

FACTORS AFFECTING THE SYNTHESIS OF RNA  
FROM EXOGENOUS PRECURSORS IN RAT KIDNEY

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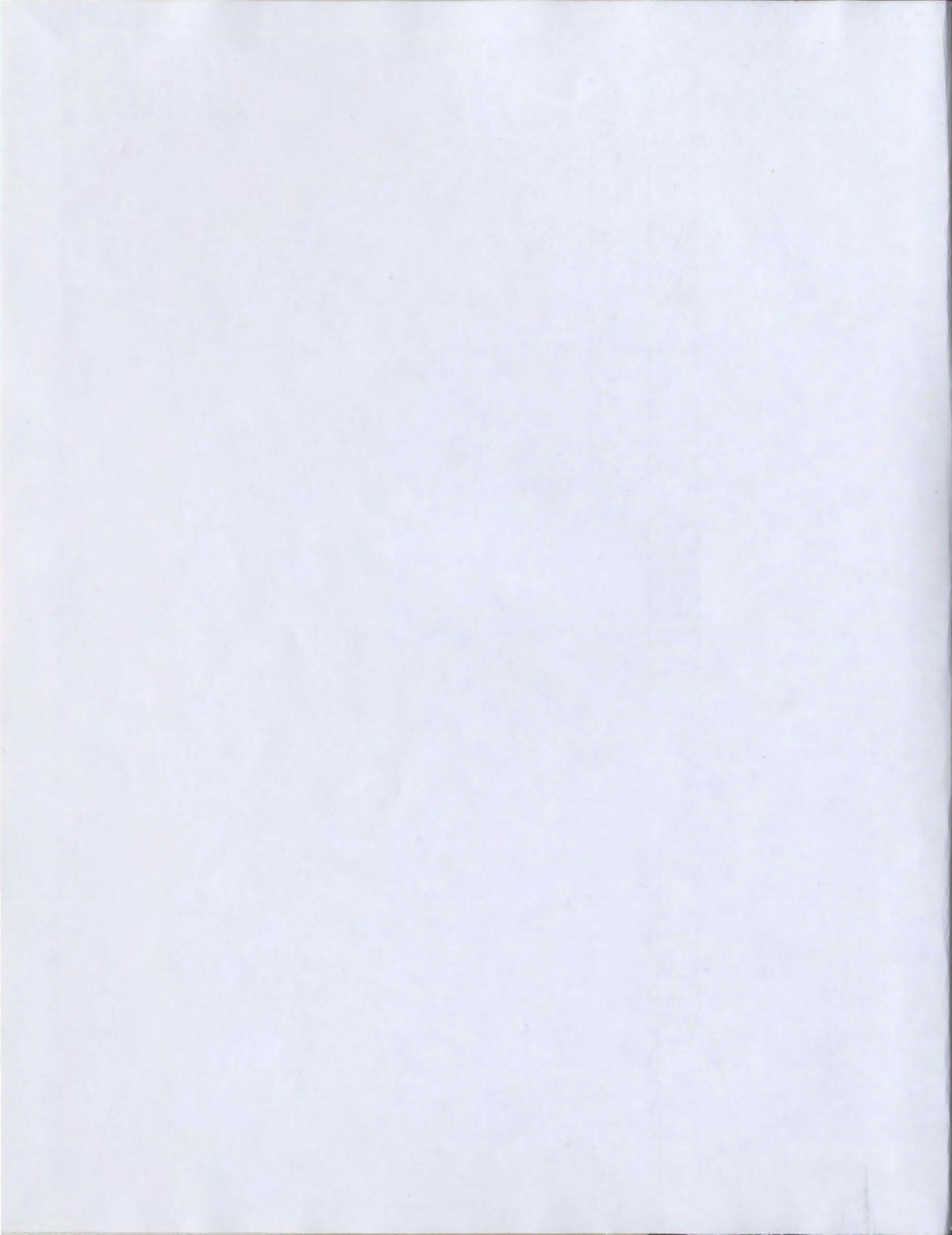
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FACTORS AFFECTING THE SYNTHESIS OF RNA FROM EXOGENOUS PRECURSORS  
IN RAT KIDNEY

A thesis

by



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the requirements of  
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FACTORS AFFECTING THE SYNTHESIS OF RNA FROM EXOGENOUS PRECURSORS  
IN RAT KIDNEY

## ABSTRACT

The effect of adrenalectomy and corticosteroid administration on precursor uptake and RNA synthesis in rat kidney was studied. A number of radioactively labeled precursors ( $^3\text{H}$ -uridine,  $^3\text{H}$ -cytidine, and  $^{14}\text{C}$ -orotic acid) were used to determine the time course of incorporation into the acid soluble pool and the alkaline hydrolyzate fractions of both the normal and adrenalectomized rats. The incorporation of these precursors into both RNA and acid soluble pool was linear for at least 20 minutes.

A general decrease in the rate of incorporation of  $^3\text{H}$ -cytidine and  $^{14}\text{C}$ -orotic acid into the alkaline hydrolyzate fraction of kidneys was observed 7 to 8 days after adrenalectomy as compared to normal animals. Administration of a single dose of aldosterone 1 or 2 hours prior to the isotope, increased the rate of incorporation into acid soluble pool as well as into the alkaline hydrolyzate fraction to a level greater than that in normal rats. Actinomycin D administration 2 hours prior to the  $^{14}\text{C}$ -orotic acid markedly decreased RNA synthesis, but had no significant effect on the uptake of precursor into the acid soluble pool. Actinomycin D plus aldosterone treatment, compared to actinomycin D alone, resulted in an increase in precursor uptake into the acid soluble pool.

These studies suggest that most of the stimulatory effect of aldosterone on the incorporation of exogenous precursor into RNA of rat kidney is attributable to an increase in the rate of precursor uptake. There may also be a genuine stimulation of RNA synthesis as aldosterone increases the RNA:DNA ratio of adrenalectomized rat kidneys to the levels

(ii)

of normal animals.

The effects of single injections of deoxycorticosterone, corticosterone and cortisol on the incorporation of  $^{14}\text{C}$ -orotic acid into the kidneys of adrenalectomized rats were also studied. Deoxycorticosterone, corticosterone stimulated the precursor incorporation into acid soluble pool but no effect was observed on the incorporation into the alkaline hydrolyzate fraction. On the other hand, cortisol administration caused a decrease in the incorporation of precursor into the alkaline hydrolyzate fraction without affecting precursor incorporation into the acid soluble pool, suggesting that it may decrease RNA synthesis in the kidneys of adrenalectomized rats.



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ABBREVIATIONS

ACTH	- adrenocorticotropic hormone
DNA	- deoxyribonucleic acid
RNA	- ribonucleic acid
m-RNA	- messenger ribonucleic acid
r-RNA	- ribosomal ribonucleic acid
TCA-cycle	- tricarboxylic acid cycle
HMP-shunt	- hexose monophosphate shunt
c-AMP	- adenosine 3',5'-cyclic monophosphate
Na-K-ATPase	- sodium, potassium activated adenosine triphosphatase
dpm	- disintegrations per minute
cpm	- counts per minute
TCA	- trichloroacetic acid
Tris	- tris (hydroxymethyl)amino methane
O.D.	- optical density
ADX	- adrenalectomized

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".....Our own wronged flesh  
May work undisturbed, restoring  
The order we try to destroy, the rhythm  
We spoil out of spite: valves close  
And open exactly, glands secrete,  
Vessels contract and expand  
At the right moment, essential fluids  
Flow to renew exhausted cells,  
Not knowing quite what has happened....."

— Nones, W. H. Auden (1)

## INTRODUCTION

Most of the cellular processes which ultimately lead to a variety of secondary manifestations such as growth, differentiation or development are initiated or regulated by hormones (2). Hormones have been defined by many workers (3-8) as chemical substances, secreted in trace amount by specific cells, and transported in the blood circulation to the organs on which their effect is produced without contributing significant amounts of energy or matter to the tissues. Some hormones exert relatively specific effects for specific target cells; other hormones are more general in both action and target.

Hormones are commonly divided into three classes according to their structure, irrespective of their physiological or biochemical functions:

(i) amino acid derivative hormones, for example, epinephrine and thyroxine, (ii) a larger group of steroid hormones, for example, the corticosteroids of the adrenal cortex and the estrogens and androgens of the ovaries and testes, and (iii) the largest group of peptide and protein hormones of which adrenocorticotrophic hormone (ACTH) and insulin are examples.

#### A. GENERAL MECHANISM OF HORMONE ACTION:

It has been shown that hormones exert a regulatory function over the biosynthesis of protein(s) in their respective target organs although the exact mechanism by which they operate is unknown. RNA synthesis has been implicated in the mechanism of action of many hormones; for example, in the action of cortisol on liver (9), cortisone on liver (10), growth hormone on liver (11), testosterone on seminal vesicle (12), ACTH on the adrenal (13) and estrogen on the uterus (14). These observations suggest a hypothetical mechanism, based upon the repressor-inducer model of bacteria (15), by which hormone action is expressed through selective control of m-RNA synthesis (16). Tata (17), however, has reported many observations which are inconsistent with this being the only mechanism of hormone action. Therefore, several theories concerning the mechanism of hormone action have been proposed.

#### B. HORMONE ACTION AT THE NON-TRANSCRIPTIONAL LEVEL:

##### *(i) Control at cell permeability*

Some hormones such as insulin (18) and ACTH (19) appear to control

protein synthesis at the translational level, by increasing the metabolic rate rather than having a direct effect on RNA synthesis. Many other reports that insulin acts at the cell membrane and is involved in membrane transport are consistent with a non-transcriptional role for insulin action (20, 21). Sharp and Leaf (22) suggested that the primary action of aldosterone is on the cell membrane, stimulating the synthesis of protein which acts like a permease in facilitating entry of sodium through the mucosal surface of the tissue.

Means and Hamilton (23, 24) observed an increased  $^3\text{H}$ -uridine uptake in the presence of estrogen as well as increased RNA synthesis in rat uterus following injection of estrogen. Agarwal *et al* (25) measured an increase in precursor uptake in the acid soluble pool of rat liver after cortisone treatment. A similar increase in RNA precursor uptake has also been reported by many other workers (26-28). Miller and Baggett (29) concluded that estrogen primarily increases the incorporation of nucleosides into nucleotide pools in mouse uterus. Billing *et al* (30) were unable to show any increased RNA synthesis by the immature rat uterus until at least 5 hours after administration of estradiol when corrections were made for increased uptake of RNA precursors. It appears from these studies that one of the mechanisms of hormone action is to increase metabolite concentration by increasing transport across the cell membrane.

*(ii) Post-transcriptional Control*

Tomkins *et al* (31) proposed a "translational repressor" mechanism to explain the superinducibility by actinomycin D of dexamethasone-induced

tyrosine transaminase. A marked increase in enzyme activity was observed following dexamethasone administration to hepatoma cell cultures, without detecting any increase in total cellular RNA or its rate of synthesis from labeled precursors (32). Reel and Kenney (33) reported that the superinduction resulted from inhibition of transaminase degradation by actinomycin D rather than an increased rate of synthesis. Thus it is still unclear and controversial whether translation repressors exist, and what their nature might be.

Studies of cellular ultrastructural changes reveal another possible category of translational control. Palmiter *et al* (34), using physical and electron microscopic techniques studied the organization of ribosomes into polysomes in the chick oviduct when either estrogen or progesterone was administered. An early assembly of polysomes was observed from preexisting ribosomes before new ribosomes entered the cytoplasm. These authors related the increased rate of protein synthesis during stimulation with an organization of ribosomes into polysomes. This observation is similar to the prolactin induced polysome assembly in rabbit mammary tissue (35) and ultrastructural changes of differentiating explants in response to hormones (36). Henshaw *et al* (37) have proposed that membrane bound ribosomes are more active than free ribosomes, which might be a mechanism to control rates and types of protein synthesized (38).

Another type of post-transcriptional control has been shown to involve RNA transport from the nucleus to the cytoplasm. Spirin (39) suggested that m-RNA may transfer from the nucleus to the cytoplasm as nucleoprotein complex (or informosomes) which provides a potential translational control point.

### C. HORMONE ACTION AT THE TRANSCRIPTIONAL LEVEL:

The most often observed result of hormone administration is a marked increase in RNA synthesis. There is still controversy over which type of RNA is synthesized during hormone action. Some investigators argue for hormone action through selective stimulation of new m-RNA (16, 40), while Tata (17) views hormone action as resulting from the synthesis of all types of RNA and especially new highly active ribosomes. This latter view of hormone action has now been more widely accepted. Jackson and Sells (41) observed no increase in m-RNA without similar increase in all classes of RNA. They suggested m-RNA and r-RNA increase in a parallel manner. A similar increase in all classes of RNA has also been reported after cortisol (42) and aldosterone (43) administration. Enhanced synthesis of ribosomal RNA has also been reported with the administration of estrogen, testosterone, growth hormone and thyroid hormones (44).

On the other hand considerable evidence has been reported regarding selective m-RNA synthesis in response to hormone action (40, 45, 46). These investigators consider that RNA which is rapidly labeled (40), extracted by hot detergent phenol (42), or high pH (46), and which is similar to DNA in composition and able to stimulate amino acid incorporation in a cell free system (47) to be m-RNA. Hydrocortisone stimulates  $^{32}\text{PO}_4$  incorporation into the hot phenol extracted RNA (46) and  $^3\text{H}$ -orotic acid incorporation into the high pH extracted RNA (45).

O'Malley and McGuire (48), using RNA-DNA hybridization, were able to show new RNA species in the chick oviduct 6 hours after progesterone administration and before ovidin synthesis. The evidence in support of

new m-RNA synthesis does not exclude additional effects on ribosome synthesis.

#### D. EFFECT OF ADRENAL INSUFFICIENCY:

Adrenal glands are vital to the maintenance of life in many living organisms. Bilateral removal of these glands results in profound physiological and biochemical changes (49-51). A variety of symptoms such as loss of appetite, muscle weakness, polyuria, low blood pressure, diarrhea, and hypoglycemia appears within a few days, depending on species. One of the most important effects of adrenal insufficiency is the failure of the kidneys to retain sodium and excrete potassium (52-55). As a result, the plasma level of potassium rises and the sodium level falls (56-60).

Unless the effects are reversed, death occurs within a few days to several weeks depending on species. The life of an adrenalectomized animal can be prolonged by supplying 1% sodium chloride in the drinking water or by administering adrenal hormones. Both of these treatments appear to reverse all the aforementioned effects.

The steroid hormones secreted by the adrenal gland may be divided into two classes: Glucocorticoids and mineralocorticoids. The most common glucocorticoids found are cortisol (man and dog) and corticosterone (man, dog and rat) (61-63). The most potent mineralocorticoid in man, dog, and rat is aldosterone (64-66).

These hormones also show some overlap in their activity; for example, cortisol and corticosterone have been shown to exert mineralocorticoid



activity whereas aldosterone shows some glucocorticoid activity (67).

#### E. ADRENOCORTICOSTEROIDS AND LYMPHOID TISSUES:

Adrenocorticosteroids have also been shown to cause involution of lymphoid tissues (68-69). Thymic involution in the rat was reported after the administration of both steroid and non-steroid hormones (70) and Mendelson and Finland (71) reported a marked decrease in the average weight of the spleen in cortisol treated mice as compared to the control animals.

These hormones have also been shown to inhibit DNA and RNA synthesis in lymphoid tissue. Knutson and Lundin (72) reported a significant decrease in the incorporation of tritiated thymidine into DNA of the thymus and spleen following cortisone treatment. A prolonged treatment of mice with cortisol or ACTH results in a decreased incorporation of labeled nucleotides into nucleic acids of the lymph nodes, thymus and spleen (73). Similarly, in the rat DNA synthesis in these tissues decreases following cortisol injection (74). RNA synthesis in isolated rabbit lymph nodes (75) and in rat thymocytes (76) is also rapidly inhibited following the administration of cortisol.

#### F. EFFECT OF ALDOSTERONE-PROPOSED MECHANISM OF ACTION:

The effect of aldosterone on sodium transport has been established in kidney and intestine (77, 78), sweat glands (79, 80), salivary glands (81), and anuran epithelia such as toad bladder (82). In addition,

aldosterone has also been shown to alter the electrolyte movement in human erythrocytes (83), in rat brain (84), and in human laryngeal carcinoma cells (85) cultured *in vitro* .

(i) *Effect on isolated toad bladder*

In 1961 Crabbé (86) observed an effect of aldosterone on sodium transport in isolated toad bladder. A latent period of about 60 to 90 minutes preceded the effect on sodium transport (87), and the duration of this latent period could not be reduced by increasing the concentration of aldosterone (86). This latent period coincides with a proposed early increase in RNA and protein synthesis following the accumulation of tritiated aldosterone in toad bladder nuclei (88-91). Rousseau and Crabbé (92) observed stimulation of a rapidly labeled RNA fraction following administration of aldosterone. Pretreatment of toad bladder with actinomycin D or puromycin decreased the effect of aldosterone on sodium transport (88, 89). The inhibitory action of actinomycin D was only observed if the tissue was exposed to inhibitor prior to the administration of aldosterone (93), suggesting that RNA and protein synthesis must precede the effect of aldosterone on sodium transport and are not secondary to intracellular sodium concentration changes (94).

Recently Rousseau and Crabbé (95) showed an increase in total nuclear RNA synthesis and  $^3\text{H}$ -uridine uptake of toad bladder after aldosterone administration but no effect was observed on cytoplasmic RNA turnover rate, m-RNA synthesis and on the pattern of protein synthesis. Sharp and

Komack (96) could not detect any stimulation of RNA metabolism unless a concentration of aldosterone 100-fold greater than that required to stimulate sodium transport was used. These studies suggest that the proposal that the effect of aldosterone on sodium transport is mediated via RNA and protein synthesis in toad bladder remains on indirect evidences.

In the toad bladder, the stimulation of sodium transport by aldosterone has also been shown to depend upon the availability of specific metabolic substrates (97, 98). Pyruvate, or compounds which yield pyruvate, enhance sodium transport in the presence of aldosterone whereas acetate and other tricarboxylic acid cycle (TCA-cycle) intermediates fail to stimulate this effect. The significance of the effect of various substrates on aldosterone enhanced sodium transport is not yet clear. Kirsten *et al* (99) have shown that the activities of the TCA-cycle enzymes are increased following the addition of aldosterone. The increases in the level of these enzyme activities was correlated with the increase in sodium transport.

Recently Kirchberger *et al* (98, 100) showed a correlation between the effect of aldosterone on sodium transport and the decrease in evolution of  $^{14}\text{CO}_2$  from 1- $^{14}\text{C}$ -glucose\* in toad bladder. The decrease in evolution of  $^{14}\text{CO}_2$  persists even in the absence of sodium and has characteristics similar to those of sodium transport with regard to the time of onset, concentration response, steroid specificity and sensitivity to

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\* Due to the combined action of the Embden-Meyerhof pathway and the tricarboxylic acid cycle,  $^{14}\text{CO}_2$  from 1- $^{14}\text{C}$ -glucose and 6- $^{14}\text{C}$ -glucose is released at equal initial rates, whereas the HMP shunt pathway initially yields  $^{14}\text{CO}_2$  only from 1- $^{14}\text{C}$ -glucose.

actinomycin D and spirolactone. It was suggested that the stimulation of sodium transport and the inhibition of the hexose monophosphate shunt (HMP shunt) pathway appears to be an effect of mineralocorticoids. In addition, these effects of aldosterone to decrease the evolution of  $^{14}\text{CO}_2$  from 1- $^{14}\text{C}$ -glucose and to stimulate sodium transport have been shown to be reproduced by adenosine 3',5'-cyclic monophosphate (c-AMP) (101). It was suggested that c-AMP might mediate the action of aldosterone, although the authors were not able to detect any change in the tissue concentration of c-AMP even after prolonged incubation with the hormone.

Hill *et al* (102) found no effect of aldosterone on the Na-K-ATPase activity of toad bladder, confirming earlier reports of Sharp and Leaf (97) and Bonting and Canady (103). The authors suggested that aldosterone induced stimulation of sodium transport in toad bladder does not appear to involve a change in the activity of Na-K-ATPase, nor its dependence on sodium.

*(ii) Effect on kidney:*

The effects of aldosterone on renal function are also well known (104-107), to increase the removal of sodium from and addition of potassium to the glomerular filtrate, presumably by affecting active transport or exchange of these ions. Aldosterone has been shown to increase the sodium resorption at the distal tubule of the nephron (108, 109). There is also a lag period of 30 to 90 minutes before an effect of aldosterone on sodium resorption is observed (104). It has been suggested that the action of aldosterone is preceded by a stimulation in the synthesis

of RNA and protein (110-112). Williamson *et al* reported that actinomycin D significantly inhibits the antinatriuretic action of aldosterone (113). However, it has also been shown that actinomycin D did not inhibit the kaliuretic response to aldosterone despite the similarity in the time course of the effect on sodium and potassium excretion (112, 113). A number of reports have appeared on the effect of aldosterone on renal RNA synthesis (110, 111). The incorporation of precursors into RNA is enhanced by aldosterone before the effect on urinary sodium is achieved. Moreover, the enhancement is inhibited by actinomycin D. Forte and Landon (43) observed an increase in the synthesis of all species of renal RNA. It is still not clear how aldosterone produces a specific effect if all species of RNA are stimulated. Recently Congote and Trachewsky (114) using RNA-DNA hybridization technique demonstrated a qualitative change in nuclear RNA of rat kidney cortex after aldosterone administration. They observed a specific increase in the hybridization activity of nuclear RNA with repetitive DNA.

(115)

Liu *et al* observed a decrease in the specific activities of some mitochondrial enzyme of rat kidney after adrenalectomy which can be restored by aldosterone treatment. Similar results have been reported by Kinne and Kirsten (116). These findings are similar to the reported increase in the activity of TCA-cycle enzymes in toad bladder after aldosterone administration (99), and also support Edelman's view that aldosterone acts via a metabolic pathway (88).

Jørgensen (117) has related the reduction in Na-K-ATPase activity to the rate of change in sodium and potassium concentration in plasma during developing adrenal insufficiency. He reported that a high sodium

intake can prevent the reduction of enzymatic activity and suggested that Na-K-ATPase is partly influenced by the sodium concentration in plasma.

Kartz and Epstein (118, 119) observed an increase in Na-K-ATPase activity when sodium resorption was increased after feeding rats with high protein diet and that Na-K-ATPase activity was reduced when sodium transport was diminished following bilateral adrenalectomy. Chignell and Titus (120) showed a decrease in the activity of Na-K-ATPase after adrenalectomy. These authors observed no change in enzyme activity after 3 hours of aldosterone treatment. Landon *et al* (121) reported a decrease in Na-K-ATPase activity in dialyzed kidney membrane of adrenalectomized and normal rats treated with aldosterone. The enzyme activity was restored to normal level in adrenalectomized rats treated with a high concentration of aldosterone. Jørgensen (122) observed that the repeated injection of aldosterone increases the activity of Na-K-ATPase in the microsomal fraction from whole kidney. These results are consistent with an increase in resorption of sodium following repeated injection of aldosterone to adrenalectomized rats.

These results in rat kidneys are inconsistent with the reported findings in toad bladder, where no effect on the activity of Na-K-ATPase was observed after aldosterone administration (97, 102, 103).

Recently Knox and Sen (123), using double label technique, reported an increase in Na-K-ATPase protein. They suggested that Na-K-ATPase may be one of the aldosterone induced proteins required for enhanced active sodium transport. These results are consistent with the hypothesis that aldosterone produces the antinatriuretic action via protein synthesis.



#### STATEMENT OF PROBLEM

It is apparent from the introductory discussion that the kidneys of adrenalectomized rats are deficient in sodium retention. This function can be largely restored by the injection of aldosterone and to a smaller extent by other adrenocorticosteroids. The action of aldosterone appears to be mediated via RNA and protein synthesis as it can be blocked by actinomycin D and puromycin. Previous workers (43, 111, 112) have also shown that aldosterone stimulates the incorporation of radioactive precursors into RNA and this has been interpreted as meaning that RNA synthesis is stimulated. However, it has become increasingly clear that hormones can modify pool sizes (124) and the rates of precursor uptake (26, 27, 29, 125); consequently, the rate of incorporation of exogenous precursors is not necessarily a valid measure of the rate of RNA synthesis.

We, therefore, examined the effect of adrenalectomy and of adrenocorticosteroids treatment on precursor uptake and acid soluble pool as well as its effect on the incorporation of precursor into acid insoluble material in an attempt to decide how much of the apparent effect is due to the pool size and uptake effects and how much represents a genuine stimulation of RNA synthesis.

## MATERIALS AND METHODS

### ANIMALS:

Male Sprague-Dawley rats, weighing 100 to 150 gms were used in these experiments unless otherwise indicated. Animals were obtained from the Medical School, Memorial University of Newfoundland, St. John's, Newfoundland, or from the Canadian Breeding Laboratories, St. Constant, Quebec. Bilateral adrenalectomy was performed by the dorsal route and adrenalectomized animals were maintained on 1% sodium chloride and Purina Laboratory Chow *ad libitum*. Adrenalectomized rats were used 7 to 8 days after being operated upon unless otherwise indicated.

### CHEMICALS:

All hormones used in this work, with the exception of aldosterone, were obtained from Sigma Chemical Co., St. Louis, Mo. Aldosterone was obtained from E. M. Reagent Div., Brinkmann Instruments Inc., Westbury, N. Y. Protosol (Tissue solubilizer) and Aquasol (Liquid scintillation counting cocktail) were purchased from New England Nuclear, Boston, Mass. Bio-solv. solubilizer BBS<sub>2</sub> and BBS<sub>3</sub> were obtained from Beckman Instruments Inc., Fullerton, California. Actinomycin D (from *Streptomyces chrysomallus*) was obtained from Sigma Chemical Co., St. Louis, Mo. All other chemicals, unless otherwise indicated were obtained from J. T. Baker Chemical Co., Phillipsburg, N. J., or Sigma Chemical Co., St. Louis Mo. All chemicals were of the analytical grade purity.

5-<sup>3</sup>H-Uridine, specific activity 25.4 curies per millimole and <sup>3</sup>H-water,

specific activity 1 millicurie per gram were purchased from New England Nuclear, Boston, Mass. 5-<sup>3</sup>H-Cytidine, specific activity 1 curie per millimole, 6-<sup>14</sup>C-Orotic acid, specific activity 60.8 millicurie per millimole and <sup>14</sup>C-Toluene, activity  $7.15 \times 10^5$  dpm per gram were purchased from Amersham/Searle, Arlington Heights, Illinois.

#### PREPARATION OF SOLUTIONS:

##### (i) Buffer---

Tris/KCl - MgCl<sub>2</sub> buffer, pH 7.4

0.04 M Tris, 0.1 M KCl, 0.004 M MgCl<sub>2</sub>, adjusted to pH 7.4 with concentrated HCl.

##### (ii) Hormones---

Aldosterone and deoxycorticosterone were prepared in a small amount of 95% ethanol and were diluted with 0.9% saline to appropriate concentrations.

Cortisol and corticosterone were prepared in 0.9% saline and were used as saline suspensions.

#### USE OF DIFFERENT LABELED PRECURSORS IN TIME COURSE STUDIES:

In these studies animals were divided into two groups: normal and adrenalectomized. In one series of experiments, each animal received an intraperitoneal injection of <sup>3</sup>H-uridine (25 µCi/100 g body weight) 5, 10, 20, 30, 40, or 75 minutes before sacrifice. In another series, each animal received an intraperitoneal injection of <sup>3</sup>H-Cytidine (25 µCi/100 g

body weight) or  $^{14}\text{C}$ -orotic acid (5  $\mu\text{Ci}/100$  g body weight) 5, 10, 20, 40 or 75 minutes before sacrifice.

#### STUDIES WITH HORMONES:

In these experiments, animals were divided into three groups: normal, adrenalectomized and adrenalectomized plus hormone treated. The animals were injected intraperitoneally with either hormone or with saline 1 or 2 hours prior to the tracer injection. The following doses of hormones were used: aldosterone (5  $\mu\text{g}/100$  g body weight), deoxycorticosterone (100  $\mu\text{g}/100$  g body weight), cortisol (2 mg/100 g body weight), corticosterone (2 mg/100 g body weight).

In one series of experiments each animal received an intraperitoneal injection of  $^3\text{H}$ -cytidine (25  $\mu\text{Ci}/100$  g body weight) 20 minutes before sacrifice.

In another series each animal received an intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5  $\mu\text{Ci}/100$  g body weight) 20 minutes before sacrifice. The 20 minutes time for isotope incorporation in these experiments was taken on the basis of time course studies. It was observed that the incorporation of  $^3\text{H}$ -cytidine and  $^{14}\text{C}$ -orotic acid into both RNA and the acid soluble pool was linear for 20 minutes but not beyond this time.

#### STUDIES WITH ACTINOMYCIN D AND ALDOSTERONE:

In these experiments, adrenalectomized rats were injected intraperitoneally with 0.9% saline, aldosterone (5  $\mu\text{g}/100$  g body weight), actinomycin D (50  $\mu\text{g}/100$  g body weight) or actinomycin D (50  $\mu\text{g}/100$  g body weight) plus aldosterone (5  $\mu\text{g}/100$  body weight) 2 hours prior to the isotope injection.

All animals received intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5  $\mu\text{Ci}$ /100 g body weight) 20 minutes before sacrifice.

TISSUE HOMOGENIZATION AND ISOLATION OF DIFFERENT FRACTIONS:

To isolate the acid soluble pool and the alkaline hydrolyzate fraction (RNA fraction), the following procedure was employed. Animals were sacrificed by cervical dislocation at the designated time. Kidneys were removed quickly and frozen in liquid nitrogen. All subsequent steps were carried out at  $0 - 4^{\circ}$  unless otherwise indicated. Frozen kidneys were homogenized in 4 ml of Tris-KCl-MgCl<sub>2</sub> buffer, pH 7.4 (126) in a Teflon glass homogenizer which was washed twice with 1.5 ml of buffer. The final volume of homogenate was made to 9 ml with buffer. 0.2 ml of 50% TCA was added to a sample (0.8 ml) of homogenate which was then diluted with 3.5 ml of 5% TCA and centrifuged for 10 minutes at 2500 rpm in an International model HN-S centrifuge in the cold room, to give acid soluble and acid insoluble fractions. The acid insoluble pellet was washed twice with 2 ml of 5% TCA, and the supernatants were combined to give the acid soluble fraction.

The lipids were then removed from the acid insoluble fraction by suspending and centrifuging in 5 ml each of ethanol, chloroform-ethanol (2:1) and ether (126). The acid insoluble defatted residue was hydrolyzed in 2.5 ml 0.3 M KOH at  $37^{\circ}$  for 16-18 hr and then acidified with 1 ml of 50% TCA. The soluble portion of this alkaline hydrolyzate was collected after centrifuging for 10 minutes at 2500 rpm. The residue was washed twice with 1 ml of 5% TCA, and the supernatants were combined to give

alkaline hydrolyzate fraction, an aliquot of which was assayed for radioactivity and RNA content.

The DNA was isolated from 0.1 ml of homogenate by Schneider method (127). The 0.1 ml homogenate was diluted with 1.9 ml of 5% TCA and heated for 15 minutes at 90°. The supernatant was collected after centrifugation and was used for DNA determination.

#### DETERMINATION OF RNA AND DNA:

An 0.5 ml aliquot of alkaline hydrolyzate was used for RNA determination. The RNA was determined by the orcinol method (128), using yeast RNA as standard.

The DNA was determined in 1 ml of extracted supernatant with diphenylamine reagent (129), using calf thymus DNA as standard.

#### MEASUREMENT OF OPTICAL DENSITY:

An 0.5 ml aliquot of acid soluble fraction was diluted to 2 ml with 5% TCA and optical density was measured at 260 m $\mu$  against 5% TCA in a Beckman DB-G spectrophotometer.

#### DETERMINATION OF RADIOACTIVITY:

Total radioactivity was measured on 0.1 ml aliquots of homogenate, solubilized with 0.4 ml of Protosol in a glass counting vial at 55° for 2 hours. The vials were brought to room temperature and the pH was adjusted to between 6 and 7 with 0.02 ml concentrated HCl. Samples were counted after adding 15 ml of Aquasol. The total radioactivity was expressed as counts per minute per mg of DNA. For measurements of radioactivity in the acid soluble pool and the alkaline hydrolyzate fraction, 0.2 ml aliquot of these fractions were counted in Aquasol. The results for acid soluble fraction and acid insoluble fraction were expressed as counts per minute per O.D. at 260 m $\mu$  and counts per minute per mg of DNA respectively. The calculation in each case was corrected for 9 ml of original homogenate.

All radioactive samples were counted in a liquid scintillation spectrophotometer (Intertechnique Model SL30). Quench corrections were performed by recounting the samples after addition of known amounts of  $^3\text{H}$ -water and  $^{14}\text{C}$ -toluene as internal standard. Counting efficiencies and background counts were as follows: for  $^{14}\text{C}$ , 73 - 76 %, 58 - 60 cpm; for  $^3\text{H}$ , 26 - 28 %, 27 - 33 cpm.

USE OF DIFFERENT CONDITIONS TO DETERMINE THE TOTAL TISSUE AND ALKALINE HYDROLYZATE FRACTION RADIOACTIVITY:

Several conditions were employed to isolate the total and alkaline hydrolyzate fraction radioactivity. In these experiments, the animals received an intraperitoneal injection of  $^3\text{H}$ -uridine (25  $\mu\text{Ci}$ /100 g body weight) 20, 40, or 150 minutes before sacrifice.

In one experiment, the method employed was similar to that described above except that the total counts were measured with or without Protosol treatment and 0.2 N NaOH or 0.3 M KOH was used to isolate the alkaline hydrolyzate fraction.

In another experiment, a number of solubilizers were employed to measure the total radioactivity from an aliquot of tissue homogenate. The different conditions used were as follows:

A. Use of Protosol, BBS<sub>2</sub>, or BBS<sub>3</sub>

0.1 ml aliquots of homogenate were solubilized with 0.4 ml of Protosol, BBS<sub>2</sub> or BBS<sub>3</sub> in a glass counting vial at 55° for 2 hours. The vials were brought to room temperature. The pH was adjusted to between 6 and 7 with concentrated HCl in the Protosol treated vials. Samples were counted after adding 15 ml of Aquasol.

B. Use of Hot TCA:

A 0.1 ml aliquot of homogenate was diluted with 0.4 ml of 20% TCA. The suspension was heated to 95° for 15 minutes, cooled and counted by adding 15 ml of Aquasol.



## RESULTS

### VALIDITY OF METHOD USED:

Table I shows the results obtained for the total tissue and alkaline hydrolyzate fraction radioactivity under different conditions.

The use of Protosol treatment\* to solubilize an aliquot of tissue homogenate for the measurement of total tissue counts appears to be necessary because the total counts were less than the total of acid soluble pool plus alkaline hydrolyzate fraction counts when Protosol treatment was avoided. The recovery of total counts with Protosol treatment was from 97 to 102 percent (Table I).

Table II shows the results obtained by the use of different solubilizer for the isolation of total tissue radioactivity.

It is clear that the total counts obtained with  $BBS_2$ ,  $BBS_3$  or hot TCA treatment are less than the total counts obtained with Protosol treatment.

The recovery of total counts with Protosol treatment was about 97 to 101 percent of the total acid soluble pool plus alkaline hydrolyzate fraction counts. There was no significant difference in efficiencies and in background counts by using different methods of sample preparation.

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\* There is a drawback by using Protosol in that it takes at least 48 hours to give constant counts (there might be a reaction between Protosol and Aquasol). However this can be overcome by adjusting the pH to between 6 and 7 with concentrated HCl before the addition of Aquasol (as has been mentioned in Materials and Methods).

TABLE I  
EFFECT OF DIFFERENT CONDITIONS ON THE ISOLATION OF TOTAL TISSUE AND ALKALINE HYDROLYZATE  
FRACTION RADIOACTIVITY OF NORMAL RAT KIDNEYS

Animal Number	<u>†Total counts</u>		††Difference (%)	<sup>a</sup> Acid soluble fraction counts	<sup>b</sup> Alkaline hydrolyzate fraction counts		Total acid soluble plus alkaline hydrolyzate fraction counts	*Total recovery (%)
	+Protosol treatment	-Protosol treatment			NaOH treatment	KOH treatment		
A	292320	235550	-18	286791	10813	-	297604	102
B	270900	200830	-26	253515	9833	-	263348	97
C	301000	235550	-22	283865	-	12537	296402	98
D	290290	229950	-21	271119	-	9011	280130	97

All animals received an intraperitoneal injection of <sup>3</sup>H-uridine (25 µCi/100 g body weight) 150 minutes before sacrifice.

† In the case of +Protosol, 0.1 ml of homogenate was solubilized with 0.4 ml Protosol as described in Materials and Methods. In the case of -Protosol, samples were counted without Protosol treatment.

<sup>a</sup> Acid soluble radioactivity is that which was not precipitated by TCA treatment.

<sup>b</sup> Alkaline hydrolyzate radioactivity is measured by hydrolyzing the acid insoluble residue with alkali as indicated.

†† Difference (%) indicates the percentage difference of counts between Protosol and no Protosol treatment.

\* Total recovery was calculated by comparing the sum of acid soluble plus alkaline hydrolyzate fraction counts with the total counts obtained by Protosol treatment of the homogenate.

Table II

EFFECT OF DIFFERENT SOLUBILIZERS ON THE MEASUREMENT OF TOTAL TISSUE RADIOACTIVITY  
OF NORMAL RAT KIDNEYS

Protosol treatment	BBS <sub>2</sub> treatment	Total Counts		Difference (%)	Hot TCA treatment	Difference (%)	Acid soluble fraction Counts	Alkaline hydrolyzate fraction Counts	Acid soluble plus alkaline hydrolyzate fraction Counts	Total recovery (%)
		BBS <sub>3</sub> treatment	Difference (%)							
6830	678600	595260	-8	-19	671310	-9	699226	47815	747041	101
1840	466110	416250	-7	-17	430380	-14	458095	27156	485251	97

peritoneal injection of <sup>3</sup>H-uridine (25μCi/100 g body weight) at the time indicated.

, BBS<sub>3</sub>, or Hot TCA treatment, 0.1 ml of homogenate was solubilized with 0.4 ml of respective solubilizer as mentioned in Materials

w and have been subtracted in all cases.

the percentage difference of Counts obtained between the Protosol treatment and the other extraction procedure. The Counts extraction procedure were compared with the Protosol treatment because of high recovery of Counts by the Protosol treatment.

Table II

EFFECT OF DIFFERENT SOLUBILIZERS ON THE MEASUREMENT OF TOTAL TISSUE RADIOACTIVITY  
OF NORMAL RAT KIDNEYS

Animal number	Time (in minutes) after injection	Total Counts							Acid soluble fraction Counts	Al hyd fr C
		Protosol treatment	BBS <sub>2</sub> treatment	Difference (%)	BBS <sub>3</sub> treatment	Difference (%)	Hot TCA treatment	Difference (%)		
A	20	736830	678600	-8	595260	-19	671310	-9	699226	4
B	40	501840	466110	-7	416250	-17	430380	-14	458095	2

Animals received an intraperitoneal injection of <sup>3</sup>H-uridine (25μCi/100 g body weight) at the time indicated.

In case of Protosol, BBS<sub>2</sub>, BBS<sub>3</sub>, or Hot TCA treatment, 0.1 ml. of homogenate was solubilized with 0.4 ml of respective solub and Methods.

Background counts were low and have been subtracted in all cases.

Difference (%) indicates the percentage difference of Counts obtained between the Protosol treatment and the other extraction obtained by the different extraction procedure were compared with the Protosol treatment because of high recovery of Counts

From these results it can be concluded that the Protosol treatment is the best condition for solubilizing the aliquot of tissue homogenate to measure the total radioactivity and 0.2 N NaOH or 0.3 M KOH can be used for the isolation of alkaline hydrolyzate fraction radioactivity. There was no significant difference in the counts of alkaline hydrolyzate fraction by hydrolyzing the acid insoluble residue with 0.2N NaOH or 0.3 M KOH (Table 1).

## TIME COURSE STUDIES WITH DIFFERENT LABELED PRECURSORS

### (A) STUDIES WITH $^3\text{H}$ -URIDINE

Table III shows the time course of incorporation of  $^3\text{H}$ -uridine into different fractions of normal rat kidneys. It is clear that the incorporation of uridine is linear for the first 20 minutes but decreases beyond this time in all fractions (Figure 1).

A similar type of time course of  $^3\text{H}$ -uridine incorporation was observed in kidneys of adrenalectomized rats (Table IV). The maximum incorporation was observed at 20 minutes in all fractions but decreases after this time (Figure 2).

It is rather difficult to compare the magnitude of  $^3\text{H}$ -uridine incorporation into kidneys of normal and adrenalectomized rats because of the small difference and inconsistent incorporation at different times after isotope injection. However it is important to note that the incorporation pattern was similar in kidneys of both normal and adrenalectomized rats (Figures 1, 2).

### (B) STUDIES WITH $^3\text{H}$ -CYTIDINE

Table V shows the time course of incorporation of  $^3\text{H}$ -cytidine into different fractions of normal rat kidneys. There is an increase in incorporation of  $^3\text{H}$ -cytidine into different fractions for at least 40 minutes. Beyond this time incorporation plateaus (Figure 3).

A similar time course of  $^3\text{H}$ -cytidine incorporation was observed in adrenalectomized rat kidneys (Table VI). The maximum incorporation was

TABLE III

TIME COURSE OF INCORPORATION OF  $^3\text{H}$ -URIDINE INTO DIFFERENT FRACTIONS  
OF NORMAL RAT KIDNEYS

Time in Minutes (after injection of isotope) <sup>a</sup>	Total counts (cpm/mg. DNA) <sup>b</sup> $\times 10^{-3}$	Acid soluble fraction (cpm/unit O.D. <sub>260</sub> mμ) <sup>b</sup> $\times 10^{-2}$	Alkaline hydrolyzate fraction (cpm/mg. DNA) <sup>b</sup> $\times 10^{-2}$	
	A.	B.	C.	C/A
5 <sub>(2)</sub>	25.99 ± 12.67	21.76 ± 10.33	4.81 ± 3.25	1.9%
10 <sub>(2)</sub>	50.20 ± 0.98	38.22 ± 1.93	8.19 ± 0.74	1.6%
20 <sub>(4)</sub>	94.62 ± 10.49	74.93 ± 5.06	22.81 ± 1.19	2.2%
30 <sub>(2)</sub>	81.19 ± 5.92	54.10 ± 7.57	19.58 ± 2.04	2.4%
40 <sub>(2)</sub>	48.97 ± 2.20	40.89 ± 1.35	11.92 ± 2.30	2.4%
75 <sub>(2)</sub>	46.20 ± 2.20	35.36 ± 0.42	12.53 ± 2.48	2.7%

All animals received an intraperitoneal injection of 5- $^3\text{H}$ -uridine (25 μCi/100 g body weight) at the time indicated.

<sup>a</sup> Number in parenthesis indicates the number of animals used.

<sup>b</sup> Mean ± standard error.

Figure 1.

Time course of incorporation of  $^3\text{H}$ -uridine into the kidneys of normal rat.

All animals received an intraperitoneal injection of  $^3\text{H}$ -uridine (25  $\mu\text{Ci}/100\text{ g}$  body weight) at the designated times before sacrifice. The different fractions were separated as mentioned in Materials and Methods.

Specific activity:

●—● Total counts (cpm/mg DNA)  $\times 10^{-3}$

★—★ Acid soluble fraction (cpm/unit O.D. 260  $\text{m}\mu$ )  $\times 10^{-2}$

⊙—⊙ Alkaline hydrolyzate fraction (cpm/mg DNA)  $\times 10^{-2}$



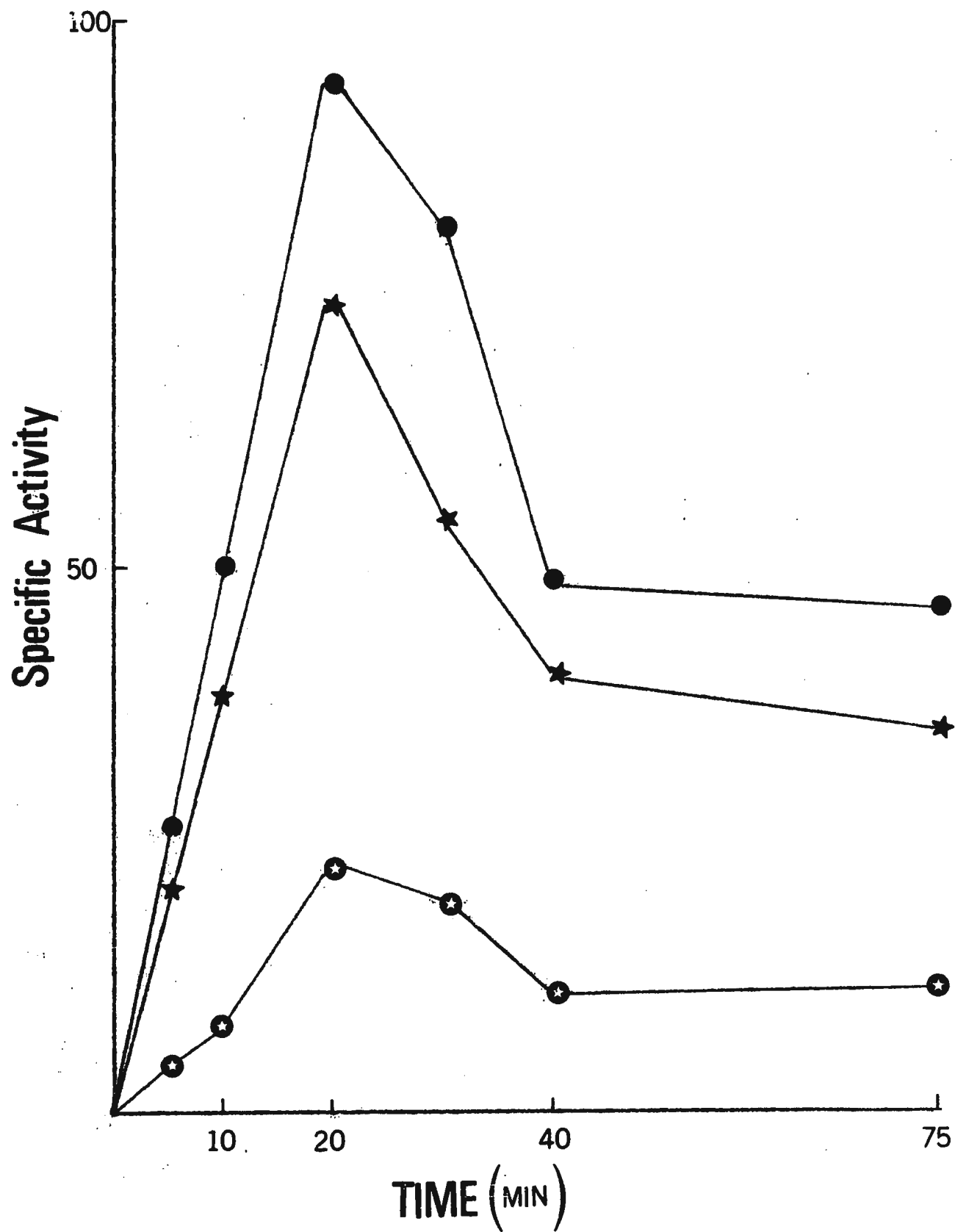


FIGURE 1

TABLE IV  
TIME COURSE OF INCORPORATION OF  $^3\text{H}$ -URIDINE INTO DIFFERENT FRACTIONS  
OF ADRENALECTOMIZED RAT KIDNEYS

Time in minutes (after injection of isotope) <sup>a</sup>	Total counts <sub>b</sub> (cpm/mg. DNA) $\times 10^{-3}$	Acid soluble fraction <sub>b</sub> (cpm/unit O.D. 260 mμ) $\times 10^{-2}$	Alkaline hydrolyzate fraction <sub>b</sub> (cpm/mg. DNA) $\times 10^{-2}$	C/A % incorporated
	A.	B.	C.	
5 (2)	58.54 ± 8.92	47.19 ± 2.12	11.13 ± 0.54	1.9%
10 (2)	73.53 ± 2.51	67.69 ± 9.15	16.95 ± 0.03	2.3%
20 (3)	88.69 ± 6.66	75.04 ± 2.06	19.65 ± 1.00	2.2%
30 (3)	75.51 ± 10.13	58.47 ± 3.96	19.14 ± 3.36	2.5%
40 (2)	62.61 ± 10.74	54.78 ± 0.30	18.36 ± 3.47	2.9%
75 (2)	55.49 ± 9.65	32.95 ± 2.10	16.76 ± 3.05	3.0%

Animals were used 7 to 8 days after being operated upon.

All animals received an intraperitoneal injection of 5- $^3\text{H}$ -uridine (25 μCi/100 g body weight) at the time indicated.

<sup>a</sup> Number in parenthesis indicates the number of animals used.

<sup>b</sup> Mean ± standard error.

Figure 2.

Time course of incorporation of  $^3\text{H}$ -uridine into the kidneys of adrenalectomized rat.

All animals received an intraperitoneal injection of  $^3\text{H}$ -uridine (25  $\mu\text{Ci}/100\text{ g}$  body weight) at the designated times before sacrifice. The different fractions were separated as mentioned in Materials and Methods.

Specific activity:

●—● Total counts (cpm/mg DNA)  $\times 10^{-3}$

★—★ Acid soluble fraction (cpm/unit O.D. 260  $\text{m}\mu$ )  $\times 10^{-2}$

⊙—⊙ Alkaline hydrolyzate fraction (cpm/mg DNA)  $\times 10^{-2}$

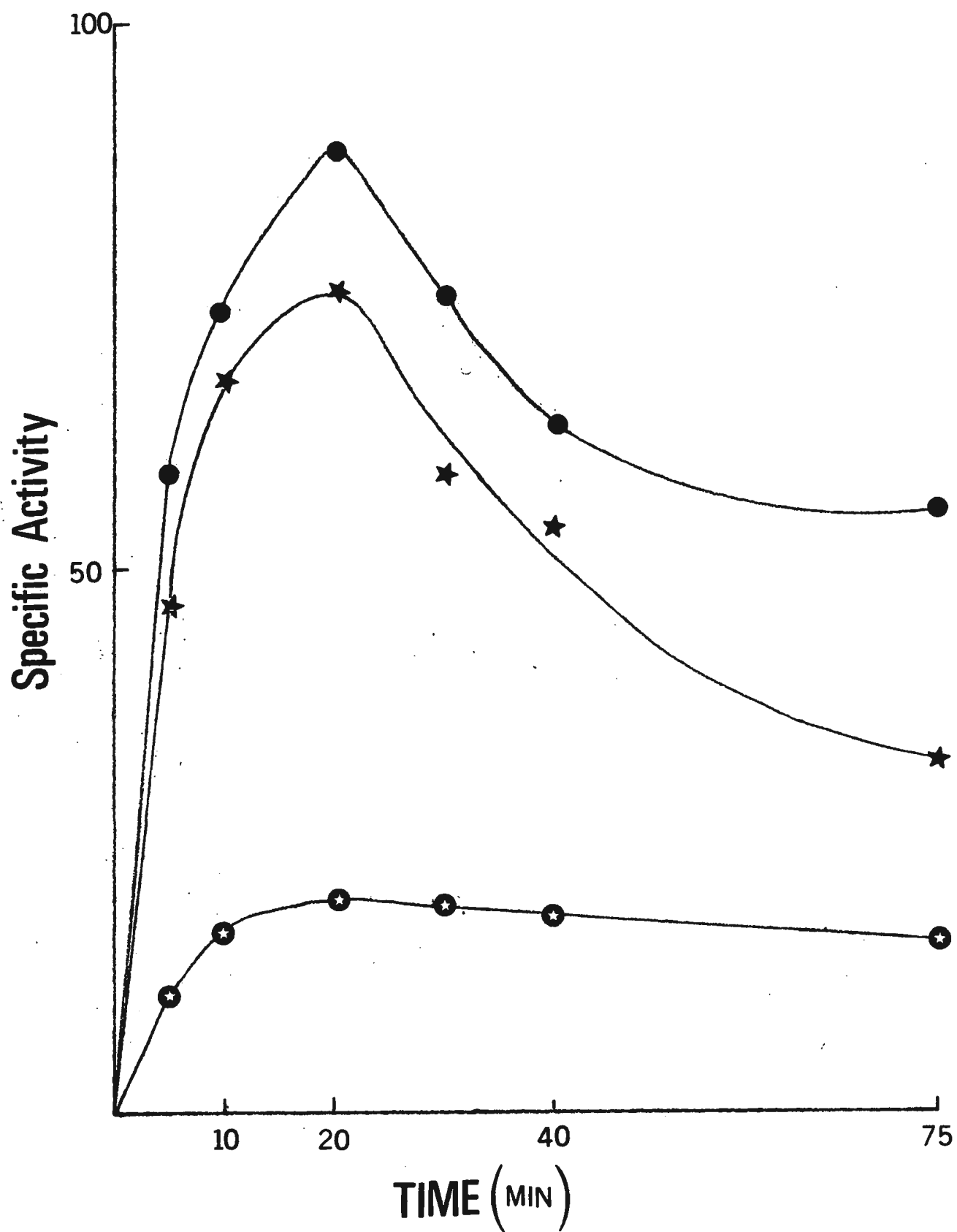


FIGURE 2

TABLE V  
TIME COURSE OF INCORPORATION OF  $^3\text{H}$ -CYTIDINE INTO DIFFERENT FRACTIONS  
OF NORMAL RAT KIDNEYS

Time in minutes (after injection of isotope) <sup>a</sup>	Total counts (cpm/mg. DNA) <sup>b</sup> $\times 10^{-4}$	Acid Soluble fraction (cpm/unit O.D. 260 m $\mu$ ) <sup>b</sup> $\times 10^{-3}$	Alkaline hydrolyzate fraction (cpm/mg. DNA) <sup>b</sup> $\times 10^{-3}$	
	A.	B.	C.	C/A
5	2.09	1.45	0.20	.95%
10 (2)	9.16 $\pm$ 0.49	7.02 $\pm$ 0.11	3.96 $\pm$ 0.44	4.3%
20 (3)	12.49 $\pm$ 1.32	8.56 $\pm$ 0.97	9.76 $\pm$ 0.81	7.8%
40 (2)	14.49 $\pm$ 2.69	10.21 $\pm$ 1.76	22.65 $\pm$ 1.53	15%
75 (2)	13.02 $\pm$ 0.17	9.11 $\pm$ 0.68	21.34 $\pm$ 5.37	16%

All animals received an intraperitoneal injection of 5- $^3\text{H}$ -cytidine (25  $\mu\text{Ci}$ /100 g body weight) at the time indicated.

<sup>a</sup> Number in parenthesis indicates the number of animals used.

<sup>b</sup> Mean  $\pm$  standard error.

Figure 3.

Time course of incorporation of  $^3\text{H}$ -cytidine into the kidneys of normal rat.

All animals received an intraperitoneal injection of  $^3\text{H}$ -cytidine (25  $\mu\text{Ci}/100\text{ g}$  body weight) at the designated times before sacrifice. The different fractions were separated as mentioned in Materials and Methods.

Specific activity:

●—● Total counts (cpm/mg DNA)  $\times 10^{-4}$

★—★ Acid soluble fraction (cpm/unit O.D. 260  $\text{m}\mu$ )  $\times 10^{-3}$

⊙—⊙ Alkaline hydrolyzate fraction (cpm/mg DNA)  $\times 10^{-3}$

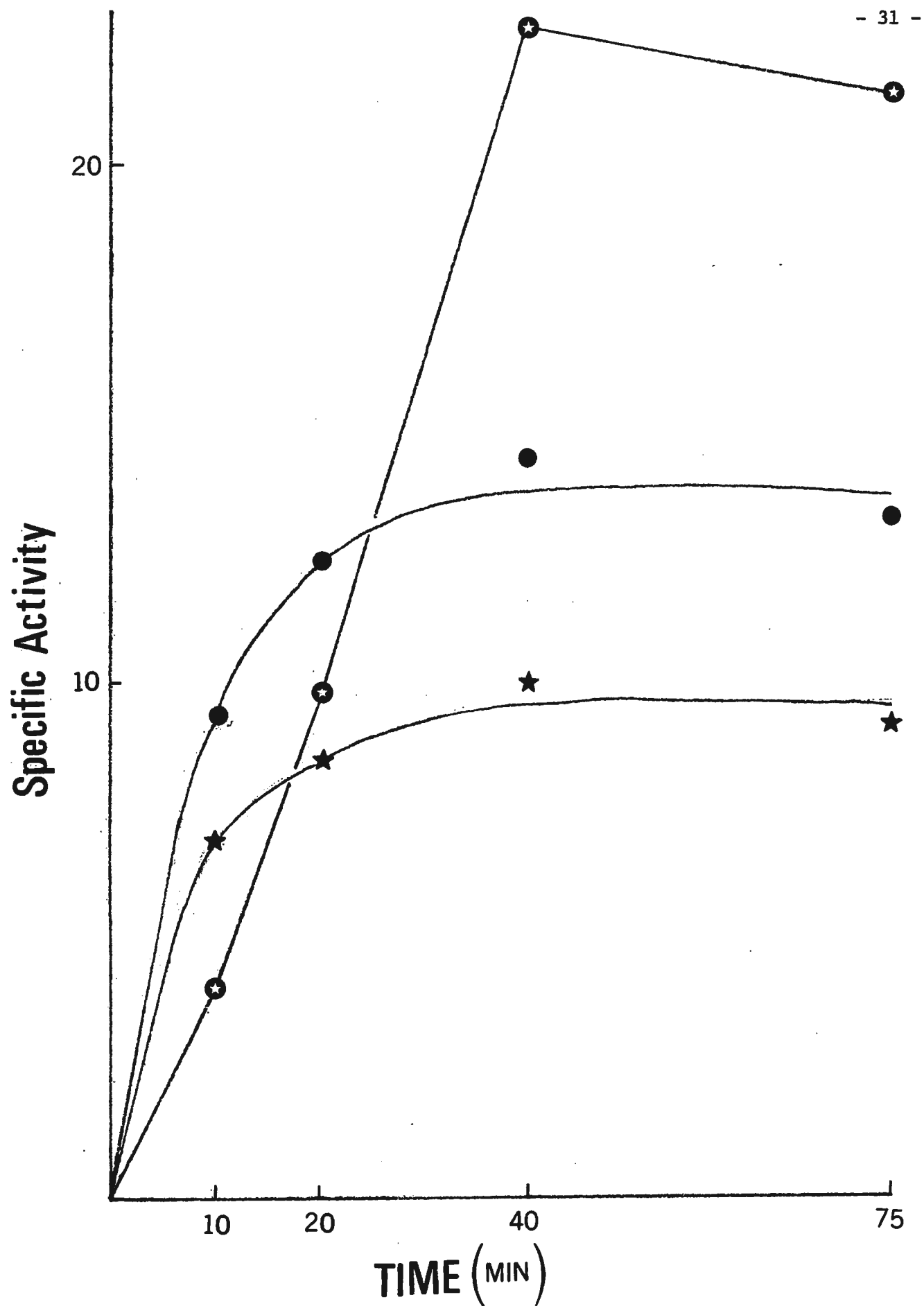


FIGURE 3

TABLE VI

TIME COURSE OF INCORPORATION OF  $^3\text{H}$ -CYTIDINE INTO DIFFERENT FRACTIONS  
OF ADRENALECTOMIZED RAT KIDNEYS

Time in minutes (after injection of isotope) <sup>a</sup>	Total Counts (cpm/mg. DNA) <sup>b</sup> $\times 10^{-4}$	Acid Soluble fraction (cpm/unit O.D. 260 m $\mu$ ) <sup>b</sup> $\times 10^{-3}$	Alkaline hydrolyzate fraction (cpm/mg. DNA) <sup>b</sup> $\times 10^{-3}$	
	A.	B.	C.	C/A
5 <sub>(2)</sub>	6.48 $\pm$ 1.20	4.47 $\pm$ 0.90	1.51 $\pm$ 0.35	2.3%
10 <sub>(2)</sub>	7.99 $\pm$ 1.54	6.73 $\pm$ 1.48	3.67 $\pm$ 0.29	4.5%
20 <sub>(3)</sub>	9.36 $\pm$ 1.62	8.19 $\pm$ 0.92	5.79 $\pm$ 0.42	6.2%
40 <sub>(2)</sub>	14.05 $\pm$ 3.66	11.23 $\pm$ 3.45	18.03 $\pm$ 5.13	13.0%
75 <sub>(2)</sub>	10.83 $\pm$ 3.25	7.43 $\pm$ 3.80	18.75 $\pm$ 0.25	17.4%

Animals were used 7 to 8 days after being operated upon.

All animals received an intraperitoneal injection of 5  $^3\text{H}$ -cytidine (25  $\mu\text{Ci}/100$  g body weight) at the time indicated.

<sup>a</sup> Number in parenthesis indicates the number of animals used.

<sup>b</sup> Mean  $\pm$  standard error.



observed at 40 minutes (Figure 4).

Comparison of the magnitude of  $^3\text{H}$ -cytidine incorporation into different fractions of normal and adrenalectomized rat kidneys shows a decrease in the incorporation of  $^3\text{H}$ -cytidine into total and alkaline hydrolyzate fraction of adrenalectomized rat kidneys at all the times.

#### (C) STUDIES WITH $^{14}\text{C}$ -OROTIC ACID

Table VII shows the time course of incorporation of  $^{14}\text{C}$ -orotic acid into normal rat kidneys. There is an increase in incorporation of  $^{14}\text{C}$ -orotic acid into different fractions with increase in time (Figure 5).

A similar time course of  $^{14}\text{C}$ -orotic acid incorporation was observed in adrenalectomized rat kidneys (Table VIII). There is a time dependent increase in incorporation into all fractions (Figure 6).

Comparison of the magnitude of  $^{14}\text{C}$ -orotic acid into different fractions of normal and adrenalectomized rat kidneys shows a general decrease in the incorporation of labeled precursor into the kidneys of adrenalectomized rats.

In all these studies, the incorporation of isotopes was linear for at least 20 minutes. In studies with  $^3\text{H}$ -uridine the incorporation beyond 20 minutes decreases whereas with  $^3\text{H}$ -cytidine and  $^{14}\text{C}$ -orotic acid there is an increase in incorporation after 20 minutes.

Table IX shows the relative efficiencies of various RNA precursors in pulse labeling of kidney RNA of normal and adrenalectomized rats. With an identical 20 minutes incorporation period labeled orotic acid and cytidine are more efficiently incorporated into RNA than the labeled uridine. In further studies with hormones only  $^3\text{H}$ -cytidine and  $^{14}\text{C}$ -orotic acid were used and a standard time of 20 minutes was chosen.

Figure 4.

Time course of incorporation of  $^3\text{H}$ -cytidine into the kidneys of adrenalectomized rat.

All animals received an intraperitoneal injection of  $^3\text{H}$ -cytidine (25  $\mu\text{Ci}/100\text{ g}$  body weight) at the designated times before sacrifice. The different fractions were separated as mentioned in Materials and Methods.

Specific activity:

●—● Total counts (cpm/mg DNA)  $\times 10^{-4}$

★—★ Acid soluble fraction (cpm/unit O.D. 260  $\text{m}\mu$ )  $\times 10^{-3}$

★—★ Alkaline hydrolyzate fraction (cpm/mg DNA)  $\times 10^{-3}$

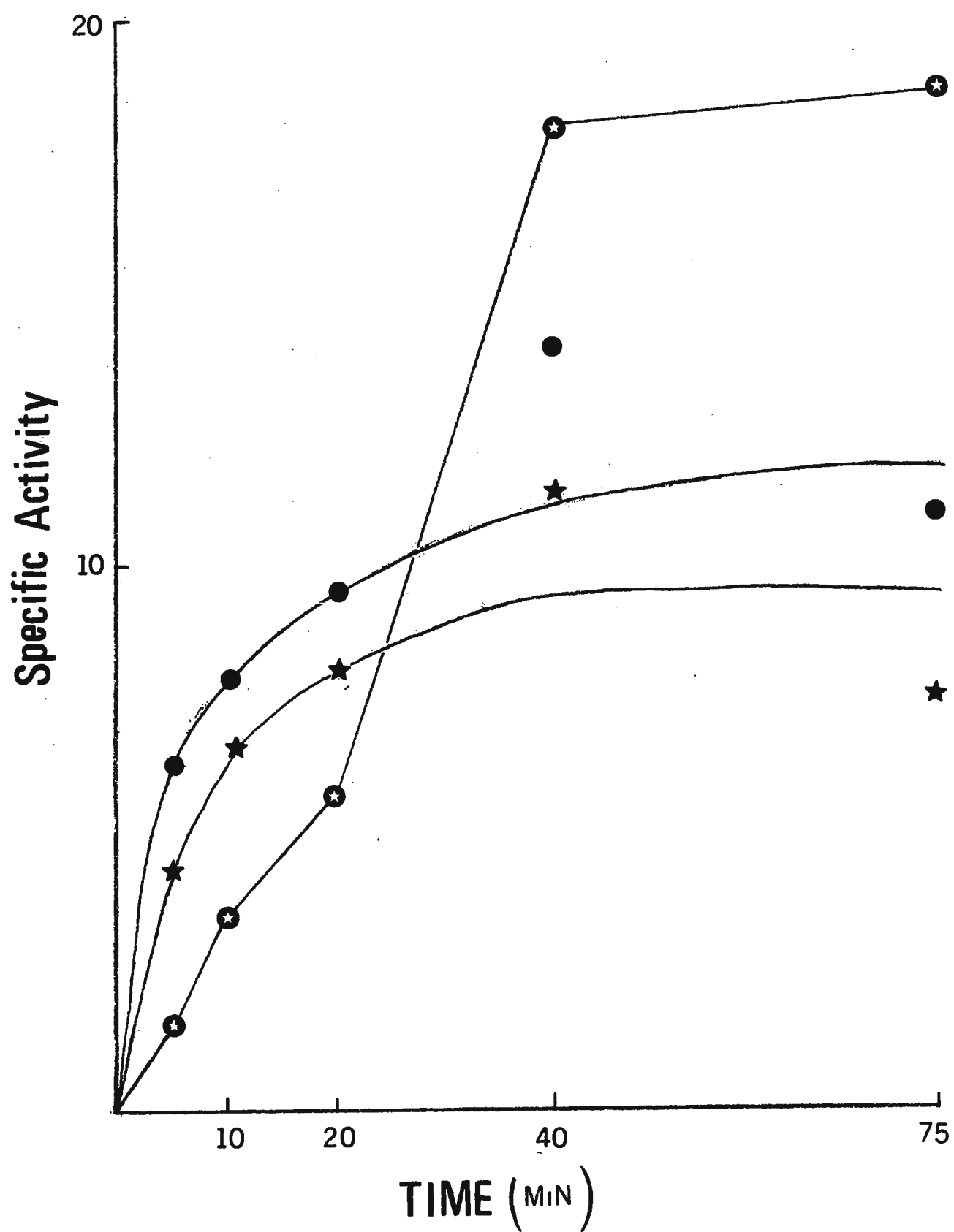


TABLE VII  
TIME COURSE OF INCORPORATION OF  $^{14}\text{C}$ -OROTIC ACID INTO DIFFERENT FRACTIONS  
OF NORMAL RAT KIDNEYS

Time in minutes (after injection of isotope) <sup>a</sup>	Total Counts (cpm/mg. DNA) <sup>b</sup> $\times 10^{-4}$	Acid Soluble fraction <sub>b</sub> (cpm/unit O.D. 260 mμ) <sup>b</sup> $\times 10^{-3}$	Alkaline hydrolyzate fraction (cpm/mg. DNA) <sup>b</sup> $\times 10^{-3}$	
	A.	B.	C.	C/A (%)
5	7.84 ± 1.39	5.25 ± 0.22	3.95 ± 2.69	5
10 <sub>(2)</sub>	11.48 ± 0.85	8.17 ± 0.69	3.25 ± 0.40	2.8
20 <sub>(5)</sub>	17.35 ± 1.59	12.64 ± 1.42	8.86 ± 0.44	5.1
40 <sub>(4)</sub>	19.35 ± 0.64	15.44 ± 0.72	12.75 ± 1.47	6.6
75 <sub>(2)</sub>	23.17 ± 0.81	18.02 ± 0.90	18.73 ± 1.30	8.1

All animals received an intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5 μCi/100 g body weight) at the time indicated

<sup>a</sup> Number in parenthesis indicates the number of animals used.

<sup>b</sup> Mean ± standard error.

Figure 5.

Time course of incorporation of  $^{14}\text{C}$ -orotic acid into the kidneys of normal rat.

All animals received an intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5  $\mu\text{Ci}/100$  g body weight) at the designated times before sacrifice. The different fractions were separated as mentioned in Materials and Methods.

Specific activity:

●—● Total counts (cpm/mg DNA)  $\times 10^{-4}$

★—★ Acid soluble fraction (cpm/unit O.D. 260  $\text{m}\mu$ )  $\times 10^{-3}$

⊙—⊙ Alkaline hydrolyzate fraction (cpm/mg DNA)  $\times 10^{-3}$

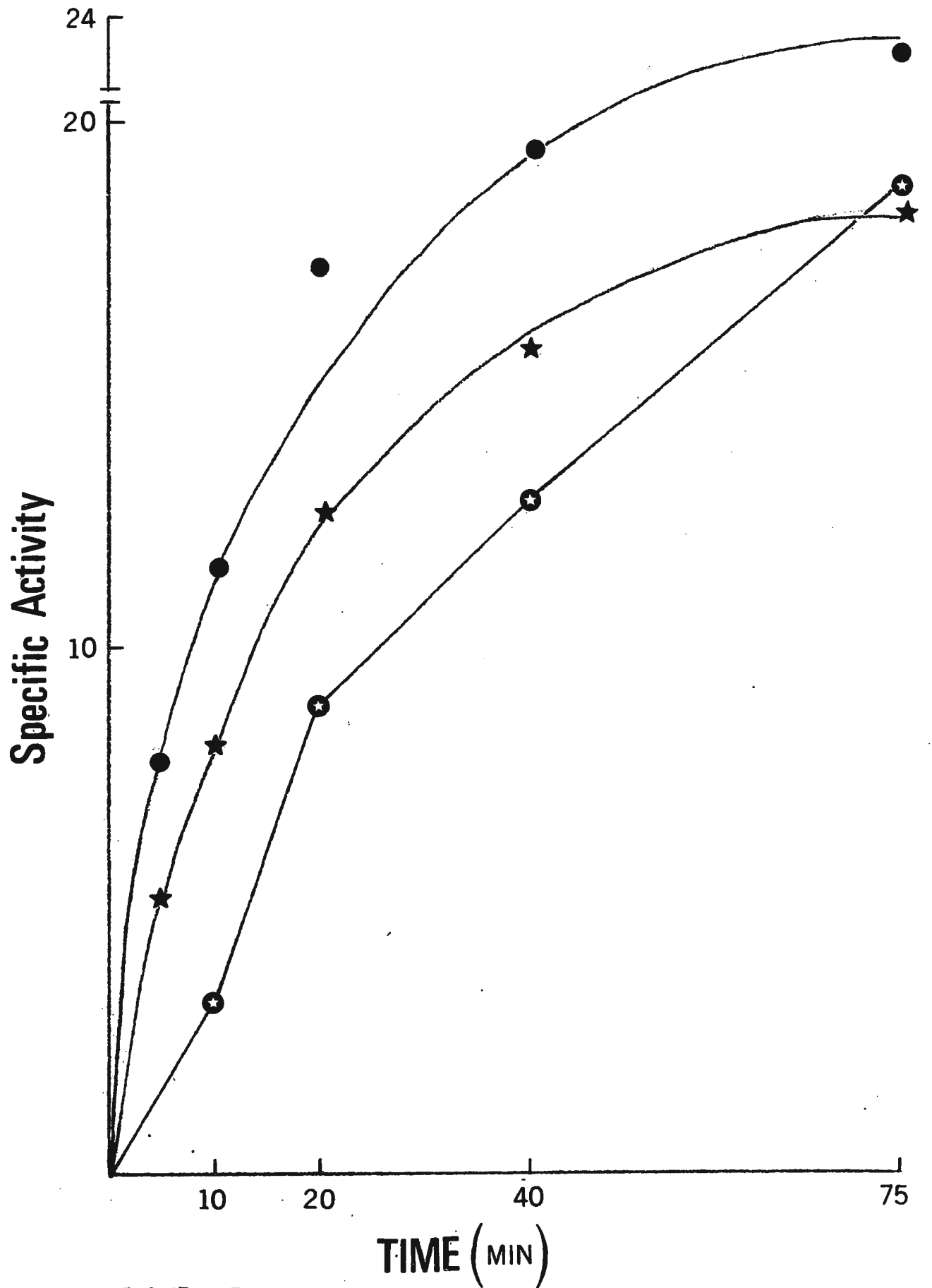


FIGURE 5

TABLE VIII

TIME COURSE OF INCORPORATION OF  $^{14}\text{C}$ -OROTIC ACID INTO DIFFERENT FRACTIONS  
OF ADRENALECTOMIZED RAT KIDNEYS

Time in minutes (after injection of isotope) <sup>a</sup>	Total Counts <sub>b</sub> (cpm/mg. DNA) $\times 10^{-4}$	Acid Soluble fraction <sub>b</sub> (cpm/unit O.D. 260 m $\mu$ ) $\times 10^{-3}$	Alkaline hydrolyzate fraction (cpm/mg. DNA) <sub>b</sub> $\times 10^{-3}$	
	A.	B.	C.	C/A
5 <sub>(2)</sub>	5.95 $\pm$ 0.78	4.21 $\pm$ 0.105	3.19 $\pm$ 0.96	5.4%
10 <sub>(3)</sub>	10.16 $\pm$ 0.581	6.56 $\pm$ 0.64	2.95 $\pm$ 0.29	3.0%
20 <sub>(6)</sub>	15.01 $\pm$ 1.13	10.94 $\pm$ 0.688	8.17 $\pm$ 0.63	5.5%
40 <sub>(3)</sub>	28.21 $\pm$ 1.20	18.54 $\pm$ 0.68	13.70 $\pm$ 0.54	4.9%
75 <sub>(3)</sub>	25.89 $\pm$ 5.35	16.19 $\pm$ 3.45	17.09 $\pm$ 2.92	6.6%

Animals were used 7 to 8 days after being operated upon.

All animals received an intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5  $\mu\text{Ci}$ /100 g body weight) at the times indicated.

<sup>a</sup> Number in parenthesis indicates the number of animals used.

<sup>b</sup> Mean  $\pm$  standard error.

Figure 6.

Time course of incorporation of  $^{14}\text{C}$ -orotic acid into the kidneys of adrenalectomized rat.

All animals received an intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5  $\mu\text{Ci}/100$  g body weight) at the designated times before sacrifice. The different fractions were separated as mentioned in Materials and Methods.

Specific activity:

●—● Total counts (cpm/mg DNA)  $\times 10^{-4}$

★—★ Acid soluble fraction (cpm/unit O.D. 260  $\text{m}\mu$ )  $\times 10^{-3}$

⊙—⊙ Alkaline hydrolyzate fraction (cpm/mg DNA)  $\times 10^{-3}$



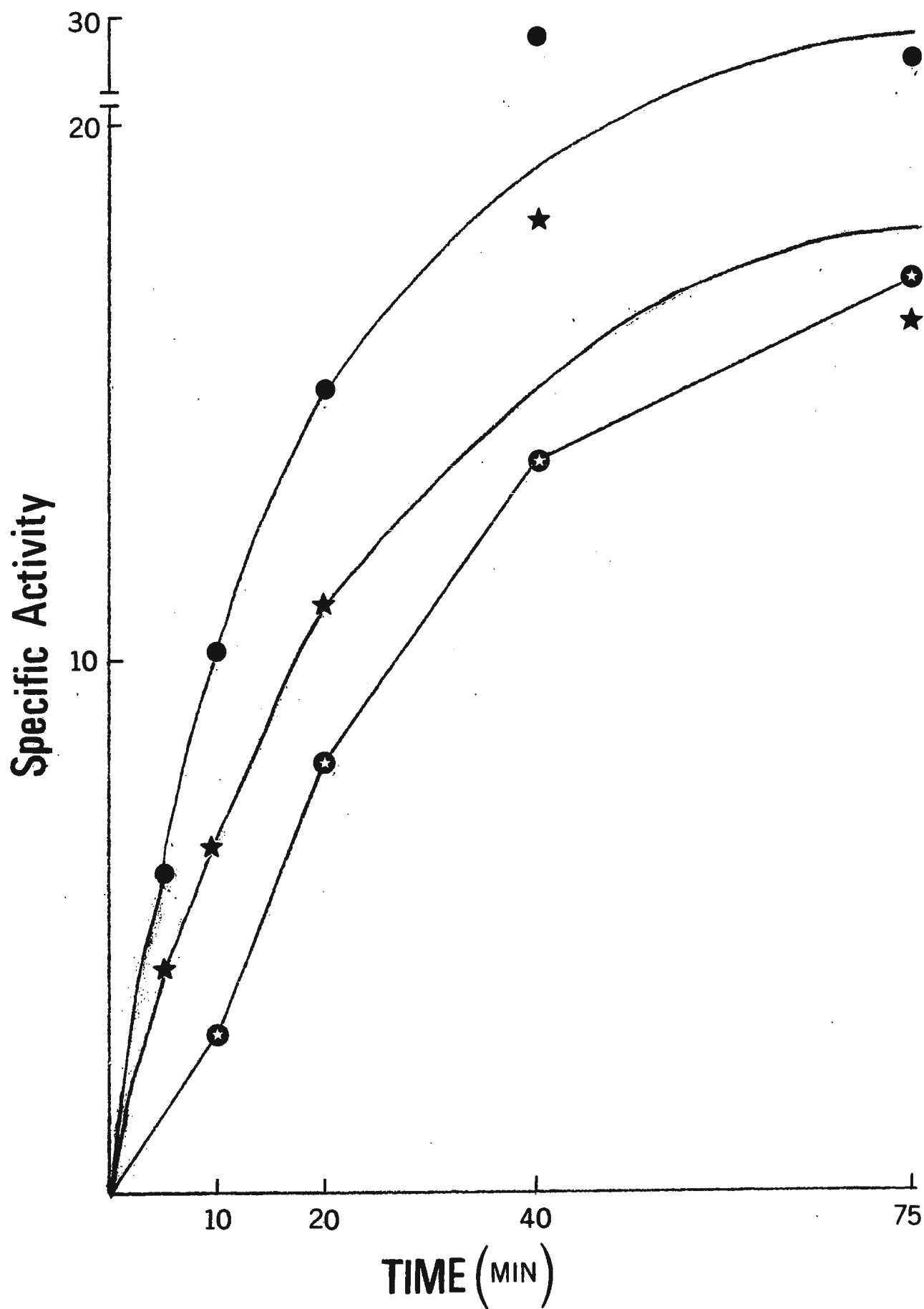


FIGURE 6

TABLE IX

## RELATIVE EFFICIENCIES OF VARIOUS RNA PRECURSORS IN PULSE LABELING OF KIDNEY RNA

Precursor	Dose <sup>a</sup>		Precursor incorporation time (minutes)	Category of animals	(A) Acid soluble fraction	(B) Alkaline hydrolyzate fraction	Incorporation efficiency B/A x 100
	µCi	µmole			(cpm/mg DNA) x 10 <sup>-3</sup>	(cpm/mg DNA) x 10 <sup>-3</sup>	
5- <sup>3</sup> H-Uridine	25	0.0009	20	Normal	98.81	2.28	2.30
	25	0.0009	20	Adrenalectomized	93.18	1.96	2.10
5- <sup>3</sup> H-Cytidine	25	0.0250	20	Normal	137.96	9.76	7.07
	25	0.0250	20	Adrenalectomized	102.48	5.79	5.64
6- <sup>14</sup> C-Orotic acid	5	0.0822	20	Normal	165.98	8.86	5.33
	5	0.0822	20	Adrenalectomized	150.52	8.17	5.42

<sup>a</sup> Amount of precursor administered per 100 g body weight.

Adrenalectomized rats were used 7 to 8 days after being operated upon.

EFFECT OF ADRENALECTOMY AND ALDOSTERONE TREATMENT ON ACID SOLUBLE POOL  
AND RNA OF RAT KIDNEYS

Table X shows the effect of adrenalectomy and aldosterone treatment on acid soluble nucleotide pool:DNA and RNA:DNA ratio of rat kidneys. There is a significant decrease in RNA:DNA ratio of adrenalectomized rats as compared to normal animals. Administration of aldosterone to adrenalectomized rats for 1 or 2 hours (and 20 minutes isotope incorporation) before sacrifice increased RNA:DNA ratio to levels even greater than normal (Figure 7). There was no change in the acid soluble nucleotide pool:DNA ratio after adrenalectomy and aldosterone treatment. It can be concluded that adrenalectomy <sup>may</sup>/cause lower rates of RNA synthesis which can be increased by aldosterone treatment.

STUDIES WITH HORMONES

Table XI shows the effect of adrenalectomy and aldosterone treatment on the incorporation of <sup>3</sup>H-cytidine into rat kidneys. There is a decrease in incorporation of precursor into total and alkaline hydrolyzate fraction after adrenalectomy. No decrease in incorporation into the acid soluble pool was observed. Administration of aldosterone either 1 or 2 hours before the isotope results in an increased incorporation of precursor into all fractions. Aldosterone treatment increases the incorporation into total and acid soluble fractions to levels even greater than normal (Figure 8). It can be concluded that aldosterone increases the rate of precursor uptake and subsequent incorporation into alkaline hydrolyzate fraction in the kidneys of adrenalectomized rats.

TABLE X

EFFECT OF ADRENALECTOMY AND ALDOSTERONE TREATMENT ON ACID SOLUBLE  
NUCLEOTIDE POOL: DNA AND RNA: DNA RATIO OF RAT KIDNEYS

Category of animals <sup>a</sup>	Acid soluble pool <sup>b</sup> :DNA <sup>c</sup>	Difference (%)	p value	RNA:DNA <sup>c</sup>	Difference (%)	p value
Normal (31)	13.98 ± 0.33	-3	N.S.	0.947 ± 0.024	-17†	<0.05
Adrenalectomized (33)	13.55 ± 0.34			0.784 ± 0.023		
Adrenalectomized plus aldosterone treated						
1 hour (9)	13.92 ± 0.32	+3	N.S.	0.947 ± 0.058	+21††	<0.05
2 hours (8)	14.61 ± 0.29	+8	N.S.	0.993 ± 0.062	+27††	<0.05

Adrenalectomized rats were used 7 to 8 days after being operated upon. Aldosterone (5 µg/100 g body weight) was injected intraperitoneally to adrenalectomized rats 1 hour and 2 hours prior to the isotope injection. All animals received an intraperitoneal injection of 5-<sup>3</sup>H-cytidine (25 µCi/100 g body weight) or <sup>14</sup>C-orotic acid (5 µCi/100 g body weight) 20 minutes before sacrifice to also study the precursor uptake (Tables XI, XII, and XIV).

<sup>a</sup> Number in parenthesis indicates the number of animals used..

<sup>b</sup> Measured as optical density at 260 mµ.

<sup>c</sup> Mean ± standard error.

p value was calculated by the student t test method.

† Normal - adrenalectomized



†† Adrenalectomized—adrenalectomized plus aldosterone treated

Figure 7.

Effect of adrenalectomy and aldosterone treatment on acid soluble nucleotide pool: DNA and RNA: DNA ratio of rat kidneys.

Adrenalectomized rats were used 7 to 8 days after being operated upon. Saline or aldosterone (5 µg/100 g body weight) was injected to adrenalectomized (ADX) rats 1 hour and 2 hours prior to the isotope injection. All animals received an intraperitoneal injection of <sup>3</sup>H-cytidine (25 µCi/100 g body weight) or <sup>14</sup>C-orotic acid (5 µCi/100 g body weight) 20 minutes before sacrifice to also study the precursor uptake (Tables XI, XII, and XIV).

Results are expressed as percentage of normal =  $\frac{\text{Experimental}}{\text{Normal}} \times 100$ .

-  - acid soluble nucleotide pool:DNA ratio
-  - RNA:DNA ratio

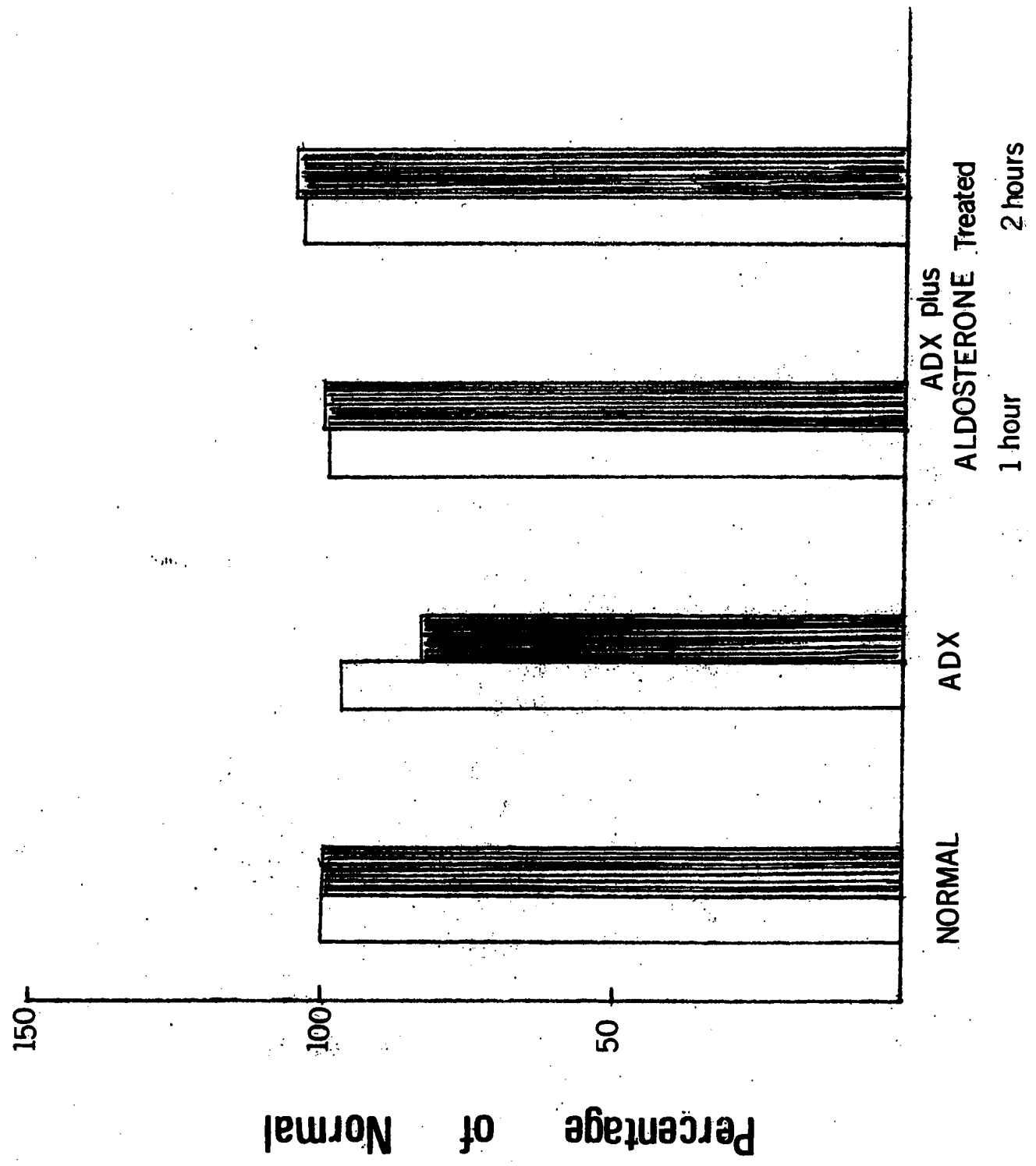


FIGURE 7

TABLE XI

EFFECT OF ADRENALECTOMY AND ALDOSTERONE TREATMENT ON THE INCORPORATION  
OF  $^3\text{H}$ -CYTIDINE INTO DIFFERENT FRACTIONS OF RAT KIDNEYS

Fraction	<sup>a</sup> Normal <sub>(3)</sub>	<sup>a</sup> Adrenalectomized <sub>(3)</sub>	†Difference (%)	<sup>a</sup> Adrenalectomized & Hormone Treatment		††Difference (%)	
				1 hr. (5)	2 hr. (4)	1 hr.	2 hr.
Total counts (cpm/mg. DNA) <sup>b</sup> x 10 <sup>-4</sup>	12.49 ± 1.32	9.36 ± 1.62	-25	14.05 ± 1.14	13.60 ± 0.67	+50	+45
Acid Soluble fraction (cpm/unit OD 260 mμ) <sup>b</sup> x 10 <sup>-3</sup>	8.56 ± 0.97	8.19 ± 0.92	-4	10.94 ± 0.66	11.09 ± 0.48	+34	+35
Alkaline hydrolyzate (cpm/mg. DNA) <sup>b</sup> x 10 <sup>-3</sup>	9.76 ± 0.81	5.79 ± 0.42	-41	8.84 ± 0.95	9.00 ± 1.77	+53	+56

Adrenalectomized rats were used 7-8 days after being operated upon. Aldosterone (5 μg/100 g body weight) was injected intraperitoneally to adrenalectomized rats 1 hour and 2 hours prior to the isotope injection. All animals received intraperitoneal injection of 5- $^3\text{H}$ -cytidine (25 μCi/100 g body weight) 20 minutes before sacrifice.

<sup>a</sup> Number in parenthesis indicates the number of animals used.

<sup>b</sup> Mean ± standard error.

† Normal - adrenalectomized

†† Adrenalectomized - adrenalectomized plus aldosterone treated




Figure 8.

Effect of adrenalectomy and aldosterone treatment on the incorporation of  $^3\text{H}$ -cytidine into different fractions of rat kidneys.

Adrenalectomized rats were used 7 to 8 days after being operated upon. Saline or aldosterone (5  $\mu\text{g}/100$  g body weight) was injected intraperitoneally to adrenalectomized (ADX) rats 1 hour and 2 hours prior to the isotope injection. All animals received an intraperitoneal injection of  $^3\text{H}$ -cytidine (25  $\mu\text{Ci}/100$  g body weight) 20 minutes before sacrifice.

Results are expressed as percentage of normal specific activity =

$$\frac{\text{Experimental}}{\text{Normal}} \times 100.$$

-  Total counts (cpm/mg DNA)
-  Acid soluble fraction (cpm/unit O.D. 260 m $\mu$ )
-  Alkaline hydrolyzate fraction (cpm/mg DNA)



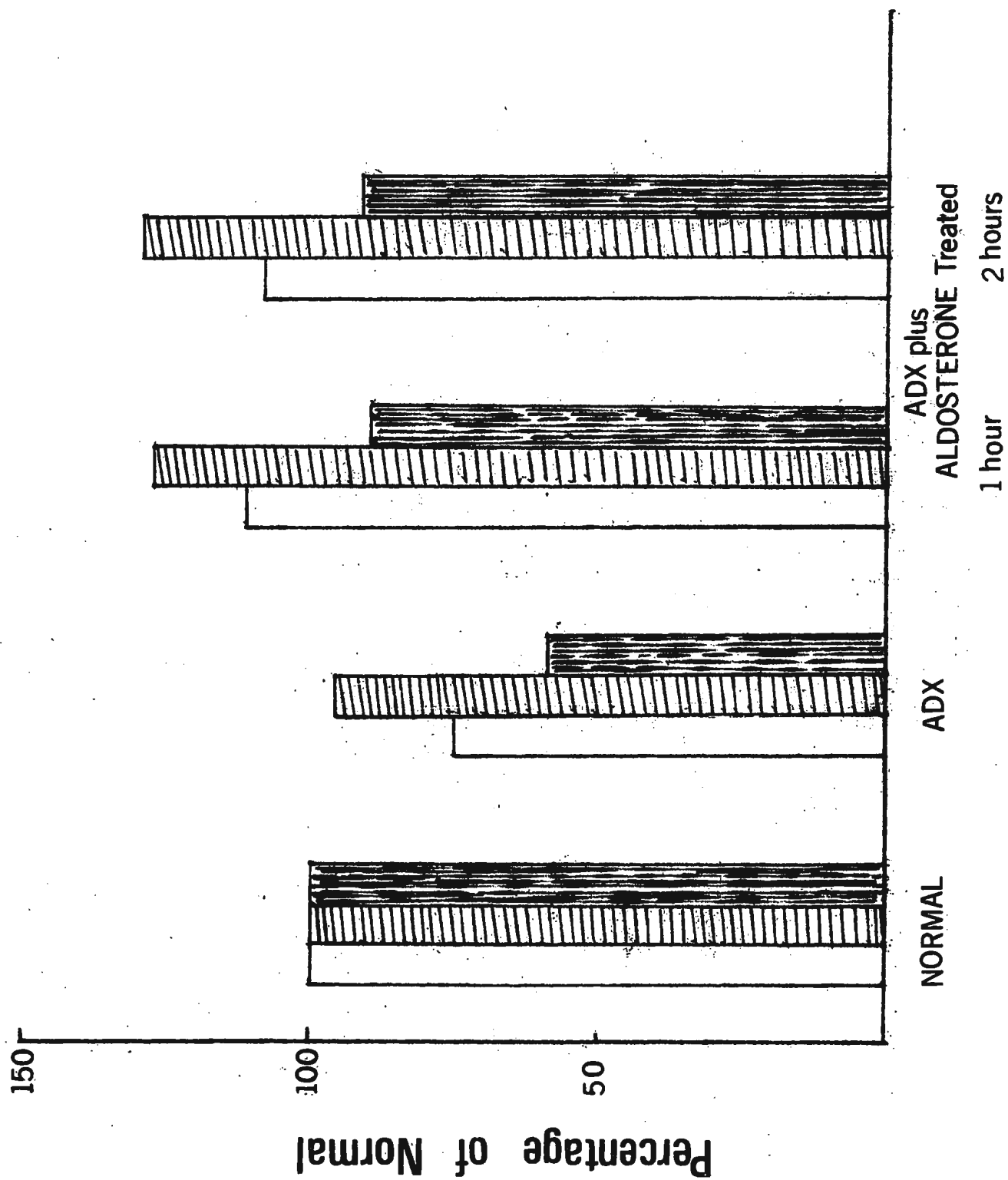


FIGURE 8

Tables XII and XIII show the effect of adrenalectomy, aldosterone, deoxycorticosterone, corticosterone and cortisol on the incorporation of  $^{14}\text{C}$ -orotic acid into different fractions of rat kidneys. There is a small general decrease in incorporation of precursor after adrenalectomy. Administration of aldosterone 1 or 2 hours before the isotope results in a marked increase in incorporation of precursor into all fractions. Administration of deoxycorticosterone or corticosterone 1 or 2 hours prior to the isotope results in an increased incorporation of precursor into total and acid soluble fractions. There was no difference observed in the incorporation of alkaline hydrolyzate fraction. On the other hand, administration of cortisol 1 or 2 hours before the isotope causes a decrease in incorporation of precursor into total and alkaline hydrolyzate fraction, whereas the incorporation into acid soluble fraction was unchanged (Figure 9). It can be concluded from these studies that aldosterone causes a marked increase in the rate of precursor uptake and subsequent incorporation into alkaline hydrolyzate fraction. There may also be a genuine increase in RNA synthesis after aldosterone treatment. Deoxycorticosterone and corticosterone cause an increase in the rate of precursor uptake whereas cortisol inhibits the uptake of precursor into the kidneys of adrenalectomized rats.

#### STUDIES WITH ACTINOMYCIN D AND ALDOSTERONE

Table XIV shows the effect of actinomycin D and aldosterone treatment on the incorporation of  $^{14}\text{C}$ -orotic acid into the kidneys of adrenalectomized rats. Administration of aldosterone 2 hours prior to the isotope results

TABLE XII  
EFFECT OF ADRENALECTOMY, ALDOSTERONE AND DEOXYCORTICOSTERONE TREATMENT ON THE  
INCORPORATION OF  $^{14}\text{C}$ -OROTIC ACID INTO DIFFERENT FRACTIONS OF RAT KIDNEYS

Fraction	<sup>a</sup> Normal (5)	<sup>a</sup> Adrenal- ectomized (6)	<sup>†</sup> Differ- ence (%)	Adrenal- ectomized and hormone treatment	<sup>a</sup> Aldosterone 1 hr (4) 2 hr (5)	<sup>††</sup> Differ- ence(%)	<sup>a</sup> Deoxycorti- costerone (3)	<sup>†††</sup> Differ- ence(%)
Total counts, (cpm/mg DNA) <sup>b</sup> x 10 <sup>-4</sup>	17.35 ± 1.59	15.01 ± 1.13	- 14	1 hr	19.63 ± 0.57	+ 31	16.30 ± 0.84	+9
				2 hrs	20.64 ± 2.42	+ 38	14.79 ± 0.97	-
Acid soluble fraction (cpm/unit OD 260 mμ) <sup>b</sup> x 10 <sup>-3</sup>	12.64 ± 1.43	10.94 ± 0.68	- 13	1 hr	14.59 ± 1.25	+ 33	15.05 ± 1.03	+38
				2 hrs	14.22 ± 1.58	+ 30	13.34 ± 0.74	+22
Alkaline hydrolyzate fraction (cpm/mg DNA) <sup>b</sup> x 10 <sup>-3</sup>	8.86 ± 0.44	8.17 ± 0.63	-8	1 hr	13.76 ± 2.42	+ 68	7.66 ± 0.17	- 6
				2 hrs	13.93 ± 3.74	+ 70	7.53 ± 0.31	- 8

Adrenalectomized rats were used 7 to 8 days after being operated upon. Aldosterone (5 μg/100 g body weight) or deoxycorticosterone (100 μg/100 g body weight) was injected intraperitoneally to adrenalectomized rats 1 hour and 2 hours prior to the isotope injection. All animals received intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5 μCi/100 g body weight) 20 minutes before sacrifice.

<sup>a</sup> Number in parenthesis indicates the number of animals used.

<sup>b</sup> Mean ± standard error.

<sup>†</sup> Normal - adrenalectomized

<sup>††</sup> Adrenalectomized - adrenalectomized plus aldosterone treated

<sup>†††</sup> Adrenalectomized - adrenalectomized plus deoxycorticosterone treated

TABLE XIII

EFFECT OF ADRENALECTOMY, CORTICOSTERONE AND CORTISOL TREATMENT ON THE INCORPORATION  
OF  $^{14}\text{C}$ -OROTIC ACID INTO DIFFERENT FRACTIONS OF RAT KIDNEYS

Fraction	<sup>a</sup> Normal (5)	<sup>a</sup> Adrenal- ectomized (6)	†Differ- ence (%)	Adrenal- ectomized and hormone treatment	<sup>a</sup> Corti- costerone (3)	††Differ ence (%)	<sup>a</sup> Cortisol (3)	†††Differ- ence (%)
Total counts (cpm/mg DNA) <sup>b</sup> x 10 <sup>-4</sup>	17.35 ± 1.59	15.01 ± 1.13	-14	1 hr.	16.59 ± 1.93	+11	13.22 ± 1.26	-12
				2 hrs.	17.14 ± 1.19	+14	12.05 ± 1.49	-20
Acid soluble fraction (cpm/unit O.D. 260 <sub>mμ</sub> ) <sup>b</sup> x 10 <sup>-3</sup>	12.64 ± 1.43	10.94 ± 0.68	-13	1 hr.	14.34 ± 2.55	+31	10.79 ± 1.00	---
				2 hrs.	15.43 ± 0.69	+41	10.92 ± 1.71	---
Alkaline hydrolyzate (cpm/mg DNA) <sup>b</sup> fraction x 10 <sup>-3</sup>	8.86 ± 0.44	8.17 ± 0.63	-8	1 hr.	8.05 ± 1.61	---	6.23 ± 1.20	-24
				2 hrs.	7.40 ± 0.82	-9	6.22 ± 1.56	-24

Adrenalectomized rats were used 7-8 days after being operated upon. Corticosterone (2 mg/100 g body weight) or cortisol (2 mg/100 g body weight) was injected intraperitoneally to adrenalectomized rats 1 hour and 2 hours prior to the isotope injection. All animals received intraperitoneal injection of  $^{14}\text{C}$ -Orotic acid (5  $\mu\text{Ci}$ /100 g body weight) 20 minutes before sacrifice.

<sup>a</sup>Number in parenthesis indicates the number of animals used.

<sup>b</sup>Mean ± standard error.

†Normal - adrenalectomized

††Adrenalectomized - adrenalectomized plus corticosterone treated

†††Adrenalectomized - adrenalectomized plus cortisol treated

Figure 9.

Effect of adrenalectomy, aldosterone, deoxycorticosterone, corticosterone and cortisol treatment on the incorporation of  $^{14}\text{C}$ -orotic acid into different fractions of rat kidney.

Adrenalectomized rats were used 7 to 8 days after being operated upon. Saline, aldosterone (5  $\mu\text{g}/100$  g body weight), deoxycorticosterone (100  $\mu\text{g}/100$  g body weight) corticosterone (2 mg/100 g body weight) or cortisol (2 mg/100 g body weight) was injected intraperitoneally to adrenalectomized (ADX) rats 1 hour and 2 hours prior to the isotope injection. All animals received an intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5  $\mu\text{Ci}/100$  g body weight) 20 minutes before sacrifice.

Results are expressed as percentage of normal specific activity =

$$\frac{\text{Experimental}}{\text{Normal}} \times 100.$$

- ☐ Total counts (cpm/mg DNA)
- ☒ Acid soluble fraction (cpm/unit O.D. 260 m $\mu$ )
- ☒ Alkaline hydrolyzate fraction (cpm/mg DNA)

FIGURE 9

Percentage of Normal

ADX plus  
Hormone  
Treatment

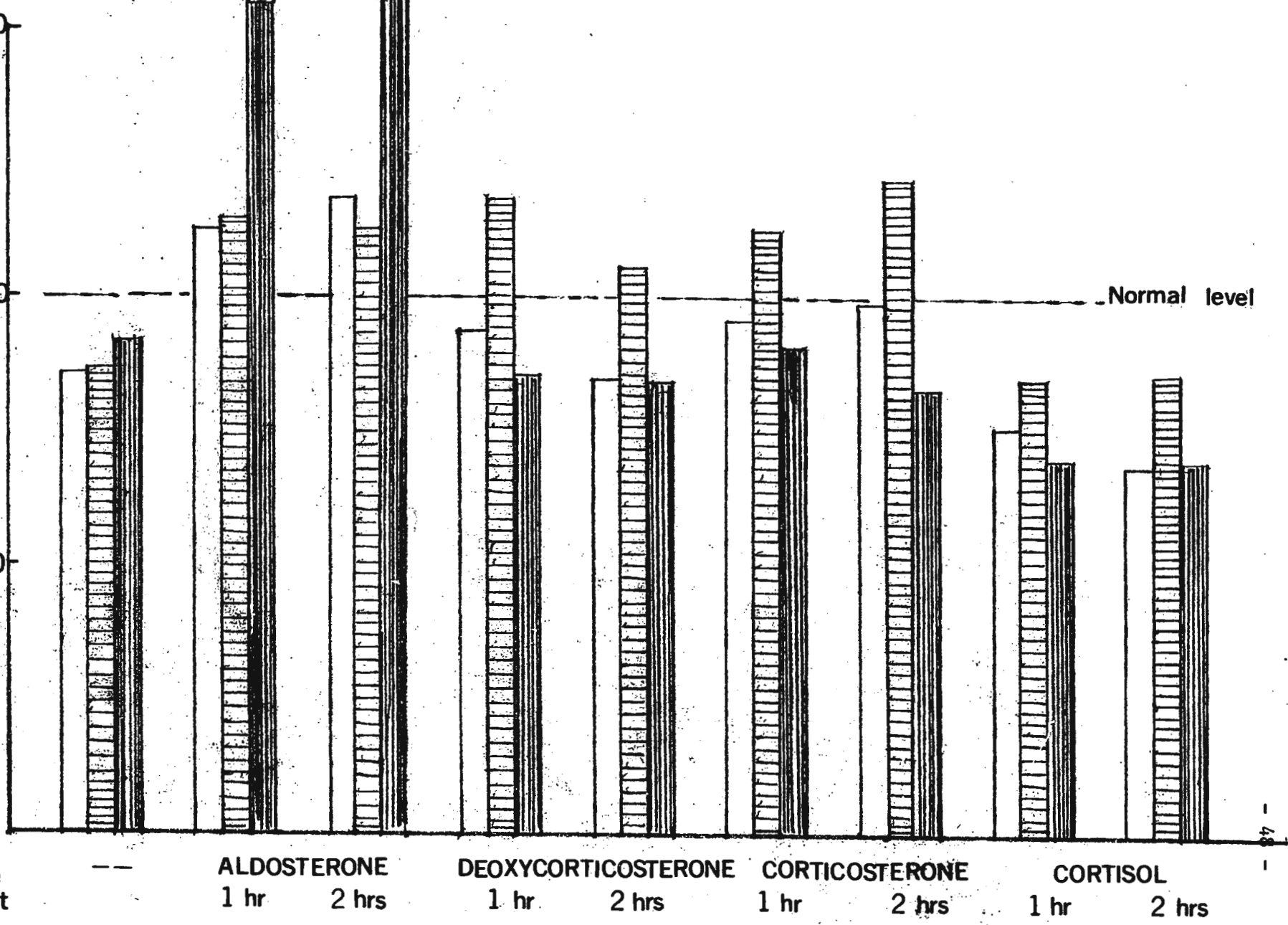


TABLE XIV

EFFECT OF ACTINOMYCIN D AND ALDOSTERONE TREATMENT ON THE INCORPORATION OF  $^{14}\text{C}$ -OROTIC ACID

INTO DIFFERENT FRACTIONS OF ADRENALECTOMIZED RAT KIDNEYS

	<sup>a</sup> Adrenalectomized (3)	<sup>a</sup> Aldosterone treated (3)	† Difference (%)	<sup>a</sup> Actinomycin D treated (3)	†† Difference (%)	<sup>a</sup> Actinomycin D plus Aldosterone treated (3)	††† Difference (%)
	15.85 ± 0.50	21.20 ± 1.26	+34	15.62 ± 0.73	---	18.39 ± 1.08	+16
in mu) <sup>b</sup>	12.03 ± 0.44	14.56 ± 0.44	+21	11.55 ± 0.63	-4	13.80 ± 0.99	+15
tion	7.23 ± 0.74	13.05 ± 1.41	+80	3.39 ± 0.18	-53	3.56 ± 0.25	-51

and 7-8 days after being operated upon. Saline, Aldosterone (5 µg/100 g body weight), Actinomycin D (50 µg/100 g body weight) (5 µg/100 g body weight) plus Aldosterone (5 µg/100 g body weight) were injected intraperitoneally 2 hours prior to the isotope. Animals received intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5 µCi/100 g body weight) 20 minutes before sacrifice.

sis indicates the number of animals used.

ror.

- adrenalectomized plus aldosterone treated
- adrenalectomized plus actinomycin D treated
- adrenalectomized plus actinomycin D and aldosterone treated

TABLE XIV

EFFECT OF ACTINOMYCIN D AND ALDOSTERONE TREATMENT ON THE INCORPORATION OF  $^{14}\text{C}$ -OROTIC ACID

INTO DIFFERENT FRACTIONS OF ADRENALECTOMIZED RAT KIDNEYS

Fraction	<sup>a</sup> Adrenalectomized (3)	<sup>a</sup> Aldosterone treated (3)	† Difference (%)	<sup>a</sup> Actinomycin D treated (3)	†† Difference (%)	<sup>a</sup> Actinomycin D plus Aldosterone treated (3)
Total Counts (cpm/mg. DNA) <sup>b</sup> x 10 <sup>-4</sup>	15.85 ± 0.50	21.20 ± 1.26	+34	15.62 ± 0.73	---	18.39 ± 1.08
Acid Soluble Fraction (cpm/unit O.D. <sub>260 mμ</sub> ) <sup>b</sup> x 10 <sup>-3</sup>	12.03 ± 0.44	14.56 ± 0.44	+21	11.55 ± 0.63	-4	13.80 ± 0.99
Alkaline hydrolyzate (cpm/mg. DNA) <sup>b</sup> fraction x 10 <sup>-3</sup>	7.23 ± 0.74	13.05 ± 1.41	+80	3.39 ± 0.18	-53	3.56 ± 0.25

All animals were used 7-8 days after being operated upon. Saline, Aldosterone (5 μg/100 g body weight), Actinomycin D (50 μg/100 g body weight) or Actinomycin D (50 μg/100 g body weight) plus Aldosterone (5 μg/100 g body weight) were injected intraperitoneally 2 hours prior to injection. All animals received intraperitoneal injection of  $^{14}\text{C}$ -orotic acid (5 μCi/100 g body weight) 20 minutes before sacrifice.

<sup>a</sup> Number in parenthesis indicates the number of animals used.

<sup>b</sup> Mean ± standard error.

† Adrenalectomized - adrenalectomized plus aldosterone treated

†† Adrenalectomized - adrenalectomized plus actinomycin D treated

††† Adrenalectomized - adrenalectomized plus actinomycin D and aldosterone treated



in an increase in incorporation of precursor into all the fractions, as shown also in Table XII. Actinomycin D treatment 2 hours before the isotope causes a marked decrease in incorporation into the alkaline hydrolyzate fraction. No such decrease in incorporation was observed in total or acid soluble fractions. Administration of actinomycin D plus aldosterone 2 hours prior to the isotope causes an increase in incorporation of total and acid soluble fraction whereas there was still a marked decrease in the incorporation of labeled precursor into alkaline hydrolyzate fraction (Figure 10). It can be concluded that aldosterone increases the rate of precursor uptake to a greater level when RNA synthesis is not inhibited by actinomycin D treatment.

Figure 10.

Effect of actinomycin D and aldosterone treatment on the incorporation of  $^{14}\text{C}$ -orotic acid into different fractions of adrenalectomized rat kidneys.

All animals were used 7 to 8 days after adrenalectomy.

Saline, aldosterone ( $5\text{ }\mu\text{g}/100\text{ g}$  body weight), actinomycin D ( $50\text{ }\mu\text{g}/100\text{ g}$  body weight) or actinomycin ( $50\text{ }\mu\text{g}/100\text{ g}$  body weight) plus aldosterone ( $5\text{ }\mu\text{g}/100\text{ g}$  body weight) were injected intraperitoneally 2 hours prior to the isotope injection. All animals received an intraperitoneal injection of  $^{14}\text{C}$ -orotic acid ( $5\text{ }\mu\text{Ci}/100\text{ g}$  body weight) 20 minutes before sacrifice.

Results are expressed as percentage of control specific activity =

$$\frac{\text{Experimental}}{\text{Control}} \times 100.$$




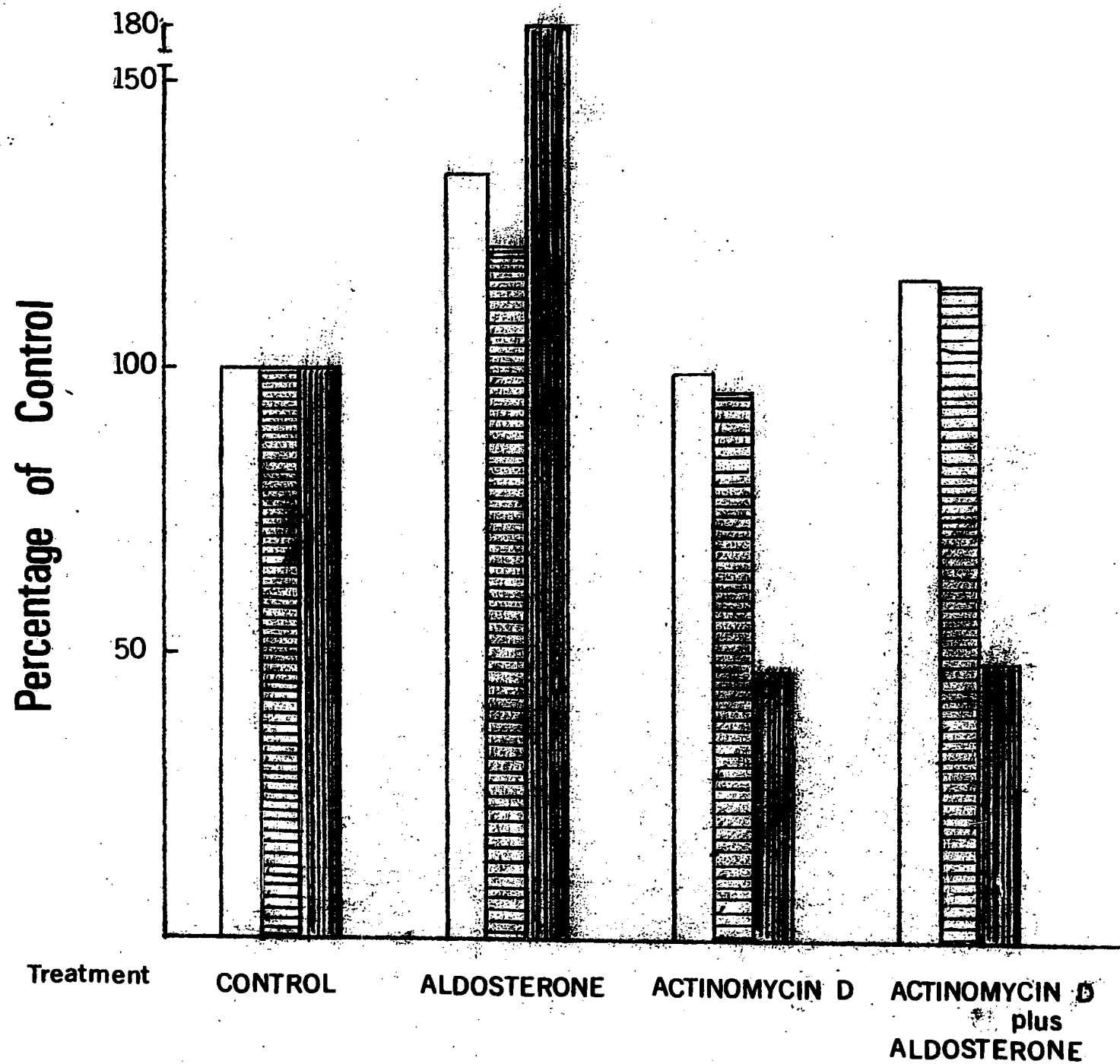
-  Total counts (cpm/mg DNA)
-  Acid soluble fraction (cpm/unit O.D. 260 mμ)
-  Alkaline hydrolyzate fraction (cpm/mg DNA)

FIGURE 10



## DISCUSSION

TIME COURSE STUDIES WITH LABELED PRECURSORS:

Many workers have used labeled nucleosides for studies of the synthesis of RNA in biological systems (90, 118, 130). The rate of incorporation of these precursors into acid precipitable material or RNA is generally taken as a measure of the rate of RNA synthesis by the cells. Nucleosides, however, are not direct precursors of RNA and it is possible, therefore, that changes in the capacity of the cell to take up or phosphorylate nucleosides to nucleoside triphosphates might cause changes in the rate of incorporation of nucleoside into RNA unrelated to the rate of RNA synthesis. There is also the possibility that the net pool of nucleotides might change. This, even if accompanied by no change in the rate of uptake of phosphorylation, results in a change in the specific activity of the pool and hence in the RNA synthesized.

Table IX shows the results in which we compared the incorporation efficiency of  $^3\text{H}$ -uridine,  $^3\text{H}$ -cytidine and  $^{14}\text{C}$ -orotic acid into RNA of normal and adrenalectomized rat kidneys.

Under experimental conditions, orotic acid and cytidine are more efficiently incorporated than uridine with an identical 20 minutes time incorporation. It seems that orotic acid and cytidine are more effective for long term incorporation studies, as is shown by the steady rise in specific radioactivity of the alkaline hydrolyzate fraction (Figures 3-6).

It is rather difficult to compare the magnitude of  $^3\text{H}$ -uridine incorporation into kidneys of normal and adrenalectomized rats because of the

inconsistent incorporation at different times after isotope injection. In this respect, uridine incorporation is different from either cytidine or orotic acid incorporation. The difficulties encountered with uridine incorporation are related to its anomalous uptake kinetics; maximum uptake was not only earlier but also far less efficient than with the cytidine and orotic acid.

The rate of uridine incorporation was almost linear up to 20 minutes and decreased markedly to lower levels after this time. The reason for this decrease in uridine incorporation is not known, but it might be a consequence of the initiation of regulatory controls either on uptake of uridine or its phosphorylation as has been suggested by Plagemann for Novikoff rat hepatoma growing cells (130).

On the other hand, the incorporation of cytidine and orotic acid increased with time up to 40 minutes and then leveled off. There was a decrease in the incorporation of these precursors into the alkaline hydrolyzate fraction of adrenalectomized rat kidneys as compared to the normal animals which suggests a slower rate of RNA synthesis in the kidneys of adrenalectomized rats.

These results emphasize the necessity of precursors of high specific radioactivity as well as the importance of the type of pyrimidine precursor employed.

#### EFFECT OF ADRENALECTOMY AND ALDOSTERONE TREATMENT ON ACID SOLUBLE

#### NUCLEOTIDE POOL AND RNA OF RAT KIDNEYS:

Several workers have demonstrated that an early response to aldosterone involves an increase in the rate of RNA synthesis, which has been

measured as an increase in the radioactive precursor incorporation into RNA (115, 112). The inhibition of RNA synthesis by actinomycin D has been found to prevent the action of aldosterone on sodium transport (110, 113). It was of interest to determine whether there is any change in RNA metabolism of adrenalectomized and aldosterone treated rat kidneys.

As shown in Table X, a significant decrease in RNA: DNA ratio was observed in the kidneys of adrenalectomized rats as compared to the normal animals. This decrease in RNA: DNA ratio can be restored by a single injection of aldosterone either 1 or 2 hours prior to the isotope injection. These findings are similar to the reported decrease in RNA:DNA ratio in seminal vesicle and prostate gland after castration and in the uterus after ovariectomy. The RNA:DNA ratio of these tissues has been restored to normal value after administration of appropriate hormones (131-134). The increase in RNA content after 1 hour of aldosterone treatment precedes the reported effect of aldosterone in increasing sodium transport (112).

In these studies, no change in acid soluble pool:DNA ratio was observed after adrenalectomy or aldosterone treatment.

The decrease in RNA:DNA ratio of adrenalectomized rats must indicate lower rates of RNA synthesis as it has been shown that RNA in various subcellular fractions has a slower turnover rate (135) and also that RNA polymerase activity is lower in the kidneys of adrenalectomized rats (136,137).

The decrease in the rate of RNA synthesis in adrenalectomized rats may result in a decrease in the rate of specific protein synthesis which could account for the decrease in sodium retention in adrenalectomized rats since

the demonstrated increase in RNA and protein synthesis induced by aldosterone treatment has been related to the increase in sodium retention (110,113).

The increase in RNA:DNA after aldosterone treatment may indicate increased rate of RNA synthesis in the kidneys of adrenalectomized rats.

These studies give further support to the postulated hypothesis that aldosterone acts through the RNA and protein synthesis.

EFFECT OF ADRENALECTOMY AND ALDOSTERONE TREATMENT ON THE INCORPORATION OF RADIOACTIVE PRECURSORS INTO RAT KIDNEYS:

Adrenalectomy causes a decrease in the incorporation of  $^3\text{H}$ -cytidine into alkaline hydrolyzate fraction. No such decrease in incorporation into the acid soluble pool was observed. Administration of aldosterone either 1 or 2 hours before the isotope results in an increased incorporation of precursor into macromolecular RNA (Table XI). However, there is also a marked stimulation of uptake of precursor into the acid soluble pool. Similar results have also been observed by using  $^{14}\text{C}$ -orotic acid as a precursor (Table XII). The increased uptake is sufficient to account for at least 65% of the stimulation of  $^3\text{H}$ -cytidine and 45% of the stimulation of  $^{14}\text{C}$ -orotic acid incorporation. There may also be a genuine increase in RNA synthesis but its magnitude is less than would be assumed if the acid insoluble incorporation alone were measured.

These studies are similar to the reported increase in incorporation of precursor into the RNA fraction. Castles and Williamson (111) reported

a stimulation by aldosterone of the *in vivo* incorporation of  $^{14}\text{C}$ -orotic acid into rat renal microsomal RNA. Their experimental design differs in several respects from the one described in this thesis. These workers injected subcutaneously a much lower dose of aldosterone (0.34  $\mu\text{g/kg}$  body weight) after the intravenous administration of  $^{14}\text{C}$ -orotic acid.

Fimognari *et al* (112) reported similar results. Following the subcutaneous injection of 2  $\mu\text{g}$  aldosterone into adrenalectomized rats, the rate of incorporation of  $^3\text{H}$ -orotic acid into various fractions of kidneys was determined *in vitro*. They reported an increase in activity in the nuclear fraction (not significant) 1 hour after hormone injection, and a significant increase was observed at 1.5 hours of hormone administration.

Forte and Landon (43) injected 22  $\mu\text{g}$  aldosterone intravenously into adrenalectomized rats and assayed for an effect of the hormone on the *in vivo* incorporation of  $^{14}\text{C}$ -orotic acid into RNA fractions obtained at different temperatures. They reported a significant stimulation in incorporation at 30 and 60 minutes after administration of aldosterone. By 2 to 3 hours after aldosterone administration the incorporation of  $^{14}\text{C}$ -orotic acid into RNA was again approaching the control levels. Their experimental design also differs from the one described in this thesis. Our design was an intraperitoneal injection of aldosterone (5  $\mu\text{g}/100\text{ g}$  body weight), followed by intraperitoneal injection of precursor 20 minutes before sacrifice.\*

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\* This experimental design is better because incorporation of exogenous precursors plateaus after about 20 minutes and hence beyond this time one is not necessarily measuring the rate of incorporation.



By keeping in mind the differences in experimental design between the reported workers and ourselves, it is of interest to note that we observed a marked stimulation in incorporation into RNA after 1 hour of hormone treatment which persists at least for 2 hours after hormone administration. Furthermore, in our studies we have also shown an increase in precursor uptake into acid soluble pool after hormone treatment.

Initially, it was felt that 5  $\mu$ g of aldosterone might represent a pharmacological dosage. However, Friedman *et al* (138, 139) injected aldosterone in doses of 5 to 10  $\mu$ g/100 g body weight. This was considered to be a reasonable maintenance dose for adrenalectomized rats (140). Fimognari *et al* (112) injected 2  $\mu$ g aldosterone subcutaneously whereas Forte and Landon (43) administered 22  $\mu$ g aldosterone intravenously into adrenalectomized rats and studied the effect of the hormone on renal RNA synthesis. The 5  $\mu$ g dose of aldosterone used in the reported studies discussed in this thesis is within the dose range reported in the literature and it is likely that 5  $\mu$ g of aldosterone is a physiological dose.

EFFECT OF ACTINOMYCIN D AND ALDOSTERONE TREATMENT ON THE INCORPORATION OF <sup>14</sup>C-OROTIC ACID IN ADRENALECTOMIZED RAT KIDNEYS:

Actinomycin D has been shown to have no effect on sodium excretion by itself, but significantly inhibits the antinatriuretic action of aldosterone (113, 112). Incorporation of <sup>14</sup>C-ototic acid into acid soluble pool and alkaline hydrolyzate fraction following actinomycin D and/or aldosterone is shown in Table XIV. Aldosterone increases the incorporation

of  $^{14}\text{C}$ -orotic acid into all fractions. When the actinomycin D treated group was compared with the control group, a significant decrease in the incorporation of precursor was observed in the alkaline hydrolyzate fraction. There was no such decrease in incorporation into the acid soluble pool. Treatment with actinomycin D plus aldosterone was still found to decrease the incorporation into alkaline hydrolyzate fraction whereas there was a small increase in precursor uptake into the acid soluble pool fraction.

These studies suggest that most of the apparent stimulatory effect of aldosterone on RNA synthesis is due to the changes in the rate of precursor uptake. This means that the previously observed effect of aldosterone (111, 112) on precursor incorporation does not necessarily indicate a stimulation of RNA synthesis. Several other reports of hormonal stimulation appear to be due to increased uptake of precursor. Means and Hamilton (23, 24) concluded that the early increase in uterine RNA synthesis in response to estrogen was due to increased uridine uptake and Billing *et al* (30) could detect no increase in RNA synthesis in uterine tissue until 5 hours after estrogen administration if correction were made for changes in the specific activity of the uridine pool. Yu and Feigelson (28) showed an increase in the orotate transport after cortisone treatment without observing any change in the size of the acid soluble pool. Miller and Baggett (29) also concluded that estrogen primarily increases the incorporation of nucleosides into nucleotide pools without changing the size of the total free nucleotide pool of mouse uterus during the first 6 hours of hormone treatment.

However, we cannot rule out the possibility that aldosterone also stimulates the RNA synthesis, particularly in the light of increased RNA:DNA ratio after aldosterone treatment (Table X). These results are also consistent with the reported increase in RNA polymerase activity following aldosterone treatment (136, 137).

EFFECT OF DEOXYCORTICOSTERONE, CORTICOSTERONE AND CORTISOL ON THE INCORPORATION OF  $^{14}\text{C}$ -OROTIC ACID INTO ADRENALECTOMIZED RAT KIDNEYS:

The adrenal corticoids, being very similar in structure show some overlap in activity. For example, liver glycogen deposition is influenced by cortisol, corticosterone, and aldosterone (67). These hormones also have the ability to influence Na/K excretion ratio (67). The effect of aldosterone, the most potent mineralocorticoid, appears to be mediated through RNA and protein synthesis (110, 112). The author is unaware of any report on the effects of deoxycorticosterone, corticosterone and cortisol on precursor incorporation in the kidneys of adrenalectomized rats.

The effects of deoxycorticosterone, corticosterone, and cortisol on the incorporation of  $^{14}\text{C}$ -orotic acid into adrenalectomized rats are shown in Tables XII and XIII. Deoxycorticosterone and corticosterone did not modify precursor incorporation into the alkaline hydrolyzate fraction for at least two hours following the hormone injection. However, these hormones do increase the precursor incorporation into acid soluble pool. These findings suggest that aldosterone has a specific effect in increasing precursor incorporation whereas deoxycorticosterone and corticosterone did not show any significant effect on the incorporation of precursor into the alkaline hydrolyzate fraction of adrenalectomized rat kidneys.

On the other hand, cortisol decreases the incorporation of  $^{14}\text{C}$ -orotic acid into total and alkaline hydrolyzate fraction (Table XIII). No change was observed in the acid soluble pool fraction. These studies suggest that cortisol may decrease RNA synthesis in rat kidneys. It is of interest because cortisol has also been shown to decrease RNA and protein synthesis in rat thymus and spleen whereas at the same time it increases RNA and protein synthesis in rat liver (141). This furnishes another striking example of how a steroid hormone can modify RNA synthesis in different tissues of the same animal in opposite directions.

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PUBLICATIONS ARISING FROM THIS WORK

1. Aldosterone stimulated RNA synthesis in the adrenalectomized rat kidney. J. Kalra and J.F. Wheldrake. Proc. Can. Fed. Biol. Soc., 15, 630 (1972).
2. Evidence that the stimulation of precursor incorporation into RNA of rat kidney is mainly an effect on uptake. J. Kalra and J.F. Wheldrake. FEBS letter, in press (1972).









