ACKNOWLEDGEMENTS

The author would like to thank Miss S. ECKHOFF and Miss K. BLANKENSTEIN as well as Mrs. D. FANCK and Miss A. SEEGER for technical assistance. This work was supported in part by the Deutsche Forschungsgemeinschaft.

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BBA 75206

WATER AND ELECTROLYTE CONTENTS OF RAT RENAL CORTICAL SLICES INCUBATED IN MEDIUM CONTAINING *p*-CHLOROMERCURIBENZOIC ACID OR *p*-CHLOROMERCURIBENZOIC ACID AND OUABAIN

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(Received July 16th, 1968)

SUMMARY

1. Slices of rat renal cortex were leached anaerobically at 0.5° and subsequently reincubated at 25° in either oxygenated ordinary medium, oxygenated medium containing 0.2 mM *p*-chloromercuribenzoic acid (PCMB) or oxygenated medium containing 0.2 mM PCMB and 10 mM ouabain.

2. The presence of PCMB caused a greater swelling of leached slices than occurred in ordinary medium alone. During reincubation there were significant losses of water, Na^+ and Cl^- and significant uptakes of K^+, though slices in medium with PCMB always contained more water, Na^+ and Cl^- and less K^+ than did slices reincubated in ordinary medium.

3. When ouabain was also present in the medium there were no appreciable differences in the water or Cl^- contents of the slices but they contained more Na^+ during reincubation and showed a net loss of K^+.

4. PCMB produced no convincing depression of O_2 uptake in ordinary medium. However, the presence of ouabain depressed O_2 uptake significantly.

5. Slices leached anaerobically at 0.5° showed no increase in their permeability to inulin when PCMB was present.

6. The results provide no convincing evidence that PCMB specifically inhibits any active process regulating cellular volume. They are more easily interpreted in terms of a change in membrane permeability. However, the results do support the hypothesis that the cellular volume is regulated by a mechanism other than a ouabain-sensitive Na^+ pump.

INTRODUCTION

The results of recent work[1–3] have suggested that renal cortical cells may regulate their water content by a mechanism other than a K^+-dependent, cardiac glycoside-sensitive, transport of Na^+, but the nature and properties of this mechanism remain to be elucidated.

BOWMAN AND LANDON[4] recently reported that the non-diuretic organic mercurial *p*-chloromercuribenzoic acid (PCMB) caused swelling of rat renal cortical slices but did not produce any significant loss of K^+ from the tissue. It is therefore possible, as their work indicated, that PCMB may preferentially interfere with a mechanism regulating cellular volume rather than affecting the cardiac glycoside-sensitive Na^+–K^+ exchange. This possibility has been explored in the present work, a preliminary account of a portion of which has appeared elsewhere[5].

METHODS

Media. The media had the following composition in mequiv/l: Na^+, 146; K^+, 5; Ca^{2+}, 2; Mg^{2+}, 2; Cl^-, 132; SO_4^{2-}, 2; acetate, 10; buffered with phosphate (8 mM) at pH 7.26. Some media also contained either PCMB (Nutritional Biochemical Corporation) 0.2 mM, or PCMB 0.2 mM and ouabain (B.D.H.) 10 mM. The ordinary medium differed from that previously used[2,6] in that the concentration of Ca^{2+} was lower. This modification avoided the formation of a visible precipitate when PCMB was dissolved in the medium.

Procedure. The procedure employed in experiments in which slices were leached at 0.5° before reincubation at 25° was similar to that previously described[2]. Inhibitors, when present, were contained in both the leaching and reincubation media.

O_2 consumption of slices was determined at 25° by standard manometric methods using Warburg manometers.

Abbreviation: PCMB, *p*-chloromercuribenzoic acid.

Analytical methods. Water, Na^+, K^+ and Cl^- were determined as previously described[2]. The extracellular space was measured with inulin as previously described[6].

Results. The results shown in the tables and figure represent the mean ± S.D. of the relevant observations. The statistical significance of differences between groups was evaluated by Student's *t* test.

RESULTS AND DISCUSSION

The extracellular space in slices leached at 0.5°

The extracellular space at 0.5° was measured, as previously described[6], in slices prepared from the kidneys of 5 rats. The measured extracellular space of 22.4 ± 1.6 % tissue wet wt. (7 slices) after 150 min in ordinary medium did not differ significantly from that reported after 120 min at 0.5° ($P > 0.20$). The space in the grossly swollen slices leached in medium with PCMB (water content 4.28 ± 0.26 kg per kg tissue dry matter, 8 slices) was 18.6 ± 1.4 % tissue wet wt.; this was significantly lower than the space in ordinary medium ($P < 0.001$) and confirms the finding reported previously[6] that gross swelling of tissue decreases the percentage of wet weight which is extracellular. In particular there was no evidence to suggest that PCMB affected the permeability of the cellular membranes to inulin. This finding agrees with that reported by BOWMAN AND LANDON[4].

O_2 uptake and composition of slices incubated at 25°

Table I shows the composition of slices after equilibration for 15 min in oxygenated ordinary medium at 25° and after subsequent incubation for 75 min at 25° in manometer flasks. Slices incubated in ordinary medium maintained a relatively constant content of water, Na^+ and Cl^-, but showed a comparatively small though significant uptake of K^+ ($P < 0.001$). In contrast slices incubated in media containing PCMB became swollen with the uptake of Na^+ and Cl^-, and, in medium with PCMB alone, lost 12 % of their K^+ ($P < 0.01$). This loss was relatively slight, however, compared with the loss of 65 % of the initial K^+ content which occurred into medium

TABLE I

THE WATER AND ION CONTENTS OF RAT RENAL CORTICAL SLICES INCUBATED FOR 75 min AT 25° IN MANOMETER FLASKS IN AN ATMOSPHERE OF O_2

Slices were first equilibrated for at least 15 min in oxygenated ordinary medium at 25°, blotted, weighed on a torsion balance and transferred to the manometer flasks, where, after equilibration for 15 min, O_2 consumption was read at 15-min intervals for a further 60 min. Two slices were placed in 3 ml of medium in each flask and these slices were subsequently analysed together. Number of observations from kidneys of eight rats are shown in parentheses.

Conditions	*Tissue contents (mequiv/kg dry matter)*				
	H_2O*	Na^+	K^+	Cl^-	$Na^+ + K^+$
After equilibration (8)	2.53 ± 0.10	265 ± 28	254 ± 20	206 ± 10	519
Ordinary medium (7)	2.63 ± 0.07	251 ± 15	291 ± 6	213 ± 10	542
Medium + 0.2 mM PCMB (8)	3.12 ± 0.13	399 ± 33	223 ± 18	281 ± 21	622
Medium + 0.2 mM PCMB + 10 mM ouabain (8)	3.19 ± 0.09	536 ± 15	90 ± 6	306 ± 10	626

* Values in kg/kg dry matter.

containing PCMB and ouabain. Ouabain had no apparent effect on the water content of the tissue ($P > 0.20$), the K^+ lost being replaced by Na^+.

The O_2 uptakes of these slices are shown in Table II. BOWMAN AND LANDON[4] have previously reported that PCMB is without effect on the O_2 consumption of rat renal cortical slices when expressed in terms of final dry weight. It was found here that slices incubated in medium containing PCMB showed a barely significant depression of O_2 uptake ($P < 0.05$) when the values were expressed in terms of initial wet weight of tissue. This difference disappeared when the results were expressed in terms of final dry weight ($P > 0.99$). This apparent difference in the results is possibly explained by the different amounts of tissue solid lost into ordinary medium and into medium containing PCMB. These losses of tissue solids can be calculated from a knowledge of the proportion of dry matter in the tissue after equilibration. Slices incubated in ordinary medium apparently lost 14.1 ± 3.3 % of their initial dry matter, whereas slices incubated in ordinary medium with PCMB lost 24.7 ± 3.7 % ($P < 0.001$). The presence of ouabain had no further effect on this loss of solid matter (25.5 ± 2.5 %; $P > 0.60$).

TABLE II

THE O_2 CONSUMPTION OF RAT RENAL CORTICAL SLICES INCUBATED FOR 75 min AT 25°

The O_2 uptake was measured at 15-min intervals for 60 min following 15 min equilibration in the manometer flasks. Before transfer to the manometer flasks slices had been equilibrated for at least 15 min at 25° in oxygenated ordinary medium. Number of observations from kidneys of eight rats are shown in parentheses.

Conditions	*O_2 consumption (µl/mg per h)*	
	Initial wet wt.	*Final dry wt.*
Ordinary medium (7)	2.68 ± 0.37	11.0 ± 1.7
Medium + 0.2 mM PCMB (8)	2.30 ± 0.20	10.9 ± 1.0
Medium + 0.2 mM PCMB + 10 mM ouabain (8)	1.85 ± 0.39	8.7 ± 1.8

The presence of ouabain depressed O_2 consumption considerably when compared on a wet weight basis with the O_2 consumption of slices in ordinary medium ($P < 0.005$) or ordinary medium containing PCMB ($P < 0.02$). The fall of 31 % in O_2 consumption produced by ouabain is of the same order as that observed by others in rabbit[7] and rat[8] renal slices incubated in balanced saline media containing ouabain.

Slices leached at 0.5° and subsequently reincubated at 25°

The results of these experiments are shown in Fig. 1. The behaviour of slices leached and reincubated in the ordinary medium was similar to that previously described[2]. In contrast slices leached in media containing PCMB swelled to a much greater extent. This increase in water content was accompanied by a corresponding increase in Na^+ and Cl^- and by a greater loss of K^+. Similar behaviour has been reported with PCMB and other mercurials by KLEINZELLER AND CORT[9].

During reincubation slices in all media showed significant losses of water and ions and these recoveries were well maintained. After 60 min, slices in ordinary

medium alone contained 16% less water and 15% less Cl^-. After the same period in oxygenated medium with PCMB slices contained 18% less water and 15% less Cl^-, and in the presence of PCMB and ouabain the losses were 15 and 14% respectively. Thus the percentage losses were similar in each medium but with PCMB the changes took place at a higher level. Slices of renal cortex reincubated in the presence of some mercurial diuretics and $HgCl_2$ have been reported to behave similarly[9].

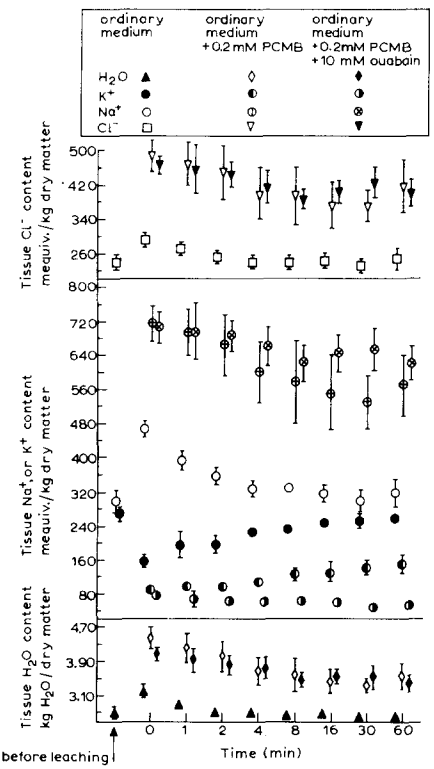

Fig. 1. Composition of rat renal cortical slices leached anaerobically at 0.5° for 150 min and then reincubated at 25° for up to 60 min either in oxygenated ordinary medium, in oxygenated medium containing 0.2 mM PCMB or in oxygenated medium containing 0.2 mM PCMB and 10 mM ouabain. Each point represents the mean ± S.D. of 6 separate observations on slices from the kidneys of 24 rats. Composition of slices at end of leaching plotted at 0.

Though the presence of 10 mM ouabain together with PCMB had no appreciable effect on the water and Cl^- contents of the slices (apart from barely significant differences in water content after leaching ($P < 0.025$) and Cl^- content after 30 min reincubation ($P < 0.025$) all other values did not differ significantly), slices reincubated with ouabain and PCMB always contained more Na^+ and less K^+. The effect on K^+ was particularly striking. There was a barely significant difference in the leached values ($P < 0.05$) but during reincubation slices continued to lose K^+ into medium with ouabain, and after 60 min showed a net loss of 31% of their K^+ content after leaching. In contrast slices reincubated with PCMB alone showed a highly significant reaccumulation of K^+ and after 60 min contained 40% more K^+ than did leached slices. This compares with the 60% increase in K^+ observed in leached slices reincubated in ordinary medium.

Slices reincubated in media containing PCMB thus retained their capacity to exchange Na^+ for K^+, a process largely inhibited by the presence of 10 mM ouabain. The effects of ouabain were therefore similar to those reported earlier[2] in ordinary medium, namely inhibition of Na^+–K^+ exchange without effect on the regulation of cellular volume.

The failure of PCMB to produce any appreciable decrease in tissue O_2 consumption, together with the significant, maintained, recovery in cellular volume which occurred during reincubation suggest that PCMB did not inhibit a mechanism concerned primarily with the regulation of cellular volume. The greater swelling which occurred when slices were leached in media with PCMB is similar to that observed by others[9] and is most easily interpreted by assuming that PCMB increased cellular permeability. The relatively small loss of K^+ (when compared with equilibrated values, Table I) from slices incubated in manometers, and the failure of slices reincubated in medium with PCMB to reaccumulate K^+ to the same extent as those reincubated in ordinary medium (Fig. 1) could also be explained by an increase in cellular membrane permeability, the active Na^+–K^+ pump being unaffected by PCMB but the passive leakage of K^+ from the cells being increased. It has been shown[4] that the concentration of PCMB used in this work is without significant effect on rat kidney membrane ATPase.

Finally, the results obtained when slices were incubated in medium containing both PCMB and ouabain fully support the conclusion reached earlier[2], and by other workers[1,3], that ouabain interferes with coupled Na^+–K^+ exchange rather than with the regulation and maintenance of cellular volume.

ACKNOWLEDGEMENTS

I wish to thank Professor J. R. ROBINSON for his advice and encouragement during this work. I also wish to thank the Medical Research Council of New Zealand for financial support.

The author is a member of the Scientific Staff of the Medical Research Council of New Zealand.

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