Effects of exercise, thyroxine, and age on corticosterone production in the male rat

Bruce William Craig
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Iowa State University, Ph.D., 1972
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Effects of exercise, thyroxine, and age on corticosterone production in the male rat

by

Bruce William Craig

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Zoology and Entomology
Major: Zoology (Physiology)

Approved:

Signature was redacted for privacy.

In Charge of Major

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For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa

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INTRODUCTION

The ability of the adrenal cortex to produce glucocorticoids has been well established, but how various factors affect the production and action of these hormones is not well known. The effects of the thyroid gland, exercise and age have been investigated, and basic trends have become evident. However, the exact role that each of these factors play in adrenal cortical activity is not clear, and many aspects concerning this problem still exist. The present study was undertaken in an attempt to answer some of the questions surrounding this research area.

Since 1934 a functional relationship between the adrenal cortex and the thyroid gland has been recognized. As early as 1897, Solis-Cohen fed thymus and adrenal preparations to hyperthyroid patients and produced a weight gain. During this same period Crary (1897) advocated using adrenal extracts to treat exophthalmic goiters. From these and other clinical studies it was discovered that malfunctions in one of these endocrine glands caused alterations in activity of the other gland. The first experimental evidence for an adrenal-thyroid association came from R. G. Hoskins (1910) who observed adrenal weight increased in guinea pigs fed desiccated thyroid gland.

Some of the early work involving the adrenal cortex and its influence over the thyroid gland has been reviewed by Ingbar and Freinkel (1955). After reviewing the literature and analyzing their own data they concluded that the glucocorticoids of the
adrenal cortex are capable of inhibiting thyroid function. More recent work by Nicoloff et al. (1970) supports these findings, and proposes that the glucocorticoids are short acting and control the thyroid via the hypothalamus.

The thyroid gland's influence over adrenal cortex activity has been summarized by Money (1955). He helped to show that hyperthyroidism speeds up glucocorticoid metabolism while hypothyroidism slows it down. This theory has been well accepted, but exactly how the thyroid exerts its influence is unknown. Steinetz and Beach (1962), in a review article, presented the most plausible theories to explain this control, but the exact mechanisms involved are still not established.

Some of the current literature dealing with the adrenal cortex-thyroid relationship discusses a reciprocal antagonism between Adrenocorticotrophin (ACTH) and Thyrotrophin (TSH) (Ducommun et al., 1966, and Retiene et al., 1968). The theory that ACTH is produced at the expense of TSH and visa-versa was first proposed by Harris (1955).

From the literature it can be seen that the adrenal cortex and the thyroid gland are interrelated, but how exercise affects this association is not well known. Most of the work done thus far deals with the effects of exercise on the adrenal cortex, and does not discuss thyroidal influences. However, basic trends can be seen in the work that has been done. Frenkel and Csalay (1962) demonstrated that daily exercise (swimming) in rats for three weeks
elevates plasma levels and secretion rates of corticosterone. They further found that if exercise is continued for six weeks corticosterone decreases. Suzuki et al. (1967) and Chin and Evonuk (1971) have been able to show that these changes in cortical activity only occur if exhaustive forms of exercise are employed. The trend then appears to be one of an increase in glucocorticoid production with short-term exhaustive exercise, and a decrease in glucocorticoid production with prolonged exhaustive exercise.

Age is another factor that plays an important role in adrenal-thyroid interrelationships. It has been established that structural changes in the adrenal cortex and thyroid gland do accompany old age (Bourne, 1967), but whether or not functional activity in these glands is also affected is still under investigation. Several workers have reported a decrease in thyroid secretion rate with age (Grad and Hoffman, 1955, and Wilansky et al., 1957). If target tissue requirements remain unchanged with advancing age, then decreasing the amount of thyroxine available would lead to a hypothyroid state. However, Grad (1969) feels that these requirements do change with age so that a hypothyroid state does not exist. Harding et al. (1961) and Shapiro and Leathen (1971) have shown a decrease in enzyme activity associated with the adrenal cortex in an aged rat.

This introduction to the factors affecting the production of corticosterones in the rat has been brief, but it has indicated that many questions concerning this problem still exist. It is
hoped that the data presented here will shed a little light on the effects of the thyroxine, exercise and age on the adrenal cortex.
LITERATURE REVIEW

Adrenal-Thyroid Relationships

General

A functional relationship between the adrenal cortex and the thyroid gland has been recognized for many years. It was first discovered on a clinical level by Solis-Cohen (1897), who found that if he fed hyperthyroid patients thymus and adrenal gland preparations a gain in body weight resulted. During the same period Crary (1897) proposed using adrenal extracts to treat exophthalmic goiter. Experimental evidence for this relationship was not presented until 1910 when R. G. Hoskins showed that guinea pigs which were fed desiccated thyroid increased their adrenal weight. These observations were strengthened by E. R. Hoskins (1916) when he repeated this experiment with rats and obtained similar results. Additional evidence for adrenal hypertrophy following thyroid feeding was provided by Herring (1920) when he demonstrated cortical enlargement occurring after such treatment.

The first report of the effects of thyroxine on the adrenal cortex came from Cameron (1923). He found that thyroxine produced adrenal hypertrophy, and that the condition could be inhibited if adrenal cortical extracts and thyroxine were given simultaneously. The relationship between these glands was further established when it was demonstrated that thyroidectomized rats
survived adrenalectomy longer than intact animals (Zwemer, 1925 and 1927).

The concept of an adrenal-thyroid relationship was well founded by 1934 (Money, 1955), but how this mutual association worked was not well understood. Since its establishment the literature on this relationship has split into two forms: the effects of the adrenal cortex on the thyroid gland, and the effects of the thyroid on the adrenal cortex.

**Adrenocortical influences**

When the physiological response of the adrenal to stress was first discovered it stimulated a great deal of research on this gland. The importance of the thyroid in maintaining the body's homeostasis made it of prime importance to the body's reaction to stress. Then when cortisone and adrenocorticotropic (ACTH) became available in the late 1940's a considerable amount of work was focused on the role that these compounds played on thyroid function. Early findings indicated that alterations in adrenocortical steroids or ACTH can cause changes in iodine metabolism. These reports were undecided as to how the adrenal cortex influenced thyroid function. Some workers felt the glucocorticoids acted on the pituitary (Hill et al., 1950), while others thought the thyroid was the site of action (Woodbury et al., 1951, and Paschakis et al., 1952). In an attempt to clarify this controlling mechanism Ingbar and Freinkel (1955) tested the effects of cortisone, hydrocortisone and ACTH on thyroxine metabolism. They found that
administration of these compounds either as replacement or in therapeutic doses do not alter circulating thyroxine degradation. In a later paper, Ingbar and Freinkel (1956) found that glucocorticoids and ACTH suppress thyrotropin (TSH) release by the hypothalamus. Their findings were supported by Beck (1958) who also discovered that the adrenal cortex modified thyroid function through its influence over the pituitary and not thyroxine metabolism.

Recent evidence by Wilber and Utiger (1969) demonstrated a suppression of TSH secretion after the administration of pharmacological doses of glucocorticoids, accompanied by a rebound of TSH after removal of therapy. They were able to show that glucocorticoids do not affect TSH release in vitro, nor do they impair the ability of the pituitary, in vivo or vitro, to respond to thyroid releasing factor (TRF). Therefore, they reasoned that glucocorticoid control was through TRF inhibition and that TSH rebound is due to a buildup of TRF during therapy. This theory was supported by Nicoloff et al. (1970), when they observed similar results. Nicoloff et al., however, found that this control was only transitory and that TRF suppression lasted only a few days. This latter observation is compatible with the concept that the glucocorticoids may exert a prolonged tonic suppression of release but are not capable of complete inhibition.
Thyroidal influences

In 1955 Money reviewed most of the available literature on thyroidal influence over adrenal function. The evidence indicated that hypothyroidism results in a decrease in adrenal function, while hyperthyroidism tends to cause the reverse. Although Money's work was substantial proof that the thyroid does, to some extent, control the adrenal cortex, his work does not explain how this is accomplished.

The thyroid could control adrenal functions through corticoid metabolism. Zarrow et al. (1957) and McCarthy et al. (1959) proposed that a hypothyroid condition causes a decrease in glucocorticoid metabolism and ACTH release, while a hyperthyroid condition causes an increase in corticoid metabolism. The results of Steinetz and Beach (1962) disagreed with Zarrow et al. and McCarthy et al. and did not implicate hypothyroidism with any impairment of the pituitary-adrenal axis. They found that hyperthyroidism decreased the volume of distribution of corticosterones in the rat, which indicates a homeostatic device to prevent concomitant hypercorticoidism.

With the use of triiodothyroxine (T₃) Melby et al. (1960) demonstrated an increase in adrenal cortex secretion. When animals were pretreated with T₃ the biological half life of a cortisone dose was shortened. It was speculated by Melby et al. that this represented an increased catabolism by the liver. They suggested that accelerated enzymatic reduction of circulating cortisol
provides the stimulus for the release of ACTH following an injection of active analogs of thyroxine (T₄). This theory was based on prior work by Yates et al. (1958) and McGuire and Tomkins (1959). Yates et al. were able to show that metabolic transformation (Ring A reduction) of cortisone was enhanced in T₃ pre-treated rats. Their work was substantiated by McGuire and Tomkins who demonstrated that Ring A reduction in steroids is dependent on available triphosphopyridine nucleotide (TPNH), which is regularly increased in rats receiving T₃ pre-treatment.

Some workers feel that the thyroid acts directly on the pituitary and its release of ACTH. As mentioned, Zarrow et al. (1957) and McCarthy et al. (1959) support the idea that the thyroid effects glucocorticoid metabolism. They also speculated that the thyroid may act through the pituitary, this being accomplished by altering ACTH synthesis or a shift in synthesis from ACTH to TSH. Lazo-Wasem (1960) published evidence that the thyroid controls ACTH synthesis. Other workers, Timiras and Woodbury (1955) and Evans et al. (1957) suggested that the rate of ACTH release was influenced by the thyroid.

Recent literature has supported a theory that would explain both the adrenal's influence over the thyroid and the thyroid's control over the adrenal. This theory concerns an antagonism between ACTH and TSH. It has been shown that when the secretion of ACTH is up, TSH secretion is down, and vice versa. Harris (1955) was the first to mention this reciprocal agreement between these
stimulating hormones. He felt that because they were both protein in nature they had a common precursor, and that only one of these hormones could utilize the precursor at one time. This idea was further strengthened by Ducommun et al. (1966) when they observed similar results. However, both Harris and Ducommun et al. noticed exceptions to this reciprocal relationship when animals were exposed to cold, hypothalamic stimulation or administration of TRF during stress. All of these treatments caused a simultaneous release of TSH and ACTH. While studying endocrine rhythms in rats, Retiene et al. (1968) were able to strengthen this theory when they found that if ACTH secretions were at their peak, TSH levels were down. In other words, the TSH levels were inversely proportionate to ACTH levels.

Exercise

Adrenocortical influences

The functional relationship between the adrenal cortex and the thyroid gland has been established, but exactly how exercise affects this association is not well understood. In the past ten years the data on exercise and adrenal function has developed some discrepancies, many of which are due to indirect assaying techniques such as adrenal ascorbic acid, eosinophil levels, or cholesterol depletion (Knouff et al., 1941, Božović and Koshial-Zwanović, 1952, and Ulrich, 1957). With the advent of fluorometric methods more sensitive glucocorticoid assays have been conducted.
Another problem found with early investigations was the wide variation in forms and intensity of exercise. Many reports used human subjects during sporting events which made interpretation difficult due to the stress factors involved (Renold et al., 1951, and Hill et al., 1956).

However, even with these conflicting reports, basic trends have become evident. In 1952 Božović and Koshial-Žwanović found that blood eosinophil counts and ascorbic acid levels of the adrenals dropped during exercise in rats. This drop occurred during the early stages of exercise and continuation of the exercise returned these parameters to normal. Božović and Koshial-Žwanovic felt their results indicated an initial increase in adrenal activity during exercise, but that the gland eventually adjusted and activity returned to normal. Similar results have been observed in plasma levels of corticosterones in the rat. Staehelin et al. (1955) noted that after 15-30 minutes of exercise the concentration of corticoids in human plasma increased, and if exercise was continued (60-120 minutes) these levels decreased. Staehelin et al. felt that as exercise continued glucocorticoid utilization increased. Using short periods of exhaustive exercise Suzuki et al. (1958) were also able to show an increase in adrenal cortical activity. Additional proof for an initial rise in adrenal cortex activity was provided by Connell et al. (1958), but they attributed the change to an increase in stress. It has been suggested by Keeney (1960) that this increase can be enhanced and
prolonged by physiological training.

In 1962 Frenkl and Csalay investigated these earlier reports that exercise can cause both an increase and a decrease in cortical activity (Božović and Koshial-Zwanović, 1952, and Staehelin et al., 1955). They confirmed these reports when they demonstrated that short-term exercise caused an increase in glucocorticoids, while prolonged exercise caused a decrease. Many other workers have obtained similar results (Masson, 1941; Haldi and Wynn, 1946; Young, 1959; Stolyarova, 1968; and Chin and Evonuk, 1971).

Cornil et al. (1965) supported Staehelin et al.'s contention that the decrease in corticosteroids during prolonged exercise was due to increased utilization of the corticosteroids. A two stage theory for the effects of exercise has been proposed by Viru and Äkke (1969). They suggested that the first stage (short-term exercise) represents an increase in glucocorticoid biosynthesis in response to exercise which causes an increase in secretion. During the second stage (prolonged exercise) the body's glucocorticoid supplies become low and biosynthesis declines in order to conserve supplies. It has been shown by Viru and Äkke that the corticoid decrease is due to a lack of stimulus (ACTH), not ability.

Suzuki and his co-workers (1967) looked at exercise intensity as a function of adrenal activity, and found that only exhaustive exercise produced results. He reported that in dogs exhaustive exercise resulted in a significant change in glucocortical secretion and that moderate exercise caused little or no change. In an
investigation by Korenskaya (1967) it was shown that exhaustive exercise is essential for a response to occur. This material supported the contention of Suzuki et al.

Some investigators have linked glucocorticoid fluctuations during exercise to enzymatic changes. Critz and Merrick (1964) and Critz and Withrow (1965) correlated adrenal cortex secretions with glutamic-oxalacetic transaminase (GOT) activity. This enzyme is responsible for converting aspartic acid to oxalacetic, a potential source of energy. In both of these works it was suggested that the adrenal corticoids regulated GOT activity.

**Thyroidal influences**

Information on the effects of exercise concerning the thyroid is limited, but it does appear as though the thyroid is involved in the body's response to exercise. It has been reported that exercise does not change the level of peripheral thyroxine (Lashof et al., 1954). However, a later investigation (Irvine, 1968) shows that peripheral thyroxine degradation does increase during exercise, and that the increase is due to deiodination of thyroxine.

In terms of thyroid activity, conflicting reports have been given by Escobar and DeEscobar (1956) and Bondy and Hagewood (1952). This investigation by Bondy and Hagewood presents evidence for an increased rate of thyroid hormone uptake during exercise, while the investigation of Escobar and DeEscobar shows no change in uptake or metabolism. Still another worker demonstrated a decrease in thyroid activity during exercise (Badrich et al., 1954). All three
of these papers used swimming as their form of exercise. The contradictions in their work may have been due to differences in water temperatures.

Dawson and Horvath (1970) felt that the decrease in plasma bound iodine (PBI) observed during swimming by Bondy and Hagewood was a result of both decreased thyroid activity (Badrich et al., 1954) and increased disappearance rate (Bondy and Hagewood, 1952) not detectable by the short term distribution of $^{131}$I labeled thyroxine (Escobar and DeEscobar, 1956). Evidence presented by Rhodes (1967) demonstrates that the amount of iodine in the thyroid depends on exercise. Recently, Kraus and Kinne (1970) have suggested that adaption to prolonged strenuous exercise is regulated by thyroid hormones.

Age

**Adrenocortical influences**

It has been demonstrated by Bourne (1967) that structural changes occur in both the adrenal cortex and thyroid gland with age. Therefore, it is reasonable to suspect that functional changes may also occur.

Little is known concerning rodent adrenal cortical function in aging, but some work has been done. Harding et al. in 1961 showed that alanine-$\alpha$-ketoglutarate transaminase activity in the rat liver was uniform during the first six weeks of life and then steadily increased until the 48th week. Cortisol treatment
increased the enzyme and adrenalectomy decreased it. Therefore, they suggested this enzyme somehow functions in metabolism and the capacity for growth, and that the adrenal cortex controls it. In 1971 Shapiro and Leathen reported a decrease with age in Δ⁵-3 hydroxysteroid dehydrogenase, the major enzyme of the steroidogenic pathway, and felt it may be related to cellular degeneration of the adrenal cortex (Jayne, 1963). These investigators also noticed a degree of similarity between the adrenal of old rats and hypothyroid rats. Wilansky et al. (1957) have concluded that hypothyroidism in senescent rats implicates the thyroid in adrenal cortical changes with aging.

It has been suggested that adrenal steroid levels remain constant in aging (Grad et al., 1967 and Friedman et al., 1969). It has been demonstrated that in cattle (Riegle and Nellor, 1967) and goats (Riegle et al., 1968) the response to ACTH declines with age. An increased adrenal corticol degeneration with age in rats was observed by Jayne (1963), and led him to suggest that age causes an impaired secretory capacity. Hunt and Hunt (1959) found a decline in glycogen content with age which implies an increased steroid metabolism. However, examination of urinary 17-ketosteroids (Kullander, 1960) and adrenal ascorbic acid (Baca and Chiodi, 1965) of rats through two years of age indicated no change in adrenal function (Rubin et al., 1961). It has been suggested by Adezali and Prando (1966) that the physiological state of the adrenal cortex in old age is characterized by the predominance
of the ACTH-corticoid and ACTH-aldosterone systems. They feel these systems have a slower rhythm of functional deterioration than other systems.

**Thyroidal influences**

The functional status of the thyroid gland in old age and its role in senescence have been subject of study for many years. A similarity between hypothyroidism and old age has been suggested (Lorand, 1904, and Shapiro and Leathen, 1971). In fact Kountz and Chieffi (1947) suggested using thyroxine for the treatment of senescence. In an early review by Carlson (1952) no convincing proof was provided that thyroid activity decreases with age. However, in 1955 Grad and Hoffman demonstrated that the secretion rate of thyroxine decreased with age. Verzar and Freydberg (1956) were also able to show a decrease in the thyroid activity in aged animals and attributed it to a decreased secretion. In 1957 Wilansky et al. suggested that the decrease in thyroid secretion was a homeostatic adaption to increased target tissue responsiveness, to impaired inactivation, or to excretion of thyroid hormones in old rats. The work of Gaffney et al. (1959, 1960, and 1962) partially agreed with Wilansky et al., but felt the homeostatic mechanisms involved caused a decrease in the space of distribution and thyroxine metabolism. Gregerman et al. (1962) viewed the age-dependent adjustment as an alteration in peripheral disposal (degradation or utilization or both) of the thyroid hormone. The similarity to hypothyroidism and age noted earlier was also studied.
by Tsuji and Ogura (1969), but they felt that a mild hypothyroid is advantageous for a long life. They based this conclusion on the assumption that a mild hypothyroid state would lower thyroxine levels and tissue metabolism which would be beneficial. Most of the literature does indicate that age causes a decrease in thyroid activity, however, a few studies such as Scazziga et al. (1968) disagree. In fact they feel there is an increase in TSH stimulation which speeds thyroid activity to compensate for a slowed thyroxine utilization in a euthyroid state.

In a 1969 paper by Grad, he states that senescence does not cause hypothyroidism because as thyroid activity declines so does the animal's metabolism. He further states that thyroid activity changes are due to alteration in the pituitary-thyroid mechanisms and the gland's sensitivity to it.
METHODS AND MATERIALS

Animals

The animals used in this study were of the Sprague-Dawley-Rolfsmeyer strain. The animals were maintained in wire mesh cages, and fed Wayne lab blox and water ad libitum. Prior to and during the experimental period the animals were kept in a temperature controlled (25± 2°C) room which was artificially lighted (8:00 a.m. to 10:00 p.m.).

Experimental Design

Two age groups were employed in this study: young animals 70 days old, and mature animals 200 days old. Four groups of animals were used in these experiments to obtain the data presented. The first two groups of animals were designated as Set 1 and run in cooperation with Herbert Naito (young animals) and Jon Story (mature animals). The corticosterone levels of the glands from these animals were analyzed. Except for age differences, the experimental design in these two groups was identical. In each group of animals five treatments were used.

1. Control — Received no treatment, thyroid intact.

2. Euthyroid — Thyroidectomized (See Appendix A) and given 1.0 ug L-thyroxine (See Appendix B) per 100gms body weight-BW/day.

3. Hyperthyroid — Thyroidectomized and given 3.5 ug
L-thyroxine/100gms BW/day.

4. Hypothyroid — Thyroidectomized and given 0.5 ug L-thyroxine/100gms/day.

5. Athyroid — Thyroidectomized with no replacement thyroxine.

The thyroxine was administered daily in subcutaneous injections of an aqueous solution. The levels of thyroxine were in the appropriate physiological range (Kumaresan and Turner, 1967). Each treatment group was split into non-exercise and exercise animals and were subjected to exhaustive exercise by swimming. The animals swam five days a week for a ten week period. Swimming was carried out in twenty-five gallon plastic barrels with the water temperature being held constant at 37±1°C with a wetting agent[^1] added to each tank. To insure exhaustive exercise lead weights amounting to 4% of the body weight were attached with tape to the tail of each animal. Exhaustion was measured as the time spent under water, and each animal was allowed to swim until he was unable to return to the surface after an 8-10 second period (Dawson and Horvath, 1970). The blood from the first sets of animals was utilized by Naito and Story in their own research, and therefore additional groups of animals (Set II) were run to obtain plasma and secretion rate data. Due to the time involved and economics only three of the previously mentioned groups were utilized.

The results obtained from the adrenal glands were responsible

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[^1]: Ivory Liquid was kindly supplied by the Proctor and Gamble Company, Cincinnati, Ohio.
for choosing these three groups. The data suggested that the level of thyroxine was not as important as the thyroxine itself, because in the three groups receiving thyroxine the greatest response came from the euthyroid group. The athyroid group gave the greatest response if all five groups were considered. Therefore, the three groups chosen were control, euthyroid and athyroid. Other than the number of treatments used the four groups of animals received identical treatment.

Experimental Procedures

Three parameters other than weight changes were measured in this study, the adrenal gland and plasma corticosterone levels, and corticosterone secretion rate. Adrenal gland and plasma levels were measured fluorometrically, and the secretion rate was measured using $^{14}$C-corticosterone and liquid scintillation. For a more detailed description of experimental procedures refer to the Appendix.

Corticosterone concentrations in adrenal glands and plasma

The animals in the first two groups were killed by decapitation. The glands were removed and cleaned of adhering fat, and then weighed on a torsion spring balance to the nearest 0.1mg. The glands were homogenized in 2.0ml of 33% ethanol, and transferred to a 5.0ml volumetric flask. The homogenate is then diluted to 5.0mls. with double distilled water and shaken (Zarrow et al., 1964). Then 1.0ml.
aliquots were transferred to a 15ml. glass stoppered centrifuge tubes, and 1.0ml. of 13% ethanol added. Determination of the corticosterone content of the glands was carried out using fluorescence (See Appendix C).

The plasma samples taken from the last two groups of animals were used to determine plasma corticosterone levels. The plasma did not need any preliminary treatment, and 1.0ml. aliquots were transferred to 15ml. glass stoppered centrifuge tubes. The final volume was again adjusted to 2.0ml. with the addition of 1.0ml. of 13% ethanol. Fluorescence was again used to determine the corticosterone levels. Duplicates were run for both the gland and plasma samples and then compared to a standard curve to determine content.

**Secretion rates**

Secretion rates were computed from metabolic clearance rates—MCR (biological half-life of corticosterone in the blood) and corticosterone levels in the plasma (James and Landon, 1968). Plasma data was obtained as shown above, and the MCR was determined with the aid of \(^{14}\)C-corticosterone and liquid scintillation (Glenister and Yates, 1961, and Fromweiler et al., 1968). The MCR was based on a series of blood samples taken over an hour's time (See Appendix A). These samples were spun down at 2000 rpm and 0.2ml. of plasma/animal was analyzed. The 0.2ml. samples were placed in liquid scintillation vials, and 1.0ml. of NCS (biological solubilizer — Amersham/Searle) was added to each vial. The cap
was replaced and the vial shaken until the solution cleared. Once clear 10ml. of toluene (containing PPO and POPOP — See Appendix B) was added to each vial. At this point the samples were ready to be counted, and were shaken and wiped clean with a kim-wipe before being placed into the counter. The counter (Packard Tricarb Counter) was set for a 14% gain and the windows set at 50-1000. Standards were prepared by adding 0.1 ug of $^{14}$C-corticosterone for young and 0.2 ug of $^{14}$C-corticosterone for mature to a scintillation vial. To each vial 1.0ml. of NCS and 10ml. toluene were added. A background vial containing only NCS and toluene was also prepared. The samples, standards, and background were allowed to cool and read for 10 minutes each.
RESULTS

Each collection of data was analyzed using analysis of variance to determine treatment, exercise, and overall age differences. In addition each group was analyzed using an LSD test to determine individual differences. The results of each LSD is shown below the table corresponding to the data presented. The statistical analysis of the data was carried out with the cooperation of Dr. D. Cox and the Statistical Department of Iowa State University, Ames, Iowa.

Three comparisons for each group of data were made. First, the treatment differences (control, euthyroid, and athyroid) were compared to each other. Secondly, exercise comparisons were made by comparing non-exercise to exercise animals. Thirdly, age comparisons were made between young and old animals of the same treatment groups.

Body and Adrenal Weights

The body-adrenal weight data is based on two sets of animals and each set will be discussed separately. The results for set 1 were obtained from the first two groups of animals and the results of set 2 came from the second two groups. The body weight data for animal sets 1 and 2 can be found in Table 1 and 2 and are compared graphically in the form of histograms (Fig. 1 and 2).
Table 1. Body weight changes for the animals of Set I (grms)

<table>
<thead>
<tr>
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<th>Mature</th>
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<tr>
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<td>NE</td>
<td>Ex.</td>
</tr>
<tr>
<td>Control</td>
<td>424.0±15.8*</td>
<td>367.0±16.8</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>412.0±15.8</td>
<td>367.0±15.8</td>
</tr>
<tr>
<td>Athyroid</td>
<td>260.0±16.8</td>
<td>285.0±15.8</td>
</tr>
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Least significant difference at $P<0.05 - 11.57$

*Mean: standard error of that mean
Table 2. Body weight changes for the animals of Set II (grms)

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<th>Young</th>
<th>Mature</th>
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<tbody>
<tr>
<td></td>
<td>NE</td>
<td>Ex.</td>
</tr>
<tr>
<td>Control</td>
<td>486.0± 13.4*</td>
<td>396.0± 14.6</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>367.0± 12.0</td>
<td>352.0± 12.4</td>
</tr>
<tr>
<td>Athyroid</td>
<td>288.0± 12.0</td>
<td>281.0± 12.0</td>
</tr>
</tbody>
</table>

Least significant difference at $P<0.05$ - 11.16

*Mean± standard error of that mean
Figure 1. Treatment effects on the body weights of the young and mature animals of Set I. The shaded bars represent exercised animals. The abscissa is divided into grams, and treatment groups are found along the ordinate.

C = Control
E = Euthyroid
A = Athyroid
Body Weights (Set 1)

Weight (grms)

Young

Mature

GROUPS

C E A

C E A
Figure 2. Treatment effects on the body weights of the young and mature animals of Set II. The shaded bars represent exercised animals. The abscissa is divided into grams, and treatment groups are found along the ordinate. The abbreviations are the same as found in Fig. 1.
Body Weights (Set 2)

Weight (grms)

Young  Mature

GROUPS
Set 1

An analysis of variance of body weight differences for the first set of animals showed a significant difference ($P<0.001$) for treatment, exercise and age. A comparison of treatment groups shows that the body weights of young, non-exercise (NE), euthyroid and athyroid animals were significantly smaller than the control animals. The only significant body weight change in the mature NE animals was a decrease in weight for the athyroid group. When comparing the young, exercise (Ex.) treatment groups no significant change in weight between the euthyroid and control animals was shown. There was, however, a significant decrease in the Ex., athyroid weight in comparison to the other two groups. Analysis of treatment differences in the mature, Ex. animals indicates that the control is significantly smaller than the other groups. Athyroid animals were the heaviest, but the difference between athyroid and euthyroid weights were not significant.

To determine the effects of exercise, each treatment group was compared to itself. This type of an analysis shows that in the young animal exercise causes a significant drop in control and euthyroid body weights, but causes an increase in athyroid body weight. Body weights of the control and euthyroid mature animals also dropped due to exercise, but instead of a weight increase in the athyroid group there was no significant change.

The effects of age can be seen when animals of like groups and treatments are compared. An analysis of the data in this manner
shows a significant increase in body weight for mature animals regardless of treatment or exercise regime.

Set 2

Treatment differences for the young, NE animals were the same as those found in the first set of animals; the control animals being the heaviest and the athyroid group the lightest. All of these body weight changes were statistically significant. In the mature, NE animals the body weights of the control animals were significantly larger than the other two groups, and the euthyroid body weight was significantly smaller than the athyroid. The weight changes within the treatment groups for the Ex. animals were the same for both age groups. These changes consisted of a significant decrease in euthyroid and athyroid weight in comparison to the control, with the athyroid weight loss being significantly larger than that of the euthyroid animals.

When a comparison was made for the effects of exercise, it was shown that exercise caused a significant decrease in body weight for the young control and euthyroid animals, but did not show a significant decrease for athyroid weights. In the mature animals exercise caused a significant decrease in weight for all three groups. Age comparisons indicated that this parameter caused a body weight increase in all groups.
**Adrenal weight**

The adrenal gland weight differences for sets 1 and 2 can be found in Tables 3 and 4 and are also represented graphically (Fig. 3 and 4).

**Set 1** The adrenal weight differences for the treatment groups were the same as body weight changes for young, NE animals. The glands of the euthyroid and athyroid animals weighed less than those of the control animals, with the greatest weight loss found in the athyroid group. Treatment comparisons of the mature, NE animals still showed the athyroid adrenal weight to be significantly smaller than both control and euthyroid groups. The euthyroid glands, instead of being smaller, were significantly larger than the control's glands. Group comparisons of Ex. animals gave results similar to the NE animals at both age levels. Except for a non-significant increase over control by the mature euthyroid group all of these changes were significant.

Comparisons for exercise between the young groups indicated that it significantly increased control and athyroid adrenal weight, while it significantly decreased euthyroid adrenal weight. The adrenal glands of all three mature groups were heavier as a result of exercise, but only the control and euthyroid changes were significant. A comparison between ages showed that except for NE control animals the age differences significantly increased adrenal weight. In the NE control group there was a slight but not significant decrease in weight.
Table 3. Adrenal weight changes for the animals of Set I (mgram)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE</td>
<td>Ex.</td>
</tr>
<tr>
<td>Control</td>
<td>$50.6\pm 3.28^*$</td>
<td>$54.7\pm 3.48$</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>$44.0\pm 3.28$</td>
<td>$41.9\pm 3.28$</td>
</tr>
<tr>
<td>Athyroid</td>
<td>$26.9\pm 3.48$</td>
<td>$34.8\pm 3.28$</td>
</tr>
</tbody>
</table>

Least significant difference at $P < 0.05 - 2.40$

*Mean*: standard error of that mean
Table 4. Adrenal weight changes for the animals of Set II (mg rms)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th></th>
<th>Mature</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE</td>
<td>Ex.</td>
<td>NE</td>
<td>Ex.</td>
</tr>
<tr>
<td>Control</td>
<td>42.7±</td>
<td>2.22*</td>
<td>44.1±</td>
<td>2.43</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>46.1±</td>
<td>1.98</td>
<td>47.5±</td>
<td>2.05</td>
</tr>
<tr>
<td>Athyroid</td>
<td>28.4±</td>
<td>1.98</td>
<td>33.7±</td>
<td>1.98</td>
</tr>
</tbody>
</table>

Least significant difference at $P < 0.05 - 1.85$

*Mean ± standard error of that mean
Figure 3. Treatment effects on the adrenal weights of the young and mature animals of Set I. The shaded bars represent exercised animals. The abscissa is divided into milligrams, and treatment groups are found along the ordinate.
Adrenal Gland Weights (Set 1)

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Young</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4. Treatment effects on the adrenal weights of the young and mature animals of Set II. The shaded bars represent exercised animals. The abscissa is divided into milligrams, and treatment groups are found along the ordinate.
Adrenal Gland Weights (Set 2)

Weight [mg/min]

Young

Mature

GROUPS
Set 2  The comparisons from this set of animals showed some differences from the first set. Treatment comparisons between young NE animals showed that the glands of euthyroid animals were significantly larger than those of the other two groups. When NE and Ex. control and athyroid young animals were compared it was found that athyroid adrenal glands were significantly smaller than control's. Mature treatment comparisons gave the same results as the young for NE animals, but not for the Ex. groups. In Ex. groups the control glands were the larger, and the athyroid the smaller, with all these differences being statistically significant.

Exercise increased adrenal weight in each group and with the exception of the young euthyroid animals these changes were significant. Without exception the mature group had significantly larger adrenals than the young.

**Medulla-cortex weight**

The medulla-cortex weights are only based on the glands from set 2; the glands from set 1 being utilized for determinations of adrenal gland content. Medulla and cortex weights can be found in Tables 5 and 6, and comparisons of weight differences are given in the form of histograms in Fig. 5 and 6.

**Medulla weight**  Analysis of the treatment differences indicated that the euthyroid animals had significantly larger medullas than the other two groups. It can be shown that the athyroid gland weight was significantly smaller than the control
Table 5. Medulla weight changes for the animals from Set II (mgrms)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th></th>
<th>Mature</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE</td>
<td>Ex.</td>
<td>NE</td>
<td>Ex.</td>
</tr>
<tr>
<td>Control</td>
<td>18.8±1.13*</td>
<td>20.0±1.24</td>
<td>16.8±1.13</td>
<td>19.5±1.48</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>21.7±1.01</td>
<td>21.7±1.04</td>
<td>20.7±1.24</td>
<td>23.4±1.59</td>
</tr>
<tr>
<td>Athyroid</td>
<td>10.9±1.01</td>
<td>12.1±1.01</td>
<td>12.9±1.18</td>
<td>15.5±1.75</td>
</tr>
</tbody>
</table>

Least significant difference at \( P < 0.05 - 0.94 \)

*Mean ± standard error of that mean
Table 6. Cortex weight changes for the animals from Set II (mgrms)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE</td>
<td>Ex.</td>
</tr>
<tr>
<td>Control</td>
<td>23.9± 1.84*</td>
<td>24.1± 2.01</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>24.4± 1.64</td>
<td>25.8± 1.70</td>
</tr>
<tr>
<td>Athyroid</td>
<td>17.5± 1.64</td>
<td>21.6± 1.64</td>
</tr>
</tbody>
</table>

Least significant difference at P< 0.05 - 1.54

*Mean± standard error of that mean
Figure 5. Treatment effects on the medulla portion of the adrenal glands from Set II animals. The shaded bars represent exercised animals. The abscissa is divided into milligrams, and treatment groups are found along the ordinate. The abbreviations are the same as those found in Fig. 1.
Medulla Weights

Weight [mg rms]

Young

C E A

Mature

C E A

GROUPS
Figure 6. Treatment effects on the cortex portion of the adrenal glands from Set II animals. The shaded bars represent exercised animals. The abscissa is divided into milligrams, and treatment groups are found along the ordinate. The abbreviations are the same as those found in Fig. 1.
gland weight. This relationship holds for both the young and mature animals regardless of exercise. Every group except one had a significant increase; the exception being the young euthyroid group which showed no significant change with exercise.

Age had a different effect on each group. In the control group there was a significant decrease with age. The medulla weights of the athyroid animals showed a significant increase with age. The medulla weight of the euthyroid animals showed both a significant increase in Ex. animals and a significant decrease in NE animals.

**Cortex weight** The effects of treatment differences on the cortex weight are not as uniform as the medulla data. In young NE animals the most significant change is in the athyroid group. The cortex of this group is significantly smaller than both the control and euthyroid. The cortex of euthyroid animals was larger than the control's, but this change was not significant. Cortex weight relationships of mature NE animals were identical to the young NE except that all of the observed changes were significant. The same pattern, euthyroid significantly larger than control and athyroid, and athyroid significantly smaller than control, was seen for the cortex weight of the young Ex. animals. The effects of treatment on the mature Ex. group changes this pattern. In these animals the control cortex is significantly larger than euthyroid and athyroid, the euthyroid cortex being slightly smaller.
than the athyroid.

Exercise caused an increase in cortex weight for each group in both age brackets. However, only the young and mature athyroid and mature control changes were significant. The age difference resulted in significant cortex weight increase for each group.

**Corticosterone Production**

The adrenal and plasma concentrations, and secretion rates for corticosterone are given in Tables 7, 8, and 9, and comparisons are found in Fig. 7, 8, and 9 in the form of histograms.

**Adrenal gland concentration**

Comparisons among the treatments showed that NE euthyroid concentrations for both ages was significantly larger than control and athyroid concentrations. The athyroid concentrations were significantly smaller than control between the NE groups. When comparing Ex. groups it was indicated that the euthyroid had the largest corticosterone concentration, while the athyroid had the smallest. Except for the difference between the control and euthyroid concentrations in mature animals these observations were significant.

Analysis of exercise effects showed that exercise caused a significant increase in adrenal gland corticosterone levels in all three of the young groups. In the mature animals the only significant increase was found among the control animals. The athyroid concentration increased but was not statistically
Table 7. Adrenal gland corticosterone concentrations (ug/ml.)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE</td>
<td>Ex.</td>
</tr>
<tr>
<td>Control</td>
<td>4.17± 0.623*</td>
<td>4.83± 0.660</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>4.79± 0.623</td>
<td>5.52± 0.623</td>
</tr>
<tr>
<td>Athyroid</td>
<td>2.54± 0.660</td>
<td>3.67± 0.623</td>
</tr>
</tbody>
</table>

Least significant difference at $P < 0.05 - 0.456$

*Mean± standard error of that mean
Table 8. Plasma corticosterone concentration (ug/ml.)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th></th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE</td>
<td>Ex.</td>
<td>NE</td>
</tr>
<tr>
<td>Control</td>
<td>0.783± 0.078*</td>
<td>0.804± 0.086</td>
<td>0.642± 0.078</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>1.063± 0.070</td>
<td>0.928± 0.072</td>
<td>1.119± 0.086</td>
</tr>
<tr>
<td>Athyroid</td>
<td>0.681± 0.070</td>
<td>0.553± 0.070</td>
<td>0.532± 0.082</td>
</tr>
</tbody>
</table>

Least significant difference at P < 0.05 - 0.065

*Mean± standard error of that mean
## Table 9. Corticosterone secretion rates in ug/ml/min

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE</td>
<td>Ex.</td>
</tr>
<tr>
<td>Control</td>
<td>10.28± 1.613*</td>
<td>9.88± 1.767</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>14.51± 1.443</td>
<td>13.38± 1.494</td>
</tr>
<tr>
<td>Athyroid</td>
<td>9.14± 1.443</td>
<td>6.71± 1.443</td>
</tr>
</tbody>
</table>

Least significant difference at $P < 0.05 - 1.348$

*Mean±: standard error of that mean
Figure 7. Treatment effects on the corticosterone concentration of the adrenal glands (Set I animals). The shaded bars represent exercised animals. The abscissa is divided into ug/ml, and treatment groups are along the ordinate. The abbreviations are the same as those found in Fig. 1.
Adrenal Gland Concentrations

Corticosterone [μg/ml]

Young

Mature

GROUPS

C E A

C E A
Figure 8. Treatment effects on the corticosterone concentration of the plasma (Set II animals). The shaded bars represent exercised animals. The abscissa is divided into ug/ml, and treatment groups are along the ordinate. The abbreviations used are the same as those found in Fig. 1.
Plasma Concentrations

Corticosterone (µg/ml)

Young

Mature

GROUPS
Figure 9. Treatment effects on corticosterone secretion rates (Set II animals). The shaded bars represent exercised animals. The abscissa is divided into ug/ml/min, and treatment groups are along the ordinate. The abbreviations used are the same as those found in Fig. 1.
Secretion Rates

Corticosterone (μg/ml/min)

Young

Mature

GROUPS

C  E  A

C  E  A
significant. In the mature euthyroid animals exercise caused a significant decrease in corticosterone levels. When compared to animals of like treatments the mature animals all showed a significant increase in corticosterone concentration.

**Plasma concentration**

Plasma results followed the same scheme as adrenal levels in regards to treatment differences. The euthyroid concentration in both age and exercise groups were significantly higher than the other treatments; athyroid plasma concentration for both age and exercise being significantly smaller than control.

The effects of exercise on plasma concentrations varied with treatment. In young control animals there was an increase in concentrations, and in the mature control animals there was a decrease. However, neither of these changes were statistically significant. The euthyroid concentrations of both young and mature animals decreased significantly with exercise. Plasma levels of young athyroid animals also underwent a significant decrease due to exercise, while the mature athyroid animals had a slight, insignificant increase in concentration.

The effects of age were also varied. For control animals there was a significant drop in plasma levels with age. The plasma concentrations in euthyroid animals increased, but only the increase among Ex. groups was significant. The change in age caused a significant drop in NE athyroid concentrations but virtually no change among athyroid Ex. groups.
**Secretion rate**

The treatment pattern obtained with adrenal and plasma concentrations can also be found in secretion rates; euthyroid rates being the highest and athyroid rates the lowest. However, not all of these changes were significant. The euthyroid secretion rates were higher than control and athyroid levels for both age and exercise groups, and the athyroid secretion rates were lower than control but not always significantly. The young, athyroid NE secretion rates were not significantly lower than control, but the mature, athyroid, NE rates were significantly lower. The young, athyroid, Ex. group was significantly lower than control but mature, athyroid, Ex. was not significantly lower.

Exercise decreased the rate of secretion in every group except the mature athyroid animals where a small insignificant increase was observed. Only two of the observed decreases were significant, that of the young athyroid and the mature control groups.

When the effects of age were investigated a similarity to the plasma data was found. In control animals an increase in age caused a significant decrease in secretion rate. With euthyroid animals the reverse was true; an increase in age caused a significant increase in secretion. Advancing age caused a decrease in athyroid secretion rate, but this decrease was only significant in the NE animals.
DISCUSSION

Glucocorticoid production by the adrenal cortex is well recognized physiologically but how various factors affect this production and the release of these hormones is not completely understood. The influence of the thyroid gland, exercise, and age have been investigated, but the exact roles played by these variables has not been established. It is the purpose of this study to help resolve some of the unanswered questions still surrounding glucocorticoid production and the effects of the thyroid gland, exercise, and age upon it. In addition to body and adrenal weight changes, adrenal cortical activity was measured using three corticoid production estimates: corticosterone levels of the gland and plasma, and secretion rates. These parameters should give an accurate measure of corticosterone synthesis and release. The results of this work can more readily be analyzed by separating it into three areas of discussion: thyroidal, exercise, and age influences.

Thyroid Gland Influence

A functional link between the adrenal cortex and thyroid gland has been recognized since 1934 (Money, 1955). Many investigators have worked on this association and their work has revealed some characteristic features of this relationship but they are not conclusive. There has been some evidence that these glands affect one another by influencing the hormonal metabolism of each other
(Woodbury et al., 1951; Paschkis et al., 1952; Zarrow et al., 1957; McGuire and Tomkins, 1959; Yates et al., 1958; McCarthy et al., 1959; and Melby et al., 1960). Other workers have shown that the controlling mechanisms involved act on the pituitary (stimulating hormone) or hypothalamus (releasing factors) (Timiras and Woodbury, 1955; Ingbar and Freinkel, 1956; Evans et al., 1957; Zarrow et al., 1957; Beck, 1958; McCarthy et al., 1959; Lazo-Wasem, 1960; Wilber and Utiger, 1969; and Nicoloff et al., 1970).

Still another theory has come into print, that of a reciprocal agreement between TSH and ACTH or their respective releasing factors (Harris, 1955; Ducommun et al., 1966; and Retiene et al., 1968).

These investigators have proven that the thyroid does influence the functional activity of the adrenal cortex. In the present study three groups of animals were set up in order to test the extent of these thyroidal influences: control-intact animals, athyroid-thyroidectomized, and euthyroid-thyroidectomized with T₄ replacement. With these three groups the effects of the thyroidectomy and T₄ on the parameters measured can be analyzed. The treatment differences will be based on comparisons of these three groups, and will be the only comparisons made in this discussion section.
Weight changes

Interpretation of weight changes between sets of animals in this study are difficult because of the variation in results. These differences are due to handling variation between groups of animals. Each group of animals within set 1 were handled by different investigators. The animals in set 2 were all handled by the same investigators, but not the same as those in set 1. Therefore, allowance for handling must be taken into account. For this reason the reliability of these weight changes are not strongly stressed. However, comparisons can and will be made, but not between sets of animals. Any comparisons between weights and concentrations or rates will be made for the animals from which both measurements were taken. In other words, the adrenal gland corticosterone concentration will only be compared to set 1 animals. Weight comparisons for any other measurement will utilize animals from the second set.

When the effect of the thyroid was totally removed (athyroid) the body weight of young animals in either set was significantly reduced. Thyroid removal did not have as uniform an effect on mature animals. Thyroidectomy of mature NE animals in set 1 did cause a significant weight loss. The same treatment caused a significant weight gain in the mature Ex. animals from set 1. In set 2 thyroidectomy (athyroid) caused a significant weight gain in mature NE animals and a significant weight loss in Ex. animals.

If T4 is given as replacement (euthyroid) the body weight is usually brought back to normal or above normal as shown by the
mature animals in set 1. These weight changes as a result of $T_4$ replacement are varied, but they do tend to indicate that $T_4$ is important in maintaining body weight. These observations are not unexpected considering the thyroid's role in metabolism.

The results for gland weights are not totally consistent with those for body weights. For instance, the adrenal weight differences for animals from set 1 agree with body weight changes, but the adrenal data for set 2 is the reverse of body weights in some cases. In both sets of animals without $T_4$ (athyroid) there was a loss in adrenal weight, and with $T_4$ (euthyroid) weights increased with the only difference being in the magnitude of the response in relation to control.

The adrenal data also indicates the importance of thyroxine in adrenal gland activity. Without $T_4$ the adrenal glands atrophied and with $T_4$ the glands were maintained. These changes are more drastic in young than old animals which might indicate that $T_4$ loses its effectiveness with age. This point will be discussed in greater detail later.

Changes in adrenal weight may or may not be indicative of cortical weight changes. Therefore, the adrenal glands of the second set of animals were weighed upon removal from the animal and then separated into medulla and cortex and weighed again. From this data it can be seen that removal of the thyroid gland tends to affect the medulla and cortex in the same manner. Except for a slight insignificant increase in cortical weight for
mature Ex. animals, the medulla-cortex portions of the gland lost weight after thyroidectomy (athyroid). When $T_4$ was given as replacement (euthyroid) the medulla-cortex weights increased.

These weight fluctuations suggest that $T_4$ is essential for adrenal growth. The maintenance of the cortex and the medulla could be the direct result of thyroxine. Another possibility has been discussed by Wurtman and Axelrod (1965 and 1966) and connects glucocorticoids to catecholamine synthesis. The results then could be interpreted two ways: the first being that exogenous $T_4$ causes an increase in glucocorticoids which in turn increase catecholamines. This proposal would depend upon a relationship between synthesis and weight change. The second interpretation would be that $T_4$ is necessary for both medulla and cortex growth and that the observed increases of these two portions of the adrenal are not necessarily related.

Corticosterone production

Adrenal weight changes are indications of how certain treatments affect an animal, but are not specific or conclusive enough to be used alone. Therefore, certain other parameters must be measured. Three such parameters have been chosen for this study, and the results of these measurements will be discussed separately. Body weights were not found to correlated with any of these parameters. If we assume that body weight changes reflect the affects of $T_4$ on metabolism and not necessarily on adrenal activity, we can omit body weight as a significant variable in the
discussion of these parameters. By doing this we can concentrate on adrenal weight and cortex weight changes and how they correlate with these measurements. As mentioned earlier the results that will be discussed here will reflect the effects of thyroidectomy and thyroxine and how they compare to control animals and one another.

**Adrenal content** Removal of the thyroid gland (athyroid) caused a significant drop in adrenal corticosterone content in all animals tested. The administration of T₄ (euthyroid) brought the concentration back to above normal levels. With the exception of the mature Ex. animals these increases were significant.

In a comparison of the adrenal weights (set 1) to adrenal concentration it can be seen that corticosterone changes do not necessarily correspond to adrenal weight changes. Some similarities can be found in the mature animals, but none are apparent in the young. However, if the same comparison is made to the animals of set 2, the results are correlated, but for reasons previously stated this comparison is not valid. If adrenal content is any indication of corticosterone synthesis, this data indicates that synthesis is not proportional to adrenal weight change.

The evidence given here suggests that the thyroid gland regulates corticosterone levels of the adrenal cortex. With adrenal weight and gland concentration data it cannot be determined whether this regulation is due to a direct action of T₄ on the cortex or
the result of secretory changes. Therefore, this point will be brought up again after the plasma and secretion rate data is discussed.

**Plasma concentration**

Plasma levels of corticosterone can be directly related to adrenal gland concentrations because they show the same pattern. With no \( T_4 \) (athyroid) or thyroidal influence, the levels are low. Injections of \( T_4 \) (euthyroid) restore the levels to above normal. The restoration in this case includes all of the groups studied.

If we compare plasma levels to adrenal weight changes (set 2) in the young animals it can be seen that they are directly proportionate. When adrenal weight increases so do the corticosterone plasma levels. The same thing is true for medulla and cortex weight changes. These phenomena could be nothing more than the effects of \( T_4 \). When the mature animals are examined this appears to be the case. The increases and decreases in plasma levels in the mature animals are not uniform and do not correspond to adrenal weight changes. The variation of adrenal weight between the sets of animals and the lack of uniform correspondence to concentration changes casts doubt on the reliability of weight fluctuation as a true estimate of treatment effects. This variation could be due to the effect of age on \( T_4 \). Due to this variation, the discussion of the remaining parameters will not be compared to adrenal weight.
**Secretion rate**  The similarity between the adrenal concentration and plasma concentration data re-emphasizes the importance of T\(_4\) influence on adrenal cortical activity. The secretion rate data follows the same pattern established by the adrenal gland and plasma results. Therefore, to get a clear idea of how the thyroid affects glucocorticoid synthesis and release, we must draw all three corticosterone estimates together for analysis.

First, let us look at the effects of these parameters on animals without thyroidal influences (athyroid). Under these conditions the adrenal gland and plasma levels drop. Secretion rate also drops which tends to show that the thyroid is needed for corticosterone release. However, the depression in secretion rate was not significant in every case. Before we can determine why some of the adrenal secretion rates for the athyroid animals were significant and some were not, we must first theorize how the system might work. With the thyroid gone the body should demand T\(_4\) which would stimulate the hypothalamus to produce TRF. The TRF in turn would cause the synthesis and release of TSH. If there is a reciprocal arrangement between ACTH and TSH for this release, as has been suggested (Harris 1955 and Ducommun et al., 1966), then in animals deprived of the thyroid and T\(_4\) (athyroid) the levels of TSH should be up and ACTH levels down. The decrease in adrenal gland and plasma concentrations suggests that this reciprocal relationship does regulate corticosterone levels. Even though the secretion rate reduction was not significant for the NE athyroid
animals it does indicate a reduction in adrenal cortical activity when the thyroid is removed. The lack of significance for the decreased secretion rates of mature, Ex., athyroid animals could be a defense mechanism by the body that overrides the ACTH-TSH relation. This mechanism might involve a stress condition similar to cold or electrical stimulation which has been shown to change the ACTH-TSH balance (Harris, 1955, and Ducommun et al., 1966). The exercise in the mature athyroid animals would act as the stress and thus cause a shift in the ACTH-TSH system towards ACTH. This would enable the animal to maintain glucocorticoids.

Secondly, let us examine thyroxine therapy (euthyroid). Here we find that secretion rates were significantly increased, even above control. This would indicate that exogenous $T_4$ is highly effective in raising corticosterone secretion rates. One explanation for this increase would also involve TSH release. The synthesis and release of TSH is regulated to some extent by $T_4$ through a negative feedback system. In a normal intact animal this feedback system would provide the animal with a means for controlling $T_4$ synthesis. If the reciprocal arrangement between ACTH and TSH is a functional one, then the exogenous $T_4$ given to euthyroid animals would act to reduce TSH and increase ACTH. The ACTH would in turn increase the glucocorticoids.

Thus far we have looked at treatment animals and have found that thyroidectomy reduced the corticoids and that $T_4$ increased them. The third group to be discussed is the control animal with
an intact thyroid. We would assume that the ACTH-TSH relationship is functioning normally. The $T_4$ concentration in a control animal should be close to or the same as the daily dose given the euthyroid group (Kumaresan and Turner, 1967). If the ACTH-TSH balance is functional in a control animal, and $T_4$ levels produced by it are the same as the euthyroid dose, then the corticosterone concentration should theoretically be the same for these two groups. The results demonstrated that corticoid levels were significantly lower in control animals when compared to the euthyroid group. This suggests that $T_4$ does not work alone in TSH regulation. It would indicate that another factor is involved which would help to keep TSH levels reduced and allow for glucocorticoid formation. However, this idea does depend on the assumption that $T_4$ levels in control animals are the same as the euthyroid group dose. If the concentrations differ, then the $T_4$ levels of control animals would be below the doses used in this study and would allow higher TSH levels to be produced.

The basic findings from these treatment comparisons are that thyroidectomy reduced corticosterone concentrations of the body and reduced secretion rates. These decreases can be reversed if $T_4$ is administered. It is felt that this decrease and increase operate through a reciprocal relationship of ACTH and TSH. The results from control animals suggest that $T_4$ might not act alone in TSH regulation.
Exercise

It has been shown by many investigators that exercise will cause an increase in glucocorticoid levels (Božović and Koshial- Žwanović, 1952; Staehelin et al., 1955; Suzuki et al., 1958; and Connell et al., 1958). In a report by Keeney (1960) it was suggested that physiological training could enhance this increase. A later report by Ingbar and Freinkel (1955) showed that short-term exercise will cause an increase in glucocorticoids, and that prolonged exercise causes a decrease. Similar results by other workers have helped to strengthen their data (Masson, 1941; Haldi and Wynn, 1946; Young, 1959; Stolyarova, 1968; Viru and Åkke, 1969; and Chin and Evonuk, 1971). Viru and Åkke (1969) have proposed a two stage theory to explain these observations. According to their data the initial increase in glucocorticoids (stage 1) was due to an increased biosynthesis which increased secretion rates, and the decrease in glucocorticoids (stage 2) was due to a decreased biosynthesis, the decrease being an attempt by the body to conserve corticoid supplies. In addition to this theory Viru and Åkke were able to show that the adrenal gland does not lose its ability to produce glucocorticoids, it just lacks a stimulus. He accomplished this by giving animals which were exposed to long periods of exercise, ACTH injections. The ACTH elevated corticoid levels, thus suggesting the lack of stimulus theory. This infers that the decrease in glucocorticoids might be due to an ACTH inhibition.

Suzuki et al. (1967) and Korenskaya (1967) have been able to
demonstrate that the intensity of physical exertion was just as important as the exercise period. They demonstrated that only exhaustive exercise was effective in changing corticoid activity.

The use of thyroxine in this study made it important to know how exercise affected the thyroid hormones. In an early report by Lashof (1954) it was shown that exercise had no effect on peripheral degradation of $T_4$. Recent reports have demonstrated that exercise increased $T_4$ degradation (Rhodes, 1967; Irvine, 1968; Dawson and Horvath, 1970; and Kraus and Kinne, 1970).

The literature shows that exhaustive exercise caused an increase in corticoids after periods of short exposure, and if exercise is prolonged a decrease occurred. It was also demonstrated that $T_4$ degradation was increased with physical exertion. Therefore, it is interesting to note how these two phenomena seemed to work together. To test this relationship the treatment groups of animals were split into NE and Ex. groups. Swimming was the form of exercise chosen, and each rat was swum to exhaustion. The animals were swum for five days a week for a ten week period. Exercise comparisons were made between NE and Ex. animals for each treatment.

**Weight changes**

Daily exercise resulted in a body weight loss for the majority of the animals. The only exception found was a weight increase in the young athyroid animals of set 1. Aside from this exception the observations demonstrated that if thyroxine is present, such as in control and euthyroid, the animal loses weight due to the exercise.
When $T_4$ is absent, as in athyroid, the body weight fluctuates. This suggests that $T_4$ might be responsible for the weight losses found in exercise animals. This seems reasonable considering the thyroid's role in metabolism. The athyroid decrease might be just a response to stress.

The body weight and adrenal weight data for exercise does not correlate. Therefore, we can again assume that body weight is indicative of metabolic change but not adrenocortical changes. The adrenal glands of exercised rats were generally heavier than non-exercise animals. There was only one exception to this increase, that being a decrease in the euthyroid animals of set 1. Except for this decrease, the data suggests that exercise increases adrenal gland activity which results in an increased adrenal weight. Examination of medulla and cortex weight changes indicates a similar weight elevation. While not all of these weight increases were significant, no decreases were observed. These observations concerning gland changes would again rely on a correlation between weight and glucocorticoid synthesis.

Corticosterone production

At this point I would like to stress the fact that comparisons to test the effects of exercise were made between NE and Ex. animals that received similar treatment. No other comparisons will be made in this section of the discussion.
Adrenal gland concentration In young animals exercise elevated the corticosterone levels of the adrenal glands, but did not disturb the basic relationship found among treatment groups. These concentration increases cannot be totally attributed to adrenal gland weight changes. The adrenal weight of control and athyroid animals increased with exercise, but euthyroid adrenal weights decreased. These adrenal gland concentrations cannot be related to previous reports because gland contents were not measured.

The pattern developed in the young animals is not followed completely by the mature animals. The mature control and athyroid gland concentrations increased with exercise, but the athyroid change was insignificant. The corticosterone level in euthyroid glands of mature animals was reduced by daily exercise. It is interesting to note that this reduction in the Ex. euthyroid animals produced an adrenal corticosterone concentration equivalent to the control group. This suggests that exercise under the influence of T₄ can raise glucocorticoid levels, but only so high.

The mature glucocorticoid concentrations did correspond more closely to adrenal weight changes, but were still not totally related to them. This data suggests that corticosterone biosynthesis was increased due to exercise. This increase did not appear to be regulated by T₄ because it occurred whether T₄ was present or not. It also seems evident that age plays a role in the effectiveness of this system. At this point in discussion it
cannot be determined if this change is a result of corticoid storage or reduced secretion.

Plasma concentration  Corticosterone levels of plasma were significantly reduced or underwent no significant change as a result of exercise. In young animals the plasma levels of euthyroid and athyroid animals were reduced, while control animals demonstrated no change in corticosterone. Except for control values the data demonstrates that exercise reduced corticosterone levels in the plasma and increased them in the glands of young animals. This would tend to indicate that exercise inhibits corticosterone secretion, thus causing the hormone to be stored. This theory would seem more plausible if corticosterone levels in the plasma of control animals had also been reduced. The absence of such a reduction and the presence of a slight increase in content suggests that secretory decreases do not necessarily increase gland content. It would seem, therefore, that exercise increases glucocorticoid biosynthesis, and that this effect is independent of its influence on secretion.

If we look at mature animals, only euthyroid plasma levels were significantly decreased due to exercise, while control and athyroid levels did not change. The control pattern for adrenal and plasma data of mature animals was the same as that of the young control animals. The euthyroid group had a decrease in gland levels. Neither gland nor plasma changes in athyroid animals was significant. From these results it would appear that age hinders the effects
of exercise.

**Secretion rate** In young animals exercise reduced the secretion rate of each group, but only the athyroid decrease was significant. These results give additional evidence that exercise affects glucocorticoid biosynthesis and secretion independently. In athyroid animals a reduction in secretion rate was coupled with a reduced plasma content and elevated gland content. In euthyroid animals there was no significant reduction in secretion, but plasma contents were reduced and gland contents were increased. With control animals there was no significant change in secretion or plasma content, but gland levels remained high. This strongly implies that the increased corticosterone levels in the adrenal gland of exercise animals is due to an increase in biosynthesis.

The results further indicate that this effect of exercise on biosynthesis is independent of its influence on secretion rate. This idea was not totally applicable to mature animals, which implies that the system involved changes with age. After age influences on the adrenal cortex are discussed this point might be resolved. If one assumes that biosynthesis and secretion of glucocorticoids are influenced by exercise in different ways, the analysis of the effects of exercise on corticoid secretion rate can be made without discussing gland content.

In the earlier discussion the control of glucocorticoid secretion was placed at the level of the hypothalamus or pituitary. It is reasonable to assume that exercise also regulates secretion
at this level. In athyroid animals with no thyroidal influence
the secretion rate and plasma levels were reduced by exercise. In
previous discussions it was brought out that the influence of a
negative feedback is removed in athyroid animals, so that the TSH
levels are allowed to increase. This elevation could inhibit ACTH
release, which would lower glucocorticoid secretion. It is possible
that exercise stimulates this TSH release, thus reducing the corti-
coids even more. In animals receiving T^4 (euthyroid) the concen-
tration of TSH does not reach the same levels as that of the
athyroid animals due to the effects of the T^4 negative feedback.
Apparently TSH levels were high enough to influence plasma corti-
costerone, but not enough to significantly reduce secretion.

There are two possible explanations for the results found
with the control animals. First, exercise might have stimulated
an increase in T^4 levels, which would have reduced TSH. This
reduction in turn would have allowed more ACTH and glucocorticoids
to be released. This would indicate that under the influence of
exercise T^4 production is increased. If the dose of T^4 given to
the euthyroid animals is truly a physiological normal dose, then
euthyroid and control levels of corticosterone should theoretically
be the same. The lack of similarity between them suggests an
additional factor influences the ACTH-TSH system. The second
explanation then could involve the T^4 analogs present in control
animals, but absent in the other groups. It has been demonstrated
that T^4 analogs can influence corticoid metabolism (Yates et al.,
1958; McGuire and Tomkins, 1959; and Melby et al., 1960). If corticosterone metabolism was stepped up it would follow that secretion would also be increased. However, the cortical metabolism (MCR) results did not seem to be affected by exercise. Thus, these results denote the possibility that $T_4$ analogs operate directly on TSH release and not through metabolism. This theory seems more logical in regards to the results given in this study.

The evidence as presented tends to indicate that $T_4$ does not work alone in its regulation of TSH release. These results help to explain how the levels of corticosterone are maintained, but do not explain why the control data deviates from previous observations. As mentioned, it has been demonstrated by other investigators that prolonged exercise decreases corticosterone levels in the plasma and decreases secretion rates (Masson, 1941; Haldi and Wynn, 1946; Young, 1959; Stolyarova, 1968; Viru and Äkke, 1969; and Chin and Evonuk, 1971). The decreases observed in these studies suggest that after an initial decrease in TSH (short-term exercise) the TSH concentrations began to rise. This increase would have caused a reduction in glucocorticoids by inhibiting ACTH. None of these workers extended their exercise period longer than six weeks. In the present study an exercise program of ten weeks was employed. Thus, the possibility does exist that animals exercised longer than six weeks lose their ability to survive on depressed glucocorticoid levels, or that these levels become too low. If this is true the lack of corticoids could represent a stress condition.
The stress in turn could override the normal ACTH-TSH balance and produce an ACTH release via a TSH reduction to elevate the corticoids. This idea is reasonable because Harris (1955) and Ducommun et al. (1966) have demonstrated that other stimuli, such as electric shock and exposure to cold, do alter the ACTH-TSH balance. The work of Viru and Åkke (1969) would also help to substantiate these findings because they showed that the glucocorticoid decrease corresponds to an ACTH decrease.

Thus, it would seem that exercise has a two-fold effect on corticosterone activity. The rate of corticoid biosynthesis appears to be directly proportionate to exercise, while corticoid secretion is indirectly proportionate to exercise under certain conditions. The data suggests that exercise is effective in lowering secretion in the absence of thyroidal influence, and that with T₄ this ability is partially hindered. It appears as though another factor is involved in TSH release, and the findings in this study suggest that T₄ analogs might be this factor.

Age

Studies on the adrenal cortex and thyroid gland have shown that as the animal ages these glands undergo structural alterations (Bourne, 1967). These changes may lead to functional deterioration with age. Rodent adrenal cortical function in aging has not been well researched, but some work has been done. One such report by Harding et al. (1961) implies that adrenal cortical hormones are
the controlling factor for alanine-\(\alpha\)-ketoglutarate transaminase activity in the rat. They feel this enzyme is involved in metabolism and the capacity for growth, and that the glucocorticoids regulate its concentration. Another paper links cellular degeneration of the adrenal cortex to decreasing levels of \(\Delta^5\)-3-hydroxysteroid dehydrogenase, the major enzyme of the steroidogenic pathway (Jayne, 1963, and Shapiro and Leather, 1971).

Investigations concerning adrenal activity and age have tended to take two positions, the first being that adrenal function does not change with age (Kullander, 1960; Rubin et al., 1961; Baca and Chiodi, 1965; Grad et al., 1967; and Friedman et al., 1969). The second position is that a decrease in function occurs with age (Hunt and Hunt, 1959, and Jayne, 1963). It was demonstrated that in cattle (Riegle and Nellor, 1967) and goats (Riegle et al., 1968) adrenal gland response to ACTH declines with age. Adezali and Prando (1966) feel that the ACTH-TSH system deteriorates with age, and that this decline is a slow rhythmic process.

Most of the literature to date implies that age causes a decrease in thyroid activity. One explanation for this decline in activity has been attributed to a decrease in thyroxine secretion (Grad and Hoffman, 1955; Verzar and Freyberg, 1956; Wilansky et al., 1957; and Gaffney et al., 1959, 1960 and 1962). Gregerman et al. (1962) feel this decline is the result of an increased peripheral degradation or utilization or both of \(T_4\).

It has been suggested that age produces a hypothyroid state
(Lorand, 1904; Kountz and Chieffi, 1947; Tsuji and Ogura, 1969; and Shapiro and Leathen, 1971). Several workers have noted a similarity between the adrenal activity of aged and hypothyroid animals (Wilansky et al., 1957; Jayne, 1963; and Shapiro and Leathen, 1971). According to Grad (1969) age does not cause a hypothyroid state because as thyroid activity declines so does the animal's metabolism. He feels that the changes in thyroid function are due to alterations of the pituitary-thyroid axis and the thyroid's sensitivity to it. Age comparisons were made between young and mature animals of the same treatment group.

**Weight changes**

In this study body weight changes with age seemed to be quite independent of treatment. Regardless of treatment received the animals in sets 1 and 2 gained body weight as a result of age, which indicates that the thyroid is not necessary for this increase. The weight gain was probably due to increased fat deposits, but without complete carcass analysis no conclusions can be made. It is again felt that these body weight changes reflect metabolic rather than adrenal alterations.

With the exception of the NE control animals in set 1, the adrenal glands also gained weight with increased age. Medulla weight showed some variation, but cortex weight was significantly elevated in aged animals. Evaluation of these results depends upon corticosterone changes and will be discussed with these measurements.
Corticosterone production

**Adrenal gland concentration**  A comparison between gland contents of young and mature animals demonstrated a large rise in corticosterone levels with age. This increase was found in every treatment group. Therefore, the absence or presence of T₄ does not seem to affect this corticosterone ratio. Neither can the high amounts of corticosterone be attributed to adrenal weight gains because the control glands lost weight and still had elevated corticoid levels.

The results of this study indicate an increased glucocorticoid biosynthesis with age. As will be seen later, this rise in adrenal gland corticosterones seems to be independent of secretion rates.

**Plasma concentration**  The plasma concentrations of the mature control animals were significantly lower than the young control animals. In the athyroid group age significantly decreased plasma corticosterones. The euthyroid plasma concentrations increased with age. The NE euthyroid difference was not significant, but did represent an increase. The deviation of these results from those of the adrenal gland data supports the theory that the biosynthesis and the secretion of glucocorticoids are each influenced differently by age. Thus, we can again disregard adrenal concentrations when discussing plasma levels and secretion rates.

The opposing results obtained in euthyroid and athyroid
animals suggests that $T_4$ is needed to maintain corticosterone levels in aging rats. Without $T_4$ (athyroid) there was a decrease in corticosterone levels, and with $T_4$ (euthyroid) there was an increase. However, only the NE decrease in athyroid animals was significant, and only the Ex. increase in euthyroid was significant. Therefore, the data demonstrates that $T_4$ might be helpful in increasing corticoids but is not totally effective. Another fact which shows that $T_4$ is not totally effective is that a decrease occurs with age in intact (control) animals.

Two groups of animals (control and euthyroid) each receiving $T_4$ gave opposing results. This plus the inconsistency noted above points to a controlling factor other than $T_4$. It would appear that the decreases observed were due to TSH influences. In the athyroid animals TSH was normally high, and there was the possibility that TSH levels were even higher in older animals. If this were so it would explain the lower corticosterone content in the plasma. In control animals the ACTH-TSH balance should be normal, but apparently age affects this relationship. In euthyroid animals the $T_4$ probably suppresses TSH and allows ACTH to be produced. Before these relationships can be analyzed the secretory rates must be discussed.

**Secretion rate** The secretory rate comparisons between young and mature animals gave results similar to plasma data. The difference is that there was a higher degree of significance in secretory changes. The control athyroid secretion rates were
significantly lower with age, and the euthyroid secretion rates were significantly higher.

If one looks at the effects of age on athyroid animals first, it can be seen that age causes a decrease in plasma levels and secretion rates of corticosterone. If we go back to the basic idea that TSH levels are high in these animals, we can theorize that age increases the TSH concentration. As the TSH levels rise, the ACTH level drops and the corticoids are decreased. This observation for plasma and secretion rate data is only valid in NE animals because the differences in Ex. animals were not significant. Therefore, exercise is causing the corticoid levels to remain stable in aged animals and does not allow the levels to drop below a certain point. It is reasonable to assume that this change occurs at the ACTH-TSH level of the control animals.

As discussed earlier, it has been demonstrated that stress can override the ACTH-TSH relationship (Harris, 1955, and Ducommun et al., 1966). From these results it seems likely that exercise in aged athyroid animals becomes a form of stress and causes the ACTH-TSH axis to be shifted to ACTH release in order to maintain the glucocorticoids. In other words, under the stress of exercise aged athyroid animals cannot tolerate glucocorticoid levels below a certain point.

In euthyroid animals a reversal of the athyroid plasma corticosterone levels and secretion rates occurred. These observations suggest that either $T_4$ is much more active in mature animals
or that mature animals are more responsive to its effect. The results for control animals do not support this idea, but do suggest that in aged animals $T_4$ levels are reduced. The decrease observed in plasma and secretion rate data indicates that TSH levels were probably being increased in these animals. The literature does support such a theory because it has been demonstrated by numerous investigators that $T_4$ activity decreases with age (Grad and Hoffman, 1955; Verzár and Freydberg, 1956; Wilansky et al., 1957; and Gaffney et al., 1959, 1960 and 1962). Thus, the reduction of $T_4$ by the aging process would allow TSH to increase and would decrease ACTH (Hunt and Hunt, 1959, and Jayne, 1963). In support of this theory it has been demonstrated that $T_4$ therapy increases the glucocorticoid levels, which implies that $T_4$ inhibits TSH release.
CONCLUSIONS

The formation and release of glucocorticoids by the adrenal cortex is modified by a variety of factors. Three major elements that influence corticosterone production in the rat are the thyroid gland, exercise and age. Under the influence of these variables corticoid production can be separated into two phases: phase one involving glucocorticoid biosynthesis, and phase two dealing with the release and subsequent establishment of plasma corticosterone levels. The thyroid gland, exercise and age have different effects on each of these phases.

Glucocorticoid biosynthesis is increased by the influence of the thyroxine, exercise and age, but does not seem to be altered by secretory or ACTH changes. It is felt that this increase in biosynthesis results in storage of the corticoids within the adrenal cortex. Exercise is the only variable that is not totally effective in increasing glucocorticoid biosynthesis. The effects of age and thyroidectomy seem to decrease the effectiveness of exercise on biosynthesis.

The effects of the thyroid gland, exercise and age on plasma levels and secretion rates of corticosterones can be analyzed more efficiently if treatment groups are discussed separately. Thyroidectomy (athyroid) caused a decrease in both plasma levels and secretion rates of corticosterones. Exercise and age also reduced glucocorticoid concentrations. It is felt that these factors influenced the corticoids by regulating TSH levels which
in turn affected the reciprocal relationship of ACTH-TSH discussed by Harris (1955) and Ducommun et al. (1966). Due to the lack of T₄ and no inhibition of TSH the levels of TSH in athyroid animals should be high. Therefore, it is reasonable to assume that these increased TSH levels decrease ACTH and the glucocorticoids. These TSH levels appear to be increased by exercise which would further decrease ACTH and the corticoids. This ACTH-TSH regulation is also probably used by the aging process to reduce glucocorticoids in athyroid rats. However, from the evidence presented it appears that mature athyroid animals are unable to cope with exercise as well as young athyroid animals. It would seem that exercise in mature athyroid animals acts like electrical stimulus or cold exposure and constitutes a stress condition (Harris, 1955, and Ducommun et al., 1966). The stress would cause a shift in the ACTH-TSH balance and enable aged animals to suppress TSH release in order to maintain certain levels of corticoids.

Thyroxine treatment (euthyroid) was able to reverse the effects of thyroidectomy to a certain degree. This influence was probably the result of the effects of T₄ negative feedback control on TSH release. The feedback would inhibit TSH release and elevate ACTH and glucocorticoids. Exercise did not affect corticosterone secretion rates in euthyroid animals, but did decrease plasma concentrations. These observations indicate that exercise either decreased the effectiveness of T₄ or speeded T₄ degradation (Rhodes, 1967; Irvine, 1968; Dawson and Horvath, 1970; and Kraus
and Kinne, 1970). Age increase caused a rise in plasma levels and secretion rates of corticosterones. It is felt that this elevation was again due to TSH suppression via the $T_4$ feedback mechanism.

The plasma concentrations and secretion rates of intact control animals which received no treatment were significantly decreased. If the ACTH-TSH reciprocal theory is correct then a corticoid reduction would be related to an ACTH decrease and TSH increase. This would indicate that $T_4$ levels are also reduced or they would have suppressed TSH release. The thyroxine levels used in euthyroid animals have been established as physiologically normal or near normal (Kumaresan and Turner, 1967). Therefore, one would expect similar results between the control and euthyroid animals. Thus, the results suggest that either the levels given euthyroid animals were not physiologically normal or TSH release was not totally controlled by $T_4$ concentrations. If factors other than $T_4$ are involved it seems logical that $T_4$ analogs from the thyroid could be responsible for this difference.

The $T_4$ analogs have been shown to be involved in corticosterone metabolisms (Yates et al., 1958; McGuire and Tomkins, 1959; and Melby et al., 1960) and are present in control animals while absent in the other groups. Age in control animals seemed to cause a deterioration of the ACTH-TSH system. The corticoids decreased with age, but the reduction cannot wholly be attributed to a $T_4$ analog influence because it was absent in athyroid animals which also had a reduction of corticoids. Therefore, the decrease could
be the result of a breakdown in the responsiveness of the ACTH-
TSH system.

It will be noted that exercise results for control animals
did not correspond to previous work that demonstrated a gluco-
corticoid decrease with prolonged exercise in intact animals
(Masson, 1941; Haldi and Wynn, 1946; Young, 1959; Stolyarova,
1968; Viru and Akke, 1969; and Chin and Evonuk, 1971). It is
felt that this difference was due to the length of the exercise
period used in this study. The extension of the exercise period
beyond the six weeks previously used apparently stressed the
animals. The stress appears to override the ACTH-TSH system,
enabling the animal to maintain the glucocorticoid levels.
SUMMARY

1. This study deals with the effects of the thyroid gland, exercise, and age on corticosterone production in the rat.

2. In order to test the effects of these variables on corticosterone production three groups of animals were established:
   a. Control — intact animals receiving no treatment;
   b. Euthyroid — thyroidectomized and given 1.0 μg/100 grams body weight of L-thyroxine/day in subcutaneous injections;
   c. Athyroid — thyroidectomized with $T_4$ therapy given.

3. Each treatment group was separated into non-exercise and exercise animals. The exercise animals were swum to exhaustion five days a week for a ten week period.

4. At the end of the exercise period corticosterone production was measured using three parameters:
   a. Adrenal gland levels of corticosterones. The glands were homogenized in alcohol, and the corticosterone extracted with dichloromethane. A fluorescent reagent was mixed with the corticosterone and the resultant florescences read on a model 111 Turner fluorometer.
   b. Plasma levels of corticosterones. The plasma was separated from the blood and subjected to the same procedure used for gland contents.
c. Secretion rates. The metabolic clearance rate or metabolic half-life of corticosterone was determined using $^{14}$C-corticosterone and liquid scintillation. The MCR and plasma data was then used to compute secretion rates.

5. The data indicated that:

a. The adrenal gland content was reduced by thyroidectomy and elevated by thyroxine. Exercise elevated the content of young animals but was not as effective in mature animals. Age was able to increase the corticosterone levels of the adrenal glands.

b. The plasma levels of corticosterone were decreased by thyroidectomy and increased by $T_4$ therapy. Exercise decreased levels in young athyroid animals but had no effect on the mature athyroid animals. The euthyroid plasma levels were raised by exercise in both age groups. The control animals showed no effects due to exercise. Age caused decreases in the plasma levels of corticosterone in control and athyroid animals and an increase in euthyroid animals.

c. The secretion rates of the athyroid group were reduced in non-exercise animals but were not significantly altered in exercise animals. In euthyroid animals the effects of $T_4$ elevated corticosterone
secretion. Exercise reduced secretion rate of the young athyroid and mature control animals but did not effect any other group. Age decreased corticosterone secretion in control and athyroid animals and increased the rate in euthyroid animals.

6. The results of this study suggest that adrenal cortical production of corticosterone by the rat consists of two independent systems. The first system being glucocorticoid biosynthesis by the adrenal cortex, and the second consisting of the maintenance of plasma levels and secretion rates of corticosterones. The thyroid gland, exercise, and age apparently have different affects on each of these systems. These variables tend to speed biosynthesis but have various effects on secretion rates and plasma. The effects vary, but all of the factors acting on secretion and plasma content operate via an ACTH-TSH reciprocal relationship.
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Surgical Procedures

**Thyroidectomy**

The animals were initially anesthetized with ether and maintained on ether throughout the operation. This was accomplished by placing a strip of gauze over the mouth and nose of the animal, the ether being applied to the gauze. Once anesthetized the animal was placed ventral side up in a dissecting pan and the head and feet immobilized. The area under the neck was shaved, and a mid-ventral incision made. The skin and underlying fascia were then pulled aside to expose the underlying muscles, which were separated with a dull probe. Once separated these muscles were held open with the aid of a retractor. This separation exposes the thyroid gland.

The thyroid is grasped with a pair of forceps, and held in place while the nervous, vascular and connective tissue holding the gland are broken with a semi-dull probe. After all connections are severed the gland is lifted out of the throat region and any bleeding that occurs stopped with cotton wads. Because of the gland's size it is removed in two parts. After all of the gland is removed and the bleeding has been stopped the muscle, fascia and skin are returned to their original positions. The skin is sutured with wound clips, and the animal returned to its cage. The animals are allowed approximately a week to recover before the
Experimental period begins. Thyroxine therapy is started five days after surgery.

**Blood sampling techniques**

In order to obtain blood samples over an hour's time, the animals were anesthetized and the carotid artery canulated. The animals were anesthetized with Nembutal, 4.5mg./100gms body weight, and secured to a dissecting pan as previously described. The neck region was then opened following the same procedures as a thyroidectomy and one of the carotid arteries isolated. The anterior portion of this exposed artery is then tied off with surgical silk. The posterior end of the vessel is closed with a bulldog clamp. A small incision just below the sutured end of the artery is made, and into this opening a piece of Intramedic polyethylene tubing is inserted and secured with surgical silk. Into the free end of the tube a blunted 25ga. needle is fastened and then attached to a three way valve. The valve is attached to a ring stand and the remaining two outlets fitted with a collecting and reserve syringe. The reserve consists of a 20 ml. syringe filled with heparin (150units/ml. of saline), and used only to prevent clot formation in the three way valve. The samples were collected in 1.0 ml. plastic syringes, and later transferred to plastic centrifuge tubes. Each collecting syringe contained 0.7 mls. of heparin, 0.5 of which was used to clear the canula between samples. The remaining 0.2 ml. was used to keep the collected sample from clotting so the plasma could be recovered.
Once the canula was in place the iliac vein of the leg was exposed and $^{14}$C-corticosterone given intravenously. In the young rats a 0.2 ml. saline solution (containing 0.1 ug of $^{14}$C-corticosterone) was given, and in the mature rat a 0.4 ml. saline solution (containing 0.2 ug of $^{14}$C-corticosterone) was given. The difference in dose reflecting the difference in weight, the mean weight of the mature animals being greater than the mean weight of the young.

After introduction of the radioactive tracer blood samples were removed at 5, 10, 15, 30, 45, and 60 minutes. Plasma samples for determining the concentration of corticosterone in the blood were removed after the last 60 min. sample. Usually 5.0 to 15.0 mls. of blood could be obtained in this way.

All blood samples were spun down for 15 min. at 2,000 rpm. and the plasma removed. With MCR samples only 0.2 mls. of plasma was taken and transferred to scintillation vials. In the case of the plasma samples all available plasma was removed and placed in test tubes (stoppered). Both the vials and test tubes were stored at 0°C until future use.

**Chemicals**

1. **Thyroxine (stock solution)**
   a. Weigh out 10mg. of L-Thyroxine (the sodium salt form was used - General Biochemical).
   b. Transfer to a one liter volumetric flask, using a 1N
NaOH solution to rinse all equipment.

c. Dilute with distilled, deionized water to approximately half its volume.

d. Using a 1N HCl solution, lower the pH to a point where the thyroxine precipitates as a white flocculent material (Approximately pH of 6). It is important not to overshoot the end point since this would result in overdilution when the injectable solution is made.

e. Dilute to volume with distilled, deionized water.

f. This stock solution can be stored in this form up to one year under refrigeration.

2. Dilution of stock solution

a. Add 1N NaOH drop by drop until the precipitate disappears. This should change the pH to about 7.

b. No further dilution is necessary, the solution is ready to be used.

c. Diluted solution will last for 48 hours.

3. Methylene dichloride purification

a. Allow dichloromethane to stand over 1/10 its volume of concentrated \( \text{H}_2\text{SO}_4 \) for three days, with occasional shaking.

b. It was then washed three times with 1/10 its volume of 2N-NaOH, and then washed three times with 1/10 its volume of distilled water.

c. Dry overnight over anhydrous sodium sulfate.

d. It was filtered and distilled through a Dufton column (the
fraction distilling between 40 and 41 degrees is collected).

4. Ethanol (absolute alcohol) purification
   a. Reflux for two hours with 5g/liter of 2,4-dinitrophenyl hydrazine and 10 ml/liter of concentrated HCl.
   b. Then twice distill through a dufton column and collect the fraction distilling at 78 degrees centigrade.

5. Fluorescence reagent: Consisted of a 3:1 ratio, of concentrated sulfuric acid $H_2SO_4$ (3) to ethanol (1). Mix the solution cautiously by running the acid down the sides of the container. Acid should be added to alcohol, and the container should be immersed in ice water (Important).

6. Corticosterone standards
   a. Dissolve 10mg. of corticosterone (Nutritional Biochemical Corporation) into 5 mls. of ethanol.
   b. Transfer the ethanol solution to a one liter volumetric flask and dilute to one liter with distilled, deionized water. This produces a stock solution of 10g/ml.
   c. The standard curve will be formed from three dilutions of the stock solution.
      (1) Pipette 25mls. of stock solution into a 100ml. volumetric flask, and fill to volume with distilled water (concentration 2.5 g/ml.).
      (2) Pipette 10mls. of stock solution to a 100ml. volumetric flask, and fill to volume with distilled water (concentration 1.0 g/ml.).
(3) Pipette 5mls. of stock into a 100ml. volumetric flask, and fill to volume with distilled water (concentration 0.5 g/ml.).

7. Toluene
   a. Contained 30.24 mg. of PPO (2,5-diphenyloxazole) as a primary fluor. (New England Nuclear).
   b. Contained 1.89 mg. of POPOP (p-Bis 2,5-phenyloxazolyl -benzene) as a secondary fluor. (New England Nuclear).

Determination of Plasma and Adrenal Corticosterone in the Rat using Fluorescence (Vander Vies, 1960)

1. Ether — wash extract by adding 5mls. of ether.
   a. Shake vigorously in vortex mixer.
   b. Centrifuge for one min. (2,000rpm).
   c. Remove top layer by aspiration.

2. Dichloromethane — extract with 4mls. of CHCl₃.
   a. Shake for 60sec. and let stand.
   b. Shake again for 30sec.
   c. Centrifuge for one min. (2,000rpm).
   d. Remove top layer by aspiration.

3. Sodium hydroxide — wash the extract with 0.25ml. 0.1N NaOH.
   a. Add NaOH and shake for 60sec.
   b. Centrifuge for one min. (2,000rpm).
   c. Remove top layer by aspiration.
4. Sodium sulfate — dry the extract.
   a. Add enough Na$_2$SO$_4$ to cover the bottom of the tube.
   b. Let stand for 5 to 10 min.
   c. At the end of this time transfer a 2.0 ml. aliquot of the sample to another clean centrifuge tube.

5. Fluorescent reagent
   a. Mix the 2.0 mls. of extract with 5mls. of fluorescent reagent.
   b. Shake for 30sec.
   c. Centrifuge for two min. (1,000rpm).
   d. Remove the top layer by aspiration.

6. One hour after shaking read the fluorescents of the acid layer. Set the primary filter at (47B) 110-813, and the secondary filter at (2A-12) 110-818.
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