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DIFFERENCES IN Na AND K TRANSPORT IN KIDNEY CORTEX AND MEDULLA INDICATED BY OUABAIN, ETHACRYNIC ACID AND OTHER INHIBITORS

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Abstract—The effect of various inhibitors, ouabain, ethacrynic acid (EA), thiomerin and *N*-ethyl maleimide (NEM) was studied on (Na + K)-ATPase in the rat kidney cortex and medulla and on sodium extrusion and potassium accumulation in slices from cortex and medulla. With each inhibitor the sensitivity of (Na + K)-ATPase in medulla was similar to that in the cortex. Mg-ATPase was more resistant than (Na + K)-ATPase to Thiomerin and NEM. Medullary Mg-ATPase was more sensitive than medullary (Na + K)-ATPase to EA. Na-extrusion and K-accumulation were completely inhibited in cortical and medullary slices by NEM. Thiomerin had no significant effect on Na extrusion or K accumulation in cortical slices; Thiomerin inhibited Na extrusion in the medulla but enhanced K-accumulation. Ouabain inhibited Na-extrusion and K-accumulation in the medulla significantly more than in the cortex. In the absence of K^+ in the medium Na extrusion persisted in the cortex at 56 per cent of maximal extrusion rate while in the medulla only 30 per cent of the maximal extrusion rate persisted. EA inhibited Na-extrusion and K-accumulation significantly more in slices of cortex than in slices of kidney medulla. It is suggested that transport of sodium in kidney cortex and medulla involve quantitatively and qualitatively different mechanisms.

Sodium transport is one of the major functions of the kidney. However, the role of sodium reabsorption differs in different parts of the kidney. Sodium reabsorption by the proximal tubules serves mostly overall sodium balance, whereas in thick ascending limb of Henle sodium reabsorption results in diluted tubular fluid. This enables excretion of a dilute urine and the sodium extracted from the lumen serves to build up the interstitial sodium gradient, necessary for the formation of concentrated urine.

Since (Na + K)-ATPase is believed to be the enzymatic basis of the sodium pump [1] the activity and properties of this enzyme, isolated from different parts of the kidney, under different conditions *in vitro*, were studied in our laboratory [2, 3]. Differences in the characteristics of this enzyme in various regions of the kidney were found: the enzyme from the papilla was more susceptible to inhibition by high concentration of urea, sodium and some other inhibitors [2–4]. Recently differences in the response of (Na + K)-ATPase in various parts of the kidney to salt loading *in vivo* were also observed [4a].

Whittembury and Proverbio, using *in vitro* incubation of kidney slices, have reported that ion movement in slices of kidney cortex of the guinea pig showed two types of transport: one inhibited by cardiac glycosides, presumably dependent on (Na + K)-ATPase activity, and another process, not inhibited by cardiac glycosides but reduced by ethacrynic acid [5].

To find out whether two different transport mechanisms existed in the kidney medulla, too, it seemed of interest to us to study the effects of various inhibitors of (Na + K)-ATPase on the enzyme activity in cortex and medulla and to compare the characteristics of sodium transport in *in vitro* incubation of slices of kidney cortex and kidney medulla.

By comparing the effect of the inhibitors on the activity of (Na + K)-ATPase on the one hand and on sodium extrusion and potassium accumulation in slices on the other, one could hope to characterize the transport mechanisms in the cortex and medulla of the kidney and their possible contribution to the different physiological processes in these parts.

MATERIALS AND METHODS

Male rats, 180–250 g, of the Hebrew University strain, were used throughout the experiments. The rats were anesthetized with ether, and were then sacrificed by cervical dislocation. The kidneys were removed immediately. After decapsulation the kidneys were cut sagittally and separated into three parts: cortex, medulla and papilla, as described previously [6].

Preparation of enzyme. Preparation of the microsomal ATPase was carried out essentially following the description of Jørgensen and Skou [7]. The three different parts of the kidney (each batch was collected from 10–20 rats) were homogenized in 10 vol. of a medium consisting of: 0.25 M sucrose, 2 mM EDTA, buffered with Tris–HCl (5 mM) to pH 7.4–7.5. The homogenate was centrifuged at 7000 *g* for 15 min; the

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supernatant was separated and the pellet was suspended and rehomogenized (same medium and same vol. as the first homogenization) and centrifuged again at 7000 *g* for 15 min. The combined supernatants were centrifuged at 48,000 *g* for 40 min.

The pellet was resuspended and rehomogenized (1:1) in a medium of the same composition. To this suspension were added 10 vol. of desoxycholate 0.067%, containing 2 mM EDTA and 25 mM Tris HCl (pH 7.0). The suspension was then incubated at room temperature for 30 min. Thereafter, centrifugation at 5600 *g* for 30 min was performed and the supernatant was collected and centrifuged at 25,000 *g* for 30 min. The pellet was suspended in a sucrose–EDTA–Tris, identical with the original homogenizing medium. This final suspension was either assayed immediately or frozen at −20 until used.

Assay of ATPase. ATPase activity was determined by the amount of inorganic phosphate (Pi) released during incubation at 37 in a shaking, thermostatic bath, as previously described [8]. All assays were run in duplicate. Pi released was studied with and without the addition of ouabain at a final concentration of 10^{-3} M, to differentiate Mg-ATPase and (Na + K)-ATPase. The basic incubation medium consisted of: NaCl 100 mM, KCl 10 mM; $MgCl_2$ 4 mM, Tris buffer 33 mM (pH 7.4), ATP 4 mM. Enzymatic activity was stopped by the addition of 10% trichloroacetic acid. Pi was determined according to the method of Fiske and SubbaRow [8]. Enzymatic protein was assayed according to Lowry *et al.* [9].

Sodium extrusion and potassium accumulation by kidney slices. Slices from cortex and medulla, weighing 30–40 mg per slice, were prepared. Enrichment of intracellular sodium and loss of cellular potassium were achieved according to the method of Munday *et al.* [10] by incubating the slices anaerobically for 12 min in a medium containing 142 mM sodium but no potassium or glucose, at 37 . Under these conditions the intracellular electrolyte composition approached the composition of the incubation medium. The composition of the incubation medium for this incubation was: 142 mM NaCl, 3 mM $CaCl_2$, 1.5 mM $MgSO_4$, 3.5 mM Sodium phosphate buffer, pH 7.4.

The sodium-loaded slices were then transferred to another medium and were incubated aerobically at 25 for 30 min, according to the method of Whittembury [11]. The composition of this medium was 112 mM NaCl, 5 mM KCl, 9 mM Na Acetate, 15 mM $NaHCO_3$, 0.6 mM NaH_2PO_4, 2.4 mM Na_2HPO_4, 1.2 mM $MgSO_4$, 0.6 mM Na_2SO_4, 1.0 mM Ca gluconate and 5.0 mM glucose. The total concentration of sodium was 143 mM, and the total osmolality 290 mOsmol. A mixture of 95% O_2 and 5% CO_2 was bubbled through the medium during the entire incubation. The pH was kept between 7.3–7.6. For reincubation in a potassium-free medium the KCl was replaced by NaCl. In experiments where sodium extrusion and potassium accumulation were determined in the presence of drugs, the drug was added to the incubation medium at final concentration as specified under Results.

Determination of sodium and potassium concentration was carried out in fresh tissue, in tissue after preincubation in anaerobic medium and in tissue after reincubation, so as to enable estimation of the changes in sodium and potassium content of the slices throughout the experiment.

Determination of sodium, potassium and water content of slices. Fresh or incubated slices were blotted on filter paper, weighed, and dried in an oven at 105 overnight, to constant wt. The difference in wt before and after drying accounted for the water content of the slice. Each slice was then immersed in 2 ml of 1 N HNO_3 and incubated (with shaking) for 48 hr at room temperature to dissolve the tissue. The concentration of sodium and potassium was determined in these samples of dissolved slices by flame photometry. Results are expressed as meq. sodium or potassium per gram dry tissue weight.

Drugs: Ouabain was purchased from Merck, Sharp and Dohme Research Lab., West Point. Ethacrynic acid was a gift of Assia Pharmaceuticals, Ramat Gan. Thiomerin was purchased from Wyeth Laboratories, Philadelphia. *N*-ethyl-maleimide was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Table 1. Inhibition by various drugs of (Na + K)-ATPase and Mg-ATPase from rat kidney cortex and medulla

	I_{50} of drug			
	(Na + K)-ATPase		Mg-ATPase	
	C	*M*	*C*	*M*
Ouabain	2.45 ± 0.17	2.95 ± 0.28		
$I_{50} \times 10^{-4}$ M	(n = 4)	(n = 4)		
Ethacrynic acid	1.9 ± 0.2	1.8 ± 0.2	0.55 ± 0.1	0.77 ± 0.1
$I_{50} \times 10^{-3}$ M	(n = 8)	(n = 8)	(n = 8)	(n = 8)
Thiomerin	0.57 ± 0.1	0.57 ± 0.08	12.0 ± 3.4	9.9 ± 4.0
$I_{50} \times 10^{-3}$ M	(n = 4)	(n = 4)	(n = 4)	(n = 4)
NEM	2.8 ± 0.4	1.9 ± 0.2	7.8 ± 2.9	6.2 ± 1.1
$I_{50} \times 10^{-3}$ M	(n = 5)	(n = 5)	(n = 5)	(n = 5)

C – Enzyme obtained from kidney cortex.
M – Enzyme obtained from kidney medulla.
I_{50} – Concentration of drug producing 50 per cent inhibition of enzyme activity.
NEM – *N*-ethyl-maleimide.
Results expressed as mean ± S.E. n = number of experiments.

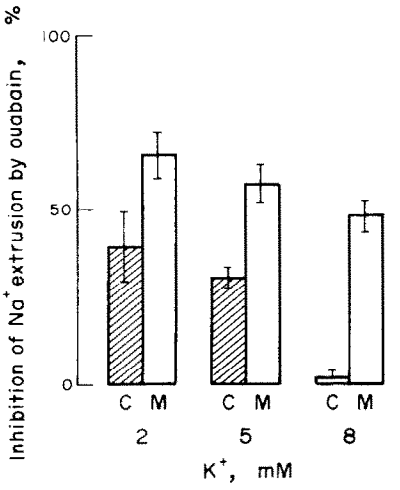

Fig. 1. Effect of ouabain on sodium extrusion from slices of rat kidney cortex and medulla. *C*—slices from cortex. *M*—slices from medulla. 2,5,8—concentration of K^+ in medium during incubation of slices. Ordinate—inhibition of sodium extrusion by ouabain (10^{-3} M) expressed as per cent of sodium extrusion in the absence of ouabain. Vertical bars—S.E. At 2 mM K^+—$n = 18$, for *C* and for *M*. At 5 mM K^+—$n = 29$, for *C* and for *M*. At 8 mM K^+—$n = 22$, for *C* and for *M*. All slices were first preincubated to cause loading of sodium, as described in Materials and Methods. Note that at each concentration of K^+ in the medium, inhibition by ouabain was more marked in the medulla than in the cortex, the largest difference being at 8 mM K^+ in the medium.

RESULTS

1. *The effect of ouabain.* The inhibitory effect of ouabain in the cortex and medulla was compared on (Na + K)-ATPase activity on the one hand, and on Na^+ extrusion and K^+ accumulation on the other hand. Table 1 shows that I_{50} values of ouabain were similar for (Na + K)-ATPase from the cortex and from the medulla. Figure 1 and Table 2 show the inhibitory effect of ouabain on Na^+ extrusion and K^+ accumulation, at different K^+ concentrations in the medium. As expected, the inhibitory effect of ouabain was antagonized by increasing concentrations of K^+ in the medium. However, at all concentrations of K^+ in the medium inhibition of Na^+ extrusion by ouabain was significantly greater in slices from the medulla than in slices from the cortex. At 8 mM K^+ in the medium Na^+ extrusion in cortical slices was not affected at all by ouabian, while in slices from the medulla sodium extrusion was inhibited by 48 per cent. Figure 2 shows the effect of ouabain on Na^+ extrusion and K^+ accumulation in slices from kidney cortex and medulla, expressed in meq./kg dry wt. It is evident that both Na^+ extrusion and K^+ accumulation were inhibited more in the medulla than in the cortex. Ouabain affects (Na + K)-ATPase and Na^+ transport to the same extent as does omission of K^+ from the medium. Figure 3 shows net Na^+ extrusion from kidney slices in the complete absence of K^+ from the medium, and sodium extrusion at increasing K^+ concentrations in the medium (up to 8 mM). In the absence of K^+ in the medium Na^+ extrusion from cortical slices was quite marked

Table 2. Inhibition of Na^+ extrusion and K^+ accumulation in slices of rat kidney cortex and medulla

	Per cent inhibition of ion transport			
	C		*M*	
	Na^+	K^+	Na^+	K^+
Ouabain ([K] in medium—2 mM)	39.4 ± 9.8 ($n = 18$)	92.3 ± 3.9 ($n = 18$)	65.8 ± 6.8 ($n = 18$)	98.7 ± 0.9 ($n = 18$)
Ouabain ([K] in medium—5 mM)	30.3 ± 3.2 ($n = 30$)	56.3 ± 3.4 ($n = 30$)	57.6 ± 5.6 ($n = 29$)	75.5 ± 3.7 ($n = 30$)
Ouabain ([K] in medium—8 mM)	0.5 ± 3.4 ($n = 22$)	39.9 ± 2.1 ($n = 24$)	48.0 ± 4.8 ($n = 22$)	54.0 ± 2.9 ($n = 24$)
Ethacrynic acid	77.4 ± 5.1 ($n = 17$)	63.3 ± 4.8 ($n = 17$)	23.3 ± 7.6 ($n = 15$)	44.3 ± 3.3 ($n = 17$)
Thiomerin	11.1 ± 3.9 ($n = 19$)	⊗15.1 ± 9.4 ($n = 19$)	22.1 ± 3.8 ($n = 19$)	⊗33.0 ± 7.2 ($n = 19$)
NEM	100.0 ± 0 ($n = 6$)	95.8 ± 4.2 ($n = 6$)	82.1 ± 8.7 ($n = 6$)	83.8 ± 7.6 ($n = 6$)

C—Ion movement in slices of kidney cortex.
M—Ion movement in slices of kidney medulla.
NEM—*N*-ethyl-maleimide.
Na^+—Net sodium extrusion after preloading of slices with Na, as described in Materials and Methods.
K^+—Net potassium accumulation in slices after washout of K by preincubation in K-free medium, as described in Materials and Methods.
Ouabain—At concentration of 10^{-3} M; ethacrynic acid at concentration of 5×10^{-3} M; thiomerin at concentration of 5×10^{-3} M; NEM at concentration of 5×10^{-3} M.
Results—Inhibition of ion transport in presence of drug expressed as per cent of transport in absence of drug (mean ± S.E.) n = number of experiments.
⊗ Enhancement of potassium accumulation above control, significant only in medulla ($P < 0.01$).

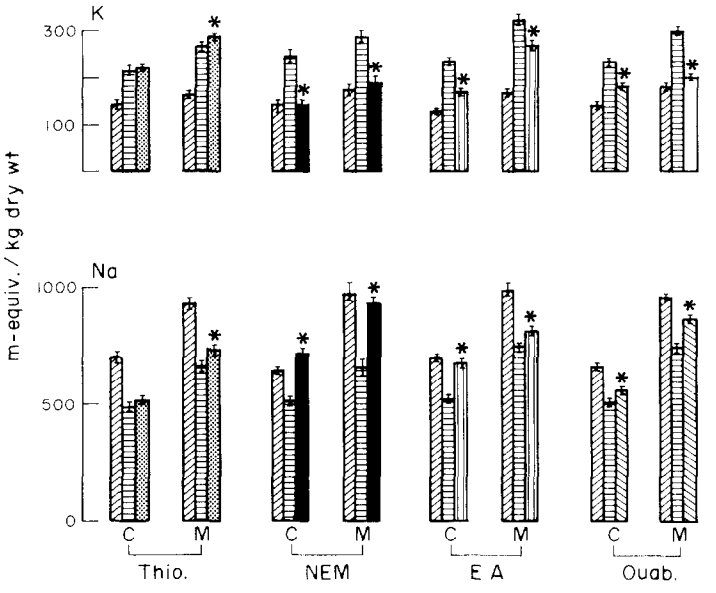

Fig. 2. Effect of different drugs on sodium extrusion and potassium accumulation in slices of rat kidney cortex and medulla. *C* slices from cortex. *M* slices from medulla. Upper panel potassium. Lower panel sodium. Thio. effect of Thiomerin (5×10^{-3} M). NEM effect of *N*-ethylmaleimide (5×10^{-3} M). EA effect of Ethacrynic acid (5×10^{-3} M). Ouab effect of ouabain (10^{-3} M). In each group of three columns, the column on the left (diagonal stripes) represents the concentration of sodium (lower panel) or potassium (upper panel) after pre-incubation of the slices in a potassium free medium, as described in Materials and Methods. The middle column of each three (horizontal stripes) represents sodium or potassium concentration in slice after re-incubation in complete medium (Krebs), in the absence of any drug. The column on the right, in each group of three, represents the sodium or potassium concentration in the slices after re-incubation in the presence of drug, as indicated below columns. Vertical bars S.E. $n = 12$ for each column of Thiomerin experiment. $n = 6$ for each column of NEM experiment. $n = 17$ for each column of EA experiment. $n = 29$ for each column of ouabain experiment. * $P < 0.025$ for ion concentration in slice after re-incubation in the presence of drug compared to re-incubation in the absence of drug (middle column of each three).

and accounted for more than 55 per cent of the maximal Na^+ extrusion observed at 8 mM K^+. Na^+ extrusion from slices of medulla was comparatively low when K^+ was omitted from the medium, and accounted for only about 30 per cent of the maximal Na^+ extrusion, found at 5 mM K^+ in the medium.

2. *Effect of ethacrynic acid (EA).* The effect of EA was studied on both (Na + K)-ATPase activity and on Na^+ extrusion and K^+ accumulation in slices of kidney cortex and medulla.

Table 1 shows that I_{50} of EA for (Na + K)-ATPase was practically the same in cortex and medulla. It is interesting to note that in the cortex and medulla, Mg-ATPase was more sensitive to inhibition by EA than (Na + K)-ATPase. This was in contrast to our observations with other inhibitors of ATPase reported previously [4]. Figure 2 and Table 2 show the inhibitory effect of EA on Na^+ extrusion and K^+ accumulation in slices from cortex and medulla. In contrast to ouabain, EA inhibited Na^+ extrusion and K^+ accumulation significantly more in slices from kidney cortex than in slices from medulla.

3. *Effect of Thiomerin.* Thiomerin is an organic mercurial diuretic. Its effect is ascribed to liberation of inorganic mercuric ions [12] which bind SH groups, apparently essential for ion transport. The effect of Thiomerin was studied both on (Na + K)-ATPase activity and on Na^+ extrusion and K^+ accumulation in slices of kidney cortex and medulla.

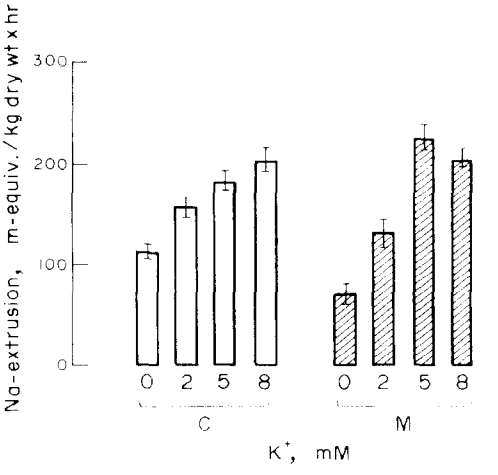

Fig. 3. Effect of potassium in medium on sodium extrusion from slices of rat kidney cortex and medulla. *C* slices from kidney cortex. *M* slices from kidney medulla. *0* incubation in a K^+-free medium. 2,5,8 incubation in the presence of 2,5,8 mM K^+ in medium. All slices were first pre-incubated, as described in Materials and Methods, to cause sodium loading of slice. Ordinate sodium extrusion during 1 hr of re-incubation in complete medium. Vertical bars S.E. $n = 6$ for experiments in K-free medium. $n = 12$ in experiments with 2 mM K^+ in medium. $n = 29$ in experiments with 5 mM K^+ in medium. $n = 22$ in experiments with 8 mM K^+ in medium.

Table 1 shows that I_{50} of thiomerin for (Na + K)-ATPase was similar in cortex and medulla. Mg-ATPase was much more resistant to inhibition by thiomerin than (Na + K)-ATPase. This is similar to results obtained previously with other inhibitors [4]. Figure 2 and Table 2 show the effect of 5 mM Thiomerin on Na^+ extrusion and K^+ accumulation in slices from cortex and medulla. In the cortex, Na^+ extrusion and K^+ accumulation were almost unaffected by thiomerin. In the medulla, Na^+ extrusion was significantly inhibited (by 22.1 ± 3.8 per cent) while K^+ accumulation was significantly enhanced (by 33.0 ± 7.2 per cent).

4. *Effect of N-ethyl-maleimide (NEM).* SH-reagents have been shown to inhibit (Na + K)-ATPase activity, thus implicating SH-groups in this enzyme as part of the active site [13]. We have compared the inhibitory effect of NEM on (Na + K)-ATPase and on Na^+ extrusion and K^+ accumulation in slices of kidney cortex and medulla. Table 1 shows that there was no difference in I_{50} of NEM between cortex and medulla for either (Na + K)-ATPase or Mg-ATPase. Both in cortex and medulla (Na + K)-ATPase was more sensitive to inhibition by NEM than Mg-ATPase. Figure 2 and Table 2 show the inhibitory effect of NEM on Na^+ extrusion and K^+ accumulation in slices from the cortex and medulla. 5 mM NEM caused total inhibition of Na^+ extrusion and K^+ accumulation in cortical slices. In the medulla the inhibition by 5 mM NEM was almost complete.

DISCUSSION

The experiments reported in this paper demonstrate differences between cortex and medulla of rat kidney in the characteristics of Na and K transport in slices incubated *in vitro*. The transport mechanism was characterized by studying the effect of various inhibitors on microsomal (Na + K)-ATPase prepared from kidney cortex and medulla and by following Na extrusion and K accumulation in slices of cortex and medulla, preloaded with Na^+ and rendered potassium-poor by preincubation *in vitro*. For each of several inhibitors studied the I_{50} for (Na + K)-ATPase from cortex was similar to that for the enzyme from medulla (Table 1). Mg-ATPase was more resistant to inhibition by all the compounds studied, except for EA, where Mg-ATPase was more sensitive than (Na + K)-ATPase.

In contrast to the similarity in (Na + K)-ATPase of cortex and medulla, striking differences between kidney cortex and medulla were observed in ion transport of slices: (1) ouabain inhibited significantly more Na^+ extrusion and K^+ accumulation in medullary slices than in cortical slices (Fig. 2); (2) in the absence of K^+ in the medium, Na^+ extrusion from cortical slices persisted to a marked extent, whereas in slices of the medulla it was significantly lower (Fig. 3).

The concept of two different transport mechanisms of sodium was also supported by the effect of ethacrynic acid on Na^+ extrusion and K^+ accumulation in slices of cortex and medulla. Figure 2 and Table 2 show that EA inhibited Na^+ extrusion and K accumulation significantly more in the cortex than in the medulla. According to Whittembury and Proverbio [5] EA is a specific inhibitor of the 'second pump' in guinea pig kidney cortex slices. While EA (at 5 mM) inhibited completely microsomal (Na + K)-ATPase from the medulla, the effect on Na-extrusion and K accumulation in slices of medulla was rather small. (At concentrations of EA lower than 1 mM no effect on Na extrusion was observed.) It should be recalled that the rat is rather insensitive to the diuretic action of EA [14]. The site of action of this diuretic is ascribed to the thick ascending limb of Henle's loop within the medulla [15]. But the major effect on transport in slices was observed in kidney cortex (Table 2 and Fig. 2). One possible mechanism for inhibition of Na extrusion in cortical slices by EA may be the accumulation of EA in kidney cortex which is a Na-dependent process [16]. Corroborating this suggestion we have recently observed that probenecid, another organic acid accumulating in kidney cortex, also inhibited net Na extrusion in cortical slices *in vitro* (Gutman, Wald and Czaczkes, unpublished observations).

Although both EA and mercurial diuretics are believed to act through SH binding [17, 18], thiomerin, showed a different pattern of action on ion movement in slices (Fig. 2 and Table 2), especially in the medulla, where Na^+-extrusion was significantly *inhibited* (by 22.1 ± 3.8 per cent) while K^+ accumulation was significantly *enhanced* (by 33.0 ± 7.2 per cent). It is evident that the effect of Thiomerin on K^+ accumulation cannot be explained by inhibition of (Na + K)-ATPase. This effect of thiomerin on K^+ accumulation in kidney slices *in vitro* corroborates clinical observations that mercurial diuretics do not cause kaliuresis *in vivo* [19, 20]. Since K^+ loss is at the luminal side of the tubular cell, the effect of thiomerin could be decreased permeability of the luminal membrane to K^+, thereby K^+ secretion is decreased.

The difference in the effects of EA and thiomerin on ion transport in kidney slices precludes the possibility that their effect is exerted through reaction with the same SH groups. However, there could be a common fundamental mechanism, e.g. reaction with some critical SH-groups. Therefore, a general SH reagent, NEM was studied. The action of NEM on Na^+ extrusion and K^+ accumulation in kidney slices was different both from that of EA and thiomerin: 5 mM NEM caused complete inhibition of Na^+ extrusion and K^+ accumulation in cortical slices as well as in slices of medulla (Fig. 2, Table 2). This may suggest that neither the effect of EA nor that of thiomerin resulted purely from SH-group binding.

Sodium transport in the cortex is related mainly to sodium balance [21]. The experiments reported here show that Na-movement in the rat kidney cortex depends only to a small extent on (Na + K)-ATPase activity. This corroborates the observation that even during ouabain infusion into the renal artery 60–70% of the filtered sodium is still reabsorbed in the proximal tubule [22]. In slices of medulla sodium extrusion depends largely on (Na + K)-ATPase activity (Table 2, Fig. 3). This may be related to the high sp. act. of medullary (Na + K) dependent ATPase [3]. The main part of the nephron located in the medulla is the thick ascending limb of Henle's loop [23]. The medulla contains those parts of the nephron implicated in urine concentration and dilution [24, 25]. It

seems, therefore, that the role of (Na + K)-ATPase in the medulla is in the concentration-dilution function. Under ouabain infusion the concentration and dilution ability of the kidney is impaired [25, 26]. However, some reports ascribe a major function to chloride transport rather than to a sodium pump in the thick ascending limb of Henle [15, 27]. It is rather difficult to find a comprehensive explanation to account for the chloride pump and the high sp. act. of (Na + K)-ATPase in the same part of the nephron, i.e. the thick ascending limb [28].

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