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The Role of Sodium-Potassium–Activated Adenosine Triphosphatase in the Reabsorption of Sodium by the Kidney *

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Summary. **In order to evaluate the possible role of sodium- and potassium-activated adenosine triphosphatase in the active transport of sodium by the renal tubules, we examined the effect of large changes in the tubular reabsorptive load of sodium on the Na-K-ATPase activity of rat kidney homogenates. Glomerular filtration and tubular reabsorption of sodium per gram of kidney tissue increased progressively after contralateral uninephrectomy. This was paralleled by an increase in Na-K-ATPase per milligram of protein in a microsomal fraction of kidney cortex. The importance of this change is underlined by the absence of simultaneous increases in other microsomal enzymes such as glucose-6-phosphatase and Mg^{++}-dependent ATPase, or in succinic dehydrogenase or glutaminase. Similar increases in Na-K-ATPase were observed when the net tubular reabsorption of sodium was increased by feeding the animals a high-protein diet or after injection of methylprednisolone. On the other hand, Na-K-ATPase was lowered when tubular transport of sodium was reduced by bilateral adrenalectomy. The results of these experiments show that renal Na-K-ATPase changes in an adaptive way when renal reabsorption of sodium is chronically increased or diminished and support the hypothesis that this enzyme system is involved in the process by which sodium is actively transported across the renal tubule.**

Introduction

The active transport of sodium and potassium across cell membranes probably utilizes energy stored in the cell in the form of adenosine triphosphate (ATP). An enzymatic property of cell membranes which accelerates the breakdown of ATP in the presence of sodium and potassium has been described in many tissues, and considerable evidence has accumulated to support the concept that a sodium- and potassium-stimulated adenosine triphosphatase (Na-K-ATPase) is directly involved with, or is part of, an active cation transport system. This enzyme, or group of enzymes, catalyzes the hydrolysis of the terminal high-energy bond of ATP in the presence of both sodium and potassium but not in the absence of either. The enzyme is located predominantly in those cellular fractions which contain the cell membranes and is selectively inhibited by digitalis glycosides which also inhibit active electrolyte transport (1).

Most of the evidence linking Na-K-ATPase to ion transport is derived from studies of coupled transfers of sodium and potassium ions in red

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blood cells or erythrocyte ghosts. A role for Na-K-ATPase in the bulk transfer of ions across epithelial cell barriers, on the other hand, is not established and has been little explored. The kidney would seem an ideal organ to test the hypothesis that Na-K-ATPase is involved in the active transport of sodium, since the enzymatic activity of kidney cell membranes is high (2, 3) and the chief work of the kidney is the active reabsorption of the sodium contained in the glomerular filtrate.

The present experiments were designed to investigate the possible connection between Na-K-ATPase and active transport of sodium by the kidney. The results indicate that an adaptive increase or decrease in Na-K-ATPase, unaccompanied by changes in other representative enzymes, can be induced when net tubular reabsorption of sodium is greatly altered. The data therefore support the concept that Na-K-ATPase is involved in some way in the active transport of sodium by the kidney.

Methods

Male albino rats of the Sprague-Dawley strain weighing 250–400 g were kept in individual metabolic cages, had free access to water, and were fed a Purina pellet rat diet unless otherwise specified.

Nephrectomy. The left kidney was removed under pentobarbital anesthesia (50 mg/kg intraperitoneally). At intervals from 24 hr to 3 wk the remaining kidney and the right kidney of control animals of the same age and weight which were fed the same diet were removed for enzyme assays. In other animals the glomerular filtration rate (GFR) and tubular reabsorption of sodium were measured as described below.

High-protein feeding. For 7 days rats were fed a diet in paste form which contained 50% protein, 31.5% carbohydrate, and 12% fat. They were compared with a control group matched for weight and age which was fed an equicaloric diet containing 20% protein, 53% carbohydrate, and 20% fat. Both diets had the same mineral and vitamin content. The amount of food eaten was weighed and found comparable in the two groups.

Methylprednisolone, 2.5 mg, in the long acting form (Depo Medrol, Upjohn Co., Kalamazoo, Mich.) was injected daily for 4 days into six rats, deep in the thigh muscles. A comparable group of six rats not receiving injections served as control. Animals of both groups were sacrificed on the 5th day, and the kidneys were homogenized for enzyme assays. In another group of five rats treated as above, GFR and tubular sodium reabsorption were measured on the 5th day and compared with untreated control animals.

Adrenalectomy was performed through a midline incision, and the animals had the choice of both water and 0.9% saline as drinking solution. 2 wk after bilateral adrenalectomy, both kidneys were removed and homogenized for enzyme assays. *Potassium depletion* was induced by feeding the rats a diet deficient in potassium for 2 wk, with unrestricted water intake. *Ammonium chloride loading* was done by substituting a 0.28 M solution of NH_4Cl for drinking water for 14 days.

Preparation of the kidney homogenate and isolation of microsomal fractions. Kidneys were removed under light ether anesthesia and immediately chilled in ice-cold isotonic saline solution. After removal of the capsule, the white medulla, and the papilla, the kidneys were blotted on filter paper and weighed. They were then homogenized in a 10/1 (v/w) solution containing 0.25 mole sucrose, 5 mmoles Na_2 EDTA, 30 mmoles histidine buffer/liter, and 0.1% sodium deoxycholate at pH 6.8. The proportion of homogenizing solution to tissue was 20/1 when only one kidney was used. Homogenization was carried out in a glass homogenizer immersed in ice with a Teflon pestle at 1725 rpm and 0.18 mm clearance, using 15–20 strokes. The homogenate was squeezed through a double layer of gauze. Part of this suspension was used immediately as the fresh whole homogenate, and the remainder was centrifuged at 10,800 *g* in a refrigerated Spinco preparative ultracentrifuge for 30 min to sediment cell debris, nuclei, and mitochondria. The supernatant was carefully removed, leaving a sufficient amount of fluid at the bottom of the tube to avoid contamination by the sediment, and centrifuged at 39,100 *g* for 30 min. The sediment, made of "heavy microsomes," was gently resuspended with a Vortex mixer in 1.5–2 ml of the original homogenizing solution without deoxycholate and the remaining supernatant centrifuged at 105,000 *g* for 60 min. The final sediment ("light microsomes") was resuspended in 1.5–2 ml homogenizing solution without deoxycholate as above. Aliquots that were not assayed immediately were stored frozen at −20°C and assayed the following day. In all experiments, assays of enzyme activity were carried out simultaneously in tissue preparations from experimental and control rats that had been processed in the same way on the same day.

Adenosine triphosphatase (ATPase). 0.1 ml of tissue suspension, which contained 0.5–1.3 mg of protein in the whole homogenate and 0.17–0.7 mg in the microsomal suspension, was used for all assays. Total ATPase activity was determined in 5 ml of a reaction mixture prewarmed at 37°C containing 100 mmoles NaCl, 20 mmoles KCl, 10 mmoles imidazole buffer, 5 mmoles $MgCl_2$, and 5 mmoles disodium adenosine triphosphate (ATP) (Sigma Chemical Co., St. Louis, Mo.)/liter at pH 7.8. The reaction was started with the addition of $MgCl_2$ and ATP, carried out for 5 min at 37°C in a shaking water bath, and terminated by the addition of 1 ml ice-cold 35% (w/v) trichloroacetic acid. The precipitated protein was discarded after centrifugation and the inorganic phosphate in the supernatant determined by the method of Fiske and Subbarow (4), the optical density being read at 660 mm in the Coleman spectrophotometer. The Na^+-K^+-stimulated portion of the kidney ATPase was defined as the difference between the inorganic phosphate liberated in

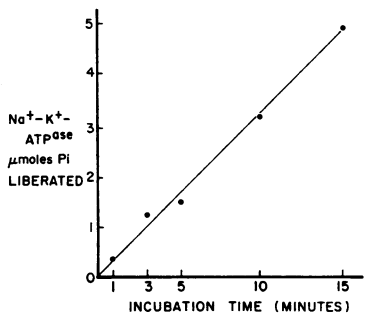

FIG. 1. ATP HYDROLYSIS SENSITIVE TO THE PRESENCE OF K^+ (NA-K-ATPASE) IS PLOTTED AS A FUNCTION OF THE TIME OF INCUBATION. 0.1 ml of "light microsomal" suspension was incubated at 37°C in a total volume of 5 ml with 5 mM Mg ATP per liter as substrate. Values shown are the mean of duplicate determinations.

the presence and in the absence of potassium. Correction was made for the spontaneous, nonenzymatic breakdown of ATP, measured as the inorganic phosphate liberated under the same experimental conditions in the absence of enzyme, which usually accounted for less than 15% of the total inorganic phosphate present.

The protein content of the tissue suspensions was determined by the method of Lowry et al. (5), using crystalline bovine albumin as standard. ATPase activity is expressed in μmoles of inorganic phosphate released per milligram of protein per hour.

Glucose-6-phosphatase. 0.1 ml of light microsomal suspension was incubated with 0.1 ml of 80 mM glucose-6-phosphate (Sigma Chemical Co., St. Louis, Mo.) solution at 37°C and pH 6.5 for 5 min in a shaking water bath, and the reaction was stopped with 2 ml of 10% trichloroacetic acid. The inorganic phosphate liberated was determined in the supernatant of this mixture by the method of Fiske and Subbarow as described above, and expressed in μmoles Pi/mg of protein per hour (6).

Succinic dehydrogenase was determined in whole homogenate by measuring the reduction of ferricyanide in the presence of succinate, according to the method of Bonner (7). The activity is expressed in arbitrary units as Δ optical density reading/mg of protein per hour.

Glutaminase activity of the whole homogenate was measured as described by Rector et al. (8) and expressed in μmoles NH_4 liberated/mg of protein per hour.

Glomerular filtration rate and sodium reabsorptive load. Rats were anesthetized with Inactin (Promonta, Hamburg, Germany) [5-ethyl-(1-methyl propyl)-2-thiobiarbituric acid], 160 mg/kg intraperitoneally, placed on a heated board at 37°C; a tracheostomy was performed, and a polyethylene PE 50 catheter was introduced in the bladder through a midline incision. Normal saline to replace surgical losses in an amount equal to 1–2% body weight was infused over 20 min through a PE 50 catheter in the jugular vein. A priming dose of 50 μc tritiated inulin (inulin-methoxy-H^3) was given rapidly intravenously followed by a sustaining infusion of 50 μc tritiated inulin in 1.2 ml/hr delivered with a Harvard constant infusion pump (model 975). After an equilibration period of 40–45 min, urine was collected under mineral oil, and the volume was measured with glass micropipettes. In each experiment four consecutive collection periods of 30 min each were obtained. Blood from the cut end of the tail was collected in heparinized capillary tubes at the beginning and the end of each collection period, and the plasma concentration of inulin and sodium was calculated as the average of two determinations. Radioactive inulin was determined in a Packard Tri-Carb scintillation counter, and plasma and urine sodium were determined with the IL flame photometer, model 143. All determinations were carried out in duplicate.

The statistical significance between mean values was assessed by Student's t test. P values less than 0.05 were considered significant.

Results

Characteristics and distribution of Na-K-ATPase of rat kidney. Under the conditions described, Na-K-ATPase activity in whole homogenate or microsomes of rat kidney was linear with time between 1 and 15 min of incubation (Fig. 1) and linear with enzyme concentration in the range 0.05–0.2 ml of tissue suspension (Fig. 2). The K_m (with Mg ATP as substrate) was approximately 0.5 mmoles Mg ATP, and the concentration of substrate necessary for maximal activity varied between 2 and 5 mmoles Mg ATP (Fig. 3). If the concentration of potassium was held at 5 mmoles in the incubating medium, and the concen-

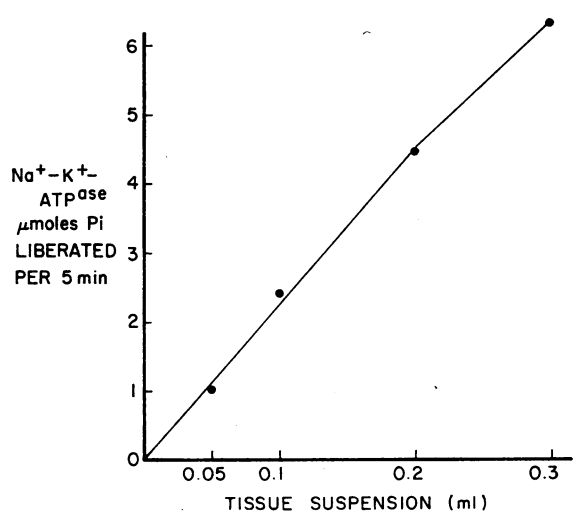

FIG. 2. NA-K-ATPASE ACTIVITY AS A FUNCTION OF THE CONCENTRATION OF ENZYME. Incubation was carried out in 5 ml of medium at 37°C for 5 min with 5 mM Mg ATP per liter as substrate.

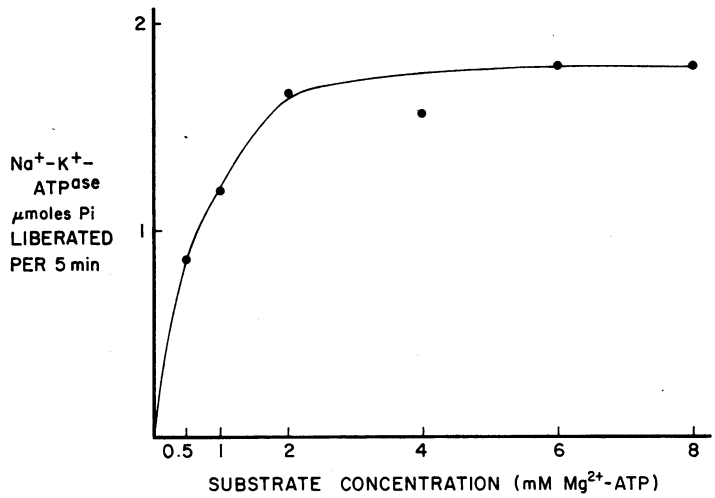

FIG. 3. NA-K-ATPASE ACTIVITY AS A FUNCTION OF THE CONCENTRATION OF SUBSTRATE. 0.1 ml of "light microsomal" suspension was incubated at 37°C for 5 min. K_m is approximately 0.5 mM Mg ATP per liter in this typical experiment.

tration of sodium varied, K_m for Na^+ was approximately 16 mmoles/liter (Fig. 4). ATPase activity was inhibited equally by omitting Na^+, K^+, both Na^+ and K^+, or by adding 2 mM ouabain to the incubating medium (Table I). Lower concentrations of ouabain do not inhibit Na-K-ATPase consistently or completely in homogenates of rat kidney.

In preliminary experiments the distribution of ATPase in the various fractions of a broken-cell homogenate was studied, and the relative activities of the sodium-potassium–activated moiety and the residual ATPase were measured in each fraction (Fig. 5). Approximately 50% of the Na-K-ATPase in the whole homogenate was found in the microsomes. The proportion of total ATPase

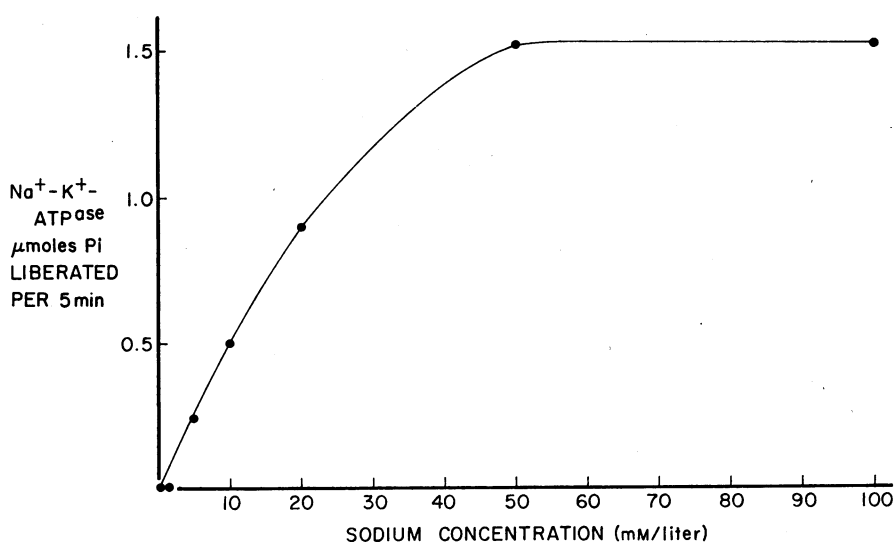

FIG. 4. NA-K-ATPASE ACTIVITY AS A FUNCTION OF SODIUM CONCENTRATION, WITH POTASSIUM CONCENTRATION KEPT CONSTANT AT 5 mM/LITER. In this representative experiment, incubation was carried out at 37°C for 5 min using 0.1 ml of "light microsomal" suspension and 5 mM Mg ATP per liter as substrate.

TABLE I

Effect of ouabain and omission of Na^+ and K^+ on ATPase activity of light microsomes of rat kidney

Composition of incubation medium*				
Na^+	Tris	K^+	Ouabain	ATPase
(mmoles)				(μmoles Pi liberated per 5 min)
100	0	20	0	2.16
100	20	0	0	1.08
0	100	20	0	1.14
0	120	0	0	1.08
100	0	20	2	1.08

* All media contained in addition 10 mmoles imidazole buffer, 5 mmoles $MgCl_2$, and 5 mmoles Na_2ATP/liter. Values are the mean of four determinations.

activated by sodium and potassium was highest in the "light microsomes," sedimenting between 39,100 and 100,000 *g*. This fraction was therefore selected for subsequent assays of the enzyme.

Effect of unilateral nephrectomy on sodium reabsorption and Na-K-ATPase in the remaining kidney (*Tables II and III, Figs. 6 and 7*). After one kidney is removed, the opposite kidney grows in size, while glomerular filtration rate and tubular reabsorption of sodium also increase. Table II illustrates that by 1 wk after nephrectomy, glomerular filtration and sodium reabsorption have

TABLE II

Changes in tubular reabsorption of sodium induced by unilateral nephrectomy and by methylprednisolone

Experiment	No.	Kidney weight	Tubular reabsorption of sodium
		g	μEq/min per g of kidney
Controls, 2 kidneys	8	2.03 ± 0.04*	173.8 ± 8.3
Nephrectomy‡ after 24 hr	8	1.19 ± 0.04 P <0.005	162.4 ± 8.4 NS
Nephrectomy after 3 days	9	1.18 ± 0.03 P <0.005	198.1 ± 12.6 NS
Nephrectomy after 7 days	8	1.42 ± 0.02 P <0.001	203.2 ± 10.2 P =0.05
Controls,§ 2 kidneys	13	2.64 ± 0.05	189.8 ± 12.9
Nephrectomy after 14–21 days	11	1.76 ± 0.04 P <0.001	230.2 ± 6.5 P <0.001
Controls, 2 kidneys	8	2.03 ± 0.04	173.8 ± 8.3
Methylprednisolone, 2 kidneys	6	2.13 ± 0.05 NS	252.5 ± 23.6 P <0.01

* SEM.

‡ In the nephrectomy experiments, results are compared to the values calculated for one kidney in the control animals.

§ Because of the large size the experimental animals reached 2–3 wk after nephrectomy, a second group of control animals of comparable size was used.

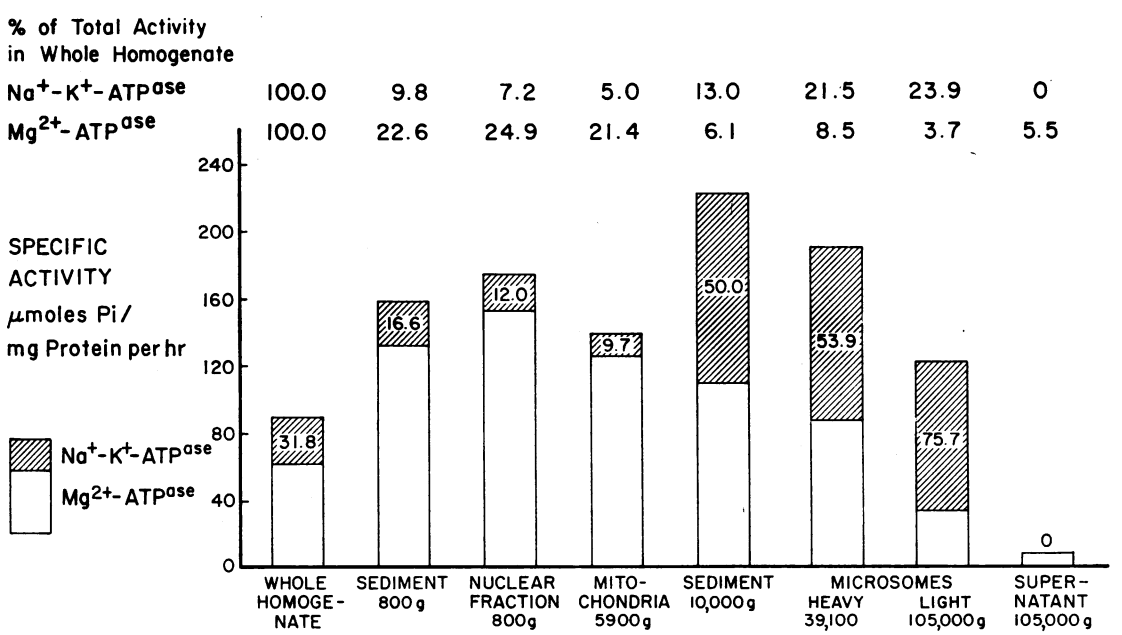

FIG. 5. DISTRIBUTION OF NA-K-ATPASE AND MG-DEPENDENT ATPASE IN DIFFERENT FRACTIONS OF A HOMOGENATE OF RAT KIDNEY. Figures in the cross-hatched bars represent per cent of total activity which is stimulated by Na and K in each fraction.

outstripped the increase in kidney size, so that thenceforth the reabsorption of sodium per gram of kidney tissue is greater than normal. The activity of Na-K-ATPase per milligram of microsomal protein rises in parallel with the increase in tubular reabsorptive work (Fig. 6, Table III). By 3 wk after nephrectomy, the specific activity of microsomal Na-K-ATPase increased by 55% over its level in control rats, at a time when sodium reabsorption per unit of kidney tissue was augmented by 21%. It should be noted that the increase in microsomal Na-K-ATPase *per kidney* was considerably greater than 55% since the kidney increased in size after contralateral nephrectomy. The relative distribution of Na-K-ATPase between the light microsomes and the rest of the broken-cell homogenate was not altered by unilateral nephrectomy.

The increase in Na-K-ATPase after nephrectomy was not accompanied by parallel changes in glutaminase and succinic dehydrogenase,[1] measured in the whole homogenate, or in two other microsomal phosphatases, glucose-6-phosphatase, and the residual, Mg-dependent, adenosine triphosphatase (Fig. 7, Table III).

Methylprednisolone (Tables II and III, Fig. 8). Since glucocorticoids are known to induce an increase in glomerular filtration (9), administration of methylprednisolone was chosen as another

[1] There was a slight but statistically significant increase (13% over control) in succinic dehydrogenase 2 wk after nephrectomy.

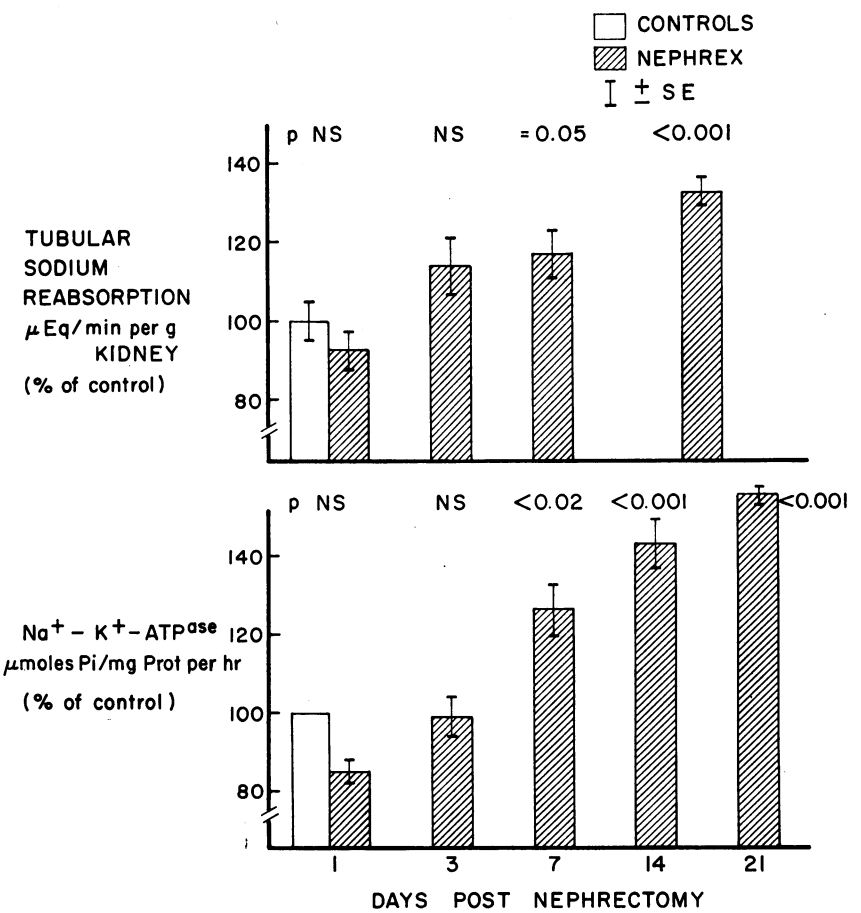

FIG. 6. THE SPECIFIC ACTIVITY OF NA-K-ATPASE IS COMPARED WITH TUBULAR REABSORPTION OF SODIUM PER GRAM OF KIDNEY (WET WEIGHT) AT INTERVALS AFTER NEPHRECTOMY. Since there was no significant difference between the tubular reabsorption of sodium at 2 and at 3 wk after nephrectomy, a single average value for these periods is given.

TABLE III

Changes in Na-K-ATPase of light microsomes and in other kidney enzymes

Experiment		No.	Kidney weight	Succinic dehydrogenase	Glutaminase	Glucose-6-phosphatase	Mg^{2+} ATPase	Na^+-K^+-ATPase
			g	*U/mg protein/hr*	*NH_4/mg protein/hr*		(*μmoles Pi/mg protein/hr*)	
Nephrectomy after 24 hr	C*	6	1.01 ± 0.03‡	2.14 ± 0.03			6.0 ± 2.7	41.2 ± 4.4
	E§	6	1.14 ± 0.02	2.37 ± 0.02			7.1 ± 3.1	34.9 ± 3.6
	P		<0.01	<0.001			NS	NS
Nephrectomy after 3 days	C	6	1.13 ± 0.03	2.67 ± 0.08			13.2 ± 1.3	45.8 ± 4.9
	E	6	1.38 ± 0.04	2.75 ± 0.17			13.1 ± 1.5	45.4 ± 2.4
	P		<0.001	NS			NS	NS
Nephrectomy after 7 days	C	12	1.23 ± 0.04	2.50 ± 0.07		30.3 ± 1.2	17.7 ± 1.8	50.3 ± 3.5
	E	12	1.40 ± 0.05	2.56 ± 0.06		29.8 ± 3.3	19.3 ± 1.6	63.1 ± 3.3
	P		<0.01	NS		NS	NS	<0.02
Nephrectomy after 14 days	C	6	1.09 ± 0.04	2.29 ± 0.05	9.6 ± 0.5	41.9 ± 1.6	21.6 ± 1.6	46.6 ± 1.7
	E	6	1.33 ± 0.02	2.56 ± 0.07	8.4 ± 0.5	39.5 ± 2.0	25.4 ± 1.9	66.7 ± 3.0
	P		<0.001	<0.025	NS	NS	NS	<0.001
Nephrectomy after 21 days	C	6	1.19 ± 0.02	2.46 ± 0.08	9.5 ± 1.3	54.2 ± 3.7	36.3 ± 1.1	50.0 ± 3.5
	E	6	1.66 ± 0.05	2.53 ± 0.07	8.5 ± 1.2	57.6 ± 4.5	42.9 ± 3.4	77.5 ± 1.4
	P		<0.001	NS	NS	NS	NS	<0.001
High-protein diet	C	12	2.55 ± 0.08	2.74 ± 0.05	6.9 ± 0.5	16.7 ± 1.9	29.7 ± 2.3	47.2 ± 2.5
	E	12	2.94 ± 0.09	2.90 ± 0.07	7.8 ± 0.3	16.2 ± 0.8	31.2 ± 2.3	59.9 ± 1.3
	P		<0.01	NS	NS	NS	NS	<0.001
Methyl-prednisolone	C	12	2.12 ± 0.04	2.47 ± 0.20	5.6 ± 0.9	30.0 ± 2.0	34.9 ± 1.9	47.0 ± 3.3
	E	12	2.30 ± 0.06	2.76 ± 0.27	6.1 ± 1.1	56.2 ± 4.5	35.3 ± 1.6	63.5 ± 2.9
	P		<0.025	NS	NS	<0.001	NS	<0.005
Adrenalectomy	C	6	2.40 ± 0.13	2.52 ± 0.05		17.2 ± 2.2	22.4 ± 1.4	41.8 ± 2.8
	E	6	1.90 ± 0.09	2.24 ± 0.08		18.6 ± 1.8	21.4 ± 1.4	26.0 ± 1.6
	P		<0.02	<0.02		NS	NS	<0.005
Potassium depletion	C	12	2.37 ± 0.09	2.30 ± 0.10	6.6 ± 0.8	28.3 ± 3.2	37.7 ± 1.1	46.4 ± 1.5
	E	12	2.70 ± 0.06	2.47 ± 0.03	26.6 ± 2.1	29.6 ± 2.3	29.2 ± 0.7	45.0 ± 1.6
	P		<0.01	NS	<0.001	NS	<0.001	NS
NH_4Cl loading	C	6	2.29 ± 0.09	2.86 ± 0.1	6.7 ± 0.7	29.0 ± 1.6	33.8 ± 1.1	44.5 ± 5.7
	E	6	2.34 ± 0.08	3.08 ± 0.07	43.1 ± 2.0	25.9 ± 1.6	28.2 ± 1.7	40.3 ± 4.4
	P		NS	NS	<0.001	NS	<0.05	NS

* Control group.
‡ SEM.
§ Experimental group.

means of increasing the delivery of filtered sodium to tubular reabsorptive sites. Injection of 2.5 mg of methylprednisolone in long acting form each day for 4 days produced an average increase of 45% in the tubular reabsorption of sodium per gram of kidney (Table II). Na-K-ATPase increased in parallel, unaccompanied by changes in Mg-ATPase or succinic dehydrogenase. Glucose-6-phosphatase increased as expected, as a result of the corticoid-induced stimulation of renal gluconeogenesis (10).

High-protein feeding (*Table III, Fig. 9*). Diets with high-protein content are known to produce marked increases in GFR in dogs and rats (11, 12). The increased filtration is presumably accompanied by an increased reabsorption of sodium. Rats fed a 50% protein mixture for 7 days showed a consistent increase in the specific activity of Na-K-ATPase, without significant changes in any of the other enzymes tested.

Adrenalectomy (*Table III, Fig. 10*). Bilateral adrenalectomy resulted in a significant decrease in kidney size and in Na-K-ATPase activity per milligram of protein in the light microsomes. Succinic dehydrogenase activity was also diminished, but Mg-ATPase and glucose-6-phosphatase remained unchanged.

Potassium depletion (*Table III*). In order to provide a control for the effects of renal hypertrophy per se, rats were fed a potassium-deficient diet for 2 wk. Potassium deficiency produces renal hypertrophy but does not increase glomerular filtration or tubular reabsorption of sodium (13). Despite an increase in kidney size comparable to

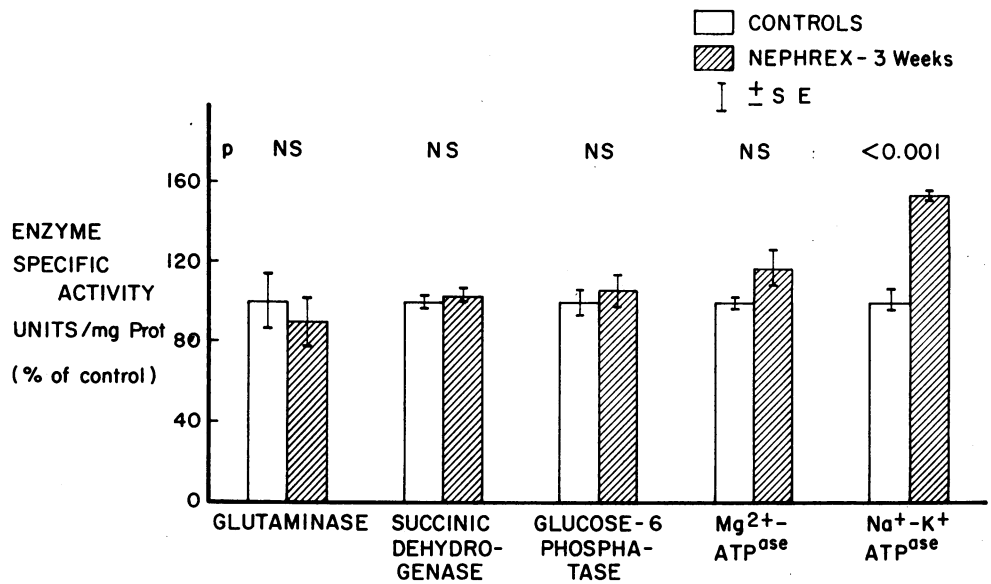

FIG. 7. SPECIFIC ACTIVITY OF RENAL ENZYMES IN CONTROL RATS AND IN RATS 3 WK AFTER UNINEPHRECTOMY. Glucose-6-phosphatase, Mg^{2+} ATPase, and Na-K-ATPase were assayed in the "light microsomal" fraction. Glutaminase and succinic dehydrogenase were assayed in the whole homogenate. Na-K-ATPase per milligram of microsomal protein increased in the remaining kidney after unilateral nephrectomy. There was no change in other enzymes.

that seen 1 wk after nephrectomy, or after a high-protein diet, and greater than that observed in rats given methylprednisolone, Na-K-ATPase of kidney microsomes was unchanged by postassium depletion. Renal glutaminase, on the other hand, rose strikingly, as others have reported (14).

Metabolic acidosis (*Table III*). The work of the kidneys in excreting acid was increased by

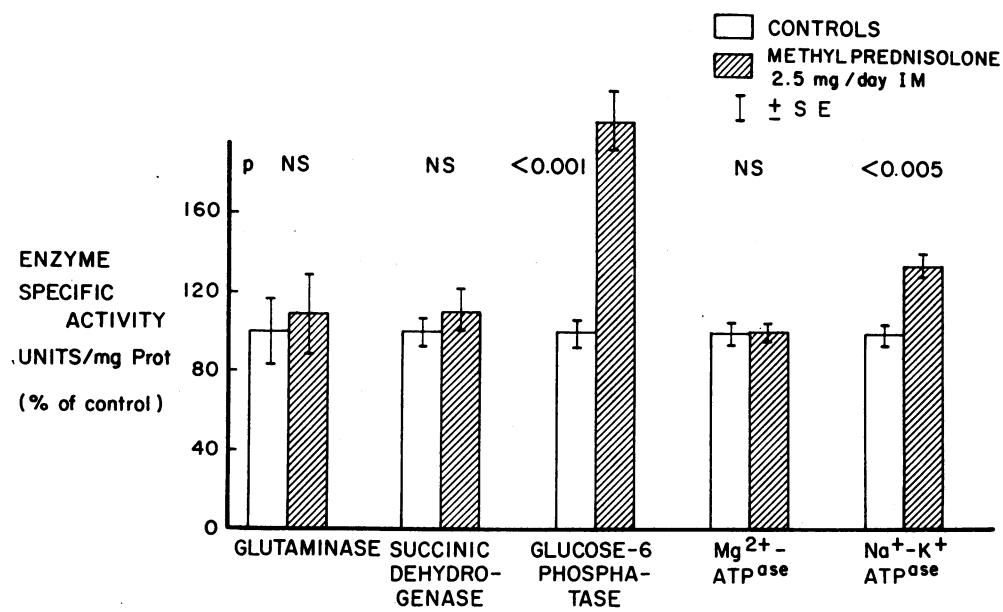

FIG. 8. CHANGES IN SPECIFIC ACTIVITY OF RENAL ENZYMES PRODUCED BY METHYLPREDNISOLONE. Na-K-ATPase and glucose-6-phosphatase were increased by this large dose of glucocorticoid.

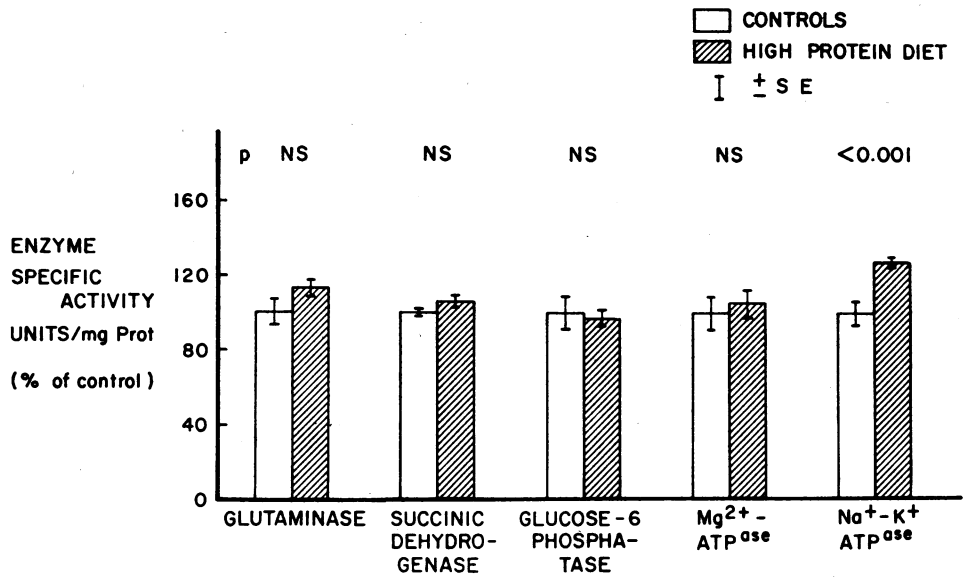

FIG. 9. SPECIFIC ACTIVITY OF RENAL ENZYMES IN RATS ON A CONTROL DIET (20% PROTEIN) AND IN RATS FED A 50% PROTEIN DIET FOR 7 DAYS. High-protein feeding increased the specific activity of Na-K-ATPase in kidney microsomes.

substituting a 0.28 M solution of ammonium chloride for drinking water for 14 days. The specific activity of renal glutaminase was greatly increased by the acid load, but Na-K-ATPase activity was not altered.

Discussion

A general method which has proved useful in elucidating the physiological role of enzymes is to correlate changes in enzymatic activity with changes in function (15, 16). If traffic over a pathway catalyzed by an enzyme is augmented, the amount of enzyme within the cell and its activity per milligram of protein often (but not always) tend to increase. With a decrease in flow through the enzymatic pathway, activity is frequently suppressed. Rate-limiting enzymes are the most responsive to such maneuvers, but enzymes which

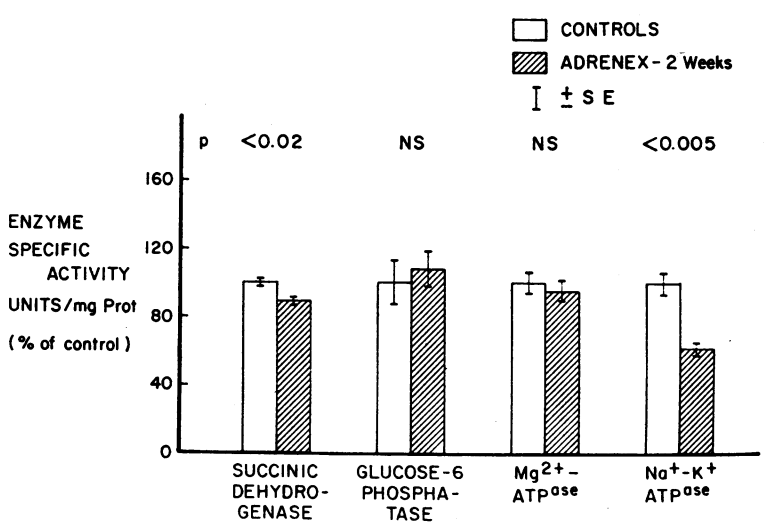

FIG. 10. SPECIFIC ACTIVITY OF RENAL ENZYMES AFTER ADRENALECTOMY. Na-K-ATPase and succinic dehydrogenase were decreased by adrenalectomy.

are not normally rate-limiting may also change. Failure to adapt when function changes, however, does not exclude a functional role for the enzyme system in question.

In the present experiments, the specific activity of sodium-potassium–activated adenosine triphosphatase of rat kidney microsomes was found to increase significantly as tubular reabsorptive work per gram of kidney tissue was augmented. This was true whether the increase in tubular reabsorption was initiated by contralateral nephrectomy, by feeding a diet high in protein, or by greatly increasing glomerular filtration rate with methylprednisolone. The change in ATPase was entirely in that fraction activated by sodium and potassium; the Mg^{++}-activated, residual moiety of ATPase remained unaltered. The stimulation of Na-K-ATPase assumes additional importance since it was not accompanied by parallel changes in certain other enzymes. The activity of succinic dehydrogenase, a mitochondrial enzyme concerned with oxidative metabolism, remained unchanged, as did glutaminase. Glucose-6-phosphatase was of special interest because it is a microsomal enzyme and also a phosphatase. The fact that its activity per milligram of protein was unchanged by uninephrectomy suggests that the increase in Na-K-ATPase produced by this procedure was not a simple consequence of microsomal proliferation in the course of renal hypertrophy.

The significance of the change induced in Na-K-ATPase was further supported by findings in the renal hypertrophy of potassium deficiency. Potassium depletion produces enlargement of the kidney without increasing filtration rate or tubular reabsorption of sodium and water (13). In this form of hypertrophy, Na-K-ATPase remained unchanged, though renal glutaminase increased markedly. Similarly, chronic ammonium chloride loading, which greatly increased the work of the kidney in excreting acid and induced a large increase in glutaminase, did not alter Na-K-ATPase. The increase in Na-K-ATPase which accompanies an increase in the tubular reabsorptive load of sodium therefore appears to be relatively specific.

Changes in the opposite direction were produced by adrenalectomy. Filtration and reabsorption of sodium are decreased by adrenalectomy (17, 18), and this produced a striking fall in Na-K-ATPase in kidney tissue as has recently been reported by others (19, 20). The residual, Mg-stimulated ATPase of kidney microsomes was unaffected. The affect of adrenalectomy on ATPase seems to be limited to the kidney, since the Na-K-ATPase of brain is unchanged by removing the adrenals.[2] Activity in kidney tissue may be restored after adrenalectomy by injecting corticosterone, but not by doses of aldosterone which are sufficient to restore mineral balance in the intact animal (19, 20). Similarly, Na-K-ATPase activity in intact rats is enhanced by methylprednisolone but not by the mineralocorticoid deoxycorticosterone nor by a salt-free diet (unpublished studies). It should be noted that the percentage increase in sodium reabsorption produced by aldosterone or deoxycorticosterone is quite small compared to the large changes produced by glucocorticoids, because of the effect of the latter upon filtration rate. Thus, 99% or more of sodium filtered at the glomerulus is normally reabsorbed, whereas the influence of aldosterone is limited to a small fraction (1%) of this. Part or all of the effect of glucocorticoids in stimulating Na-K-ATPase activity may therefore be secondary to changes in filtration rate and tubular reabsorption rather than to a direct effect of the hormone upon cellular production or degradation of the enzyme. The impression gained from all these experiments is that Na-K-ATPase changes in an adaptive way when renal reabsorption of sodium is chronically increased or diminished. The data therefore support the hypothesis that this enzyme system is closely involved in the process by which sodium is actively transported across the renal tubule.

Na-K-ATPase was detected in highest concentrations in "microsomes" prepared from fragmented cells, and it was here that changes in enzymatic activity, when found, were most marked. The localization of Na-K-ATPase activity in these small cell fragments is not easily correlated a priori with cation transport since there is no direct evidence that the endoplasmic reticulum participates in the active movement of cations in the same way as the plasma membrane of the cell does. This dilemma has been clarified by the elegant

[2] Gallagher, B. G., and G. H. Glaser. Seizure threshold, adrenalectomy, and sodium-potassium-stimulated ATPase in rat brain. Submitted for publication.

experiments of Wallach and his coworkers on the Na-K-ATPase of Ehrlich ascites tumor cells (21, 22). Microsomes prepared from these cells contain substantial amounts of Na-K-ATPase. On density gradient separation, this enzymatic activity is found to coincide with the peak of distribution of surface-cell antigens, as determined by an immunological technique using intact cells. The conclusion drawn by these investigators is that small fragments of plasma membrane sediment, are harvested with the "microsomes," and provide the bulk of the Na-K-ATPase activity in the "microsomal" fraction of cell homogenates. The "microsomes" prepared in the present experiments probably represent a mixture of cell membrane fragments, some of which contain enzyme and some of which do not. It is therefore not possible to be entirely certain whether changes noted in the specific activity of Na-K-ATPase reflected the specific activity of purified plasma membranes or a change in the proportion of enzyme-containing membrane to nonenzyme-containing material.

The role of sodium-potassium–activated adenosine triphosphatase in supplying energy from ATP for transfers of Na^+ and K^+ across cell membranes has in the past been supported chiefly by experiments with human red blood cells and red cell ghosts (23–25). In these cells, active transport of Na^+ and K^+ in opposite directions across the plasma membrane are linked stoichiometrically to each other and to the hydrolysis of ATP (26). A logical role for Na-K-ATPase in the bulk net transport of sodium across epithelial membranes, however, poses special difficulties. Organs like the toad bladder, the teleost gill, and the kidney transport sodium ions in one direction across a sheet of cells but do not transport potassium ions actively (at least in large amounts) in the other direction. In this respect they are not analogous to the red cell membrane. It is therefore especially interesting that active transport from mucosa to serosa by the toad bladder ceases when K^+ is omitted from the solution bathing the serosal surface (27), that ouabain inhibits Na^+ transport when applied to the serosa (28), and that the inhibitory action of ouabain, as with red cells and red cell ATPase, is mitigated by increasing the concentration of K^+ in the serosal medium (29). In the kidney, the fact that potassium is not actively transported into the tubular lumen (30) is not a crucial objection to the participation of a membrane pump analogous to that of the red cell. The sodium pump is thought to be located on the basal surface of the renal tubular cell (31). As Na^+ is pumped out, K^+ might be reciprocally transferred into the cell, only to leak out again through the basilar membrane, which is known to be many times more permeable to K^+ than the luminal surface of the tubular cell (32). That sodium reabsorption from tubular urine is to some degree dependent upon the presence of K^+ at the basilar surface of tubular cells is suggested by the fact that perfusion of amphibian kidneys with potassium-free solutions greatly reduces sodium absorption while increasing the excretion of sodium in the urine (33).

An intriguing group of facts relating ATPase to transport in the kidney deals with the efficacy of ATPase inhibitors as diuretics. Ouabain injected into the tubular lumen reduces proximal tubular absorption in stopped flow perfusions of single nephrons in Necturus (34). Ouabain also inhibits net sodium reabsorption when infused into the renal portal vein of the frog (33) and the chicken (35) and when injected into the renal artery of the dog (36). Three powerful diuretic drugs are also potent inhibitors of Na-K-ATPase, both in vivo and in vitro: mercurials, ethacrynic acid, and furosemide (3, 37–41). Furthermore, ATPase is inhibited in the kidney in vivo at concentrations of the drugs roughly similar to those producing diuresis. A clear connection between their diuretic properties and their ability to inhibit ATPase, however, is blurred by several considerations. Mercury in the proper concentration inhibits many enzymes, probably because of its affinity for —SH groups. Moreover, nondiuretic mercurial compounds also inhibit Na-K-ATPase (38, 42). Ethacrynic acid inhibits Na-K-ATPase in rat kidneys, yet in this species it does not increase sodium excretion (40). Analogues of ethacrynic acid may be prepared which do not inhibit ATPase, yet are diuretic.[3] Many other diuretic compounds which inhibit reabsorption of Na^+ by the kidney have no effect upon ATPase in the test tube (37–39).

The intimate nature of the connection between

[3] Welt, L. G. Personal communication.

Na-K-ATPase and sodium transport is not clear from the present experiments. The conclusion that there is a connection rests entirely upon the parallelism observed between the reabsorptive work of the kidney and the specific activity of the enzyme system in kidney microsomes. It should be emphasized that the correlation between enzymatic activity and ion transport does not imply an identity between the Na-K-ATPase and the ion pump. The magnitude of the changes observed was limited by the feasibility of inducing large alterations in filtration rate per gram of kidney tissue. The largest increment in this parameter was seen after injection of methylprednisolone and amounted to about 50%. In certain other organs which transport sodium actively, the physiological range of activity is wider. The gill of the euryhaline teleost, *Fundulus heteroclitus,* for example, increases its active transport of sodium by many orders of magnitude when the fish is transferred from fresh to salt water (43). Under these circumstances it has recently been shown that the Na-K-ATPase content of the gill is multiplied several times (44). The induction of Na-K-ATPase activity when sodium transport is greatly increased may therefore be a general biological phenomenon. If this proves to be correct, it may help to establish an important link between the biochemical properties of cell fragments and the active movement of ions across epithelial membranes.

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