Effects of age, exercise, graded thyroxine levels and adrenocorticotropic hormone upon the in vitro secretion of corticosterone in the male rat

John Franklyn Pritchett
Iowa State University

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Effects of age, exercise, graded thyroxine levels and adrenocorticotropic hormone upon the in vitro secretion of corticosterone in the male rat

by

John Franklyn Pritchett

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

DOCTOR OF PHILOSOPHY

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In Charge of Major Work

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For the Graduate College

Iowa State University
Ames, Iowa

1973
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INTRODUCTION

The ability of mammalian adrenal cortices to elaborate and secrete glucocorticoids is governed by an intricate system aligned with the hypothalamic-hypophyseal-adrenocortical axis. Because of the extreme complexity encountered when dealing with the interactions among these components, many of the finer points concerning physiological mechanisms lack a firm basis of experimental evidence. However, a general working scheme has been elucidated.

According to current concepts, the adrenal cortex produces glucocorticoids in response to adrenocorticotrophic hormone (ACTH), a polypeptide of adenohypophyseal origin. The regulation of ACTH release by the pituitary gland is, in turn, governed by two major factors. The first of these involves the production of corticotrophin releasing factor (CRF) by discreet centers within the hypothalamus. This substance transverses the pituitary portal system and elicits the release of ACTH from the adenohypophysis, which in turn stimulates corticosteroidogenesis.

The second facet of the regulation of ACTH release, and thus the production of glucocorticoids, involves a negative feedback system involving the titer of circulating glucocorticoids and the adenohypophysis and/or hypothalamus. Increased amounts of glucocorticoids appear to inhibit either CRF release or ACTH release or both. Conversely, diminished production of glucocorticoids by the adrenal cortices with a concomitant decrease in the vascular concentration of
these hormones seems to increase both CRF release and ACTH secretion. In response, the adrenal cortices increase corticosteroid production.

The complexity of the preceding model of the regulation of corticosteroidogenesis is compounded with the realization that various factors may interact at any of the three levels (e.g., hypothalamic, adenohypophyseal, adrenal cortical) to alter the basic regulatory schemes. Among these factors are age, exercise, and level of thyroid activity.

A moderate amount of literature exists on age-adrenal cortical interactions. Bourne (1967) has reported significant structural changes in the adrenal cortex in response to age. The functional significance of these alterations has yet to be clarified. In clinical studies involving individuals ranging in age from nineteen years to ninety-five years, the diurnal rhythm of excretion of corticosteroids was found to be abolished in aged subjects (Pincus, 1959). In a study of the responsiveness of the adrenal cortex to exogenous ACTH and to stresses eliciting the release of endogenous ACTH in young and old men, Pincus (1950) reported a decrease in the absolute amount of corticosteroid urinary metabolites with age. Handler (1959) has cited additional evidence indicating decreases in both urinary and plasma adrenal steroid levels with age and suspects that this is indicative of lower ACTH secretion. Decreases in enzymatic activity associated with corticosteroidogenesis have also been reported (Harding, et al., 1961; Shapiro and Leathen, 1971).
It is therefore apparent from the preceding introductory considerations that age plays a role in influencing adrenal cortical activity. However, the mechanism of this interaction has yet to be substantiated.

Exercise of an exhaustive nature is also known to alter adrenal cortical activity. It has been reported that various exercise regimens either increase (Frenkl and Csalay, 1962) or decrease adrenal cortical secretory activity (Chin and Evonuk, 1971). Additional influences of exercise in laboratory animals on the adrenal cortex have been cited by Dawson and Horvath (1970). Cortical activity is inversely related to the duration of the exercise program. It appears that short term exercise programs increase cortical activity while long term exercise decreases indices of cortical activity. Few attempts have been made to clarify the actual mechanism of the exercise interaction.

A wealth of evidence exists concerning the influence of the thyroid gland on indices of adrenal cortical activity. From the extensive reviews by Honey (1955) and Steinetz and Beach (1962), it was generally concluded that cortical function is influenced by thyroid function. This was evidenced by an increase of cortical indices in a hyperthyroid state and a decrease in these parameters in the hypothyroid animal. No completely acceptable mechanism for this control has been presented although recent evidence (Ducommun, et al., 1966 and Retiene, et al., 1968) has suggested interactions between thyroid stimulating hormone (TSH) and ACTH.
Although it is apparent that each of the preceding factors may alter adrenal cortical activity, the simultaneous interaction of these factors on cortical function has, with one exception (Craig, 1972), yet to be investigated in depth. It was therefore the purpose of this study to investigate the interactions of age, exercise, thyroid level, and *in vitro* ACTH administration upon adrenal cortical activity.
LITERATURE REVIEW

Thyroid-Adrenal Cortical Interactions

Adrenal cortical function and the metabolism of adrenal steroid hormones is significantly altered by changes in thyroid function. After an extensive review of the literature, Money (1955) concluded that hypothyroidism resulted in atrophy of the zona fasciculata while hyperthyroidism caused an increase in cortical function.

This hypothesis has been supported by the observations that urinary excretion of corticosteroid metabolites is decreased in hypothyroid animals (Felber, et al., 1959; Peterson, 1958, and Williams, et al., 1957). Studies of additional parameters of adrenal cortical activity in the hypothyroid animal revealed a decreased titer of circulating corticosteroids (Eik-Nes and Brizzee, 1956; Peterson, 1958) and a decreased response to adrenocorticotrophic hormone (Eik-Nes and Brizzee, 1956; Williams, et al., 1957; Felber, et al., 1959). Steinetz and Beach (1962) have reported that adrenal cortices removed from hypothyroid animals produced less corticosteroid in vitro than did glands from normal animals. In addition, Craig (1972) has shown that thyroidectomy decreased both adrenal and plasma concentrations of corticosterone.

Reports of alterations of adrenal cortical activity in hyperthyroid animals are somewhat contradictory. Increases in urinary corticosteroid metabolites have been shown (Felber, et al., 1959; Peterson, 1958; Jakobson, 1958). Peterson (1958, 1959) has also demonstrated an
increase in the adrenal secretory rate of both cortisol and corticosterone. Hilton, et al. (1962) have reported an increase in the blood concentration of corticotrophin in hyperthyroid animals. Increases in adrenal weight, adrenal content of corticosterone, and plasma levels of corticosterone have been reported in thyroidectomized, thyroxine treated animals (Craig, 1972).

Other investigators suggest a slightly different picture of adrenal cortical activity in the hyperthyroid animal. Mikulaj and Nemeth (1958) demonstrated a decrease in adrenal cortical response to exogenous corticotrophin in hyperthyroid animals when compared to euthyroid controls. Although the apparent differences in cortical response to the hyperthyroid state have yet to be resolved, a consideration of possible mechanisms of thyroidal control of cortical function may clarify the situation.

Because in vitro cortical preparations from hyperthyroid animals produced corticosteroids at near normal levels, thyroidal influences might be manifested via the pituitary gland (Steinetz and Beach, 1962). These investigators demonstrated that thyroid treatment caused no increase in adrenal weight in hypophysectomized animals. Blood concentrations of corticotrophin are elevated in hyperthyroid animals (Hilton, et al., 1962). McCarthy, et al. (1959) have proposed that thyroid hormones may influence adrenal cortical function by modifying corticotrophin synthesis. This opinion has been supported by Lazo-Wasem (1960). Timiras and Woodbury (1955) and Evans, et al. (1957) have reported that the rate of corticotrophin release, rather than synthesis, was altered by thyroid gland.
While the precise mechanism of thyroid hormone influence upon corticotrophin production and release by the adenohypophysis has yet to be determined, an interesting hypothesis has been proposed (Harris, 1955). Since corticotrophin (ACTH) and thyroid stimulating hormone (TSH) concentrations fluctuate inversely with one another, a possibility exists that both peptides are elaborated from the same precursor. The reciprocity involving TSH and ACTH had also been noted in later reports (Ducommun, et al., 1966; Retiene, et al., 1968). In the hyperthyroid animal, the production of TSH would be inhibited by high titers of thyroid hormone and the precursor peptide-ACTH synthetic pathway would be promoted, thus giving rise to increased levels of circulating ACTH.

While it may be concluded that thyroidal control of cortical function is manifested in part via the pituitary, it is not safe to assume that this is the only point of regulation. Zarrow et al. (1957) have demonstrated that corticoid metabolism at the cellular level is modified by thyroid level. These workers reported an increase in corticoid metabolism in hyperthyroid animals and a decrease in corticoid metabolism in hypothyroid animals. Several workers have shown corticoid half life in the blood is reduced in hyperthyroid animals and increased in hypothyroid animals (Brown, et al., 1958; Gabrilove and Weiner, 1962; Peterson, 1958; Peterson, 1959). This is not surprising as thyroid hormones enhance the metabolism of many substances.

After a review of various studies on the metabolism of adrenal
steroids, Rall, et al., (1964) have concluded that thyroid hormones increase the catabolism of corticoids, with the result that the pituitary is stimulated to secrete more ACTH, which in turn causes increased cortical secretion. It is important to note that although hyperthyroidism may increase adrenal cortical function, it does not necessarily cause hyperadrenalism.

Thus adrenal cortical function is probably modified by thyroid function. The mechanism of interaction may involve either the pituitary, via TSH-ACTH interplay, or the direct effects of thyroid hormone upon corticosteroid catabolism, or, quite possibly, both.

Age-Adrenal Cortical Interactions

Bourne (1959), in a review of the effects of aging upon histological aspects of endocrine glands, has noted marked changes in the adrenal cortex with age. Jayne (1963) has noted similar structural changes and has indicated that these changes might possibly impair cortical function. Their results support to some extent the findings of Pincus (1959) in which the diurnal rhythm of excretion of corticoid metabolites was abolished with age. Other investigations (Pincus, et al., 1955; Romanoff, et al., 1957, 1958, 1959) demonstrated a decline in urinary corticoid metabolites with age, but showed that after either cortisol or ACTH therapy, excretion patterns are not significantly different with respect to age. Thus it would appear that age influences either the titer of ACTH or the ability of the adrenal cortex to respond to ACTH.

In a study of the responsiveness of the adrenal cortex to
exogenous ACTH and to stresses eliciting the release of endogenous ACTH, Pincus (1950) reported a decrease in the absolute amount of corticosteroid urinary metabolites with age. Because the responsiveness of the adrenal cortex to ACTH may reflect the recent history of exposure of the gland to endogenous ACTH (Jenkins, et al., 1955; Renold, et al., 1952), these results suggest a diminished secretion rate of ACTH with age. Handler (1959) has cited additional evidence of decreases in both urinary and plasma corticosteroid levels with age and proposed that these changes were reflections of diminished ACTH secretion. More recently, Riegle and Nellor (1967) and Riegle, et al., (1968) have reported a decrease in responsiveness of cortical tissue to ACTH with age.

Other studies have also indicated cortical alterations with age. Hunt and Hunt (1959) observed a depletion in liver glycogen with age and interpreted this to be related to increased catabolism of adrenal steroids. Shapiro and Leathen (1971) reported the decrease with age of Δ5-3 hydroxysteroid dehydrogenase, a key enzyme in the biosynthetic pathway of corticosteroids. Finally Craig (1972) has reported both a significant increase in cortical weight and an increase in glandular corticosterone concentration with age.

Despite all of the evidence to the contrary, some workers suggest that little or no adrenal cortical alterations occur with age. Kullander (1960) and Baca and Chiodi (1965) have reported little change in adrenal function with age as measured by urinary metabolites and adrenal ascorbic acid.
Exercise-Adrenal Cortical Interactions

Little doubt exists that exercise influences adrenal cortical activity. It is equally apparent that the type of exercise regimen utilized, the duration and the conditions under which exercise is carried out modifies the extent of adrenal involvement.

Bozovic and Koshial-Zwanovic (1952) have reported eosinopenia and adrenal ascorbic acid depletion in rats during short-term exercise programs. Ascorbic acid depletion under similar circumstances has been reported by Critz (1966), Critz and Withrow (1965) and Geber, et al. (1966). Keeney (1960) has also reported eosinopenia in animals exercised utilizing an acute training regimen. Staehelin, et al. (1955) and Suzuki, et al. (1958) have shown increased plasma corticosteroid values in the human and dog in response to short-term exercise. Kimeldorf and Baum (1954) have reported thymic involution after short-term exhaustive exercise in rats and have suggested that this is indicative of accelerated adrenal cortical output. Badrich, et al. (1955) have reported that short-term exercise increased cortical uptake of radioactive phosphorus, once again suggesting heightened cortical activity.

Conversely, Ingbar and Freinkel (1955) have reported that long-term exercise decreased indices of cortical activity, namely plasma corticoid levels. Similar results have been obtained by Young (1959), Viru and Akke (1969) and Chin and Evonuk (1971).

One index of adrenal cortical activity is apparently not in agreement with the hypothesis that chronic exercise decreases cortical
activity. Adrenal hypertrophy has long been accepted as a parameter of heightened corticosteroidogenesis (Selye, 1936; Cramer and Horning, 1939; Noble and Collip, 1941; Dougherty and White, 1943). Several workers have reported an increase in adrenal weight with chronic exercise (Eranko, et al., 1962; Frenkl and Csalay, 1962; Gollnick, 1963; Gollnick, et al., 1967; Craig, 1972), a finding which seems inconsistent with reports of decreased cortical activity during chronic exercise. Frenkl and Csalay (1962) believe, however, that increases in adrenal size did not necessarily portend heightened cortical activity under conditions of prolonged exercise.

Specific mechanisms for the responses of the adrenal cortex to exercise lack a firm basis in experimental fact. However, several possibilities exist for the apparent biphasic response of the adrenal cortex in response to exercise. Connell, et al. (1958) proposed that initial increases in cortical activity were due to stress. McDermott, et al. (1950) have shown that conditions which activate the sympathico-adrenal medullary system (stress) also evoke increased secretion of ACTH. These workers demonstrated that adrenal demedullation partially inhibited cortical response to stressful stimuli. Farrell and McCann (1952) have also demonstrated that epinephrine activates the pituitary-adrenal cortical axis as judged by a decrease in adrenal cortical ascorbic acid and an increase in blood ACTH.

The apparent decline in cortical activity after prolonged exercise has been explained in two different ways. Cornil, et al. (1965) and Staehelin, et al. (1955) have proposed that decreases in
plasma corticosteroids are the result of enhanced utilization. Viru and Akke (1969) take the position that decreased levels of corticosteroids were the result of a lack of corticotrophin in chronically exercised animals.

**Adrenocorticotrophin-Adrenal Cortical Interaction**

Detailed investigations have been made of the structural and functional changes of the adrenal cortex in response to various doses of adrenocorticotrophin (ACTH) given for different lengths of time. Astwood (1955) summarized the effects as: (1) increased secretion of corticosteroids, (2) depletion of ascorbic acid and cholesterol, and (3) cortical hypertrophy.

The action of ACTH upon the adrenal cortex is extremely rapid since intravenous injection in rats increased the secretion rate within ten minutes (Bush and Ferguson, 1953). In the human, an increase in plasma corticosteroids has been detected within fifteen minutes after ACTH treatment (Bayliss and Steinbeck, 1954; Bliss, et al., 1954).

The duration of enhanced cortical secretion after a single injection of ACTH varies with species, but apparently activation outlasts the stimulus by an appreciable period of time (Astwood, 1955). In the human, a single intravenous ACTH injection led to a maximal cortical response within one hour (Bliss, et al., 1954) while in the dog increased response was detected for no longer than fifteen minutes (Nelson and Hume, 1954). Following hypophysectomy, secretion of corticosterone into the adrenal venous effluent declined to one-half normal value within one hour to one-tenth normal within two hours (Sweat and Farrell, 1954).
Adrenocorticotrophin is one of the few hormones which has been studied extensively in in vitro situations. When added to adrenal slices, ACTH has been reported to increase respiration, reduce ascorbic acid, and increase the rate of corticosteroidogenesis (Astwood, 1955). Stimulation of corticosteroidogenesis by ACTH has been noted in rat adrenal slices as well as whole rat adrenals in vitro (Saffran, et al., 1952) and slices of bovine glands (Haynes, et al., 1952).

It is of interest to note that the rate of corticosteroidogenesis in the absence of ACTH was enhanced by the addition of acetoacetate, acetate, or glycerol, but in the presence of these agents, ACTH may cause a decrease in synthesis (Hoffman and Davidson, 1953). Also all of the steroid synthesized in vitro by the whole or sliced adrenal of the rat appeared in the incubation medium and none accumulated in the tissue (Saffran, et al., 1952), implying that secretion occurred in the absence of circulation of blood (Astwood, 1955).

Many investigations have revealed that the steroids secreted by the adrenal cortex following ACTH stimulation are qualitatively the same in both in vivo and in vitro situations (Bush, 1952; Bush, 1953; Bush and Ferguson, 1953). The notable exception is the rabbit. Extended ACTH therapy resulted in hydrocortisone becoming the major secretory product, whereas, without ACTH therapy, corticosterone was the preponderant steroid produced (Kass, et al., 1954).

The response of the adrenal cortex to ACTH is greatly modified by the state of the gland at the time of stimulation. Atrophic glands
resulting from prolonged therapy with corticosteroids failed to respond immediately to endogenous or exogenous ACTH (Astwood, 1955). Conversely, glands made hyperplastic by exposure of several days or longer to high ACTH concentrations produced more corticosterone in response to a given concentration of ACTH than did resting or atrophic glands (Renold, et al., 1952; Jenkins, et al., 1955). Thus the responsiveness of the adrenal cortex to exogenous ACTH reflected the recent history of exposure of the gland to endogenous ACTH.
MATERIALS AND METHODS

Animals

Male rats of the Sprague-Dawley-Rolfsmeyer strain were utilized in this study. All animals were housed in colony cages, six to eight animals per cage, in a temperature controlled (25 ± 2° C) room. Photoperiod was regulated to fourteen hours of light and ten hours of darkness per day. The animals were maintained on Wayne Lab Blox and tap water given ad libitum throughout the investigation. Upon arrival, the animals were allowed to acclimate to their surroundings for a period of five to seven days.

Experimental Design

A two by two by five factorial design was utilized in this study. The animals were subdivided into two age groups: young animals, 70 days of age at the initiation of experimental procedures, and mature animals, 200 days of age at the initiation of experimental procedures. Each age group was, in turn, subdivided into an exercised sub-group and a non-exercised sub-group. Each sub-group was divided into five treatment categories:

1. Control—Received no treatment, thyroid intact.
2. Athyroid—Thyroidectomized, no L-thyroxine replacement (see Appendix A).
3. Hypothyroid—Thyroidectomized, L-thyroxine replacement at the rate of 0.5 micrograms per 100 grams of body weight per day (see Appendix B).
4. Euthyroid—Thyroidectomized, L-thyroxine replacement at the rate of 3.5 micrograms per 100 grams of body weight per day.

5. Hyperthyroid—Thyroidectomized, L-thyroxine replacement at the rate of 14.0 micrograms per 100 grams of body weight per day.

Finally, a sub-plot was developed to measure the effects of ACTH. One gland from each animal was incubated \textit{in vitro} in the presence of ACTH while the other gland was incubated with no ACTH present in the incubation medium.

**Experimental Procedures**

**Body weights**

All animals were weighed once each week to the nearest gram. These body weights were utilized to calculate exercise loads as well as dosage levels of either exogenous thyroxine solution or placebo.

**Thyroxine administration**

Thyroxine was administered subcutaneously on a daily schedule for seventy consecutive days. Concentrations of the aqueous hormonal solution were adjusted so that no animal received more than one milliliter of solution per day. Control and athyroid animals were injected with equivalent volumes of placebo (water) each day for the same period of time.

**Exercise**

Exercise was accomplished through swimming the appropriate animals to exhaustion five days per week for ten consecutive weeks.
Swimming was carried out in twenty-five gallon plastic containers with water temperature held to 35 ± 1°C. A wetting agent was added to each container to decrease buoyancy created by air trapped in the fur of the animals. Lead weights equivalent to 4% of the body weight were taped to the tail of each animal to motivate vigorous swimming. Exhaustion was interpreted to occur at the point where the animal submerged and was unable to return to the surface during a ten-second period (Dawson and Horvath, 1970).

**Corticosterone secretion rate**

At the end of the seventy-day treatment period all animals were sacrificed and the in vitro secretory rate of corticosterone was determined. The determination involved two steps: incubation of the adrenal glands in a suitable medium for a set period of time and subsequent analysis of the incubation medium for corticosterone content.

**Incubation (see Appendix C)**

Because it has been established that a diurnal rhythm exists with respect to the elaboration and secretion of corticosteroids, all incubation procedures were carried out between 1900 and 2300 hours. Each animal was anesthetized with sodium pentobarbital (35 milligrams per kilogram of body weight). Approximately one hour after administration of the anesthetic, the adrenals were removed from the animal and placed on a filter paper moistened with incubation medium in a Petri dish.

---

1Ivory Liquid was supplied by the Procter and Gamble Company, Cincinnati, Ohio.
After each gland was trimmed of adipose tissue, it was weighed to the nearest milligram on a torsion balance. Each gland was then carefully cut into quarters and placed in a Warburg flask containing either two milliliters of incubation medium or two milliliters of incubation medium containing 250 milliunits of ACTH (Nutritional Biochemicals) per milliliter. The left and right adrenal glands from each animal were alternated insofar as ACTH exposure was concerned. Each flask was then charged with an oxygen-carbon dioxide mixture (95% oxygen--5% carbon dioxide; Matheson) and incubated for 90 minutes at 37°C. At the end of the incubation period, the medium was removed to a small screw-capped vial and stored at -10°C in an upright freezer.

**Corticosterone determination (see Appendix D)**

Each vial was removed from the freezer and allowed to remain at room temperature for one hour. The contents were then thoroughly mixed with a vortex mixer. Two 0.5 milliliter aliquots were then removed for steroid analysis. Corticosterone content was measured fluorometrically.
RESULTS

The data were analyzed using least squares analysis of variance to determine the main effects as well as interactions of age, exercise, thyroxine levels and adrenocorticotropic hormone (ACTH) upon the variables adrenal weight, amount of corticosterone secreted per gland per hour (absolute secretion rate, ASR) and amount of corticosterone secreted per 100 milligrams of glandular weight per hour (relative secretion rate, RSR). The statistical analysis of the data was carried out with the cooperation of the Statistical Laboratory, Iowa State University, Ames, Iowa.

Tabular summaries of the data are provided in this section. For the analysis of variance of the parameters studied, see Appendices E, F, and G.

The results are presented in the following manner. First, the data on age, exercise and age-exercise interaction will be considered. This will be followed by a presentation of the data on thyroid level, age-thyroid level interaction, and exercise-thyroid level interaction. Finally, the findings on ACTH effects and the interactions of age, exercise, and thyroid level with ACTH will be given.

Age Effects

The main effects of age on the parameters studied are summarized in Table 1. Adrenal glands from mature animals weighed significantly more ($P < 0.01$) than glands from young animals. However, glands from young animals secreted substantially more corticosterone, both from the standpoint of ASR ($P < 0.005$) and RSR ($P < 0.001$).
Table 1. Effects of age on mean adrenal weight,\(^a\) absolute secretion rate and relative secretion rate of corticosterone

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Mature</th>
<th>Difference(^b)</th>
<th>Probability of a greater F-value(^c)</th>
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<td>Adrenal weight (mg.)</td>
<td>27.93(^d)</td>
<td>29.69</td>
<td>1.76</td>
<td>(P &lt; 0.01)</td>
</tr>
<tr>
<td>Absolute secretion rate ((\mu g/\text{hr.}))</td>
<td>4.35</td>
<td>3.60</td>
<td>0.75</td>
<td>(P &lt; 0.005)</td>
</tr>
<tr>
<td>Relative secretion rate</td>
<td>15.67</td>
<td>12.17</td>
<td>3.50</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>((\mu g/\text{hr.}/100 \text{ mg. gland}))</td>
<td></td>
<td></td>
<td></td>
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</table>

\(^a\)Single gland weight.

\(^b\)Difference between young and mature values.

\(^c\)From least squares analysis of variance (see Appendices E, F and G).

\(^d\)Each value represents the mean of 80 observations.
Exercise Effects

The main effects of exercise upon the parameters studied are summarized in Table 2. Exercise significantly increased both glandular weight \((P < 0.005)\) and the quantity of corticosterone secreted by each gland \((P < 0.005)\). Exercise did not significantly change the amount of corticosterone secreted per unit of glandular weight.

Age-Exercise Interaction

In an attempt to discern whether exercise effects were uniform in both young and mature animals, the interaction of these two factors on the variables chosen was examined. The results are summarized in Table 3.

The effect of exercise on mean adrenal weight was significantly different \((P < 0.025)\) in a comparison of young and mature animals. In young animals, exercised glands averaged 1.57 milligrams heavier while in mature animals exercise resulted in a mean increase in glandular weight of 7.09 milligrams.

Increase in corticosterone secretion per gland was slightly greater in mature animals than in young animals in response to exercise but the difference in response was not statistically significant. In a consideration of the amount of corticosterone secreted per 100 milligrams of glandular weight in response to exercise, young animals exhibited a slight increase \((0.62 \text{ micrograms})\) while mature animals showed a slight decrease \((0.24 \text{ micrograms})\). These responses were not significantly different.
Table 2. Effects of exercise on adrenal weight,\textsuperscript{a} absolute secretion rate and relative secretion rate of corticosterone

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-exercised</th>
<th>Exercised</th>
<th>Difference\textsuperscript{b}</th>
<th>Probability of a greater F-value\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal weight (mg.)</td>
<td>26.69\textsuperscript{d}</td>
<td>30.94</td>
<td>4.25</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Absolute secretion rate ((\mu)g./hr.)</td>
<td>3.68</td>
<td>4.29</td>
<td>0.61</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Relative secretion rate ((\mu)g./hr./100 mg. gland)</td>
<td>13.82</td>
<td>14.09</td>
<td>0.27</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Single gland weight.

\textsuperscript{b}Difference between non-exercised and exercised values.

\textsuperscript{c}From least squares analysis of variance (see Appendices E, F and G).

\textsuperscript{d}Each value represents the mean of 80 observations.
Table 3. Age-exercise interaction on adrenal weight, absolute secretion rate and relative secretion rate of corticosterone

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th></th>
<th></th>
<th>Mature</th>
<th></th>
<th></th>
<th>Probability of a greater F-value&lt;sup&gt;b&lt;/sup&gt; (young vs. mature)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Ex.&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Difference&lt;sup&gt;e&lt;/sup&gt;</td>
<td>NE</td>
<td>Ex.</td>
<td>Difference</td>
<td></td>
</tr>
<tr>
<td>Adrenal weight (mg.)</td>
<td>27.14&lt;sup&gt;f&lt;/sup&gt;</td>
<td>28.71</td>
<td>1.57</td>
<td>26.24</td>
<td>33.33</td>
<td>7.09</td>
<td>P &lt; 0.025</td>
</tr>
<tr>
<td>Absolute secretion rate (μg/hr.)</td>
<td>4.12</td>
<td>4.58</td>
<td>0.46</td>
<td>3.24</td>
<td>3.98</td>
<td>0.74</td>
<td>Not significant</td>
</tr>
<tr>
<td>Relative secretion rate (μg/hr./100 mg. gland)</td>
<td>15.35</td>
<td>15.97</td>
<td>0.67</td>
<td>12.29</td>
<td>12.05</td>
<td>0.24</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

<sup>a</sup>Single gland weight.

<sup>b</sup>From least squares analysis of variance for interaction (see Appendices E, F and G).

<sup>c</sup>NE = Non-exercised.

<sup>d</sup>Ex. = Exercised.

<sup>e</sup>Difference between exercised and non-exercised values.

<sup>f</sup>Each value represents the mean of 40 observations.
Effects of Thyroxine Level

The effects of the various thyroxine levels on the variables under study were analyzed and the results are summarized in Table 4. Adrenal weights were significantly different ($P < 0.005$) when analyzing the effects of various thyroid levels.

Examination of the data in Table 4 reveals a possible relationship between adrenal weight and thyroxine replacement level. Analysis of the regression of adrenal weight on thyroxine level revealed that increasing thyroxine levels significantly ($P < 0.001$) increased adrenal weight. The regression analysis was carried out after omitting the intact control from consideration since the level of endogenous thyroid secretions was not subject to control.

The ASR varied significantly ($P < 0.001$) with thyroid level. Examination of Table 4 reveals that the intact control mean was not significantly different from the hyperthyroid mean. Hypothyroid animals exhibited a significantly greater ($P < 0.05$) secretion rate than athyroid animals, euthyroid means were significantly greater than hypothyroid means ($P < 0.05$) and hyperthyroid animals showed a significantly greater ($P < 0.05$) secretion rate than euthyroid animals. Excluding the intact control as before and analyzing the regression of secretion rate per gland on thyroxine level showed that increasing thyroxine levels significantly increased ($P < 0.001$) secretion rate per gland.

Analysis of the amount of corticosterone secreted per unit of glandular weight indicated that varying thyroxine levels significantly
Table 4. Effects of thyroxine level on adrenal weight,\textsuperscript{a} absolute secretion rate and relative secretion rate of corticosterone.

<table>
<thead>
<tr>
<th></th>
<th>Athyroid</th>
<th>Hypothyroid</th>
<th>Control</th>
<th>Euthyroid</th>
<th>Hyperthyroid</th>
<th>LSD\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal weight (mg.)</td>
<td>20.02\textsuperscript{c}</td>
<td>26.26</td>
<td>30.08</td>
<td>31.27</td>
<td>36.02</td>
<td>5.04</td>
</tr>
<tr>
<td>Absolute secretion rate (\textmu g/hr)</td>
<td>2.62</td>
<td>3.51</td>
<td>4.98</td>
<td>4.13</td>
<td>4.67</td>
<td>0.48</td>
</tr>
<tr>
<td>Relative secretion rate (\textmu g/hr/100 mg. gland)</td>
<td>13.21</td>
<td>13.23</td>
<td>16.89</td>
<td>13.37</td>
<td>13.02</td>
<td>0.81</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Single gland weight.

\textsuperscript{b}Least significant difference at P < 0.05.

\textsuperscript{c}Each value represents the mean of 32 observations.
Table 5. Age-thyroid level interaction on adrenal weight\textsuperscript{a}

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Young</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athyroid</td>
<td>18.83\textsuperscript{b}</td>
<td>21.23</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>25.41</td>
<td>27.93</td>
</tr>
<tr>
<td>Control</td>
<td>27.99</td>
<td>32.17</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>30.11</td>
<td>32.67</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>37.18</td>
<td>34.84</td>
</tr>
</tbody>
</table>

Least significant difference at $P < 0.05 = 4.62$

\textsuperscript{a}Single gland weight.
\textsuperscript{b}Each value represents the mean of 16 observations.

changed ($P < 0.001$) the secretion rate. Reference to Table 4 reveals that the source of this variation lies in the intact control. Utilization of least significant difference analysis at the five per cent level showed the intact control to be different from each of the other treatment groups. However, there was no statistical difference between any of the remaining four groups.

Age-Thyroxine Level Interaction

The interaction of age and thyroid level was analyzed in an attempt to determine whether young animals responded differently to different levels of thyroxine than did mature animals. The results are summarized in Tables 5, 6, and 7.
Table 6. Age-thyroxine level interaction on absolute secretion rate of corticosterone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Young</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athyroid</td>
<td>2.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.43</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>3.84</td>
<td>3.17</td>
</tr>
<tr>
<td>Control</td>
<td>5.54</td>
<td>4.42</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>4.46</td>
<td>3.73</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>5.09</td>
<td>4.25</td>
</tr>
</tbody>
</table>

Least significant difference at \( P < 0.05 = 0.69 \)

<sup>a</sup>Each value represents the mean of 16 observations.

No significant interaction was present in a consideration of glandular weight (Table 5). In both young and mature animals, adrenal weight increased in response to increasing thyroxine levels. The same was true with reference to the quantity of corticosterone secreted per gland (Table 6).

Significant interaction (\( P < 0.001 \)) between age and thyroid level was present when the quantity of corticosterone secreted per unit weight of the gland was analyzed (Table 7). Close scrutiny of the data revealed that the source of the interaction involved the intact control animals. Young intact control animals seemed to secrete more corticosterone per unit of glandular weight than did mature intact
Table 7. Age-thyroxine level interaction on relative secretion rate of corticosterone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Young</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athyroid</td>
<td>14.89a</td>
<td>11.53</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>15.18</td>
<td>11.29</td>
</tr>
<tr>
<td>Control</td>
<td>19.89</td>
<td>13.89</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>14.77</td>
<td>11.66</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>13.65</td>
<td>12.41</td>
</tr>
</tbody>
</table>

Least significant difference at $P < 0.05 = 1.27$

*Each value represents the mean of 16 observations.

controls. Further, elimination of intact controls from the analysis results in no significant age-thyroid level interaction with respect to the amount of hormone secreted per unit of glandular weight.

Exercise-Thyroxine Level Interaction

In an attempt to determine whether varying thyroxine levels had the same effect in exercised and non-exercised animals, the interaction of these two factors was examined. The results are summarized in Tables 8, 9, and 10.

No significant interaction was present in a consideration of glandular weight (Table 8). Both exercised and non-exercised glands increased in weight with increasing thyroxine levels. Conversely
Table 8. Exercise-thyroxine level interaction on adrenal weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Non-exercised</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athyroid</td>
<td>17.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.91</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>24.95</td>
<td>28.39</td>
</tr>
<tr>
<td>Control</td>
<td>27.29</td>
<td>32.86</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>29.58</td>
<td>33.08</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>34.46</td>
<td>37.57</td>
</tr>
</tbody>
</table>

Least significant difference at \( P < 0.05 = 4.60 \)

<sup>a</sup>Single gland weight.

<sup>b</sup>Each value represents the mean of 16 observations.

Exercise increased glandular weight at all thyroxine levels studied, although not significantly \( (P < 0.10) \).

Significant interaction \( (P < 0.001) \) existed between exercise and thyroxine level when the absolute secretion rate of corticosterone was considered (Table 9). Non-exercised animals did not show the same response to increasing thyroxine levels as did exercised animals. It will be recalled that both exercise and increasing thyroxine levels increased the absolute secretion rate of corticosterone. The interaction appears in a consideration of the pattern of increase. Non-exercised hyperthyroid animals showed no significant increase over
Table 9. Exercise-thyroxine level interaction on absolute secretion rate of corticosterone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Non-exercised</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athyroid</td>
<td>2.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.99</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>3.25</td>
<td>3.77</td>
</tr>
<tr>
<td>Control</td>
<td>4.20</td>
<td>5.76</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>4.47</td>
<td>3.78</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>4.22</td>
<td>5.12</td>
</tr>
</tbody>
</table>

Least significant difference at $P < 0.05 = 0.69$

<sup>a</sup>Each value represents the mean of 16 observations.

Euthyroid non-exercised animals while exercised hyperthyroid animals exhibited a significant ($P < 0.05$) increase over exercised euthyroid animals. Another source of interaction involves the intact controls. In the non-exercised group they were not significantly different from either the euthyroid or hyperthyroid group. However, in the exercised group, intact controls showed a significant ($P < 0.05$) increase over all but the hyperthyroid group, with the latter difference approaching significance ($P < 0.10$).

Significant interaction ($P < 0.001$) was also present in an analysis of the amount of corticosterone secreted per unit of
Table 10. Exercise-thyroxine level interaction on relative secretory rate of corticosterone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Non-exercised</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athyroid</td>
<td>13.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.24</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>13.15</td>
<td>13.31</td>
</tr>
<tr>
<td>Control</td>
<td>15.37</td>
<td>18.42</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>14.97</td>
<td>11.66</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>12.91</td>
<td>13.64</td>
</tr>
</tbody>
</table>

Least significant difference at $P < 0.05 = 1.27$

<sup>a</sup>Each value represents the mean of 16 observations.

Glandular weight (Table 10). Glands from exercised intact animals produced more corticosterone per unit of glandular weight than did glands from the non-exercised intact control group.

Effects of ACTH

Table 11 summarizes the influence of ACTH on both ASR and RSR. Because ACTH was administered to the in vitro preparation after glandular weight was determined, it had no bearing on this parameter. Therefore, further consideration of glandular weight as a variable will be omitted. Administration of ACTH to the in vitro preparation significantly increased both ASR ($P < 0.005$) and RSR ($P < 0.001$).
Table 11. Effects of ACTH on absolute secretion rate and relative secretion rate of corticosterone

<table>
<thead>
<tr>
<th></th>
<th>No ACTH</th>
<th>ACTH</th>
<th>Difference$^a$</th>
<th>Probability of a greater F-value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute secretion rate ($\mu$g/hr.)</td>
<td>3.26$^c$</td>
<td>4.70</td>
<td>1.44</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Relative secretion rate ($\mu$g/ hr./100 mg. gland)</td>
<td>11.28</td>
<td>16.59</td>
<td>5.31</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

$^a$ACTH value minus non-ACTH treated value.

$^b$From least squares analysis of variance.

$^c$Each value represents the mean of 80 observations.
Age-ACTH Interaction

To determine whether both young and mature glands responded in the same manner to ACTH, the interaction of these two factors on absolute and relative secretion rates was examined. The results are presented in Table 12.

Young animals exhibited a significantly ($P < 0.005$) greater increase in absolute secretion rate in response to ACTH than did mature animals. Young animals also showed a significantly ($P < 0.001$) greater response to ACTH insofar as relative secretives rates were concerned.

Exercise-ACTH Interaction

Because it was of interest to ascertain whether exercise influenced the response of the adrenal cortex to ACTH, the interaction of these factors was analyzed. The results are presented in Table 13.

In exercised animals, ACTH caused a significantly ($P < 0.025$) greater increase (1.74 micrograms) in absolute secretion rate than in non-exercised animals (1.13 micrograms). In addition ACTH treatment resulted in a significantly ($P < 0.05$) greater increase in relative secretion rate in the exercised animal (6.06 micrograms) when compared to the non-exercised animal (4.46 micrograms).

Thyroxine Level-ACTH Interaction

Because both age and exercise influenced the response of the adrenal cortex to ACTH and since these factors also interact insofar as thyroid level is concerned, the data were analyzed for possible
Table 12. Age-ACTH interaction on absolute secretion rate and relative secretion rate of corticosterone

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Mature</th>
<th>Probability of a greater F-value&lt;sup&gt;b&lt;/sup&gt; (young vs. mature)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No ACTH</td>
<td>ACTH</td>
<td>Difference&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Absolute secretory rate (μg/hr)</td>
<td>3.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.26</td>
<td>1.85</td>
</tr>
<tr>
<td>Relative secretory rate (μg/hr/100 mg. gland)</td>
<td>12.13</td>
<td>19.11</td>
<td>6.98</td>
</tr>
</tbody>
</table>

<sup>a</sup>ACTH treated value minus non-ACTH treated value.

<sup>b</sup>From least squares analysis of variance (see Appendices F and G).

<sup>c</sup>Each value represents the mean of 40 observations.
Table 13. Exercise-ACTH interaction on absolute secretion rate and relative secretion rate of corticosterone

<table>
<thead>
<tr>
<th></th>
<th>Non-exercised</th>
<th></th>
<th>Exercised</th>
<th></th>
<th>Probability of a greater F-value&lt;sup&gt;b&lt;/sup&gt; (non-exercised vs. exercised)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No ACTH</td>
<td>ACTH</td>
<td>Difference&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No ACTH</td>
<td>ACTH</td>
</tr>
<tr>
<td>Absolute secretory rate (µg/hr.)</td>
<td>3.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.24</td>
<td>1.13</td>
<td>3.41</td>
<td>5.15</td>
</tr>
<tr>
<td>Relative secretory rate (µg/hr./100 mg. gland)</td>
<td>11.54</td>
<td>16.10</td>
<td>4.46</td>
<td>11.02</td>
<td>17.08</td>
</tr>
</tbody>
</table>

<sup>a</sup>ACTH treated value minus non-ACTH treated value.

<sup>b</sup>From least squares analysis of variance (see Appendices F and G).

<sup>c</sup>Each value represents the mean of 40 observations.
thyroxine level-ACTH interaction. The results are presented in Tables 14 and 15.

Analysis of the data on absolute secretion rates (Table 14) revealed a highly significant ($P < 0.005$) interaction between thyroxine level and ACTH treatment. Intact control animals exhibited a much greater response to ACTH than did any of the thyroidectomized-thyroxine treated animals. Little difference existed between athyroid, or euthyroid animals insofar as response to ACTH in terms of additional corticosterone produced. Hyperthyroid animals responded to ACTH to a decidedly lesser degree than other treatment groups. Considering the increase due to ACTH treatment in each thyroxine treated group as a percentage of the non-ACTH treated level, the degree of ACTH-induced increase in ASR was inversely proportional to thyroxine level.

Analysis of relative secretion rates (Table 15) showed significant ($P < 0.001$) interaction of ACTH and thyroxine level. As with the absolute secretion rate, relative secretion rates increased to the greatest degree in intact control animals in response to ACTH treatment. The increase in relative secretion rate due to ACTH was progressively less as the level of thyroxine was increased.
Table 14. Thyroxine level-ACTH interaction on absolute secretion rate of corticosterone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No ACTH</th>
<th>ACTH</th>
<th>Difference&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Percentage increase&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.24</td>
<td>2.52</td>
<td>67.7%</td>
</tr>
<tr>
<td>Athyroid</td>
<td>1.95</td>
<td>3.28</td>
<td>1.23</td>
<td>63.2%</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>2.86</td>
<td>4.15</td>
<td>1.29</td>
<td>45.1%</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>3.51</td>
<td>4.71</td>
<td>1.20</td>
<td>36.8%</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>4.27</td>
<td>5.07</td>
<td>0.80</td>
<td>18.7%</td>
</tr>
</tbody>
</table>

Probability for a greater F-value for interaction:<sup>d</sup> P < 0.005

<sup>a</sup>ACTH treated values minus non-ACTH treated values.

<sup>b</sup>(ACTH treated value minus non-ACTH value) / (non-ACTH value X 0.01).

<sup>c</sup>Each value represents the mean of 16 observations.

<sup>d</sup>From least squares analysis of variance (see Appendix F).
Table 15. Thyroxine level-ACTH interaction on relative secretion rate of corticosterone

<table>
<thead>
<tr>
<th>Condition</th>
<th>Micrograms per hour per 100 milligrams of gland</th>
<th>Percentage increase&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No ACTH</td>
<td>ACTH</td>
</tr>
<tr>
<td>Control</td>
<td>12.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.17</td>
</tr>
<tr>
<td>Athyroid</td>
<td>10.04</td>
<td>16.38</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>10.85</td>
<td>15.62</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>11.20</td>
<td>15.41</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>11.70</td>
<td>14.36</td>
</tr>
</tbody>
</table>

Probability of a greater F-value for interaction:<sup>d</sup> P < 0.001

<sup>a</sup>ACTH treated value minus non-ACTH treated value.

<sup>b</sup>(ACTH treated value minus non-ACTH treated value) / (non-ACTH treated value X .01).

<sup>c</sup>Each value represents the mean of 16 observations.

<sup>d</sup>From least squares analysis of variance (see Appendix G).
DISCUSSION

It is a demonstrable fact that corticosterone is the major glucocorticoid produced both in vivo and in vitro by the rat adrenal cortex (Bush, 1952; Bush, 1953; Bush and Ferguson, 1953). However, the specific effects of age, exercise, graded thyroxine levels, and ACTH, as well as the interactions of these factors, have yet to be completely resolved. Therefore, the influence of the aforementioned factors upon the variables of adrenal weight, amount of corticosterone secreted per adrenal gland (absolute secretion rate or ASR) and amount of corticosterone secreted per 100 milligrams of adrenal weight (relative secretion rate or RSR) will be considered in this discussion.

The influence of age as well as age-ACTH interaction will be discussed first. This will be followed by a consideration of the effects of exercise and thyroid levels and the interaction of ACTH with these factors. Finally age-exercise, age-thyroid and exercise-thyroid interactions upon the variables under study will be considered.

Age Effects and Age-ACTH Interaction

Mature animals were found to have significantly larger adrenal glands than young animals. However, glands from young animals secreted substantially more corticosterone, both from an absolute as well as relative standpoint.

In a consideration of glandular weight, these findings substantiate the work of Craig (1972) in which he reported a significant increase in glandular weight with age. He also reported an age
related increase in glandular content of corticosterone as well as an age related increase in cortical, but not medullary, weight. Since it has been established that mature animals have a greater body mass than do young animals, it is not unreasonable to assume that larger adrenal glands in mature animals reflect a general increase due to size.

However, the fact that mature glands, even though larger, secreted at a decreased rate is a point of interest. Many workers (Pincus, 1950; Romanoff, et al., 1957, 1958, 1959; Pincus, et al., 1955; Handler, 1959; Riegle, et al., 1968) have reported age related decreases in cortical function. The specific cause has yet to be determined, but an age related decrease in either (1) ACTH (Pincus, 1950, Riegle and Nellor, 1967; Riegle, et al., 1968) or (2) a key enzyme utilized in corticosteroidogenesis (Shapiro and Leathen, 1971) have been considered as possibilities.

Within the context of this investigation, glands from young animals exhibited significantly greater responses to ACTH stimulation in terms of both ASR and RSR than did glands from mature animals. Because it has been demonstrated that the response to exogenous ACTH stimulation reflects the recent history of the gland to endogenous ACTH (Renold, et al., 1952; Jenkins, et al., 1955), the decline in secretion rate with age may be due in part to depression of ACTH concentration. Should this be the case, the mechanism through which age decreases ACTH is, as yet, unknown.
Exercise Effects and Exercise-ACTH Interaction

Exercise significantly increased both glandular weight and the amount of corticosterone secreted per gland. Exercise did not, however, increase the amount of hormone secreted per unit of glandular weight. The increase in secretion rate was proportionate to the increase in glandular size.

That exercise increases the weight of the adrenal gland is a well documented fact (Eranko, et al., 1962; Craig, 1972). Furthermore, most of this weight increase is due to an increase in cortical mass (Eranko, et al., 1962; Craig, 1972). Thus it was not surprising to find that larger glands produced more hormone.

This latter finding is not consistent with the reports of others that prolonged exercise decreases corticosteroidogenesis (Chin and Evonuk, 1971; Craig, 1972; Young, 1959). It is of interest to note that previous interpretation of the level of cortical function was based for the most part upon plasma levels of corticosteroids and not upon the actual output of the gland. Indeed, Cornil, et al. (1965) and Staehelin, et al. (1955) have proposed that plasma levels of corticosteroids may be decreased by exercise through enhanced utilization. Should this be the case, diminished corticosteroi levels should in turn elicit ACTH release which would then stimulate, rather than inhibit, cortical activity. A consideration of the response of glands from exercised animals to ACTH stimulation is therefore in order at this point.
Under the conditions of this investigation, ACTH stimulation caused a significantly greater increase in absolute as well as relative secretion rates in glands from exercised animals when compared to their non-exercised counterparts. This fact strongly suggests that exercise increases the release of ACTH, and thus adrenal size and secretion rate. Whether this release is effected indirectly via decreasing plasma corticoids as proposed by Cornil et al. (1965) and Staehelin, et al. (1955) or directly through some action upon the hypothalamic-adenohypophyseal axis is not known.

It is of interest at this point to note a possible parallel of exercise to stress. Since prolonged exhaustive exercise may be defined as a stressful situation, and since stressful conditions are known to elevate plasma ACTH levels, the possibility that exercise manifests its stimulatory effects upon the adrenal cortex, at least in part, via the sympathico-adrenal medullary system cannot be ruled out.

Thyroid Effects and Thyroid-ACTH Interaction

Adrenal weights were directly proportional to the level of thyroxine replacement in thyroidectomized animals. In the case of the intact control group, adrenal weights were not significantly different from the euthyroid-replaced group. This substantiates the work of Craig (1972) in which he reported decreases in adrenal mass in response to thyroidectomy and increases in adrenal mass as a result of thyroxine replacement in the thyroidectomized animal.
In addition, the absolute secretion rate was also proportional to the level of thyroxine replacement in thyroidectomized animals. Since glandular weights also increased in these animals, one should not expect a great difference in the amount of corticosterone secreted per unit weight of gland in each of the groups. This was the case because there was no significant difference in relative secretion rates when comparing any of the four thyroidectomized groups.

It would appear that glandular secretion rate in these animals was a function of glandular size which in turn was regulated in some manner by thyroxine level. This is consistent with the findings of Williams, et al., (1957), Peterson, (1958), Felber, et al., (1959), and Craig (1972).

A question arises in a consideration of the intact control animals. Although intact controls had mean adrenal weights which were not significantly different from thyroidectomized, euthyroid-replaced animals, the amount of corticosterone secreted by the control glands was greater than in any of the thyroxine treated groups. This in turn resulted in a significantly greater relative secretion rate in the control group. Control animals produced more corticosterone per unit of glandular mass than did any of the athyroid or thyroidectomized-thyroxine replaced animals.

Specifically why this response occurred is not known although a degree of speculation is possible. It has been established that thyroxine (T-4) is not the only active thyroid substance elaborated
by the thyroid. Tri-iodothyronine (T-3) is also produced by the thyroid and is believed by many to possess a biological activity greater than that of thyroxine.

Should this be the case, intact control animals are not strictly comparable to thyroidectomized-thyroxine replaced animals since replacement involved only thyroxine. In other words, T-3, which was present in the intact animal, was not replaced in any of the thyroxine treated animals. That T-3 influences adrenal cortical secretion has been demonstrated by Melby, et al., (1960). These workers showed an acute rise in cortical secretion in the dog following T-3 treatment but not with thyroxine treatment. It is therefore possible that optimal adrenal cortical secretion rates were dependent upon both T-3 and thyroxine rather than upon the latter substance alone.

Because it has been suggested that ACTH levels might be influenced in various thyroid states (Ducommun, et al., 1966; Retiene, et al., 1968) the interaction of ACTH and thyroid level upon cortical secretion rates should be considered. Once again the response of intact control glands to ACTH stimulation was much greater, both from a relative and absolute standpoint, than in any of the thyroidectomized groups. Because the only difference between the intact group and the thyroidectomized-thyroxine replaced groups was the existence of an intact thyroid, we must again assume that some factor in addition to thyroxine was necessary for optimal functioning of the pituitary-adrenal cortical axis. Although the nature of this substance is not discernible within the context of the present study,
it could very well be the thyroxine analog T-3.

In a consideration of the cortical response of athyroid and thyroidectomized-thyroxine replaced animals, an interesting pattern was observed. Although non-stimulated secretion rates were directly proportional to thyroid level, the magnitude of increase due to ACTH stimulation was inversely related to thyroid level. Athyroid animals exhibited a much greater increase than did thyroxine replaced animals while the hyperthyroid group displayed the smallest response to ACTH.

Initially these findings seem to be in conflict with the proposed reciprocity between TSH and ACTH. It will be recalled that factors which decrease TSH (i.e., high levels of thyroxine) may increase ACTH levels with a concomitant increase in cortical secretion. In this investigation, since the response (i.e., percentage increase of secretion) of the cortex to ACTH is less in hyperthyroid animals than in animals in lower thyroid states, it might be initially concluded that the level of ACTH in the hyperthyroid animals is diminished. This is contrary to other findings concerning the interaction of thyroid level and ACTH.

Indeed, such a conclusion would not be consistent with the fact that, in this investigation, increasing thyroxine levels increased both glandular size and secretion rate. According to the theory of reciprocal action of TSH-ACTH, an animal in the athyroid state should have smaller glands and a lower secretion rate than either euthyroid or hyperthyroid animals. Such was the case. Therefore, because
cortical activity is elevated in proportion to thyroid level, an increased amount of ACTH may have been present.

However, glands under the influence of elevated concentrations of ACTH should respond to exogenous ACTH stimulation to a greater extent than glands influenced by lower levels of endogenous ACTH. Such was not the case and seems wholly contradictory to the idea that elevated thyroxine levels elevate ACTH levels.

Although this seeming contradiction cannot be resolved on the basis of present findings, some discussion of these results is in order. Assuming that elevated cortical function in hyperthyroid animals was due to elevated ACTH levels and that diminished cortical secretion in athyroid animals was a function of decreased ACTH, and, further, assuming that the response of the gland to exogenous ACTH stimulation was an indirect measure of endogenous ACTH, then why did athyroid glands show a greater percentage of increase in cortical secretion as a result of ACTH stimulation than did hyperthyroid glands?

It has been reported that in in vitro preparations of cortical tissue, ACTH in the presence of acetate, glycerol, or acetoacetate will not increase cortical secretion (Hoffman and Davidson, 1953). And since the cortical tissue of hyperthyroid animals is in an accelerated state of metabolism (as indicated by an increased secretory rate), it is not unreasonable to assume that the concentration of acetate may be increased. This in turn would lead to a diminished response to exogenous ACTH.
Another possible explanation involves the role of thyroxine within the adrenal cortex. It is well established that cortical metabolism is elevated by thyroxine. Should this be the case, it would be logical to assume that the metabolic degradation of ACTH in cortical tissue would be enhanced. Since the concentration of exogenous ACTH administered was held constant over the various thyroid levels, should it be more rapidly metabolized in the hyperthyroid animal, its effect would not be as pronounced in the hyperthyroid preparation as in preparations from animals in lower thyroid states. Indeed, it has been reported that cortical response to ACTH is reduced in hyperthyroid animals (Felber, et al., 1959; Mikulaj and Nemeth, 1958).

A third possibility involves a readjustment of the adrenal cortex to the absence of thyroid analogs other than thyroxine. It has been suggested in this study that a thyroid factor (or factors) other than thyroxine was necessary for optimal response of the cortex to ACTH stimulation since intact control glands produced more steroid and responded to exogenous ACTH to a significantly greater degree than did thyroidectomized euthyroid-replaced glands. It would appear that the capacity of the cortex in thyroidectomized thyroxine-replaced animals to produce steroids is diminished. It has been established that increasing thyroxine levels enhances steroid production in the thyroidectomized animal, it is possible that glands from animals treated with high thyroxine levels may have been already producing steroids at close to their maximum capacity. Therefore exogenous ACTH
stimulation should produce a smaller degree of increase in steroid production in the thyroidectomized hyperthyroid-replaced gland than in glands from animals replaced with lower levels of thyroxine. In other words, glands from hyperthyroid animals may have been stimulated to their maximum capacity by exogenous ACTH from a level slightly below maximum capacity. Thus the percentage increase would not have been as great as at lower thyroid levels. Whether any of these factors play a role in the diminished response of the hyperthyroid gland to exogenous ACTH stimulation cannot be determined at this time.

Age-Exercise Interaction

In an attempt to determine whether the effects of exercise upon cortical parameters were the same in both young and old animals, the interaction of these factors was examined. It will be recalled that aging increased adrenal weights but that in the mature animal both absolute and relative secretion rates were below those observed in young animals. It is believed that this was due in part to an age related drop in ACTH. Furthermore, exercise significantly increased both glandular size and absolute secretion rate, presumably through increasing ACTH levels.

The influence of exercise upon adrenal weight is significantly greater in mature animals. Exercise increased glandular size approximately four times as much in mature animals as compared to their young counterparts. However, mature glands did not significantly increase corticoid secretion in response to exercise when compared to young glands. Indeed, glands from young exercised animals exhibited
a greater response to exogenous ACTH when compared to their non-exercised controls than did glands from mature exercised animals. These findings confirm those of Craig (1972).

The interpretation of these results was difficult. Why does the mature gland grow larger in response to exercise and not appreciably increase its response to ACTH insofar as secretion is concerned? These results imply that exercise increases ACTH levels in both young and mature animals. Glands from mature animals, although larger did not increase corticosteroid output. This, in turn, suggests that the mature gland was unable to respond to ACTH via corticosteroidogenesis due to some age related deficiency in the steroidogenic pathway. This is a distinct possibility in view of the recent report of Shapiro and Leathen (1971) in which an age related decline of $\Delta^5$-3 hydroxysteroid dehydrogenase was detected. This enzyme occupies a key position in the cortical steroidogenic system.

Age-Thyroid Level Interaction

In view of the fact that adrenal cortical function is dependent upon both the age as well as the thyroid level of the animal, the simultaneous influence of these factors upon parameters of cortical activity is of interest. Aging apparently decreases cortical activity either through depressing ACTH levels or by decreasing enzymatic activity within the cortex. Thyroxine, in turn, may stimulate cortical activity via the pituitary by either directly or indirectly increasing the levels of ACTH. The question arises as to
whether or not thyroxine therapy elicits the same cortical responses in both young and mature animals.

No significant interaction was detected in a consideration of adrenal weights or the absolute amount of corticosterone secreted. Increasing thyroxine levels increased both of these parameters in both young and mature animals. However, considering the amount of corticosterone secreted per unit of glandular weight, significant interaction did exist between age and thyroid level. Mature animals responded differently at the various thyroxine levels when compared to young animals. Close analysis of the data revealed that the source of this difference was in the intact control groups.

In young intact controls, relative secretion rates are significantly greater than in either athyroid or thyroidectomized-thyroxine replaced animals. The magnitude of this difference was significantly lower when comparing mature intact controls in a like manner. It has already been suggested that the difference in response of intact controls may have been due to the fact that some thyroid factor, possibly tri-iodothyronine, was exerting an effect on corticosteroidogenesis and that this factor was present in greater quantities in young animals or that the adrenal cortices were not as responsive to this factor in mature animals.

Several workers have demonstrated that thyroid secretion rate is diminished with age (Grad and Hoffman, 1955; Verzar and Freydberg, 1956; Gregerman, et al., 1962). Should this be the case, decreased thyroxine and triiodothyronine levels would elicit accelerated TSH
release. Assuming a reciprocal relationship between TSH and ACTH levels would be reduced thus giving lower levels of corticosteroid secretion. A consideration of the response of glands from both young and mature intact control animals to exogenous ACTH would appear in order at this time.

Young intact control glands responded to ACTH stimulation to a greater degree than did glands from mature intact controls. In the case of young animals the increase was from 13.67 micrograms of corticosterone per 100 milligrams per hour (basal secretory rate) to 26.11 micrograms per 100 milligrams of adrenal weight per hour (ACTH stimulated level). This reflects a ninety-one percent increase. Values for mature intact controls showed an increase from 11.56 micrograms to 16.24 micrograms for a forty-nine percent increase. Once again assuming that the response to exogenous ACTH is an indication of the level of endogenous ACTH, it may be concluded that ACTH levels in the mature intact control animals were decreased. Thus it is possible that decreased thyroid secretion rates with age influence the secretion rate of the adrenal cortex via the pituitary and ACTH.

Exercise-Thyroid Level Interactions

The next point to be considered in this discussion is the interaction between exercise and thyroid level. It was of interest to determine to what extent exercise altered the response of the adrenal cortex to the various thyroid levels.

No significant interaction existed in a consideration of
adrenal weight. Glands from both exercised and non-exercised animals increased in weight in response to increasing thyroid levels. Conversely, exercise increased glandular weight at all thyroid levels studied. Since it has already been demonstrated in this investigation that both exercise and increasing thyroid levels act to increase glandular weight, presumably through elevation of ACTH concentration, these results were to be expected.

Significant interaction was present between thyroid level and exercise when considering, either the absolute or relative secretion rates of corticoserone. As before, the source of interaction involved the response of the intact control animal to exercise. Exercise increased cortical secretion rates to a much greater extent in intact control animals than in any of the thyroidectomized thyroxine replaced groups.

Thus it is once again suggested that some active thyroid principal other than thyroxine is necessary for the full expression of exercise upon the adrenal cortex. And as before, assuming that this principal acts via the pituitary and ACTH, we should expect a greater response to exogenous ACTH in the exercised, thyroid intact control. This proved to be the case as exogenous ACTH elicited an eighty-four percent increase in secretory rate in glands from exercised animals as compared to a fifty-six percent increase in non-exercised, thyroid-intact animals.
CONCLUSIONS

The function of the adrenal cortex of the male rat as measured by weight as well as in vitro secretion rate of corticosterone is modified by age, exercise, thyroid state, and ACTH as well as by interactions among these factors. Collectively, the effects may be manifested through either (1) altering ACTH levels via a reciprocal TSH-ACTH system in which factors which decrease TSH increase ACTH and vice versa, (2) altering ACTH levels by reducing plasma levels of glucocorticoids which in turn elicits increases in ACTH, (3) altering the response of the adrenal cortex to ACTH, or (4) directly affecting steroidogenic pathways within the adrenal cortex.

Aging decreases the in vitro secretion rate of corticosterone since young animals displayed higher secretion rates than did mature animals. Since glands from young animals also exhibited a significantly greater response to exogenous ACTH than did glands from mature animals, it is concluded that the age related decrease in secretion of corticosterone is due, at least in part, to an age related decrease in ACTH or an age related decrease in responsiveness to ACTH.

Exercise increased both adrenal weight and the in vitro secretion of corticosterone. It is assumed that exercise enhanced peripheral utilization of corticoids with a resultant drop in plasma levels of these substances. This in turn elicited ACTH release from the pituitary which in turn enhanced cortical weight and secretion rate. That ACTH levels were elevated in exercised animals is suggested by
the fact that glands from exercised animals exhibited a greater response to exogenous ACTH stimulation than did glands from non-exercised animals.

Adrenal weight and secretion rate was influenced by thyroid level. Increasing thyroxine levels increased both adrenal weight and the secretion rate of corticosterone. It is believed that these effects were manifested through a reciprocal interaction between TSH and ACTH in that increasing thyroxine levels decreased TSH which in turn possibly led to an increase in ACTH with a resultant increase in adrenal weight and secretion rate. It is also suggested that high thyroxine levels inhibited the full in vitro expression of ACTH upon the adrenal cortex since in vitro cortical secretion rates in hyper-thyroid animals showed a smaller increase in response to ACTH stimulation than did glands from animals at lower thyroid levels.

It is further concluded that thyroxine is not the only thyroid principle required for the full expression of ACTH upon the adrenal cortex. Because intact control animals exhibited both greater secretion rates of corticosterone and greater responses to exogenous ACTH stimulation than did thyroidectomized, thyroxine replaced animals, it is suggested that some thyroid factor in addition to thyroxine was required.

Mature animals did not respond to exercise in the same manner as young animals. In young animals exercise resulted in an increased peripheral utilization of corticoids which in turn enhanced ACTH release. That ACTH levels were elevated is indicated by the fact
that glands from young exercised animals exhibited a greater response to exogenous ACTH stimulation than did glands from young non-exercised animals.

Mature exercised animals did not show significant increases in secretion rates in response to exogenous ACTH stimulation although glands from mature animals did show a greater increase in weight in response to exercise than did their young counterparts. This indicates that although exercise may enhance ACTH, the mature gland was unable to respond, possibly because of an age related change in the corticosteroidogenesis.

An age related decrease of an active thyroid principal, possibly tri-iodothyronine, necessary for optimal cortical secretion occurred. Since glands from young intact controls exhibited both greater secretion rates and increases in secretion rates as a response to exogenous ACTH than did glands from mature intact controls, and since this difference was not significant in thyroidectomized, thyroxine-replaced animals, it is concluded that some thyroid factor other than thyroxine influences cortical secretion rates and that this factor decreased with age.

The same thyroid principal is also believed to influence the response of the cortex to exercise. Exercise increased secretion rates in intact control glands to a greater extent than in any of the thyroidectomized thyroxine-replaced groups. It is further concluded that this factor in part influenced cortical activity via ACTH since
the response of glands from exercised intact controls to exogenous 
ACTH stimulation was much greater than the response to the same 
stimulus in non-exercised intact control animals.
SUMMARY

1. This investigation dealt with the effects of age, exercise, the thyroid gland, and adrenocorticotropic hormone upon the in vitro secretion of corticosterone in the male rat.

2. Animals were divided into two age groups: young (70 days of age at the beginning of the experimental period) and mature (200 days of age at the beginning of the experimental period).

3. Each age group was subdivided into five thyroid treatment groups: intact control (no treatment), athyroid (thyroidectomized), hypothyroid (thyroidectomized and injected daily with thyroxine at a rate of 0.5 micrograms per 100 grams of body weight), euthyroid (thyroidectomized and injected daily with thyroxine at a rate of 14.0 micrograms per 100 grams of body weight per day).

4. Each treatment group was subdivided into an exercised and non-exercised group. Exercised animals were swum to exhaustion five days per week for a ten week period.

5. At the end of the exercise period, the animals were sacrificed, glandular weights were recorded and the in vitro secretion rate of corticosterone, either in the presence or absence of exogenous ACTH was determined.

6. Analysis of the data revealed:
   a. Aging increased adrenal weight but decreased the secretion rate of corticosterone.
   b. Glands from young animals exhibited much greater response to ACTH than did glands from mature animals.
c. Exercise increased both adrenal weight and the secretion of corticosterone.
d. Glands from exercised animals responded to a greater extent to ACTH stimulation than did glands from non-exercised animals.
e. Adrenal weights and secretory rates were directly proportional to thyroid level because increasing thyroid level increased these parameters.
f. Glands from hyperthyroid animals did not respond to ACTH to as a great an extent as did glands from animals of lower thyroid levels.
g. Intact control animals were not strictly comparable to thyroidectomized, thyroxine-replaced animals because their secretion rates and responses to exogenous ACTH were much greater than those of any thyroxine treated group.
h. Young animals responded to exercise to a significantly greater degree than did mature animals.
i. Thyroid levels promoted adrenal cortical function to a greater degree in young animals than in mature animals.
j. Exercise induced greater increases in indices of adrenal cortical function in young animals when compared to mature animals.

7. These results suggest that both exercise and thyroxine increased the in vitro secretion of corticosterone by increasing ACTH. Age decreased the in vitro secretion rate through a direct effect upon the adrenal cortex and by decreasing an active thyroid substance
other than thyroxine. It is further suggested that this same thyroid substance was necessary for the full expression of exercise effects upon the adrenal cortex. Finally, it was indicated that thyroid and exercise effects were mediated via ACTH concentrations while age effects may have involved ACTH concentrations as well as the responsiveness of the adrenal cortex to this hormone.
BIBLIOGRAPHY


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To my wife Jane, to my children Chris and Lindsey, and to Dr. Kenneth Ottis of the Department of Zoology-Entomology, Auburn University, I wish to express my appreciation for their love, encouragement, and understanding. And finally to my associates at Lambuth College, Mr. Kenneth Kramer, Mr. Lloyd Mabry, Mr. Russell Keller, Mr. Wayne Johnson, Miss Nancy Meadows, and especially Miss Gayle Emro, for their aid, encouragement, and friendship during the preparation of the manuscript, very special thanks are extended.
APPENDIX A: THYROIDECTOMY

Animals were anesthetized and maintained with ether. The cutaneous region ventral to the thyroids was shaven, a mid-line incision was made and the glands were exposed through careful dissection of the subcutaneous tissues.

The glands were carefully dissected free of surrounding elements and were removed from the animal. The incision was closed with wound clips and the animal was returned to its cage. Approximately one week was allowed for recovery before initiation of the experimental period. Thyroxine injections were commenced five days after the surgical procedures.
APPENDIX B: THYROXINE SOLUTIONS

1. Preparation of the stock solution.
   a. Fourteen milligrams of L-thyroxine (as the sodium salt) were
diluted with approximately 500 milliliters of distilled
deonized water in a one liter volumetric flask.
   b. The pH of this solution was lowered by adding 1N HCL, a drop
at a time, until the thyroxine precipitates.
   c. The solution was then diluted to one liter with distilled
deonized water. Final concentration was 14 micrograms of
thyroxine per milliliter.
   d. This solution is stable under refrigeration for periods up
to one year.

2. Dilution of the stock solution for injection.
   a. A small quantity of the stock solution was removed and 1N NaOH
was added drop by drop until the precipitated thyroxine went
into solution. An aliquot of this solution was removed and
utilized for injections at the 14 microgram rate.
   b. The remaining solution was further diluted to obtain the 0.5
and 3.5 microgram injection solutions.
   c. Diluted solutions remain active for up to forty-eight hours.
APPENDIX C: IN VITRO INCUBATION PROCEDURES

1. Incubation Medium (Umbreit, et al., 1964)
   a. Stock solutions were prepared according to the following procedures and stored under refrigeration.

   (1) NaCl  4.5 grams/500 milliliters distilled water
   (2) KCl  1.73 grams/150 " " "
   (3) CaCl₂·2H₂O  2.43 grams/150 " " "
   (4) KH₂PO₄  3.17 grams/150 " " "
   (5) MgSO₄·7H₂O  5.73 grams/150 " " "
   (6) NaHCO₃  1.95 grams/150 " " "

   b. Preparation of the medium

   Aliquots of the various stock solutions were mixed in the following proportions:

   100 parts solutions 1
   4 " " 2
   3 " " 3
   1 " " 4
   1 " " 5
   21 " " 6

   Two hundred milligrams of glucose was added per 100 milliliters of incubation medium. Where ACTH stimulation was desired it was added to the incubation medium so as to give a concentration of 250 milliunits ACTH per milliliter of medium.
2. Incubation procedure
   a. Rapidly remove the adrenal glands from the animal and place on a filter paper moistened with medium (no ACTH present) in a Petri dish. The dish should be kept on ice.
   b. Trim all adipose tissue from the glands and weigh on an analytical balance to the nearest one-tenth of a milligram.
   c. Carefully cut each gland into quarters. Segments from one gland are placed into two milliliters of incubation fluid in one Warburg flask while segments of the other gland are added to two milliliters of incubation fluid containing ACTH in a second Warburg flask. (Note: the right and left glands were alternated insofar as ACTH stimulation was concerned).
   d. Each flask was gassed with 95% O₂ plus 5% CO₂ for sixty seconds and then incubated with shaking for ninety minutes at thirty-seven degrees centigrade.
   e. At the end of the incubation period, the medium from each flask was removed to a four milliliter screwcapped vial and frozen until further analysis.
APPENDIX D: FLUOROMETRIC DETERMINATION OF CORTICOSTERONE
(Vander Vies, 1960; Zarrow, et al., 1964; Craig, 1972)

1. Reagents

a. Dichloromethane purification

(1) Allow the dichloromethane to stand over one-tenth its volume of concentrated H₂SO₄ for not less than three days. Occasional shaking is desirable.

(2) Wash the dichloromethane three times with one-tenth its volume of 2N NaOH and then wash three times with one-tenth its volume of distilled water.

(3) Dry for not less than twenty-four hours with anhydrous sodium sulfate.

(4) Distill through a Dufton column, collecting that fraction which comes over between 40-41°C.

b. Ethanol purification

Ethanol was purified by twice distilling it in a Dufton column and collecting the fraction which came over at 78°C.

c. Fluorescent reagent

Three parts of concentrated H₂SO₄ are added to one part of purified ethanol. Mixing should be carried out slowly in an ice bath.

d. Corticosterone standard solutions

(1) A stock solution is prepared by weighing out 25 milligrams of corticosterone (Nutritional Biochemicals) and dissolving
in 100 milliliters of purified ethanol. The stock solution is stored under refrigeration.

(2) Standard solutions of corticosterone are prepared as follows:

(a) Remove the stock solution from the refrigerator and allow to come to room temperature.

(b) Remove a one milliliter aliquot and dilute to twenty-five milliliters with distilled water. This solution contains ten micrograms corticosterone per milliliter.

(c) Prepare solutions containing 1.0, 2.5, 5.0 and 7.5 micrograms per milliliter by further dilution with distilled water. Utilize these solutions for determination of a standard curve.

2. Corticosterone determination procedures

a. Allow the frozen incubation medium to come to room temperature (approximately one hour is required).

b. Remove duplicate 0.5 milliliter aliquots to stoppered centrifuge tubes for steroid analysis.

c. Extract with five milliliters of purified dichloromethane by shaking for sixty seconds. Allow the layers to separate and shake again for thirty seconds.

d. Centrifuge at 2,000 RPM for two minutes. Remove the aqueous (top) layer by aspiration.
e. Shake the extracts with 200 microliters of 0.1N NaOH for sixty seconds, then centrifuge for sixty seconds at 2,000 RPM.

f. Remove the NaOH by aspiration. (Note: Don't delay as NaOH destroys the steroid). Dry the extract for ten minutes with anhydrous sodium sulfate.

g. Mix two milliliters of the dried extract with five milliliters of fluorescent reagent by shaking for thirty seconds.

h. Centrifuge for two minutes at 1,000 RPM, then remove the organic (top) layer by aspiration.

i. One hour after mixing, reading the fluorescence utilizing a Turner fluorometer and the following filters:

   Primary 110-813 (47B)

   Secondary 110-818 (214012)
APPENDIX E: ANALYSIS OF VARIANCE FOR THE VARIABLE ADRENAL WEIGHT
Table 16. Analysis of variance for the variable adrenal weight

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
<th>Probability of a greater F-value</th>
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</thead>
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<tr>
<td>Age effects</td>
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<td>123.31</td>
<td>3.2</td>
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</tr>
<tr>
<td>Exercise effects</td>
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<td>717.69</td>
<td>18.4</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
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<td>313.58</td>
<td>8.0</td>
<td>P &lt; 0.025</td>
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<td>1127.83</td>
<td>29.0</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
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<td>51.91</td>
<td>1.3</td>
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</tr>
<tr>
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<td>15.65</td>
<td>0.4</td>
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</tr>
<tr>
<td>Residual</td>
<td>19</td>
<td>744.89</td>
<td>39.01</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

*N.S. = Not significant.
APPENDIX F: ANALYSIS OF VARIANCE FOR VARIABLE
ABSOLUTE SECRETION RATE
Table 17. Analysis of variance for variable absolute secretion rate

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
<th>Probability of a greater F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1</td>
<td>22.37</td>
<td>22.37</td>
<td>26.2</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Exercise</td>
<td>1</td>
<td>14.95</td>
<td>14.95</td>
<td>17.3</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Age-exercise</td>
<td>interaction</td>
<td>1</td>
<td>0.14</td>
<td>0.14</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Thyroid level</td>
<td>4</td>
<td>114.23</td>
<td>28.56</td>
<td>33.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Age-thyroid level</td>
<td>interaction</td>
<td>4</td>
<td>1.98</td>
<td>0.49</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Exercise thyroid</td>
<td>level interaction</td>
<td>4</td>
<td>21.34</td>
<td>5.33</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Residual 1</td>
<td>1</td>
<td>16.34</td>
<td>0.86</td>
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<td>---</td>
</tr>
<tr>
<td>ACTH effects</td>
<td>1</td>
<td>81.75</td>
<td>81.75</td>
<td>160.0</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Age-ACTH interaction</td>
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<td>6.89</td>
<td>13.5</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Exercise ACTH</td>
<td>interaction</td>
<td>1</td>
<td>3.28</td>
<td>3.28</td>
<td>P &lt; 0.025</td>
</tr>
<tr>
<td>Thyroid level-ACTH</td>
<td>interaction</td>
<td>4</td>
<td>13.15</td>
<td>3.28</td>
<td>P &lt; 0.025</td>
</tr>
<tr>
<td>Residual 2</td>
<td>102</td>
<td>52.04</td>
<td>0.51</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

*N.S. = Not significant.
APPENDIX G: ANALYSIS OF VARIANCE FOR VARIABLE
RELATIVE SECRETION RATE
<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
<th>Probability of a greater F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td>484.83</td>
<td>484.83</td>
<td>161.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Exercise</td>
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</tr>
<tr>
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<td>2.0</td>
<td>N.S.*</td>
</tr>
<tr>
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<td>348.37</td>
<td>87.09</td>
<td>29.2</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Age-thyroid level interaction</td>
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<td>100.10</td>
<td>25.02</td>
<td>8.3</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Exercise-thyroid level interaction</td>
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<td>168.34</td>
<td>42.08</td>
<td>14.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
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</tr>
<tr>
<td>ACTH effects</td>
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<td>1119.99</td>
<td>500.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Age-ACTH interaction</td>
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<td>109.36</td>
<td>21.8</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
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<td>21.99</td>
<td>4.4</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Thyroid level-ACTH interaction</td>
<td>4</td>
<td>164.36</td>
<td>41.09</td>
<td>8.2</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Residual 2</td>
<td>102</td>
<td>518.42</td>
<td>5.08</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

*N.S. = Not significant.