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THE EFFECTS OF ETHACRYNIC ACID ON THE ELECTROLYTE AND WATER CONTENTS OF RAT RENAL CORTICAL SLICES

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SUMMARY

1. Slices of rat renal cortex were leached anaerobically at 0.5° and reincubated at 25° in oxygenated medium containing ethacrynic acid. In 0.5 mM ethacrynic acid slices showed definite, and in 0.75 mM probable, initial recovery in volume during reincubation which was subsequently not maintained. Slices reincubated in 1 mM ethacrynic acid continued to swell without significant initial recovery.

2. Slices reincubated in oxygenated medium with 1 mM ethacrynic acid and slices reincubated anaerobically with 1 mM ethacrynic acid and 10 mM iodoacetate became significantly more swollen than slices reincubated anaerobically with 10 mM iodoacetate alone.

3. O_2 consumption of slices incubated at 25° in medium containing ethacrynic acid was markedly depressed and slices swelled. The presence of 10 mM ouabain together with 1 mM ethacrynic acid did not decrease O_2 consumption further though it resulted in a lower tissue K^+ content.

4. Slices leached anaerobically and reincubated in oxygenated medium with 0.01 mM 2,4-dinitrophenol showed initial recovery in volume which was not maintained. Slices leached and reincubated with 0.1 mM and 1 mM 2,4-dinitrophenol showed no appreciable recovery.

5. The results were consistent with the possibility that ethacrynic acid inhibits cellular metabolism, rather than with the suggestion that ethacrynic acid specifically inhibits a mechanism primarily concerned in the regulation of cellular volume.

6. Although ethacrynic acid probably increased the permeability of cellular membranes to K^+ it was not possible to decide whether it also specifically interfered with linked Na^+–K^+ transport or, instead, produced loss of tissue K^+ as a result of inhibition of metabolism.

INTRODUCTION

There is now evidence that the regulation of cellular volume in renal tissue occurs by a mechanism independent of a conventional Na^+–K^+-linked, ouabain-sensitive, cation pump[1–4]. The nature of this process remains to be firmly established.

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KLEINZELLER and co-workers[1,5–7] have suggested that a contractile or elastic mechanism may contribute to the regulation of cellular volume, while WHITTEMBURY[3] has postulated that renal tissue may possess a Na^+ pump insensitive to cardiac glycosides. The suggestion[8] that erythrocytes possess a second Na^+ pump and that this pump is insensitive to ouabain but inhibited by the diuretic ethacrynic acid raised the possibility that a second Na^+ pump in renal tissue, if such indeed exists, might be sensitive to ethacrynic acid. It has also been suggested recently that ethacrynic acid inhibits active Na^+ transport in toad oocytes[9]. While this work was in progress a report by WHITTEMBURY[10] suggested that the regulation of cellular volume in slices of guinea pig renal cortex was indeed specifically inhibited by ethacrynic acid. The results of the present work, however, suggest that, while the maintenance of normal cellular volume in slices of rat renal cortex is prevented by ethacrynic acid, this effect is more likely to be the consequence of interference with cellular metabolism and, perhaps, some increase in cellular membrane permeability, than to be due to any specific inhibition of a mechanism concerned primarily with the regulation of cellular volume.

METHODS

Media

The media had the following composition in mequiv/l: Na^+, 146; K^+, 5; Ca^{2+}, 5; Mg^{2+}, 2; Cl^-, 134; SO_4^{2-}, 2; acetate, 10 buffered with phosphate (8 mM) at pH 7.26. Some media also contained ethacrynic acid (Merck, Sharp and Dohme), ouabain (B.D.H.), or 2,4-dinitrophenol. In some media 10 mM sodium acetate was replaced by 10 mM sodium iodoacetate. Media with dinitrophenol also contained 5 mM glucose. The pH of each medium containing ethacrynic acid was adjusted to that of ordinary medium by the addition of 1 M NaOH where required.

Procedure

The procedure in experiments in which slices were leached at 0.5° before reincubation at 25° was similar to that previously described[2]. As before inhibitors were present throughout both leaching and reincubation. O_2 consumption of slices was determined at 25° by standard manometric methods using Warburg manometers as described previously[11]. The analytical methods have been described elsewhere[2].

Results

The results shown in the tables and figures represent the mean ± S.D. of the relevant observations. The statistical significance of differences between groups was evaluated by Student's *t* test. The intracellular values, where provided, were derived using a value of 26 % wet wt. of tissue for slices of normal or slightly increased water content (water content 2.50–3.50 kg water per kg tissue dry matter) and a value of 19 % wet wt. of tissue for grossly swollen slices (3.80–4.50 kg water per kg tissue dry matter). The justification for assuming these values has been discussed elsewhere[12]. Though the distinction between the groups is somewhat arbitrary, important comparisons within groups have been made using the same percentage value for the extracellular space.

RESULTS

Slices leached at 0.5° and subsequently reincubated at 25°

In preliminary experiments it was established that concentrations of ethacrynic acid less than 0.1 mM had no apparent effect on the composition of the slices while concentrations above 1 mM appeared to cause a marked loss of tissue solid and gross tissue swelling. The water content of slices leached for 150 min and subsequently reincubated for 60 min in medium containing 2 mM ethacrynic acid were 3.67 and 5.40 kg water per kg tissue dry matter respectively (one observation), and in medium containing 10 mM ethacrynic acid the water contents were 6.80 and 7.30 kg water per kg tissue dry matter respectively (mean of two observations). It was therefore decided to examine in more detail the effects of 0.5, 0.75 and 1 mM ethacrynic acid and the results are illustrated in Fig. 1.

The slices leached in media containing ethacrynic acid became swollen to the same extent and this swelling was of the same order as that observed in other experi-

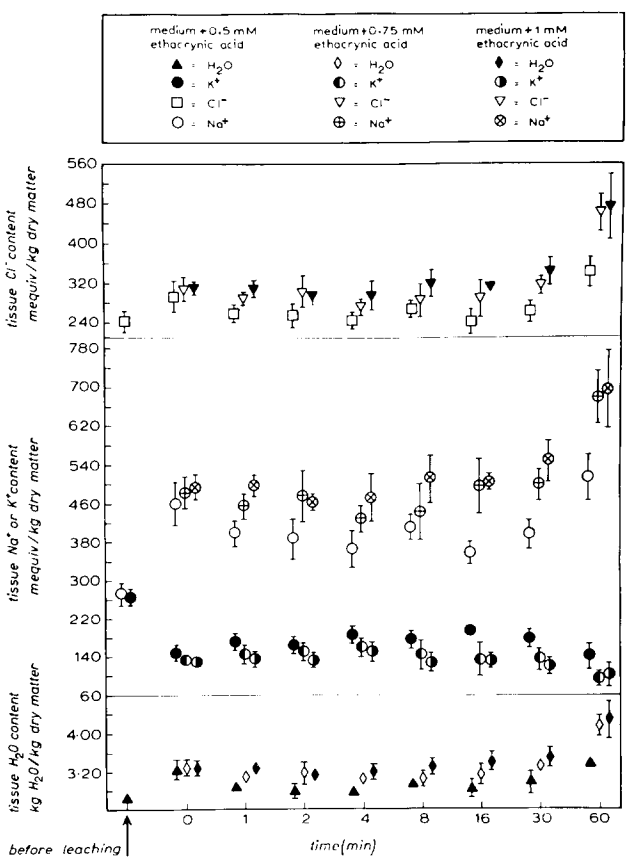

Fig. 1. Composition of rat renal cortical slices leached anaerobically at 0.5° for 150 min and then reincubated at 25° in oxygenated medium containing either 0.5, 0.75 or 1 mM ethacrynic acid. Each point represents the mean ± S.D. of 5 separate observations on slices from the kidneys of 22 rats. Composition of slices at end of leaching plotted at 0.

ments where slices have been leached in ordinary medium[2]. This swelling was accompanied by the uptake of Na^+ and Cl^- and loss of K^+.

During subsequent reincubation, slices in medium containing 0.5 mM ethacrynic acid showed initial significant recovery in cellular volume with losses of water, Na^+ and Cl^-, the water and Cl^- contents after 4 min reincubation being not significantly different from those of slices before leaching ($P > 0.10$, $P > 0.70$ respectively). There was also some net uptake of K^+, though, in contrast to the behaviour observed in ordinary medium[2], the K^+ content never returned to the level observed in slices before leaching. This inhibition of K^+ accumulation was reflected in the behaviour of tissue Na^+. Though there was a significant net loss of Na^+ ($P < 0.001$) after 4 min, the tissue Na^+ content also never returned to the level found in slices before leaching. This recovery in cellular volume was relatively well maintained for the first 30 min of reincubation but during the next 30 min slices became swollen with the uptake of Na^+ and Cl^- and loss of K^+, so that after 60 min reincubation their composition was similar to that of slices after leaching. This behaviour is in marked contrast to the well maintained recovery observed in previous experiments[2] in slices reincubated in ordinary medium, ordinary medium containing ouabain and K^+-free medium.

The water content of slices leached and reincubated in medium containing 0.75 mM ethacrynic acid probably recovered in the early minutes of reincubation, the water contents after 1, 4 and 8 min all being significantly lower than those observed in leached slices ($P < 0.05$, $P < 0.01$, $P < 0.05$ respectively). There was also some loss of Na^+ and Cl^- initially, though only the values after 4 min ($P < 0.02$, $P < 0.025$ respectively) were significantly different. Also, a small, barely significant, net uptake of K^+ had occurred after 4 min ($P < 0.05$). This apparent tendency for some small initial recovery in cellular volume and ionic composition was not maintained as reincubation continued, and after 60 min slices were considerably swollen, with uptake of Na^+ and Cl^- and loss of K^+ ($P < 0.001$ in each case compared with leached values).

Slices leached and reincubated in medium containing 1 mM ethacrynic acid showed no appreciable recovery in composition but continued to gain water, Na^+ and Cl^- throughout reincubation. These slices too showed a tendency to lose K^+, though after 60 min reincubation their K^+ content was not significantly different from that of leached tissue ($P > 0.05$).

O_2 uptake and composition of slices incubated at 25°

Table I shows the O_2 uptake and Table II the composition of slices of rat renal cortex incubated in manometer flasks at 25° for 75 min either in ordinary medium, ordinary medium containing 1 mM ethacrynic acid or ordinary medium containing 1 mM ethacrynic acid and 10 mM ouabain. It was found that the presence of 1 mM ethacrynic acid reduced O_2 consumption by 54% (on a final tissue dry wt. basis). WHITTEMBURY[10] in guinea pig, and JONES AND LANDON[13] in rabbit, have reported a decrease of approx. 56% in O_2 consumption of slices of renal cortex incubated in medium containing 1 mM ethacrynic acid. Unlike the effect in guinea pig renal slices[10], the presence of ouabain, together with ethacrynic acid did not depress O_2 consumption further.

In contrast to the slices incubated in ordinary medium, which maintained a normal tissue composition, slices incubated in medium containing ethacrynic acid

TABLE I

THE EFFECTS OF 1 mM ETHACRYNIC ACID AND 1 mM ETHACRYNIC ACID + 10 mM OUABAIN ON O_2 CONSUMPTION OF RAT RENAL CORTICAL SLICES

Slices were incubated at 25° in oxygenated ordinary medium and then transferred to medium in Warburg manometers where they remained for 75 min at 25°. After 15 min equilibration the O_2 consumption was measured over the next 60 min. Two slices were present in each manometer (approx. 50 mg wet wt. of tissue) and each value represents the mean ± S.D. of 6 separate observations on slices from kidneys of 6 rats.

Conditions	*Oxygen consumption*	
	μl/mg/h initial wet wt.	*μl/mg/h final dry wt.*
Ordinary medium	2.28 ± 0.29	9.2 ± 1.1
Medium + 1 mM ethacrynic acid	0.90 ± 0.25	4.2 ± 1.2
Medium + 1 mM ethacrynic acid + 10 mM ouabain	0.99 ± 0.31	4.4 ± 1.6

TABLE II

THE EFFECTS OF 1 mM ETHACRYNIC ACID AND 1 mM ETHACRYNIC ACID + 10 mM OUABAIN ON TISSUE WATER AND ELECTROLYTE CONTENTS OF RAT RENAL CORTICAL SLICES INCUBATED IN WARBURG MANOMETERS

Details of experimental procedure given in Table I.

Conditions	*Tissue water content (kg/kg dry matter)*	*Tissue Na^+ content**	*Tissue Cl^- content**	*Tissue K^+ content**	*Intracellular K^+ concn. (mequiv/l intracellular water)*
Before manometry	2.60 ± 0.19	259 ± 23	236 ± 32	250 ± 14	147 ± 9
Ordinary medium	2.67 ± 0.11	236 ± 9	218 ± 9	292 ± 6	168 ± 7
Medium + 1 mM ethacrynic acid	3.84 ± 0.14	600 ± 34	404 ± 22	110 ± 18	36 ± 6
Meidum + 1 mM ethacrynic acid + 10 mM ouabain	3.60 ± 0.07	587 ± 26	390 ± 15	72 ± 7	24 ± 3

* mequiv/kg dry matter.

became swollen with the uptake of Na^+ and Cl^- and loss of K^+. Slices in medium containing ethacrynic acid and ouabain also swelled, though they contained slightly less water after 75 min ($P < 0.005$). They also lost more K^+, losing 71 % of their initial K^+ compared with the loss of 56 % from slices in medium with ethacrynic acid alone. This difference in K^+ content was highly significant ($P < 0.001$), as was the difference between the calculated intracellular K^+ concentrations ($P < 0.005$). It therefore appears that in rat, as in guinea pig[10], the presence of ouabain produces a greater loss of K^+ from renal slices than does ethacrynic acid alone This is presumably due to inhibition of active Na^+–K^+ exchange by ouabain. Yet the addition of ouabain did not produce any further significant reduction in O_2 consumption. This finding may simply reflect the small O_2 consumption related to the decreased active K^+ transport under these experimental conditions. Alternatively it may suggest that energy derived from anaerobic metabolism, rather than from aerobic metabolism, was supporting K^+ transport in the presence of ethacrynic acid. It has been shown that anaerobic metabolism probably supports some K^+ uptake[14].

Effects of ethacrynic acid on tissue K^+ and tissue water

The effects of ethacrynic acid on tissue K^+ and tissue water were examined in further experiments in which the effects of ethacrynic acid were compared with the effects of inhibition of active K^+ transport by ouabain and of inhibition of cellular metabolism by anaerobic incubation with iodoacetate (Table III).

Slices leached in medium containing iodoacetate or medium containing ouabain had the same K^+ content ($P > 0.05$). However, slices in medium with ethacrynic acid lost significantly more K^+ than did slices in medium with iodoacetate ($P < 0.001$) or ouabain ($P < 0.001$). The presence of ouabain together with ethacrynic acid had no further effect on the K^+ content of the slices after leaching ($P > 0.60$) the K^+ content being again significantly lower than in medium with iodoacetate ($P < 0.005$) or ouabain alone ($P < 0.001$). Ethacrynic acid therefore caused a greater loss of K^+ from tissue than did suppression of active K^+ transport by anaerobic incubation, chilling and either iodoacetate or ouabain.

After reincubation for 60 min the intracellular K^+ concentration of slices in medium with ethacrynic acid did not differ significantly ($P > 0.20$) from that of slices with iodoacetate for although their intracellular K^+ content was significantly higher ($P < 0.001$) so was their water content ($P < 0.001$).

In a further experiment the effects of ethacrynic acid on slices whose metabolism was largely inhibited by anaerobic incubation with iodoacetate (Table IV) confirmed that the increased loss of K^+ from slices leached with ethacrynic acid was not due

TABLE III

THE EFFECTS OF 1 mM ETHACRYNIC ACID, 10 mM OUABAIN, 1 mM ETHACRYNIC ACID + 10 mM OUABAIN, AND 10 mM IODOACETATE ON TISSUE WATER AND ELECTROLYTE CONTENTS OF RAT RENAL CORTICAL SLICES AFTER LEACHING ANAEROBICALLY AT 0.5° FOR 150 min AND THEN REINCUBATING AT 25° FOR 60 min

O_2 was bubbled through media during reincubation except for medium with iodoacetate through which N_2 was bubbled. Before leaching slices were incubated in oxygenated ordinary medium at 25° for at least 15 min. Each value represents the mean ± S.D. of 8 separate observations on slices from the kidneys of 8 rats.

Conditions	*Tissue water content (kg/kg dry matter)*	*Tissue Na^+ content**	*Tissue Cl^- content**	*Tissue K^+ content**	*Intracellular K^+ concn. (mequiv/l intracellular water)*
Leached tissue					
Medium + 1 mM ethacrynic acid	3.41 ± 0.14	527 ± 16	325 ± 16	119 ± 6	51 ± 3
Medium + 10 mM ouabain	3.16 ± 0.09	451 ± 15	287 ± 18	156 ± 15	72 ± 8
Medium + 1 mM ethacrynic acid + 10 mM ouabain	3.25 ± 0.08	502 ± 11	308 ± 14	116 ± 17	52 ± 9
Medium + 10 mM iodoacetate	3.26 ± 0.13	460 ± 18	322 ± 19	143 ± 10	64 ± 4
Reincubated tissue					
Medium + 1 mM ethacrynic acid	4.65 ± 0.41	766 ± 84	542 ± 71	93 ± 20	25 ± 8
Medium + 10 mM ouabain	2.68 ± 0.20	368 ± 35	240 ± 27	173 ± 13	98 ± 14
Medium + 1 mM ethacrynic acid + 10 mM ouabain	4.21 ± 0.17	748 ± 32	485 ± 29	56 ± 8	16 ± 3
Medium + 10 mM iodoacetate	3.96 ± 0.18	676 ± 35	498 ± 44	70 ± 7	22 ± 2

* mequiv/kg dry matter.

TABLE IV

THE EFFECTS OF 1 mM ETHACRYNIC ACID OR 10 mM OUABAIN IN MEDIUM CONTAINING 10 mM IODOACETATE, AND MEDIUM CONTAINING 10 mM IODOACETATE ALONE, ON THE TISSUE WATER AND ELECTROLYTE CONTENTS OF RAT RENAL CORTICAL SLICES AFTER LEACHING ANAEROBICALLY AT 0.5° FOR 150 min AND THEN REINCUBATING ANAEROBICALLY AT 25° FOR 60 min

Before leaching slices were incubated in oxygenated ordinary medium at 25° for at least 15 min. Each value represents the mean ± S.D. of 6 separate observations on slices from the kidneys of 5 rats.

Conditions	*Tissue water content (kg/kg dry matter)*	*Tissue Na^+ content**	*Tissue Cl^- content**	*Tissue K^+ content**	*Intracellular K^+ concn. (mequiv/l intracellular water)*
Leached tissue					
Iodoacetate medium + ethacrynic acid	3.47 ± 0.12	503 ± 16	359 ± 19	132 ± 8	55 ± 4
Iodoacetate medium + ouabain	3.20 ± 0.11	439 ± 22	322 ± 15	169 ± 13	78 ± 6
Iodoacetate medium	3.26 ± 0.21	433 ± 28	314 ± 30	175 ± 8	79 ± 6
Reincubated tissue					
Iodoacetate medium + ethacrynic acid	4.40 ± 0.10	752 ± 19	536 ± 14	76 ± 5	21 ± 1
Iodoacetate medium + ouabain	3.85 ± 0.09	643 ± 43	465 ± 28	70 ± 11	22 ± 3
Iodoacetate medium	4.08 ± 0.08	671 ± 28	467 ± 30	83 ± 10	25 ± 4

* mequiv/kg dry matter.

to any interference by ethacrynic acid with metabolism at this temperature for although the composition of slices leached in medium with iodoacetate or medium with iodoacetate and 10 mM ouabain did not differ significantly, the slices leached with iodoacetate and ethacrynic acid together contained significantly less K^+ ($P < 0.001$). After reincubation for 60 min, slices with ethacrynic acid and iodoacetate contained significantly more water ($P < 0.001$) than did slices reincubated with iodoacetate alone. This observation suggests that the increased water content in slices reincubated with ethacrynic acid (Tables III and IV) resulted from an increase in membrane permeability rather than from any effect of ethacrynic acid on cellular metabolism.

It was of interest that in these experiments slices reincubated with ouabain in medium containing ethacrynic acid or iodoacetate (Tables III and IV) contained significantly less water than slices reincubated with ethacrynic acid ($P < 0.02$) or iodoacetate ($P < 0.001$) alone, though the absolute differences were small. Similarly, slices incubated in manometers with ethacrynic acid and ouabain became less swollen than those incubated with ethacrynic acid alone ($P < 0.005$, Table II). This apparent effect of ouabain on cellular swelling remains to be investigated but the conclusion[1-3] that recovery in cellular volume is brought about by a mechanism other than a Na^+–K^+-linked, ouabain-sensitive, cation pump appears to be unaffected.

The effects of 2,4-dinitrophenol on the composition of slices leached at 0.5° and reincubated at 25°

The initial recovery, with subsequent swelling, when leached slices were reincubated in media containing either 0.5 or 0.75 mM ethacrynic acid was reminiscent

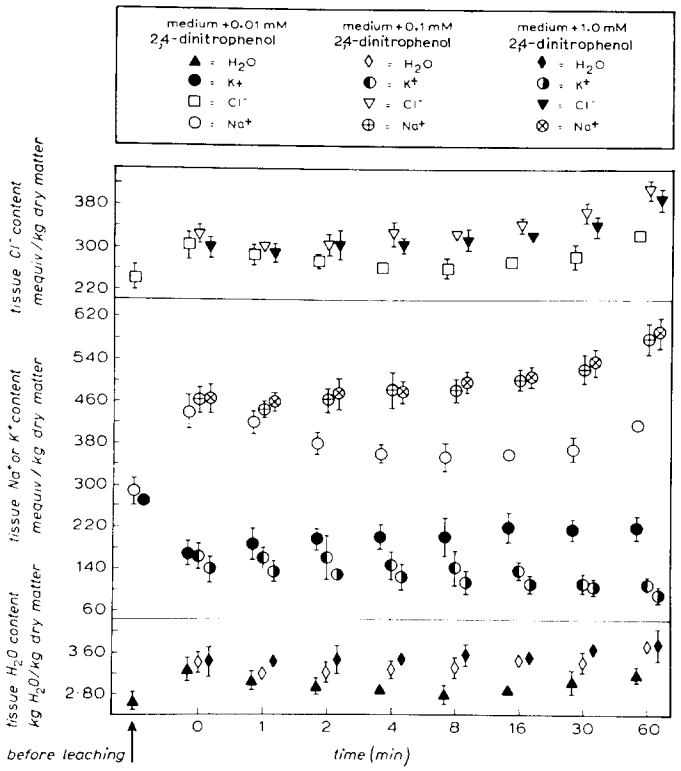

Fig. 2. Composition of rat renal cortical slices leached anaerobically at 0.5° for 150 min and then reincubated at 25° in oxygenated ordinary medium containing 5 mM glucose and either 0.01, 0.1 or 1 mM 2,4-dinitrophenol. Each point represents the mean ± S. D. of 5 separate observations on slices from the kidneys of 20 rats. Composition of slices at end of leaching plotted at 0.

of the behaviour observed when slices were reincubated anaerobically with or without ouabain[14]. Such behaviour was also seen when slices were leached and reincubated in ordinary medium containing 0.01 mM dinitrophenol. The results of experiments with 0.01, 0.1 and 1.0 mM dinitrophenol are shown in Fig. 2. All slices became swollen to the same extent during leaching. During reincubation slices in medium containing 0.01 mM dinitrophenol showed an initial loss of water which was maintained for 16–30 min after which they became swollen again. This recovery was associated with loss of Cl^-. After 8 min the water and Cl^- contents of the slices did not differ significantly from those found in slices before leaching ($P > 0.20$, $P > 0.10$ respectively). Though there was a considerable loss of Na^+ and uptake of K^+ slices never regained the Na^+ or K^+ contents of tissue which was not leached ($P < 0.001$ in each case). 0.1 mM and 1.0 mM dinitrophenol virtually prevented restoration of cellular water, Na^+ or Cl^- contents and slices in these media continued to swell during reincubation. They also lost K^+ throughout reincubation.

DISCUSSION

The effect of ethacrynic acid on cellular volume

Slices became swollen after incubation in medium containing 1 mM ethacrynic acid (Table II) or after reincubation in media containing ethacrynic acid (Fig. 1),

and their O_2 consumption was decreased in medium with 1 mM ethacrynic acid (Table I). This behaviour might be expected were ethacrynic acid specifically inhibiting the regulation of cellular volume. Partial inhibition of such a mechanism might allow some initial recovery of cellular volume during reincubation, and any such recovery should subsequently be well maintained. However, slices reincubated in either 0.5 or 0.75 mM ethacrynic acid were unable to maintain an initial recovery of cellular volume. This behaviour though inconsistent with that predicted were a volume regulating mechanism specifically inhibited, was very similar to that observed when slices were incubated under conditions in which metabolism was partially, rather than completely, suppressed. Such an effect is illustrated in Fig. 2 where leached slices reincubated in medium containing 0.01 mM dinitrophenol showed initial recovery in cellular volume which was not subsequently maintained. This type of behaviour has also been found in slices reincubated anaerobically[14]. These results are therefore consistent with the possibility that ethacrynic acid in these concentrations was inhibiting cellular metabolism, rather than inhibiting a specific mechanism regulating cellular volume. There is evidence that ethacrynic acid in similar concentrations interferes with mitochondrial respiration[13,17,18]. The findings reported by WHITTEMBURY[10] in guinea pig cortical slices, though interpreted as evidence that ethacrynic acid specifically inhibited cellular volume regulation, are also consistent with the possibility that ethacrynic acid interfered with cellular metabolism.

Alternatively, the initial recovery of leached slices might have occurred before inhibition of volume regulation by ethacrynic acid became effective. However, the following observations make this unlikely. After 150 min at 0.5° ethacrynic acid should have become distributed throughout the tissue, and in fact 1 mM ethacrynic acid prevented any significant recovery from the beginning of reincubation. Furthermore, slices leached at 0.5° in 1 mM ethacrynic acid contained significantly less K^+ than those leached in medium containing ouabain or iodoacetate (Table III) suggesting that ethacrynic acid had an effect on tissue at 0.5°. Although the water and Cl^- contents of slices in 0.5 mM ethacrynic acid returned, during the early period of reincubation, to values not significantly different from those in slices before leaching, their K^+ content showed only incomplete recovery. This must reflect activity of ethacrynic acid in these early minutes. Finally, slices reincubated in 0.5 mM ethacrynic acid maintained a relatively constant composition for 30 min before swelling. It seems unlikely that 0.5 mM ethacrynic acid took 30 min to exert a specific effect on volume regulation when twice the concentration was effective from the beginning of reinbation.

If the effect of ethacrynic acid was due to interference with metabolism, then, with 1 mM ethacrynic acid, insufficient energy appeared to be available for any initial restoration of cellular water and ion contents. Yet the O_2 consumption of slices incubated in 1 mM ethacrynic acid was not entirely abolished, the uptake of O_2 being about 40% of the level measured in slices in ordinary medium (Table I). Either little or no energy from this residual O_2 consumption became available to the mechanisms concerned with regulating volume and ionic composition or, alternatively this O_2 consumption did not yield energy to the cell. GORDON[18] demonstrated that ethacrynic acid uncoupled mitochondrial oxidative phosphorylation, but the fact that ethacrynic acid depresses rather than stimulates the O_2 uptake of intact cells suggests that this is not its only effect.

As well as any effect on cellular metabolism, 1 mM ethacrynic acid possibly increased the permeability of the cellular membranes to ions, for slices became significantly more swollen during reincubation with ethacrynic acid alone, and with ethacrynic acid and iodoacetate together, than they did during reincubation in media containing enough iodoactetate to completely suppress metabolism[14] (Tables III and IV). This suggestion is supported by the observations in preliminary experiments where 2 and 10 mM ethacrynic acid caused considerably greater swelling, and also by the behaviour of K^+ in slices leached in ethacrynic acid, discussed below.

The effect of ethacrynic acid on cellular K^+

Slices incubated in medium containing ethacrynic acid lost K^+ (Table II), and did not regain it during reincubation of leached tissue (Fig. 1, Table III). Ethacrynic acid has a similar effect on the K^+ content of slices of guinea pig renal cortex[10]. This loss of K^+ might be the result either of (i) a specific inhibition of active K^+ uptake, (ii) a decreased availability of energy for active transport, or (iii) an increase in passive K^+ loss from the cells, which might result either from an increased permeability of cellular membranes to K^+, a depolarisation of the cellular membranes, or a decrease in the affinity of specific binding sites for K^+ on intracellular macromolecules[15,16].

From the present results it is not possible to distinguish clearly between these possibilities. The observation (Table III) that ethacrynic acid in medium at 0.5° produced a greater loss of K^+ from slices than did iodoacetate alone, and the fact that this loss of K^+ was unaffected by the presence of ouabain (Table III) or iodoacetate (Table IV) suggests that ethacrynic acid increased passive K^+ loss at this temperature.

There is evidence that ethacrynic acid may specifically inhibit Na^+–K^+-linked membrane ATPase[19,21] which many believe to be intimately associated with active ion movements across cellular membranes[22]. The additive effect of ouabain and ethacrynic acid together on the K^+ content and concentration in slices incubated in manometers (Table II) and in slices reincubated at 25° (Table III) would suggest that ethacrynic acid by itself did not inhibit all active K^+ transport at 25° but does not allow a distinction to be made between specific inhibition of ATPase and interference by ethacrynic acid with the amount of energy available for active K^+ uptake. At least in some tissues ethacrynic acid appears not to inhibit a Na^+–K^+-linked membrane ATPase, for DANIEL[23] reported that, though it inhibited extrusion of Na^+ from rabbit uterine muscle, ethacrynic acid did not prevent K^+ uptake, a process thought to depend upon glycolysis rather than oxidative metabolism in this tissue[24].

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