling in choline Ringer (see Discussion), an experiment was performed to examine tissue water content in guinea-pig slices depleted before exposure to choline Ringer. Table 8 shows that some increase in tissue water and chloride occurred in potassium-depleted slices in contrast to the behaviour of the control tissue which had not been potassium-depleted.

Incubation of renal cortical slices in caesium or rubidium Ringer

Slices of rat or guinea-pig renal cortex were leached and reincubated in either sodium or caesium Ringer, with or without iodoacetamide. In both species there was a greater cellular swelling in caesium Ringer than in

TABLE 7. The behaviour of guinea-pig renal cortical slices incubated for up to 3 hr in media with or without ethacrynic acid, 1 mM (n = 8 for each group)

	Water content (kg/kg dry wt.)	Sodium content	Potassium content	Chloride content
		(m-mole/kg dry wt.)		
Equilibrated	3·12 ± 0·10	233 ± 10	308 ± 13	221 ± 6
Incubated in sodium Ringer				
60 min	2·84 ± 0·11	202 ± 8	328 ± 16	207 ± 9
180 min	2·83 ± 0·05	225 ± 3	325 ± 8	215 ± 4
Incubated in sodium Ringer + ethacrynic acid				
10 min	2·92 ± 0·09	244 ± 8	313 ± 13	219 ± 8
30 min	3·05 ± 0·10	275 ± 9	300 ± 13	229 ± 8
60 min	3·54 ± 0·11	462 ± 18	203 ± 7	335 ± 13
120 min	4·62 ± 0·17	679 ± 34	158 ± 13	541 ± 28
180 min	5·80 ± 0·27	935 ± 43	93 ± 5	792 ± 40
Incubated in choline Ringer				
60 min	2·52 ± 0·04	21 ± 2	259 ± 9	172 ± 3
180 min	2·46 ± 0·07	17 ± 2	197 ± 12	183 ± 5
Incubated in choline Ringer + ethacrynic acid				
10 min	2·63 ± 0·08	34 ± 5	285 ± 11	199 ± 7
30 min	2·77 ± 0·05	28 ± 3	285 ± 10	191 ± 6
60 min	2·82 ± 0·12	42 ± 10	192 ± 10	244 ± 14
120 min	3·57 ± 0·16	21 ± 3	132 ± 13	415 ± 35
180 min	4·53 ± 0·19	25 ± 5	69 ± 5	633 ± 32

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly transferred to the oxygenated media shown where they remained up to 180 min. Since the full time course of behaviour in sodium and choline Ringer had already been investigated (Table 3), only 60 and 180 min analyses were made of controls in media without ethacrynic acid.

sodium Ringer during leaching (Table 9). Restoration of cellular volume occurred as expected in metabolizing slices in oxygenated sodium Ringer at 25° C. There was little evidence of any metabolically dependent volume regulation in metabolizing slices in caesium Ringer in either species. One unexpected finding was the greater swelling of metabolizing rat renal cortical slices in caesium Ringer when compared with slices from the same group of animals whose metabolism was inhibited. This was also observed in rat slices incubated in rubidium Ringer (Table 10) and has been found

in rat slices incubated in potassium Ringer (K. R. Cooke, unpublished observations). It was not observed in guinea-pig slices in caesium or rubidium Ringer. One possible explanation for this observation is that caesium, rubidium and potassium ions enter the cells to some extent by a

TABLE 8. The behaviour of rabbit renal cortical slices depleted of potassium and incubated in choline Ringer (n = 8 for each group)

	Water content (kg/kg dry wt.)	Sodium content	Potassium content	Chloride content
		(m-mole/kg dry wt.)		
Equilibrated	3·40 ± 0·07	295 ± 9	308 ± 7	313 ± 6
Incubated for 60 min at 30° C				
Sodium Ringer	3·19 ± 0·06	260 ± 8	327 ± 5	311 ± 10
Potassium-free sodium Ringer + 10 mM ouabain	2·85 ± 0·05	458 ± 10	129 ± 5	295 ± 8
Transferred from sodium Ringer at 30° C to sodium Ringer at 25° C				
Incubated 30 min	3·20 ± 0·04	252 ± 6	321 ± 4	303 ± 6
60 min	3·22 ± 0·09	253 ± 8	334 ± 8	307 ± 8
120 min	3·35 ± 0·12	263 ± 15	350 ± 8	311 ± 13
180 min	3·14 ± 0·04	243 ± 6	321 ± 10	294 ± 2
Transferred from sodium Ringer at 30° C to choline Ringer at 25° C				
Incubated 30 min	2·94 ± 0·07	22 ± 1	271 ± 7	261 ± 6
60 min	2·85 ± 0·06	18 ± 2	231 ± 14	249 ± 6
120 min	2·89 ± 0·04	24 ± 4	241 ± 4	262 ± 13
180 min	2·94 ± 0·03	14 ± 1	207 ± 6	269 ± 5
Transferred from potassium-free sodium Ringer + ouabain at 30° C to choline Ringer at 25° C				
Incubated 30 min	2·59 ± 0·09	43 ± 3	140 ± 4	253 ± 8
60 min	2·78 ± 0·07	32 ± 1	145 ± 6	275 ± 9
120 min	2·99 ± 0·04	18 ± 1	138 ± 4	296 ± 9
180 min	3·30 ± 0·04	16 ± 1	133 ± 4	351 ± 6

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly transferred either to oxygenated sodium Ringer (controls) or to oxygenated potassium-free sodium Ringer containing 10 mM ouabain to deplete them of potassium at 30° C. After 60 min, control slices were transferred either to oxygenated sodium or choline Ringer at 25° C. Slices depleted of potassium were transferred to oxygenated choline Ringer at 25° C.

carrier mechanism which depends for its integrity and/or activity on cellular metabolism. Some support for this suggestion comes from the observation (A. D. C. Macknight, unpublished observations) that, even in the presence of 150 mM potassium, ouabain in the medium slowed the rate of swelling of rat renal cortical slices in a potassium Ringer.

Table 10 shows the water and chloride contents of preincubated rat and guinea-pig slices leached and reincubated in rubidium Ringer. Slices in

TABLE 9. The behaviour of equilibrated rat or guinea-pig renal cortical slices leached and reincubated in sodium or caesium Ringer (n = 7–8 for each group)

	Sodium Ringer		Sodium Ringer + 1 mM iodoacetamide		Caesium Ringer		Caesium Ringer + 1 mM iodoacetamide	
	Water content (kg/kg dry wt.)	Chloride content (m-mole/kg dry wt.)	Water content (kg/kg dry wt.)	Chloride content (m-mole/kg dry wt.)	Water content (kg/kg dry wt.)	Chloride content (m-mole/kg dry wt.)	Water content (kg/kg dry wt.)	Chloride content (m-mole/kg dry wt.)
Rat slices								
Equilibrated	2·72 ± 0·03	229 ± 4	—	—	—	—	—	—
Leached and reincubated leached 150 min	3·34 ± 0·09	332 ± 14	3·39 ± 0·11	328 ± 14	3·59 ± 0·11	335 ± 10	3·84 ± 0·10	391 ± 10
Reincubated								
2 min	—	—	—	—	3·51 ± 0·06	343 ± 6	3·71 ± 0·07	383 ± 9
10 min	2·69 ± 0·08	245 ± 12	3·29 ± 0·16	323 ± 14	3·87 ± 0·11	421 ± 15	3·93 ± 0·03	408 ± 4
30 min	—	—	—	—	4·80 ± 0·07	553 ± 9	4·02 ± 0·05	441 ± 7
60 min	2·63 ± 0·04	252 ± 5	4·14 ± 0·11	510 ± 13	5·18 ± 0·12	652 ± 12	4·47 ± 0·07	551 ± 22
Guinea-pig slices								
Equilibrated	3·04 ± 0·07	235 ± 6	—	—	—	—	—	—
Leached and reincubated leached 150 min	3·29 ± 0·07	268 ± 11	3·30 ± 0·06	279 ± 12	3·65 ± 0·05	292 ± 8	3·63 ± 0·04	292 ± 9
Reincubated								
2 min	—	—	—	—	3·48 ± 0·07	271 ± 9	3·75 ± 0·6	323 ± 10
10 min	3·15 ± 0·05	253 ± 8	3·30 ± 0·05	277 ± 5	3·48 ± 0·04	272 ± 7	3·64 ± 0·07	317 ± 7
30 min	—	—	—	—	3·63 ± 0·08	315 ± 13	4·05 ± 0·08	376 ± 10
60 min	3·11 ± 0·05	234 ± 7	3·73 ± 0·06	371 ± 4	4·13 ± 0·05	409 ± 11	4·36 ± 0·07	425 ± 7

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly transferred to the media shown, at 0·5 °C, gassed with nitrogen where they remained 150 min. They were then reincubated in media of the same composition in which they had been leached, for up to 60 min at 25° C, either oxygenated (media without iodoacetamide) or with nitrogen bubbled through the medium.

TABLE 10. The behaviour of equilibrated rat or guinea-pig renal cortical slices leached and reincubated in sodium or rubidium Ringer (n = 7–8 for each group)

	Sodium Ringer		Sodium Ringer + 1 mM iodoacetamide		Rubidium Ringer		Rubidium Ringer + 1 mM iodoacetamide	
	Water content (kg/kg dry wt.)	Chloride content (m-mole/kg dry wt.)	Water content (kg/kg dry wt.)	Chloride content (m-mole/kg dry wt.)	Water content (kg/kg dry wt.)	Chloride content (m-mole/kg dry wt.)	Water content (kg/kg dry wt.)	Chloride content (m-mole/kg dry wt.)
Rat slices								
Equilibrated	2·83 ± 0·11	263 ± 11	—	—	—	—	—	—
Leached and reincubated leached 150 min	3·29 ± 0·11	353 ± 17	3·39 ± 0·20	337 ± 26	4·40 ± 0·17	499 ± 15	4·49 ± 0·05	571 ± 7
Reincubated								
2 min	—	—	—	—	4·53 ± 0·11	521 ± 9	4·57 ± 0·08	450 ± 8
10 min	2·83 ± 0·08	270 ± 7	3·31 ± 0·13	357 ± 17	4·93 ± 0·11	608 ± 14	4·74 ± 0·17	578 ± 11
30 min	—	—	—	—	5·18 ± 0·12	663 ± 12	4·80 ± 0·13	607 ± 12
60 min	2·77 ± 0·04	270 ± 4	4·95 ± 0·25	661 ± 38	5·74 ± 0·07	739 ± 13	5·19 ± 0·08	658 ± 19
Guinea-pig slices								
Equilibrated	3·03 ± 0·05	231 ± 7	—	—	—	—	—	—
Leached and reincubated leached 150 min	3·45 ± 0·08	305 ± 11	3·70 ± 0·09	344 ± 15	4·76 ± 0·10	488 ± 10	4·78 ± 0·08	522 ± 10
Reincubated								
2 min	—	—	—	—	4·69 ± 0·11	494 ± 14	4·85 ± 0·09	539 ± 8
10 min	3·17 ± 0·07	311 ± 8	3·37 ± 0·10	340 ± 10	4·64 ± 0·11	533 ± 10	5·31 ± 0·11	585 ± 11
30 min	—	—	—	—	5·68 ± 0·19	682 ± 28	5·44 ± 0·10	632 ± 16
60 min	2·86 ± 0·08	254 ± 8	4·31 ± 0·09	450 ± 7	5·91 ± 0·21	737 ± 23	5·95 ± 0·15	676 ± 16

All slices were first equilibrated in oxygenated sodium Ringer at 25° C. They were then randomly transferred to the media shown, at 0·5° C, gassed with nitrogen where they remained 150 min. They were then reincubated in media of the same composition in which they had been leached, for up to 60 min at 25° C, either oxygenated (media without iodoacetamide) or with nitrogen bubbled through the medium.

rubidium Ringer swelled more during leaching. Again there was no evidence to suggest any metabolically dependent volume-regulating mechanism which could extrude rubidium, and thereby chloride and water, from the cells.

DISCUSSION

The experimental results presented in this paper reveal the following.

(*a*) Slices of rabbit and guinea-pig renal cortex incubated in oxygenated choline Ringer did not swell over 3 hr at 25° C; instead there was an initial small decrease in tissue water with losses of sodium, potassium and chloride (Tables 2 and 3). Some at least of that sodium lost from the non-inulin space must have been replaced by choline for the losses of 'cellular' (i.e. non-inulin) cations (sodium plus potassium), exceeded the loss of diffusible anion (Cl).

(*b*) Slices of rabbit and guinea-pig renal cortex incubated in choline Ringer under conditions of metabolic inhibition by iodoacetamide swelled gradually over 3 hr at 25° C, though again there may have been some small initial decrease in cellular volume (Tables 2 and 3).

(*c*) Slices of rat renal cortex incubated in choline Ringer swelled over 3 hr whether or not metabolism was inhibited (Table 1).

(*d*) Slices from all species lost as much potassium at the same rate in choline Ringer as they did in choline Ringer containing ouabain. Metabolic inhibition caused a further loss of potassium (Tables 1–3).

(*e*) Slices of rat and rabbit renal cortex leached in choline Ringer showed a smaller increase (rat) or a decrease (rabbit) in tissue water when compared with slices leached in sodium Ringer. During 60 min reincubation in choline Ringer tissue water tended to increase but the changes were small. Loss of tissue water was observed in sodium Ringer (Tables 4 and 5).

(*f*) Slices of rat and guinea-pig cortex leached in caesium or rubidium Ringer swelled more than those leached in sodium Ringer, the swelling in rubidium Ringer being greater than that in caesium Ringer. Reincubation under metabolically favourable conditions did not reverse this swelling. Instead, further swelling occurred, the rate of such swelling in the guinea-pig slices being unaffected by metabolism. In the rat slices however, swelling occurred more rapidly in metabolizing slices (Tables 9 and 10).

These experiments were performed to investigate the hypothesis that cellular volume regulation involves a mechano-chemical mechanism which utilizes metabolic energy to exclude ions and water from the cells by means other than a sodium pump (Kleinzeller, 1972). As support for this hypothesis it has been claimed that cells in rabbit renal cortical slices maintain their volume as well in Ringer in which sodium has been replaced by choline, lithium or Tris as they do in sodium Ringer (Kleinzeller &

Knotková, 1964*a*; Kleinzeller, 1972). Our results do not support this hypothesis, for volume regulation should be seen also in media containing rubidium and caesium rather than sodium, as well as in media in which sodium was completely replaced by potassium (Cooke, 1975). Furthermore, slices from all species should have maintained their water contents when incubated in choline Ringer, but rat renal cortical slices swelled in choline Ringer as they do also in lithium Ringer (Cooke, 1976*a*, *b*). A mechano-contractile mechanism playing an important and fundamental role in cell volume regulation is unlikely to function in some species but not in others.

What alternative explanations are there for the relative constancy of tissue water content in slices incubated in choline and lithium Ringer (Cooke, 1976*a*, *b*) and for the swelling in caesium, rubidium and potassium Ringer? It is clear that such constancy of volume in choline and lithium Ringer does not involve the sodium pump directly, for the sodium–potassium ATPase is stimulated neither by choline nor by lithium (Hokin & Dahl, 1972; Skou, 1975), the medium is sodium-free, the cellular sodium is very low and it changes very little throughout the experiments. Furthermore, ouabain which is a specific inhibitor of the sodium–potassium ATPase, did not increase the loss of potassium from slices incubated in choline Ringer, supporting the claim that the substitution of choline for sodium had already led to maximum inhibition of the sodium–potassium ATPase.

However, to explain these results, it is not necessary to postulate either a mechano-chemical mechanism nor a sodium pump. The results fit a pattern whereby ions to which the membrane is relatively less permeable (as judged by the rate of swelling when metabolism is inhibited), i.e. choline and lithium (Cooke, 1976*a*, *b*), cause little or no swelling, whereas the more permeable caesium and rubidium (and potassium, Cooke, 1975) cause swelling. Such behaviour is in fact predicted from the theory of colloid osmotic cellular swelling (Leaf, 1956) and the pump-leak hypothesis formulated by Post & Jolly (1957) and developed by Tosteson & Hoffman (1960). The steady-state volume is determined by the rate of ion transport by the pump and the rate of net ion leakage across the cells, the latter reflecting both the electrochemical potential gradient affecting the ions and the membrane permeability to them. As Stein (1967) has pointed out, so long as cellular potassium is held in the steady state at a concentration greater than that required for electrochemical equilibrium (and this seems to be true in renal cortical slices, Whittembury, 1965; Proverbio & Whittembury, 1975) the rate of change in cellular volume when metabolism is inhibited, or, as in the present experiments, when extracellular sodium is replaced by another cation, will reflect the difference between the rate at which cellular cations (mainly potassium but also sodium) and chloride

diffuse from the cells and the rate at which extracellular cation and chloride diffuse into the cells (if chloride is distributed at electrochemical equilibrium in the metabolizing cells at a steady state then the rate of volume change will reflect only the difference between the rate of cation loss and of cation gain). In the first minutes after changing medium cation composition, an extremely large electrochemical gradient must exist driving sodium from the cell and the new extracellular cation into the cell. The relative permeabilities of the membrane to these cations must initially influence the cellular volume. Thereafter, so long as potassium (and chloride) leave the cells as fast as cation (and chloride) enter, volume will remain constant. If potassium (and chloride) are lost more rapidly than cation (and chloride) enter, cellular volume will decrease (e.g. rabbit and guinea-pig renal cortical cells in choline Ringer in the present experiments); if potassium (and chloride) are lost more slowly than cation (and chloride) are gained, cellular volume will increase (e.g. slices in caesium, rubidium and potassium). It is important to stress that ultimately, as the potassium (chloride) gradient initially created by cellular metabolism is depleted, cells must swell no matter what the major extracellular cation (so long as the membrane has any permeability to it) as a consequence of the colloid osmotic force exerted by cellular macromolecules to which the membrane is impermeable (Leaf, 1956).

This hypothesis would explain the behaviour of metabolizing renal cortical slices to choline, lithium, caesium, rubidium or potassium Ringer. It would mean that the rate of entry (a reflexion of both the electrochemical gradient and of the membrane permeability) of choline together with chloride was slower than, of lithium about the same as, and of potassium, rubidium and caesium greater than, the rate of loss of potassium and chloride from the cells (after the first minutes cellular sodium–medium cation exchange had become minimal and could not contribute measurably thereafter to the changes in cellular volume).

One has then to explain the increased water content of metabolically inhibited rabbit and guinea-pig renal cortical slices incubated in choline Ringer. Such swelling is, as discussed earlier, unlikely to reflect inhibition of an energy-dependent mechanism regulating cellular volume. An alternative explanation, in agreement with the hypothesis that has just been developed, is that metabolic inhibition increases membrane permeability to cations. This would mean that the rate of entry of choline (and chloride), would be increased and the inevitable gain of extracellular cation with chloride and water could no longer be offset by cellular potassium loss. The mechanisms which might be involved in increased membrane permeability during metabolic inhibition remain speculative but there is some evidence suggesting an important role for calcium in regulating membrane perme-

ability (Romero & Whittam, 1971; Trump, Strum & Bulger, 1974) and it is known that cellular calcium increases when metabolism is inhibited as a result perhaps of inhibition of an energy-dependent calcium pump present in renal cells (Kinne-Saffran & Kinne, 1974).

The observations presented in this paper are consistent with the suggestion that the relative maintenance of cellular volume in choline Ringer reflects a balance between passive potassium loss and choline uptake. Addition of PCMB (a chemical known to increase membrane permeability), to the medium, resulted in cellular swelling (Table 6), as did ethacrynic acid (Table 7) one of whose effects, at least, is to increase membrane permeability (Macknight, 1969). Depletion of cellular potassium, by initial incubation in potassium-free Ringer with ouabain, also resulted in cellular swelling (Table 8) in slices transferred to choline Ringer. (Though potassium depletion might of itself result in metabolic inhibition, oxygen consumption measurements (Cooke, 1976*c*) do not support this hypothesis in renal cortical tissue.) A similar cellular swelling is eventually seen in slices depleted of potassium and incubated in potassium-free sodium Ringer (Macknight, 1968*a*; K. R. Cooke, 1976*c*).

The present results have been interpreted in terms of the conventional model which attributes the regulation of cellular volume to a balance between cation influx and efflux – a balance maintained normally by metabolically dependent sodium extrusion which offsets the colloid osmotic swelling force of the cellular macromolecules. There has however been much experimental support in recent years for the possibility that regulation of cellular volume involves a ouabain-insensitive mechanism (Daniel & Robinson, 1971; Kleinzeller & Knotková, 1964*a*, *b*; Macknight, 1968*a*; Macknight *et al.* 1974; Maude, 1969; Whittembury, 1968). The nature of such a mechanism however remains controversial. What evidence do the present results provide about this possibility? The present work does not support the mechano-chemical hypothesis (Kleinzeller, 1972). It also suggests that considerable caution must be exercised in interpreting results of experiments with cardiac glycosides of the type shown in Tables 1–3. In these experiments ouabain did not cause cellular swelling in sodium Ringer, metabolic inhibition did. But these results would be as consistent with the hypothesis developed above for slices exposed to choline Ringer as they would with the hypothesis of ouabain-insensitive energy-dependent volume regulation.

The strongest experimental evidence favouring energy-dependent cardiac glycoside insensitive volume regulation comes from leaching-reincubation experiments in which ouabain failed to prevent the restoration of cellular volume in slices incubated in sodium Ringer. Such volume recovery when metabolism was restored to swollen slices could not result

simply from changes in membrane permeability. It reflects extrusion of sodium, chloride and water (Kleinzeller & Knotková, 1964*a*; Macknight, 1968; Whittembury, 1968) and the generation of a more negative (intracellular compared to medium) membrane potential (Proverbio & Whittembury, 1975). Further work is required to investigate this seemingly ouabain-insensitive sodium extrusion. However, the present experiments lend no support to the suggestion that an ethacrynic acid-sensitive sodium pump is involved (Whittembury, 1968). There is considerable evidence that ethacrynic acid inhibits sodium transport non-specifically as a result of its inhibition of cellular metabolism (Epstein, 1972; Macknight, 1969; Poat *et al.* 1970) and that it is this metabolic inhibition which causes cellular swelling (Macknight, 1969). This interpretation receives strong support from the results shown in Table 7, which were obtained using slices of guinea-pig renal cortex, the species studied by Whittembury and his associates. Two points emerge. Firstly, there is no dissociation of cellular swelling from cellular potassium loss in tissue exposed to 1 mM ethacrynic acid (a concentration similar to that used by Whittembury). Secondly, slices incubated in choline Ringer with ethacrynic acid became markedly swollen, a swelling which could not be ascribed to inhibition of a ouabain-insensitive, ethacrynic acid-sensitive, sodium pump, for there was no sodium in the medium. Results such as these illustrate the non-specific effects of ethacrynic acid at these concentrations and the caution required in interpreting results obtained with this diuretic in renal cortical slices.

In summary, no evidence is provided from experimental results presented in this paper to support a mechano-chemical mechanism regulating cellular volume. It is suggested that caution is required in interpreting the results of experiments in which cardiac-glycosides are used to inhibit active sodium transport as favouring the concept of glycoside-insensitive regulation of cellular volume.

We are grateful to Dr K. R. Cooke for helpful and stimulating discussions during which many of the ideas presented here evolved, and for his permission to cite unpublished observations. This work was supported by the Medical Research Council of New Zealand.

REFERENCES

COHEN, P. P. (1945). Methods of preparing animal tissues. In *Manometric Techniques*, ed. UMBREIT, B. H. & STAUFFER, J. F. Minneapolis: Burgess.

COOKE, K. R. (1975). Water and ion contents of rat renal cortical slices incubated in isosmotic media with different potassium concentrations. *Proc. Univ. Otago med. Sch.* **53**, 61–62.

COOKE, K. R. (1976*a*). Water and ion contents of rat renal cortical slices leached and reincubated in isosmotic media with lithium replacing sodium. *Proc. Univ. Otago med. Sch.* **54**, 10–12.

COOKE, K. R. (1976*b*). Effects of incubating rat and rabbit renal cortical slices in media containing lithium. *Proc. Univ. Otago med. Sch.* **54**, 12–14.

COOKE, K. R. (1976*c*). Oxygen consumption and water and ion content of rat renal cortical slices incubated in potassium-free media with and without ouabain. *Proc. Univ. Otago med. Sch.* **54**, 36–37.

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

DANIEL, E. E. & ROBINSON, K. (1971). Effects of inhibitors of active transport on ^{22}Na and ^{42}K movements and on nucleotide levels in rat uteri at 25° C. *Can. J. Physiol. Pharmac.* **49**, 178–204.

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

EPSTEIN, R. W. (1972). The effects of ethacrynic acid on active transport of sugars and ions and on other metabolic processes in rabbit kidney cortex. *Biochim. biophys. Acta* **274**, 128–139.

HOKIN, L. E. & DAHL, J. L. (1972). The sodium–potassium adenosinetriphosphatase. In *Metabolic Pathways*, vol. VI, ed. HOKIN, L. E. New York: Academic Press.

KINNE-SAFFRAM, E. & KINNE, R. (1974). Localization of a calcium-stimulated ATPase in the basal-lateral plasma membranes of the proximal tubule of the rat kidney cortex. *J. Membrane Biol.* **17**, 263–274.

KLEINZELLER, A. (1972). Cellular transport of water. In *Metabolic Pathways*, vol. VI, ed. HOKIN, L. E. New York: Academic Press.

KLEINZELLER, A. & KNOTKOVÁ, A. (1964*a*). The effect of ouabain on the electrolyte and water transport in kidney cortex and liver slices. *J. Physiol.* **175**, 172–192.

KLEINZELLER, A. & KNOTKOVÁ, A. (1964*b*). Electrolyte transport in rat diaphragm. *Physiologia bohemoslov.* **13**, 317–326.

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

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

MCIVER, D. J. L. & MACKNIGHT, A. D. C. (1974). Extracellular space in some isolated tissues. *J. Physiol.* **239**, 31–49.

MACKNIGHT, A. D. C. (1968*a*). Water and electrolyte contents of rat renal cortical slices incubated in potassium-free media and media containing ouabain. *Biochim. biophys. Acta* **150**, 263–271.

MACKNIGHT, A. D. C. (1968*b*). Water and electrolyte contents of rat renal cortical slices incubated in medium containing *p*-chloromercuribenzoic acid or *p*-chloromercuribenzoic acid and ouabain. *Biochim. biophys. Acta* **163**, 500–505.

MACKNIGHT, A. D. C. (1969). The effects of ethacrynic acid on electrolyte and water content of rat renal cortical slices. *Biochim. biophys. Acta* **173**, 223–229.

MACKNIGHT, A. D. C., PILGRIM, J. P. & ROBINSON, B. A. (1974). The regulation of cellular volume in liver slices. *J. Physiol.* **238**, 279–294.

MAIZELS, M. & REMINGTON, M. (1958). Mercaptomerin and water exchange in cortex slices of rat kidney. *J. Physiol.* **143**, 275–282.

MAUDE, D. L. (1969). Effects of K and ouabain on fluid transport and cell Na in proximal tubule *in vitro*. *Am. J. Physiol.* **210**, 1199–1206.

POAT, P. C., POAT, J. A. & MUNDAY, K. A. (1970). The site of action of the diuretic ethacrynic acid on rat kidney and liver tissue. *Comp. gen. Pharmac.* **1**, 400–408.

POST, R. L. & JOLLY, P. C. (1957). The linkage of sodium, potassium and ammonium active transport across the human erythrocyte membrane. *Biochim. biophys. Acta* **25**, 118–128.

PROVERBIO, F. & WHITTEMBURY, G. (1975). Cell electrical potentials during enhanced sodium extrusion in guinea-pig kidney cortex slices. *J. Physiol.* **250**, 559–578.

ROMERO, P. J. & WHITTAM, R. (1971). The control by internal calcium of membrane permeability to sodium and potassium. *J. Physiol.* **214**, 481–507.

SKOU, J. C. (1965). Enzymatic basis for active transport across cell membrane. *Physiol. Rev.* **45**, 596–617.

SKOU, J. C. (1975). The ($Na^+ + K^+$) activated enzyme and its relationship to transport of sodium and potassium. *Q. Rev. Biophys.* **7**, 401–434.

STEIN, W. D. (1967). *The Movement of Molecules across Cell Membranes*, pp. 242–253. New York and London: Academic Press.

TOSTESON, D. C. (1964). Regulation of cell volume by sodium and potassium transport. In *The Cellular Functions of Membrane Transport*, ed. HOFFMAN, J. F., pp. 3–22. New Jersey: Prentice Hall.

TOSTESON, D. C. & HOFFMAN, J. F. (1960). Regulation of cell volume by active cation transport in high and low potassium sheep red cells. *J. gen. Physiol.* **44**, 169–194.

TRUMP, B. F., STRUM, J. M. & BULGER, R. E. (1974). Studies on the pathogenesis of ischemic cell injury. I. Relationships between ion and water shifts and cell ultrastructure in rat kidney slices during swelling at 0–4 degrees C. *Virchows Arch. path. Anat.* **16**, 1–34.

WHITTAM, R. & WILLIS, J. S. (1963). Ion movements and oxygen consumption in kidney cortex slices. *J. Physiol.* **168**, 158–177.

WHITTEMBURY, G. (1965). Sodium extrusion and potassium uptake in guinea-pig kidney cortex slices. *J. gen. Physiol.* **48**, 699–717.

WHITTEMBURY, G. (1968). Sodium and water transport in kidney proximal tubular cells. *J. gen. Physiol.* **51**, 303–314*S*.

WHITTEMBURY, G. & PROVERBIO, F. (1970). Two modes of Na extrusion in cells from guinea-pig kidney cortex slices. *Pflügers. Arch. ges. Physiol.* **316**, 1–25.

WOODBURY, J. W. (1965). The cell membrane: ionic and potential gradients and active transport. In *Physiology and Biophysics*, ed. RUSH, T. H. & PATTON, H. D. Philadelphia and London: W. B. Saunders.