Title

Relationships between thyroid hormone and glucocorticoids in the regulation of renal $Na^+ + K^+$ -ATPase activity

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RELATIONSHIPS BETWEEN THYROID HORMONE AND GLUCOCORTICOIDS IN THE REGULATION OF RENAL $Na^+ + K^+$-ATPase ACTIVITY

by

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THESIS

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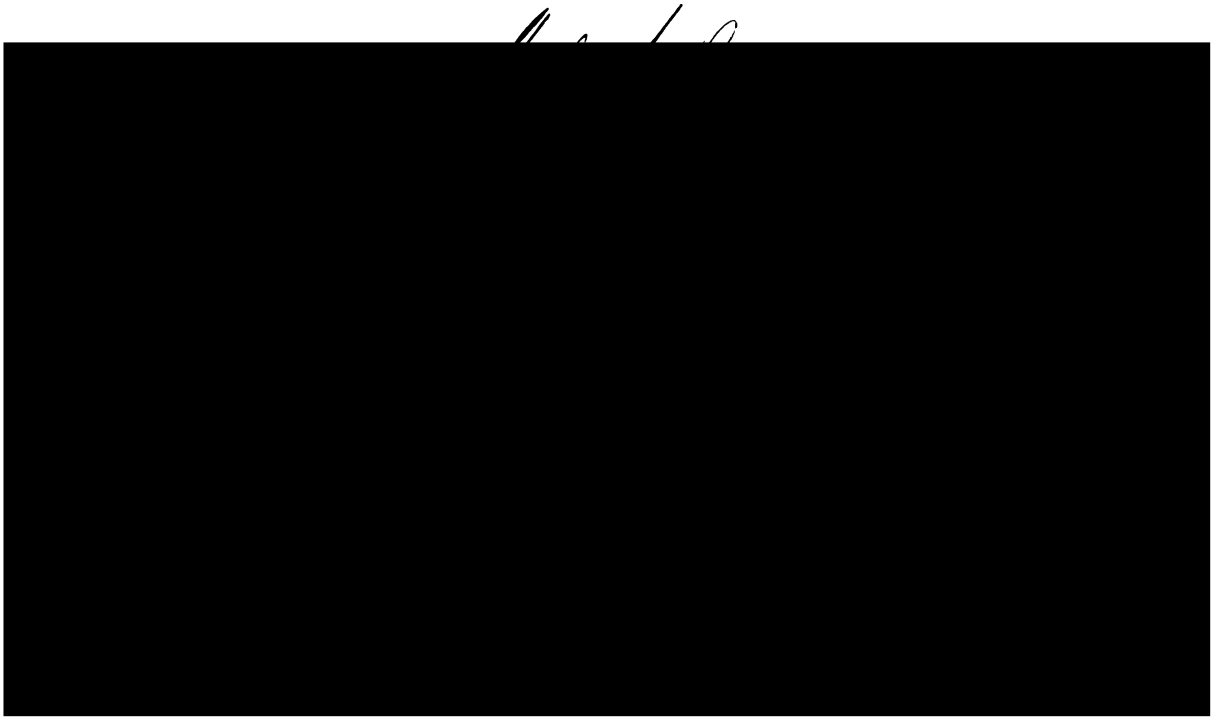

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I would like to thank Ella Highland, Diana Marver, Chu-Shek Lo and Isidore S. Edelman for their continued critical help and concern for me and my work.

Underlying virtually all the physiological activities as well as the development of higher organisms, there is a complex regulatory and integrative network which uses specific compounds as chemical signals emitted by certain cells and interpreted by others. And it is clear even to the layman today that the development, functioning and survival of complex organisms would hardly be conceivable were it not for the existence of these regulatory chemical interactions between cells, tissues and organs.

J. Monod

No man is an island, entire of itself; every man is a piece of the continent, part of the main.....

J. Donne

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INTRODUCTION

Face these cold facts: in order to survive and evolve, you are faced with a sudden need to coordinate cellular processes for maintaining a constant internal environment. The protovertebrates, who left the salt water to invade the fresh water, and the terrestrial forms, who took the first real steps for mankind, to deal with such environmental difficulties as cold air and scarcity of water, encased their tissues in a hairy epithelium and developed an elaborate renal system for filtering body fluids which included an ultimate tubular network for reabsorbing needed nutrients, electrolytes and water. Hardly the most practical of organ systems (it seems energetically wasteful to reabsorb 99% of a filtrate) but the human kidney nevertheless is able to respond promptly to electrolyte and fluid changes in the body.

The sodium and potassium-activated adenosine triphosphotase, (Na+ + K+ ATPase), intimately related to the transport of sodium and potassium across epithelial membranes of numerous tissues (1), is considered to play a role in the mechanism whereby the kidney reabsorbs filtered sodium (2,3,4). Both cortical and medullary renal tissues are rich in Na+ + K+ ATPase activity and the infusion of cardiac glycosides such as ouabain, a known inhibitor of the Na+ + K+ ATPase, into the renal artery of dogs leads to a profound decrease in the capacity to concentrate and dilute the urine, manifested by a decrease in free water clearance. A significant natruresis occurs at the same time that there

is a marked reduction in the activity of cortical and medullary Na+ + K+ ATPase (5,6).

Surgical removal of various hormone producing glands and subsequent replacement of depleted hormones can influence Na+ + K+ ATPase activity and sodium reabsorption in the kidney. Adrenalectomy produces a 46% decrease in Na+ + K+ ATPase activity (7-10) and a 2% decrease in sodium reabsorption (11). Because of the high filtration rate this 2% decrement in reabsorption is large enough to account for the increased sodium excretion observed after adrenalectomy. The injection of glucocorticoids (7,8,12,13) and mineralocorticoids (8,14-16) raises Na+ + K+ ATPase activity and aldosterone is effective in preventing the sodium wasting following adrenalectomy (17).

Thyroidectomy also produces significant decreases in Na+ + K+ ATPase activity (18,19) and influences renal hemodynamics and salt and water excretion. Hypothyroid rats drink more water and excrete more urine (20), than normals and have a decreased urinary concentrating ability (21), waste sodium and die when fed a sodium free diet (22). These facts have led numerous investigators to study both the direct action of triiodothyronine (T_3) on Na+ + K+ ATPase activity and the interaction of T_3 with the adrenal steroids. In early experiments, Bogoroch and Timiras (24) postulated that stress depressed thyroid activity. Fortier et al (23) observed that a stress induced ACTH secretion was accompanied by a decrease in TSH secretion. These investigators suggest

that the hypothalamic-pituitary-adrenal axis can be inversly related to the hypothalamic-pituitary-thyroid axis so that the result of thyroidectomy is a rise in thyroid stimulating hormone (TSH) and a subsequent depression of adreno-corticotropic hormone (ACTH) production (23,24). T_3 affects glucocorticoid metabolism by regulating levels of the glucocorticoid transport protein, CBG, (23,25) and conversly, increases in the levels of glucocorticoids increase T_3 production (26). Furthermore, both hormones utilize the RNA synthetic machinery necessary for enzyme production which is responsible for many of their anabolic and catabolic expressions(27,28).

It seemed possible then, that T_3 and corticosterone (also referred to as Kendall's compound B, or simply, 'B')might be involved permissively at a production, transport, or cellular level in the regulation of Na+ + K+ ATPase activity in the kidney. Previously, Edmonds, Thompson and Marriot (29) reported that T_3 is necessary (presumably permissive) to observe the increase in electrical potential differences in the rat colon elicited by an intravenous dose of aldosterone. A single dose of T_3 given to a hypothyroid rat 10-16 hours before aldosterone restored the potential difference response to normal.

My study was designed to compare the relative effectiveness of corticosterone, the most abundant glucocorticoid in the rat, on renal Na+ + K+ ATPase activity in the presence and absence of thyroid hormones and conversly the effective-

ness of T_3 on Na+ + K+ ATPase activity in the presence and absence of the adrenal steroids in the hope of clarifying the mechanism of renal complications encountered following thyroidectomy; complications which resemble the renal failure induced when cardiac glycosides inhibit the Na+ + K+ ATPase activity in the kidney.

MATERIALS AND METHODS

Initially I was interested in performing successful thyroidectomies and adrenalectomies, keeping the animals healthy until depleted of circulating hormone levels, then preparing an enzyme preparation sensitive enough to detect changes in Na+ + K+ ATPase activity. Sprague Dawley Rats, 200-240 gms, were divided into four groups; Group 1 to be thyroidectomized, Group 2 to be adrenalectomized, Group 3 to be thyroadrenalectomized and Group 4 left as normal controls. These animals were fed a Purina rat chow diet and given tap water ad libidum. All rats were housed in a temperature controlled room (72°F) lighted from 6 AM to 6 PM.

Thyroidectomy. On day one, Group 1 and 3 animals were anaesthetized using a 3.6% chloralhydrate solution injected intraperitoneally (1 ml/100 gm body wt). Occasionally the stomach or bladder was injected in which case the animal remains awake and an additional 0.5 ml was injected intraperitoneally. A one inch cut was made along the mid ventral line from the chin to the beginning of the pectoral muscles, and the skin clamped to the side. Fat and connective tissue were tweezed apart revealing the sternohyoid muscle sur-

rounding the trachea and flanked on both sides by the bulbous submaxillary glands. The muscle was cut longitudinally and clamped, revealing the red thyroid tissue and anterior yellow parathyroid gland resting on the trachea like a saddle. The isthmus, tissue connecting the two thyroid lobes, was severed and each lobe was removed individually. Parathyroids remain intact. There are no internal sutures, the sternohyoid muscle anastomoses, and a staple closes the outer skin. The surgery lasts only ten minutes but often there was considerable bleeding from this vascular organ, up to one ml of blood may be lost. The recurrent laryngeal nerve was severed at times leaving the animal with respiratory problems. Damage to the parathyroids leads to hypocalcemic tetany within four days, an excitatory affect of low Ca++ levels on nerve and muscle cells. These animals were easily spotted and were eliminated from the group.

Adrenalectomy. Seven days following thyroidectomy, Group two and Group three animals were adrenalectomized. Group two animals anaesthetized using 3.6% chloralhydrate injected as 1% body weight, and Group three injected as 0.9% body weight. The adrenal glands are well encapsulated and by using a loop tweaser were isolated and excised without leaving tissue to regenerate. If the capsule was crushed during removal, with the possibility of leaving adrenal tissue, the rat was eliminated from the group. Adrenalectomized rats received 0.9% saline in place of tap water. Controls received tap water. The addition of

saline to normal rats (8,30,15) and to adrenalectomized rats (8) produced no difference in Na+ + K+ ATPase activity when compared respectively to normal and adrenalectomized rats receiving tap water.

Tissue Preparation.

The time required for hormone depletion to be evident after surgery was estimated from the oxygen consumption studies performed by Asano (31) on thyroidectomized rats and the Na+ + K+ ATPase studies performed by Titus (8) on adrenalectomized rats. Oxygen consumption is an indication of metabolic activity and is related to thyroid output (32). It has been estimated that from 20 to 45% of the resting QO_2 is devoted to active Na+ extrusion (52). There is a rapid decrease in oxygen consumption during the week after thyroidectomy (31), reflecting in part a decreased Na+ + K+ ATPase activity. Both Chignell and Titus (8) and Jorgensen (7) have shown significant decreases in Na+ + K+ ATPase activity three days following adrenalectomy.

Ten days following thyroidectomy, three days following adrenalectomy, rats were sacrificed by decapitation and bled. Then the kidneys were perfused via the abdominal aorta with 20 ml of ice cold 0.9% saline to clear the kidney of trapped blood which contains significant quantities of protein. The kidneys were removed and dissected free of adipose and connective tissue and placed on a filter paper soaked with 0.9% ice cold saline. Renal cortical (brown) tissue and outer medulla (red) tissue were dissected out. The

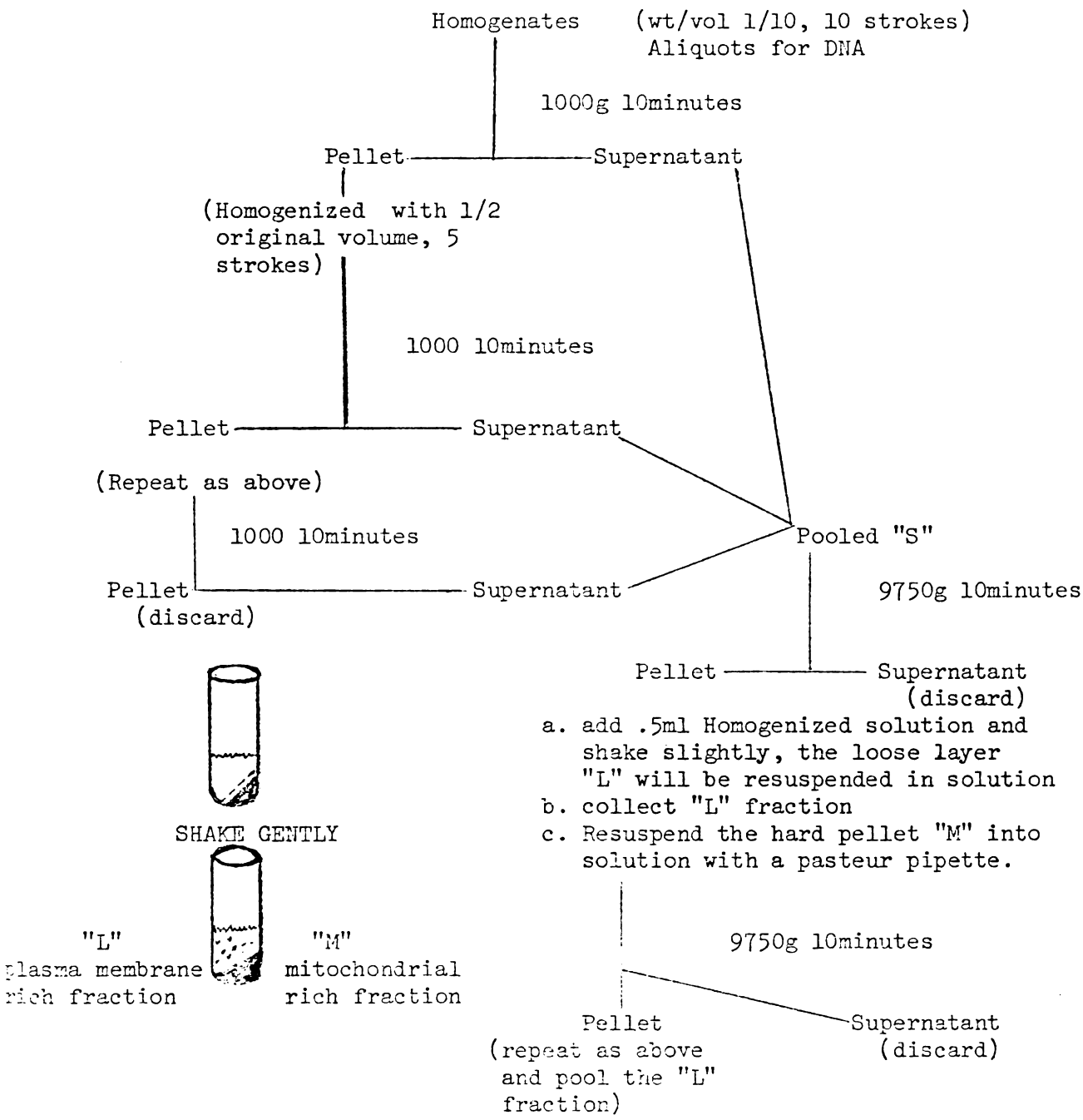
inner medulla (papilla) was previously demonstrated to be unresponsive to T_3 and was discarded (33). The respective zones were pooled from both kidneys and weighed. The tissue was then placed into a homogenizing medium (1 gm wet weight/10 ml medium) and homogenized in a teflon glass potter Elvehjem homogenizer (ca. 10 strokes). The homogenizing medium contained 0.3 molar D-L-Histidine, 0.5 m molar free acid EDTA and 0.25 molar sucrose. Aliquots were taken from the homogenates for DNA determination. The homogenates were then centrifuged at 1000 g for 10 minutes in a Sorval SS-34 rotor. The supernatant "S" fraction crude homogenate was drawn off carefully and stored on ice. The pellet containing nuclei, unbroken cells and connective tissue was resuspended in 0.5 ml of homogenizing solution, homogenized 5 strokes and respun at 1000 g. Supernatants were pooled, and the pellet wash repeated once more. The "S" fraction for crude homogenate studies were pooled and frozen overnight and Na+ + K+ ATPase and Mg++ ATPase activities were assayed the following day.

To obtain a plasma membrane rich fraction, instead of freezing overnight the pooled "S" fraction was centrifuged at 10,000 g for ten minutes. At the end of the centrifugation, the supernatant was discarded. The pellet contained two layers, an upper white cloudy layer ("L" fraction) composed of plasma membrane vesicles and a bottom layer composed of mitochondria. The "L" layer is loose and can be resuspended in 0.5 ml homogenizing solution with gentle shaking. This "L" suspension was collected with a pasteur

pipette and saved. The M layer was resuspended in 1 ml homogenizing solution and centrifuged at 10,000 g, 10 minutes. Another "L" fraction was collected. "L" fractions were pooled and frozen overnight. The entire procedure is diagrammed in Fig. 1.

Tissue Assay. The crude homogenate was left at room temperature to defrost and samples for protein determination were collected before diluting the "S" sample 1 to 3 with homogenizing solution. 0.05 ml of this enzyme solution was added to incubating tubes resting in a water bath at 38°C. The incubating tubes contained in a total volume of 1 ml, either (1) 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 1 mM EDTA, (2) the above with 3 mM Ouabain, (3) 3 mM $MgCl_2$, 1 mM EDTA. The hydrolysis was started with the addition of 0.1 ml of 3 mM ATP (tris buffered) and allowed to run 15 minutes at 38°C then stopped by the addition of ice cold 30% trichloroacetic acid and placement in ice. The tubes were next spun in a Sorval B2 centrifuge at 7,000 RPM for 5 minutes. One ml. aliquots of the supernatant were assayed for inorganic phosphate by the method of Fiske and Subbarow (34). Na+ + K+ ATPase activity was defined as the difference between inorganic phosphate released in the presence of Mg, Na, K and the presence of ouabain, Mg, Na and K, or of Mg alone. The addition of ouabain and the omission of Na + K produced the same results so that ouabain was used in the remaining experiments. The protein content of the whole homogenate was determined by the method of Lowry et al (35) using

Fig. 1 Diagrammatic Representation of the Na+ + K+ ATPase and Mg++ ATPase Enzyme Preparation.*



standards of bovine albumin dissolved in homogenizing solution. Results were expressed as micromoles per liter of inorganic phosphate released per milligram protein per hour and are given as the mean ± standard error. The number of separate observations (n) represent individual animals included in that group. Statistical analysis of the results was made using the students t test.

All chemicals employed were reagent grade. Corticosterone Tris-ATP and Ouabain were purchased from Sigma Chemical Co., St. Louis, Mo. D-L-Histidine (free base) was purchased from Mann Research Lab., N.Y., N.Y. EDTA was obtained from S.T. Baker Chem. Co., Phillipsburg, N.Y. Tris (base) was purchased from Schwartz-Mann Chem. Co., Orangeburg, N.Y. T_3, L-3,3',5 triiodothyronine, free base, was purchased from Calbiochem., San Diego, Ca.

Initial Results and Modifications The unique cation requirements of the Na+ + K+ ATPase make it possible to estimate the total amount of this enzyme in rat kidney crude homogenate. At the same time levels of Mg++ ATPase can be calculated which provides a second enzyme system for comparision to changes in the Na+ + K+ ATPase. There were no differences in Mg++ ATPase activity when calculated using ouabain in the medium or eliminating Na and K from the medium (Fig. 2).

Fig. 3 shows the effects of thyroidectomy or adrenalectomy on Na+ + K+ ATPase activity in rat kidney cortex and medulla crude homogenates. There were no significant changes

Fig. 2 The Effect of Ouabain and Cations on the ATPase Activities of Rat Kidney Membrane Rich Fraction. Incubating tubes contained 100 mM Tris buffer, 1mM Edta, 3mM ATP. $MgCl_2$ was 3mM, NaCl was 100mM, KCl was 20mM, and Ouabain, 3mM. The results are means ± SE. N = 7 assays.

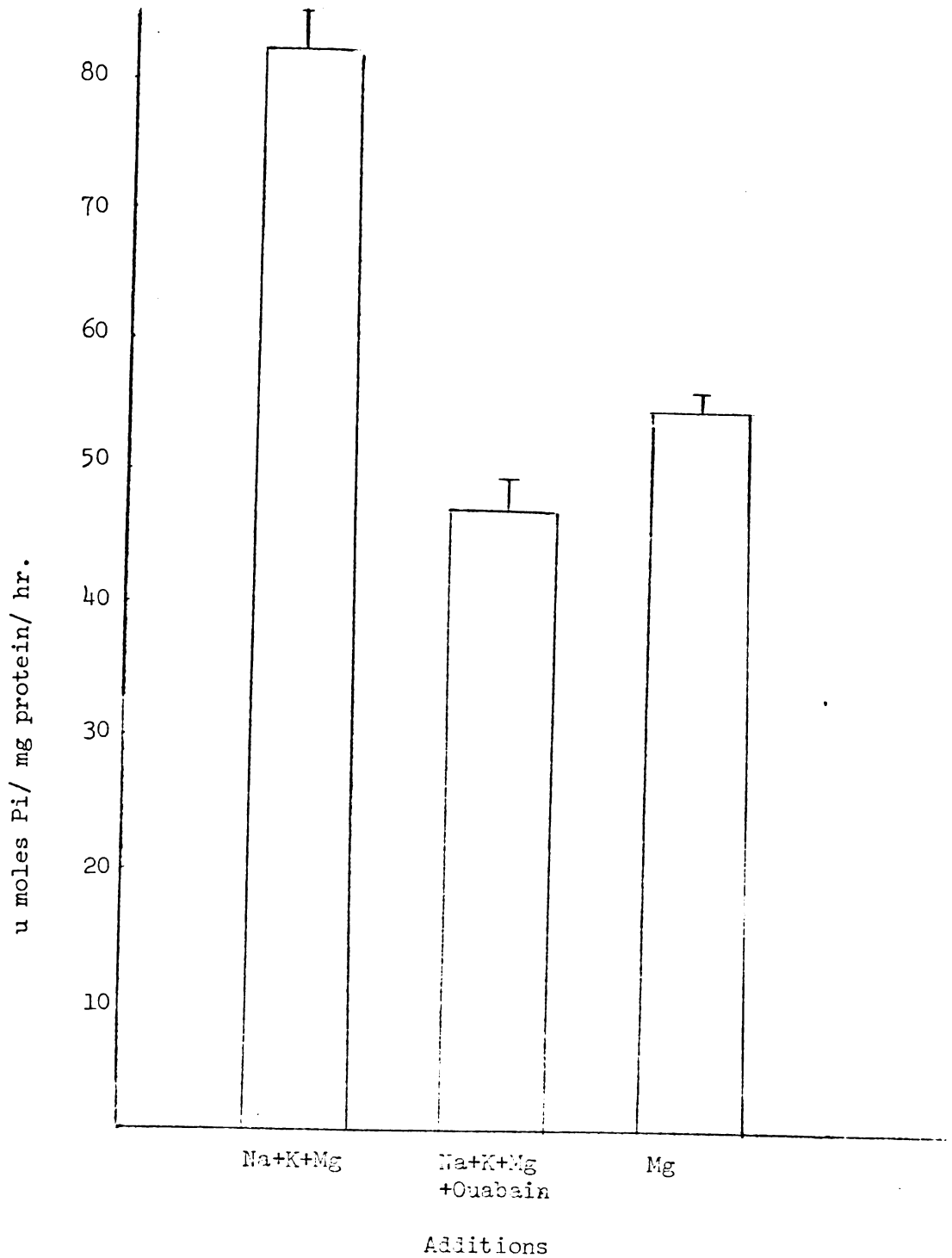
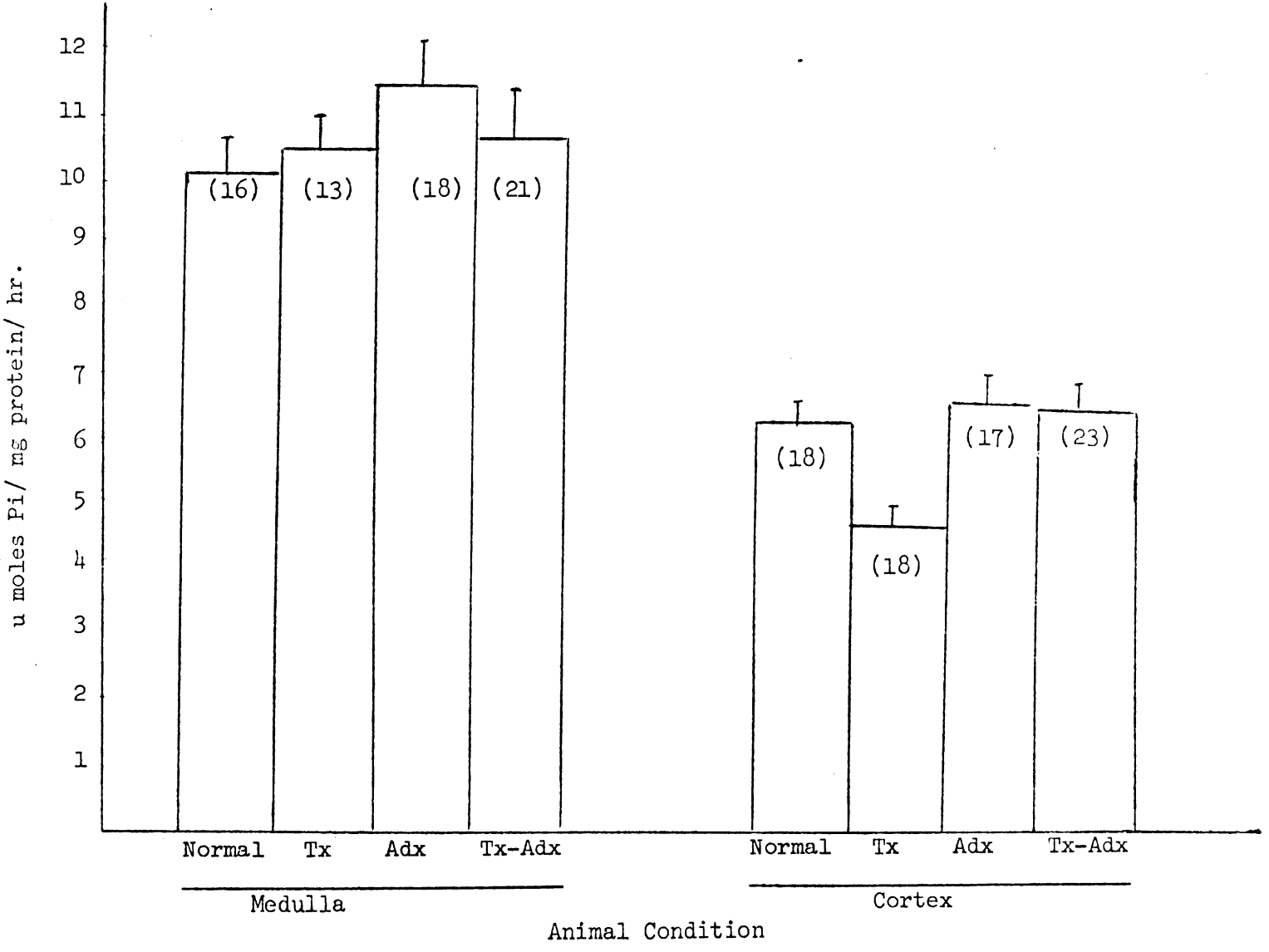


Fig. 3 Effect of Surgical Gland Removal on the Na+ + K+ ATPase in Rat Kidney Medulla and Cortex Crude Homogenate. Thyroidectomy decreased Na+K+ ATPase in cortex, p = .001. Values are means ± SE. ( ) = number of animals used.



in Na+ + K+ ATPase activity of medulla 9 days after thyroidectomy or 3 days after adrenalectomy. This was disappointing considering that significant changes have been reported previously, following both thyroidectomy (18) and adrenalectomy (8,9,15) in whole homogenate preparations of medulla. There was a 26% decrease nine days after thyroidectomy in the cortex whole homogenate which was statistically significant (6.52 ± 0.26 vs. 4.80 ± 0.28, $p = .001$) and demonstrated that a whole homogenate preparation was sensitive enough to detect changes in this fraction. There were no significant changes however, in the cortex sample from either the adrenalectomized or thyroadrenalectomized groups when compared to normal controls. The specific ATPase activity of the medulla was twice the ATPase activity of the cortex using protein as a standard. This purportedly reflects the intense reabsorptive capacity of the ascending limb of Henle located in the medulla fraction (3).

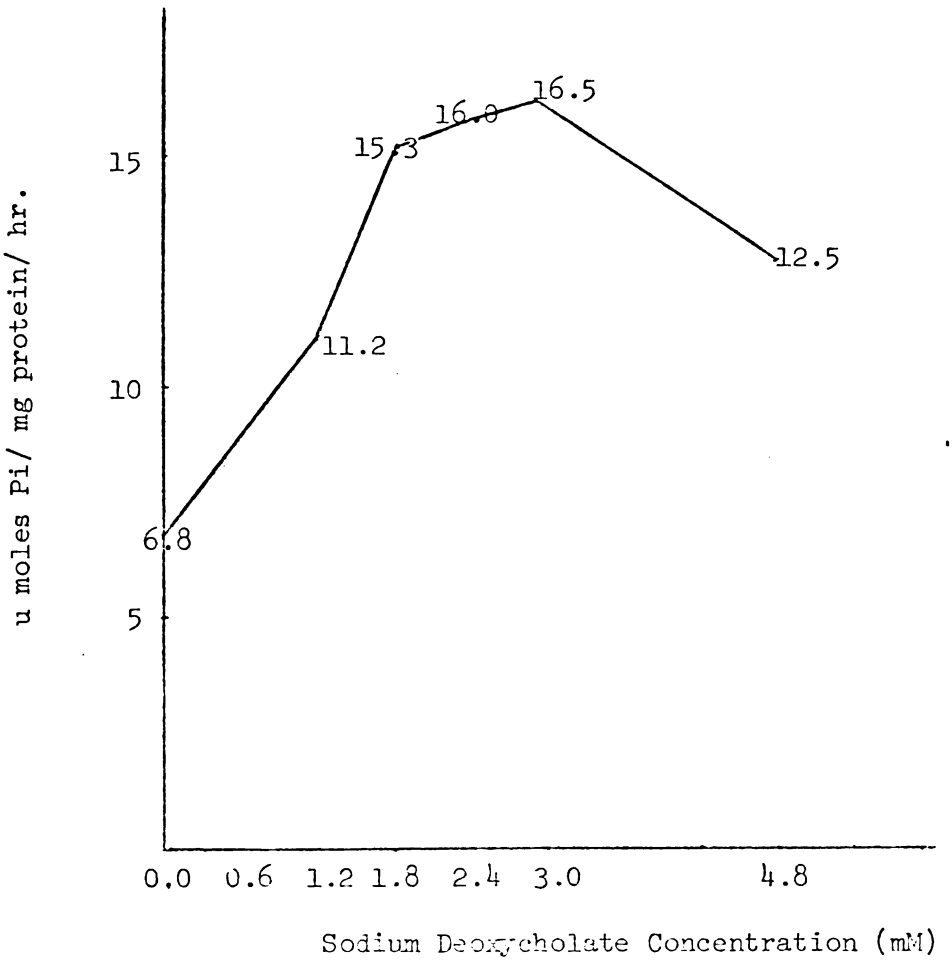
To test further whether adrenalectomy modifies Na+ + K+ ATPase activity, sodium deoxycholate (DOC), a detergent, was added to the homogenizing solution. Jorgensen (7) has observed 35 to 46% reduction in Na+ + K+ ATPase activity following adrenalectomy in preparations treated with DOC where his changes in fresh preparations were small. He attributes this change in Na+ + K+ ATPase of DOC treated homogenates to exposure of latent sites in the preparation. The removal of protein may lead to opening of vesicular structures resulting in free access of the substrate and

and activators to their respective sites on the membrane (36). Jarnefeldt (37) suggests that the detergent removes bound sodium and potassium ions from rat microsomal preparations which have become sensitive to the addition of exogenous cations.

High concentrations of DOC can destroy enzyme activity and it is necessary to find a potentiating concentration and not exceed this concentration during homogenization. I added varying concentrations of DOC from 6 mM to 48 mM to the total homogenizing medium in a volume ratio of 1:10. A 0.1 gram tissue sample was homogenized in 0.9 ml homogenizing solution and 0.1 ml detergent. A final concentration of 2.4 mM was sufficient to more than double the activity of $Na^+ + K^+$ ATPase while there were no significant changes in Mg^{++} ATPase. (The results are shown in Fig. 4). This concentration has been cited in the literature (8) as 1 mg per ml.

Before repeating the experiments on the effects of thyroidectomy and adrenalectomy and starting the hormone replacement studies one further modification was made. T_3 (38) and corticosterone (39) have known anabolic effects and if they increase $Na^+ + K^+$ ATPase activity while increasing the total protein content of the cell changes in $Na^+ K^+$ ATPase activity, measured with protein as the standard, might be masked. For this reason DNA was used as a standard and was

Fig. 4 Changes in Na+ + K+ ATPase Activity Following Homogenization of Cortex Tissue (1 gm wet wt in 10 ml medium) with Varying Concentrations of Sodium Deoxycholate. Homogenizing solution contained 0.3 M DL-Histidine, 5 mM free acid EDTA, 0.25 M Sucrose. The volume of Sodium Deoxycholate was 0.10 of the homogenizing solution.



assayed by the method of Burton (40). Results were expressed as micromoles per liter of inorganic phosphate hydrolyzed per mg DNA per hour.

Surgical Gland Removal and Hormone Replacement Studies. Male Sprague-Dawley rats weighing 200-250 grams were used in all experiments. Animals were divided into three groups and enzyme activities were measured simultaneously from each group.

Group 1:

Thyroidectomized rats
Thyroidectomized + T_3
Thyroidectomized Shams

Group 2:

Adrenalectomized rats
Adrenalectomized + Corticosterone (B)
Adreanalectomized Shams

Group 3:

Thyroadrenalectomized rats
Thryoadrenalectomized + Corticosterone
Thyroadrenalectomized + T_3
Thyroadrenalectomized + T_3 + Corticosterone
Thyroadrenalectomized Shams

In each experiment, age matched shams were identically handled and studied simultaneously. Instead of injected hormones, shams received appropriate quantities of diluent. Thyroidectomies and adrenalectomies were performed as described previously. Hypothyroidism in rats is characterized by a reduction in both oxygen consumption and growth rate (53). Daily weight changes were recorded following thyroidectomy as an indication of successful surgery. Rats that continued to gain weight at a similar rate to sham controls were eliminated from the group. All rats were maintained on a purina

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chow and tap water ad libidum unless adrenalectomized in which case 0.9% saline was substituted for tap water. All rats were housed in a temperature controlled room, lit from 6 AM to 6 PM. Thyroidectomized rats were sacrificed ten days following surgery, adrenalectomized rats six days following surgery. There was a four day recover period between thyroidectomy and adrenalectomy in Group 3.

T_3 was injected in doses of 50 mg/100 gm b.w. dissolved in dilute NaOH at 96, 48, and 24 hours prior to sacrifice. Corticosterone was injected in doses of 250 ug/100 gm b.w./8 hrs. for three days prior to sacrifice (54), dissolved in 2.5% ethyl alcohol. This schedule corresponds to a physiological dose rate. All injections were subcutaneous, to avoid injecting the bladder or stomach. In later experiments, Group 3 animals received 3.0 ml (.9%) saline/200 gm b.w./8 hrs. for three days prior to sacrifice in an attempt to halt sudden weight loss and death which was seen in thyroadrenalectomized rats receiving thyroid hormone replacement (discussed later). A summary of the final surgery and injection pattern is as follows:

	Tx				Adx		B/T_3	B	B/T_3	Sacrifice
day	1	2	3	4	5	6	7	8	9	10

The success of this injection schedule for stimulating Na+ + K+ ATPase has been reported previously for the injection of T_3 to thyroidectomized rats (33) and corticosterone to adrenalectomized rats (8). The medulla and cortex of hypothyroid rats respond at different times to T_3 so that

injections were administered both at 72 and 24 hrs. prior to sacrifice (33). A crude homogenate "S" fraction preparation was performed as described previously with the addition of 1% DOC (0.1% final conc.) to the homogenizing medium.

RESULTS

Effect of Surgical Gland Removal on the Na+ + K+ ATPase Activity in the Renal Cortex Crude Homogenate.

The bulk of cortical tissue consists mainly of promixal convolutions a site where isosmotic sodium reabsorption takes place (3). Approximately 60-75% of the ultrafiltrate is reabsorbed by various mechanisms by the end of the proximal tubule.

As shown in Table 1 and Fig. 5, there was a 16% decrease in Na+ + K+ ATPase activity of renal cortex in throidectomized rats, ten days following surgery, this was significant compared to sham controls ($p = .072$). This slight decrease was disappointing compared to the 26% decrease observed previously after thyroidectomy using protein as a standard and eliminating the DOC wash. There was a 27% decrease in Na+ + K+ ATPase activity six days after adrenalectomy which was significant ($p = 0.001$) and a 45% decrease after thyroadrenalectomy ($p = 0.001$) when compared to sham controls. This 45% decrease was equivalent to the sum of the decreases observed after each individual surgery. There were no significant Mg++ ATPase changes following either thyroidectomy or adrenalectomy as shown in Table 2 and Fig 6a. Small changes in DNA content could not account for changes in enzyme activity (table 1a).

Table 1 Effects of Surgical Gland Removal and Hormone Replacement on Renal Na+ + K+ ATPase Activity.

	Na+ + K+ ATPase u moles Pi/ mg DNA/ hr.	
	Cortex	Red Medulla
Group 1		
Thyrodectomized Sham	83.9 ± 4.1 (8)	47.4 ± 3.3 (9)
Thyroidectomized	75.3 ± 6.1 (16)	55.0 ± 5.2 (15)
Thyroidectomized + T_3	112.7 ± 4.8 (11)	60.1 ± 6.2 (12)
Group 2		
Adrenalectomized Sham	94.2 ± 15.2 (6)	58.6 ± 9.2 (6)
Adrenalectomized	65.1 ± 15.2 (18)	41.3 ± 3.6 (18)
Adrenalectomized + Corticosterone	89.2 ± 5.4 (17)	51.2 ± 4.0 (19)
Group 3		
Throadrenalectomized Shams	90.1 ± 6.2 (15)	55.2 ± 3.7 (18)
Thyroadrenalectomized	49.2 ± 3.6 (18)	33.5 ± 3.1 (19)
Thyroadrenalectomized + Corticosterone	65.2 ± 5.6 (14)	43.2 ± 4.0 (15)
Throadrenalectomized +T_3	84.6 ± 7.7 (13)	38.0 ± 3.4 (14)
Thyroadrenalectomized + Corticosterone + T_3	106.6 ± 5.0 (12)	43.3 ± 5.3 (13)
Total Shams (Groups 1,2 and 3)	89.2 ± 4.5 (29)	53.7 ± 2.7 (33)

Values are means ± SE. Numbers of observations are in parentheses. Corticosterone was injected, 250 ug 100 gm body wt/ 8 hrs, for 3 days. T_3 was injected 50 ug/ 100 gm body wt/ for 2 doses. Rats were adrenalectomized for 6 days, thyroidectomized for 10 days.

Table 1a Effects of Surgical Gland Removal and Hormone Replacement on DNA Content of Kidney Tissue Crude Homogenate.

u grams DNA/.150ml whole homogenate

	Cortex	Red Medulla
Group I		
Thyroidectomized Sham	43 ± 2.0 (5)	70 ± 5.0 (6)
Thyroidectomized	55 ± 4.0 (9)	81 ± 5.0 (14)
Thyroidectomized + T_3	46 ± 4.0 (9)	68 ± 4.0 (10)
Group 2		
Adrenalectomized Sham	55 ± 7.0 (4)	80 ± 5.0 (4)
Adrenalectomized	52 ± 1.0 (9)	73 ± 4.0 (14)
Adrenalectomized + Corticosterone	50 ± 3.0 (8)	76 ± 2.0 (15)
Group 3		
Thyroadrenalectomized Shams	45 ± 3.0 (11)	68 ± 3.0 (14)
Thyroadrenalectomized	55 ± 4.0 (8)	99 ± 6.0 (12)
Thyroadrenalectomized + Corticosterone	59 ± 4.0 (14)	87 ± 3.0 (12)
Thyroadrenalectomized + T_3	58 ± 4.0 (11)	86 ± 5.0 (9)
Thyroadrenalectomized + Corticosterone + T_3	55 ± 2.0 (7)	87 ± 5.0 (12)

All tissue samples were homogenized in a 1:10 weight to volume ratio. Values are means ± SE. Numbers of observations are in parentheses. Corticosterone was injected, 250/ug 100 gm body wt/8 hrs, for 3 days. T_3 was injected 50 ug/100 gm body wt/ for 2 doses. Rats were adrenalectomized for 6 days, thyroidectomized for 10 days.

Fig. 5 Effect of Surgical Gland Removal on the Na+ + K+ ATPase in Rat Kidney Cortex Crude Homogenate. Thyroidectomy decreased Na+ + K+ ATPase activity. P=0.072. Adrenalectomy decreased Na+K ATPase, P=0.001. Thyroadrenalectomy decreased Na+K ATPase, P=0.001. Values are means ± SE. ( ) = number of animals used. Percentage decreases compared to a combined group of all shams.

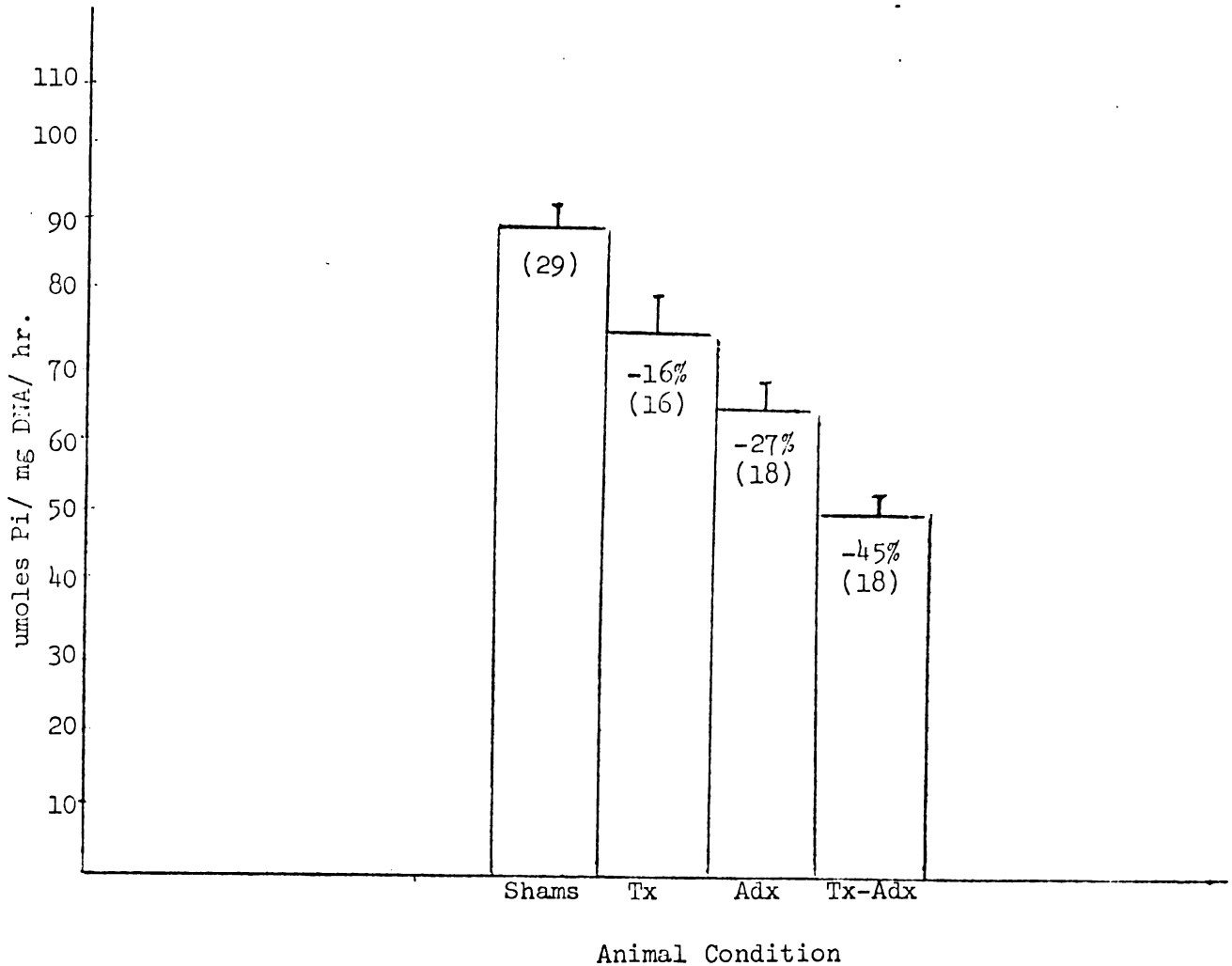
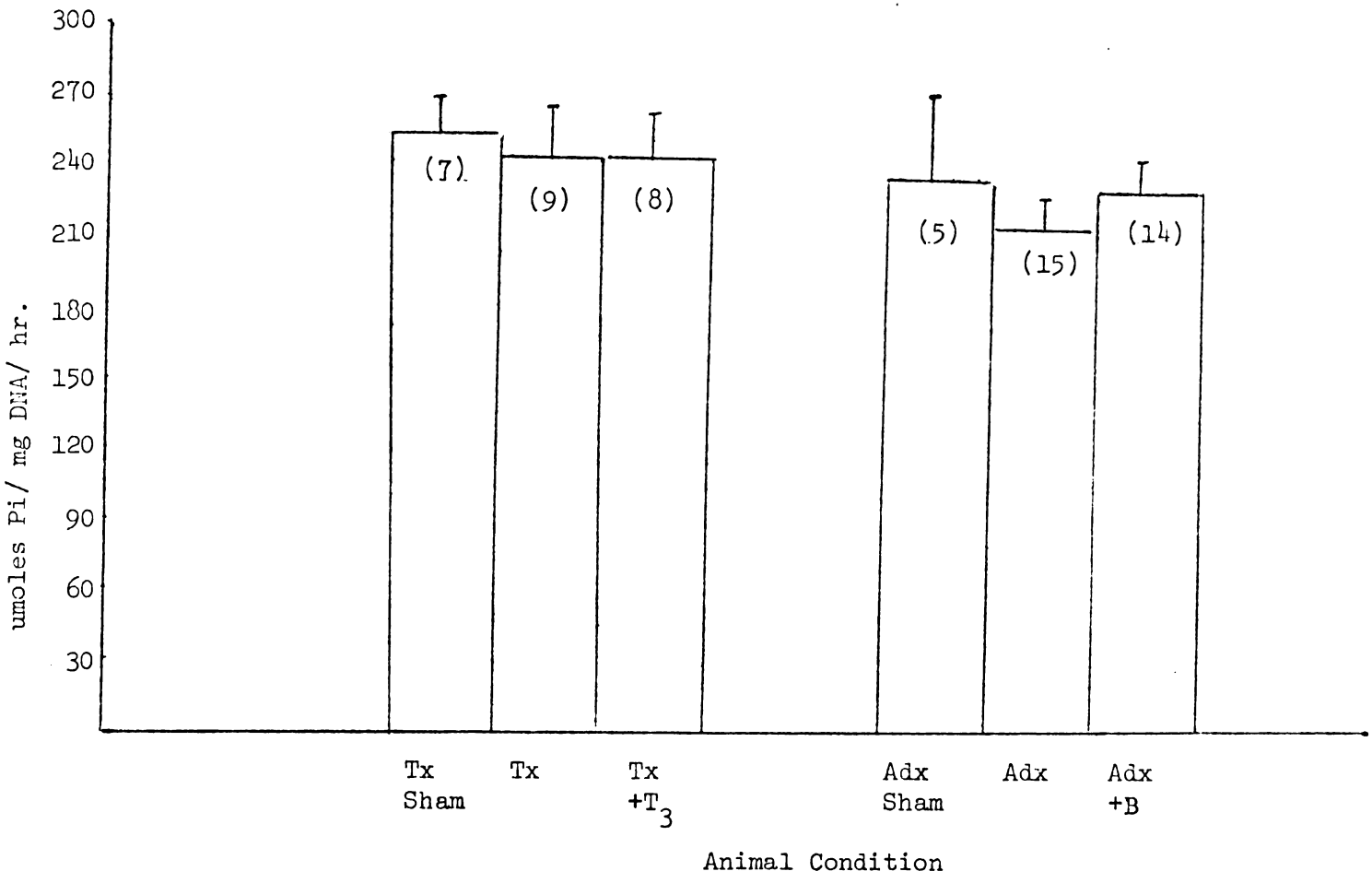


Table 2 Effects of Surgical Gland Removal and Hormone Replacement on Renal MG++ ATPase Activity.

	MG++ ATPase umoles Pi/mg DNA/hr.	
	Cortex	Red Medulla
Group 1		
Thyroidectomized	254.0 ± 15.0 (7)	83.1 ± 4.7 (7)
Sham		
Thyroidectomized	246.6 ± 18.4 (9)	77.0 ± 4.3 (9)
Thyroidectomized + T_3	246.1 ± 19.2 (8)	98.4 ± 9.8 (8)
Group 2		
Adrenalectomized	236.7 ± 26.8 (5)	89.2 ± 13.0 (5)
Sham		
Adrenalectomized	215.5 ± 9.2 (15)	87.5 ± 5.6 (16)
Adrenalectomized + Corticosterone	234.4 ± 19.0 (14)	91.8 ± 3.6 (17)

Values are means ± SE. Numbers of observations are in parentheses. Corticosterone was injected, 250/ug 100 gm body wt/ 8 hrs, for 3 days. T_3 was injected 50 ug/ 100 gm body wt/ for 2 doses. Rats were adrenalectomized for 6 days, thyroidectomized for 10 days.

Fig. 6a Effect of Surgical Gland Removal and Hormone Replacement on the Mg^{++} ATPase Activity in Rat Kidney Cortex Crude Homogenate. There were no significant changes in any of the groups. Values are means ± SE ( ) = number of animals in each group.



Effects of Hormone Replacement on the Na+ + K+ ATPase Activity in the Cortex Crude Homogenate Following Surgical Gland Removal.

Rats thyroidectomized for a total of ten days, receiving injections of T_3 (50 ug/100 gm b.w.) at 72 and 24 hrs. prior to sacrifice showed a 50% increase in Na+ + K+ ATPase activity of the cortex (75.3 $\pm$ 6.8 v. 112.7 $\pm$ 4.0) which was significant ($p = 0.001$) (Fig. 6). Rats (Group 2) adrenalectomized for a total of 6 days, receiving corticosterone (250 ug/100 gm b.w./8 hr.), showed a 31% increase in Na+ + K+ ATPase activity ($p = 0.001$) which returned adrenalectomized levels back to normal (89.2 $\pm$ 5.4 v 94.2 $\pm$ 15.2) (Fig. 7). This stimulating effect of glucocorticoids on Na+ + K+ ATPase activity has been reported previously (8,15).

In thyroadrenalectomized animals, the addition of T_3 (50 ug/100 gm b.w.) raised Na+ + K+ ATPase levels 72% ($p = 0.001$) which was the same absolute increase produced by the addition of T_3 to thyroidectomized rats (Group 1), 37.4 u moles Pi/mg DNA/hr. v 35.4 u moles Pi/mg DNA/hr. (Fig. 6). Corticosterone increased thyroadrenalectomized Na+ + K+ ATPase levels of the cortex 33% ($p = 0.01$) and again this absolute increase was not significantly different from the addition of corticosterone to adrenalectomized rats, (Group 2), 16.0 u moles Pi/mg DNA/hr. v. 24.1 u moles Pi/mg DNA/hr. (Fig. 7).

The addition of both corticosterone and T_3 to a thyroadrenalectomized rat (Fig. 8) produced a 117% increase in

Fig. 6 Effect of Surgical Gland Removal and Hormone Replacement on the Na+ K+ ATPase in Rat Kidney Cortex Crude Homogenate. T_3 (50 ug/100 gm body wt) increased Na+K ATPase in hypothyroid rats, P = 0.001, and in thyroadrenalectomized rats, P = 0.001. Values are means ± SE. ( ) = number of animals used.

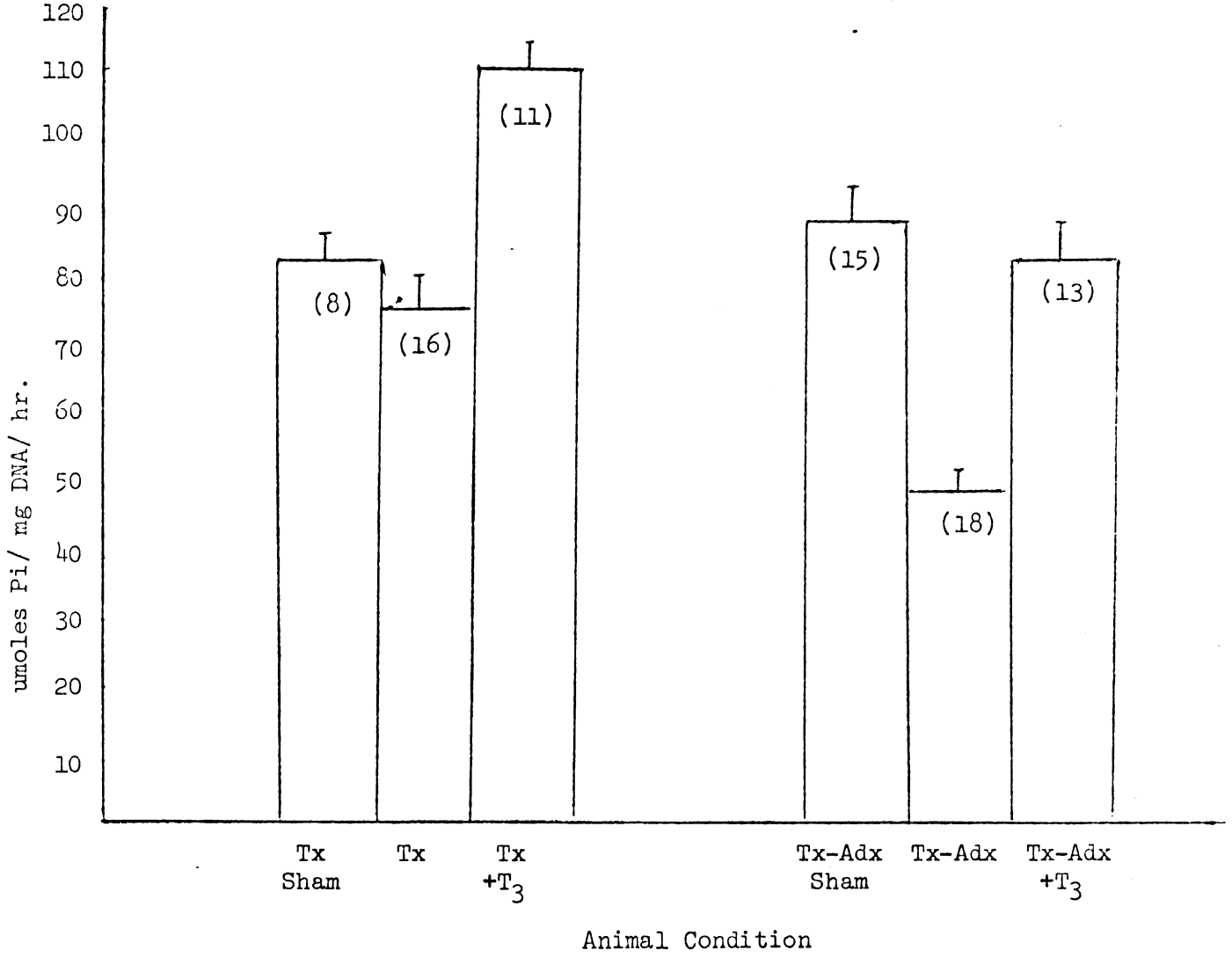


Fig. 7 Effects of Surgical Gland Removal and Hormone Replacement on the Na+ + K+ ATPase in Rat Kidney Cortex Crude Homogenate. Adrenalectomy decreased Na+K ATPase activity, P=0.026. Corticosterone, 250 ug/ 100 gm body wt., increased adrenalectomized levels, P=0.003. Thyroadrenalectomy decreased Na+K ATPase activity, P=0.02. Values are means ± SE. ( ) = number of animals used.

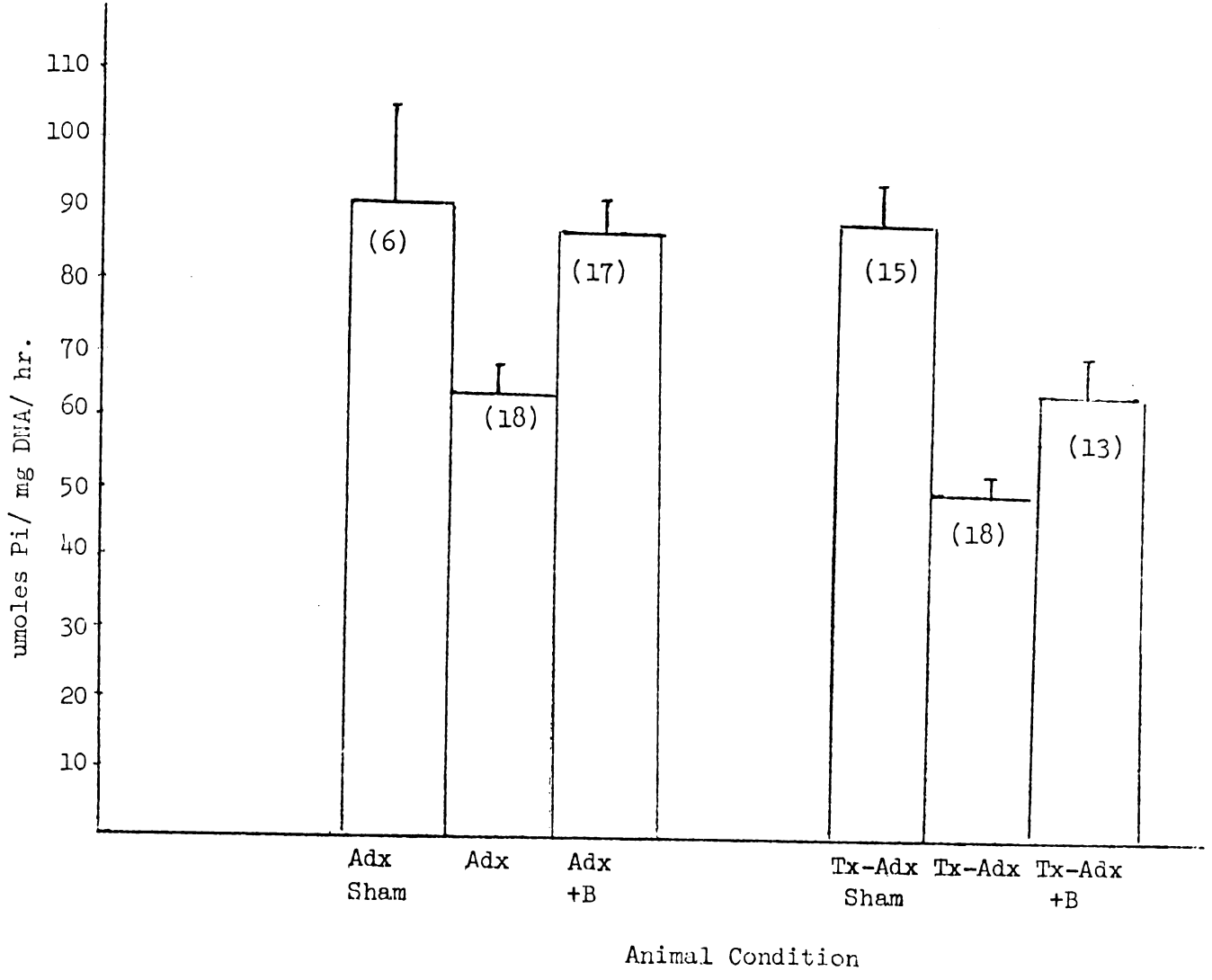
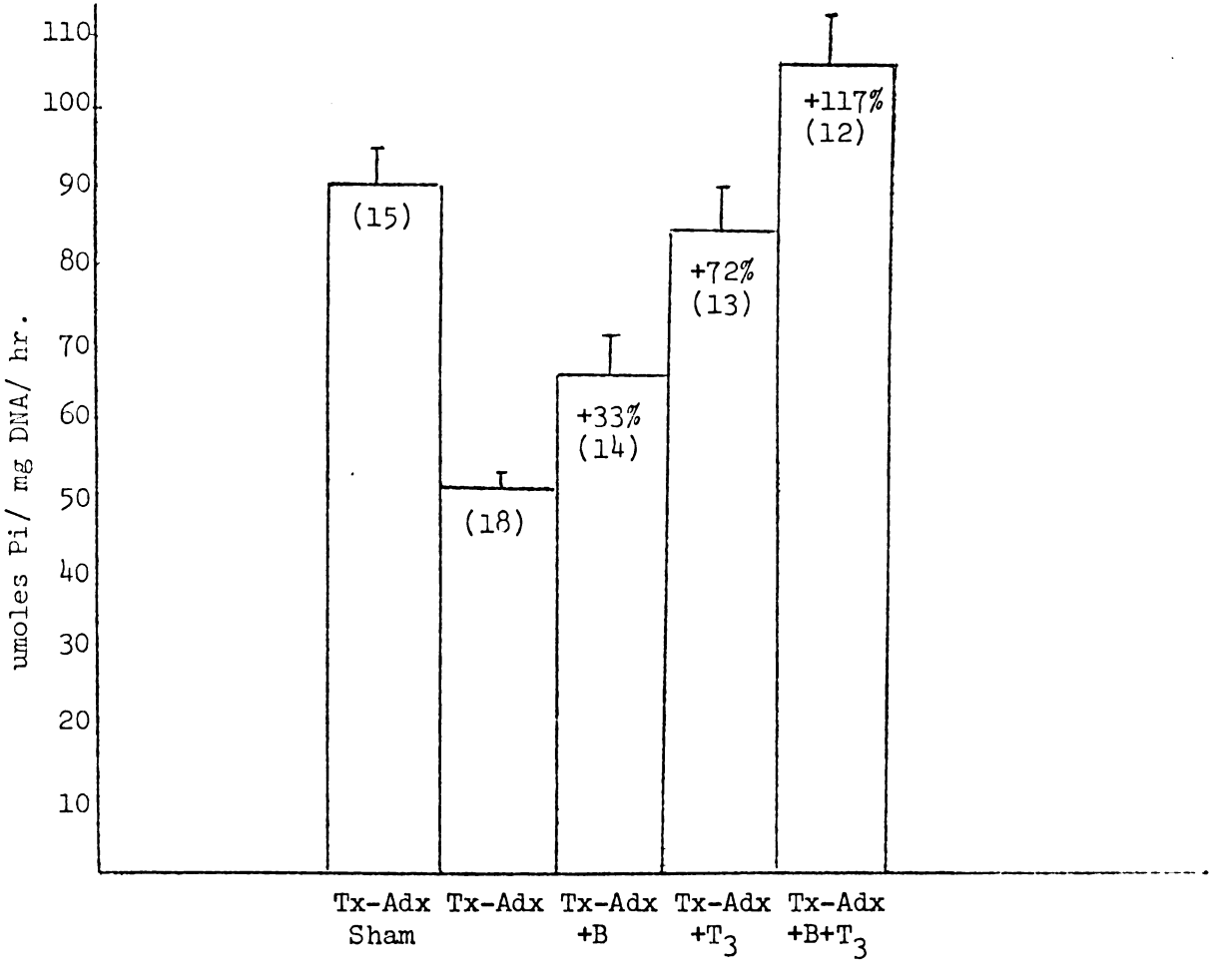


Fig. 8 Effect of Surgical Gland Removal and Hormone Replacement on the Na++ K+ ATPase in Rat Kidney Cortex Crude Homogenate. Corticosterone, 250 ug/ 100gm body wt., increased Na+K ATPase in thyroadrenalectomized rats, P=0.01, T_3, 50ug/ 100gm body wt., increased Na+K ATPase in thyroadrenalectomized rats, P=0.001, T_3 + Corticosterone increased Na+K ATPase in thyroadrenalectomized rats, P=0.001. Values are means ± SE. ( ) represents number of animals used. Percentage increases compared to thyroadrenalectomized level.



Na+ + K+ ATPase. This increase was not significantly different from the 105% increase produced when T_3 and corticosterone were injected individually and then their percentage increases added together.

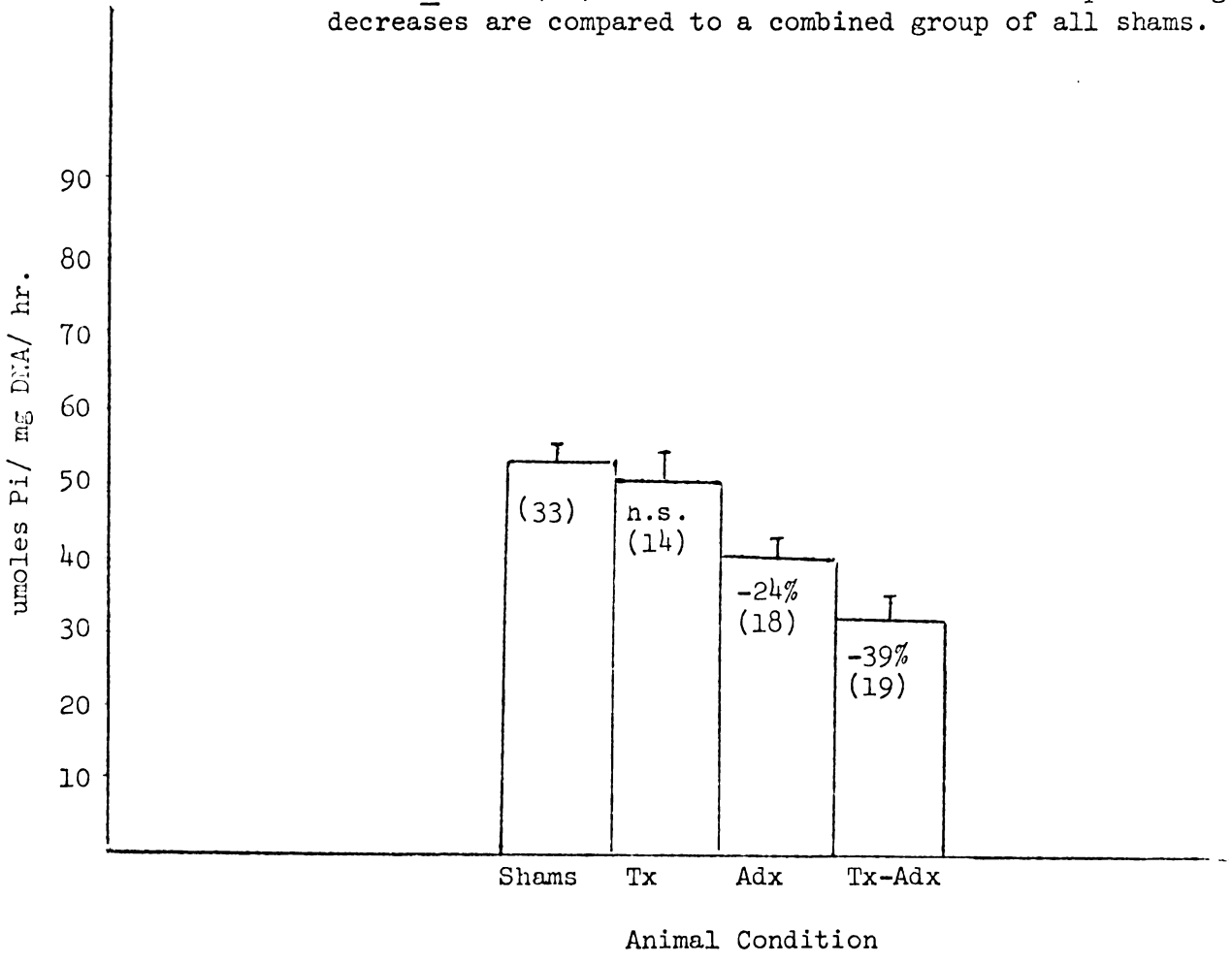
There were no changes in Mg++ ATPase activity following the administration of T_3 to a thyroidectomized rat and the adminstration of corticosterone to an adrenalectomized rat as shown in Table 2 and Fig. 6a.

The Effect of Surgical Gland Removal on the Na+ + K+ ATPase Activity in the Renal Medulla Crude Homogenate. Groups 1,2,3.

The red medulla, the zone of tissue between the papilla and cortex of the kidney, consists primarily of ascending and descending loops of Henle and is functionally involved in actively transporting electrolytes from the tubule to the interstitium but is relatively impermeable to water. This creates a hypertonic interstitium and a hypotonic urine reentering the cortex of the kidney via the distal tubule. Both factors are important in determining the final concentration of the urine. Martinez-Maldonado et al (3) infusing cardiac glycosides into the renal artery of dogs, concluded that a natruresis only occurred when the medullary Na+ + K+ ATPase activity was affected. Blocking simply the cortex Na+ + K+ ATPase activity did not produce a natruresis (3).

In my experiments, thyroidectomy had no effect on Na+ + K+ ATPase activity (Fig. 9). Adrenalectomy induced a 24% decrease in Na+ + K+ ATPase activity and thyroadrenal-

Fig. 9 Effect of Surgical Gland Removal on the Na+ + K+ ATPase in Rat Kidney Medulla Crude Homogenate. Adrenalectomy decreased Na+K ATPase activity, P=0.05. Thyroadrenalectomy decreased Na+K ATPase activity, P=0.01. Values are means ± SE. ( )= number of animals used. The percentage decreases are compared to a combined group of all shams.



lectomy induced a 39% decrease when compared to sham controls, however, this was not significantly different from the drop induced by adrenalectomy alone. There were no changes in Mg++ ATPase following thyroidectomy or adrenalectomy as shown in Table 2 Fig. 10a.

Effects of Hormone Replacement on Na+ + K+ ATPase Activity in the Medulla Crude Homogenate Following Surgical Gland Removal. Groups 1,2,3.

The administration of T_3 at 72 and 24 hrs. prior to sacrifice produced no significant changes in a hypothyroid rat (Fig. 10). Similarly the addition of T_3 to a thyroadrenalectomized rat produced no significant changes in Na+ + K+ ATPase activity (Fig. 11).

Corticosterone 250 ug/100 gm b.w. raised Na+ + K+ ATPase activity 24% in an adrenalectomized rat but this was not significant ($p = .077$). However, corticosterone produced a significant increase ($p = 0.05$) when administered to thyroadrenalectomized rats (Group 3). The reason for these differences in response is uncertain. If a greater number of animals were used in Group 2 a significant increase in the response may have been obtained despite the variability in this population.

Administration of both corticosterone and T_3 to thyroadrenalectomized rats produced the same increase (25%) in Na+ + K+ ATPase activity as the injection of corticosterone alone. However, this rise was not statistically significant ($p = 0.10$), again possibly reflecting the relatively large standard errors in the assays of crude

Fig. 10a Effect of Surgical Gland Removal and Hormone Replacement on the Mg++ ATPase Activity in Rat Kidney Medulla Crude Homogenate. There were no significant changes in any of the groups. Values are means ± SE ( ) = number of animals used.

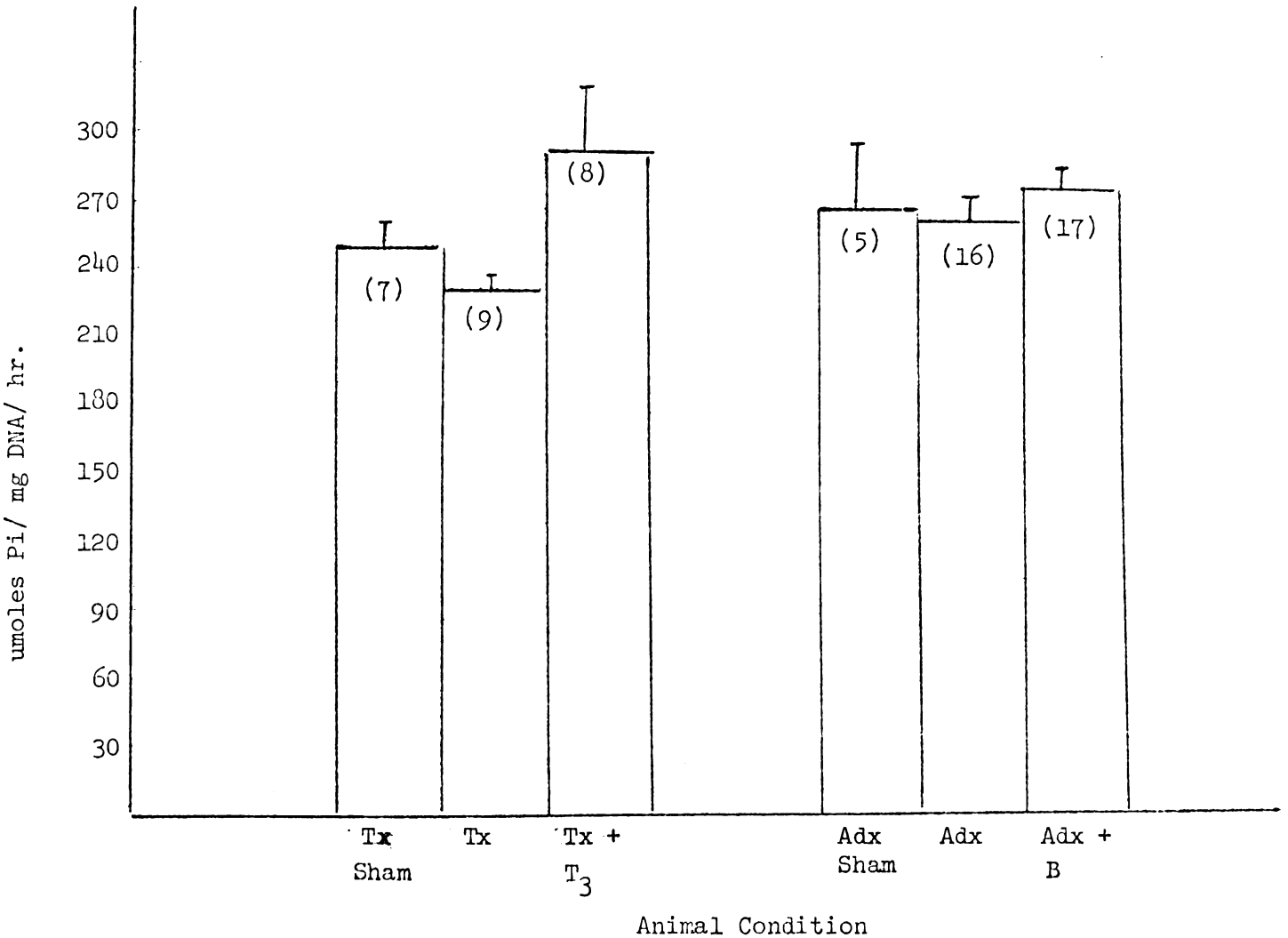


Fig. 10 Effect of Surgical Gland Removal and Hormone Replacement on the Na+ + K+ ATPase in Rat Kidney Medulla Crude Homogenate. Adrenalectomy decreased Na+K ATPase activity, P=0.05. Values are means ± SE. ( )= number of animals used.

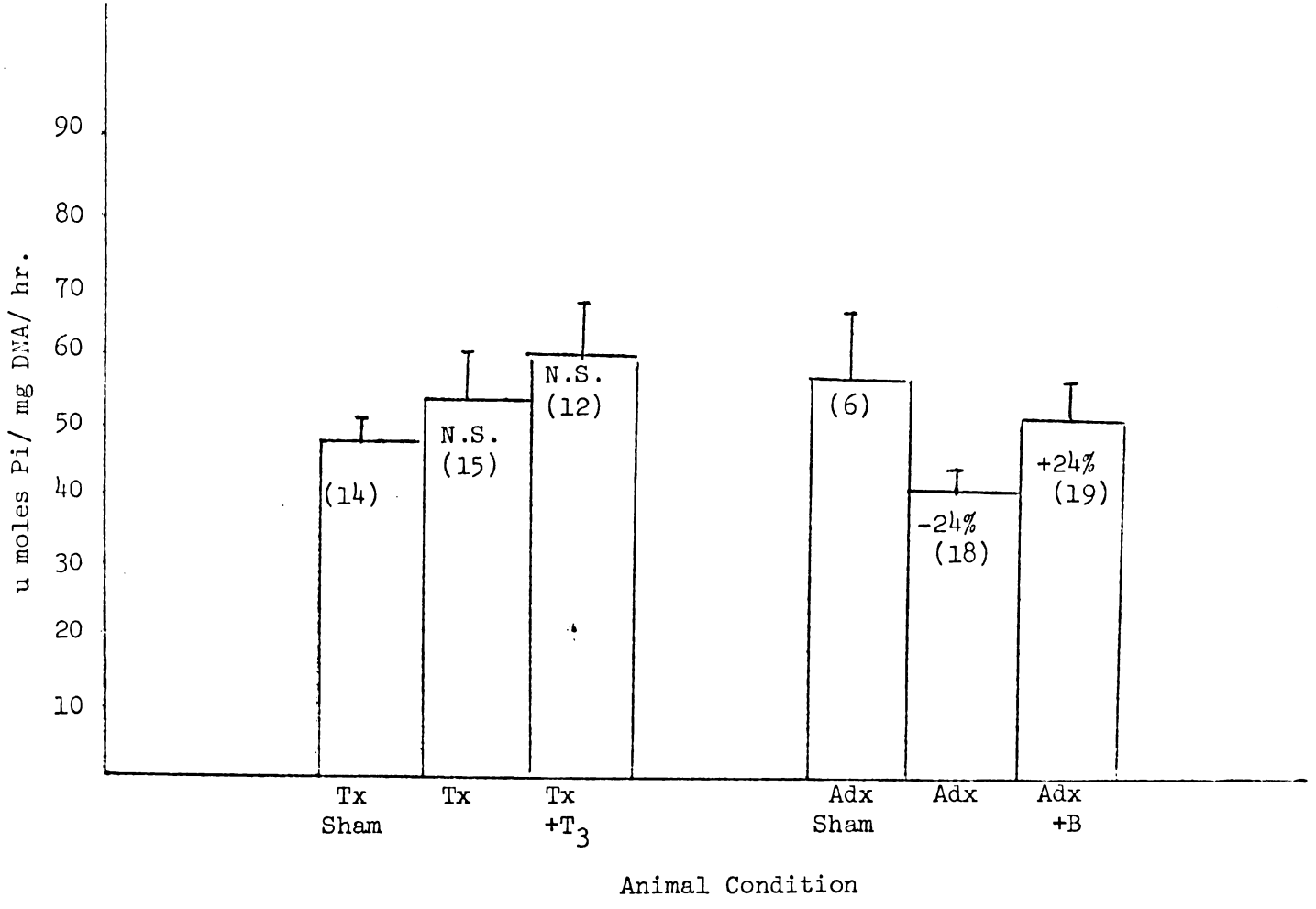
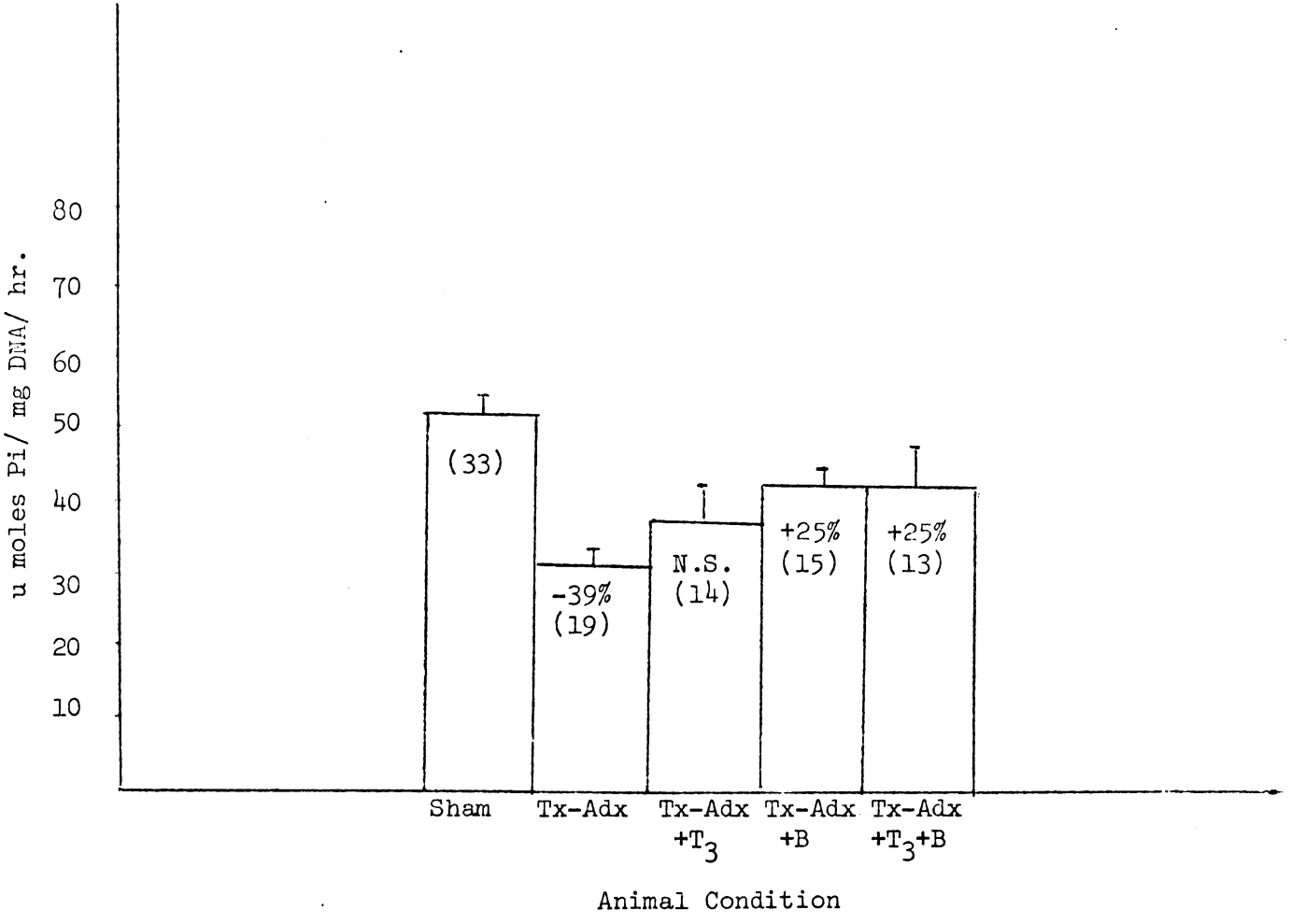


Fig. 11 Effect of Surgical Gland Removal and Hormone Replacement on the Na+ + K+ ATPase in Rat Kidney Medulla Crude Homogenate. Corticosterone, 250 ug/ 100 gm body wt., increased Na+K ATPase activity in thyroadrenalectomized rats, P=0.05. All other hormone injections produced no significant changes. Values are means ± SE. ( ) = number of animals used.



homogenate preparations.

After the eighth week of experimentation, I noticed that thyroadrenalectomized rats receiving T_3 replacement were losing weight, primarily after the second injection, and that the death rate was greater than in Group 3 animals not given T_3. A computation showed the following data.

Table 2. Animal condition during injection period.

Animal Group	Average Weight Change During 5 Day Injection Period (grams)	% Deaths During 5 Day Injection Period, 3 doses T_3	% Deaths During 4 Day Injection Period, 2 doses T_3, 0.9% Saline 3ml/200 gm b.w./8hrs.
Tx Sham	+31.5	1.	
Tx	+10.3	11.8	
Tx + T_3 (50ug/100gm b.w.)	+ 3.4	12.5	
Tx-Adx	+ 2.0	7.1	10.5
Tx-Adx + B (250ug/100gm b.w.)	+ 7.4	21.4	16.7
Tx-Adx + T_3 + B	- 3.2	14.3	13.3
Tx-Adx + T_3	-14.1	52.9	33.3

Thyroidectomized rats recover from surgery and remain at a constant weight for the duration of the injection period. The thyroadrenalectomized rats receiving T_3 lost, on the average, 14.1 gram during the 5 day injection period and died at a much greater rate than either thyroidectomized rats receiving T_3 or thyroadrenalectomized rats receiving T_3 and B. This rapid weight loss suggested a fluid loss and possibly death due to dehydration. To compensate for the presumed dehydration all group 3 animals received, in addition to the hormone

replacement, 9 ml (0.9%) saline/200 gm b.w./day during the injection period. The number of injections of T_3 was reduced from 3 to 2 and the injection period shortened by a day as described previously. These changes reduced the percentage of deaths from 52.9% to 33.3% in this group while the death rate in the other groups remained nearly constant. The weight loss continued, however, even during the injection of saline, suggesting that the reduced injection period may have contributed more to the survivial rate of these animals.

Administration of saline to normal and adrenalectomized animals has previously been shown to have no effect on Na+ + K+ ATPase activity (8,30,15). In Table 3, Group 3 rats receiving saline and hormones are compared to those receiving hormones alone: No significant differences in Na+ + K+ ATPase activity were seen.

Effects of T_3, 2 ug/100 gm b.w., on Na+ + K+ ATPase Activity and Kidney Growth.

The injection of 50 ug T_3/100 gm b.w. has been criticized as pharmacological, not representing the normal rat thyroid output of 0.13-0.2 ug T_3/100 gm. b.w. I decided to test and compare a much smaller dose of T_3, 2 ug/100 gm b.w., by injecting this amount for two weeks into hypothyroid rats and then observing changes in body weight, kidney growth, and ATPase activity.

Male Sprague-Dawley rats weighing 200-220 gms were thyroidectomized as described previously and maintained on a Purina chow diet in a temperature controlled room (72 F). After two weeks, daily weight changes were recorded and

Table 3 The Effect of Surgical Gland Removal and The Effect of Corticosterone and T_3 on Renal Na+ + K+ ATPase in Rats receiving 0.9% Saline in Addition to the Hormone Replacement.

Na+ + K+ ATPase Acvitity
u moles Pi/ mg DNA/ hr.

Cortex	Tx-ADX	+ B 250ug/100gm	+T_3 50ug/ 100gm
Saline,3ml(.9%)/200gm injection	42.2 ± 6.8 (5)	49.5 ± 16 (3)	84.8 ± 13 (5)
Without Saline	52.3 ± 4.3 (13) NS	69.5 ± 5.2 (11) NS	83.9 ± 11 (7) NS
Medulla			
Saline, 3ml (0.9%)/200gm.	34.9 ± 5.7 (6)	40.0 ± 5.8 (4)	34.3 ± 4.0 (6)
Without Saline	32.9 ± 3.7 (13) NS	44.4 ± 5.1 (11) NS	40.8 ± 5.1 (8) NS

Saline, 3ml(0.9%)/200gm body wt/8hrs, for three days produced no significant changes in any group. Corticosterone (B) was injected as 250ug/100gm body wt/8hrs for three days. T_3 was injected as 50ug/100gm body wt/ for two doses in the saline group and three doses in the non saline group. All values are means ± SE.

after four weeks, 5 hypothyroid rats were given daily injections of T_3 and seven hypothyroid rats injections of dilute NaOH the diluent. Six weeks after thyroidectomy all animals were decapitated and blood from the hypothyroid rats that received the diluent and normal rats was collected for protein-bound-iodine studies. I wanted further evidence that successful surgical thyroidectomy, evidenced by weight change, could also be correlated to lower PBI levels.

Enzyme preparations were performed as described for a crude homogenate using a DOC wash and DNA as a standard, on the cortex tissue only.

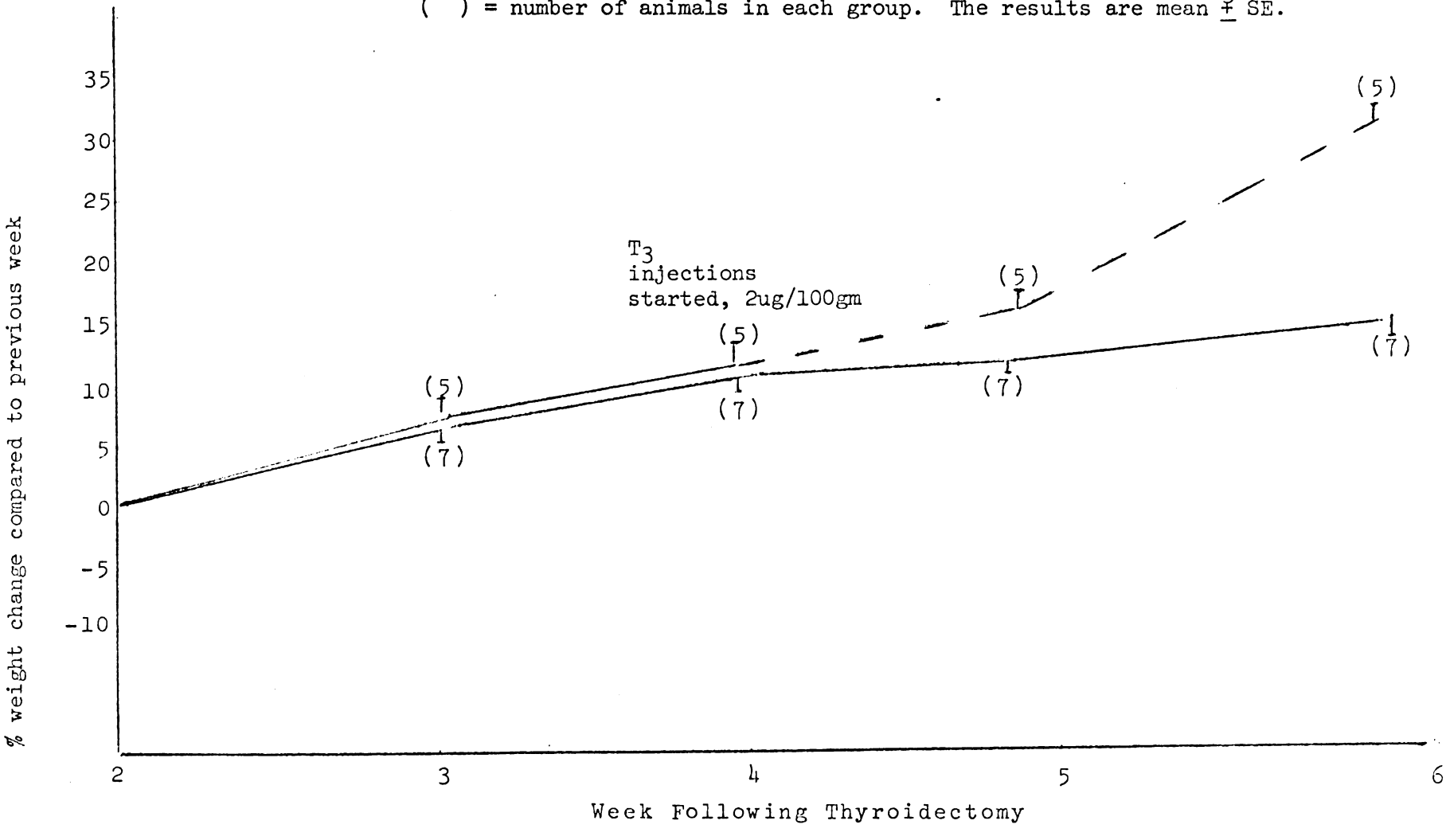
Results. Changes in body weight, kidney wet weight, PBI, and Na+ + K+ ATPase activity following six weeks of thyroidectomy and two weeks of T_3 injections (2 ug/100 gm. b.w.) are listed in Table 4. The low PBI in hypothyroid rats when compared to normal values indicates that surgical thyroidectomy reduced hormone levels in the blood and the usual gain in body weight in these rats. The hypothyroid rats receiving 2 ug T_3/100 gm b.w. resumed weight gain during the second week of injections (fig. 12) at a rate similar to normal rats. In contrast, hypothyroid rats receiving injections of the diluent failed to gain weight. Kidney wet weight was significantly lower in hypothyroid rats than in normals (1.79 v 4.10) but the administration of T_3 for two weeks increased kidney weight by 50%, to 2.6 g. Na+ + K+ ATPase activity was significantly lower (50%) in hypothyroid rats given the diluent compared to normals ($p = 0.001$) whereas those receiving injection of 2 ug of T_3/day had normal

Table 4 Effects of T_3, 2 ug/ 100 gm body wt/ day, for 2 weeks on Kidney Wet Weight, Body Weight, Na+ + K+ ATPase Activity, of Hypothyroid Rats.

Group	PBI MCG/DL	N	Kidney Wet Weight grams	% Change in Body Weight	Na+ + K ATPase Activity umoles Pi/ mg DNA/hr.
Normal	4.2 ± 0.5	4	4.1 ± .37	16.4 ± 1.7	101.0 ± 9.4
Hypothyroid + Diluent	1.5 ± .28 (n = 4)	7	1.8 ± .13	1.6 ± .11	59.3 ± 7.2
Hypothyroid + T_3		5	2.7 ± .14	16.1 ± 1.6	112.5 ± 3.8

Thyroidectomy decreased PBI, P = 0.003, kidney wet weight, P = P.001, Na+ + K+ ATPase activity, P = 0.009 compared to normal rats. T_3, 2ug/ 100 gm body wt., increased kidney wet weight, P = 0.001, and increased Na+ + K+ ATPase activity, P = 0.001 in hypothyroids. Changes in body weight recorded during second week of T_3 injections. Values are means ± SE.

Fig. 12 The Effect of T_3. 2ug/100gm b.w./day, on the Renal Cortex Na+ + K+ ATPase in Hypothyroid Rats. ------ = hypothyroids + T_3 ( ) = number of animals in each group. The results are mean ± SE.



Na+ + K+ ATPase levels (112.5 ± 3.8 v 101.0 ± 9.4). Moreover the 2 ug dose given for two weeks raised Na+ + K+ ATPase activity to the same absolute value as the 2 doses of 50 ug/100 gm b.w. given over a three day period (112.46 ± 3.8 v. 112.7 ± 4.8).

DISCUSSION

The renal response to hormones is one way the body responds to changes in internal fluid and electrolyte content. Even in the lower forms such as the fish, corticosteroids promote increased outflow of sodium from the gills and therefore make an important contribution to the exchange of ions and water between the animal and the external medium (42). In man, ever since Addison described "Addison's disease" as early as 1849 it has been known that adrenalectomy causes numerous renal and metabolic complications: Specifically; (1) a loss of Na+ in the urine (2) decreased blood volume, renal plasma flow, and glomerular filtration rate, (3) a tendency to hypoglycemia, (4) poor resistance to shock and infection (5) poor fat mobilization and various psychic changes (17). Adrenal cortical hormones can reverse these effects. These steroids have been classified into various categories (mineralocorticoid, glucocorticoid) depending on their predominant effects. These two types of steroids are not as functionally independent as the names imply. Glucocorticoids improve the impaired water excretion

capacity found in primary myxedema (43), and stimulate renal Na+ + K+ ATPase activity, an important enzyme in Na+ reabsorption following adrenalectomy (58)(8). Jorgensen (14) and Chignell and Titus (8) studied the effects of administered adrenal steroids, and found that aldosterone, a mineralocorticoid, increased ATPase levels following adrenalectomy to a level midway between adrenalectomized and normal levels but corticosterone returned the activity to normal. My results substantiate the effects of glucocorticoids on Na+ + K+ ATPase activity in the renal cortex. A physiologic dose of corticosterone of 250 u gram/100 gm b.w./8 hrs. over three days restored Na+ + K+ ATPase activity to normal following adrenalectomy.

Similar to adrenal insufficiency, hypothyroidism has profound effects on kidney function in the rat. Renal plasma flow and glomerular filtration rate are markedly reduced (41,30,44) and hypothyroid rats exhibit a limited urinary concentrating capacity (21). There is also renal impairment in the conservation of sodium so that 50% of the animals fed low sodium diet die within two weeks following surgery (22,45). These observations have led numerous investigators to study the interrelations between thyroid hormones and adrenal steroids (23,26) and the subsequent effects on kidney function (22,46) especially renal sodium reabsorption. Fregly and Taylor (22) suggested that thyroidectomy decreased renal production of renin and also decreased tubular sensitivity to aldosterone so that only large doses of deoxycorticosterone acetate (DOCA), 250 ug/100 gm b.w., and aldosterone,

0.5 ug/100 gm b.w., had a salt retaining effect during isotonic loading. Straw and Taylor (46) demonstrated a decreased rate of aldosterone secretion in hypothyroid rats and attributed this to the poor salt handling in these animals. Pretreatment of thyroadrenalectomized animals with aldosterone however, did not abolish the sodium wasting (41,48). Fractional excretion of Na^+ in response to saline loading is 3.4 times greater in hypothyroid rats (41) so that at the conclusion of a diuresis some animals excrete greater than 45% of a filtered sodium load. Adrenalectomized animals undergoing a mannitol diuresis excrete only 9% of a filtered sodium load (41). This information suggests there are other reasons besides the depressed aldosterone secretion to explain poor sodium reabsorption in hypothyroidism.

Renal sodium reabsorption is known to be influenced by a number of factors such as glomerular filtration rate, extracellular fluid volume, plasma sodium concentration and certain physical factors such as oncotic pressure in the peritubular capillary network (47). Plasma sodium concentration and extracellular fluid volume (measured as inulin space) remain the same or decrease slightly after thyroidectomy (30) and therefore seem unlikely to affect sodium reabsorption. An exaggerated natruresis can also occur with no changes in glomerular filtration rates during a hypertonic saline loading to a hypothyroid rat (41).

Hemodynamic and physical factors can also influence salt excretion (47). Koehn, Shindler and Stanton (50), demonstrated

that aortic perfusion pressure is reduced by 17% in radiothyroidectomized rats, and Osario and Zadunaisky (55) demonstrated that the clearance of Diodrast, an indication of renal plasma flow, was decreased 66% in conjunction with a 33% reduction in clearance of inulin which signifies an increased filtration fraction. The observed increase in plasma protein concentration (56,57) in conjunction with an increased filtration fraction suggests that peritubular capillary protein concentration is increased in the hypothyroid rat. These conditions would facilitate an increased reabsorption and therefore can not explain the decrease in tubular sodium reabsorption during hypothyroidism.

Michael et al (30) performed clearance and micropuncture studies to locate and define the mechansism of poor salt handling in hypothyroid rats. Fractional proximal reabsorption of sodium assessed from proximal tubular fluid to plasma ratios of inulin was found to be decreased by 28% in hypothyroid rats. This technique relies on the principle that the ultrafiltrate at the beginning of the proximal tubule is isotonic with plasma and as salt and water are reabsorbed, the concentration of inulin in the tubule increases as a direct indication of reabsorption. In this case the 28% decrease in (TF/P) inul. ratios indicates a decreased proximal tubular reabsorption. These results led this group of scientists to suggest that the decrease in fractional reabsorption occurred in the proximal segment and was due to the chronic deficiency of thyroid hormone itself and not to

such changes as GFR, RPF or extracellular volume. In fact Ismael-Beigi and Edelman (19) have reported an effect of T_3 on a sodium reabsorptive mechanism when they showed a 69% increase in Na+ + K+ ATPase activity after three subcutaneous injections of T_3 (50 μg/100 gram b.w.). Furthermore, Na+ + K+ ATPase activities in liver and diaphragm were also significantly increased by administration of T_3 (51). My results confirm the action of T_3 on the Na+ + K+ ATPase activity in the cortex of the kidney. Thyroidectomy produced decreases of 16,24 and 50% in enzyme activity and the injection of T_3 increased enzyme activity by at least 50%. Moreover, at a dose rate of 2 ug/100 gm. b.w./day, T_3 administered to hypothyroid animals for two weeks, increased enzyme activity by 92% (59.3 ± 7.2. v. 112.5 ± 3.8).

The fact that both glucocorticoids and thyroid hormones stimulate Na+ + K+ ATPase activity, responsibile in part for sodium reabsorption in the kidney, led me to investigate the action of T_3 on Na+ + K+ ATPase activity in the presence and absence of corticosterone and conversly the action of corticosterone on this enzyme activity in the presence and absence of T_3. The prospect of some interaction was made more interesting by (1) the work of Edmonds et al (29) who demonstrated that there is little effect of aldosterone on short circuit current in the descending colon of hypothyroid rats until a single small dose of T_3 is given prior to aldosterone infusion and (2) by the suggestion of an inverse relationship between ACTH and TSH secretion (23,26). This proposed

relationship is based on the observation that stress induced ACTH secretion is accompanied by decreased T.S.H. secretion and that T.R.F. induced T.S.H. secretion is associated with a concomittant decrease in stress induced ACTH secretion. In addition, inhibition of ACTH with dexamethasone has been reported to enhance TSH secretion in response to cold or T.R.F.

Both hormones also have in common, a dependency on RNA synthesis and general protein synthetic machinery for their expression. Actinomyosin D, cyclohexamide, and puromycin prevent glucocorticoid and T_3 stimulated increases in numerous enzymes (58,29). Prior to their interaction with the nucleus, these hormones are involved with populations of cytoplasmic receptors (proteins) another possible focus for synergistic interaction (58,60).

By surgically removing the thyroid and adrenal glands from a single animal, then keeping the animal healthy during a period of time sufficient to depress circulating hormone levels, I could then restore T_3 and corticosterone separately and observe their effects on Na+ + K+ ATPase activity. In the cortex, T_3, (50 ug/100 gm b.w.) administered to a thyro-adrenalectomized rat produced the same absolute increase as T_3 administered to a thyroidectomized rat. Corticosterone, 250 ug/100 gm b.w., increased Na+ + K+ ATPase activity to the same extent in a thyroadrenalectomized rat as in an adrenalectomized rat. These results suggest parallel independent pathways for these hormonal effects on the Na+ + K+

ATPase activity. This conclusion is further substantiated by the fact that the percentage increase observed after both T_3 and corticosterone were administered to a thyroadrenalectomized rat was equal to the sum of the increases after administering T_3 and corticosterone individually.

The results in the medulla are not as easily interpreted. Thyroid hormone seems to have little effect on medulla Na+ + K+ ATPase activity. Thyroidectomy produced no significant decrease in enzyme activity and the injection of T_3, (50 ug/100 gm b.w.), to either a thyroidectomized or thyroadrenalectomized rat produced no significant increases. Furthermore, thyroadrenalectomy reduced the activity to the same extent as adrenalectomy (Table 1). The injection of corticosterone, 250 ug/100 gm b.w., stimulated activity only in a thyroadrenalectomized rat, not an adrenalectomized rat ($p = 0.077$). The injection of both T_3 and corticosterone to a thyroadrenalectomized rat produced the same 25% increase as the injection of simply corticosterone, suggesting that corticosterone is singularly involved with medullary Na+ + K+ ATPase activity.

These data provide an explanation for the exaggerated natruretic response of hypothyroid rats but do not indicate the existence of a permissive effect between thyroid hormone and corticosterone on renal Na+ + K+ ATPase activity. Both hormones stimulate this enzyme in the cortex equally as well in the absence of the other. A substantial decrease in activity is found after thyroidectomy and adrenalectomy suggesting that basal amounts of these hormones are necessary for

SUMMARY

1. Thyroidectomy and adrenalectomy decrease Na+ + K+ ATPase activity in the renal cortex crude homogenate.
2. Both T_3 and corticosterone can restore lost cortical Na+ + K+ ATPase activity to normal in thyroidectomized and adrenalectomized rats respectively.
3. The injection of T_3 and corticosterone to a thyroadrenalectomized rat produced an increase in Na+ + K+ ATPase activity similar to the sum of the increases after T_3 and corticosterone are injected individually. This suggests parallel independent pathways for these hormonal effects on ATPase activity.
4. T_3 has little effect on medulla Na+ + K+ ATPase activity. Thyroidectomy produced no significant decrease and thyroadrenalectomy produced the same decrease as adrenalectomy alone.
5. Corticosterone increased Na+ + K+ ATPase activity in a thyroadrenalectomized rat in the medulla crude homogenate. Corticosterone and T_3 together raised Na+ + K+ ATPase activity in the medulla crude homogenate to the same extent as corticosterone alone. This suggests that corticosterone is singularly involved with the medulla Na+ + K+ ATPase.

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