BBA 76071

EFFECT OF LOW pH AND HIGH CO_2 TENSION OF THE CELL VOLUME OF RABBIT RENAL CORTEX SLICES

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(Received May 29th, 1972)

SUMMARY

1. Slices of rabbit renal cortex were leached anaerobically at 1 °C for 2 h, and subsequently reincubated at 25 °C in an oxygenated medium at pH 6.2. The pH was controlled by a bicarbonate buffer system or by a Tris–*N*-tris (hydroxymethyl)-methyl-2-aminoethanesulfonate (TES) buffer mixture.

2. The cells were able to control the cell volume both in the presence and the absence of ouabain (0.5 mM), when the pH was controlled by the Tris–TES buffer mixture.

3. When the pH was controlled by the bicarbonate buffer system (pH 6.2) the cells were able to control the cell volume in presence of ouabain (0.5 mM) whereas a marked cellular swelling took place in the absence of ouabain.

4. Bicarbonate buffer caused a decrease in the cellular pH and in the passive membrane permeability to $^{42}K^+$.

5. The results are compatible with the contention that the cell volume is controlled by two different mechanisms, an ouabain-sensitive and an ouabain-insensitive mechanism.

INTRODUCTION

In a previous paper we have described the effect of external pH (varying from 6.2 to 8.2) on the cell volume in rabbit kidney cortex slices[1]. It was shown that the inhibition of the Na^+ pump by ouabain or in the absence of external Na^+ did not abolish the control of cell volume at pH 6.2 and pH 7.2, whereas a marked cellular swelling at pH higher than 7.5 was found. The evidence indicated that the cell volume was controlled by two different mechanisms: (1) an ouabain-sensitive mechanism which controlled the cell volume at high pH (the leak and pump system[2]), and (2) an ouabain-insensitive mechanism which regulated the cell volume at low

Abbreviations: TES, *N*-tris(hydroxymethyl)methyl-2-aminomethanesulfonate; DMO, 5,5-dimethyl-2,4-oxazolidinedione.

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pH. The aim of the present paper is to describe an unexpected swelling mechanism of kidney cortex cells incubated in a bicarbonate buffer medium at pH 6.2.

METHODS AND MATERIALS

Media

The bicarbonate-buffered Na^+ saline had the following composition in mM: Na^+, 135; K^+, 6.75; Li^+, 6.75; Ca^{2+}, 2.9; Mg^{2+}, 1.35; Cl^-, 126.1; HCO_3^-, 13.5; acetate, 6.75; SO_4^{2-}, 1.35; and $H_2PO_4^-$, 1.35. The Tris–TES (*N*-tris (hydroxymethyl)methyl-2-aminomethanesulfonate) buffered Na^+ saline was prepared by substituting the $NaHCO_3$ by a equivalent amount of a Tris–TES buffer mixture[3]. Li^+ saline was prepared by substituting all Na^+ by Li^+ and K^+-free saline by substituting all K^+ by Na^+. The pH of the bicarbonate-buffered saline was adjusted by varying the composition of the gas phase. At pH 6.2 the gas phase contained 50 % O_2 and 50 % CO_2, at pH 7.2 95 % O_2 and 5 % CO_2, and at pH 8.2 99.5 % O_2 and 0.5 % CO_2.

Materials

All the experiments were carried out with previously leached kidney cortex slices. The slices were leached for 2 h at 1 °C in media of the compositions given above except that the buffer (Tris–TES or bicarbonate) was replaced by NaCl. Slices incubated in Li^+ saline were leached in Li^+ saline. Details for the incubation of slices were given previously[3].

Analytical procedures

As in previous papers from this laboratory the cell volume was given in terms of kg water/kg dry wt. Na^+, K^+ Li^+, ATP, protein, and inulin space were determined as described in a previous paper[1]. The Na^+, K^+, and Li^+ contents of the slices are given in mequiv/kg dry wt. The distribution of $^{36}Cl^-$ and 5,5-dimethyl-2,4-[2-^{14}C]-oxazolidinedione ([^{14}C]DMO) was obtained as described by Kleinzeller *et al.*[3]. The analytical values obtained allowed the calculation of the following data: The apparent ionic concentrations in the cell water and the media (subscripts i and o, respectively), and the apparent Nernst potentials (E with subscripts of the appropriate ionic species). On the basis of previous data[4], $E_{^{36}Cl^-}$ was taken to be identical with the membrane potential. The $[H^+]_i$ was calculated from $[[^{14}C]DMO]_i/[[^{14}C]DMO]_o$ at the determined pH_o.

The influx of $^{42}K^+$ was studied by first bringing the slices to a steady-state level of their cellular components (aerobic preincubation at 25 °C for 2 h 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 $^{42}K^+$ are expressed as percent of tissue K^+.

The wash-out technique[5] was employed to study the efflux of $^{22}Na^+$. Slices were first loaded with $^{22}Na^+$ by aerobic incubation in labeled saline (1 μCi/ml) for 2 h. Blotted slices were then placed into tubes with nylon netting, and the wash-out of $^{22}Na^+$ into a series of 50-ml tubes each containing 10 ml saline vigorously aerated (with 100 % O_2 in Tris–TES buffered Na^+ saline and with 50 % O_2 and 50 % CO_2 in bicarbonate-buffered Na^+ saline) was followed. The activity in the tissue (after loading and after wash-out) and in the tubes was then determined by scintillation spectrometry.

Materials

All reagents used were of analytical grade. $H^{36}Cl$ and [^{14}C]DMO and [^{3}H]-inulin were obtained from New England Nuclear Corporation, Boston, Mass. $^{22}NaCl$ from Amersham Radiochemical Centre, England and ^{42}KCl from A.E.K., Risø, Denmark.

RESULTS

Effect of pH on the steady-state water content of kidney cortex slices

The data in Tables I and II show the tissue water content of kidney cortex slices incubated for 2 h at 25 °C in Na^+ saline at pH 6.2, 7.2, and 8.2, with and without 0.5 mM ouabain. The pH in the experiments shown in Table I was controlled by a Tris–TES buffer mixture, whereas the pH in the experiments shown in Table II was controlled by a bicarbonate buffer system. If one compares the steady-state water content of kidney cortex slices incubated at the different pH values in the presence of Tris–TES buffer (Table I) with the water content of the slices incubated at the same pH in presence of bicarbonate buffer (Table II) one observes that there was no marked difference in the water content, except at pH 6.2 in the absence of ouabain. Furthermore, it was observed that the inhibition of the Na^+ pump by ouabain did not abolish the control of the cell volume at pH 6.2 and 7.2, whereas a marked cellular swelling was observed at pH 8.2 in both salines. The effect of pH on this ouabain-insensitive volume control has been analysed in a previous report[1].

As compared with values for tissue water in slices incubated at pH 6.2 in Na^+ saline buffered with a mixture of Tris–TES (2.96 kg water/kg dry wt), the tissue was swollen when incubated in a bicarbonate buffer medium at the same pH (3.69 kg water/kg dry wt). Ouabain (0.5 mM) abolished this swelling effect of bicarbonate (3.02 kg water/kg dry wt). This swelling effect of bicarbonate was specific for this

TABLE I

EFFECT OF pH AND OUABAIN ON THE WATER CONTENT OF KIDNEY CORTEX SLICES

Slices were incubated for 2 h at 25 °C in Tris–TES-buffered Na^+ salines of indicated pH, without (control) or with 0.5 mM ouabain. Values are the means ± S.E. of six samples from three rabbits.

Saline	*kg water/kg dry wt*		
	pH 6.2	*pH 7.2*	*pH 8.2*
Control	2.96 ± 0.04	2.83 ± 0.02	3.09 ± 0.05
Ouabain	2.84 ± 0.05	3.10 ± 0.12	4.56 ± 0.08

TABLE II

EFFECT OF pH AND OUABAIN ON THE WATER CONTENT OF KIDNEY CORTEX SLICES

Slices were incubated for 2 h at 25 °C in bicarbonate-buffered Na^+ saline of indicated pH, without (control) or with 0.5 mM ouabain. Values are the means ± S.E. of six samples from two rabbits.

Saline	*kg water/kg dry wt*		
	pH 6.2	*pH 7.2*	*pH 8.2*
Control	3.69 ± 0.04	2.95 ± 0.04	2.85 ± 0.06
Ouabain	3.02 ± 0.04	3.14 ± 0.03	4.20 ± 0.14

system, since no swelling was observed at pH 6.2 when a phosphate buffer system was used (data not given here).

That the observed swelling at pH 6.2 was not caused by inhibition of ATP synthesis was verified by measuring the amount of ATP present in the slices after the incubation. In Na^+ saline with bicarbonate buffer (pH 6.2) the amount of ATP was 13.0 ± 0.4 mmoles/kg protein, in Na^+ saline with Tris–TES buffer (pH 6.2) the amount of ATP was 14.6 ± 1.5 mmole/kg protein.

Changes in cell water and ion content during incubation at pH 6.2

Fig. 1 shows the changes in tissue water, Na^+ and K^+ content as a function of time during incubation at pH 6.2 (bicarbonate buffer). The slices were leached for 2 h in Na^+ saline with bicarbonate buffer (pH 6.2) before the incubation. During the first 10 min of the incubation a small recovery in the cell volume was observed together with a highly significant extrusion of Na^+ and an uptake of K^+. Thereafter the cells continued to swell. Together with the swelling a net increase in the total $Na^+ + K^+$ content was observed. The net increase in the total $Na^+ + K^+$ content was caused by uptake of K^+.

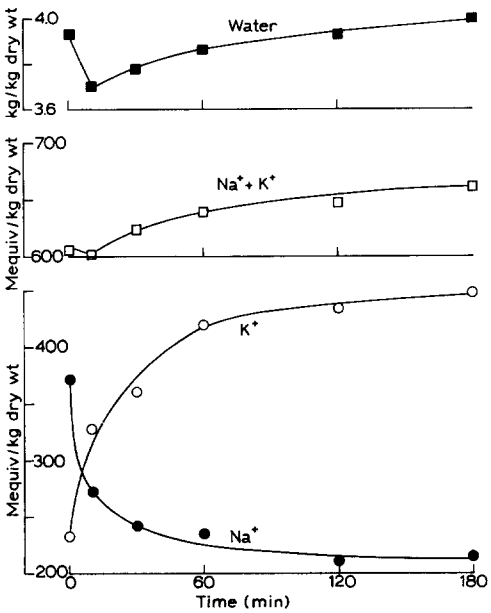

Fig. 1. Changes in tissue water and Na^+ and K^+ content during incubation in bicarbonate-buffered Na^+ saline (pH 6.2) at 25 °C. Slices were leached for 2 h at 1 °C in bicarbonate-buffered Na^+ saline (pH 6.2) before the incubation. ○—○, mequiv K^+/kg dry wt; ●—●, mequiv Na^+/kg dry wt; □—□, mequiv $(Na^+ + K^+)$/kg dry wt; ■—■, kg water/kg dry wt. Values are the means of two analyses. The curves are representative for three experiments.

Effect of various experimental conditions on the ion and water distribution in kidney cortex slices incubated at pH 6.2

Tables III and IV (c,d,e) show the water and ion contents of kidney cortex slices incubated for 2 h at pH 6.2 (bicarbonate buffer) under different conditions

which caused an inhibition of active Na^+ transport. 0.5 mM ouabain, K^+-free saline and Li^+ saline were used as inhibitors. Two series of control experiments were performed. In one series the pH was controlled by a Tris–TES buffer mixture (Tables III and IV, a), and in the other by a bicarbonate buffer system (Tables III and IV, b). The following points are of particular interest: (1) The level of tissue water and K^+ was low in presence of Na^+ transport inhibitors (Table III, c,d,e) (*i.e.* inhibitors of Na^+ transport inhibited the swelling caused by the bicarbonate buffer system at pH 6.2). (2) Slices incubated in Na^+ saline with bicarbonate buffer had a higher K^+ content per kg dry wt (*i.e.* per cell) than slices incubated in Na^+ saline with Tris–TES buffer (Table II, a,b). The data in Table IV (a,b) show that the use of bicarbonate buffer instead of Tris–TES buffer had no effect on the membrane potential ($E_{^{36}Cl^-}$) but did reduce the cellular pH; a reduction of the cellular pH was also observed in Li^+ saline (*cf.* Table IV, e and f). In the presence of Na^+ transport inhibitors a significant reduction of the membrane potential was observed (Table IV, c,d,e). Since the apparent Nernst diffusion potential E_{H^+} was opposite in sign to $E_{^{36}Cl^-}$, extrusion

TABLE III

EFFECT OF VARIOUS EXPERIMENTAL CONDITIONS ON THE ION AND WATER CONTENT OF KIDNEY CORTEX SLICES INCUBATED AT pH 6.2

Slices were incubated for 2 h at 25 °C in: (a) Na^+ saline, (b) Na^+ saline, (c) Na^+ saline *plus* 0.5 mM ouabain, (d) K^+-free saline and (e) Li^+ saline. The pH was controlled with a Tris–TES buffer mixture or a bicarbonate buffer system. Values are the means ± S.E. of six samples from two rabbits.

Expt	*Buffer*	*Water (kg/kg dry wt)*	*K^+ (mequiv/kg dry wt)*	*Na^+ (mequiv/kg dry wt)*
a	Tris–TES	2.98 ± 0.05	365 ± 15	169 ± 4
b	Bicarbonate	3.81 ± 0.13	431 ± 20	195 ± 8
c	Bicarbonate	3.15 ± 0.11	201 ± 11	327 ± 15
d	Bicarbonate	3.21 ± 0.05	260 ± 8	266 ± 9
e	Bicarbonate	2.90 ± 0.04	172 ± 11	350 ± 7*

* mequiv Li^+/kg dry wt.

TABLE IV

EFFECT OF VARIOUS EXPERIMENTAL CONDITIONS ON THE INTRACELLULAR pH AND THE MEMBRANE POTENTIAL OF KIDNEY CORTEX SLICES INCUBATED AT pH 6.2

Slices were incubated for 2 h at 25 °C in: (a) Na^+ saline, (b) Na^+ saline, (c) Na^+ saline *plus* 0.5 mM ouabain, (d) K^+-free saline, (e) Li^+ saline, and (f) Li^+ saline. The pH was controlled with a Tris–TES buffer mixture or a bicarbonate buffer system. Portions of salines were labeled with $^{36}Cl^-$ or [^{14}C]DMO. All values are the means ± S.E. of six analyses (two animals).

Expt	*Buffer*	*E_{H^+} (mV)*	*pH_i*	*$E_{^{36}Cl^-}$ (mV)*	*$E_{H^+} - E_{^{36}Cl^-}$ (mV)*
a	Tris–TES	37.7 ± 0.6	6.87 ± 0.006	−25.9 ± 1.5	63.6 ± 1.6
b	Bicarbonate	26.8 ± 1.1	6.59 ± 0.009	−25.1 ± 1.4	51.9 ± 1.8
c	Bicarbonate	18.4 ± 1.3	6.47 ± 0.017	−16.2 ± 0.8	34.6 ± 1.5
d	Bicarbonate	20.2 ± 0.5	6.51 ± 0.006	−19.2 ± 1.9	39.4 ± 2.0
e	Bicarbonate	20.8 ± 1.0	6.49 ± 0.012	−20.5 ± 1.0	41.3 ± 1.4
f	Tris–TES		6.94 ± 0.011		

of that ion took place against an electrochemical gradient. Active transport of H^+ *in vitro* in kidney cortex slices (Tris–TES buffer) has previously been demonstrated by Kleinzeller *et al.*[3]. Since $E_{^{36}Cl^-}$ can be taken as a measure of the membrane potential, the steady-state electrochemical potential for a given ion is then determined by the difference E_{ion} *minus* $E_{^{36}Cl^-}$. From Table IV it follows that the electrochemical potential for H^+ ($E_{H^+} - E_{^{36}Cl^-}$) in Na^+ saline (bicarbonate-buffered) was 51.9 mV; in the presence of ouabain 34.6 mV; in K^+-free saline 39.4 mV; and in Li^+ saline 41.3 mV. Thus the electrochemical potential for H^+ was reduced by inhibition of active Na^+ transport. This indicates that a part of the H^+ extrusion was in some way coupled to Na^+ transport *e.g. via* Na^+–H^+ exchange mechanism[6].

The effect of bicarbonate buffer on the steady-state exchange of $^{42}K^+$ in Na^+ saline plus 0.5 mM ouabain

In order to contribute to the understanding of the enhanced K^+ accumulation in the presence of bicarbonate buffer in kidney cortex slices, the apparent steady-state exchange of $^{42}K^+$ in Na^+ saline with 0.5 mM ouabain was compared at pH 6.2 in Na^+ saline buffered by either a Tris–TES mixture or bicarbonate (Fig. 2). It has been shown previously that the kinetics of K^+ exchange in kidney cortex slices can

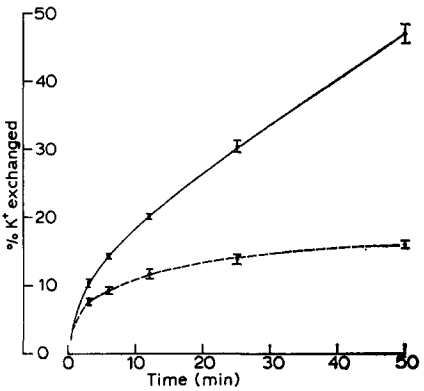

Fig. 2. The effect of bicarbonate buffer on the steady-state exchange of $^{42}K^+$ in kidney cortex slices in the presence of 0.5 mM ouabain. ●--●, slices incubated in bicarbonate-buffered Na^+ saline in the presence of 0.5 mM ouabain; ●—●, slices incubated in Tris–TES buffered Na^+ saline in the presence of 0.5 mM ouabain. Ordinate, % K^+ in the slices which had exchanged with $^{42}K^+$ in the media. Medium taken as 100 % $^{42}K^+$. The tissue was first preincubated in the respective salines at 25 °C for 2 h, then transferred to the respective salines labelled with $^{42}K^+$ and incubated further for varying periods of time. After incubation, tissue K^+ and $^{42}K^+$ were determined. Values are the means ± S.E. of six analyses (two rabbits).

be graphically resolved into two first-order components[7]. However, a part of the flux into the fast exchanging compartment results from the K^+ present in the extracellular space (the inulin space). The inulin space in these experiments contained 4 % of the total K^+ in the slices. Since the K^+ present in the extracellular space exchanges at a very high rate[8] one can treat this part as a blank value. If this is done the experimental points of Fig. 2 agree well with curves representing the following empirical functions:

In Tris–TES-buffered Na^+ saline

$$P = 4 + 6\,[1 - \exp(-0.365)t] + 90\,[1 - \exp(-0.0106)t]$$

In bicarbonate-buffered Na^+ saline

$$P = 4 + 7\,[1 - \exp(-0.173)t] + 89\,[1 - \exp(-0.0012)t]$$

where t is time in min.

The water and K^+ contents of the slices in bicarbonate-buffered Na^+ saline were 2.97 kg water/kg dry wt and 157.7 mequiv K^+/kg dry wt, and in Tris–TES-buffered Na^+ saline 2.84 kg water per kg dry wt and 150.8 mequiv K^+/kg dry wt. Since the cell volume and the K^+ content of the slices were nearly identical, the K^+ fluxes were proportional to the rate constants.

Comparing the rate constants for K^+ exchange in slices incubated in bicarbonate-buffered Na^+ saline with the rate constants for K^+ exchange in slices incubated in Tris–TES-buffered Na^+ saline one observes: (1) the flux into the fast exchanging compartment (which contained 6 or 7 % of the total K^+ in the slices) was reduced by about 50 %; (2) the flux into the slow exchanging compartment (which contained about 90 % of the total K^+ in the slices) was reduced by about 90 %. Thus the passive K^+ flux into the cells was significantly reduced in bicarbonate–buffered Na^+ saline as compared to cells incubated in Tris–TES buffered Na^+ saline.

Na^+ efflux

The effect of bicarbonate buffer on the efflux of Na^+ from kidney cortex slices was investigated by means of the washing-out technique[5]. The results of a typical experiment are shown in Fig. 3 and the mean values of the rate constants of

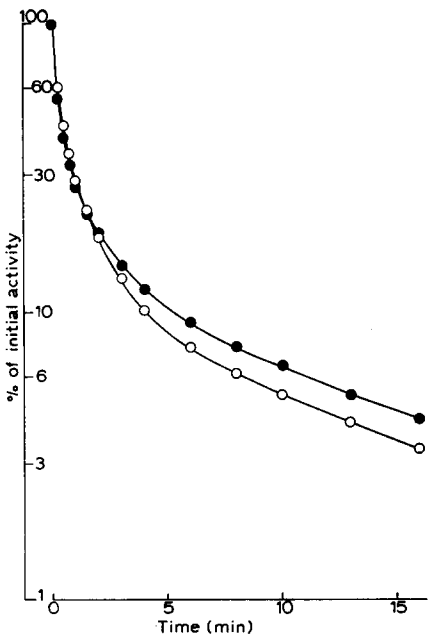

Fig. 3. Effect of bicarbonate buffer on the efflux of $^{22}Na^+$ from kidney cortex slices. Slices were first loaded with $^{22}Na^+$ by aerobic preincubation at 25 °C in bicarbonate-buffered Na^+ saline (●—●), or in Tris–TES-buffered Na^+ saline (○—○). Then the washing-out was followed at 25 °C. Ordinate, % of initial activity in the slices.

four experiments are given in Table V. It follows from these data that the bicarbonate buffer had no effect on the very fast efflux component (k_1) whereas the two slow efflux components (k_2 and k_3) were barely significantly reduced. Evidence has been presented earlier in favour of the view that the two slow efflux components correspond to the intracellular components[5,8] and the very fast component to the extracellular space[5,8]. The wash-out was performed after 2 h of incubation where the Na^+ content of the slices incubated in Tris–TES-buffered Na^+ saline was in a steady state[3]. From Fig. 1 it appears that the Na^+ content of slices incubated in bicarbonate-buffered Na^+ saline for 2 h was near steady state. The Na^+ influx is equal to the Na^+ efflux during steady state. The Na^+ content and the cellular Na^+ concentration of the slices used in the wash-out experiments were: (1) in bicarbonate-buffered Na^+ saline 263.6 ± 7.8 mequiv Na^+/kg dry wt and 39.5 ± 2.5 mM, and (2) in Tris–TES-buffered Na^+ saline 234.0 ± 3.9 mequiv Na^+/kg dry wt and 45.9 ± 2.1 mM. Thus both the cellular Na^+ concentration and the slow rate constants (k_2 and k_3, Table V) were reduced when the slices were incubated in bicarbonate-buffered Na^+ saline. Accordingly, the slight reduction in Na^+ efflux was caused by a reduction in passive Na^+ flux into the cells.

TABLE V

EFFECT OF BICARBONATE BUFFER (pH 6.2) ON THE RATE CONSTANTS OF STEADY-STATE EFFLUX OF $^{22}Na^+$ FROM KIDNEY CORTEX SLICES

For experimental conditions, see Fig. 3. Values are the means of four paired experiments ± S.E.

Buffer	*Rate constants*		
	k_1 (min^{-1})	k_2 (min^{-1})	k_3 (min^{-1})
Tris–TES	2.9 ± 0.3	0.62 ± 0.02	0.079 ± 0.003
HCO_3^-	2.6 ± 0.3	0.45 ± 0.02	0.064 ± 0.004
Significance of difference	$0.8 > P > 0.7$	$0.1 > P > 0.05$	$0.05 > P > 0.025$

DISCUSSION

It has been shown above that kidney cortex slices incubated at pH 6.2 in bicarbonate-buffered Na^+ saline swell as compared to slices incubated at pH 6.2 in a Tris–TES-buffered Na^+ saline (*cf.* Tables I and II). The swelling of the slices was accompanied by a simultaneous accumulation of K^+ (Fig. 1). The fact that the swelling and the K^+ uptake were inhibited by (Na^+–K^+)-pump inhibitors (Table III) indicates that the swelling was caused by K^+ accumulation. Furthermore, it was shown that both the passive K^+ and Na^+ permeabilities were reduced in bicarbonate-buffered Na^+ saline as compared to Tris–TES-buffered Na^+ saline (Figs 2 and 3).

According to the leak and pump hypothesis[2], the cell volume is controlled by the following parameters: (1) permeability to Na^+ and K^+, (2) the amount of nonpenetrating ions, and (3) the rate of the coupled (Na^+–K^+)-pump.

The following hypothesis (which is based on the leak and pump hypothesis) would explain the swelling observed at pH 6.2. Na^+ is pumped actively out of the cells and K^+ is pumped actively into the cells *via* a coupled (Na^+–K^+)-pump. The rate

of the Na^+, K^+ pump is determined by the Na^+ concentration in the cells (*i.e.* the passive Na^+ influx). Since the Na^+ pump is a coupled Na^+, K^+ pump, it appears that the active uptake of K^+ depends on the passive Na^+ influx. However, the passive K^+ efflux is reduced relatively more than the passive Na^+ influx. Therefore, the cells have to accumulate K^+, and consequently Cl^- and water, and swell. Thus it was this active uptake of KCl which provided the energy necessary to overcome the "ouabain-insensitive cell volume control mechanism". The data in Table IV show that the intracellular pH not only was a function of the extracellular pH but also dependent on the type of buffer used. Struyvenberg *et al.*[9] showed in their study of the acid–base behavior of separated canine renal tubule cells that "Cell acidity varied in direct linear relation to extracellular acidity when the latter was changed by systematic variation of either P_{CO_2} or extracellular $[HCO_3^-]$, but P_{CO_2} had a significantly greater effect." Thus the data in Table IV and the data of Struyvenberg *et al.*[9] showed that an increase in P_{CO_2} (at constant pH_0) caused a decrease in the cellular pH. Struyvenberg *et al.*[9] did suggest that the decreased pH_i was caused by a specific effect of CO_2 on the cell membrane. However, the decrease in pH_i could also be due to the cell membrane being very permeable to CO_2. Thus H^+ in a CO_2 (bicarbonate buffer) containing medium could enter the cells both as H^+ via leak and as CO_2 across the cell membrane. The CO_2 in the cells would cause a decrease in the pH_i ($CO_2 + H_2O \rightarrow H_2CO_3$). According to this hypothesis it was an increased flux of H^+ into the cells which caused the decrease in pH_i.

It has been shown in previous papers[1,3] that K^+ permeability decreased with decreasing pH_0. The decrease in passive K^+ permeability observed in slices incubated in bicarbonate-buffered Na^+ saline could then be due to the decreased pH_i caused by the bicarbonate buffer. Therefore, one might conclude that the K^+ permeability depended more on the intracellular pH than on the extracellular pH. Since the decrease in passive Na^+ permeability was somewhat lower than the decrease in K^+ permeability, one might suggest that it was the pH_0 which was the major determinant of Na^+ permeability. However, the data in Table IV indicate that there also was a Na^+–H^+ exchange mechanism in the cells. Therefore, Na^+ could enter the cells *via* two pathways, namely *via* the "leak" and *via* the Na^+–H^+ exchange mechanism. Accordingly, the passive Na^+ flux through "leak" could be reduced by the same amount as the passive K^+ efflux; but the decreased pH_i would increase Na^+ influx *via* the Na^+–H^+ exchange mechanism. This might be the reason why K^+ permeability was reduced relatively more than Na^+ permeability.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Prof A. Kleinzeller and to the whole staff of the Department of Physiology, The School of Medicine, University of Pennsylvania, for their hospitality, which enabled him to carry out a considerable part of the experimental work described here. The work was supported by Grant AM-12619 awarded to Prof. A. Kleinzeller. I also wish to acknowledge support from the Danmark Amerika Fondet, and for the Fullbright Travel grant.

REFERENCES

1 G. Rorive, R. Nielsen and A. Kleinzeller, *Biochim. Biophys. Acta*, 266 (1972) 376.
2 D. C. Tosteson and J. F. Hoffman, *J. Gen. Physiol.*, 44 (1960) 169.

3 A. Kleinzeller, D. A. Ausiello, J. A. Almendares and A. H. Davies, *Biochim. Biophys. Acta*, 211 (1970) 293.
4 A. Kleinzeller, A. Knotkova and J. Nedvidkova, *J. Gen. Physiol.*, 51 (1968) 326 (s).
5 A. Kleinzeller, K. Janacek and A. Knotkova, *Biochim. Biophys. Acta*, 59 (1962) 239.
6 R. F. Pitts and R. S. Alexander, *Am. J. Physiol.*, 144 (1945) 239.
7 R. J. Whittam and R. E. Davies, *Biochem. J.*, 56 (1954) 445.
8 A. Kleinzeller and A. Knotkova, *Physiol. Bohemoslovenica*, 16 (1967) 214.
9 A. Struyvenberg, R. B. Morrison and A. S. Relman, *Am. J. Physiol.*, 214 (1968) 1155.