Plasma corticosterone concentrations and in vitro adrenal secretion rates in aging rats: effect of physical training and acute swimming

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Plasma corticosterone concentrations and in vitro adrenal secretion rates in aging rats: Effect of physical training and acute swimming

by

James Alan Severson

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>27</td>
</tr>
<tr>
<td>RESULTS</td>
<td>30</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>46</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>60</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>63</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>64</td>
</tr>
<tr>
<td>APPENDIX A: INCUBATION MEDIUM</td>
<td>72</td>
</tr>
<tr>
<td>APPENDIX B: GLUCOCORTICOID ANALYSIS BY COMPETITIVE PROTEIN-BINDING RADIOASSAY</td>
<td>73</td>
</tr>
</tbody>
</table>
INTRODUCTION

The importance of adrenocortical steroid hormones as metabolic adaptors is a well-recognized fact. The glucocorticoids are essential for the induction of enzymes that are key points in metabolic pathways and through their permissive actions enhance the effectiveness of other hormones (Thompson and Lippmann, 1974).

Adrenocortical response to ACTH has been shown to decline during aging in several species, including humans. In general, plasma glucocorticoid hormone concentrations were unchanged as humans and other animals aged. However, the ability of the adrenal cortex to respond to ACTH or other stressors by increasing plasma glucocorticoid concentrations was decreased during aging (Grad et al., 1967; Hess and Riegle, 1970). Data from several species have indicated that, in older animals, the adrenal cortex was very close to being maximally stimulated by ACTH at all times in order to maintain plasma glucocorticoid concentrations at levels similar to those of younger animals. Other studies have indicated that older animals have difficulty in regulating ACTH secretion (Riegle and Hess, 1972; Riegle, 1973; Britton et al., 1975).

Interest has existed in the relationship between glucocorticoid hormones and exercise since it was demonstrated that these hormones could increase the work capacity of adrenalectomized and intact rats (Ingle et al., 1951; 1952). Since then, numerous studies have examined this relationship under a variety of conditions and have resulted in varying conclusions. Physical-training has been shown to increase the maximal work capacity of humans (Hartley et al., 1972b) and rats (Buuck and Tharp,
1971), even though post-exercise plasma glucocorticoid concentrations were generally lower in exercise-trained subjects (Tharp, 1975). Physical-training may reduce the need for glucocorticoids during exercise through the well-known effects of these hormones on energy metabolism (Gordon, 1967; Oscai and Holloszy, 1971). However, the necessity of the adrenal cortex for the adaptation to chronic exercise has been put into question by subsequent data (Berdanier and Moser, 1972). Adrenocortical responsiveness to ACTH has been shown to increase (Pritchett, 1973; Frenkl et al., 1975) and decrease (Tharp and Buuck, 1974), as a result of exercise-training.

This study was designed to investigate the combined effects of physical-training and age on the adrenocortical function of male rats. Plasma corticosterone concentrations were determined after an acute swim and in nonswum controls. The in vitro responsiveness of the adrenal glands of exercise-trained rats to ACTH also was determined.
REVIEW OF LITERATURE

Hypothalamic-Pituitary-Adrenal System

The hypothalamic-pituitary-adrenal system has been shown to be a mechanism through which animals adapt to various metabolic situations and stressors. A stressor has been defined as any internal or external stimulus that ultimately results in an increased secretion of corticotrophin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and glucocorticoids above those levels found in unstimulated animals. Within the past 15 years, numerous studies have been reported concerning the function of this system. This is not intended to be a summarization of current data, but rather a brief overview of this complicated area. For additional information in this area, the reader is referred to several recent review articles from which the following discussion of the hypothalamic-pituitary-adrenal system was taken (Garren et al., 1971; Gill, 1972; King and Mainwaring, 1974; Sayers et al., 1974; Schulster, 1974; Thompson and Lippmann, 1974; Yates et al., 1974; Feldman, 1975; Halkerston, 1975; Leung and Munck, 1975; Reichlin et al., 1976).

Corticotrophin-releasing hormone

CRH has been shown to be stored in synaptosome granules of neurosecretory neurons of the hypothalamic median eminence. The release of CRH from its site of storage, into the hypothalamic-hypophyseal portal vascular system has been established as the final common pathway for neural control of ACTH secretion. Various neural pathways, neural transmitters and hormones acting on the brain have been shown to affect CRH secretion.
Stimulatory neural pathways appeared to enter the hypothalamus anteriorly. Electrical stimulation of the amygdaloid-septal complex increased ACTH secretion. An ascending cholinergic neuronal system that originated in the substantia nigra and ventral tegmental area of the midbrain, and projected through the hypothalamus and subthalamus to basal forebrain areas, also has been shown to be stimulatory. Glucocorticoid-sensitive neurons in the hypothalamus and midbrain were stimulated by acetylcholine and inhibited by norepinephrine and dopamine, suggesting cholinergic pathways to be stimulatory and noradrenergic pathways to be inhibitory to CRH secretion. Implantation of atropine in the anterior hypothalamus blocked the adrenocortical response to surgery and ether stress.

Inhibitory neural pathways appeared to be slower acting than stimulatory pathways and entered the hypothalamus posteriorly. These pathways also were involved with the amygdala and septum. Electrical stimulation of the hippocampus inhibited CRH secretion. Another inhibitory pathway arose in the rostral, basal preoptic region and entered the medial basal hypothalamus.

Hormonal influence on CRH secretion was inhibitory and stimulatory. Glucocorticoids and possibly ACTH feedback inhibited CRH secretion. CRH has been suggested to be involved in short-loop feedback of its own secretion. Glucocorticoid feedback inhibition appeared to be the most physiologically significant hormonal influence.

Hypothalamic CRH content and plasma ACTH concentrations exhibited circadian rhythms of about 24 hours duration. Presumably, this rhythm was due to neural input signals to the median eminence. The period of
highest secretion corresponded to the start of the daily activity cycle of the animal.

Adrenocorticotropic hormone

In response to stimulation by CRH, the anterior pituitary gland secreted ACTH. This 39 amino acid polypeptide was synthesized and stored in corticotrophs within the pairs distalis and pars intermedia of the anterior pituitary. Vasopressin, angiotensin and direct sympathetic stimulation of the anterior pituitary gland also resulted in the stimulation of ACTH secretion. However, CRH was the only definite stimulus for ACTH secretion. Glucocorticoids acted at the level of the pituitary gland to decrease or abolish the CRH-stimulated secretion of ACTH.

The actions of ACTH on the adrenal gland included:

1. Stimulation of adrenal corticosteroid synthesis and content;
2. Increased blood flow through the adrenal gland;
3. Increased adrenal gland weight by a trophic effect;
4. Acceleration of adrenocortical phosphate turnover and hydrolysis of cholesterol esters;
5. Stimulation of glycogenolysis and glucose oxidation in the adrenal gland;
6. Decreased adrenocortical ascorbic acid, lipid and cholesterol content.

The initial event in the action of ACTH was its stereospecific binding to its hormone receptor on the plasma membrane of the adrenocortical cell. ACTH entry into the cell was not required for the stimulation of steroidogenesis. The interaction of ACTH and the
receptor generated a signal which may have been the result of a conformational change in the membrane components. The signal of hormonal activation was detected, and was then transmitted across the plasma membrane to activate adenyl cyclase. Calcium was required for the activation of adenyl cyclase, but not for ACTH binding to the receptor. The binding of ACTH to the receptor was specific for ACTH. Modifications in the peptide that resulted in decreased binding to the receptor caused decreased activation of adenyl cyclase and decreased steroidogenesis.

The activation of adenyl cyclase resulted in an increased conversion of adenosine triphosphate (ATP) to adenosine 3',5'-cyclic monophosphate (cyclic AMP). The rise in intracellular cyclic AMP concentrations, resulting from ACTH stimulation, preceded any increase in steroidogenesis. Cyclic AMP appeared to be the only cyclic nucleotide, of any physiological significance, to interact with the receptor protein. When cyclic AMP was bound to the receptor protein, it was protected from phosphodiesterase inactivation by conversion to 5'-adenosine monophosphate (5'-AMP). In adrenocortical cells, cyclic AMP-dependent protein kinase was located in the endoplasmic reticulum and consisted of two subunits: the regulatory receptor subunit and a catalytic protein kinase subunit. The cyclic AMP-receptor functioned as an inhibitor of the protein kinase. The binding of cyclic AMP to the receptor subunit resulted in the dissociation of the cyclic AMP-bound receptor from the protein kinase subunit. When free of the inhibitory action of the receptor subunit, the protein kinase became fully activated.

Adrenal cholesterol esterase may have been activated by a cyclic AMP-dependent protein kinase mediated phosphorylation. This resulted in the increased conversion of fatty acid-esterified cholesterol to free
cholesterol, the substrate for steroidogenesis. Purified adrenal ribosomes were an excellent substrate for cyclic AMP-dependent protein kinase. Serine and threonine residues of specific ribosomal protein bands were phosphorylated on each ribosomal subunit. The phosphorylation of ribosomal proteins may have regulated the participation of ribosomes in protein synthesis. Protein synthesis was required for the stimulation of steroidogenesis by ACTH. Puromycin blocked protein synthesis at the level of translation of messenger RNA, and blocked ACTH or cyclic AMP-induced stimulation of steroidogenesis. Cyclic AMP also may have induced the synthesis of a labile protein at the level of translation. This protein increased the conversion of cholesterol to pregnenolone, the initial step of steroidogenesis, by binding and carrying cholesterol to its site of side-chain cleavage on the mitochondrial cytochrome P-450 enzyme system. Cycloheximide and puromycin, but not actinomycin, inhibited steroidogenesis, indicating another instance in which cyclic AMP promoted steroidogenesis at the level of translation.

The rate-limiting step of steroidogenesis was side-chain cleavage, which involved the conversion of cholesterol to pregnenolone and isocapraldehyde within the mitochondrion. ACTH and cyclic AMP stimulated side-chain cleavage by several mechanisms, which were mentioned previously. Following side-chain cleavage, pregnenolone exited from the mitochondrion and entered the soluble microsomal cell fraction, where it was sequentially converted to progesterone and 11-deoxycorticosterone. Transfer of 11-deoxycorticosterone into the mitochondrion was required for its conversion to corticosterone, the main glucocorticoid of the rat. Once synthesized, corticosterone was secreted into the adrenal venous
Chronic ACTH treatment caused gross increases in adrenal gland weight, protein and nucleic acid content of the adrenal gland. Increases in the entire enzyme pathway for steroid production were not only maintained by ACTH, but also were sequentially increased by elevated concentrations of ACTH. After ACTH administration there was an increased in vitro protein synthetic capacity that appeared to be due to changes in the cytosol, microsomes and isolated polysomes.

**Glucocorticoids**

Plasma glucocorticoids circulated either unbound (free) or bound to plasma proteins. Generally, at physiological concentrations, less than ten per cent of the circulating glucocorticoids were unbound. The binding of glucocorticoids prevented their interaction with target tissue and their enzymatic conversion to inactive metabolites in the liver. Glucocorticoid binding to plasma proteins was at two distinct types of binding sites:

1. Albumin, a low affinity, high capacity binding site;
2. Corticosteroid-binding globulin (CBG or transcortin), a low capacity binding site, with a binding affinity for glucocorticoids that was three orders of magnitude greater than that of albumin.

Unbound plasma glucocorticoids entered cells by passive diffusion. For glucocorticoids to have had an effect on cell function, glucocorticoid receptors must have been present in that cell. The presence of receptors in a cell designated it as a responsive or target cell. Glucocorticoid
receptors are protein in nature and have been found in liver, brain, heart, skeletal muscle, adipocytes, pituitary, retina, osteoclasts, lung, uterus, testes and spleen of a variety of species.

The binding of glucocorticoids to a receptor was necessary for hormone action. Most of the biological actions of glucocorticoids appeared to be mediated by a receptor mechanism. There was a direct correlation between hormone-binding to receptors and biological activity of the hormone. Binding to cytoplasmic receptors was stereospecific and of high affinity, but noncovalent and saturable. It appeared that the receptor was able to distinguish between steroids of varying biological actions, i.e. glucocorticoids, mineralocorticoids, androgens, estrogens and progesterone. The interaction of the active steroid with the receptor initiated a temperature-dependent activation of the steroid-receptor complex involving a conformational change in the complex. The activated complex was then rapidly transferred to the nucleus. Within the nucleus, it appeared that DNA may have been the acceptor for the steroid-receptor complex and that chromosomal proteins may have acted to restrict the binding of the complex to specific regions of DNA. Once bound to DNA, the steroid-receptor complex initiated the synthesis of messenger RNAs that coded for specific proteins. These proteins caused effects that were specific for each target tissue. Specific glucocorticoid effects included:

1. Stimulation of hepatic gluconeogenesis by induction of the synthesis of key enzymes, i.e. tyrosine-glutamate aminotransferase, phosphoenol-pyruvate carboxykinase and pyruvate carboxylase;
2. Inhibition of glucose oxidation in adipocytes;
3. Enhanced fatty acid mobilization by permitting the expression of the lipolytic activity of catecholamines.

Adrenocortical Function in Aging

One of the many proposed theories of aging has suggested that physiological aging was the result of a loss of endocrine function with time (Gusseck, 1972). Reports concerning adrenocortical function during the aging process have stated that plasma concentrations of adrenocortical hormones may either decrease or remain constant with time. Adrenocortical secretion and the ability to respond to various stressing agents appeared to decline with increasing age. This indicated that the ability of the adrenal gland to respond to ACTH was altered during aging.

Plasma cortisol and corticosterone concentrations have been reported to be similar in men and women aged 25 and 72 (Grad et al., 1967). Young persons excreted more urinary corticosteroids than older individuals during the six hours following awakening, the most active period of adrenocortical secretion. Elderly individuals may have adapted by decreasing their glucocorticoid distribution volume in order to maintain the same plasma concentrations of cortisol and corticosterone in the face of a decreased adrenocortical secretory capacity.

Decreased adrenocortical secretory capacity during aging also was indicated by mineralocorticoid secretion. Aldosterone secretion rates in 27 year-old males were twice that of 77 year-olds (Flood et al., 1967). The older individuals also had lower plasma concentrations of aldosterone.
and a lower metabolic clearance of aldosterone.

In guinea pigs, circulating concentrations of cortisol and 17-hydroxycorticosteroids decreased with age (Good et al., 1956; Thornton et al., 1962). As measured by cannulation of the adrenal vein, old guinea pigs secreted less cortisol than young animals.

A comparison of kittens and adult cats showed no difference in the corticosterone secreted into the adrenal venous blood (Illett and Lockett, 1969). However, the adult cats had higher cortisol concentrations in the same samples of venous effluent.

Mice (C57BL/6J and DBA/2J strains) at 30 months-of-age had the same plasma corticosterone concentrations as two month-old mice (Eleftheriou, 1974). This study also reported that, in both strains of mice, there was a progressive, declining adrenocortical response to ACTH with increasing age. Plasma concentrations of corticosterone reported by Eleftheriou were contrary to those reported by Grad and Khalid (1968) for C57BL/6J mice. Grad and Khalid found that circulating corticosterone concentrations were highest at two months-of-age and then declined until mice were 26 months-old. Eleftheriou (1974) suggested that differences in housing conditions may have resulted in the differences observed in data for the same strain of mice. Eleftheriou housed mice one per cage for one week prior to the experiment to prevent the interaction of mice, which could have accounted for the elevated plasma corticosterone concentrations in the study of Grad and Khalid.

Resting plasma concentrations of cortisol and corticosterone remained unchanged from three months to 13 years-of-age in Holstein-Friesian bulls (Riegle and Nellor, 1967). Venous infusion of 10 units
(U) of ACTH per 100 pounds body weight resulted in a greater increase in plasma glucocorticoids in bulls less than four years-of-age than in bulls older than eight years. Normal concentrations of circulating glucocorticoids and decreased adrenocortical responsiveness to ACTH infusion suggested that the adrenal cortex of older cattle may have been less responsive to endogenous ACTH. Higher levels of plasma ACTH may have been required in older cattle to maintain normal glucocorticoid concentrations. The decreased adrenocortical responsiveness to ACTH infusion indicated that the bulls' adrenocortical tissues were normally near maximal stimulation by endogenous ACTH.

Similar results to those obtained from bulls also have been reported for non-lactating Toggenburg dairy goats (Riegle et al., 1968). Resting plasma concentrations of cortisol and corticosterone were similar in goats between one and nine years-of-age. Venous ACTH infusion (30 U) resulted in smaller increases in plasma cortisol concentrations in goats older than six years than for those seen in goats five years-of-age and younger. Repeated ACTH stimulation of the adrenal tissue of older goats did not result in a more responsive adrenal cortex.

Resting plasma corticosterone concentrations were unchanged as male and female rats aged from four to 25 months (Hess and Riegle, 1970). Subcutaneous injection of ACTH (four U per 100 grams body weight) or ether stress resulted in a greater increase in plasma glucocorticoid concentrations in young rats than in old. These data showed that the functional reserve capacity of the adrenal glands of old rats was 70 per cent of that of young rats. This demonstrated in another species of animal that in order to maintain similar plasma glucocorticoid
concentrations during aging, the adrenal gland must be nearly maximally stimulated \textit{in vivo} by ACTH. Histological data also have indicated that the adrenal glands of older rats had a lower functional reserve capacity (Lewis and Wexler, 1974). The adrenal cortex of four month-old rats exhibited lipid depletion from inner cortical zones and an apparent collapse of the fascicular structure, indicating an exhausted condition or a reduced ability to respond to ACTH stimulation.

Chronic depot injection of ACTH (gelatin vehicle) resulted in a more responsive adrenal cortex to acute ACTH injection or ether stress in four and 24 month-old rats (Hess and Riegle, 1972). The biological half-life of corticosterone in young rats was less than that of older rats, but the corticosterone distribution volume was unchanged. Chronic ACTH treatment did not affect the biological half-life of corticosterone or the corticosterone distribution volume. These data did not support the idea of adrenal exhaustion in young or old rats. After prolonged adrenocortical activation, the hypothalamic-pituitary ACTH-secretory mechanism responded in the following manner to elevated plasma glucocorticoid concentrations:

1. Increased responsiveness of young and old adrenal cortices to direct stimulation;

2. Decreased responsiveness to stress in young rats.

The age-related change in hormone sensitivity suggested that the adrenocortical control mechanism had undergone alterations, which had altered the precision of adrenocortical regulation.

Arteriosclerotic breeder female rats had a lowered metabolic clearance rate of corticosterone and a production rate of corticosterone
that was half that of virgin rats (Saroff and Wexler, 1969). Plasma levels of corticosterone in breeder rats were 25 per cent lower than that of virgin control rats. This indicated that the adrenal gland and other physiological factors were responsible for the decreased production rate of corticosterone in arteriosclerotic breeder female rats.

Five month-old rats exhibited a greater depression of adrenocortical responsiveness than that observed in 27 month-old rats in response to dexamethasone suppression of the hypothalamic-pituitary-adrenal system (Riegle and Hess, 1972). Riegle (1973) has shown that chronic restraint stress resulted in a decreased ability of five and 25 month-old rats to respond to ether stress. However, younger rats exhibited a greater depression of adrenocortical responsiveness. Plasma corticosterone concentrations of restraint-adapted rats following ACTH injection were greater in younger rats, again indicating that the ability of the adrenocortical system to respond to stress was decreased as animals age. Both studies suggested that the feedback-sensitive site of the adrenocortical system in old rats may have been less sensitive to endogenous or exogenous glucocorticoids.

Once corticosterone was available to the liver of aging rats and mice, the induction of tyrosine amino-transferase (TAT) activity was similar at all ages examined (Finch et al., 1969; Adelman and Freeman, 1972; Britton et al., 1975). However, in two month-old rats, hepatic TAT increased in response to ACTH injection more rapidly than in an 18 month-old rat (Adelman and Freeman, 1972). The time course of increases in hepatic TAT activity was not altered as rats aged from two to 24 months. Increases in plasma corticosterone concentration in 24 month-old
rats were delayed in response to an acute fast (Britton et al., 1975). This also was seen in old C57BL/6J mice under the same conditions. Failure of plasma glucocorticoid concentrations to increase in old animals during a fast was not due to an enhanced rate of utilization of hormone from the blood or a reduced capacity of the adrenal cortex to secrete corticosterone in response to exogenous ACTH, but rather a deficiency in the regulation of ACTH in aging animals.

**Adrenocortical Function in Response to Exercise**

Adrenocortical response to exercise has been shown to produce variable results. The role of glucocorticoids in exercise and training has been reviewed previously (Dawson and Horvath, 1970; Tharp, 1975). The conclusions of these reviewers can be summarized in the following manner:

1. The principal functions of glucocorticoids during exercise are the stimulation of gluconeogenesis and the mobilization of fatty acids and amino acids from body stores;

2. The administration of exogenous glucocorticoids produced significant increases in the work produced by isolated muscle and by intact animals;

3. Light to moderate exercise loads produced increases, decreases or no change in plasma glucocorticoid concentrations, depending on the degree of psychological and physiological stress involved;

4. In moderate to exhaustive exercise, the plasma glucocorticoid concentrations progressively increased. Exhaustion produced a
decrease in plasma glucocorticoid concentrations in animals, which may have represented a defense mechanism to prevent the depletion of body resources;

5. Chronic exercise-training induced adrenocortical hypertrophy and usually a smaller rise in plasma glucocorticoid concentrations during an acute exercise bout than that obtained with nontrained subjects. Resting glucocorticoid concentrations frequently increased initially during training, but returned to normal as the trained state was reached;

6. Changes in glucocorticoid response during training appeared to be produced by decreased responsiveness of the adrenal cortex to ACTH stimulation, and possibly by adaptation of the hypothalamus-pituitary system, which decreased the ACTH released in response to stress;

7. The many combinations of psychological and physiological stress present in different exercise regimens probably accounted for the variety of glucocorticoid responses reported in the literature.

Points 3, 4, 5 and 6 will be discussed further.

Human studies

Human exercise studies have been complicated by psychological factors, which were difficult to separate from the effect of muscular exercise. Renold et al. (1951) examined adrenocortical function in college oarsmen by measuring eosinophil counts before and after training sessions and competitive racing. Their results indicated that in well-trained individuals, emotional stress, either alone or in combination
with muscular activity, may have led to adrenocortical stimulation. Eosinophil counts and urinary 17-hydroxycorticosteroid secretion also have been examined in trained rowing crews before and after practice sessions, time trials and a competitive race (Hill et al., 1956). The time trials and competitive race were of approximately equal physical exertion. The distance of each race was four miles. The excretion of 17-hydroxycorticosteroids on practice days (physical exertion was roughly comparable to time trial and race days) was not elevated over control, nonexercise days. There was evidence of significant adrenocortical activation on time trial and race days.

Analysis of plasma cortisol and aldosterone in men following the Boston Marathon race (26 miles 385 yards) showed a three-fold increase in plasma cortisol concentrations and a six-fold increase in plasma aldosterone concentrations (Newmark et al., 1976). An elevation of serum potassium concentrations has been observed after the Boston Marathon, which could have caused the increase in plasma aldosterone concentrations (Rose et al., 1970). Newmark et al., (1976) suggested that the elevations were the result of multiple factors stimulating aldosterone secretion and a submaximal stimulation of ACTH secretion.

A significant elevation of plasma cortisol concentrations was reported in veteran male athletes after a 1500-meter and a 5000-meter race (Sutton and Casey, 1975). Cortisol concentrations were higher following the 5000-meter race than after the 1500-meter race. No significant correlations were observed between age and basal plasma cortisol concentrations or age and plasma cortisol concentrations following the 5000 meter race. It was suggested that the elevation of
plasma cortisol concentrations was a combination of physical and psychological stimuli acting on the pituitary via the cerebral cortex and hypothalamus resulting in ACTH and cortisol secretion.

Resting plasma cortisol concentrations have been reported to be similar in racing cyclists and nontrained controls (Johnson et al., 1974). Plasma cortisol concentrations were found to be higher in cyclists than controls immediately after subjecting individuals to work loads up to 75 per cent of their maximum work capacity. However, five minutes after the cessation of exercise, plasma cortisol concentrations were similar in cyclists and controls. The elevation of plasma cortisol concentrations in young men has been reported to occur only at heavy work loads (75 per cent of the maximum work capacity). Resting values were restored within 60 minutes after exercise (Hartley et al., 1972a). A seven-week training program resulted in a 14 per cent increase in the maximal work capacity, but plasma cortisol concentrations were unaffected by physical-training (Hartley et al., 1972b). These changes were suggested to produce a sparing effect on muscle glycogen during exercise and recovery.

Frenkl et al. (1969) reported a lower elevation in plasma cortisol concentrations in water polo players following a 60-minute exercise than in nontrained controls. In the trained individuals, the plasma cortisol concentration returned to normal by three hours after cessation of the exercise, but that of nontrained individuals was twice normal levels at this time. The results indicated that the difference in the stress reaction between trained and untrained individuals was due to a smaller and less sustained rise in ACTH secretion in trained subjects. The disappearance rate of prednisolone, a synthetic glucocorticoid, was
elevated in trained individuals (Frenkl et al., 1970). The reduction of circulating lymphocytes was more rapid in trained individuals, but by four hours after prednisolone administration, the lymphocyte count was the same in trained and untrained subjects. This led the authors to suggest that a higher rate of glucocorticoid elimination may not have meant a reduction in effect, and that tissue sensitivity to the glucocorticoid also had to be considered. Subsequent data also indicated a change in the cortisol turnover rate or utilization during exercise (Raymond et al., 1972). This investigation reported a decline in plasma cortisol concentrations after five and 20 minutes of walking on a treadmill at a speed of three miles per hours of 16 and 32 per cent, respectively. The lowered cortisol concentration was maintained for 30 minutes following the 30-minute walk. Urinary 11-hydroxycorticosteroid excretion has been reported to be twice that of resting rates after 30 minutes of moderately severe exercise (Bellet et al., 1969).

Elevation of plasma cortisol concentrations during exercise was delayed until energy expenditure exceeded 60 per cent of the individual's maximum work capacity (Davies and Few, 1973; 1974). In light exercise, the plasma cortisol concentration decreased by 40 per cent and remained at this level during a 60-minute recovery period. The plasma cortisol concentration was increased by 70 percent in heavy exercise and was elevated further by the tenth minute of recovery, so that it was twice resting concentrations. The concentration declined steadily after the tenth minute of recovery. Davies and Few concluded that a critical level of exercise and body temperature had to be reached before plasma cortisol concentrations would be elevated. These critical values were
estimated as 60 per cent of the individual's maximum oxygen uptake and a body temperature of 37.2 °C. The rate of removal of $^3$H-cortisol from the plasma also indicated that the elevation of plasma cortisol observed during exercise was due to an increased secretion of cortisol and not a decreased rate of cortisol removal from the plasma. Disappearance of the tritium label during exercise was found to be within the normal range for humans at rest (Davies and Few, 1973), but the half-life of cortisol was found to increase four-fold during recovery (Davies and Few, 1973). Few (1974) surmised that exercise increased the rate of cortisol uptake by peripheral tissues and that when the work load exceeded a critical level, stimulation of the adrenal cortex resulted in a massive secretion of cortisol. The secretion of cortisol elevated plasma concentrations which, in turn, promoted cortisol entry into tissues. After exercise, cortisol returned from the tissues into the plasma. Elevated cortisol entry into interstitial and intracellular fluids during light exercise also has been suggested by Cornil et al. (1965).

Animal studies

A prominent effect observed during the chronic exercise of animals was an increase in adrenal gland weight with physical-training. This effect has been observed in animals trained in swimming (Frenkl and Csalay, 1962; Craig, 1972; Pritchett, 1973; Frenkl et al., 1975) and running (Dieter, 1969; Ring et al., 1970; Buuck and Tharp, 1971; Song et al., 1973; Tharp and Buuck, 1974). Rats trained by daily swimming were reported to have significant adrenal gland enlargement after three weeks and the enlargement was more pronounced after six weeks (Frenkl
and Csalay, 1962). Rats trained by treadmill running demonstrated adrenal gland enlargement after four weeks (Tharp and Buuck, 1974). Mature, exercise-trained rats had significantly larger adrenal cortices and medullas than nontrained controls (Craig, 1972). Buuck and Tharp (1971) reported that adrenocortical enlargement was limited to the zona fasciculata, the primary adrenocortical zone for glucocorticoid synthesis and secretion. No significant changes were found in the width of the zona glomerulosa or zona reticularis.

Song et al. (1973) investigated changes within the adrenal gland resulting from exercise-induced enlargement. Exercise-training resulted in an increased total DNA and RNA content of the adrenal gland. Changes in nucleic acid content were due to the increase in total adrenal gland size, because the amount of DNA and RNA per gram of gland was similar to that of the nontrained rats. The increase in adrenal gland weight appeared to result from hypertrophy and hyperplasia.

In nontrained, adult male rats, acute bouts of running or swimming resulted in a doubling of plasma corticosterone concentrations (Dieter et al., 1972; Poland et al., 1975). Light exercise did not change the basal cortisol secretion rate in untrained dogs, as measured in adrenal venous effluent blood (Suzuki et al., 1967). However, moderate non-exhaustive running caused a brief increase in the secretion rate of cortisol after the cessation of exercise. Complete exhaustion resulted in a five-fold increase in the cortisol secretion rate.

Resting plasma corticosterone concentrations were similar in rats exercise-trained by swimming (Frenkl and Csalay, 1970; Frenkl, 1971; Craig, 1972; Craig and Griffith, 1976) and running (Buuck and Tharp,
1971). Variations to this general response pattern have been reported. A twelve-week swim-training regimen resulted in elevated resting plasma corticosterone concentrations in rats after three weeks of training, followed by a progressive decline in resting corticosterone concentrations until the twelfth week (Frenkl et al., 1975). Resting plasma corticosterone concentrations were less than that of nontrained control rats at weeks six and twelve. Resting plasma corticosterone concentrations of rats trained by daily exhaustive swimming for six weeks were significantly less than that of nontrained controls and rats trained by moderate swimming (Chin and Evonuk, 1971). These two reports suggested that the decline in plasma concentrations resulted from exhaustion of the adrenal cortex as a result of continued stimulation by ACTH. Resting plasma corticosterone concentrations of rats trained by treadmill-running were elevated above control values until six weeks of training had elapsed (Buuck and Tharp, 1971). After six weeks, resting plasma corticosterone concentrations were similar. The elevation of resting plasma corticosterone concentrations prior to six weeks of training was suggested to be the result of one or a combination of the following:

1. Inability of liver enzymes to convert circulating corticosterone to an inactive form;

2. Inability of body cells to remove corticosterone from the blood, and thereby allowing it to accumulate in the plasma;

3. Continued adrenocortical secretion of corticosterone at rest to replenish liver and glycogen stores during rest periods.
In general, exercise-trained rats responded to an acute exercise with less of an elevation in plasma corticosterone concentrations than that observed in nontrained controls (Frenkl et al., 1968; Dieter, 1969; Frenkl et al., 1969; Frenkl, 1971; Frenkl et al., 1975). The elevation reported to occur in circulating hormone concentrations in nontrained rats was generally about twice that of trained rats. The elevated plasma corticosterone concentrations in trained rats have been reported to return to resting levels within 90 minutes following exercise (Frenkl et al., 1969). However, nontrained levels were twice resting concentrations three hours after an acute swim. The response of the exercise-trained rat was less marked and of shorter duration. Circulating lymphocyte counts also were reduced more in nontrained rats than in trained rats in response to an acute swim (Frenkl et al., 1968). However, another study reported that eosinophil depression in trained rats was dependent upon the duration of the acute swimming exercise (Keeney, 1959).

Exceptions have been reported to the generalization that exercise-trained rats respond to an acute exercise with less of an elevation of plasma corticosterone concentrations than that of nontrained controls. Following an acute swim, plasma corticosterone concentrations were similar in rats swim-trained for three and six weeks and rats swum for the first time (Frenkl and Csalay, 1970). An acute bout of swimming elevated plasma corticosterone concentrations above control values after three weeks of chronic swim-training (Frenkl et al., 1975). The response to acute swimming in rats trained six and twelve weeks was less than that of nontrained controls. Following an exhaustive treadmill run, plasma
corticosterone concentrations were similar in nontrained controls and rats trained by eight weeks of daily treadmill running (Buuck and Tharp, 1971). Trained rats were able to run almost seven times longer than nontrained controls. The maximum secretory capacity of the adrenal cortex was not changed with training, but the trained rat could run longer. Adrenalectomy abolished the effect that training had on work ability and myocardial adaptation to exertion. This suggested that the effect of training was partly mediated through improved adrenocortical function (Korge and Roosson, 1975).

Conflicting results have also been reported concerning in vitro adrenocortical responsiveness to ACTH with exercise-training. Adrenal glands were more responsive to ACTH stimulation in rats trained by daily swimming for six (Frenkl et al., 1975) and ten weeks (Pritchett, 1973). Three weeks of swim-training resulted in an increased adrenocortical responsiveness to ACTH in vitro (Frenkl and Csalay, 1962) and an elevated adrenal gland concentration of ATP (Frenkl and Csalay, 1970). After six weeks of training, the in vitro response of adrenal glands of exercise-trained rats to ACTH was less than that of controls, while adrenal gland ATP concentrations were similar to that of control rats (Frenkl and Csalay, 1962; 1970). Adrenal glands from exercise-trained rats became decreasingly responsive to ACTH in vitro during an eight week training program by treadmill running and were significantly less responsive than adrenal glands from nontrained control rats at six and eight weeks (Tharp and Buuck, 1974). Adrenal glands from trained rats were not exhausted, which appeared to shift the cause of decreased plasma corticosterone response to intra-adrenal changes and
alterations in the hypothalamus-pituitary-adrenal system.

An extensive amount of data has suggested that exercise-trained animals secrete less ACTH during acute exercise than nontrained controls. This would account for plasma corticosterone concentrations being higher in nontrained controls than in trained animals in response to acute exercise. In guinea pigs, three five-minute sets of swimming resulted in an increased total corticosteroid and cortisol content of the adrenal glands (Viru and Akke, 1969). Repeated five-minute swim sets, until the guinea pigs were exhausted, resulted in a depression of plasma cortisol concentrations to half of control values. Plasma and adrenal gland cortisol concentrations were greater in guinea pigs injected with ACTH prior to exhaustive swimming. This suggested that the adrenal glands were not exhausted, and that ACTH secretion was decreased as the guinea pigs neared exhaustion.

Data obtained from rats also have contradicted the idea of adrenal gland exhaustion in exercise-trained rats, and have suggested a decrease in endogenous secretion of ACTH (Frenkl et al., 1975). The half-life of plasma corticosterone was decreased by exercise-training (Frenkl et al., 1969). Elimination of exogenously administered prednisolone was elevated at rest and after acute exercise in exercise-trained rats (Frenkl and Csalay, 1970). The apparent cause of this response was an enhanced rate of steroid turnover by tissues, or increased tissue binding with exercise-training, or both. In conjunction with these data, Frenkl et al. (1969) suggested that the exercise-trained rat responded to acute exercise with a smaller and less sustained rise in ACTH secretion. Following an exhaustive swim, elevated plasma corticosterone
concentrations in nontrained control rats were twice that of exercise-trained rats (Frenkl et al., 1968). However, ACTH injection prior to exhaustive swimming resulted in similar elevations of plasma corticosterone concentrations and similar depressions of circulating lymphocyte numbers.

Surgical stress and histamine injection resulted in data similar to that elicited by acute swimming: exercise-trained rats responded with less of an increase in plasma corticosterone concentrations than nontrained controls (Frenkl, 1971). However, the response to ether stress, epinephrine injection or Salmonella endotoxin injection was similar in trained and nontrained rats. Frenkl speculated that stressors that utilized the same hypothalamic-pituitary pathways as those activated by muscular effort were less effective in causing elevations in plasma corticosterone concentrations in exercise-trained rats. He termed it a stimulus specificity in the endocrine response. The pituitary-adrenal system of the well-trained and well-adapted organisms acquired the ability to control steroid turnover according to the existing needs of the body, and, within limits, independent of the actual functional state of the adrenal cortex (Frenkl and Csalay, 1970).

The data presented seem to indicate that the adaptation of adrenocortical function to regular muscular exertion is the result of a complex adjustment at several levels: ACTH is not mobilized readily, consequently the adrenocortical response is diminished and the rate of removal of glucocorticoids is increased.
MATERIALS AND METHODS

Male Sprague-Dawley rats were maintained at 25 ± 3 °C in a room with a controlled light-dark cycle (14 hours light, 10 hours dark). Tap water and Teklad rat chow were provided ad libitum.

A 3x2x2 factorial experimental design was employed. Rats were 100, 200 and 300 days old at the beginning of the experimental period. Within each age group, rats were divided into exercise-trained (Ex) and nonexercised control (C) groups. Ex rats were run on a motor-driven treadmill (Jette et al., 1969; Auth, 1975) according to the following conditions:

1. The treadmill was set at an eight degree incline;
2. Belt speed was 15 meters per minute;
3. Rats were run 30 minutes per day and 5 days per week;
4. Length of the exercise-training period was 10 weeks.

At the end of the training-program, rats within each treatment were further divided into swim (S) and nonswim (NS) groups. Ex-S and C-S rats were subjected to an acute swim in 35 ± 2 °C water in 25 gallon plastic barrels with lead weights amounting to four per cent of body weight attached to the tail of each rat. Rats were individually swum at a water depth of about 20 inches. Rats were removed from the water when they could not surface for a 10-second period (Dawson and Horvath, 1970). Within one minute of removal from the water, rats were anesthetized with an intraperitoneal injection of sodium pentobarbitol (Nembutol, Abbot Laboratories, 25 mg/kg). NS rats also were anesthetized with sodium pentobarbitol.
Blood was removed from the jugular vein using a heparinized, plastic syringe following the induction of anesthesia. The blood was transferred to a heparinized, glass centrifuge tube and centrifuged for 10 to 15 minutes. The plasma was removed and stored at -20 °C until analyzed.

Adrenal glands were removed as soon after death as possible, dissected free of visible fat, weighed and divided into quarters. By random determination, one gland from each rat was incubated in 2 ml Krebs-Ringer bicarbonate medium containing 2 mg glucose per ml (Appendix A); this gland served as a control to represent the in vivo adrenal gland corticosterone secretion rate at the time of sacrifice (Saffran et al., 1952; van der Vies, 1960). This was designated as the unstimulated adrenocortical secretion rate. The other gland from each pair was incubated in 2 ml Krebs-Ringer bicarbonate medium containing 2 mg glucose per ml and 250 millinuits (mU) ACTH per ml. This dose of ACTH was sufficient to cause maximal corticosterone secretion by the gland. This was designated as the maximal adrenocortical secretion rate. All incubations were conducted with shaking for 60 minutes in a 95% O₂-5% CO₂ atmosphere at 37 °C. Following incubation, the medium was stored at -20 °C until analyzed.

All rats were autopsied after adrenal gland incubation was begun. Rats that exhibited gross pathological lesions (i.e. otitis media, respiratory infection or tumors) were not considered to be physiologically normal and were excluded from all analyses. Therefore, data were not biased by the inclusion of rats that might have exhibited an altered stress response.
Total glucocorticoid content of plasma and incubation medium was determined by competitive protein-binding radioassay (Murphy, 1967) utilizing the modifications of Cameron and Scarisbrick (1973) and Topel (personal communication; Appendix B). Corticosterone (Sigma Chemical Co.) was used as a standard in all assays.

Data were analyzed in consultation with the Iowa State University Statistical Laboratory using the Statistical Analysis System (Barr and Goodnight, 1971). The MEANS procedure was used to calculate means of all variables within each experimental group. The ANOVA procedure was used to calculate the analysis of variance for unequal treatment cell numbers. The residual mean square obtained from this procedure was used as the best estimate of variance (Snedecor and Cochran, 1967). Comparisons were made using Student's t-test for unequal cell numbers.
RESULTS

For the convenience of the reader, this section has been divided into the following subsections: final body weight, paired adrenal gland weight, plasma corticosterone concentration, absolute in vitro corticosterone secretion rate and relative in vitro corticosterone secretion rate. Each variable will be referred to in its own subsection by an abbreviation, which will be used only in that subsection. In addition, the treatment groups will be referred to as C for control rats and Ex for exercise-trained rats. Nonswim and swim groups will be designated as NS and S, respectively. Results will be discussed in the following order in each subsection: control nonswim (C-NS) versus control swim (C-S), exercise-trained nonswim (Ex-NS) versus exercise-trained swim (Ex-S), C-NS versus Ex-NS and C-S versus Ex-S.

Final Body Weight

Means, standard errors and the residual mean square for final body weight (FBW) are presented in Table 1. Statistical comparisons were made using the residual mean square as the best estimate of variance and are presented in Table 3.

The FBW of C-NS rats at 170 days-of-age was significantly greater than that of C-S rats (p<0.001). A highly significant increase in FBW was observed between 170 and 270 days in C-NS and C-S groups (p<0.001 for both groups). At 270 days-of-age, the FBW of C-NS rats was less than that of C-S rats (p<0.001). Between 270 and 370 days, the FBW was unchanged in C-NS rats (p>0.05), but that of C-S rats decreased (p<0.001). The FBW of C-NS and C-S rats was not significantly different at 370 days.
Ex-NS rats at 170 days-of-age were heavier than Ex-S rats of the same age (p<0.001). In both groups, the FBW increased between 170 and 270 days (p<0.001 for both groups), and at 270 days-of-age, the FBW of Ex-NS and Ex-S rats was similar (p>0.05). The FBW was unchanged between 270 and 370 days in Ex-NS and Ex-S rats (p>0.05 for both groups), but at 370 days-of-age the FBW of Ex-NS rats was less than that of Ex-S rats (p<0.01).

The FBW of C-NS rats at all three ages was significantly greater than that of Ex-NS rats of the same age (p<0.001 at all three ages). Similar results were found for C-S and Ex-S rats at 270 and 370 days-of-age (p<0.001 and p<0.05, respectively). At 170 days-of-age, the FBW of C-S and Ex-S rats was similar (p>0.05).

Rats were chosen for the NS or S groups at the completion of the 10-week training program, and were not weight-matched. For this reason, the obvious differences in FBW were observed. The body weights of NS and S groups were pooled to attempt to eliminate the biases that the selection of rats for NS and S groups may have given to the data. Means and standard errors for the pooled FBW are presented in Table 1. Statistical comparisons for the pooled FBW are presented in Table 4.

At all three ages, C rats were significantly heavier than Ex rats of the same age (p<0.001 at all three ages). A highly significant increase in FBW was observed between 170 and 270 days-of-age in C and Ex rats (p<0.001 for both groups). The FBW of C rats decreased between 270 and 370 days (p<0.001), while that of Ex rats was unchanged during this time period (p>0.05). Cursory examination of body weights suggested
Table 1. Means and standard errors for final body weight.

<table>
<thead>
<tr>
<th></th>
<th>Nonswim (NS) (g)</th>
<th>Swim (S) (g)</th>
<th>NS and S combined (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>444 ± 8a (7)b</td>
<td>385 ± 13 (10)</td>
<td>409 ± 11 (17)</td>
</tr>
<tr>
<td>270 days</td>
<td>486 ± 11 (6)</td>
<td>520 ± 11 (9)</td>
<td>506 ± 9 (15)</td>
</tr>
<tr>
<td>370 days</td>
<td>478 ± 14 (9)</td>
<td>468 ± 12 (13)</td>
<td>472 ± 9 (22)</td>
</tr>
<tr>
<td>Exercise (Ex)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>407 ± 10 (13)</td>
<td>378 ± 5 (16)</td>
<td>391 ± 6 (29)</td>
</tr>
<tr>
<td>270 days</td>
<td>449 ± 8 (15)</td>
<td>454 ± 7 (17)</td>
<td>452 ± 5 (32)</td>
</tr>
<tr>
<td>370 days</td>
<td>441 ± 11 (9)</td>
<td>457 ± 10 (12)</td>
<td>450 ± 7 (21)</td>
</tr>
</tbody>
</table>

Residual mean square (s^2) = 152

a Mean ± standard error of the mean.
b Number of rats per treatment group.

that exercise-training resulted in a depression of body weights and that normal growth occurred between 170 and 270 days-of-age.

Exercise-training resulted in a depression of FBW.

Paired Adrenal Gland Weight

Means, standard errors and the residual mean square for paired adrenal gland weight (PAG) are presented in Table 2. Statistical comparisons for PAG were made using the residual mean square as the best estimate of variance and are presented in Table 3.

The PAG of C-NS and C-S rats was similar at 170 days-of-age (p>0.05). Between 170 and 270 days, the PAG of C-NS and C-S rats increased (p<0.001 for both groups), but at 270 days, the PAG of C-NS rats was greater than that of C-S rats (p<0.01). The PAG decreased
Table 2. Means and standard errors for paired adrenal gland weight.

<table>
<thead>
<tr>
<th></th>
<th>Nonswim (NS) (mg)</th>
<th>Swim (S) (mg)</th>
<th>NS and S combined (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control (C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>47.3 ± 4.4a (7)b</td>
<td>48.1 ± 2.4 (10)</td>
<td>47.8 ± 2.2 (17)</td>
</tr>
<tr>
<td>270 days</td>
<td>59.3 ± 3.5 (6)</td>
<td>54.0 ± 2.1 (9)</td>
<td>56.1 ± 1.9 (15)</td>
</tr>
<tr>
<td>370 days</td>
<td>53.3 ± 3.6 (9)</td>
<td>50.6 ± 1.8 (13)</td>
<td>51.7 ± 1.8 (22)</td>
</tr>
<tr>
<td><strong>Exercise (Ex)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>64.0 ± 1.4 (13)</td>
<td>61.6 ± 1.8 (16)</td>
<td>62.7 ± 1.2 (29)</td>
</tr>
<tr>
<td>270 days</td>
<td>63.9 ± 1.7 (15)</td>
<td>67.2 ± 1.7 (17)</td>
<td>65.6 ± 1.2 (32)</td>
</tr>
<tr>
<td>370 days</td>
<td>60.9 ± 3.8 (9)</td>
<td>66.0 ± 3.0 (12)</td>
<td>63.8 ± 2.4 (21)</td>
</tr>
</tbody>
</table>

Residual mean square (s^2) = 6.2

^Mean ± standard error of the mean.

*bNumber of rats per treatment group.

between 270 and 370 days in C-NS and C-S rats (p<0.001 and p<0.01, respectively). However, the PAG of C-NS rats was still greater than that of C-S rats at 370 days-of-age (p<0.05).

Ex-NS rats at 170 days-of-age had a greater PAG than that of Ex-S rats (p<0.05). The PAG was unchanged between 170 and 270 days in Ex-NS rats (p>0.05), while that of Ex-S rats increased (p<0.001). At 270 days-of-age, the PAG of Ex-NS rats was less than that of Ex-S rats (p<0.001). The PAG of Ex-NS rats decreased between 270 and 370 days (p<0.01), but no change occurred in the PAG of Ex-S rats (p>0.05). At 370 days, the PAG of Ex-NS rats was greater than that of Ex-S rats (p<0.001).

The PAG of C-NS rats was significantly less than that of Ex-NS rats at 170, 270 and 370 days-of-age (p<0.001, p<0.01 and p<0.001,
Table 3. Statistical comparisons made using Student's t-test for final body weight and paired adrenal gland weight without combining nonswim and swim values (ns=not significant at p<0.05, *=p<0.05, **=p<0.01, ***=p<0.001).

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Final body weight</th>
<th>Paired adrenal gland weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>170 C-NS and 170 C-S</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>270 C-NS and 270 C-S</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>370 C-NS and 370 C-S</td>
<td>ns</td>
<td>*</td>
</tr>
<tr>
<td>170 Ex-NS and 170 Ex-S</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>270 Ex-NS and 270 Ex-S</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>370 Ex-NS and 370 Ex-S</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>170 C-NS and 170 Ex-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 C-S and 170 Ex-S</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>270 C-NS and 270 Ex-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>270 C-S and 270 Ex-S</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>370 C-NS and 370 Ex-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>370 C-S and 370 Ex-S</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>170 C-NS and 270 C-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 C-NS and 370 C-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>270 C-NS and 370-C-NS</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>170 C-S and 270 C-S</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 C-S and 370 C-S</td>
<td>***</td>
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</tr>
<tr>
<td>270 C-S and 370 C-S</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 Ex-NS and 270 Ex-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 Ex-NS and 370 Ex-NS</td>
<td>***</td>
<td>**</td>
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<tr>
<td>270 Ex-NS and 370 Ex-NS</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>170 Ex-S and 270 Ex-S</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 Ex-S and 370 Ex-S</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>270 Ex-S and 370 Ex-S</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

respectively). Similar results were obtained for the comparison of C-S and Ex-S rats (p<0.001 for all three ages).

Like the final body weight, the PAG of NS and S groups was pooled, because rats were chosen for these groups at the end of the experimental
period. Means and standard errors for the pooled PAG are presented in Table 2. Statistical comparisons for PAG are presented in Table 4.

Table 4. Statistical comparisons made using Student's t-test for final body weight and paired adrenal gland weight with nonswim and swim values combined (ns=not significant at $p<0.05$, *=p<0.05, ***=p<0.001).

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Final body weight</th>
<th>Paired adrenal gland weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>170 C and 170 Ex</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>270 C and 270 Ex</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>370 C and 370 Ex</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 C and 270 C</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 C and 370 C</td>
<td>***</td>
<td>***</td>
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<tr>
<td>270 C and 370 C</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 Ex and 270 Ex</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 Ex and 370 Ex</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>270 Ex and 370 Ex</td>
<td>ns</td>
<td>*</td>
</tr>
</tbody>
</table>

At all three ages, the PAG of C rats was significantly less than that of Ex rats ($p<0.001$). The PAG of C and Ex rats at 270 days was significantly greater than that of 170 and 370 day-old rats ($p<0.001$ for all comparisons).

Exercise-training resulted in an elevation of the paired adrenal gland weight.

**Plasma Corticosterone Concentration**

Means, standard errors and the residual mean square for plasma corticosterone concentration (PCC) are presented in Table 5. Statistical comparisons for PCC were made using the residual mean square as the best estimate of variance and are presented in Table 6.
Table 5. Means and standard errors for plasma corticosterone concentration.

<table>
<thead>
<tr>
<th></th>
<th>Nonswim (NS) (ug·100 ml plasma⁻¹)</th>
<th>Swim (S) (ug·100 ml plasma⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>29.9 ± 2.3⁹ (7)</td>
<td>36.3 ± 2.6 (10)</td>
</tr>
<tr>
<td>270 days</td>
<td>32.5 ± 2.0 (5)</td>
<td>41.8 ± 2.0 (9)</td>
</tr>
<tr>
<td>370 days</td>
<td>39.3 ± 2.7 (8)</td>
<td>44.0 ± 1.5 (13)</td>
</tr>
<tr>
<td>Exercise (Ex)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>30.8 ± 1.2 (13)</td>
<td>39.3 ± 1.5 (16)</td>
</tr>
<tr>
<td>270 days</td>
<td>30.5 ± 2.0 (15)</td>
<td>38.0 ± 1.4 (17)</td>
</tr>
<tr>
<td>370 days</td>
<td>36.3 ± 2.6 (9)</td>
<td>40.8 ± 2.5 (12)</td>
</tr>
</tbody>
</table>

Residual mean square (s²) = 6.7

aMean ± standard error of the mean.
bNumber of rats per treatment group.

Comparison of the PCC of C-NS and C-S rats demonstrated that acute swimming resulted in an elevation of PCC at all three ages (p<0.001 at all three ages). The PCC of C-NS rats increased between 170 and 270 days and between 270 and 370 days (p<0.05 and p<0.001, respectively). The PCC of C-S rats increased between 170 and 270 days (p<0.001), but was unchanged between 270 and 370 days-of-age (p>0.05).

The PCC of Ex rats also was significantly elevated by acute swimming (p<0.001 at all three ages). The PCC of Ex-NS rats at 170 and 270 days-of-age was similar (p>0.05), but the PCC of Ex-NS at 370 days was greater than at 270 days (p<0.001). At all three ages, the PCC of Ex-S rats was similar (p>0.05) for all comparisons.
Table 6. Statistical comparisons made using Student's t-test for plasma corticosterone concentration (ns=not significant at p<0.05, *=p<0.05, **=p<0.01, ***=p<0.001).

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Plasma corticosterone concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>170 C-NS and 170 C-S</td>
<td>***</td>
</tr>
<tr>
<td>270 C-NS and 270 C-S</td>
<td>***</td>
</tr>
<tr>
<td>370 C-NS and 370 C-S</td>
<td>***</td>
</tr>
<tr>
<td>170 Ex-NS and 170 Ex-S</td>
<td>***</td>
</tr>
<tr>
<td>270 Ex-NS and 270 Ex-S</td>
<td>***</td>
</tr>
<tr>
<td>370 Ex-NS and 370 Ex-S</td>
<td>***</td>
</tr>
<tr>
<td>170 C-NS and 170 Ex-NS</td>
<td>ns</td>
</tr>
<tr>
<td>170 C-S and 170 Ex-S</td>
<td>*</td>
</tr>
<tr>
<td>270 C-NS and 270 Ex-NS</td>
<td>ns</td>
</tr>
<tr>
<td>270 C-S and 270 Ex-S</td>
<td>**</td>
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<tr>
<td>370 C-NS and 370 Ex-NS</td>
<td>ns</td>
</tr>
<tr>
<td>370 C-S and 370 Ex-S</td>
<td>**</td>
</tr>
<tr>
<td>170 C-NS and 270 C-NS</td>
<td>*</td>
</tr>
<tr>
<td>170 C-NS and 370 C-NS</td>
<td>***</td>
</tr>
<tr>
<td>270 C-NS and 370 C-NS</td>
<td>***</td>
</tr>
<tr>
<td>170 C-S and 270 C-S</td>
<td>***</td>
</tr>
<tr>
<td>170 C-S and 370 C-S</td>
<td>***</td>
</tr>
<tr>
<td>270 C-S and 370 C-S</td>
<td>ns</td>
</tr>
<tr>
<td>170 Ex-NS and 270 Ex-NS</td>
<td>ns</td>
</tr>
<tr>
<td>170 Ex-NS and 370 Ex-NS</td>
<td>***</td>
</tr>
<tr>
<td>270 Ex-NS and 370 Ex-NS</td>
<td>***</td>
</tr>
<tr>
<td>170 Ex-S and 270 Ex-S</td>
<td>ns</td>
</tr>
<tr>
<td>170 Ex-S and 370 Ex-S</td>
<td>ns</td>
</tr>
<tr>
<td>270 Ex-S and 370 Ex-S</td>
<td>ns</td>
</tr>
</tbody>
</table>

There was no significant difference between the PCC of C-NS and Ex-NS rats at all three ages (p>0.05 at all three ages).

The PCC of C-S rats was less than that of Ex-S rats at 170 days (p<0.05). At 270 and 370 days-of-age, the PCC of C-S rats was greater.
than that of Ex-S rats (p<0.01 at both ages).

The resting PCC was similar at all ages and acute swimming resulted in an age-dependent elevation of the PCC of Ex and C rats.

Absolute In Vitro Corticosterone Secretion Rate

Absolute in vitro adrenal gland corticosterone secretion rates were expressed as ug·hr$^{-1}$ and represent a measure of the total corticosterone secretion of the gland irrespective of adrenal gland weight. Means, standard errors and the residual mean square for the absolute secretion rates are presented in Table 7. Statistical comparisons for this variable were made using the residual mean square as the best estimate of variance and are presented in Table 9.

Unstimulated absolute in vitro corticosterone secretion rate

The unstimulated absolute in vitro corticosterone secretion rate (UASR) of C-NS rats at 170 days-of-age was significantly less than that of C-S rats (p<0.001). The UASR of C-NS rats increased between 170 and 270 days (p<0.001), while that of C-S rats decreased (p<0.01), so that at 270 days-of-age, the UASR of C-NS rats was greater than that of C-S rats (p<0.001). Between 270 and 370 days-of-age, the UASR of C-NS and C-S rats declined (p<0.001 and p<0.05, respectively) and the UASR of both groups was similar at 370 days (p>0.05).

The UASR of Ex-NS rats at 170 days-of-age was greater than that of Ex-S rats (p<0.001). The UASR of Ex-NS rats at 270 days was less than that of Ex-NS rats at 170 days (p<0.001). No change in the UASR of Ex-S rats was observed between 170 and 270 days (p>0.05), so that the
Table 7. Means and standard errors for absolute in vitro adrenal gland corticosterone secretion rate.

<table>
<thead>
<tr>
<th></th>
<th>N(^a)</th>
<th>Unstimulated rate (ug·hr(^{-1}))</th>
<th>Maximal rate (ug·hr(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control (C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonswim (NS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>6</td>
<td>2.09 ± 0.39(^b)</td>
<td>2.75 ± 0.52 **</td>
</tr>
<tr>
<td>270 days</td>
<td>6</td>
<td>4.06 ± 0.38</td>
<td>4.78 ± 0.42 **</td>
</tr>
<tr>
<td>370 days</td>
<td>9</td>
<td>2.84 ± 0.23</td>
<td>3.70 ± 0.34 ***</td>
</tr>
<tr>
<td>Swim (S)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>10</td>
<td>3.49 ± 0.17</td>
<td>4.85 ± 0.44 ***</td>
</tr>
<tr>
<td>270 days</td>
<td>9</td>
<td>3.11 ± 0.20</td>
<td>4.47 ± 0.21 ***</td>
</tr>
<tr>
<td>370 days</td>
<td>13</td>
<td>2.85 ± 0.18</td>
<td>4.13 ± 0.41 ***</td>
</tr>
<tr>
<td><strong>Exercise (Ex)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonswim (NS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>13</td>
<td>4.08 ± 0.29</td>
<td>6.22 ± 0.49 ***</td>
</tr>
<tr>
<td>270 days</td>
<td>15</td>
<td>2.83 ± 0.18</td>
<td>3.85 ± 0.16 ***</td>
</tr>
<tr>
<td>370 days</td>
<td>9</td>
<td>2.84 ± 0.27</td>
<td>3.71 ± 0.30 ***</td>
</tr>
<tr>
<td>Swim (S)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days</td>
<td>16</td>
<td>2.93 ± 0.29</td>
<td>4.68 ± 0.36 ***</td>
</tr>
<tr>
<td>270 days</td>
<td>17</td>
<td>2.79 ± 0.17</td>
<td>4.46 ± 0.29 ***</td>
</tr>
<tr>
<td>370 days</td>
<td>12</td>
<td>3.30 ± 0.24</td>
<td>4.58 ± 0.33 ***</td>
</tr>
<tr>
<td>Residual mean square (s(^2))</td>
<td>0.067</td>
<td>0.165</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Number of rats per treatment group.

\(^b\)Mean ± standard error of the mean.

**Significantly different from unstimulated rate at p<0.01.

***Significantly different from unstimulated rate at p<0.001.

UASR of Ex-NS and Ex-S rats was similar at 270 days-of-age (p>0.05).
The UASR of 270 and 370 day-old Ex-NS rats was similar (p>0.05), but the UASR of 370 day-old Ex-S rats was greater than that of 270 day-old Ex-S rats (p<0.001). At 370 days-of-age, the UASR of Ex-NS rats was
less than that of Ex-S rats (p<0.001).

At 170 days-of-age, the UASR of C-NS rats was less than that of Ex-NS rats (p<0.001). The UASR of C-NS rats was greater than that of Ex-NS rats at 270 days (p<0.001). The UASR of C-NS and Ex-NS rats was similar at 370 days-of-age (p>0.05).

C-S rats had a greater UASR than that of Ex-S rats at 170 and 270 days-of-age (p<0.001). However, at 370 days, the UASR of C-S rats was less than that of Ex-S rats (p<0.001).

Maximal absolute in vitro corticosterone secretion rate

The maximal absolute in vitro corticosterone secretion rate (MASR) of C-NS rats at 170 days-of-age was less than that of C-S rats (p<0.001). The MASR of C-NS rats increased between 170 and 270 days (p<0.001), but the MASR of C-S rats was similar at these two ages (p>0.05). At 270 days-of-age, the MASR was similar in C-NS and C-S rats (p>0.05).

Between 270 and 370 days, the MASR of C-NS rats decreased (p<0.001), while no change was observed in the MASR of C-S rats during this period (p>0.05). The MASR of C-NS rats was less than that of C-S rats at 370 days (p<0.05).

The MASR of Ex-NS rats was greater than that of Ex-S rats at 170 days-of-age (p<0.001). At 270 days, the MASR of Ex-NS rats was greater than at 170 days (p<0.001), while the MASR of Ex-S rats was similar at 170 and 270 days-of-age (p>0.05). The MASR of Ex-NS rats was less than that of Ex-S rats at 270 days-of-age (p<0.001). Between 270 and 370 days-of-age, no change was observed in the MASR of Ex-NS and Ex-S rats (p>0.05 for both comparisons). At 370 days, the MASR of Ex-NS rats was less than that of Ex-S rats (p<0.001).
The MASR of C-NS rats was less than that of Ex-NS rats at 170 days-of-age (p<0.001). At 270 days, the MASR of C-NS rats was greater than that of Ex-NS rats (p<0.001). The MASR of C-NS and Ex-NS rats was similar at 370 days-of-age (p>0.05).

At 170 and 270 days-of-age, the MASR of C-S rats was similar to that of Ex-S rats (p>0.05). However, at 370 days, the MASR of C-S rats was less than that of Ex-S rats (p<0.01).

Table 7 contains comparisons of UASR and MASR for each treatment group. These comparisons were made using Student's t-test for unequal variance and unequal cell numbers. For all comparisons, the UASR was significantly less than the MASR (p<0.01, at least, for all comparisons).

No obvious trend was detected in the absolute secretion rates.

Relative In Vitro Corticosterone Secretion Rate

Relative in vitro adrenal gland corticosterone secretion rates were expressed as ug·hr⁻¹·100 mg gland⁻¹. This accounted for the influence of glandular weight on the secretion of corticosterone. Means, standard errors and the residual mean square for this variable were made using the residual mean square as the best estimate and are presented in Table 8. Statistical comparisons for this variable were made using the residual mean square as the best estimate and are presented in Table 9.

Unstimulated relative in vitro corticosterone secretion rate

The unstimulated relative in vitro adrenal gland corticosterone secretion rate (URSR) of C-NS rats was less than that of C-S rats (p<0.001). However, at 270 days, the URSR of C-NS rats was greater than
Table 8. Means and standard errors for relative in vitro adrenal gland corticosterone secretion rate.

<table>
<thead>
<tr>
<th></th>
<th>N^a</th>
<th>Unstimulated rate</th>
<th>Maximal rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(ug·hr⁻¹·100 mg gland⁻¹)</td>
<td>(ug·hr⁻¹·100 mg gland⁻¹)</td>
</tr>
<tr>
<td>Control (C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonswim (NS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days 6</td>
<td>8.51 ± 1.32^b</td>
<td>12.11 ± 2.39 ***</td>
<td></td>
</tr>
<tr>
<td>270 days 6</td>
<td>14.82 ± 1.96</td>
<td>15.71 ± 1.38 ns</td>
<td></td>
</tr>
<tr>
<td>370 days 9</td>
<td>10.43 ± 1.36</td>
<td>13.97 ± 1.64 ***</td>
<td></td>
</tr>
<tr>
<td>Swim (S)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days 10</td>
<td>14.94 ± 1.53</td>
<td>21.61 ± 2.65 ***</td>
<td></td>
</tr>
<tr>
<td>270 days 9</td>
<td>11.89 ± 0.91</td>
<td>16.66 ± 1.16 ***</td>
<td></td>
</tr>
<tr>
<td>370 days 13</td>
<td>11.40 ± 0.71</td>
<td>16.64 ± 1.81 ***</td>
<td></td>
</tr>
<tr>
<td>Exercise (Ex)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Nonswim (NS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days 13</td>
<td>13.04 ± 1.03</td>
<td>19.68 ± 1.57 ***</td>
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<td>270 days 15</td>
<td>8.79 ± 0.41</td>
<td>12.18 ± 0.35 ***</td>
<td></td>
</tr>
<tr>
<td>370 days 9</td>
<td>9.17 ± 0.53</td>
<td>12.94 ± 1.35 ***</td>
<td></td>
</tr>
<tr>
<td>Swim (S)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170 days 16</td>
<td>9.63 ± 0.61</td>
<td>15.29 ± 1.16 ***</td>
<td></td>
</tr>
<tr>
<td>270 days 17</td>
<td>8.61 ± 0.51</td>
<td>13.06 ± 0.91 ***</td>
<td></td>
</tr>
<tr>
<td>370 days 12</td>
<td>10.05 ± 0.68</td>
<td>14.64 ± 1.29 ***</td>
<td></td>
</tr>
<tr>
<td>Residual mean square (s^2) =</td>
<td>0.941</td>
<td>1.896</td>
<td></td>
</tr>
</tbody>
</table>

^aNumber of rats per treatment group.

^bMean ± standard error of the mean.

ns = not significant at p<0.05.

***Significantly different from unstimulated rate at p<0.001.

that of C-S rats (p<0.001). Between 170 and 270 days-of-age, the URSR of C-NS rats increased (p<0.001) and that of C-S rats decreased (p<0.001) to cause this difference. Between 270 and 370 days, the URSR of C-NS rats decreased significantly (p<0.001), but that of C-S rats was unchanged...
Table 9. Statistical comparisons made using Student's t-test for absolute in vitro adrenal gland corticosterone secretion rate and relative in vitro adrenal gland corticosterone secretion rate (ns=not significant at p<0.05, *=p<0.05, **=p<0.01, ***=p<0.001).

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Absolute secretion rate</th>
<th>Relative secretion rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>unstimulated</td>
<td>maximal</td>
</tr>
<tr>
<td>170 C-NS and 170 C-S</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>270 C-NS and 270 C-S</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>370 C-NS and 370 C-S</td>
<td>ns</td>
<td>*</td>
</tr>
<tr>
<td>170 Ex-NS and 170 Ex-S</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>270 Ex-NS and 270 Ex-S</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>370 Ex-NS and 370 Ex-S</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 C-NS and 170 Ex-NS</td>
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<td>***</td>
</tr>
<tr>
<td>170 C-NS and 170 Ex-S</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>270 C-NS and 270 Ex-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>270 C-S and 270 Ex-S</td>
<td>**</td>
<td>ns</td>
</tr>
<tr>
<td>370 C-NS and 370 Ex-NS</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>370 C-S and 370 Ex-S</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>170 C-NS and 270 C-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 C-NS and 370 C-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>270 C-NS and 370 C-NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>170 C-S and 270 C-S</td>
<td>**</td>
<td>ns</td>
</tr>
<tr>
<td>170 C-S and 370 C-S</td>
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<td>***</td>
</tr>
<tr>
<td>270 C-S and 370 C-S</td>
<td>*</td>
<td>ns</td>
</tr>
<tr>
<td>170 Ex-NS and 270 Ex-NS</td>
<td>***</td>
<td>***</td>
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<tr>
<td>170 Ex-NS and 370 Ex-NS</td>
<td>***</td>
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<tr>
<td>270 Ex-NS and 370 Ex-NS</td>
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<td>170 Ex-S and 270 Ex-S</td>
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<td>ns</td>
</tr>
<tr>
<td>170 Ex-S and 370 Ex-S</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>270 Ex-S and 370 Ex-S</td>
<td>***</td>
<td>ns</td>
</tr>
</tbody>
</table>
p>0.05), so that at 370 days-of-age, there was no significant difference between these two groups.

The URSR of Ex-NS rats was greater than that of Ex-S rats at 170 days (p<0.001). The URSR of both groups decreased between 170 and 270 days (p<0.001 and p<0.05, respectively) and at 270 days were not significantly different from each other (p>0.05). No change occurred in the URSR of Ex-NS rats between 270 and 370 days-of-age (p>0.05), but that of Ex-S rats increased during this time (p<0.01). The URSR of Ex-NS and Ex-S rats was similar at 370 days-of-age (p>0.05).

The URSR of C-NS rats was less than that of Ex-NS rats at 170 days-of-age (p<0.001). At 270 and 370 days, the URSR of C-NS rats was greater than that of Ex-NS rats (p<0.001 at both ages).

At all three ages, the URSR of C-S rats was greater than that of Ex-S rats (p<0.001, p<0.001 and p<0.01, respectively).

Maximal relative in vitro corticosterone secretion rate

The maximal relative in vitro adrenal gland corticosterone secretion rate (MRSR) of C-NS rats at 170 days-of-age was less than that of C-S rats (p<0.001). The MRSR of C-NS rats increased between 170 and 270 days (p<0.001), while that of C-S rats decreased (p<0.01). No significant difference was observed in the MRSR at 270 days-of-age between C-NS and C-S rats (p>0.05). Between 270 and 370 days, the MRSR of C-NS rats decreased (p<0.01), while that of C-S rats was unchanged (p>0.05). At 370 days-of-age, the MRSR of C-NS rats was less than that of C-S rats (p<0.001).
The MRSR of Ex-NS rats at 170 days was greater than that of Ex-S rats (p<0.001). Between 170 and 270 days-of-age, the MRSR of both groups decreased (p<0.001 for both groups), and were not significantly different from each other at 270 days (p>0.05). The MRSR of Ex-NS rats was unchanged between 270 and 370 days-of-age (p>0.05), while during this time period the MRSR of Ex-S rats increased (p<0.05). At 370 days-of-age, the MRSR of Ex-NS rats was less than that of Ex-S rats (p<0.01).

The MRSR of C-NS rats was less than that of Ex-NS rats at 170 days-of-age (p<0.001). The MRSR of C-NS rats was greater than that of Ex-NS rats at 270 days (p<0.001). At 370 days-of-age, the MRSR of C-NS and Ex-NS rats was similar (p>0.05).

The MRSR of C-S rats was greater than that of Ex-S rats at 170, 270 and 370 days-of-age (p<0.001, p<0.001 and p<0.01, respectively).

Table 8 contains comparisons of URSR and MRSR for each treatment group. With only one exception, the URSR was less than the MRSR in all groups (p<0.001 for all comparisons). The URSR and MRSR of C-NS rats at 270 days-of-age were not significantly different (p>0.05).

In general, relative secretion rates decreased between 170 and 270 days and then were unchanged between 270 and 370 days-of-age.
Verification of Exercise-Training

Changes in final body weight and paired adrenal gland weight indicated that a training effect was obtained in Ex rats at all three ages. Pooled final body weights of C rats were significantly larger than those of Ex rats in all age groups. This has been reported previously for exercise-trained rats by a number of investigators (Craig, 1972; Long, 1974; Auth, 1975). In this study, exercise-training resulted in an 11 per cent depression of body weight at 270 days-of-age. This was in agreement with Story (1972), who reported that exercise-training had a greater effect on the body weight of older rats.

Rats at 270 and 370 days-of-age had significantly greater final body weights than rats at 170 days. Singh and Kanungo (1968) and Story (1972) reported that growth in rats occurred until about 210 days-of-age. After 210 days, the rate of body weight gain decreased with increasing age. In this study, the final body weight of 270 day-old Ex rats was the same as Ex rats that were 370 days-of-age, but C rats demonstrated a seven per cent decrease in final body weight between 270 and 370 days-of-age. This may have been due to the death of heavier rats or a depression of food intake in rats between 270 and 370 days which resulted in a loss of body weight.

At all three ages, Ex rats exhibited significantly heavier adrenal glands than those of C rats. The adrenal gland enlargement in response to exercise-training has been reported by a number of investigators (Buuck and Tharp, 1971; Song et al., 1973) and has been shown to be
produced mainly by the enlargement of the zona fasciculata, the primary adrenocortical region involved in glucocorticoid synthesis (Thompson and Lippmann, 1974). Presumably, adrenocortical hypertrophy in response to exercise-training was the result of chronic adrenocortical stimulation by ACTH (Selye, 1956).

Adrenocortical hypertrophy and body weight depression in exercise-trained rats, when compared to nontrained controls, was indicative of a successful training program, as has been described previously (Tharp and Buuck, 1974).

Plasma Corticosterone Concentration

Plasma corticosterone concentrations of C-NS and Ex-NS rats were similar at all three ages. This observation demonstrated that the Ex rats did adapt to exercise-training. Prior to three or four weeks of chronic physical-training, the resting plasma corticosterone concentrations of exercised rats have been shown to be elevated above those of nonexercised control rats (Buuck and Tharp, 1971; Frenkl et al., 1975). However, after three of four weeks of exercise-training, plasma corticosterone concentrations returned to control values or were even less than that of control rats. Resting plasma corticosterone concentrations of Ex rats were lower than those of C rats in this study at 270 and 370 days-of-age, but were not significantly different from those of C rats.

Age-related changes in resting plasma corticosterone concentrations represented an interesting trend that was contrary to almost all of the data previously reported for aging rats. Resting plasma corticosterone
concentrations in senescent rats have been reported to be similar to those of young or mature rats (Hess and Riegle, 1970; 1972; Riegle and Hess, 1972). However, in this study, a progressive increase in plasma corticosterone concentrations was observed in C-NS rats between 170 and 370 days-of-age. The plasma corticosterone concentration of Ex-NS rats increased between 270 and 370 days-of-age. All NS rats were removed from their cages, carried to another room, weighed and injected with anesthetic before they were sacrificed. These events which preceded sacrifice may have acted to elevate plasma corticosterone concentrations in older rats. Plasma corticosterone concentrations in rats have been reported to be elevated for at least two hours following handling and weighing (Barrett and Stockham, 1963). However, rats at all three ages were treated similarly, which seemed to eliminate this possibility. On the other hand, this may have been the normal resting plasma corticosterone concentration of these rats. Eleftheriou (1974) reported that the resting plasma corticosterone concentrations of C57BL/6J mice at nine months-of-age was greater than that of two month-old mice. However, another report concerning aging C57BL/6J mice has shown that the resting plasma corticosterone concentrations at two months-of-age were three-fold greater than at eight months-of-age (Grad and Khalid, 1968). Britton et al. (1975) reported that the resting plasma corticosterone concentrations were greater in 12 month-old rats than in two month-old rats. Obviously, two month-old rats were still rapidly growing and 12 month-old rats were past their peak growth period. An analogous situation existed in this study. As mentioned in the previous section, rats stop growing near 210 days-of-age. Possibly, as maturity was reached in these rats, a
developmental change occurred in their hypothalamic-pituitary-adrenal system which elevated plasma corticosterone concentrations above those levels observed in growing rats (170 days). This suggested change in the hypothalamic-pituitary-adrenal system was not altered by exercise-training.

Results obtained in this study demonstrated that with increasing age, exercise-training produced a variability in the plasma corticosterone increase observed in response to acute swimming. The elevation of plasma corticosterone concentration in Ex-S rats above that of C-S rats at 170 days-of-age was similar to that reported by Sakellaris and Vernikos-Danellis (1975). They reported that rats adapted to restraint-stress or cold exposure and then exposed to a second stressor responded with an increased drive on their hypothalamic-pituitary-adrenal system when compared to rats that were exposed only to the second stressor. In this study, this response was reversed at 270 and 370 days, so that C-S rats had a greater plasma corticosterone concentration than that of Ex-S rats. The rats used in the restraint-stress study were 60 days-old at the start of the experiment and therefore, were still rapidly growing rats, like the 170 day-old rats in this experiment. It was possible that exercise-training, like restraint-stress or cold exposure, could have altered the means by which a rat responded to a second stressor, like acute swimming. Because this phenomenon did not occur at 270 or 370 days-of-age, this indicated that this increased response with exercise-training was age-dependent.

It has been shown that the magnitude of the adrenocortical response of exercise-trained rats to a stressor was dependent upon which
hypothalamic-pituitary pathways were activated in response to the stressor. The pathways which were activated in response to muscular effort were suggested to be less effective in eliciting increases in the plasma corticosterone concentration of exercise-trained rats than in nontrained controls (Frenkl, 1971). The investigator suggested that the hypothalamic-pituitary pathways activated during exercise-training became less sensitive to muscular exertion. The results obtained in the present study could be interpreted to mean that these pathways were possibly hypersensitive to muscular exertion in the 170 day-old Ex rats.

The elevation of plasma corticosterone concentrations at 270 and 370 days-of-age in response to acute swimming was similar to the response pattern reported by Frenkl et al. (1968) and Frenkl (1971). These data indicated that exercise-training resulted in an adaptation of the hypothalamus or pituitary so that, in response to a similar intensity of exercise, exercise-trained rats secreted less ACTH than nontrained controls.

Exercise-training has been reported to result in an improved efficiency of energy metabolism (Gordon, 1967; Oscai and Holloszy, 1971). Exercise-trained rats exhibited an increased ability to obtain energy by cellular respiration through an elevation of enzymes of fatty acid oxidation and the citric acid cycle. Endurance exercise-training resulted in an adaptive increase in the capacity of skeletal muscle to regenerate ATP aerobically. Coupling factor 1 also was increased to a point where it was not a rate-limiting step in ATP regeneration. A tighter coupling of ATP regeneration to electron transfer would result in a greater amount of ATP formed for the same amount of substrate
catabolized in an exercise-trained rat. By this mechanism, the demand for glucose and fatty acids would be lowered and the need for glucocorticoids for the stimulation of gluconeogenesis was diminished. In this study, it could be argued that the 170 day-old Ex rats were unable to adapt to exercise-training by improving the efficiency of their energy metabolism. To compensate for this inability to adapt, the 170 day-old Ex-S rats were forced to increase their plasma corticosterone concentration, and, as a result, substrate flow to skeletal muscle was increased. Liver glycogen degradation during exercise has been reported to occur almost completely independent of hormonal regulation and indicated that glycogenolysis could be stimulated by local metabolites or oxygen supply (Gollnick et al., 1970). Liver glycogen has been shown to be a more important source of glucose during prolonged exercise than muscle glycogen (Baldwin et al., 1973). However, glucocorticoids were necessary for a more complete depletion of liver glycogen stores.

**In Vitro Corticosterone Secretion Rates**

Maximal absolute *in vitro* adrenal gland corticosterone secretion rates were an indication of the ability of the adrenal gland to respond to a supraphysiological stimulation by ACTH (van der Vies, 1960). Absolute secretion rates represented the total secretory output of the adrenal gland irrespective of the influence of glandular weight. It has been shown that factors which influenced the adrenal gland *in vivo* also will be manifested *in vitro* (van der Vies, 1960). Therefore, unstimulated absolute secretion rates were an indication of the secretory activity of the adrenal glands at the time of sacrifice. Maximal relative secretion
rates demonstrated the secretory efficiency of the gland, because the secretion rate was expressed per 100 milligrams of gland weight. Unstimulated relative secretion rates may have been influenced by a variety of factors: plasma ACTH concentration at the time of gland removal, other in vivo influences on glandular secretion and the efficiency of glandular secretion. Because of these complications, unstimulated relative secretion rates were not considered to be very beneficial to the interpretation of these data. Therefore, maximal relative and maximal and unstimulated absolute secretion rates will be discussed in this section in the same order as they were presented in the previous section: C-NS versus C-S, Ex-NS versus Ex-S, C-NS versus Ex-NS and C-S versus Ex-S.

Maximal relative secretion rates indicated that at 170 and 370 days-of-age, in vivo factors increased the secretory efficiency of the adrenal glands of the C-S rat. Maximal absolute secretion rates also bear this out. The maximal absolute secretion rate of C-S rats was greater than that of C-NS rats at 170 and 370 days-of-age. A progressive decline in the ability of the adrenal glands of C-S rats to respond to ACTH also was observed between 170 and 370 days. This decline also was observed in the absolute secretion rate of C-S rats between 270 and 370 days-of-age. Age-related reductions in adrenal gland responsiveness to ACTH have been reported previously for rats and mice (Hess and Riegle, 1970; Eleftheriou, 1974). These reported changes were first noticed at about 370 days-of-age in the rat.

Unstimulated secretion rates of C-S rats, both absolute and relative, demonstrated the same response pattern as the corresponding
maximal secretion rate at all three ages, but unstimulated secretion rates were significantly less than maximal rates. The facts suggested that the same amount of ACTH was secreted by the pituitary of C-S and C-NS rats at each age, but that differences in adrenal gland secretory efficiency resulted in the variations observed in unstimulated absolute secretion rates. The apparent turnover rate of corticosterone was altered in response to acute swimming as C rats aged from 170 to 370 days. The unstimulated absolute secretion rate of C-S rats progressively decreased between 170 and 370 days-of-age, while that of C-NS rats varied over a wide range. At 270 and 370 days-of-age, the apparent turnover rate of corticosterone in C-S rats was less than that of C-NS rats, resulting in the higher plasma corticosterone concentrations observed at these ages. At 170 days-of-age, the elevated plasma corticosterone concentration probably was the result of the elevated unstimulated absolute secretion rate.

In Ex rats at 170 days-of-age, maximal absolute and relative secretion rates suggested that an in vivo factor, or the lack of this in vivo factor, acted to decrease the secretory efficiency of the adrenal glands of Ex-S rats. For this reason, the elevation of plasma corticosterone concentrations at this age presumably was due to extra-adrenal mechanisms. At 270 days, the maximal relative secretion rates of Ex-NS and Ex-S rats were similar, even though the Ex-S adrenal gland was capable of secreting more corticosterone. This indicated that the pituitary gland of the Ex-S rats secreted less ACTH in order to maintain the same steroid secretion at 270 days-of-age. Even though corticosterone secretion rates were similar, plasma corticosterone concentrations were
greater in Ex-S rats. It appeared that, at this age, the enlarged adrenal glands of Ex rats required less ACTH for the same amount of corticosterone secreted and that elevations in plasma corticosterone were due to decreased turnover of corticosterone in the Ex-S rat. This was contradictory to reports in the literature. The half-life of plasma glucocorticoids in exercise-trained rats has been reported to be lower than that of nontrained controls (Frenkl et al., 1969; Frenkl and Csalay, 1970).

At 370 days-of-age, the maximal relative and absolute secretion rates of Ex-S rats were greater than that of Ex-NS rats. Acute swimming, at this age, resulted in an increased efficiency of adrenal gland corticosterone secretion in the Ex rat. Also, the unstimulated absolute secretion rate of Ex-S rats was greater than that of Ex-NS rats, so that the elevated secretion rate in Ex-S rats could have accounted for the elevated plasma corticosterone concentration at 370 days.

The responsiveness of the adrenal glands of exercise-trained rats to ACTH was variable with increasing age. At 170 days-of-age, the responsiveness of the adrenal glands of Ex-NS rats was greater than that of C-NS rats. This was reversed at 270 days, so that the maximal relative secretion rate of the C-NS rats was greater than that of Ex-NS rats. Frenkl and Csalay (1962) reported that the in vitro secretion of corticosterone increased after three weeks of exercise-training, but after six weeks of exercise training, the secretion rate was less than that of nontrained controls. Tharp and Buuck (1974) reported a depression of the ACTH responsiveness of adrenal glands of exercise-trained rats after six weeks of training, but no elevation in responsiveness was
observed prior to this time. Adrenal glands from exercise-trained rats have been reported to be more responsive to ACTH after a 10-week training program (Pritchett, 1973). Unstimulated, resting corticosterone secretion rates have been reported to be greater than (Pritchett, 1973) and less than that of nontrained controls (Craig, 1972) following a 10-week training program.

At 370 days-of-age, it appeared that exercise-training was not capable of increasing the efficiency of corticosterone secretion. At all three ages, total steroid secretion, irrespective of glandular weight, also reflected this change. However, at each age, plasma corticosterone concentrations were similar in C-NS and Ex-NS rats, even though the unstimulated absolute secretion rates of the adrenal glands varied over a wide range. It appeared that corticosterone turnover was elevated in the Ex-NS rat at 170 days, but that it was decreased at 270 days. At 370 days-of-age, the unstimulated absolute secretion rates of Ex-NS and C-NS rats were similar and therefore, the plasma corticosterone concentrations were similar.

The C-S rat, at all ages, was able to increase its efficiency of corticosterone secretion in response to ACTH stimulation above that of Ex-S rats. However, total absolute secretion in response to ACTH stimulation showed that the Ex-S rat secreted similar amounts of corticosterone at 170 and 270 days-of-age. At 370 days, the Ex-S rat was able to secrete more corticosterone than the C-S rat. Clearly, this was the influence of elevated adrenocortical size as a result of exercise-training. Exercise-training also maintained adrenocortical responsiveness to ACTH with increasing age in response to acute muscular
exertion. C-S rats exhibited a progressive decline in ACTH responsiveness with increasing age.

Unstimulated absolute secretion rates indicated that extra-adrenal mechanisms were controlling plasma corticosterone concentrations in Ex-S and C-S rats. Unstimulated total steroid secretion in C-S rats was greater than that of Ex-S rats at 170 and 270 days-of-age. At 170 days, the plasma corticosterone concentration was greater in Ex-S rats than in C-S rats, but the opposite occurred at 270 days-of-age. Steroid turnover appeared to be altered in response to acute exercise in the C rat. In the Ex-S rat, no change occurred in the unstimulated absolute secretion rate or in the plasma corticosterone concentration between 170 and 270 days-of-age. The plasma corticosterone concentration increased between 170 and 270 days in C-S rats, but the unstimulated absolute corticosterone secretion rate decreased during this time. This indicated that the apparent steroid turnover was decreased in the 270 day-old rat, when compared to the 170 day-old, in order for the plasma corticosterone concentration to increase. Between 270 and 370 days-of-age, the secretion rate of C-S rats decreased, that of Ex-S rats increased and the plasma corticosterone concentrations were unchanged. This suggested that, at 370 days, the Ex-S rat increase its metabolic clearance rate of corticosterone and as a result, plasma corticosterone concentrations were unchanged.

So far in this discussion of in vitro adrenal gland function, an in vivo factor has been referred to as a means by which the glandular secretory efficiency was increased. Decreased ACTH secretion from the pituitary gland has also been mentioned to occur in response to acute
exercise in exercise-trained rats in this and other studies (Viru and Akke, 1969; Frenkl and Csalay, 1970; Chin and Evonuk, 1971; Tharp and Buuck, 1974). Epinephrine may be involved in both processes. Changes in epinephrine secretion with age possibly were responsible for the variability observed in the in vitro adrenal gland secretion of corticosterone in the present investigation. In view of the close physiological relationship between glucocorticoids and epinephrine within the adrenal medulla (Wurtman and Axelrod, 1965; Maling et al., 1966), such a suggestion does not seem implausible. Reasons for this suggestion follow.

Epinephrine has been suggested to play a role in the activation of the pituitary-adrenal system (Nakai et al., 1973). Exercise-trained and nontrained control rats responded to identical injected doses of epinephrine with similar increases in their plasma corticosterone concentration (Frenkl, 1971). However, rats trained by exhaustive swimming exhibited lower resting plasma epinephrine concentrations than nontrained controls (Chin and Evonuk, 1971). Trained human subjects exhibited decreased sympathetic nervous system activity during exercise (Hartley, 1975). In the present study, exercise-trained rats were accustomed to a daily regimen of physical stress and even though swimming was a novel experience, they still responded with a smaller release of epinephrine from their adrenal medullas than did nontrained controls. This also has been proposed by Chin et al. (1973). The catecholamine-sensitive peripheral tissues of exercise-trained rats may have been more sensitive to catecholamines than controls and therefore, less catecholamines were secreted. Adaptation of central sympathetic
mechanisms also has been shown to occur with exercise-training (Gollnick, 1967). This was shown to result in local sympathetic control of fat mobilization, rather than requiring the presence of circulating catecholamines. Adrenal medullary catecholamines also were reported not to be essential for controlling glycogenolysis during exercise (Gollnick et al., 1970). Consequently, in this study, through reduced catecholamine secretion in the exercise-trained rat, the efficiency of glandular corticosterone secretion may not have been elevated to its full potential during acute swimming. However, if tissue sensitivity to catecholamines was increased with exercise-training and fat mobilization and glycogenolysis were more dependent on local control, then circulating corticosterone concentrations did not need to be elevated to the extent of that in nontrained controls.

Wurtman and Axelrod (1955) have shown that in the rat, corticosterone was necessary to induce the synthesis of phenylethanolamine-N-methyl transferase (PNMT). PNMT catalyzed the conversion of norepinephrine to epinephrine within the adrenal medulla. This established a necessity of adrenocortical hormones for the synthesis of circulating catecholamines. Adrenal demedullated and pharmacologically sympathectomized rats were able to achieve only 40 per cent of the increase in plasma corticosterone concentration of intact rats during an exhaustive exercise bout (Maling et al., 1966). This suggested that adrenocortical glucocorticoid secretion was dependent upon adrenomedullary catecholamines. A "push-pull" mechanism may be occurring in the adrenal gland so that adrenocortical glucocorticoid secretion and adrenomedullary catecholamine secretion are dependent upon each other. Maximal relative and absolute secretion rates,
in this study, fit this assumption of an adrenocortical-adrenomedullary interaction in C rats at all three ages. In the C rat, acute swimming resulted in a stimulation of corticosterone and possible epinephrine secretion. Because both hormones were dependent upon each other, the efficiency of corticosterone secretion was enhanced in the C rat. Ex rats at 270 and 370 days-of-age responded like C rats. Ex rats at 170 days-of-age, on the other hand, responded to acute swimming with less of an elevation in adrenomedullary epinephrine secretion and therefore, the efficiency of adrenocortical secretion was not increased. The response of Ex rats presumably reflected an alteration in the central sympathetic and the pituitary-adrenal mechanisms with increasing age.

A strong argument can be made for the interaction of epinephrine and corticosterone secretion in the rat. It appeared that changes in epinephrine secretion could have resulted in the changes in in vitro corticosterone secretion observed in this study.
SUMMARY

Plasma corticosterone concentrations and \textit{in vitro} adrenal gland corticosterone secretion rates were measured following an acute bout of swimming in exercise-trained and nontrained control male rats of three ages. Initially, rats were divided into groups according to a 3x2 factorial experimental design for age (100, 200 and 300 days-of-age) and treatment (exercise-trained (Ex) and control (C)). Ex rats were exercise-trained by running on a motor-driven treadmill for a 10-week experimental period, five days per week for 30 minutes daily. The treadmill was set at an eight degree incline and at a speed of 15 meters per minute. C rats served as sedentary controls.

At the conclusion of the 10-week experimental period, Ex and C groups were further divided into nonswim (NS) and swim (S) groups. All S rats were subjected to an acute bout of swimming immediately prior to being anesthetized for blood sampling. NS rats also were anesthetized prior to blood sampling, but were not swum.

Following the induction of anesthesia, blood was removed from the jugular vein and plasma was prepared. Adrenal glands were removed from the rats and were incubated individually; one in media alone and the other in media containing an amount of ACTH capable of maximally stimulating the gland.

Corticosterone content of plasma and incubation media was determined by competitive protein-binding radioassay.

The results of this study were as follows:

1. The final body weight of Ex rats was depressed below that of C rats following 10 weeks of exercise-training.
2. Paired adrenal gland weight of Ex rats was elevated above that of C rats following 10 weeks of exercise-training. This coupled with the depression of final body weight indicated that a successful training program was employed in this study.

3. Resting plasma corticosterone concentrations (Ex-NS and C-NS) were similar at all three ages. A significant elevation in resting plasma corticosterone concentrations was observed between 270 and 370 days-of-age.

4. Acute swimming resulted in significant elevations in the plasma corticosterone concentrations of Ex and C rats.

5. Plasma corticosterone concentrations of Ex-S rats were higher than that of C-S rats at 170 days-of-age. However, at 270 and 370 days, the plasma corticosterone concentration of Ex-S rats was less than that of C-S rats. An age-dependent alteration in the hypothalamus-pituitary-adrenal system or an inability of the skeletal muscle enzyme systems to adapt to exercise-training.

6. In vitro adrenal gland corticosterone secretion rates were age-dependent and suggested that changes in plasma corticosterone concentrations in exercise-trained rats were due to a combination of the efficiency of glandular secretion of corticosterone, the amount of ACTH reaching the adrenal cortex and the ability of the rat to alter corticosterone turnover in response to age-dependent changes in the other two factors.
7. A change in epinephrine secretion from the adrenal medulla was suggested as a cause of the altered adrenocortical responsiveness.
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APPENDIX A: INCUBATION MEDIUM

1. The following quantities of stock solution or their multiples were mixed to make Krebs-Ringer bicarbonate medium (Dawson et al., 1969):
   a. 100 ml 0.9% NaCl
   b. 4 ml 1.15% KCl
   c. 3 ml 1.62% CaCl₂·2H₂O
   d. 1 ml 2.11% KH₂PO₄
   e. 1 ml 3.80% MgSO₄·7H₂O
   f. 21 ml 1.30% NaHCO₃ (made fresh prior to use)

2. pH of the media was adjusted to 7.4, if necessary.

3. Glucose (dextrose, Fischer Scientific Co.) was added to give a concentration of 2 mg/ml.

4. ACTH (ICN Life Sciences Division) was added to give a concentration of 250 mU/ml. Media containing ACTH was used only for maximal adrenocortical stimulation.
APPENDIX B: GLUCOCORTICOID ANALYSIS BY COMPETITIVE PROTEIN-BINDING RADIOASSAY

(Murphy, 1967; Cameron and Scarisbrick, 1973)

1. Handling of glassware

   All glassware used in the analysis was cleaned in the following manner:
   a. washed thoroughly in Alconox detergent (Alconox, Inc.) and water and rinsed in tap water;
   b. soaked for at least six hours in a $\text{H}_2\text{SO}_4$-$\text{K}_2\text{Cr}_2\text{O}_7$ bath to remove soap residues;
   c. soaked for at least 30 minutes in a 2M HCl bath to remove residual $\text{K}_2\text{Cr}_2\text{O}_7$;
   d. carefully rinsed at least 10 times each in tap water and distilled, deionized water;
   e. rinsed twice in double distilled, deionized water containing 10 ug EDTA per liter.

2. Preparation of materials used in the analysis

   a. Outdated female human plasma was obtained from the Blood Bank at Mary Greeley Memorial Hospital in Ames. The plasma from four subjects was pooled, divided into 4 ml aliquots and stored at -20 °C until required.
   b. Florisil (60-100 mesh, Fischer Scientific Co.) was washed approximately 10 times in double distilled, deionized water to remove any fine particles, washed twice in absolute ethanol and dried overnight at 120 °C.
The Florisil was stored in a desiccator until required.

c. Tritiated corticosterone \((1,2,6,7-^{3}\text{H}(N))\)-corticosterone, New England Nuclear Corp.) was delivered in benzene and ethanol \((9:1,\text{v/v})\). Upon arrival, the benzene and ethanol were evaporated to dryness in a stream of filtered, desiccated air and resuspended in absolute ethanol at a concentration of 50 uCi per ml. This solution was stored at \(-20^\circ\text{C}\) until required.

3. Preparation of corticosteroid-binding globulin (CBG) solution
   a. Fresh CBG-solution was prepared prior to each assay run.
   b. 1.5 ml human plasma was diluted to 100 ml in double distilled, deionized water containing 10 ug EDTA per liter.
   c. 15 g Florisil was added.
   d. The CBG-solution and Florisil were tightly covered and warmed to \(40^\circ\text{C}\) in a water bath.
   e. The Florisil was removed from the CBG-solution by vacuum filtration.
   f. 5 uCi of \((1,2,6,7-^{3}\text{H}(N))\)-corticosterone in absolute ethanol was added slowly to prevent precipitation of the CBG.
   g. The CBG-solution was cooled at \(10^\circ\text{C}\) or less in an ice-water bath for at least one hour prior to its use.

4. Competitive protein-binding radioassay for corticosterone
   a. Extraction of corticosterone
i. Triplicate 100 µl quantities of plasma or 50 µl quantities of incubation medium were extracted with 500 µl or 1000 µl of ethyl acetate (Fischer Scientific Co.), respectively, in a 12x75 mm glass culture tube.

ii. The aqueous and aliphatic layers were mixed thoroughly using a Vortex mixer.

b. Preparation of ethyl acetate supernatant for analysis

i. A 100 µl quantity of supernatant from the extracted plasma or 50 µl of that from the extracted incubation medium was transferred to a second 12x75 mm glass culture tube.

ii. Triplicate 100 µl quantities of corticosterone standards in ethyl acetate were pipetted into 12x75 mm glass culture tubes. Standards contained 0, 2.5, 5.0, 7.5, 10.0 and 15.0 ng corticosterone.

iii. Extraction media and standards were evaporated to dryness at 40 °C in a stream of filtered, desiccated air.

c. Addition of CBG-solution

i. 1000 µl of CBG-solution was pipetted into each tube and mixed using a Vortex mixer.

ii. Tubes were warmed to 40 °C for five minutes in a water bath to facilitate the binding equilibrium between free and bound corticosterone.
iii. Tubes were then cooled at 10°C or less in an ice-water bath for 10 minutes.

d. Separation of free and bound corticosterone

i. 40 mg Florisil was added to each tube using a spoon as previously described by Murphy (1967).

ii. Florisil was immediately mixed with the CBG-solution for exactly 30 seconds using a Vortex mixer.

iii. Tubes were placed in an ice-water bath at 10°C or less for exactly five minutes.

iv. 500 ul of the supernatant was pipetted into a liquid scintillation counting vial.

v. 500 ul of CBG-solution also was prepared for counting. This was arbitrarily designated as 100 per cent of the tritium label bound to the CBG (100% bound).

vi. 10 ml of counting media was added. This media was of the following composition:

   a.) 5 g PPO (2, 5-diphenyloxazole, New England Nuclear Corp.)

   b.) 100 ml Biosolv BBS-3 (Beckman Instruments Inc.).

   c.) Toluene (Fischer Scientific Co.) to 1 liter.
5. Sample counting and handling of counting data
   a. Prepared counting vials were counted to a one per cent counting error on a Beckman LS-250 liquid scintillation spectrometer at a gain setting of 330. Counting efficiency was approximately 36 per cent, as determined by a commercially prepared quenched standard set and automatic external standardization.
   b. Raw counting data were converted from counts per minute (cpm) to per cent of the tritium label bound (% bound) by the following calculation:

   \[
   \% \text{ bound} = \frac{\text{Sample cpm} - \text{Background cpm}}{100\% \text{ bound cpm} - \text{Background cpm}} \times 100\%
   \]

   c. Standard values were plotted on graph paper as % bound versus ng corticosterone. Values (% bound) obtained for plasma and incubation media were read from this curve.
   d. Appropriate corrections were made for dilution during analysis.