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Research Article

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Sodium- and Potassium-Activated ATPase

A POSSIBLE TARGET OF ALDOSTERONE

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ABSTRACT Na-K-ATPase activity was measured with an ultramicromethod in single portions of the proximal and distal convolution and of the thick ascending limb of Henle from adrenalectomized rats and after treatment with 5 μg aldosterone per 100 g body wt. The activity in all tubular structures returned to normal within 1 h after injection. This rapid activation of Na-K-ATPase induced by hormone was completely prevented by actinomycin D and cycloheximide. It appears that this aldosterone effect on Na-K-ATPase requires an intact protein synthetic process.

INTRODUCTION

The action of aldosterone on sodium transport is apparently mediated by stimulation of DNA-dependent synthesis of RNA and the subsequent *de novo* synthesis of proteins (1). One of the ways in which the aldosterone-induced protein (AIP)[1] might regulate sodium transport is by increasing the activity of the sodium pump. It is generally assumed that the sodium-potassium-activated ATPase (Na-K-ATPase) is the enzymatic equivalent of the sodium pump (2). Attempts to show an effect of aldosterone on the enzymatic equivalent of the sodium pump, the Na-K-ATPase (2), in broken cells or isolated membrane preparations from kidney and toad bladder have thus far provided disappointing results (3–5). Injection of a single dose of aldosterone (5 μg and less per 100 g rat) into adrenalectomized rats evoked a decrease in urinary Na/K ratio within 2 h (6). By comparison, a supramaximal dose of the steroid was necessary to obtain an effect on Na-K-ATPase activity in the kidney that could be detected not earlier than 6 h after injection (3, 4, 7, 8). The discrepancy between the dosage and time required for the electrolyte effect and the enzyme effect led to the conclusion that these two effects are not directly related.

Assaying Na-K-ATPase in microdissected tubular portions from lyophilized kidney slices of adrenalectomized rats, we found a specific activity 75% below normal in the thick ascending limb of Henle and in the distal convolution as early as 6 h after adrenal excision (9). This rapid decline in activity was in contrast to the known delayed fall in Na-K-ATPase in microsomal membranes (3). Since various methods for measuring NA-K-ATPase yield results with different specific activity, we have reinvestigated the effect of aldosterone on the activity of Na-K-ATPase in renal tubular cells. Adrenalectomized rats were given a single low dose of aldosterone, and Na-K-ATPase was measured in one isolated portion of the thick ascending limb of Henle 1 h later. This site of the nephron was chosen because it is known that changes in Na-K-ATPase after adrenalectomy and after aldosterone are more pronounced in the outer medulla than in the cortex (8, 9). In addition isolated portions of the proximal and distal convolution were examined.

METHODS

General. Each group consisted of four male Wistar rats weighing 200 g. The rats were adrenalectomized 10 days before treatment and had free access to 0.9% saline and water. The completeness of adrenalectomy was checked with the water loading test (10). 5 μg aldosterone per 100 g rat was given intraperitoneally 60-90 min before the kidneys were removed. 10 μg actinomycin D or 4 mg cyclo-

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[1] *Abbreviations used in this paper:* AIP, aldosterone-induced protein; Mg-ATPase, ouabain-insensitive magnesium-activated ATPase; Na-K-ATPase, sodium-potassium-activated ATPase; PAS, periodic acid-Schiff; P_i, inorganic phosphate.

TABLE I

Characteristics in the Identification of Functional Segments of the Rat Nephron

Structure	Localization		PAS staining			Lyophilized section
	Cortex	Medulla (outer)	Brush border	Cytoplasm	Nuclei	
Proximal tubule						
Pars convoluta	Adjacent glomeruli		Deep red	Bright red	Basal, round	*
Pars recta	Peripheral glomeruli		Deep red	Pale red	Basal, round	*
Distal tubule						
Pars convoluta	Adjacent glomeruli		Absent	Bright red	Apical, oblong	*
Thick ascending limb		Inner stripe	Absent	Brown-violet	Apical, oblong	Closed lumen
Collecting duct						
Cortical	Peripheral glomeruli		Absent	Deep red	Dispersed, round	*
Medullary		Inner stripe	Absent	Deep red	Basal, round	Open lumen

* Not characteristic.

heximide per rat was injected intraperitoneally 1 h before aldosterone treatment in designated experiments.

Tissue preparation. After decapitation the kidneys were removed, and a small cone of 2 mm thickness was cut from each kidney (11). The cones were mounted upright on a tissue holder and plunged into liquid nitrogen within 1 min of sacrifice. The renal tissue was sliced in a cryostat (−25°C), one of two consecutive sections (16 μm) was lyophilized (10^{-2} torr) overnight, and the other one was stained with periodic acid-Schiff (PAS) (12).

Microdissection. The PAS-stained section served as a guiding map for identifying the desired tubular structures in the lyophilized section. In a controlled temperature (18°C) and humidity (40%) room single portions of the thick ascending limb and of the proximal and distal convolutions were dissected from the lyophilized section with the use of a dissecting microscope. The thick ascending limb of Henle was isolated from the inner stripe of the outer medulla. In the PAS-stained section (Table I) the thick ascending limb reveals cells with a brown-violet cytoplasm and longitudinally arrayed nuclei. The medullary collecting duct, however, demonstrates cells with a shining-red cytoplasm and round nuclei that are localized to the cell base. In the lyophilized section the thick ascending limb is recognizable by its relatively large wall and collapsed lumen. The medullary collecting duct has a remarkable thin wall in the lyophilized section and usually a patent lumen.

The proximal and distal convolution was dissected from the surrounding glomeruli. In the PAS-stained section the deep-red color of the brush border permits easy differentiation between the proximal and distal convolutions. Special attention was given to the distinction between the proximal convolution and the proximal straight portion by the localization of the former at the periphery of the glomerulus.

The distal convolution was distinguished from the cortical collecting duct by its localization near the glomerulus. The cross section of the distal convolution reveals nuclei arranged in a star-shaped configuration in the luminal area. The cortical collecting duct displays dispersed nuclei in cross section and is localized peripheral to the glomerulus.

Chemical measurements. The samples were weighed on a quartz fiber balance (13). The dry wt of one sample was approximately 10 ng each for the thick ascending limb and for the proximal convolution and 5 ng for the distal convolution. Samples of 10 ng were divided into two halves; one half served for Na-K-Mg-ATPase (total), the other one for ouabain-insensitive magnesium-activated ATPase (Mg-ATPase). One sample of the distal convolution was required for each ATPase. The ATPase was measured with the oil-well technique utilizing an enzymatic cycling reaction (14). All operations for loading and pipetting were performed under a stereomicroscope.

Wells (4 × 4 mm) in a room temperature Teflon block were loaded with the dry weighed samples of lyophilized tissue. 400 nl of ATPase reagent was added; *Na-K-Mg-ATPase:* Tris-HCl 0.1 M, pH 7.4; NaCl 55 mM; KCl 5 mM; $MgCl_2$ 2 mM; EDTA 0.1 mM; bovine serum albumin 0.05%. *Ouabain-insensitive Mg-ATPase:* NaCl 60 mM; ouabain 10^{-3} M. The loaded wells were covered with oil droplets, and the Teflon block was then cooled. 200 nl of ATP-disodium salt were added, resulting in a final ATP concentration of 2 mM. The ATPase reaction was started in a water bath at 37°C. 10 min later the reaction was stopped by heating the block at 100°C for 3 min. The enzyme activity was linear with respect to time and protein concentration (12). Into a second cooled Teflon block 200 nl of the incubated medium was pipetted into 1 μl reagent under oil for assaying *inorganic phosphate* (P_i). The mixture contained imidazole-HCl 0.05 M, pH 6.9; glycogen 0.08%; NADP 0.3 mM; 5'-AMP 0.01 mM; EDTA 1 mM; $MgCl_2$ 0.5 mM; glucose-6-phosphate dehydrogenase 1 μg/ml; phosphoglucomutase 3 μg/ml; glycogen phosphorylase 190 μg/ml; and bovine serum albumin 0.05%. The reaction was initiated at 37°C and ran to completion in 15 min. 400 nl of the incubation medium was then removed and added to 250 μl 0.04 N NaOH and 0.05 mM cysteine and incubated for 10 min at 60°C to destroy excess NADP. Thereafter 6 μl was pipetted into 25 μl *cycling reagent,* containing: Tris-HCl 0.1 M, pH 8.0; oxoglutarate 5 mM; glucose-6-phosphate 1 mM; ADP 0.3 mM; NH_4-acetate 25 mM; glutamate dehydrogenase 150 μg/ml; glucose-6-phosphate dehydrogenase 35 μg/ml; and bovine serum albumin 0.02%. After an incubation time of 60 min at 37°C the cycling reaction was stopped by boiling for 2 min. The formed 6-phosphogluconate was determined by adding 200 μl of *indicator reagent,* containing: Tris-HCl 0.02 M, pH 8.0; NADP 100 μM; 6-phosphogluconate dehydrogenase 100 μg/ml; EDTA 100 μM; $MgCl_2$ 3.25 mM; NH_4-acetate 64 mM; and bovine serum albumin 0.05%. The formed NADPH was measured fluorometrically with a Farrand photofluorometer (Farrand Optical Co., Inc., Valhalla, N. Y.) equipped with Corning filters no. 5860 as the primary and nos. 4308 and 3389 as the secondary combination.

Calculations. The enzyme activity is expressed in moles

of P_i liberated per kilogram dry wt of tissue per hour at 37°C (MKH units). Na-K-ATPase was computed by subtracting ouabain-insensitive ATPase from Na-K-Mg-ATPase. All measurements were carried out in either duplicate or triplicate, and the results are expressed as a mean±SD followed by the number of observations (n). Statistical analysis of the data was carried out with Student's t test.

Materials. The chemicals were all of the highest grade available. All enzymes and cofactors were purchased from C. F. Boehringer & Soehne GmbH, Mannheim, Germany, except glycogen phosphorylase which was obtained from Sigma Chemical Co., St. Louis, Mo. Aldosterone was a gift from Ciba-Geigy, Basel, Switzerland. Actinomycin D and cycloheximide were purchased from Serva, Heidelberg, Germany.

RESULTS

Distribution of ATPase activity (Table II). The normal distribution of ATPase activity was measured in single segments from subcapsular nephrons. The Na-K-ATPase activity was found to be highest in the distal convolution and lowest in the proximal convolution. An intermediate value was found in the thick ascending limb of the loop of Henle. Mg-ATPase activity did not differ significantly in the distal tubule and ascending limb but was diminished in the proximal convolution. These results are in good agreement with our previous measurements in pooled tubular segments determined by a different, less sensitive method (11).

Response to adrenalectomy (Table III). Animals adrenalectomized 10 or more days before study and maintained on a normal diet with free access to saline and water demonstrated a significant depression in Na-K-ATPase activity as compared to paired controls. This effect was apparent in all functional segments of the nephron examined and varied from a 78% decrease in activity in the distal convolution ($P < 0.01$) to a 48% decrement ($P < 0.01$) in the enzyme activity of the thick ascending limb of the loop of Henle. In addition, adrenal extirpation produced a complete disappearance of measurable Na-K-ATPase activity in the proximal convolution. In contrast to these profound effects on the Na-K-ATPase fraction there were no significant changes in the ouabain-insensitive Mg-ATPase activity in any of the tubular segments.

TABLE II

Distribution of ATPase Activity in Isolated Nephron Segments

Structure	Na-K-ATPase	Mg-ATPase
Proximal convolution	1.5±0.3 (5)*	5.5±0.3 (5)
Distal convolution	7.8±2.0 (5)	6.9±1.4 (5)
Thick ascending limb of Henle	4.2±1.2 (7)	7.7±1.1 (7)

* Enzyme activity in moles of P_i liberated per kilogram dry wt of tissue per hour at 37°C (MKH units) ±SD. The number of analyses is in parentheses.

Effect of aldosterone and inhibitors of protein synthesis (Tables III and IV). The administration of a single low dose of aldosterone (6, 15) given in vivo produced a complete restoration of Na-K-ATPase activity (Table III). This effect was apparent as early as 1 h after the administration of hormone. Our results thus correlate well temporally with the observed time lag in the rat of the renal excretion of sodium and potassium previously reported (15, 16). This effect on Na-K-ATPase was present in all tubular segments of the nephron previously affected by adrenalectomy. As was the case with the latter effect this action of the mineralocorticoid was confined only to the Na-K-ATPase fraction of enzyme activity.

The rapid restoration of enzyme activity after aldosterone differs from the longer delays previously reported (3, 4, 7). Since this delay is consistent with the mediation of the hormone effect through an alteration in protein synthetic rate (1, 17), studies were performed to investigate whether a similar mechanism was involved in the presence of the shorter lag time reported here. Inhibitors of protein synthesis of different mechanisms were employed in sufficient dose to block protein synthesis by more than 95% (18, 19) and to inhibit other inducible enzymes (20). These drugs produced no effect alone but completely suppressed the restoration of Na-K-ATPase by aldosterone after adrenalectomy (Table IV). This effect was apparent in all tubular segments examined although the exceedingly low activity in the proximal convolution prevented meaningful evaluation of change in activity at this site. These results are con-

TABLE III

Effect of Adrenalectomy and Aldosterone on ATPase Activity

	Adrenalectomy		Adrenalectomy + aldosterone*	
Structure	Na-K-ATPase	Mg-ATPase	Na-K-ATPase	Mg-ATPase
Proximal convolution	0.6±0.8 (17)‡	5.1±1.1 (17)	1.4±0.5 (18)	4.8±0.8 (18)
Distal convolution	1.7±2.2 (20)	7.7±1.1 (20)	7.9±3.2 (15)	6.0±1.6 (15)
Thick ascending limb of Henle	2.0±1.3 (8)	8.8±2.4 (8)	4.0±1.7 (10)	6.6±1.7 (10)

* A single dose of 5 μg aldosterone per 100 g rat.

‡ Moles P_i per kilogram dry wt per hour at 37°C ±SD. The number of analyses is in parentheses.

TABLE IV

Effect of Inhibitors of Protein Synthesis on Aldosterone Restoration of ATPase Activity

Structure	Inhibitors alone		Adrenalectomy + inhibitor + aldosterone	
	Na-K-ATPase	Mg-ATPase	Na-K-ATPase	Mg-ATPase
Proximal convolution*	1.4±0.6 (12)‡	4.6±1.0 (12)	0.6±1.1 (14)	4.9±1.0 (14)
Distal convolution*	8.0±1.0 (10)	6.5±1.4 (10)	1.8±2.8 (14)	6.4±1.2 (14)
Thick ascending limb of Henle§	4.8±2.3 (8)	7.9±1.0 (8)	1.8±1.4 (12)	6.9±1.4 (11)

* 4 mg cycloheximide per rat 1 h before 5 μg aldosterone.

‡ Moles P_i per kilogram dry wt per hour at 37°C±SD. The number of analyses in in parentheses.

§ 10 μg actinomycin D per rat 1 h before 5 μg aldosterone.

sistent with the notion that this rapid effect of aldosterone is similar in character to those previously reported (1) and is dependent upon the presence of an intact protein synthetic process.

DISCUSSION

These experiments demonstrate a mineralocorticoid modulation of the activity of Na-K-ATPase along the rat nephron. During the period of experimentally induced adrenal insufficiency, as manifested by an inability to excrete a water load, Na-K-ATPase activity was significantly depressed. In addition, enzymatic activity was completely restored within 1 h of a single low dose of aldosterone, a time when a measurable physiologic response to the drug occurs. Despite these apparent correlations between function and enzymatic activity, previous studies have either failed to demonstrate a similar response or have required exceedingly high dosages of mineralocorticoid for extended periods of time to achieve an effect (3, 4, 7).

We believe that the principle reason for this discrepancy can be attributed to the difference in methodology employed. Previous investigators have utilized broken cell preparations in combination with techniques of subcellular fractionation, often in the presence of particle solubilization agents to measure a purified fraction of ATPase activity (2–4, 7). These procedures result in an enrichment of the enzyme specific activity either by concentrating the enzyme in a single fraction and/or by unmasking latent sites of activity. It is conceivable that these techniques result in a disruption of the in vivo relationship between *in situ* regulated enzymatic activity and potential total activity as measured in a highly purified enzyme preparation. In addition assay conditions are altered to achieve optimum activity usually containing ATP in a concentration of 3 mM and more, and sodium and potassium in concentrations of 130 mM and 20 mM, respectively. The Na-K-ATPase activity obtained from highly purified membrane fractions covers an amount of enzyme activity which exceeds that in our preparation.

In our enzyme preparation kidney tissue is frozen, sliced, and lyophilized. Single microdissected tubular portions from the lyophilized section are directly put into the reagent without any treatment with detergents. The reaction is carried out with 2 mM ATP, which is approximately equimolar to the content of the nucleotide in the living renal epithelia (21). In addition sodium and potassium of only 50 and 5 mM concentrations are used, which also corresponds with the distribution of the two cations at the inner and outer side of the plasma membrane (22).

In our study the aldosterone effect on Na-K-ATPase activity could be detected in the proximal and distal tubules (Table III). Micropuncture studies confirm that the sites of aldosterone action are the distal convolution and the collecting duct (23). There is still considerable confusion as to whether aldosterone promotes sodium reabsorption in the proximal convolution and in the thick ascending limb of Henle. One group of investigators reported a correction of impaired sodium reabsorption at both tubular sites by aldosterone (15, 23). This could not be confirmed by others (24, 25).

The discrepancy between some micropuncture data and our results may suggest that the mineralocorticoid-dependent Na-K-ATPase change is unrelated to sodium transport. In studies on the isolated thick ascending limbs of Henle from rabbit kidney, the demonstration of a positive potential difference (3-5 mV) at the luminal site is consistent with an active electrogenic chloride pump rather than active sodium transport (26). We know, however, that in a highly purified microsomal membrane fraction from the rabbit outer medulla, where the thick ascending limb is the main component, the highest specific Na-K-ATPase activity was reported (27). Obviously species differences and methodological techniques must be kept in consideration.

Aldosterone seems to act on Na-K-ATPase specifically and not on plasma membranes in general, because the ouabain-insensitive Mg-ATPase remained unchanged. Protein synthesis is involved in the rapid steroid-dependent activation of Na-K-ATPase as shown in Table

IV. Actinomycin D and cycloheximide inhibit completely the restoration of Na-K-ATPase by aldosterone. Neither antibiotic has any influence on the Na-K-ATPase activity of tubular portions from kidney tissue of normal rats when injected 2 h before the animals are sacrificed.

Micropuncture studies have shown that both antibiotics prevented the normalization of the impaired tubular sodium reabsorption after aldosterone application in adrenalectomized rats (15). In addition recent experiments revealed that spironolactone in a concentration of 14 mg per 100 g rat inhibited the mineralocorticoid-dependent activation of Na-K-ATPase in both the proximal and distal convolutions of adrenalectomized rats (data not shown).

Several possible interpretations of the present results should be considered. Firstly, rapid activation might be due to a hormonally induced augmentation of Na-K-ATPase synthesis. This would imply a turnover of the enzyme with a short half-life which seems, however, unlikely since studies with highly purified membranes and isolated tubules from the outer medulla of rat kidney revealed that changes in the total activity of Na-K-ATPase require more than 2 h (8, 9).

Secondly, aldosterone may change the membrane structure. It was shown that aldosterone within 1 h alters fatty acid metabolism of membrane phospholipids by stimulating the conversion of pyruvate into fatty acids (28). Thus, a change in the lipid environment of the Na-K-ATPase could account for the rapid activation that we observed.

Thirdly, aldosterone might induce the synthesis of a new protein (AIP). AIP may act like deoxycholate, unmasking a fraction of the Na-K-ATPase present in renal tissue of adrenalectomized rats. The observed lag period of the hormonally induced Na-K-ATPase response and the inhibition of this enzymatic response by inhibitors of protein synthesis support this concept. One can calculate that the microsomal membranes of the outer medulla from adrenalectomized rats contain as much as ten times more Na-K-ATPase (250 μmol P_i/mg protein/h) than we measure in single portions of the distal tubule (8–25 μmol P_i/mg protein/h) from normal rats. Thus stimulation of net synthesis of Na-K-ATPase is not necessary to explain the aldosterone-induced restoration of activity in isolated tubular portions.

The activity of Na-K-ATPase which we determine with our technique appears to change very rapidly and probably without any increase or decrease in the amount of enzyme present. It might be that this activity is regulated by aldosterone and that somehow it is connected more closely with the reabsorptive process of sodium than assumed so far (29).

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REFERENCES

1. Edelman, I. S., and D. D. Fanestil. 1970. Mineralocorticoids. *In* Biochemical Actions of Hormones. G. Litwack, editor. Academic Press, Inc., New York. **1**: 324.
2. Skou, J. C. 1972. The relationship of the (Na-K)-activated enzyme system to transport of sodium and potassium across the cell membrane. *Bioenergetics.* **4**: 203.
3. Jørgensen, P. L. 1969. Regulation of the Na-K-activated ATP hydrolyzing enzyme system in rat kidney. II. The effect of aldosterone on the activity in kidneys of adrenalectomized rats. *Biochim. Biophys. Acta.* **192**: 326.
4. Hendler, E. F., J. Torretti, L. Kupor, and F. H. Epstein. 1972. Effects of adrenalectomy and hormone replacement on Na-K-ATPase in renal tissue. *Am. J. Physiol.* **222**: 754.
5. Hill, J. H., N. Cortas, and M. Walser. 1973. Aldosterone action and sodium- and potassium-activated adenosine triphosphatase in toad bladder. *J. Clin. Invest.* **52**: 185.
6. Wiederholt, M., H. Stolte, J. P. Brecht, and K. Hierholzer. 1966. Mikropunktionsuntersuchungen über den Einfluss von Aldosteron, Cortison und Dexamethason auf die renale Natrium Resorption adrenalektomierter Ratten. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **292**: 316.
7. Landon, E. J., N. Jazob, and L. Forte. 1966. Aldosterone and sodium-potassium-dependent ATPase activity of rat kidney membranes. *Am. J. Physiol.* **211**: 1050.
8. Jørgensen, P. L. 1972. The role of aldosterone in the regulation of Na-K-ATPase in rat kidney. *J. Steroid Biochem.* **3**: 181.
9. Schmidt, U., and U. C. Dubach. 1971. Sensitivity of Na-K-adenosine triphosphatase activity in various structures of the rat nephron: studies with adrenalectomy. *Eur. J. Clin. Invest.* **1**: 307.
10. Stolte, H., J. P. Brecht, M. Wiederholt, and K. Hierholzer. 1968. Einfluss von Adrenalektomie und Glucocortikoiden auf die Wasserpermeabilität corticaler Nephronabschnitte der Rattenniere. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **299**: 99.
11. Schmidt, U., and U. C. Dubach. 1969. Activity of (Na-K)-stimulated adenosine triphosphatase in the rat nephron. *Pfluegers Arch. Eur. J. Physiol.* **306**: 219.
12. Schmidt, U., and U. C. Dubach. 1972. Quantitative Histochemie am Nephron. *Prog. Histochem. Cytochem.* **2**: 185.
13. Lowry, O. H. 1953. The quantitative histochemistry of the brain. *J. Histochem. Cytochem.* **1**: 420.
14. Schmidt, U., and U. C. Dubach. 1971. Na-K-stimulated adenosine triphosphatase: intracellular localization within the proximal tubules of the rat nephron. *Pfluegers Arch. Eur. J. Physiol.* **330**: 265.
15. Hierholzer, K., and H. Stolte. 1969. The proximal and distal tubular action of adrenal steroids on Na reabsorption. *Nephron.* **6**: 188.

16. Kirsten, R., and E. Kirsten. 1972. Redox state of pyridine nucleotides in renal response to aldosterone. *Am. J. Physiol.* **223**: 229.
17. Lahav, M., T. Dietz, and J. S. Edelman. 1973. The action of aldosterone on sodium transport: further studies with inhibitors of RNA and protein synthesis. *Endocrinology.* **92**: 1685.
18. Stewart, B. W. 1973. Effect of cycloheximide on dimethylnitrosamine-induced polyribosome disaggregation in rat liver. *Chem.-Biol. Interactions.* **6**: 81.
19. Chu, L. L. H., and J. S. Edelman. 1972. Cordycepin and α-Amanitin: Inhibitors of transcription as probes of aldosterone action. *J. Membr. Biol.* **10**: 291.
20. Hash, J. H. 1972. Antibiotic mechanisms. *Annu. Rev. Pharmacol.* **12**: 35.
21. Rea, C., and S. Segal. 1972. ATP content of rat kidney cortex slices: relation to α-aminoisobutyric acid uptake. *Kidney Int.* **2**: 101.
22. Ling, G. N., C. Miller, and M. M. Ochsenfeld. 1973. The physical state of solutes and water in living cells according to the association-induction hypothesis. *Ann. N. Y. Acad. Sci.* **204**: 6.
23. Hierholzer, K., M. Wiederholt, H. Holzgreve, G. Giebisch, R. M. Klose, and E. E. Windhager. 1965. Micropuncture study of renal transtubular concentration gradients of sodium and potassium in adrenalectomized rats. *Pfluegers Arch. Ges. Physiol. Menschen Tiere.* **285**: 193.
24. Lynch, R. E., E. G. Schneider, L. R. Willis, and F. G. Knox. 1972. Absence of mineralocorticoid-dependent sodium reabsorption in dog proximal tubule. *Am. J. Physiol.* **223**: 40.
25. Sakai, F., and Y. Murayama. 1971. Effect of aldosterone and cortisol on Henle's loop in the adrenalectomized rat's kidney. *Jap. J. Pharmacol.* **21**: 23.
26. Burg, M. B., and N. Green. 1973. Function of the thick ascending limb of Henle's loop. *Am. J. Physiol.* **224**: 659.
27. Jørgensen, P. L., and J. C. Skou. 1969. Preparation of highly active ($Na^+ + K^+$) ATPase from the outer medulla of rabbit kidney. *Biochem. Biophys. Res. Commun.* **37**: 39.
28. Goodman, D. B. P., J. E. Allen, and H. Rasmussen. 1971. Studies on the mechanism of action of aldosterone: hormone-induced changes in lipid metabolism. *Biochemistry.* **10**: 3825.
29. Schmidt, U., H. Schmid, B. Funk, and U. C. Dubach. 1974. The function of Na-K-ATPase in single portions of the rat nephron. *Ann. N. Y. Acad. Sci.* **242**: 489.