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EFFECT OF pH ON THE WATER AND ELECTROLYTE CONTENT OF RENAL CELLS

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SUMMARY

The effect of external pH (varying from 6.2–8.2) on the water and electrolyte contents of preparations of rabbit kidney cortex (slices and isolated tubules) was studied:

1. In sodium saline, increasing external pH produced primarily a decrease in cell K^+. High pH increased both the passive membrane permeability for $^{42}K^+$, and also the compartmentation of K^+ within the cells.

2. Inhibition of the Na^+ pump by ouabain (0.5 mM) or absence of external Na^+ (lithium, Tris, and choline salines) did not abolish the control of cell volume at pH 6.2 and 7.2, whereas a marked cellular swelling at pH higher than 7.5 was found. The ouabain-insensitive (and Na^+-independent) volume control at pH 6.2 was dependent on metabolism, since it was inhibited by 0.1 mM dinitrophenol and anaerobiosis.

3. Involvement of Ca^{2+} in the ouabain-insensitive volume control was demonstrated by showing that the absence of Ca^{2+}, ouabain, or absence of Na^+, produced a marked swelling at pH 7.2. This phenomenon was accompanied by increased extracellular space, due to a swelling of the basal tubular membrane.

4. A correlation between cellular swelling and a decrease in cell ATP was presented.

5. The results are compatible with the mechanochemical hypothesis for the ouabain-insensitive (and Na^+-independent) control of cell volume. It is suggested that in this mechanism, cell (membrane) ATP and Ca^{2+} are determinants of the physical properties of the membrane.

INTRODUCTION

Convincing evidence has been repeatedly presented to show that in addition to the leak-and-pump mechanism an ouabain-insensitive system is involved in the control

Abbreviation: TES, *N*-tris-(hydroxymethyl)-aminomethane sulfonate.

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of the volume of kidney and diaphragm cells (see, *e.g.* refs 1–7). However, the nature of this mechanism is subject to considerable discussion (see, *e.g.* ref. 8). Studying the effect of pH on sugar uptake by kidney cortex slices, it was noticed[9] that the cells were able to control their volume also in the absence of Na^+, *i.e.* in lithium saline, in the lower pH range between 6.2 and 7.4, whereas at pH values up to 8.2 a marked swelling took place. These observations are not compatible with the leak-and-pump hypothesis.

Kidney cortex slices are a rather complex experimental material. In the absence of a satisfactory preparation of isolated cells, kidney tubules prepared as described by Burg and Orloff[10] allow one to some extent to avoid difficulties, particularly concerning diffusional pathways in the tissue. Therefore, the effect of external pH on the water and electrolyte composition of this preparation was also studied. Evidence will be presented here that a Na^+-independent and ouabain-insensitive control of the cell volume, operative in renal tubular cells in the lower pH range 6.2–7.2, is abolished by raising pH to values higher than 7.4. Ca^{2+} and ATP are involved in this mechanism of volume control.

METHODS AND MATERIALS

Tissue preparations

Isolated kidney tubules were prepared essentially as described by Burg and Orloff[10] by digesting diced rabbit cortex with collagenase. A few modifications were introduced: Ice-cold saline (10 ml) was injected into the renal artery of each kidney prior to the perfusion of collagenase solution. With the exception of the digestion step, all the preparation procedures were conducted on ice.

The suspension of tubules (final concentration usually 20 mg wet wt/ml) was incubated usually for 1 h at 25 °C in vessels described by Burg and Orloff[10] and aerated with O_2. For analyses, 2 ml of suspension was transferred into tubes similar to those described by Burg and Orloff and centrifuged 10 min at 0 °C at 10000 rev./min (HB 4 Sorvall horizontal rotor), in an RC 2B refrigerated centrifuge. The supernatant and the white top layer of cells were removed by suction. The pellet of tissue was directly pushed into a teflon vial and weighed.

Kidney cortex slices were prepared and handled as previously described[2,9].

Salines

The basic saline of the Krebs–Ringer type was prepared from isotonic stock solutions as described earlier[9], using lithium acetate (6.7 mM, final concn) as substrate. 3 % calf serum (Flow Laboratories, Rockville, Md., U.S.A.) was systematically added to salines employed for the suspension of isolated tubules.

Variations of saline pH between 6.2 and 8.2 were obtained by using appropriate mixtures of 0.308 Tris and TES (*N*-tris-(hydroxymethyl)-aminomethane sulfonate); the final concentration of the buffer was 13.6 mM. This buffer was selected because it offers advantages as compared with other buffer systems used for similar experiments. It has been shown that bicarbonate and pCO_2 themselves affect the ionic composition of both kidney tubules and diaphragm[11–13]. These interactions between bicarbonate, pCO_2 and pH effects add further complications to the interpretation of the results. On the other hand, phosphate buffer cannot be used in the alkaline

range without Ca^{2+} precipitation. Finally, using the above buffer system, variations of pH could be achieved without modification of the Na^+, K^+ and Cl^- concentrations. It should be pointed out, however, that the effect of pH on the cellular metabolism could to some extent vary with the buffer system used in the saline[14].

Experimental and analytical procedures

Details for the incubation of slices were given previously[9].

In addition to the determination in the slices or tubules of tissue water, inulin space, Na^+, K^+, Li^+ and Cl^- (for details see ref. 9), Ca^{2+} was also assayed in the 0.1 M HNO_3 extract of the tissue. The Ca^{2+} concentration was determined by atomic absorption spectrometry (EEL instrument, Halstead, England) using appropriately diluted samples of the tissue extract to which $LaCl_3$ (35 mM final concn) was added. All data for electrolytes are given in mequiv/kg tissue dry wt. The extracellular (inulin) space is expressed in ml/kg wet wt.

In some experiments, tissue inorganic phosphate (P_i) and ATP were also determined. Here, the tissue was first deproteinized with ice-cold $HClO_4$ (5 %, w/v), the tube contents homogenized, and the protein pellet spun off at 0 °C in a refrigerated centrifuge at 10000 × *g*. The supernatant was assayed for P_i (ref. 15) and ATP (ref. 16). The protein pellet was dissolved in 1 M NaOH and in the suitably diluted solution protein was determined by the method of Lowry *et al.*[17]. The results are expressed in mmoles P_i or ATP per kg tissue protein.

Tissue respiration at 25 °C was measured by the conventional Warburg technique; O_2 was used as the gaseous phase in the vessels. The results are given as QO_2 (μl O_2 used per mg tissue dry wt h).

The influx of $^{42}K^+$ was studied by first bringing slices to a steady-state level of their cellular components (aerobic preincubation at 25 °C in the respective salines) and subsequently transferring the tissue for varying time intervals into salines containing $^{42}K^+$ (0.01 μCi/ml). The tissue was then extracted with 0.1 M HNO_3 and in the extract total K^+ and $^{42}K^+$ is expressed as percent of tissue K^+.

The effect of external osmolarity on the steady-state level of tissue water in kidney cortex slices was studied using variations of the saline osmolarity with mannitol as previously described[18]. [^{14}C]Mannitol was added and the steady-state space of mannitol was used to estimate the extracellular water in these experiments.

Data presented below are the means of at least 3 analyses. Where more than 5 analyses were carried out, means ± S.E. are presented.

Materials

All reagents used were of analytical grade. [^{3}H]Inulin and [^{14}C]mannitol were obtained from New England Nuclear Corp., Boston, Mass. 2,4-Dinitrophenol, Tris, and TES were purchased from Sigma Co., St. Louis, Mo.

RESULTS

Comparison of water and electrolytes content of isolated tubules and slices of rabbit kidney cortex

The steady-state water and ionic composition of isolated tubules and kidney slices after aerobic incubation at 25 °C are rather similar (Table I). However, several

TABLE I

COMPARISON OF IONIC AND WATER CONTENT OF ISOLATED TUBULES AND RABBIT RENAL CORTEX SLICES

Steady-state values for aerobic incubation (O_2) in sodium saline at pH 7.2 are given. Values for tubules are the means ± S.E. ($n = 22$). Values for slices are the means ± S.E. for 6 analyses (3 animals).

	Tubules	*Slices*
Water (kg/kg dry wt)	3.53 ± 0.08	2.83 ± 0.02
Na^+ (mequiv/kg dry wt)	221 ± 11	189 ± 6
K^+ (mequiv/kg dry wt)	321 ± 8	339 ± 10
Cl^- (mequiv/kg dry wt)	237 ± 7	220 ± 2
Ca^{2+} (mequiv/kg dry wt)	29.3 ± 1.2	51.7 ± 7.5
Inulin space (ml/kg wet wt)	277 ± 11	236 ± 17

discrepancies have to be pointed out. The water content of the tubules is higher; as indicated by the greater inulin space; this difference is partly due to a higher amount of extracellular water trapped in the pellet of tubules. The K^+ content, which is one of the best indicators for the viability of a tissue *in vitro*, is practically the same in both preparations. No definite explanation for the lower Ca^{2+} content in the tubules can be offered. However, it has to be pointed out that the slices were systematically stored at 0 °C for 2.5 h before incubation. Höfer and Kleinzeller[19] have shown that by maintaining the slices at 0 °C in an isotonic saline containing 2 mM Ca^{2+}, an uptake of Ca^{2+} by the tissue took place, and that this increase was slowly reversible on incubation at 25 °C. The treatment of the tissue prior to incubation may contribute to differences in the Ca^{2+} content of slices and the tubule preparation.

The similarity of the ionic composition of the tubules and the slices, as well as the stability of their water and cation composition of tubules during incubation periods up to 3 h suggests that their cellular metabolism was intact and that the membrane structure and function of the isolated tubules were not impaired, confirming the conclusions of other authors[10,12].

Since variations of experimental conditions produced qualitatively identical (and quantitatively very similar) results with both tissue preparations, data selected for presentation below are appropriate to both.

Effect of pH on the water and ionic composition of isolated tubules

In the absence of ouabain (controls), increasing pH from 6.2 to 8.2 produced an insignificant effect on the content of water, Na^+ and Cl^-, and on the inulin space of isolated tubules (Table II); similar results were previously reported for kidney cortex slices[9]. The marked decrease in the K^+ content produced by increasing pH has also been described in slices. A new observation is the considerable increase in tissue Ca^{2+} which takes place at pH 8.2. This phenomenon appears to be due to a precipitation in the cells of Ca^{2+} as a phosphate[20]. Direct measurements of the steady-state tissue P_i (in mmoles/kg tissue protein) showed a small but significant increase when the pH was raised from 6.2 (29.5 ± 2.9 mmoles P_i) to 8.2 (39.7 ± 2.3 mmoles P_i). Furthermore, no increase of tissue Ca^{2+} was observed when experiments were carried out in phosphate-free salines (Table III).

TABLE II

EFFECT OF pH AND OUABAIN ON THE ELECTROLYTE AND WATER CONTENT OF ISOLATED KIDNEY TUBULES

Tubules were incubated aerobically (O_2) at 25 °C for 1 h in salines of indicated pH, without (control) or with ouabain (0.1 mM). Values are the means ± S.E. of at least 8 samples from two tube preparations.

		pH 6.2		*pH 7.2*		*pH 8.2*	
		Control	*Ouabain*	*Control*	*Ouabain*	*Control*	*Ouabain*
Water	(kg/kg dry wt)	3.60 ± 0.11	3.28 ± 0.10	3.64 ± 0.10	3.88 ± 0.04	3.91 ± 0.21	5.89 ± 0.10
Na^+	(mequiv/kg dry wt)	258 ± 17	340 ± 10	236 ± 10	408 ± 3	314 ± 12	699 ± 10
K^+	(mequiv/kg dry wt)	314 ± 5	141 ± 1	292 ± 11	84 ± 7	219 ± 8	87 ± 3
Cl^-	(mequiv/kg dry wt)	257 ± 14	242 ± 3	201 ± 26	341 ± 3	273 ± 13	597 ± 5
Ca^{2+}	(mequiv/kg dry wt)	25.8 ± 0.6	26.7 ± 0.6	29.2 ± 1.4	31.5 ± 1.2	72.4 ± 5.0	130.9 ± 3.0
Inulin space	(ml/kg wet wt)	282 ± 13	282 ± 13	277 ± 11	277 ± 11	313 ± 14	313 ± 14

TABLE III

EFFECT OF PHOSPHATE-FREE SALINE ON THE Ca^{2+} CONTENT OF ISOLATED KIDNEY TUBULES

Tubules were incubated aerobically for 1 h at 25 °C in salines of indicated pH in the presence (1 mM, control) and absence of phosphate. Values of tissue Ca^{2+} (mequiv/kg dry wt) are the mean ± S.E. of 8 determinations.

	Tissue Ca^{2+} mequiv/kg dry wt	
Saline pH	*Control*	*Phosphate-free saline*
7.2	30.9 ± 0.5	30.4 ± 0.5
8.2	53.1 ± 1.1	29.9 ± 1.1

It is generally assumed that the cell volume is controlled by the ratio between the rate constant of pumping and leakage for bulk cations, *i.e.* Na^+ and K^+. This leak-and-pump hypothesis[21,22] would thus predict that an inhibition of the pump and/or increased leak should produce a cellular swelling. In order to study the effect of pH on cell volume regulation, the tissue was incubated at different pH values at conditions where the pump is supposed to be completely inhibited by ouabain (0.1, 0.5 and 1 Mm) and/or where the membrane permeability is assumed to be increased, *i.e.* in Ca^{2+}-free saline[24].

Table II shows the effect of ouabain on the water and ionic composition of isolated tubules as a function of external pH. The following observations are pertinent. At all pH values the cardiac glycoside manifested its inhibitory effect on the Na^+ pump by increasing tissue Na^+ and decreasing K^+. However, a marked cellular swelling was observed only at external pH values higher than 7.4, whereas at pH 6.2 the water content of the tubule preparation was even significantly lower than in the control without ouabain. The swelling effect of ouabain at pH 8.2 was considerable and increased with the duration of the incubation; the values given in Table II therefore do not represent a steady state.

The ratio between the Na^+ taken up (Na_t) and the K^+ lost (K_l) after 1 h of incubation at different pH with or without ouabain, expresses the relationship between the movements of these two cations. Taking the difference in the value for the Na^+ and the K^+ content, it may be computed from Table II that this ratio increased from 0.48 at pH 6.2 to 0.82 at pH 7.2, and to a value of 2.90 at pH 8.2. Thus, at pH 6.2, ouabain produced a net loss of cation from the tissue, whereas, at pH 8.2 the sum ($Na^+ + K^+$) per unit dry wt increased. This relationship between Na^+ and K^+ movements may have contributed to the fact that, as compared to the control, ouabain at pH 6.2 did not produce an increase in tissue Cl^-. Ca^{2+} was not considered in this calculation, since it was assumed above that the increased tissue level of this cation at high pH may be osmotically inactive.

The observations reported here may also explain the apparent discrepancy between the reported swelling effect of ouabain on isolated kidney tubules[24], as opposed to slices; minor differences in the pH appear to be responsible.

It has been shown previously that at pH 7.4 the absence of saline Ca^{2+} did not affect the steady state tissue level of water while increasing tissue Na^+ and decreasing K^+ (ref. 15). It was therefore of interest to compare the effect of pH and ouabain in the absence of Ca^{2+}, particularly since the absence of Ca^{2+} is known to increase the passive permeability of the cell membrane[23]. The results are summarized in Table IV. It will be noted that at low external concentration of Ca^{2+} a marked swelling of tissue took place at pH 8.2, whereas in the lower pH range, the values for the tissue water stayed in the same range as those obtained in the presence of Ca^{2+} (Table II). Low external Ca^{2+} produced a significant increase in Na^+, and a decrease in tissue K^+. These changes, similar to those produced by ouabain in presence of Ca^{2+}, were more marked at pH 8.2. As to tissue Ca^{2+}, a comparison between Tables II and IV shows that incubation of the tubules at low $[Ca^{+2}]_0$ produced lower levels of Ca^{2+} at all pH values. It will also be noticed that at pH 8.2 and low $[Ca^{2+}]_0$ the tissue Ca^{2+} was considerably higher than values at lower saline pH and in fresh tissue; serum present in the suspension medium obviously served as a source of Ca^{2+} here.

TABLE IV

EFFECT OF pH AND OUABAIN ON THE ELECTROLYTE AND WATER CONTENT OF ISOLATED KIDNEY TUBULES INCUBATED IN Ca^{2+}-FREE SALINES

Tubules were incubated aerobically (O_2) at 25 °C for 1 h in Ca^{2+}-free salines (final Ca^{2+} concentrations approx. 0.1–0.2 mM) of indicated pH, without (control) and with 0.1 mM ouabain. Values are the means ± S.E. of at least 8 samples from two tubule preparations.

		pH 6.2		*pH 7.2*		*pH 8.2*	
		Control	*Ouabain*	*Control*	*Ouabain*	*Control*	*Ouabain*
Water	(kg/kg dry wt)	3.83 ± 0.17	3.60 ± 0.17	3.47 ± 0.28	4.93 ± 0.43	6.39 ± 0.34	7.57 ± 0.28
Na^+	(mequiv/kg dry wt)	301 ± 10	373 ± 25	249 ± 14	566 ± 10	712 ± 34	934 ± 18
K^+	(mequiv/kg dry wt)	280 ± 4	123 ± 6	229 ± 14	75 ± 6	106 ± 8	92 ± 5
Cl^-	(mequiv/kg dry wt)	236 ± 13	293 ± 26	234 ± 22	446 ± 14	606 ± 44	797 ± 14
Ca^{2+}	(mequiv/kg dry wt)	19.2 ± 1.6	18.0 ± 0.7	18.3 ± 1.8	23.0 ± 1.6	58.5 ± 4.6	68.2 ± 3.5
Inulin space	(ml/kg wet wt)	282 ± 13	282 ± 13	277 ± 11	277 ± 11	403 ± 7	404 ± 5

TABLE V

THE EFFECT OF pH ON THE WATER AND K^+ CONTENT OF ISOLATED KIDNEY TUBULES INCUBATED IN Na^+-FREE SALINE

Tubules were incubated aerobically (O_2) for 1 h at 25 °C in Tris salines of indicated pH, with and without Ca^{2+} added. Values are the means ± S.E. of 8 samples.

Saline pH	*Saline Ca^{2+} (mM)*	*Water (kg/kg dry wt)*	*K^+ (mequiv/kg dry wt)*
6.2	2.5	3.32 ± 0.07	127 ± 1
6.2	None	3.52 ± 0.11	118 ± 5
7.2	2.5	3.80 ± 0.15	90 ± 2
7.2	None	4.87 ± 0.52	86 ± 2
8.2	2.5	4.03 ± 0.32	87 ± 3
8.2	None	8.15 ± 0.47	89 ± 5

At low external Ca^{2+} concn, ouabain produced drastic changes of the water and electrolyte content of the tubule preparation, particularly at pH 7.2 and 8.2, whereas at pH 6.2 no effects on the water content could be observed. The marked swelling found at pH 7.2 should be contrasted with the lack of effect on tissue water seen when the tubules were incubated in saline containing ouabain *plus* Ca^{2+} (Table II), although under both sets of conditions ouabain inhibited the exchange of Na^+ and K^+.

The absence of Ca^{2+} also affected the stoichiometric ratio between Na^+ taken up and K^+ lost; as compared with the above values found in the controls (2.5 mM Ca^{2+}), these ratios were considerably higher: 1.75 at pH 7.2, and as high as 9.9 at pH 8.2.

In the absence of Ca^{2+}, the inulin space was found to be markedly increased at pH 8.2 to values of 403 ml/kg wet wt both in the presence or absence of ouabain, whereas at low pH, the inulin space was identical with that found in the presence of Ca^{2+} (277 ± 11 ml/kg tissue wet wt). The considerable swelling in the region of the basal membrane (Fig. 1) obviously related only to the absence of external Ca^{2+}, appears to account for a major portion of the observed increase of the inulin space.

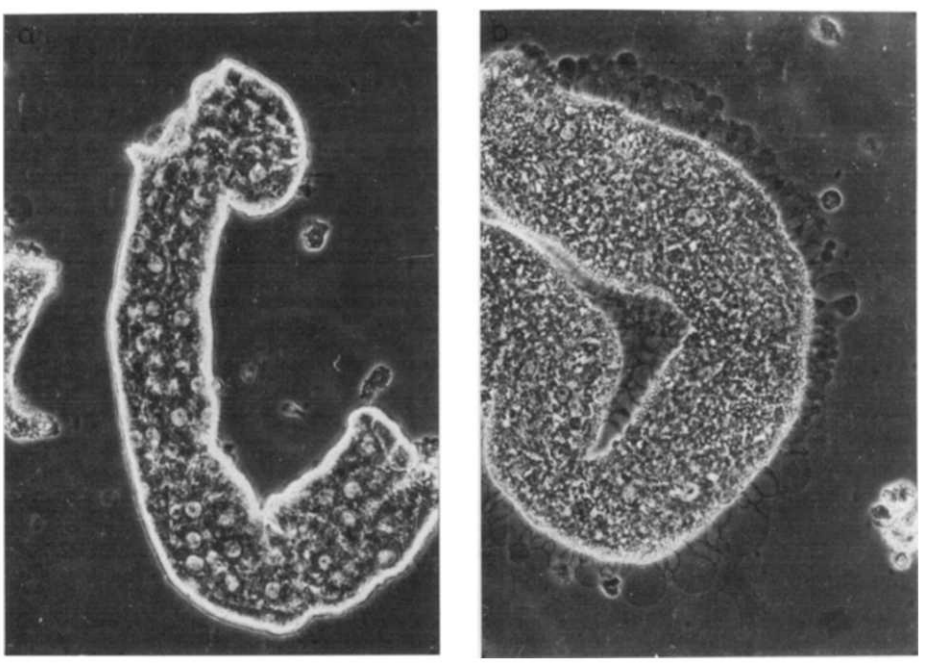

Fig. 1. Phase contrast micrographs of kidney cortex tubules. (a) Tubules incubated aerobically 1 h at 25 °C, pH 7.2. (b) Tubules incubated aerobically 1 h at 25 °C in Ca^{2+}-free saline at pH 8.2.

The results reported above showed that particularly at pH 6.2, the kidney cells were capable of controlling their volume by a mechanism which was insensitive to both ouabain and external Ca^{2+}. It was therefore interesting to test whether this cell volume control at pH 6.2 was specific for Na^+. The effect of pH on the water content of isolated tubules was tested in Na^+-free (lithium or Tris saline), and Cl^--free

medium (isethionate saline) in presence and absence of Ca^{2+}. In lithium or Tris saline, in the absence of Ca^{2+}, the tissue water varied with increasing pH in the same way as in sodium saline, in the presence of ouabain, *i.e.* a swelling observed at pH 7.2 and particularly at pH 8.2, but not at pH 6.2 (Table V). These results obtained with tubules again paralleled the observations made using kidney slices[9].

The similarity of the responses of the system(s) responsible for the cell volume control to variations of pH and $[Ca^{2+}]_0$ in the absence of Na^- and under conditions when the Na^+ pump was inhibited by ouabain warrants the conclusion that the studied phenomenon is both ouabain-insensitive and independent of Na^+.

The replacement of the permeable Cl^- by the impermeable isethionate anion prevented the cellular swelling at pH 7.2 (ouabain *plus* absence of Ca^{2+}) and partly reduced the swelling at pH 8.2 produced by ouabain and/or absence of Ca^{2+} (see Fig. 2). These results are consistent with the above observations that cellular swelling is associated with a net entry of equivalent amounts of cations and permeable anions as an isotonic solution; consequently, the swelling was prevented by the presence of an impermeable bulk anion. At pH 8.2 when some swelling was observed in the Cl^--free saline, measurements of the inulin extracellular space showed that the increased water and electrolyte content of the tissue could be accounted for by the appearance of swollen structures at the basement membrane (Fig. 1).

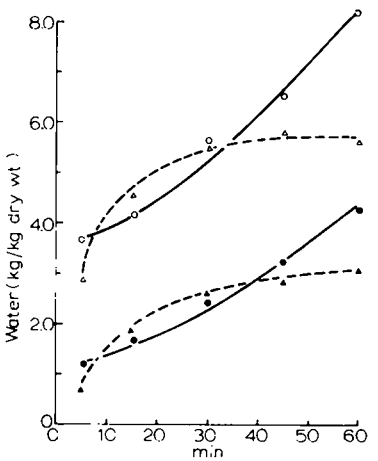

Fig. 2. Tissue water and extracellular space in kidney tubules incubated in Ca^{2+}-free saline at pH 8.2. Tissue water (○, △), inulin extracellular space (●, ▲), both in kg water/kg tissue dry wt. All values are the mean of 3 analyses. Salines: ○, ●, standard composition; △, ▲ Cl^- free (isethionate) medium.

The ouabain-insensitive control of cell volume of pH 6.2 was found to be metabolically dependent since 0.1 mM 2,4-dinitrophenol produced a rather similar increase in the water and Na^+ content of isolated tubules at all pH values tested (Table VI). As observed for the incubation at 0 °C, inhibition of tissue metabolism by 2,4-dinitrophenol produced a significant increase of Ca^{2+} at all pH values tested.

Finally, it was of interest to know whether free reversibility can be demonstrated for the observed pH effect on the volume control in cells where the Na^+ pump is blocked. In such experiments tissue preparations were first loaded with Na^+,

TABLE VI

EFFECT OF 0.1 mM 2,4-DINITROPHENOL ON THE WATER AND IONIC CONTENT OF ISOLATED TUBULES

Tubules were incubated aerobically for 1 h at 25 °C at different pH values. Values are the mean ± S.E. of 8 analyses from two tubule preparations.

		pH 6.2		*pH 7.2*		*pH 8.2*	
		Control	*Dinitrophenol*	*Control*	*Dinitrophenol*	*Control*	*Dinitrophenol*
Water	(kg/kg dry wt)	3.60 ± 0.11	4.75 ± 0.06	3.64 ± 0.10	4.56 ± 0.21	3.91 ± 0.21	4.98 ± 0.07
Na^+	(mequiv/kg dry wt)	258 ± 7	550 ± 7	236 ± 10	535 ± 16	314 ± 12	543 ± 10
K^+	(mequiv/kg dry wt)	314 ± 5	62.6 ± 2.5	292 ± 11	66.8 ± 8	219 ± 8	80.1 ± 2.4
Cl^-	(mequiv/kg dry wt)	257 ± 14	524 ± 11	201 ± 26	440 ± 14	273 ± 13	480 ± 17
Ca^{2+}	(mequiv/kg dry wt)	25.8 ± 0.6	38.6 ± 2.3	29.2 ± 1.4	34.8 ± 1.2	72.4 ± 5.0	80.6 ± 2.5

TABLE VII

THE EFFECT OF 0.5 mM OUABAIN ON TISSUE WATER AND ELECTROLYTE CONTENTS OF RENAL CORTICAL SLICES AFTER 20 min OF INCUBATION

Slices were maintained for 2.5 h in ice-cold sodium saline (A). After leaching the slices were incubated for 20 min at 25 °C under aerobic conditions in the presence of 0.5 mM ouabain. In condition B, the pH was 6.2 and in condition C, the pH was 8.2. The means ± S.E. of 6 measurements and the difference ± S.E. of the difference are presented.

Conditions	*Water (kg/kg dry wt)*	*K^+ (mequiv/kg dry wt)*	*Na^+ (mequiv/kg dry wt)*	*PO_4^{3-} (mmoles/kg dry wt)*	*Ca^{2+} (mmoles/kg dry wt)*	*Li^+ (mmoles/kg dry wt)*	*Cl^- (mmoles/kg dry wt)*
After leaching (A)	3.87 ± 0.02	219.5 ± 4.5	399 ± 10	80	54.2 ± 1.5	25.7 ± 0.5	323 ± 6
pH 6.2 (B)	3.20 ± 0.04	164.8 ± 1.3	414 ± 15	30	51.7 ± 7.5	25.0 ± 0.4	270 ± 9
pH 8.2 (C)	3.19 ± 0.08	100.3 ± 1.0	436 ± 9	40	60.4 ± 1.8	30.6 ± 0.6	277 ± 10
(B−A)	− 0.67 ± 0.04	− 54.7 ± 4.7	+ 15 ± 18	50	− 2.5 ± 7.6	− 0.7 ± 0.6	− 53 ± 11
(C−A)	− 0.68 ± 0.09	− 119.2 ± 4.6	+ 37 ± 13	40	+ 6.2 ± 2.3	+ 4.9 ± 0.8	− 46 ± 12

Cl^- and water by incubation in ice-cold saline for 2.5 h at pH 7.2 (the medium was only slightly buffered), and subsequently incubated aerobically at 25 °C in ouabain-containing salines of standard composition at either pH 6.2 or 8.2. At the time interval indicated on Fig. 3, portions of the tissue were removed from their respective incubation media, blotted, and transferred to salines of different pH.

Fig. 3 shows results for tissue water obtained with slices. It will be noticed that at pH 6.2 the tissue rapidly extruded water and reached within 30 min the steady-state value reported in Table II. On transfer of these slices from saline at pH 6.2 to saline at 8.2 the tissue swelled. Slices incubated directly at pH 8.2 at first also extruded water but subsequently (after 30 min) started to swell. This swelling process was not stopped at 120 min incubation, and was not freely reversible: transfer of the swollen slices to saline at pH 6.2 did not produce a renewed shrinking, although the actual swelling process was stopped.

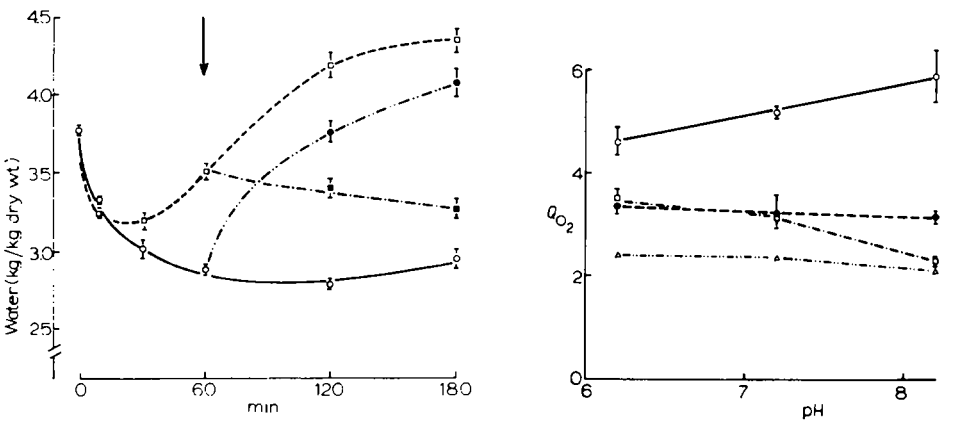

Fig. 3. Reversibility of the pH effect on tissue water of kidney cortex slices incubated in sodium-salines *plus* 0.5 mM ouabain. Slices were first loaded with water, Na^+ and Cl^- by pre-incubation for 2.5 h in ice-cold saline at pH 7.2, then incubated aerobically (O_2) at 25 °C in sodiumsalines containing 0.5 mM ouabain at pH 6.2 (○) or pH 8.2 (□). The arrow indicates the time when portions of the tissue were transferred from pH 6.2 to pH 8.2 (●) or from pH 8.2 pH 6.2 (■). All values are the mean of 6 analyses ± S.E. (2 rabbits).

Fig. 4. Effect of pH on the respiration of slices of kidney cortex. Values of Qo_2 (μl O_2 per mg tissue dry wt·h) at 25 °C, ± S.E. where more than 6 determinations were made. Media: ○, sodium saline; ●, sodium saline *plus* 0.5 mM ouabain; □, lithium saline; △, Tris saline.

Table VII presents data for the electrolyte changes taking place in the tissue concomitant with the extrusion of water in the first 20 min incubation at both pH values. As a consequence of being inhibited by ouabain, no net accumulation of K^+ occurred in the tissue, and the extrusion of water took place as a practically isotonic solution of electrolytes, as reported previously[1-3,6,25]. Thus, during incubation at pH 8.2 a net loss of 119 mequiv K^+ and an actual gain of 37 mequiv Na^+ took place, *i.e.* a net total of 82 mequiv ($Na^+ + K^+$) were lost from the cells together with 0.68 kg water. Therefore, the electrolytes were extruded from the cells as an 82/0.68 = 122 mM solution. As a result, the apparent concentrations of bulk cell cations changed as follows: $[K^+]_i$ decreased from 80.5 mM after leaching at 0 °C to 42 mM after incubation, whereas $[Na^+]_i$ actually increased from 91 to 140 mM. As for anions, a net loss of 93 mequiv ($Cl^- + P_i$) took place under these conditions.

The analytical data obtained at pH 6.2 are not as convincing, and this aspect may require further elucidation.

Essentially identical results were obtained when such experiments were carried out in the absence of Na^+ (lithium salines).

The simplest interpretation of such results would be the assumption that pH 8.2 did not block the actual ouabain-insensitive extrusion of water (and electrolytes) from the tissue, but affected some other process, *e.g.* the supply of metabolic energy, or possibly the passive permeability of the membrane.

Effect of pH on some metabolic processes in kidney cortex tissue

Fig. 4 shows that increasing pH from 6.2 to 8.2 appeared to increase slightly the O_2 uptake of slices in sodium saline (control). The Q_{O_2} of the tissue in sodium saline *plus* 0.5 mM ouabain, lithium saline and Tris saline was lower than for the controls, in agreement with other reports[26], and no major differences were found under these conditions. Also, no marked effect of pH on the O_2 uptake was noticed under conditions where the Na^+ pump was inhibited, or Na^+ was absent.

Next, the effect of pH on the ATP content of the tissue was studied. The results are summarized in Table VIII and Fig. 5. The values of ATP in fresh slices of rabbit kidney cortex were found to be 13.8 ± 0.5 μmoles/g tissue protein (n = 30), in reasonable agreement with the data available for the rat kidney cortex[27]. Leaching of the tissue at 0 °C produced a marked drop of the ATP level. On aerobic incubation of the slices in standard sodium saline at all pH values tested the ATP level returned to values observed in fresh tissue. However, in the presence of ouabain, or absence of external Ca^{2+}, the ATP content was similar to that of the controls only under conditions where no tissue swelling took place (*e.g.* pH 6.2 or 7.2 in the presence of Ca^{2+}), whereas low values of ATP were associated with marked tissue swelling (pH 8.2 in the presence of ouabain and/or absence of external Ca^{2+}). The single exception in this respect is the relatively high ATP content at pH 7.2 in Ca^{2+}-free saline in the presence of ouabain. Fig. 5 demonstrates a correlation between the

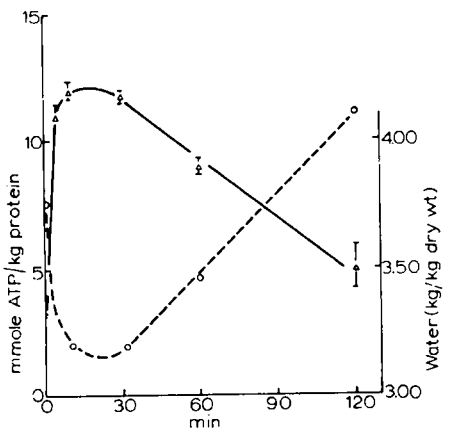

Fig. 5. The relationship between tissue water and ATP in slices of kidney cortex incubated at pH 8.2 in sodium saline containing 0.5 mM ouabain. For conditions of the experiment see legend to Fig. 3. ○, kg water/kg dry wt; △ mmoles ATP per kg tissue protein. All values are the means of 6 analyses ± S.E., 3 rabbits.

tissue levels of water and ATP under experimental conditions described in Fig. 3. It will be noticed that during the extrusion of water from loaded slices at pH 8.2 (0.5 mM ouabain present) a net formation of ATP took place; the renewed swelling after 30 min of incubation was then associated with a rapid drop of the tissue ATP level.

TABLE VIII

STEADY-STATE VALUES OF ATP IN SLICES OF KIDNEY CORTEX INCUBATED IN DIFFERENT SALINES

Slices were first maintained for 2.5 h in ice-cold salines, then aerobically (O_2) preincubated for 45 min and incubated for 60 min at 25 °C in the respective media. Values given in μmoles ATP per g tissue protein, ± S. E. where more than 5 analyses were carried out. Value after leaching at 0 °C: 2.7 μmoles ATP per g.

Medium *Saline pH:*	*6.2*	*7.2*	*8.2*
Sodium saline	14.6 ± 1.5	13.2 ± 1.1	11.3 ± 0.6
Sodium saline *plus* 0.5 mM ouabain	13.9 ± 1.4	11.1 ± 1.2	5.1 ± 0.9
Sodium saline, Ca^{2+}-free		10.7	3.4
Sodium saline, Ca^{2+}-free, *plus* 0.5 mM ouabain		11.3	6.5
Lithium saline		13.4	6.6

The above results thus established a relationship between the tissue levels of water and ATP. However, one observation points against a simple interpretation of the above result, *i.e.* that tissue swelling is produced by a decrease of metabolic energy available for the ouabain-insensitive extrusion of water and electrolytes. If this were so, one would expect also other active transport processes to be depressed under conditions where tissue swelling occurs. This is not the case. As compared with controls (at pH 6.2 and 7.2) the active accumulation of 2-deoxyglucose in renal cells was actually enhanced at pH 8.2 in sodium saline *plus* 0.5 mM ouabain, or in Na^+-free media[9].

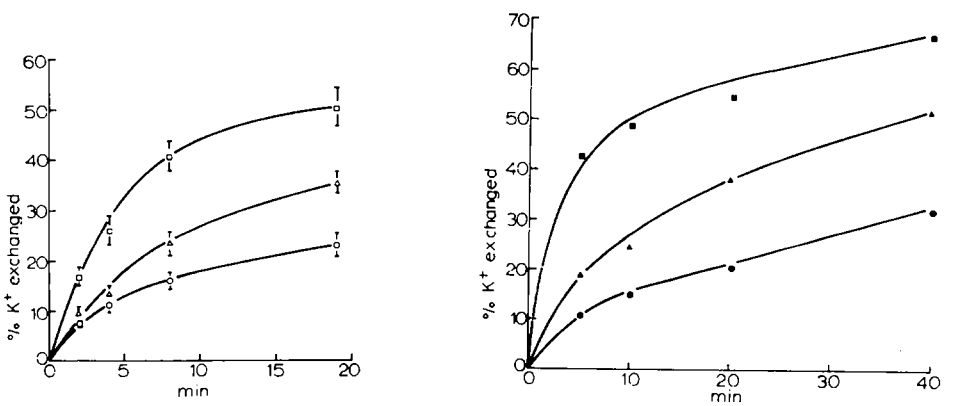

Fig. 6. The effect of pH on the steady-state exchange of $^{42}K^+$ in kidney cortex slices. a). Slices incubated in sodium salines (open symbols); b). Slices incubated in sodium salines containing 0.5 mM ouabain (full symbols). The tissue was first preincubated in the respective salines at 25 °C for 2 h, then transferred into the respective salines labelled with $^{42}K^+$ (0.1 μCi/ml) and incubated further for varying periods of time. After incubation, tissue K^+ and $^{42}K^+$ was determined ○, ●, pH 6.2; △ ▲, pH 7.2; □, ■, pH 8.2. The means of 6 analyses ± S.E. (3 rabbits) in series a, and of 4 analyses (2 rabbits) in series b, are given.

The effect of pH on the steady-state exchange of $^{42}K^+$

In order to contribute to the understanding of the effect of pH on the K^+ level in kidney cortex tissue (Section 2), the apparent steady-state ^{42}K exchange in sodium saline without (control) and with 0.5 mM ouabain was studied at different pH values. The results are shown in Figs 6a and 6b. It has been shown previously that the kinetics of K^+ exchange in slices[28] and isolated tubules[24] of kidney cortex can be graphically resolved into two first order components. The experimentally determined points in sodium saline *plus* ouabain (Fig. 6b) agreed well with curves representing the following empirical functions.

$$\text{pH } 6.2 \quad P = 12\,(1 - \exp(-0.187)t) + 88\,(1 - \exp(-0.006)t)$$
$$\text{pH } 7.2 \quad P = 24\,(1 - \exp(-0.217)t) + 76\,(1 - \exp(-0.010)t)$$
$$\text{pH } 8.2 \quad P = 42\,(1 - \exp(-0.495)t) + 58\,(1 - \exp(-0.013)t)$$

where t is time (min).

The data show that the exchange rate increased with increasing pH. Furthermore, the amount of K^+ in the fast exchanging component also increased with increasing pH, whereas the amount in the slowly exchanging component decreased. Since identical changes were also observed in the controls, these results suggest that the variations of the rate of K^+ transport as a function of external pH are due to either an increase of membrane permeability for K^+, or a K^+ exchange independent of the ouabain-sensitive Na^+ pump. From the rate constants and the total amount of K^+ present in both compartments derived from the uptake curve, the influx of K^+ into the slices was calculated. The values were: at pH 6.2, 5.5 μequiv K^+ per min per kg dry wt; at pH 7.2, 7.9 μequiv; at pH 8.2, 21.8 μequiv K^+ per min per kg dry wt.

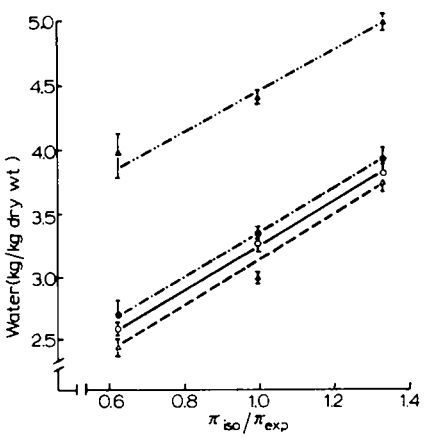

Fig. 7. The osmometric behaviour of renal cells incubated at different pH. Slices were incubated in salines differing from the standard media by a lower Na^+ concentration (95 mM), the osmolarity being adjusted with mannitol from 220 to 425 mosM. Media: ○, △, pH 6.2; ●, ▲, pH 8.2; ○, ●, standard sodium salines; △, ▲, Ca^{2+}-free sodium salines containing 0.5 mM ouabain. The values are expressed in kg water/kg dry wt, after correction for the contribution of mannitol to the dry wt (based on the determination of [^{14}C]mannitol in the tissue). Abscissa: reciprocal of the relative osmolarity, π, the subscripts iso and exp denoting isotonic and experimental conditions, respectively. All data are the means of 6 analyses ± S.E. (3 rabbits).

The effect of pH on the osmometric properties of kidney cortex cells

It has been demonstrated previously[18] that the cells of kidney cortex behave as rather imperfect osmometers. Fig. 7 shows that variations of external pH did not affect the osmometric behavior of the cells (using slices); this result suggests that within the pH range studied, no major effects on the physical and/or chemical compartmentation of water took place. A marked deviation from the controls was found only at pH 8.2 in the presence of ouabain and absence of Ca^{2+}, when the 'non-compressible space', *i.e.* on the plot, the intersect of the line with the ordinate was considerably increased. Such result is consistent with the observed swelling of the basal membrane, reported above (Fig. 1).

DISCUSSION

The results reported here provide further insight into the effects of pH, Ca^{2+} and inhibition of the Na^+ pump by ouabain or absence of external Na^+ on the steady-state water and electrolyte level in kidney cortex cells. Three aspects arising from the above findings, *i.e.* (1) the relationship between external pH and cell K^+, (2) the properties of the ouabain-insensitive transport system, and (3) the effect of pH on the regulation of cell volume, will now be considered in some detail.

Effect of pH on cellular K^+

Data given above show that with increasing external pH, the tissue K^+ markedly decreased irrespective of whether the Na^+ pump was operative (Table II, control), inhibited by ouabain (Table II), or the absence of Na^+ (Table IV), as well as under conditions where the cellular permeability was increased by the absence of external Ca^{2+} (Table III). Moreover, at least in the controls in sodium saline (Table II), this dependence of cell K^+ on external pH was not associated with marked changes of cell Na^+ and Cl^-. With respect to this phenomenon, kidney cortex differs from the diaphragm where with increasing pH an increase of cell K^+ was found[11].

When considering the possible mechanism(s) of this relationship between external pH and cell K^+, the following possibilities have to be assessed:

(a) A simple Donnan effect of external pH does not appear to explain the observed data. It has been shown previously[9] that with increasing saline pH, the apparent intracellular pH of renal cells also increased. Consequently, the dissociation of the non-diffusible intracellular anions, as well as the Donnan ratio of freely permeable ions (*e.g.* K^+, H^+ or Cl^-), would also be expected to raise. The results do not bear out such prediction.

(b) The electrochemical gradient established by the active extrusion of H^+ from renal cells was found to decrease with increasing pH (ref. 9). A coupling between the extrusion of H^+ and an accumulation of cell K^+ (*i.e.* a K^+–H^+ exchange system) might contribute to the observed pH effect. Alternatively, increasing pH might directly inhibit the activity of a specific K^+ pump; the existence of such a transport system was indicated by previous studies[18].

(c) The size of the intracellular pool of osmotically and electrochemically less active ("bound") K^+ might be affected by pH Evidence for this possibility stems from ^{42}K-uptake studies which showed that in kidney cortex slices, increasing external pH produced an increase in the rate of ^{42}K uptake simultaneously with

a decrease in the slowly exchangeable fraction. A pH-dependent accumulation of anionic metabolites in the kidney cells demonstrated by Simpson[14] might be involved here.

(d) Finally, these modifications in the K^+ content could be the result of the increase of the permeability to K^+ shown by the $^{42}K^+$ uptake.

The ouabain-insensitive mechanism for the regulation of cell volume

According to the leak-and-pump hypothesis, the cell volume is controlled by the ratio of the rate of leakage of bulk cations into the cells, and the rate of their extrusion (pumping) out of the cells[21,22]; the cell membrane is assumed to be freely permeable to water. A corollary of this hypothesis (see, *e.g.* Tosteson and Hoffman[22]) is that if the pump is inhibited, the cells should swell; in particular, the rate of swelling should depend on the ratio between K^+ or Na^+ rate of leakage. Our results show clearly that at pH 6.2 and 7.2, inhibition of the Na^+–K^+ exchange pump by ouabain, or an increase of the passive permeability of the membrane by incubation in Ca^{2+}-free saline did not affect the volume of kidney tubule cells. Similar observations have already been presented for kidney slices[1-7,28-31], and will also be shown for diaphragm cells[32]. Furthermore, it has been repeatedly shown that ouabain did not inhibit the metabolically dependent extrusion of water as an isotonic solution of NaCl from the cells previously loaded at 0 °C with Na^+, Cl^- and water, although the cardiac glycoside prevented the reaccumulation of K^+ in these cells[1,2,28-31]. It has to be implied that, in addition to the leak-and-pump system, another mechanism is involved in the regulation of cell volume.

The results presented above concerning the effect of pH on the ionic and water content of renal cells provide some additional information about the nature of the mechanisms involved in the cell volume regulation.

At pH 6.2 and 7.2 in presence of ouabain, or in Ca^{2+}-free saline, kidney cells were able to control their cell water content, and the mechanism(s) involved in this control was found to be dependent on the cellular metabolism since 2,4-dinitrophenol produced a marked swelling at all pH values tested[33]. Moreover, the system involved was shown not to be specific for Na^+, since this volume control occurred also in Na^+-free (lithium, Tris or choline) salines using either kidney cortex slices or isolated tubules; here the above cations were found to be extruded together with the available anion, Cl^- (see refs 1 and 9, and Table V).

This volume control at pH 6.2 in the absence of external Na^+ was found to be dependent on cell metabolism in view of the observation[33] that 0.1 mM 2,4-dinitrophenol produced a marked swelling of the tissue. Experiments carried out with labelled choline and Tris (to be reported elsewhere) showed that these cations readily enter the cells, replacing Na^+. Marked differences in the swelling of the tissue in the above salines in the presence of 2,4-dinitrophenol may reflect differing rates of entry of these cations into the cells. At an external pH higher than 7.5 an inhibition of the Na^+ pump produced the swelling postulated by the leak-and-pump hypothesis; it is surmized that under these conditions the ouabain-insensitive mechanism of volume control was inoperative.

In a leak-and-pump system (see above) characterized by a high permeability of the membrane to the bulk electrolytes (evidenced in the case of renal cells by the fast exchangeability of the cellular Na^+, K^+ and Cl^-, and fast net changes of these

ions down their electrochemical gradients when the active transport system is blocked by cold or some metabolic inhibitors, see, *e.g.* refs 1 and 3) it is the active transport of electrolytes (and water) which is the major determinant of the steady-state level of tissue water (*i.e.* regulation of cell volume). Therefore, in the context of the subsequent discussion, the regulation of the cell volume may be taken as a manifestation of the metabolically dependent extrusion of electrolytes and water. If, however, some of the bulk electrolytes are impermeable, the contribution of the leak to the volume control has to be reevaluated; accordingly, the use of the impermeable isethionate ion prevented the swelling of the cells under conditions where in chloride saline a massive swelling occurred (Fig. 2).

The above results are compatible with the view that an ouabain-insensitive, metabolically dependent extrusion of electrolytes and water is involved in the control of cell volume. The following characteristics of the ouabain-insensitive mechanism arise from a consideration of previous and present studies (ref. 32): (1) The transport of water takes place as a practically isotonic solution of bulk cellular electrolytes at a constant electrochemical gradient; (2) The ouabain-insensitive extrusion of water and electrolytes is dependent on metabolic energy; (3) The system is not specific for Na^+; (4) External K^+ is not required for the operation of this mechanism; (5) The ouabain-insenstive transport system is inhibited by external pH higher than 7.5; and, (6) Ca^{2+} and ATP (and/or other adenosine nucleotides) are involved.

Several hypotheses have been advanced as to the mechanism of the ouabain-insensitive control of cell volume:

(a) Ouabain does not completely inhibit the Na^+–K^+ exchange pump; in the absence of external K^+, the 'cryptic' pump could operate by a reaccumulation of K^+ leaked out of the cells through the intercellular spaces[8].

(b) In addition to the ouabain-sensitive Na^+–K^+ exchange system, Na^- is actively extruded by an ouabain-insensitive, electrogenic Na^+ pump II which is characterized by its sensitivity to ethacrynic acid; such a pump has been suggested for erythrocytes[34] and kidney cortex cells[25,30,31]. According to Sachs[35], this ouabain-insensitive Na^+ transport could be brought about by the classical (Na^+–K^+) pump if it is assumed that in the presence of ouabain the system could still bring about a Na^+–Na^+ exchange. However, this would not explain a net ouabain-insensitive extrusion of Na^+ (and Cl^-) from the cells.

Both these hypotheses have in common the assumption that the hydrostatic pressure gradient across the cell membrane is negligible, *i.e.* the extrusion of electrolytes by the pump balances the excess (intracellular) osmotic pressure due to Donnan forces; thus, the activity of water in both the intracellular and extracellular compartments is assumed to be practically equal.

(c) A mechanochemical system brings about an ouabain-insensitive extrusion of an isotonic solution of bulk intracellular ions[1,2]. Recent observations, particularly on erythrocytes, added weight to the mechanochemical hypothesis: A contractile system in the membrane phase of the red blood cell has been suggested[36] in which ATP and Ca^{2+} are involved; a Ca^{2+}-activated ATPase, isolated from erythrocyte ghosts, appears to participate in this system as well as contractile protein[37–43]. Similar evidence for the role of ATP and Ca^{2+} in the control of cell volume has been also obtained with Ehrlich ascites tumor cells[41,45] and isolated kidney tubules[45]. No

clear indications for the presence of an ouabain-insensitive system for the control of cell volume has been found in liver cells[1].

This view assumes the possibility of a major hydrostatic gradient across the cell membrane. From the equation:

$$\bar{V}\Delta p = -(RT)\,\Delta \ln a_w$$

relating the partial molal volume of water, $\bar{V}$, the pressure gradient Δp across the membrane dividing two compartments, and the gradient of water activity, Δa_w, across the membrane (see *e.g.* refs 47 and 33), it follows that a decrease in volume at constant a_w is bound to produce a corresponding increase in the pressure gradient; alternatively, the activity of cell water would have to drop with decreasing cell volume.

The results presented here are not compatible with the 'cryptic pump' hypothesis (*i.e.* incomplete inhibition of the Na^+–K^+ exchange pump) in view of the following: (1) A 5- and 10-fold increase of the ouabain concentration did not produce additional effects on the water and ionic distribution in kidney tissue (details not given here), in agreement with similar observations of Whittembury *et al.*[25]. (2) The concentration of ouabain (0.5 mM) used, completely blocked the Na^+-dependent uptake of α-methylglucoside in slices[48]. (3) An identical control of cell volume was found in complete absence of Na^+. (4) A difference in the degree of inhibition of the (Na^+–K^+) pump as a function of pH can hardly explain the variation in the Na_t/K_1 ratio observed, unless it is assumed that pH affects mainly the ouabain-insensitive and K^+-independent exchange of Na^+, or that pH affects the stoichiometry of the (Na^+–K^+) pump. (5) Finally, pH did not markedly affect the uptake of ouabain by kidney cortex slices (data not presented here in detail).

The lack of specificity for Na^+ also strongly argues against the concept of an ouabain-insensitive, electrogenic Na^+ pump being responsible for the volume control. By definition, a pump would be expected to exhibit substrate specificity for which no evidence has been obtained in the above experiments.

The above experiments appear to add weight to the mechanochemical hypothesis in that they demonstrate the involvement of Ca^{2+} and ATP; the ouabain-insensitive system of volume control in renal cells has many aspects similar to the contractile mechanism postulated for erythrocytes and Ehrlich ascites tumor cells. Further evidence for the involvement of Ca^{2+} and ATP in the regulation of the volume of renal cells, and a possible participation of a Ca^{2+}-activated nucleotidase in this process will be presented elsewhere[46]. The role of Ca^{2+} and ATP in the regulation of the cell volume may also represent a feasible explanation for such observations as the decrease of cell swelling at 0 °C by external Ca^{2+} (under these conditions, tissue ATP stays high) and the curious phenomenon first observed by Dr E.C. Foulkes (personal communication) and confirmed in this laboratory, that preliminary aerobic incubation of slices at 25 °C prevented their subsequent swelling at 0 °C.

No evidence is available so far which would allow a choice to be made between the various possibilities by which a mechanochemical system could bring about a volume control, *i.e.*: (a) A contractile system which by squeezing out of the cell an isotonic solution of electrolyte would increase the gradient of hydrostatic pressure across the membrane. Here, cell (membrane) Ca^{2+} and/or ATP (possibly also ADP

or AMP) would contribute to the stiffening of the membrane phase at low pH, preventing swelling, and have a plasticizing effect at pH higher than 7.5, thus allowing cells to swell owing to the colloid osmotic pressure. (b) Alternatively, pH-induced conformational changes of cellular proteins would alter the compartmentation of intracellular ions, thus affecting the activity of water rather than the pressure on the membrane. Indications of such effects have been obtained for cell K^+ (see Fig. 6).

Whatever the detailed mechanism, the mechanochemical system for cell volume control would still have to operate against the passive influx of ions (and water) into the cells due to the existing osmotic and electrochemical gradients across the membrane. Thus, this system is also visualized as a 'leak-and-pump'; it differs from the original concept in that not only the ouabain-sensitive Na^+ pump (involving $(Na^+–K^+)$-ATPase), but also a cation-nonselective mechanism is operative. The involvement of Ca^{2+} and ATP in this second mechanism, taken in conjunction with available data for the erythrocyte and ascites tumor cells, indicate that the physical properties of the membrane are a determinant in the regulation of the cell volume.

The effect of pH on the ouabain-insensitive regulation of cell volume

The possible mechanisms by which the ouabain-insensitive (and Na^+-independent) system for cell volume control is affected by external pH warrants an appraisal.

In the given system, the following possibilities have to be considered: The swelling effect of external pH higher than 7.5 might be produced either by an increase in the passive leak, or by an inhibition of the ouabain-insensitive extrusion of the electrolyte solution; in the latter case, an attempt should be made to distinguish between possible inhibitory effects directly on the extruding mechanism, or, alternatively, on the supply of energy for this process.

The simple argument that the swelling at pH higher than 7.5 in Na^+-free salines or in the presence of ouabain is brought about by a pH effect on the energy source, appears to be invalidated by some data given above, *i.e.* the fact that, as compared with values for lower pH, the O_2 uptake (Fig. 4) and also the active transport of some sugars[9] were not inhibited at pH 8.2 although the ATP level in the cells was decreased (Table VIII).

The time course of the swelling effect observed at pH 8.2 in sodium salines containing ouabain (Figs 3 and 5) or in the absence of Na^+ indicate that the actual extrusion mechanism was intact as long as the ATP level in the cells did not drop below a critical level. In keeping with the above discussion, at low cellular ATP the physical properties of the membrane would then allow the major swelling observed on further incubation. However, data obtained concerning the pH effect on the exchange of tissue K^+ (Fig. 6) represent an argument that external pH also affects the passive permeability of the cell membrane. Evidence indicating an effect of pH on the permeability of muscle cells has been presented[49–51], the changes being in the same direction as those observed here. Moreover, an increased permeability of the cells, particularly to Cl^-, postulated on the basis of data obtained on muscle[49–51], would also be in accord with the results presented here, *i.e.* the observation that substitution of an impermeable anion for Cl^- suppressed the tissue swelling under all experimental conditions tested (Fig. 2), and that the Cl^- content

of the tissue was not markedly affected by ouabain and Ca^{2+}-free saline at low pH (Tables II and III). It remains a matter of conjecture whether such permeability changes may also be related to the Ca^{2+} and ATP metabolism of the membrane; such a view is plausible in the light of recent data[52-54] concerning interactions of Ca^{2+} and ATP with membrane components. Alternatively, an effect of pH on dissociable groups ($-NH_3^+$ or $-COO^-$) of membrane components might produce changes in the membrane permeability.

It is obvious that more information is required to elucidate the detailed mechanism(s) by which pH affects the control of cell volume.

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REFERENCES

1 A. Kleinzeller and A. Knotková, *J. Physiol.*, 175 (1964) 172.
2 A. Kleinzeller and A. Knotková, *Physiol. Bohemoslovenica*, 13 (1964) 317.
3 A. Kleinzeller, *Arch. Biol. (Liège)*, 76 (1965) 217.
4 G. Whittembury, *J. Gen. Physiol.*, 48 (1965) 699.
5 J. S. Willis, *J. Gen. Physiol.*, 49 (1966) 1221.
6 A. D. Macknight, *Biochim. Biophys. Acta*, 150 (1968) 263.
7 G. Whittembury, *J. Gen. Physiol.*, 51 (1968) 3035.
8 J. S. Willis, *Biochim. Biophys. Acta*, 163 (1968) 506.
9 A. Kleinzeller, D. A. Ausiello, J. A. Almendares and A. H. Davis, *Biochim. Biophys. Acta*, 211 (1970) 293.
10 M. B. Burg and J. Orloff, *Am. J. Physiol.*, 203 (1962) 327.
11 S. Adler, A. Roy and A. S. Relman, *J. Clin. Invest.*, 44 (1965) 8.
12 A. Struyvenberg, R. B. Morrison and A. S. Relman, *Am. J. Physiol.*, 214 (1968) 1155.
13 S. Adler, *J. Clin. Invest.*, 49 (1970) 1647.
14 D. P. Simpson, *J. Clin. Invest.*, 46 (1967) 225.
15 C. F. Fiske and Y. SubbaRow, *J. Biol. Chem.*, 66 (1925) 375.
16 H. Adam, in H. U. Bergmeyer, *Methoden der Enzymatischen Analyse*, Verlag Chemie, Weinheim, Germany, 1962, p. 539.
17 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 183 (1951) 265.
18 A. Kleinzeller, J. Nedvídková, *Biochim. Biophys. Acta*, 135 (1967) 286.
19 M. Höfer and A. Kleinzeller, *Physiol. Bohemoslovenica*, 12 (1963) 425.
20 P. J. Goodford, *J. Physiol.*, 192 (1967) 145.
21 R. L. Post and P. C. Jolly, *Biochim. Biophys. Acta*, 25 (1957) 118.
22 D. C. Tosteson and J. F. Hoffman, *J. Gen. Physiol.*, 44 (1960) 169.
23 A. Kleinzeller, A. Knotková and J. Nedvídková and A. Knotková, *J. Gen. Physiol.*, 51 (1968) 326 (s).
24 M. B. Burg, E. F. Grollman and J. Orloff, *Am. J. Physiol.*, 206 (1964) 483.
25 G. Whittembury and F. Proverbio, *Pflügers Arch.*, 316 (1970) 1.
26 R. J. Whittam and J. S. Willis, *J. Physiol.* 168 (1963) 158.
27 M. J. Weidemann, D. A. Hems and H. A. Krebs, *Biochem. J.*, 115 (1969).
28 R. J. Whittam and R. E. Davies, *Biochem. J.*, 56 (1954) 445.
29 J. A. Willis, *Biochim. Biophys. Acta*, 163 (1968) 516.

30 A. D. Macknigh', *Biochim. Biophys. Acta*, 163 (1968) 500.
31 F. Proverbio, J. W. L. Robinson and G. Whittembury, *Biochim. Biophys. Acta*, 211 (1970) 327.
32 G. Rorive and A. Kleinzeller, *Biochim. Biophys. Acta*, in the press.
33 A. Kleinzeller, in L. E. Hokin, *Metabolic Pathways*, Vol. 6, Academic Press, New York, in the press.
34 J. F. Hoffman and F. N. Kregenow, *Ann. N. Y. Acad. Sci.*, 137 (1966) 586.
35 J. R. Sachs, *J. Gen. physiol.*, 57 (1971) 259.
36 R. I. Weed P. L. LaCelle and E. W. Merrill, *J. Clin. Invest.*, 48 (1969) 795.
37 P. Wins and E. Schoffeniels, *Arch. Int. Physiol. Biochim.*, 74 (1966) 812.
38 A. S. Rosenthal, F. N. Kregenow and H. L. Moses, *Biochim. Biophys Acta*, 196 (1970) 254.
39 T. Ohnishi, *J. Biochem. (Tokyo)*, 52 (1962) 307.
40 J. Palek, W. A. Curby and F. J. Lionetti, *Am. J. Physiol.*, 220 (1971) 19.
41 S. Marchesi, E. Steers, V. T. Marchesi and T. W. Tillack, *Biochemistry*, 9 (1969) 50.
42 T. W. Tillack, S. Marchesi, V. T. Marchesi and E. Steers, *Biochim. Biophys. Acta*, 200 (1970) 125.
43 J. Palek, W. A. Curby and F. J. Lionetti, *Am. J. Physiol.*, 220 (1971) 1028.
44 C. C. Stewart, G. Gasic and H. G. Hempling, *J. Cell. Physiol.*, 73 (1969) 125.
45 H. G. Hempling, C. C. Stewart and G. Gasic, *J. Cell. Physiol.*, 73 (1969) 133.
46 G. Rorive and A. Kleinzeller, in preparation.
47 J. Dainty, *Symp. Soc. Exp. Biol.*, 19 (1965) 329.
48 A. Almendares and A. Kleinzeller, *Arch. Biochem. Biophys.*, 145 (1971) 511.
49 O. F. Hutter and A. E. Warner, *J. Physiol.*, 189 (1967) 403.
50 O. F. Hutter and A. E. Warner, *J. Physiol.*, 189 (1967) 427.
51 O. F. Hutter and A. E. Warner, *J. Physiol.*, 189 (1967) 445.
52 R. F. Palmer and V. A. Posey, *J. Gen. Physiol.*, 55 (1970) 90.
53 Y. N. Cha, B. C. Shin and K. S. Lee, *J. Gen. Physiol.*, 50 (1971) 202.
54 P. J. Romero and R. Whittam, *J. Physiol.*, 214 (1971) 481.