Effect of Adrenal Steroids on a Na\(^+\) - and K\(^+\)-requiring Adenosine Triphosphatase from Rat Kidney

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COLIN F. CHIGNELL AND ELWOOD TITUS

From the Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Bethesda, Maryland 20014

SUMMARY

In the rat, kidney adenosine triphosphatase activity levels in the presence of sodium and potassium ((Na\(^+\) + K\(^+\))-ATPase) fall slowly after adrenalectomy, reaching a minimum on the 6th or 7th postoperative day. Kinetic data from kidney microsomal preparations indicate that this result from a decrease in enzyme levels rather than an alteration in the $K_v$ values for ATP, sodium, or potassium.

The administration of aldosterone (10 $\mu$g) to adrenalectomized rats produced no detectable changes in kidney (Na\(^+\) + K\(^+\))-ATPase levels after 3 hours, even though maximal sodium retention may be observed at that time. However, corticosterone, when given in doses equivalent to the adrenal output, brought the kidney (Na\(^+\) + K\(^+\))-ATPase levels of adrenalectomized rats back to normal in 2 to 3 days.

The transport of sodium and potassium ions across cell membranes against an electrochemical gradient is an energy-dependent active process. In the squid axon, energy for active cation transport can be supplied by adenosine triphosphatase or one of its precursors, e.g. phosphoenolpyruvate or arginine phosphate (1). The transport of sodium and potassium ions across the red cell membrane is also dependent upon ATP as an energy source (2, 3). It therefore seems reasonable that cell membranes contain a system capable of converting chemical energy into a form in which it can be used for cation transport. When cellular organization is destroyed, this system is thought to manifest itself as a Mg\(^{2+}\)-dependent adenosine triphosphatase (ATPase, EC 3.6.1.4) which can be stimulated by the simultaneous presence of sodium and potassium ions. The existence of (Na\(^+\) + K\(^+\))-ATPase was first demonstrated by Skou (4) in a microsomal fraction from crab peripheral nerve, and it has subsequently been found in mammalian tissues, particularly those with nervous or secretory functions (5). Well documented evidence now exists to support Skou's original hypothesis that (Na\(^+\) + K\(^+\))-ATPase could provide an enzymatic basis for the active transport of cations across cell membranes (6, 7).

In the kidneys of warm blooded animals active transport apparently carries sodium ions from the glomerular filtrate back into the circulation, since perfusion of the organ with cardio glycosides causes loss of sodium (8, 9). In the normal rat essentially all of the sodium in glomerular filtrates is reabsorbed, and after adrenalectomy about 98% (10). Because of the high filtration rates this 2% decrement in reabsorption is large enough to account for the increased sodium excretion observed after adrenalectomy. Restoration of the deficient reabsorption in the distal tubule appears to be the mechanism by which aldosterone exerts its antinatriuretic effect in the adrenalectomized animal (11). This effect can be abolished by pretreating the animal with actinomycin D (12). Edelman, Bogoroch, and Porter (13, 14) have convincingly demonstrated that in the toad bladder a ribonucleic acid-mediated synthesis of protein precedes the increase of sodium transport caused by aldosterone. Further complications are introduced by the observations of Lockett and Roberts (15) that growth hormone is required for the full expression of the aldosterone effect. In any case it would appear that the steroid is stimulating the synthesis of an enzyme system involved in the active transport of sodium. If (Na\(^+\) + K\(^+\))-ATPase is the enzyme involved, it might be expected that changes in its level would be either so small or so localized anatomically that detection of these changes would be difficult. In view of these observations it is somewhat surprising that Landon and Forte (16) observed a very marked decrease in the (Na\(^+\) + K\(^+\))-ATPase activities of kidney microsomes from rats which had been adrenalectomized or treated with aldactone, an aldosterone antagonist. Since so little is known of the effects of steroids on this enzyme, it seemed advisable to examine the levels of the kidney ATPase after the administration of various adrenocortical steroids before embarking on further studies of the hormonal control of transport. A preliminary report of this work has already appeared (17).

EXPERIMENTAL PROCEDURE

Corticosterone, d-aldosterone, and hydrocortisone were obtained from Calbiochem. Growth hormone (NIH-GH-S-3 ovine, 1 U.S.P. unit per mg) was kindly supplied by Dr. Peter Condliffe. Terminally labeled ATP (specific activity, 5 to 10 mC per $\mu$ mole) was prepared by the method of Pfleiderer (18) as modified by Gibbs, Roddy, and Titus (19) and was diluted with
carrier ATP obtained from Sigma to give a specific activity of 10 to 20 μC per mmole. All other chemicals were obtained from commercial sources and were of reagent grade. Both normal and adrenalectomized rats (male, Sprague-Dawley) were purchased from Hormone Assay Laboratories and were fed Purina rat chow. Adrenalectomized animals were allowed a 5% glucose-1% sodium chloride solution ad libitum. In preliminary experiments several groups of adrenalectomized rats were maintained on tap water for 24 to 48 hours before removal of the kidneys. Since levels of kidney ATPase were not different from those of animals receiving NaCl, it seemed unnecessary to maintain precise control of salt intake. Single doses of aldosterone (10 μg) were administered intravenously in 0.2 ml of 0.9% NaCl solution; controls received NaCl only. Growth hormone (100 μg) was given intramuscularly in 0.2 ml of 0.9% NaCl adjusted to pH 9.0. In the chronic experiments aldosterone (5 and 10 μg), corticosterone (1 and 2 μg) and hydrocortisone (0.08 and 0.15 mg) were given intramuscularly in sesame oil (0.1 ml). Controls received sesame oil (0.1 ml) only.

The rats were sacrificed by a blow on the head, and the kidneys were removed and dissected free of adipose and connective tissue. They were then homogenized for 1 min (1200 rpm) in 0.2 M sucrose-0.005 M EDTA-0.1% sodium deoxycholate, pH 6.8, with the use of an all-glass Tenbroeck homogenizer (0.004- to 0.006-inch clearance), then further diluted with the same buffer. Such preparations hydrolyzed ATP at the rate of 3 to 4 μmoles per mg of kidney tissue (20 to 30 μmoles per mg of protein) when assayed in the presence of 6 mM MgCl₂, 20 mM KCl, 100 mM NaCl, 2 μM ATP, and 0.1 μM imidazole-70% KOH. Approximate half of this activity was sodium and potassium sensitive.

Assay of Tissue ATPase—Incubation tubes contained 1 mg of kidney tissue (or 0.1 mg of microsomal protein) in a final volume of 1.0 ml and were shaken in a Dubnoff bath at 37° for 4 min. The reaction was started by the addition of enzyme to the tubes and stopped by the addition of 0.5 ml of cold phosphate reagent (20). The latter was prepared fresh for each experiment by adding 2 parts of 10% ammonium molybdate to 1 part of 0.1 M H₂SO₄ and 1 part of 0.1 M hydroxylmung acid. The tubes were immediately immersed in ice to prevent further hydrolysis of the substrate. Incubated blanks were prepared by adding the enzyme immediately after the acid reagent. Aliquots of the ATPase (specific activity 10 to 20 μC per mmole) solution were hydrolyzed completely by heating with 6 N HCl in a boiling water bath for 30 min, then cooled, and phosphate reagent was added. This enabled calculation of the absolute amount of ATP hydrolyzed in each tube. All solutions were then shaken vigorously with 3.0 ml of isobutyl alcohol for 20 sec and then centrifuged briefly. Aliquots (1.0 ml) of the organic phase were counted with 5 ml of phosphor solution. The latter was prepared by mixing 3 parts of ethanol with 7 parts of a toluene solution containing 5% 2,5-diphenyloxazole and 0.005% β-bis-[2-(phenylxazolyl)]benzene.

Kidney Microsomal Fraction—The whole kidney homogenates were centrifuged at 37,000 × g for 40 min. The supernatant was then centrifuged at 100,000 × g for 120 min. The pellet was resuspended in half the original volume of histidine buffer (0.08 M histidine-0.25 M sucrose-0.001 M EDTA, pH 6.8) and stored at -20°. This procedure has been found to produce a preparation of endoplasmic reticulum free of intact mitochondria and large mitochondrial fragments (21). Fresh microsomal preparations hydrolyzed ATP at the rate of 60 to 70 μmoles per hour per mg of protein; half of this activity was sodium and potassium sensitive. Approximately 30% of the (Na⁺ + K⁺)-ATPase activity present in the whole homogenates was recovered in the microsomal fraction.

Protein Determinations—These were carried out by the method of Lowry, Rosebrough, Farr, and Randall (22).

Kidney ATP Determinations—Five adrenalectomized rats (120 to 140 g, 7th postoperative day) were injected intravenously at zero time with 10 μg of aldosterone dissolved in NaCl (0.2 ml). Controls received NaCl (0.2 ml) only. After 3 hours the rats were sacrificed by a blow on the head, and the kidneys were removed quickly into liquid nitrogen. This technique was found to produce kidney ATP levels not significantly different from those obtained in rat kidneys which had been completely immersed in liquid nitrogen.

The frozen kidneys were dissected free from adhering adipose and connective tissue and weighed quickly on a torsion balance. Each kidney was pulverized in a tissue disintegrator (23) in the presence of 1 ml of 0.3 M perchloric acid, and the mixture was allowed to thaw and was then treated with a further 4 ml of cold 0.3 M perchloric acid. After standing at 0° for 15 min, the extract was washed into a homogenizer with 10 ml of cold distilled water, briefly homogenized, and then centrifuged for 10 min at 3000 × g. The supernatant was carefully decanted into a chilled beaker containing 10 ml of cold 0.2 M glycylglycine buffer (adjusted to pH 8.0 with 1.0 N KOH) and the pH was adjusted to 7.5 with 1.0 N KOH. After centrifugation of the precipitated KClO₄, the extract was quantitatively transferred to a chilled 50-ml volumetric flask and made up to volume with distilled water. Aliquots of this neutralized extract were assayed for ATP by the luciferin-luciferase method (24).

RESULTS

The unique cation requirements of (Na⁺ + K⁺)-ATPase make it possible to estimate the total amount of this enzyme in rat kidneys by assaying the activity of whole homogenates. Fig. 1 shows the effects of cations and ouabain on the crude rat kidney preparation used in this investigation. A high concentration of ouabain (1 μM) was used because the rat enzyme is very insensitive to this glycoside. In contrast to the rat kidney microsomal preparation of Landon and Norris (21), dialysis was not necessary for the demonstration of (Na⁺ + K⁺)-ATPase activity. This was undoubtedly due to the inclusion of deoxycholate in the homogenising medium. Järncfält (26) has reported that this detergent removes bound sodium and potassium ions from rat brain microsomal preparations which then become sensitive to the addition of exogenous cations.

Both the kidney microsomes (Fig. 2) and the whole homogenates release inorganic phosphate from ATP in a linear fashion with respect to enzyme concentration up to 8 min of incubation provided that not more than 20% of the substrate is hydrolyzed. The ATPase activities of the crude homogenates are reported in micromoles of P1 formed per hour per mg of wet kidney weight. This method of expressing the results is valid since neither adrenalectomy nor the administration of steroids appears to alter the degree of kidney hydration (17, 26). This was confirmed by measurement of the protein content of the homogenates by the method of Lowry et al. (22). In a typical experiment,
C. F. Chignell and E. Titus

![Figure 1](image-url)

**Fig. 1.** The effect of ouabain and cations on the ATPase activities of rat kidney homogenates. Tubes contained 0.1 M imidazole, pH 7.2, and 2 mM ATP. MgCl₂ was 0 mM, NaCl, 100 mM; KCl, 20 mM; and ouabain, 1 mM. The results are the mean values of preparations from five animals with the standard error represented by a horizontal bar.

(Experiment 6, Table II), kidney homogenates from the control adrenalectomized rats contained 0.77 ± 0.03 mg of protein per ml. Kidney homogenates from the rats receiving hydrocortisone (0.08 mg twice daily for 5 days) contained 0.75 ± 0.02 mg of protein per ml, whereas those receiving aldosterone (5 µg twice daily for 5 days) contained 0.76 ± 0.02 mg of protein per ml.

The whole homogenates were found to lose Mg⁺⁺-ATPase activity slowly on standing at 0°C, while the (Na⁺ + K⁺)-ATPase levels remained unchanged (Fig. 3). This decay of Mg⁺⁺-ATPase has been observed by other workers (27) and may account for the different levels of this enzyme observed in experiments carried out on different days (see Table I). Within one experiment, however, the Mg⁺⁺-ATPase levels were found to be constant, thus providing a steady base line from which to measure (Na⁺ + K⁺)-ATPase activity. All measurements of (Na⁺ + K⁺)-ATPase activities were made within 2 hours of the preparation of the homogenate.

Adrenalectomy caused a 40 to 50% reduction in the (Na⁺ + K⁺)-ATPase activities of rat kidney homogenates without significantly affecting the Mg⁺⁺-ATPase levels (Experiments 1 and 4, Table I). Landon and Forte (16) have also found lower (Na⁺ + K⁺)-ATPase levels in dialyzed kidney microsomal preparations from adrenalectomized rats. As may be seen from Fig. 4, the fall of kidney (Na⁺ + K⁺)-ATPase levels after adrenalectomy is very slow in weanling rats, reaching the previously observed level (Experiment 4, Table I) on the 7th postoperative day. Kidney (Na⁺ + K⁺)-ATPase levels in older rats (120 to 140 g) also fall slowly after adrenalectomy, reaching a minimum on the 6th or 7th postoperative day. Other experiments (17) have shown that the fall in (Na⁺ + K⁺)-ATPase activity occurs to the same extent in both the cortex and the medulla. Microsomal preparations from the kidneys of normal and adrenalectomized rats reflect the same differences observed in the whole homogenates (Fig. 2).

Although maximal sodium retention may be observed 3 hours after the administration of aldosterone (10 µg intravenously) to an adrenalectomized rat (28), no change in (Na⁺ + K⁺)-ATPase levels could be detected at that time (Table I). Growth hormone (100 µg intramuscularly), which has been reported to be necessary for the complete restoration of sodium reabsorption in rate (15), did not alter (Na⁺ + K⁺)-ATPase levels when given alone or in combination with aldosterone (10 µg intravenously). Increased doses of aldosterone up to 100 µg per rat were also without effect (17). Epinephrine (10 µg subcutaneously)...
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**TABLE I**

Effect of adrenalectomy and acute administration of steroids on rat kidney (Na\(^+\) + K\(^+\))-ATPase

Aliquots of the whole kidney homogenates (containing 1 mg of kidney, wet weight) were incubated at 37° for 4 min with 2 mM ATP and 0.1 M imidazole-HCl, pH 7.2. MgCl\(_2\) was 6 mM; KCl, 20 mM; and NaCl, 100 mM. The (Na\(^+\) + K\(^+\))-ATPase activity was calculated by subtracting the enzyme activity obtained in the presence of MgCl\(_2\) alone (Mg\(^++\)-ATPase) from that obtained with MgCl\(_2\), NaCl, and KCl. Numbers in parentheses indicate the days after adrenalectomy. Results are expressed as the mean from five animals followed by the standard error. Rats received the dose of drug indicated 3 hours before sacrifice.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mg(^++)-ATPase</th>
<th>(Na(^+) + K(^+))-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Adrenalectomized (7)*</td>
<td>1.53 ± 0.22</td>
</tr>
<tr>
<td>Normal*</td>
<td>1.98 ± 0.12</td>
<td>2.37 ± 0.10</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Adrenalectomized (11)*</td>
<td>2.05 ± 0.04</td>
</tr>
<tr>
<td>Adrenalectomized (11), aldosterone, 10 µg, i.v.</td>
<td>2.00 ± 0.04</td>
<td>1.04 ± 0.04*</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>Adrenalectomized (6)*</td>
<td>1.35 ± 0.05</td>
</tr>
<tr>
<td>Adrenalectomized (6), aldosterone, 10 µg, i.v. + growth hormone, i.m.</td>
<td>1.20 ± 0.00</td>
<td>1.28 ± 0.04*</td>
</tr>
<tr>
<td>Adrenalectomized (6), growth hormone, i.m.</td>
<td>1.42 ± 0.08</td>
<td>1.07 ± 0.07*</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>Adrenalectomized (9)</td>
<td>1.77 ± 0.09</td>
</tr>
<tr>
<td>Normal*</td>
<td>1.95 ± 0.06</td>
<td>2.49 ± 0.05</td>
</tr>
</tbody>
</table>

* Weight, 120 to 140 g.

b \(p < 0.001\).

c \(p > 0.05\).

d \(p < 0.01\).

e Weight, 50 to 70 g (weanlings).

o Weight, 120 to 140 g.

Fig. 1. The effect of adrenalectomy on rat kidney ATPase levels. Weanling rats were adrenalectomized on day zero and then sacrificed on the days indicated. The ATPase activities of the whole kidney homogenates were estimated with ATP, MgCl\(_2\), NaCl, KCl, and buffer concentrations as in Fig. 1. Each point is the mean from five animals with the standard error represented as a vertical line. ○, Mg\(^++\)-ATPase; ●, (Na\(^+\) + K\(^+\))-ATPase.

**TABLE II**

Effect of chronic administration of steroids on rat kidney (Na\(^+\) + K\(^+\))-ATPase

Incubation conditions were the same as in Table I. Rats received the dose of drug indicated intramuscularly twice daily for 5 days. Activity was measured on the 5th day. Numbers in parentheses indicate the days after adrenalectomy at the beginning of the experiment. Results are expressed as the mean from five rats followed by the standard error.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Mg(^++)-ATPase</th>
<th>(Na(^+) + K(^+))-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Adrenalectomized (14)*</td>
<td>2.20 ± 0.04</td>
</tr>
<tr>
<td>Adrenalectomized (14), corticosterone, 2.0 mg</td>
<td>2.20 ± 0.04</td>
<td>2.27 ± 0.10*</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Adrenalectomized (12)*</td>
<td>1.59 ± 0.06</td>
</tr>
<tr>
<td>Adrenalectomized (12), hydrocortisone, 0.15 mg</td>
<td>1.77 ± 0.04</td>
<td>1.18 ± 0.04*</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>Adrenalectomized (15)*</td>
<td>2.15 ± 0.08</td>
</tr>
<tr>
<td>Adrenalectomized (15), aldosterone, 10 µg</td>
<td>2.15 ± 0.03</td>
<td>1.60 ± 0.08*</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>Adrenalectomized (5)*</td>
<td>1.35 ± 0.03</td>
</tr>
<tr>
<td>Adrenalectomized (5), aldosterone, 100 µg</td>
<td>1.35 ± 0.04</td>
<td>1.05 ± 0.08*</td>
</tr>
<tr>
<td>Experiment 5</td>
<td>Adrenalectomized (7)*</td>
<td>1.61 ± 0.05</td>
</tr>
<tr>
<td>Adrenalectomized (7), hydrocortisone, 0.08 mg</td>
<td>1.64 ± 0.08</td>
<td>2.30 ± 0.05*</td>
</tr>
<tr>
<td>Adrenalectomized (7), corticosterone, 1.0 mg</td>
<td>1.55 ± 0.10</td>
<td>3.11 ± 0.05*</td>
</tr>
<tr>
<td>Experiment 6</td>
<td>Adrenalectomized (7)*</td>
<td>2.04 ± 0.16</td>
</tr>
<tr>
<td>Adrenalectomized (7), aldosterone, 5 µg</td>
<td>2.06 ± 0.09</td>
<td>1.98 ± 0.10*</td>
</tr>
<tr>
<td>Adrenalectomized (7), hydrocortisone, 0.08 mg</td>
<td>2.29 ± 0.08</td>
<td>2.95 ± 0.10*</td>
</tr>
</tbody>
</table>

* Weight, 120 to 140 g.

b \(p < 0.001\).

c \(p > 0.05\).

d \(p < 0.01\).

e Weight, 50 to 70 g (weanlings).
Aldosterone was given twice daily in a dose (10 μg) equivalent to four times the reported daily adrenal output (32). The results from these experiments may be found in Table II. It may be seen that corticosterone brought the (Na⁺ + K⁺)-ATPase levels back to normal in the older rats (120 to 140 g) while hydrocortisone was without effect. Aldosterone significantly increased kidney (Na⁺ + K⁺)-ATPase levels at both doses tested but in neither case were normal levels attained.

In the weanling adrenalectomized rats, corticosterone also significantly increased (Na⁺ + K⁺)-ATPase levels (Table II). The effect of hydrocortisone in weanling rats is not clear since in one experiment (Na⁺ + K⁺)-ATPase levels were increased (Experiment 6, Table II) while no significant change could be demonstrated in other experiments (Experiment 5, Table II). Aldosterone had no effect at the dose level tested. It will be noted that in these experiments the (Na⁺ + K⁺)-ATPase levels in the control adrenalectomized weanling rats (Table II and Fig. 5A) were slightly higher than those observed previously (Table I and Fig. 4). This discrepancy could not be accounted for and may represent the variation between different batches of animals.

It may be seen from Fig. 5B that in the older adrenalectomized rats (120 to 140 g) the response of kidney (Na⁺ + K⁺)-ATPase to exogenous corticosterone is not apparent until the 2nd day.

![Fig. 5. The effect of corticosterone on rat kidney ATPase levels. The weanling rats (A) were given 1 mg of corticosterone intramuscularly twice daily, while the older rats (B) received 2 mg twice daily. Injections were commenced on day zero, and the rats were sacrificed on the days indicated. All animals were 7 days postoperative at the commencement of the experiment. ATPase activities of the whole kidney homogenates were estimated with ATP, MgCl₂, NaCl, KCl, and buffer concentrations as in Fig. 1. Each point is the mean from five animals with the standard error represented as a vertical line. O, (Na⁺ + K⁺)-ATPase; ●, Mg⁺⁺-ATPase.](http://www.jbc.org/)

![Fig. 6. Rate of Pᵢ release as a function of ATP concentration plotted according to the method of Lineweaver and Burk. MgCl₂, NaCl, KCl, and buffer concentrations were as in Fig. 1. A, microsomes from adrenalectomized weanling rats (sacrificed on the 7th postoperative day). B, microsomes from normal weanling rats. Each tube contained 0.018 mg of microsomal protein. The lines of best fit were calculated by the method of least squares. O, (Na⁺ + K⁺)-ATPase; ●, Mg⁺⁺-ATPase.](http://www.jbc.org/)

Normal kidney (Na⁺ + K⁺)-ATPase levels were observed on the 3rd day. The response of weanling adrenalectomized rats to corticosterone was slightly faster, reaching a maximum on the 2nd day (Fig. 5A).

To ascertain the effect of adrenalectomy on the kinetic behavior of rat kidney (Na⁺ + K⁺)-ATPase, the \( K_m \) values for ATP, sodium, and potassium were measured. These determinations were made on microsomal preparations rather than whole homogenates since the latter showed pronounced changes in activity on freezing and thawing. The microsomes, on the other hand, were stable for several months when stored at -20°. Kinetic data for kidney microsomes (Na⁺ + K⁺)-ATPase from normal and adrenalectomized rats are presented in Figs. 6 and 7 and summarized in Table III. The linearity of the Lineweaver-Burk plot for ATP (Fig. 6) suggests that the interaction of this substrate with (Na⁺ + K⁺)-ATPase can adequately be described by simple Michaelis-Menten kinetics. Adrenalectomy results in a reduction of the specific activity (\( V_{max} \)) of the preparation while the \( K_m \) for ATP is not significantly altered (Table III). The \( Mg^{++} \)-ATPase is not influenced by adrenalectomy (Table III). It is of interest to note that for \( Mg^{++} \)-ATPase the \( K_m \) of ATP is 3 to 4 times lower than for (Na⁺ + K⁺)-ATPase. This is in contrast to previous reports for beef and guinea pig brain microsomal preparations (19, 33).

Lineweaver-Burk plots for Na⁺ and K⁺ showed a pronounced upward bending, indicating a deviation from simple Michaelis-Menten kinetics. Reliable estimates of the \( K_m \) value for these...
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The data for ATP were calculated from the Lineweaver-Burk plots (Fig. 7) while the Na+ and K+ data were taken from the corresponding Hill plots (Fig. 8). Values for Vmax expressed as micromoles of Pi formed per hour per mg of protein.

![Diagram](https://example.com/diagram.png)

**Fig. 7. Hill plots for Na+ and K+.** A and C, microsomes from normal weanling rats; B and D, microsomes from adrenalectomized weanling rats (sacrificed on the 7th postoperative day). Each tube contained 0.18 mg of microsomal protein. The lines of best fit were calculated by the method of least squares.

### Table III

Kinetic data for kidney microsomal preparations from normal and adrenalectomized weanling rats

<table>
<thead>
<tr>
<th>Substrate</th>
<th>(Na+ + K+)-ATPase</th>
<th>Mg++-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Adrenalectomized</td>
</tr>
<tr>
<td>ATPa</td>
<td>km (mM)</td>
<td>Vmax (pmol/h/mg)</td>
</tr>
<tr>
<td>Na+</td>
<td>0.29</td>
<td>33.9</td>
</tr>
<tr>
<td>K+</td>
<td>2.0</td>
<td>46.2</td>
</tr>
<tr>
<td>ATPb</td>
<td>0.07</td>
<td>3.0</td>
</tr>
<tr>
<td>Na+</td>
<td>22.7</td>
<td>48.4</td>
</tr>
<tr>
<td>K+</td>
<td>2.0</td>
<td>46.2</td>
</tr>
</tbody>
</table>

a Kidney microsomal Preparation 1.
b Kidney microsomal Preparation 2.
c Apparent Km ("K") values.

two cations were not possible from these plots although the corresponding Vmax values could be determined with reasonable accuracy. With these values it was possible to determine the apparent Km value ("K") for Na+ and K+ by making use of the Hill equation (34).

\[
\log \frac{V_m}{V - 1} = \log "K" - (n) \log s
\]

When \( V_m/V - 1 \) is plotted against \( \log s \), a straight line is obtained from which it is possible to estimate the apparent Km value ("K"). It has been suggested that when \( n > 1 \) there is cooperative interaction between two or more binding sites for the same ligand (35). As may be seen from Fig. 7, the Hill equation adequately describes the interaction of Na+ and K+ with (Na+ + K+)-ATPase. The values obtained for \( n \) are greater than unity (Fig. 7), which is in agreement with the current concept of an allosteric interaction between (Na+ + K+)-ATPase and Na+ and K+. Similar results have been reported by Squires (36) for a rat brain microsomal preparation. If it may be assumed that the apparent Km values for Na+ and K+ adequately describe the interaction of these cations with (Na+ + K+)-ATPase, then adrenalectomy appears to produce no change (Table III).

No increase in kidney ATP levels could be detected in adrenalectomized rats 3 hours after the administration of aldosterone (10 μg, i.v.). In a typical experiment the kidneys from five treated rats contained 1.09 ± 0.06 μmoles of ATP per g of frozen tissue while kidneys from five controls contained 1.08 ± 0.09 μmoles of ATP per g.

**DISCUSSION**

The (Na+ + K+)-ATPase activity of rat kidney homogenates is significantly lowered by adrenalectomy (Table I). Kinetic data obtained from microsomal preparations seem to indicate that this is the result of a decrease in enzyme levels rather than an alteration in the Km values for ATP, sodium, or potassium. In the rat, kidney (Na+ + K+)-ATPase levels fall slowly after adrenalectomy, reaching a minimum on the 6th or 7th postoperative day. Thus, it would appear that the enzyme may be turning over very slowly. It is also possible that the messenger RNA concerned with synthesis de novo is very long lived.

The administration of aldosterone (10 μg) to adrenalectomized rats produced no change in kidney (Na+ + K+)-ATPase levels after 3 hours even though maximal sodium retention may be observed at that time (28). If aldosterone exerts its antinatriuretic effect by control of (Na+ + K+)-ATPase, the induced changes must be very small or very highly localized. Experiments with the toad bladder (37) have suggested that aldosterone may control the supply of metabolic energy to the cation-transporting mechanism. Figmonari, Kasbekar, and Edelman (38), for example, have reported that if the toad bladder is incubated with aldosterone (7 x 10^-8 M), the level of ATP is increased by 30%. In the mouse ascites tumor cell, it has been shown that active transport is linearly related to the intracellular level of ATP (39). Since Feldman, Wende, and Kessler (40) have shown that aldosterone (100 μg intraperitoneally) increases the levels of cytochrome oxidase and succinic dehydrogenase in rat kidney, it seemed possible that this steroid could control active transport by altering the level of ATP in the tubule cells. No increase in kidney ATP levels was observed, however, after the administration of aldosterone (10 μg intravenously) to adrenalectomized rats. It should be pointed out, however, that the doses used by Feldman et al. were high enough for the glucocorticoid effects of aldosterone to be seen.

The data provided here do indicate that, in the rat, corticosterone, and not aldosterone, is responsible for maintaining kidney (Na+ + K+)-ATPase levels. The physiological significance of this finding is not at present clear, although it is known that corticosterone is the main steroid secreted by the rat adrenal (31). It would be of interest, therefore, to investigate the role of corticosterone in other species, e.g. hamster, dog, where hydrocortisone is the main component of adrenal secretion.

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REFERENCES
1. Dunham, E. T., Physiologist, 1, 23 (1957).
Effect of Adrenal Steroids on a Na\(^+\)- and K\(^+\)-requiring Adenosine Triphosphatase from Rat Kidney
Colin F. Chignell and Elwood Titus


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