THE EFFECTS OF ELECTRICAL STRESS AND/OR PHYSICAL ACTIVITY ON THE HISTOLOGY OF THE HEART, THYROID, AND ADRENAL MEDULLA IN ADULT MALE ALBINO RATS

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ABSTRACT

THE EFFECTS OF ELECTRICAL STRESS AND/OR PHYSICAL ACTIVITY ON THE HISTOLOGY OF THE HEART, THYROID, AND ADRENAL MEDULLA IN ADULT MALE ALBINO RATS

by Kenneth Douglas Coutts

The purpose of this study was to investigate the effects of an anxiety-producing situation and/or physical activity on the incidence of myocardial damage, the catecholamine concentration of the adrenal medulla, and the epithelial cell height of the thyroid gland. Relative thyroid weight was also examined, and compared to the cell-height measurement.

Eighty adult-male albino rats were randomly assigned to ten groups, including two control groups. Eight experimental groups received different combinations of an electric-shock treatment and/or a forced-exercise treatment. These treatments were applied for one-half hour a day, five days a week for a period of ten weeks. Half of the animals were housed in small sedentary cages with the remaining half kept in cages which permitted the animals to walk or run in a revolving drum at will.

The histologic findings on the hearts were determined by subjective evaluation of a transverse section from the middle third of the ventricles. The catecholamine concentration of the adrenal medulla was evaluated by a

histospectophotometric measurement of a potassium dichromate staining reaction. Thyroid epithelial cell height was determined by tracing the outer and inner cell border of several follicles from each animal with subsequent quantification. Body weight and trimmed, wet weight of the thyroid gland permitted calculation of relative thyroid weight for all animals.

Since no obvious heart damage was found in any animals, no statistical treatment of these data was warranted. A reexamination of the heart sections for more subtle histological changes was then made. A check-list of these findings provides exploratory data in this area.

Analysis of variance, with the Scheffe multiple comparison method when appropriate, was used for statistical evaluation of the thyroid and adrenal medulla variables. The level of significance for all statistical procedures was set at twenty-five per cent.

The limitations and design of the study prevented any definitive conclusions about the results. An hypothesized phasic response of the thyroid gland to stress was suggested by the data and previous information. Further evidence, such as serial data collection, was recommended to test this hypothesis. It was also recommended that the level or period of stress be increased to elicit a testable incidence of heart damage, and that the method used to determine adrenal medulla catecholamine concentration be discarded due to a large sampling error. The more subtle changes in heart histology had no apparent significance, as the treatments did not appear to alter the frequency of these findings.

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By

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Dedicated to my wife, Nancy and our daughter, Carol who are the major source of inspiration and challenge in my life.

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CHAPTER I

INTRODUCTION

Previous investigations indicate a probable positive relationship between anxiety and the genesis of degenerative cardiovascular disorders (61,69,84); whereas, physical activity has an apparent prophylactic effect (10, 11,34,42,43,47,73,77,86).

One theory, regarding the relationship of anxiety to heart damage, is that the adrenergic reflexes of man to present anxiety-producing situations are inappropriate and result in an excessive secretion of catecholamines in relation to the musculo-motor responses(61). The oxygen-wasting action of excessive amounts of catecholamines in the coronary circulation is believed to be a major factor in the precipitation of cardiac necroses from anxiety situations (45, 55,56,61).

Two general theories may be proposed as to the protective action of physical activity in anxiety related cardiopathies. One is that physical exercise, by providing a musculo-motor outlet, reduces the degree and length of exposure of cardiac tissue to catecholamine action (61). Selye (76), in his theory of cross-resistance proposes the other. Selye contends that physical activity, or other moderate, well tolerated stressors, may act in a manner analagous to a vaccine in stress-related cardiopathies.

Purpose

Most of the previous investigations, which support the hypothesized relationship of anxiety and exercise to heart disease, have either been pharmacological in nature or limited in terms of the number of treatment groups used and/or variables measured. The purpose of the present study was to obtain data regarding the roles of the thyroid gland and adrenal medulla in the relationships of anxiety and exercise to stress-induced cardiopathies. It is an attempt to evaluate the usefulness of nonpharmacological procedures in studying the above relationships. To accomplish this purpose, the epithelial cell height of the thyroid gland, the catecholamine concentration of the adrenal medulla, and the incidence of microscopic, myocardial changes were measured in adult, male albino rats. Eight different groups of animals were subjected to the stresses of anxiety and/or forced exercise with two additional groups serving as controls during a ten-week experimental period.

Definition of Terms

Anxiety.--The response of the animals to periodic electric shocks in confined quarters.

Physical Activity.--Two types of physical activity were employed in this study.

1. Voluntary activity consisted of spontaneous walking or running in a revolving drum.
2. Forced exercise consisted of periods of mandatory swimming with overload.

Limitations of the Study

1. The sample size was smaller than the size calculated as necessary to achieve the desired level of statistical confidence.
2. The results cannot be directly applied to human beings.
3. The length of the experimental period was somewhat arbitrary and therefore not necessarily optimal for obtaining significant results.
4. Discussion of the etiology of heart damage is limited to the parameters measured and experimental treatments employed.

CHAPTER II

REVIEW OF RELATED LITERATURE

Relationship of Anxiety and Exercise to Heart Disease

Epidemiological studies.--In numerous survey studies, the relationship of anxiety and exercise has been investigated. Russek (69,70,71) and Russek and Zohman (72), found a high association between the incidence of heart disorders and occupational stress, while the exercise habits of the subjects had no apparent effect on the incidence of heart disorders. Taylor (82), in a review of epidemiological studies, drew the following conclusions: (a) exercise, if it does deter the development of coronary heart disease, operates in populations with advanced atherosclerosis, and (b) that exercise is presently of minor significance compared to the scope of the problem, since the mortality ratio between sedentary and active sub-populations is in the range of 1.5 to 2.0. Morris and Crawford (42), in studying 3,800 autopsy reports, found no relationship between the degree of occupational physical activity and the incidence of coronary atherosclerosis; however, there was a definite correlation between the incidence of ischemic myocardial fibrosis and lack of exercise. Wolffe (86), however, showed that 300 athletes, all past middle age, had less evidence of athersclerosis than 300 executives. While this evidence is confusing, if not conflicting, the variations in populations sampled and criteria for diagnosis of specific disorders may account for some of the differences in results.

From their results, Morris and Crawford (42) hypothesized that exercise may be protective, men with cardiovascular disease may be less active because of their debility, or some indirect factor may be responsible for the observed relationship. In a comparison of patients under treatment for myocardial infarction and controls, Kasanen, Kallio and Forsstrom (30) noted that the patients were more sedentary and had higher levels of anxiety in their occupations and avocations. The fact that occupations with a high physical activity requirement are usually associated with a low level of mental anxiety, and vice versa, points up the logic for assuming that either exercise, anxiety or both are related to the occurrence of cardiovascular diseases. The limitations of epidemiological studies do not permit clarification of this problem.

Laboratory studies.--Closely controlled laboratory studies are helpful in defining more precisely the roles of anxiety and exercise in the production of cardiovascular disorders. Raab, Chaplin and Bajusz (61), found that emotional stimuli, with limited opportunity for physical responses, were more cardiotoxic in wild than domestic rats. Their results support the role of emotional stimuli in the genesis of heart disease. In addition, the authors proposed that a less blunted "fight or flight" reflex in the wild animals, causing a greater discharge of catecholamines with no opportunity to fight or take flight, was responsible for the greater toxicity of emotional stress. Rats subjected to Faradization of four volts to exhaustion from one to six times showed signs of developing myocardial damage (28).

As a specific example of Selye's (76) theory of cross-resistance, Bajusz and Selye (3) demonstrated that the cardiac necroses produced by a variety of stressors, including exercise, cold, noradrenaline, and bone fracture, in sensitized rats could be prevented by pretreatment with some of the same stressors.

While survey and laboratory work point to possible relationships between anxiety, exercise, and heart damage, the establishment of cause-effect bonds is dependent upon the determination of the intermediary mechanisms responsible for the relationship.

Effects of Anxiety on Cardiac Function

In a study on effort syndrome patients, patients with anxiety states--but no effort syndrome, and controls, Jones and Mellersh (29) showed a significant differentiation between both types of patients and controls in their response to exercise. The similarity in the exercise responses of effort syndrome and anxiety patients implies a similarity in their inefficiency during activity and a relative hypoxic condition in the heart. Cohen, White and Johnson (13) report data supporting the relatively low work capacity and efficiency of effort syndrome patients and additional evidence that effort syndrome is inherited with Mendelian dominance. Raab (55), in an excellent review of hormonal and neurogenic cardiovascular disorders makes the following statement.

> If special attention is paid to the frame of mind in which physical exertions are undertaken by patients with angina pectoris, it will not infrequently be found that pain occurs only or prevailingly under the aggravating influence of mental tension or mere awareness of purpose and that much heavier muscular work, devoid of such connotations, can be performed with relative ease.

Mascitelli-Coriandoli, Boldrini and Citterio (40) noted that rats subjected to high intensity light and sound at irregular intervals for two to five hours per day for five weeks, had higher relative heart weights, higher lipid concentrations, and reduced metabolic stores, such as glycogen, ATP, and creatine phosphate in the heart. This effect is comparable to that seen in thyroxinized animals and indicates a possible role of the thyroid hormones in reducing the reserve capacity of the heart in response to chronic emotional stimuli.

Hickam, Cargill and Golden (27) found that anxiety has an effect on the circulation similar to small doses of epinephrine which implicates epinephrine as a plausible intermediary in the relationship of anxiety to heart disease.

Effects of Exercise on Cardiac Function

Schimert and Schwalb (74) and Mellerowicz (41), in reviewing the effect of exercise on the cardiovascular system point out the relative efficiency and the higher reserve capacity of the trained heart act as deterents to hypoxic conditions during rest and under stress. Taylor, et al. (83), working from the other side of the question, noted that a sedentary existence led to findings indicative of inefficiency and lowered capacity of the oxygen transport system.

Raab (57,58) established that there was a decreasing sympathetic tone with increasing exercise habits as measured by the resting heart rate and isometric contraction period. This shift in autonomic control of the heart could be changed in either direction by participation in a physical training program or prolonged lack of exercise. In discussing the autonomic control of the heart, Kraus and Raab (35) and Raab (53,56) postulate that the more efficient heart of an active person, by increased vagal tone, results in a decrease of potentially pathogenic catecholamines in the heart.

Eckstein (16), Petren, et al. (50,51,52) and Roberts and Wearn (68) provide anatomical evidence that prolonged physical training reduces the probability and/or extent of cardiac ischemia since this treatment increases the capillary to muscle fiber ratio in the heart. However, in a study on young mice forced to exercise by daily treadmill running or swimming, for periods up to fourteen weeks, Kitamura (33) noted a decrease in the capillary to fiber ratio, below control values, after an initial increase in this parameter during the first seven weeks. Excessive exercise in these young animals resulted in hemorrhage and infiltration of the myocardium with atrophy and fibrosis of myocardial fibers following termination of exercise. The conflicting results regarding the cardiac capillary to fiber ratio response to exercise indicates a need for more research. The data obtained to the present time indicate a possible difference in response of this parameter with variations in age, species, and/or intensity and duration of the exercise. Additional evidence for a cardiopathic role of exercise is supplied by Perttala (49) and Kipshidze (32), who found that animals on an atherogenic diet subjected to exercise had more extensive myocardial damage than animals just receiving the diet treatment. Animals on a normal diet that were forced to exercise exhibited no major heart lesions.

The evidence presented relating exercise and heart disease appears to be two sided. That is, exercise has been shown to prevent or foster the occurrence of heart damage.

The preventive aspects of exercise involve the observed changes in parameters which reflect an increased efficiency and capacity of the cardiovascular system as adaptations to chronic strenuous activity. These adaptations to chronic exercise would tend to prevent the occurrence of ischemic and/or hypoxic conditions in the myocardium.

The precipitation of heart damage as a result of exercise has only been observed when the work loads were excessive or other factors limiting the cardiac blood and/or oxygen supply were present. Thus the problem involving exercise and heart disease may be a question of how much exercise is needed to reap beneficial effects without approaching pathogenic levels. The influence of atherosclerosis, age and other factors on the fine line between beneficial and pathogenic levels of exercise merely complicates the problem.

Effect of Stress on Thyroid Function

The response of the thyroid gland to various stressors was studied by Williams, Jaffe and Kemp (85) in rats. They noted an initial decrease in I-131 uptake followed by an increase. Paschkis, et al.(46) found that formalin injection resulted in a similar initial decrease. In measuring the PBI response to a sequential stress of cold, exercise and fasting in normal and thyroidectomized rats, Bondy and Hagewood (6) found an initial decrease in this parameter also. The fact that thyroidectomized animals maintained on thyroxine showed the same response as normal animals led them to conclude that the decrease was at least partially due to an increased destruction of circulating thyroid hormone. By studying adrenalectomized and hypophysectomized animals, Badrick, et al. (1) were able to show that the initial decrease in thyroid function and subsequent increase were not dependent upon the adrenals or pituitary, and therefore, were likely under vasomotor control. Brown-Grant, Harris and Reichlin (9) confirmed the apparent lack of adrenal involvement in the thyroid's response to a variety of emotional and physical stresses. Del Conte, Ranello and Stux (14), however, report conflicting evidence regarding the role of the pituitary in the stress response of the thyroid since electroshock caused an increase in blood thyrotrophin and loss of granulation in the beta cells of the hypophysis in guinea pigs.

In an extensive human study, Reiss (67) noted a significant relationship between improvements in psychiatric status and thyroid function in hospital patients. The majority of these patients were hyperthyroid.

The response of the thyroid to the specific stress of exercise was studied by Bogoroch and Timiras (5), who noted a relatively small decrease in thyroid I-131 uptake when compared to changes elicited by formalin injections and spinal cord transection. Khorol (31), in a study of young athletes, found that physical exertion resulted in a decrease in thyroid I-131 uptake with a compensatory rise 24-72 hours after the stress. The observed decrease and subsequent increase was greatest in highly trained subjects.

Utilizing thyroid weight as a measure of function, Steinhaus, Hoyt and Rice (79) observed an increase in this parameter in dogs following chronic exposure to exercise while Donaldson (15) and Hatai (24) noted a decrease in albino rats.

In summary, the initial response of the thyroid to stress appears to involve a decrease in iodine uptake, mediated by vasomotor activity, coupled with an increased destruction of circulating thyroid hormones. This drop in blood thyroxine is a possible explanation for the subsequent compensatory increase of thyroidal activity since it could account for the observed increase in secretion of thyrotrophic hormone by the pituitary.

The response of the thyroid to chronic stress is not well established. Exercise has yielded conflicting results while emotional stress is more frequently associated with hyperthyroidism.

Effects of Stress on Catecholamine Activity

Using bioassay techniques, Fleetwood and Diethelm (19) found a positive correlation between degree of anxiety and plasma norepinephrine. Cannon and Britton (12) discovered that a combination of physical activity and emotional excitement caused a greater pulse rate increase in a denervated heart assay procedure, than the response elicited by either of the treatments separately. With acute bouts of muscular work, Gray and Beetham (20) noted a marked rise in plasma norepinephrine with only slight increase in epinephrine levels. These parameters returned to normal 15-30 minutes after the exercise bout. This evidence indicates that there is no apparent difference in catacholamine response to acute bouts of anxiety and exercise with a partially additive response when both treatments are administered simultaneously.

Raab (58), however, was able to demonstrate a proportionate relationship between the degree of physical activity and the neurovegatative status of the heart. Raab, et al. (64) found that more active subjects tended to have a lower resting pulse rate and a longer isometric contraction period in the age range of 17 to 50. Atropine administration caused the same absolute increase in heart rate in these subjects. Heart acceleration in response to epinephrine injection was slightly less in the more active subjects, but after atropine, epinephrine had a greater effect in the active subjects. They concluded that chronic physical activity increases the parasympathetic control of the heart and limits the sympathetic stimulation and/or response during stress. A possible tendency for physical activity to counteract the sympathetic overactivity of emotionally excitable people was noted by Raab and Krzywanek (63) in a study of the resting sympathetic tone and its response to mild sensory and mental stress in 200 apparently healthy men.

The response of the adrenal medulla to chronic exercise was studied by Eranko, Kazronen and Raisanen (17), who swam adult albino rats to exhaustion daily for periods of up to 81 days. A visual judgement of staining reactions indicated that the concentration of norepinephrine and epinephrine in the cells of the adrenal medulla were not affected by the treatment. Volume measurements, however, indicated an increase in total medullary volume with a greater absolute increase in iodate negative tissue and a larger relative increase in the iodate positive tissue in response to the swimming. According to Selye's (76) concept of a triphasic response to stress, these animals were probably in the resistance stage since he reports a loss of chromaffin granules from the adrenal medulla during the alarm reaction stage, normal granulation during resistance, and a final depletion during exhaustion. It is apparent, therefore, that stress increases the capacity of the adrenal medulla by volume changes rather than changes in concentration.

Interrelationship of Thyroid and Adrenal Medulla Glands and Hormones

In an early review of thyroid-suprarenal relationships, Herring (26) states that hyperthyroidism is associated with an increased load of chromaphil tissue. Earlier work by Herring (25), however, indicates a possible sex difference since male rats showed increased suprarenal adrenalin while females had a diminished amount after thyroid feeding. To add to the confusion, Kuriyama (37) observed no difference in the epinephrine content of adrenals between animals fed desiccated thyroid and control animals. Nogoescu, et al. (44) observed an increased adrenalin secretion, an increased reserve capacity of the adrenal medulla, and a decreased peripheral degradation of adrenalin in hyperthyroid subjects. In a recent review of adrenal medullary-thyroid relationships, Harrison (23) notes that the majority of evidence indicates normal urine and serum levels of catecholamines in hyperthyroidism, but that an increased sensitivity to adrenalin is present in this condition. In the adrenal medulla, treatment with thyroid hormone decreased the catecholamine content while thyrotrophic hormone had the opposite effect.

Studying more specific effects of a thyroid-catecholamine relationship, Brewster, et al. (8) noted that a sympathetic block abolished the normal increase in heart rate, oxygen consumption, cardiac index, and effective ventricular stroke work in thyroid-fed dogs. Bray (7), using various drugs and surgical treatments affecting thyroid and adrenal medulla functions, concluded that while some functional relationship exists, the adrenergic component predominated in control of the heart rate while the thyroid gland was the comparatively major factor regarding growth and BMR. Lutolf (39), in a series of investigations, observed an increase in the force and rate of contraction of the mammalian heart in response to adrenalin which was exaggerated by 1:20,000 solution of thyroxin. Heart damage occurred with a dilution of 1:2,000. It is apparent that thyroid hormone sensitization of the heart to adrenalin may lead to heart damage. The fact that Kurland, Hammond and Freedberg (38) noted that hyperthyroid animals had an increased concentration in comparison to euthyroid controls indicates that the sympathetic system may compensate for this change in sensitivity. When this compensation was overridden, Kroneberg and Huter (36) and Peltola (48) observed that animals pretreated with thyroxin were more sensitive to adrenalin than controls as measured by the LD_{50} dose. The role of the heart in this finding was not ascertained.

From the above literature, it can be seen that thyroid hormones and catecholamines are intimately related in their metabolism and actions. The synergistic action of these substances on the heart is of particular significance to the present study.

Relation of Thyroid Gland and Catecholamines to the Heart

Raab (55), in summarizing the potentially pathogenic phenomena of catecholamines in the heart, lists the neurosecretion of adrenalin with its hypoxia producing action and the potentiation of this action by thyroid hormones and mineralcorticoids as the primary factors. Repeated epinephrine injections have led to degenerative changes in heart muscle ranging from hyaline degeneration to necroses and scar formation which are intensified by thyroid hormone. Anoxic myocardial necrosis, including infarction, similar to that produced by epinephrine injection, has been elicited in animals by forced exercise and Faradization. Strenuous exercise, emotions, and similar conditions associated with excessive adrenosympathetic secretion have been identified as precipitating factors in most cases of non-occlusive myocardial necrosis. In specific studies reported in the literature, Bajusz and Raab (2) noted that subcutaneous injections of epinephrine in rats produced a decline or complete loss of phosphorylase, glycogen and potassium in areas of the heart where necrotic lesions are usually found. Regan (65) applied sustained left coronary infusion of l-epinephrine to a closed chest, anesthetized dog. He observed an early increase in contractility of the heart, increased R.Q., appearance of lactate, decreased glucose and free fatty acid extraction, and increased uptake of triglycerides, potassium and phosphate. One hour later, heart contractility declined, ions were released, and the altered lipid transport persisted for about three hours. Raab (59) hypothesized that anoxia, caused by exaggerated catecholamine action in vulnerable myocardial cell groups, may result in altered cell membrane permeability, permitting the loss of intracellular potassium which initiates the process of cell destruction. Selye (75) cites several sources, including evidence for cardiac damage on potassium deficient diets, to support the possible key position of potassium depletion in the process of heart degeneration. He feels, however, that mineralcorticoids are more important than catecholamines in this ion shift.

The case for catecholamine involvement with thyroid facilitation in heart degeneration is too strong to relegate it to a minor role. Oester (45) evaluating the incidence of aortic sclerosis after injection of various substances, found that both thyroxine and epinephrine were essential to produce epinephrine atherosclerosis since thyroxine injections in intact animals and epinephrine injections in thyroidectomized rabbits failed to produce sclerosis while epinephrine injections in intact animals resulted in an 80-90 per cent incidence of aortic sclerosis. Raab (60) noted that patients with essential hypertension or angina pectoris had abnormal increases in blood catecholamines in response to exercise. Work on rats indicated a fairly constant fatal heart concentration of catecholamines. In further work, Raab (54) found that thyroxine pretreatment in rats lowered the fatal concentration of catecholamines in the heart while thiouracil had the opposite effect. Raab, et al. (64) observed that antiadrenergic drugs diminished the severity of stress-induced myocardial lesions. A higher incidence of myocardial fibrosis in intact animals was noted by Gross and Greenberg (21) when comparing the response of intact and thyroidectomized animals to adrenalin and thyroid extract treatment. In dogs, Szakacs and Cannon (80) noted a dose dependent difference in cardiac lesions produced by continuous infusion of norepinephrine. Szakacs, Dimmettee and Cowart (81), in a review of pathologic lesions associated with catecholamines, proposed that the massive hemorrhagic mitral valves observed with large doses of norepinephrine are a result of mechanical factors while lesions, such as focal areas of necrosis, associated with therapeutic or small doses of norepinephrine were the result of metabolic factors.

The specific contribution of the adrenal medulla in heart lesions has not been studied. However, Bhagat (4), in comparing adrenal demudullated rats to sham operated controls, noted that demedullation significantly retarded the restoration of cardiac catecholamine levels after depletion while it had no effect on the final concentration.

This last section of review has attempted to show the separate and combined roles of catecholamines and thyroid hormones in the production of cardiovascular pathology, and some of the hypothesized mechanisms, such as potassium depletion, for this relationship. It should be noted that these studies were primarily pharmacological in nature and aid in establishing fruitful areas for clinical and physiological research.

Summary

Figure 1 presents the general picture provided by the evidence and hypotheses in the literature. The present investigation was designed to add information to this area by examining a number of the relationships in single study on the same animals.

Exercise
Acute Chronic

Anxiety

Catecholamines

Thyroid Gland

Myocardial Hypoxia

Myocardial Damage

Stimulatory ———
Inhibitory -----

Fig. 1.--Summary of Literature Review

CHAPTER III

EXPERIMENTAL DESIGN AND METHODS

This study was conducted to determine the effects of anxiety-producing stress and/or physical activity on the incidence and extent of cardiac necrosis with concomitant evaluation of relationships to structural and functional variations of the adrenal medulla and thyroid gland in laboratory animals.

Treatment Groups

The following ten treatment groups of animals were used:

1. A sedentary control group (S) received no special treatment during the experimental period, and was housed in standard individual sedentary cages for small animals. These cages are 24 cm. long by 18 cm. wide by 18 cm. tall.
2. A sedentary forced-exercise group (SF) was also confined in sedentary cages. Each animal in this group was forced to swim for thirty minutes, five days per week, in an individual metal cylindrical tank having a diameter of 28 cm. and a height of 75 cm. (water depth 70 cm.) A weight equal to two per cent of the animal's body weight was attached to the base of his tail during the swimming period.

3. A sedentary anxiety group (SA) was housed in sedentary cages and placed in individual electrical-stress cages for thirty minutes, five days per week. Each electrical-stress cage is constructed of 1/4 inch clear plastic and has a stainless steel rod grid serving as a floor. These cages are 17 cm. long by 17 cm. wide by 11 cm. tall. During the electrical-stress period each animal received a 0.36 sec. D.C. electrical shock of approximately 0.4 milliamps every fifteen seconds. This intensity of stimulation had been found prevously to be disturbing but noninjurious.
4. A sedentary forced-exercise plus anxiety group (SFA) was housed in sedentary cages and received both the swimming and electrical-stress treatments, in that order, five days per week.
5. A sedentary anxiety plus forced-exercise group (SAF) was housed in sedentary cages and received both the electrical-stress and swimming treatments, in that order, five days per week.
6. A voluntary exercise control group (V) received no special treatment during the experimental period, but was housed in individual spontaneous-exercise cages. Each of these cages has a standard individual sedentary cage for living quarters

plus a freely revolving activity wheel, 13 cm. in width and 35 cm. in diameter, which allows the animal to rest or exercise at will. An automatic counter records each revolution of the wheel.

7. A voluntary, forced-exercise group (VF) was housed in spontaneous-exercise cages and received the swimming treatment five days per week.
8. A voluntary exercise, anxiety group (VA) was housed in spontaneous-exercise cages and received the electrical-stress treatment.
9. A voluntary, forced-exercise plus anxiety group (VFA) was housed in spontaneous-exercise cages and received both the swimming and the electrical stress treatments, in that order, five days per week.
10. A voluntary exercise, anxiety plus forced-exercise group (VAF) was housed in spontaneous-exercise cages and received both the electrical-stress and swimming treatments, in that order, five days per week.

Sample

One hundred young adult (76 days old) male rats (Sprague-Dawley strain) were housed in spontaneous-exercise cages for fifteen days to permit adjustment to the laboratory and stabilization of voluntary-activity patterns. Activity records were kept during the final three days of this period. To obtain a relatively homogeneous sample, those animals with activity records in the highest and lowest deciles were eliminated from the study.

The remaining 80 animals were kept in spontaneous-exercise cages for an additional nine days. During this period 3 ml. blood samples and BMR measurements were taken by other investigators interested in studying the treatment effects on various blood and metabolic parameters.

The animals were randomly assigned to one of the ten treatment groups, and experimental procedures were begun when the animals were 101 days old. The treatments were continued for ten weeks, five days per week. The treatments were halted two days per week and food was withheld one day per week to permit weekly measurement of the BMR of all animals. Otherwise, all animals received water and a commercial diet ad libitum throughout the experiment. During the final week of treatments, a second blood sample was taken from all animals.

Measurements

Within five days after cessation of the experimental period, all animals were sacrificed using ether anesthesia. At this time, body weight was taken for each animal and numerous tissues, including heart, thyroid and adrenals were excised, trimmed and wet weights obtained.

One adrenal from each animal was treated according to the method of Eranko and Raisanen (18) for the determination of the intensity of the chromaffin reaction. The only significant changes in procedure were the use of a number 58 Wratten filter, 40 micron sections and seven determinations per animal. A test-retest, Pearson product moment reliability coefficient of only -0.05 was obtained from duplicate determinations on sixteen animals. Eranko and Raisanen (18) obtained a correlation coefficient of 0.947 between this histochemical technique and a chemical method for determining adrenal medulla catecholamine concentration, with the average of five to eight animals under the same experimental treatment representing one observation. They concluded that this pooling of data was necessary to compensate for the variability due to sampling error; however, they made no quantitative statement regarding the reliability of the technique. The original work in developing this technique was carried out on controls and animals subjected to reserpine injections or exhaustive exercise, and the difference in catecholamine concentrations of these animals may have been great enough to compensate for or decrease the variability in measurements on individual animals. The difference in treatment of animals is a possible explanation for the apparent discrepency in reliability of this technique.

The thyroids and hearts from all animals were fixed in 10 per cent formalin after they were trimmed and weighed. These tissues were subsequently embedded in paraffin, sectioned at a thickness of 7 microns, placed on glass slides and stained with hematoxylin and eosin.

The thyroid slides were then projected onto a piece of translucent, frosted acetate paper at a linear magnification of 750X by means of a Bausch and Lomb Photomicrographic Equipment, Model L. The outer and inner cell border of seven follicles from each animal were traced on two or more sheets of acetate paper with subsequent retracing with a planimeter to determine the area within the inner and outer cell borders. Assuming all follicles to be spherical, the difference between the radius of the outer cell border and the radius of the inner border was then calculated as a measurement of thyroid epithelial cell height (66). An inter-investigator coefficient of objectivity for cell height was calculated from duplicate data on sixteen animals and found to be 0.83.

Determination of the presence or absence of myocardial changes was made by microscopic examination of a histological section from the middle third of the ventricles from each animal. Since there was no obvious cardiac damage in any of the animals, a check-list of more subtle histological changes, including presence of Anitchkow cells, sarcoplasmic vacuolization or granulation, pleomorphic fiber nuclei, fragmentation of the fibers, differential eosin staining of fibers and relative number of sarcolemmal cells, was made to explore any possible significance of these parameters.

Missing Data

Three animals were eliminated from the study during the experimental period. One animal from the VFA group drowned; one from the SAF group was sacrificed due to hind limb paralysis and inability to continue treatments; and one animal from the SFA group was found dead in his cage. These animals were frozen until the end of the study when measurements were taken on all animals. The data from these animals, where available, are included in the appendices, but were excluded from the statistical evaluations.

In addition to the loss of the animals noted above, a number of measurements were not possible on the remaining animals due to improper handling of tissues. A total of four thyroid cell height measurements, one thyroid weight, and one heart evaluation were lost in this manner.

Statistical Procedures

The heart data were not statistically analyzed. The thyroid and adrenal medulla parameters were statistically evaluated by the unequal, one-way analysis of variance procedure (22). Since every treatment group did not have the same number of animals at the end of the experiment, the Scheffe method (22) for multiple comparisons was used to determine which groups of animals were significantly different when the results of the analysis of variance procedure warranted further statistical treatment of the data.

Since the purpose of this study was to obtain data and derive hypotheses concerning fruitful areas for future research, the level of significance for the statistical tests was arbitrarily set so there was a twenty-five per cent chance of rejecting a true null hypothesis. The null hypothesis in all tests was equality of treatment effects.

CHAPTER IV

RESULTS AND DISCUSSION

The results of this study are the effects of the treatments on changes in cardiac histology, the catecholamine concentration of the adrenal medulla, the epithelial cell height of the thyroid gland, and relative weight of the thyroid.

Heart Parameters

An initial examination of the heart sections revealed no obvious heart lesions in any of the animals. A subsequent pathologist's report on twenty of the sections confirmed this finding and indicated that the presence of Anitchkow cells and other more subtle changes in some of the animals were the only noteworthy signs of any disorder. The remaining heart slides were subsequently reevaluated for the presence or absence of these phenomena. This reevaluation of the heart sections was exploratory in nature and carried out to see if any relationship existed between the treatments and the histological changes observed. A summary of the histological findings is presented in Table I. Complete data are given in Appendix E.

Adrenal Medulla

Table II shows the mean values for optical density of the staining reaction for the treatment groups. Table III gives the analysis of variance table for these optical density measurements. The calculated value of the F statistic was not significant at the prescribed level, therefore no further analysis of these data was warranted.

TABLE I

SUMMARY OF HISTOLOGICAL FINDINGS IN THE HEART (Frequencies)

Group	N	Anitchkow Cells	Vacuolization of Sarcoplasm	Pleomorphic Nuclei	Granular Sarcoplasm	Fragmentation of Fibers	Differential Eosin Stain of Fibers	Increased Number of Sarcolemma Cells
S	8	3	1	0	0	2	0	2
SF	8	2	0	1	1	2	0	2
SA	7	3	0	3	0	4	0	1
SFA	7	4	0	0	0	1	0	0
SAF	7	3	1	0	0	2	1	2
V	8	5	1	1	1	3	0	2
VF	8	4	0	2	1	2	0	2
VA	8	7	0	1	0	0	0	0
VFA	7	4	0	0	0	1	0	4
VAF	8	4	0	2	0	2	0	4

TABLE II

ADRENAL MEDULLA CATECHOLAMINE
CONCENTRATION-GROUP MEANS
(Optical Density)

Group	N	Mean	Standard Deviation
S	8	0.305	0.0345
SF	8	0.315	0.0339
SA	8	0.273	0.0290
SFA	7	0.282	0.0769
SAF	7	0.300	0.0261
V	8	0.275	0.0270
VF	8	0.316	0.0295
VA	8	0.338	0.1532
VFA	7	0.295	0.0371
VAF	8	0.303	0.0321

TABLE III

ADRENAL MEDULLA CATECHOLAMINE
CONCENTRATION-ANALYSIS
OF VARIANCE

Source of Variance	df	Sum of Sq.	Mean Sq.	F Value
Between Groups	9	0.02893	0.00321	0.8519
Within Groups	67	0.25281	0.00377	
Total	76	0.28174		

Thyroid Epithelial Cell Height

Representation of group means and the analysis of variance for thyroid epithelial cell height are presented in Tables IV and V respectively. Since the F value for this variable was significant, further statistical analysis was utilized in an attempt to find the treatments which had a significant effect.

The Scheffe method for multiple comparisons was used in further analysis of the thyroid measurement with the significance level for all contrasts retained at twenty-five per cent. None of the contrasts involving the comparison of two group means were significant. The results of contrasts made on various combinations of groups are shown in Table VI. The sedentary vs. voluntary contrast compares all animals housed in sedentary cages to all animals housed in voluntary cages, regardless of other treatments. The forced vs. non-forced contrast is a similar comparison for the swimming treatment. Since the housing variable did not have a significant effect on thyroid epithelial cell height, the groups were compared on the basis of other treatments irrespective of housing condition. Additional contrasts on the basis of a stress rating seemed logical. Animals receiving both the anxiety and forced exercise treatments were designated as the high stress group; animals subjected to only one of these treatments were put in the medium stress group; and animals which did not receive either of these stressors were placed in the low stress group.

Relative Thyroid Weight

Tables VII and VIII show the group means and analysis of variance for relative thyroid weight respectively. Comparison of individual groups by the Scheffe contrast method are presented in Table IX while contrasts of combined groups similar to those compared for thyroid epithelial cell height are in Table X.

TABLE IV

THYROID EPITHELIAL CELL HEIGHT-GROUP MEANS (Microns)

Group	N	Mean	Standard Deviation
S	8	10.1275	1.30213
SF	8	10.6088	1.30921
SA	8	11.1300	0.96638
SFA	7	9.9514	1.74058
SAF	6	9.1133	2.05620
V	7	10.2600	1.61991
VF	8	10.5838	1.02004
VA	8	10.3062	1.02419
VFA	6	9.5100	0.83848
VAF	7	9.1671	1.03923

TABLE V

THYROID EPITHELIAL CELL HEIGHT-ANALYSIS OF VARIANCE

Source of Variance	df	Sum of Sq.	Mean Sq.	F Value
Between Groups	9	27.06849	3.00761	1.7212*
Within Groups	63	110.08757	1.74742	
Total	72	137.15607		

*Significant at the 0.25 level of confidence.

TABLE VI

THYROID EPITHELIAL CELL HEIGHT-SCHEFFE CONTRASTS FOR COMBINED GROUPS

Contrast	Critical Value	Observed[1] Value
Sedentary vs. Voluntary	1.06	+ 0.25
Forced vs. Non-Forced	1.06	- 0.58
S plus V vs. SF plus VF	0.82	- 0.41
S plus V vs. SA plus VA	0.82	- 0.53
S plus V vs. SFA plus VFA	0.86	+ 0.44
S plus V vs. SAF plus VAF	0.86	+ 1.05*
SF plus VF vs. SA plus VA	0.51	- 0.12
SF plus VF vs. SFA plus VFA	0.86	+ 0.85
SF plus VF vs. SAF plus VAF	0.86	+ 1.46*
SA plus VA vs. SFA plus VFA	0.86	+ 0.97*
SA plus VA vs. SAF plus VAF	0.86	+ 1.58*
SFA plus VFA vs. SAF plus VAF	0.89	+ 0.61
High vs. Medium Stress	1.20	- 1.21*
High vs. Low Stress	1.48	- 0.74
Medium vs. Low Stress	1.42	+ 0.47

*Significant at the 0.25 level of confidence

[1]The observed value is the difference between the means for the groups compared. A plus sign indicates that the groups listed on the left had a higher mean than the other groups in the contrast. A minus sign indicates the opposite occurrence.

TABLE VII

RELATIVE THYROID WEIGHT-GROUP MEANS (mg./100 gms.)

Group	N	Mean	Standard Deviation
S	8	5.437	0.7489
SF	8	6.580	0.8160
SA	8	5.231	0.9965
SFA	7	6.530	1.1733
SAF	7	5.822	0.4504
V	8	5.308	0.8456
VF	8	6.191	0.9197
VA	8	5.595	1.1733
VFA	7	6.117	0.9335
VAF	7	6.330	1.8379

TABLE VIII

RELATIVE THYROID WEIGHT-ANALYSIS OF VARIANCE

Source of Variance	df	Sum of Sq.	Mean Sq.	F Value
Between Groups	9	17.65658	1.96184	1.8217*
Within Groups	66	71.07577	1.07691	
Total	75	88.73235		

*Significant at the 0.25 level of confidence.

TABLE IX

RELATIVE THYROID WEIGHT-SCHEFFE CONTRASTS FOR INDIVIDUAL GROUPS

Contrast	Critical Value	Observed Value[a]
S vs SF	0.89	- 1.14*
S vs SA	0.89	+ 0.21
S vs SFA	0.92	- 1.09*
S vs SAF	0.92	- 0.39
S vs V	0.89	+ 0.13
S vs VF	0.89	- 0.75
S vs VA	0.89	- 0.16
S vs VFA	0.92	- 0.68
S vs VAF	0.92	- 0.89
SF vs SA	0.89	+ 1.35*
SF vs SFA	0.92	+ 0.05
SF vs SAF	0.92	+ 0.76
SF vs V	0.89	+ 1.27*
SF vs VF	0.89	+ 0.39
SF vs VA	0.89	+ 0.98*
SF vs VFA	0.92	+ 0.46
SF vs VAF	0.92	+ 0.25
SA vs SFA	0.92	- 1.30*
SA vs SAF	0.92	- 0.59
SA vs V	0.89	- 0.08
SA vs VF	0.89	- 0.96*
SA vs VA	0.89	- 0.36
SA vs VFA	0.92	- 0.89
SA vs VAF	0.92	- 1.10*
SFA vs SAF	0.95	+ 0.71
SFA vs V	0.92	+ 1.22*
SFA vs VF	0.92	+ 0.34
SFA vs VA	0.92	+ 0.93*
SFA vs VFA	0.95	+ 0.41
SFA vs VAF	0.95	+ 0.20
SAF vs V	0.92	+ 0.51
SAF vs VF	0.92	- 0.37
SAF vs VA	0.92	+ 0.23
SAF vs VFA	0.95	- 0.29
SAF vs VAF	0.95	- 0.51
V vs VF	0.89	- 0.88
V vs VA	0.89	- 0.29
V vs VFA	0.92	- 0.81
V vs VAF	0.92	- 1.02*
VF vs VA	0.89	+ 0.60
VF vs VFA	0.92	+ 0.07
VF vs VAF	0.92	- 0.14
VA vs VFA	0.92	- 0.52
VA vs VAF	0.92	- 0.73
VFA vs VAF	0.95	- 0.21

*Significant at the 0.25 level of confidence.

[a]The observed value is the difference between the means for the groups compared. A plus sign indicates that the groups listed on the left had a higher mean than the other groups in the contrast. A minus sign indicates the opposite occurrence.

TABLE X

RELATIVE THYROID WEIGHT-SCHEFFE CONTRASTS FOR COMBINED GROUPS

Contrast	Critical Value	Observed[1] Value
Sedentary vs Voluntary	1.78	+ 0.01
Forced vs Non-forced	0.82	+ 0.87*
S plus V vs SF plus VF	0.63	- 1.01*
S plus V vs SA plus VA	0.63	- 0.04
S plus V vs SFA plus VFA	0.65	- 0.95*
S plus V vs SAF plus VAF	0.65	- 0.70*
SF plus VF vs SA plus VA	0.63	+ 0.97*
SF plus VF vs SFA plus VFA	0.65	+ 0.06
SF plus VF vs SAF plus VAF	0.65	+ 0.31
SA plus VA vs SFA plus VFA	0.65	- 0.91*
SA plus VA vs SAF plus VAF	0.65	- 0.66*
SFA plus VFA vs SAF plus VAF	0.67	+ 0.25
High vs Medium Stress	0.92	+ 0.30
High vs Low Stress	1.12	+ 0.83
Medium vs Low Stress	1.09	+ 0.53

*Significant at the 0.25 level of confidence.

[1]The observed value is the difference between the means for the groups compared. A plus sign indicates that the groups listed on the left had a higher mean than the other groups in the contrast. A minus sign indicates the opposite occurrence.

Discussion

The fact that no significant myocardial lesions were present in these animals indicates that the stress was not sufficient in time and/or degree to elicit the damage which has been found in previous studies. The changes observed in cardiac histology were minor, and the significance of these findings is questionable at the present time. All of the changes noted are found routinely in autopsies of "normal" animals, and many of the phenomena are believed to be the result of variations in technique rather than actual alterations in the heart.

The measurements of thyroid epithelial cell height and relative weight provide the most interesting results of this study. One specific observation of note is that treatments which had a statistically significant effect on both parameters, affected one parameter differently than the other. For example, the combined group of S plus V had a higher epithelial cell height than the SAF plus VAF group, while the relative thyroid weights showed the reverse relationship. Two other contrasts had significant differences for both parameters, and followed this same pattern. Table XI, showing the rank order of the treatment groups for both parameters, indicates that almost all treatment groups with a low epithelial cell height had a high relative thyroid weight, and vice versa. The only exceptions to this pattern are the SF and VF groups. These groups were comparatively high on both parameters.

A second look at Table XI indicates that, in general, the groups receiving both the anxiety and forced exercise stresses had low epithelial cell heights and high relative thyroid weights. The animals receiving neither of the stresses or only the anxiety treatment showed the opposite trend.

Since previous investigations have indicated no variation in the response of the thyroid to different stressors, including exercise and emotional stress, the assumption of such variation in response to explain the results of the present study is not warranted. The most tenable hypothesis which is consistent with the present and previous data would appear to be a phasic, possibly triphasic, response of the thyroid gland to stress. Under this hypothesis, the animals receiving no stress or the anxiety stress, with a low relative thyroid weight and high epithelial cell height, would be in the initial phase of response. The animals forced to swim represent the second phase of the response with comparatively high values for both parameters. Animals subjected to both the swimming and anxiety treatments would be in third phase of the response, and have a high relative thyroid weight and low epithelial cell height. It cannot be determined if these data present all phases of the thyroid's response to stress, and therefore it must be left to future research to fill the gaps in the data and test the hypothesis.

TABLE XI

RANK ORDER OF THYROID PARAMETERS BY TREATMENT GROUPS[1]

Group	Epithelial Cell Height	Relative Weight
S	6	8
SF	2	1
SA	1	10
SFA	7	2
SAF	10	6
V	5	9
VF	3	4
VA	4	7
VFA	8	5
VAF	9	3

[1]Rank #1 indicates highest cell height and heaviest relative weight.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The purpose of this study was to evaluate the effects of emotional and physical stresses on changes in cardiac histology, the catecholamine concentration of the adrenal medulla, the epithelial cell height of the thyroid gland, and relative thyroid weight.

Eighty adult male albino rats were randomly assigned to ten treatment groups which received different combinations of an electric shock treatment and/or a forced exercise treatment. Half of the animals were housed in small sedentary cages and the rest in voluntary activity cages.

The experimental period lasted for ten weeks with the treatments applied for one-half hour a day, five days a week.

The incidence of myocardial changes was determined by subjective evaluation of a histological section from the middle third of the ventricles. The catecholamine concentration of the adrenal medulla was evaluated by a histospectophotometric measurement of a staining reaction. Thyroid epithelial cell height was determined by tracing the outer and inner cell border of several follicles with subsequent measurement and calculation. Relative thyroid weight was obtained by dividing the wet weight of the trimmed thyroid by the total body weight.

Analysis of variance, with use of the Scheffe method for multiple comparisons when warranted, was employed for the thyroid and adrenal medulla variables.

Conclusions

The formation of speculative hypotheses based on the data obtained in this investigation as well as previous information was deemed the most appropriate form for conclusions in this study.

Heart changes.--The apparent lack of any heart lesions as a result of the experimental treatments is contradictory to previous investigations of a similar nature. The most defensible hypothesis in this situation is that the experimental treatments, as defined and used in this study, were of insufficient severity and/or length to elicit cardiac damage. The more subtle changes in histology noted in this study tend to confirm this belief since the treatments had no apparent effect on them.

Adrenal medulla.--The only conclusion possible regarding the adrenal medulla catecholamine concentration is that the lack of reliability of the technique used makes it inappropriate for ascertaining small differences in concentration. The use of recently developed, more sophisticated histospectophotometric equipment would not appear to correct the limitations of this technique since the major proportion of the variability seems to be the result of sampling error.

Thyroid parameters.--A phasic response of the thyroid gland to stress can be hypothesized from the data on thyroid epithelial cell height and relative weight. If such a response exists, the logical sequence would be as follows: The initial or control "phase" may be characterized by a comparatively high cell height and a low relative weight. The next "phase" observed would be represented by high values for both parameters. The last "phase" observed might indicate a reversal of the initial phase. Further evidence, however, is needed to adequately test this hypothesis.

Recommendations

1. The use of the histospectophotometric technique for the determination of the adrenal medulla catecholamine concentration should be limited to investigations in which a greater range of concentration values are found due to the variability in sampling.
2. The severity and/or length of time of stressors should be increased until a satisfactory incidence of heart damage is elicited.

3. A more quantitative measurement of heart damage, which will permit the use of more powerful statistical procedures, should be utilized.
4. The evaluation of serial data on thyroid function should permit more conclusive statement regarding the presence or absence of a phasic stress response by this gland.

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APPENDICES

APPENDIX A

ADRENAL MEDULLA CATECHOLAMINE CONCENTRATION (Optical Density)

Animal #	Determination 1	2	3	4	5	6	7
Group S							
1	0.215	0.268	0.181	0.240	0.292	0.197	0.197
2	0.367	0.310	0.268	0.292	0.337	0.284	0.337
3	0.357	0.260	0.292	0.357	0.409	0.398	0.276
4	0.328	0.248	0.288	0.328	0.367	0.256	0.362
5	0.244	0.323	0.284	0.288	0.252	0.357	0.292
6	0.328	0.347	0.314	0.276	0.272	0.372	0.392
7	0.252	0.280	0.387	0.367	0.301	0.319	0.252
8	0.272	0.403	0.332	0.342	0.301	0.276	0.352
Group SF							
1	0.310	0.357	0.229	0.226	0.208	0.260	0.301
2	0.222	0.314	0.426	0.347	0.310	0.314	0.377
3	0.357	0.301	0.409	0.276	0.377	0.272	0.328
4	0.367	0.357	0.420	0.319	0.403	0.398	0.367
5	0.215	0.310	0.260	0.372	0.252	0.218	0.284
6	0.288	0.229	0.323	0.409	0.314	0.310	0.362
7	0.305	0.314	0.342	0.248	0.284	0.342	0.357
8	0.264	0.314	0.319	0.260	0.382	0.352	0.288
Group SA							
1	0.347	0.204	0.292	0.222	0.301	0.284	0.276
2	0.292	0.305	0.276	0.342	0.284	0.337	0.288
3	0.268	0.284	0.260	0.357	0.347	0.284	0.319
4	0.174	0.197	0.211	0.226	0.244	0.272	0.201
5	0.244	0.233	0.268	0.237	0.248	0.229	0.256
6	0.218	0.244	0.268	0.280	0.268	0.392	0.280
7	0.264	0.222	0.297	0.268	0.288	0.319	0.367
8	0.301	0.280	0.292	0.222	0.240	0.301	0.260
Group SFA							
1	0.140	0.092	0.100	0.086	0.137	0.089	0.125
2*							
3	0.310	0.367	0.323	0.403	0.292	0.357	0.226
4	0.362	0.444	0.305	0.297	0.310	0.268	0.284
5	0.264	0.301	0.347	0.310	0.284	0.352	0.297
6	0.222	0.377	0.301	0.305	0.211	0.284	0.292
7	0.268	0.337	0.222	0.280	0.403	0.392	0.276
8	0.352	0.332	0.328	0.305	0.310	0.264	0.280

*Animal died during the experiment

APPENDIX A--Continued

Animal #	Determination 1	2	3	4	5	6	7
Group SAF							
1	0.332	0.240	0.377	0.328	0.284	0.347	0.403
2	0.328	0.301	0.342	0.268	0.252	0.377	0.332
3	0.280	0.337	0.292	0.328	0.305	0.319	0.426
4	0.237	0.272	0.276	0.301	0.297	0.332	0.280
5*							
6	0.301	0.305	0.226	0.305	0.301	0.252	0.260
7	0.252	0.252	0.319	0.288	0.382	0.347	0.280
8	0.181	0.211	0.301	0.222	0.264	0.362	0.276
Group V							
1	0.323	0.208	0.226	0.272	0.305	0.328	0.215
2	0.288	0.276	0.347	0.284	0.256	0.260	0.301
3	0.284	0.328	0.301	0.323	0.264	0.301	0.319
4	0.164	0.240	0.229	0.248	0.229	0.208	0.215
5	0.256	0.328	0.292	0.187	0.284	0.352	0.276
6	0.260	0.137	0.240	0.301	0.332	0.301	0.297
7	0.268	0.237	0.319	0.244	0.276	0.292	0.248
8	0.328	0.403	0.268	0.284	0.276	0.310	0.260
Group VF							
1	0.301	0.438	0.211	0.284	0.444	0.174	0.357
2	0.310	0.301	0.420	0.301	0.357	0.409	0.301
3	0.328	0.244	0.284	0.319	0.319	0.387	0.398
4	0.288	0.357	0.367	0.276	0.297	0.305	0.288
5	0.310	0.323	0.305	0.414	0.342	0.276	0.392
6	0.260	0.332	0.337	0.367	0.280	0.284	0.222
7	0.305	0.328	0.272	0.229	0.204	0.222	0.226
8	0.377	0.332	0.332	0.248	0.382	0.387	0.342
Group VA							
1	0.276	0.347	0.276	0.272	0.292	0.310	0.218
2	0.168	0.226	0.280	0.252	0.323	0.222	0.305
3	0.244	0.233	0.284	0.268	0.229	0.260	0.305
4	0.276	0.398	0.292	0.332	0.260	0.292	0.292
5	0.248	0.268	0.288	0.284	0.260	0.284	0.280
6	0.244	0.469	0.319	0.288	0.292	0.319	0.280
7	0.323	0.244	0.305	0.301	0.244	0.332	0.319
8	0.495	0.620	0.688	0.757	0.838	0.782	0.810

*Animal died during the experiment

APPENDIX A--Continued

Animal #	Determination 1	2	3	4	5	6	7
Group VFA							
1*							
2	0.362	0.337	0.264	0.155	0.256	0.218	0.264
3	0.367	0.342	0.301	0.387	0.301	0.280	0.328
4	0.469	0.284	0.292	0.414	0.314	0.276	0.432
5	0.420	0.233	0.218	0.268	0.218	0.297	0.301
6	0.218	0.301	0.305	0.297	0.272	0.284	0.432
7	0.292	0.276	0.194	0.215	0.240	0.310	0.197
8	0.237	0.260	0.292	0.456	0.284	0.222	0.292
Group VAF							
1	0.264	0.233	0.276	0.319	0.305	0.301	0.268
2	0.211	0.268	0.201	0.260	0.276	0.314	0.284
3	0.438	0.305	0.260	0.244	0.244	0.323	0.226
4	0.276	0.328	0.314	0.276	0.403	0.319	0.367
5	0.222	0.323	0.237	0.248	0.256	0.319	0.297
6	0.143	0.310	0.268	0.409	0.276	0.409	0.357
7	0.377	0.248	0.352	0.319	0.502	0.323	0.314
8	0.260	0.352	0.377	0.372	0.328	0.420	0.403

*Animal died during the experiment

APPENDIX B

TEST-RETEST RELIABILITY DATA FOR ADRENAL MEDULLA CATECHOLAMINE CONCENTRATION (Optical Density)

Animal #	Determination						
	1	2	3	4	5	6	7
Test							
1	0.215	0.268	0.181	0.240	0.292	0.197	0.197
2	0.140	0.092	0.100	0.086	0.137	0.089	0.125
3	0.301	0.438	0.211	0.284	0.444	0.174	0.357
4	0.264	0.233	0.276	0.319	0.305	0.301	0.268
5	0.332	0.314	0.264	0.284	0.377	0.367	0.237
6	0.328	0.184	0.237	0.244	0.292	0.284	0.387
7	0.438	0.297	0.337	0.342	0.352	0.450	0.310
8	0.332	0.240	0.377	0.328	0.284	0.347	0.403
9	0.244	0.252	0.252	0.323	0.332	0.256	0.337
10	0.328	0.301	0.342	0.268	0.252	0.377	0.332
11	0.292	0.233	0.328	0.292	0.268	0.328	0.377
12	0.292	0.264	0.319	0.342	0.357	0.342	0.323
13	0.323	0.208	0.226	0.272	0.305	0.328	0.215
14	0.237	0.328	0.398	0.352	0.319	0.305	0.301
15	0.280	0.337	0.292	0.328	0.305	0.319	0.426
16	0.367	0.310	0.268	0.292	0.337	0.284	0.337
Retest							
1	0.301	0.264	0.301	0.292	0.268	0.337	0.297
2	0.310	0.284	0.268	0.305	0.310	0.301	0.367
3	0.229	0.347	0.204	0.301	0.292	0.310	0.301
4	0.256	0.284	0.280	0.314	0.310	0.305	0.342
5	0.357	0.347	0.414	0.305	0.292	0.387	0.377
6	0.237	0.244	0.305	0.319	0.276	0.332	0.280
7	0.310	0.233	0.357	0.264	0.372	0.314	0.272
8	0.305	0.240	0.292	0.222	0.292	0.372	0.244
9	0.229	0.260	0.268	0.319	0.347	0.272	0.256
10	0.260	0.337	0.314	0.347	0.372	0.352	0.450
11	0.284	0.276	0.319	0.305	0.367	0.398	0.284
12	0.264	0.288	0.314	0.284	0.284	0.260	0.337
13	0.244	0.357	0.268	0.222	0.218	0.233	0.229
14	0.256	0.226	0.319	0.280	0.191	0.256	0.248
15	0.252	0.201	0.276	0.256	0.260	0.284	0.264
16	0.392	0.256	0.268	0.244	0.332	0.284	0.233

APPENDIX C

AREA OF OUTER AND INNER CELL BORDER OF THYROID FOLLICLES (Square inches)[a]

Animal #	Follicle # 1	2	3	4	5	6	7
Group S							
1 Outer	1.79	1.15	3.41	1.64	1.74	3.49	1.68
Inner	0.61	0.45	1.56	0.37	0.30	1.54	0.75
2 Outer	2.34	2.18	1.82	1.72	2.40	2.77	2.23
Inner	0.70	0.83	1.02	0.28	0.84	0.85	0.81
3 Outer	1.19	1.70	4.35	2.69	1.37	0.80	3.24
Inner	0.30	0.93	3.15	1.37	0.28	0.18	1.97
4 Outer	1.70	1.32	4.25	2.14	1.77	1.82	2.88
Inner	0.78	0.28	2.03	0.87	0.57	0.70	1.07
5 Outer	3.55	1.23	6.10	1.46	1.65	3.72	2.47
Inner	2.40	0.30	4.11	0.60	1.02	2.24	1.28
6 Outer	2.53	2.27	0.65	3.39	2.91	4.00	2.03
Inner	1.19	0.56	0.22	1.35	1.79	2.11	0.88
7 Outer	3.70	1.12	2.13	2.13	2.89	2.47	2.55
Inner	1.46	0.54	0.81	0.78	1.45	0.92	0.95
8 Outer	1.73	1.91	2.35	4.27	3.37	1.34	2.24
Inner	0.75	0.71	0.77	2.47	1.78	0.47	1.03
Group SF							
1 Outer	3.37	4.38	2.37	2.68	3.01	2.03	2.55
Inner	1.78	1.10	1.22	1.59	1.85	1.08	1.28
2 Outer	1.62	1.00	2.78	2.90	3.62	1.69	1.91
Inner	0.46	0.19	1.32	1.38	1.55	0.72	1.00
3 Outer	2.17	3.43	2.41	1.74	2.19	3.76	3.17
Inner	0.81	1.50	0.53	0.44	0.63	1.86	1.07
4 Outer	1.88	4.43	1.52	1.77	2.29	1.49	2.03
Inner	0.76	2.41	0.54	0.57	0.87	0.57	0.70
5 Outer	4.47	2.82	2.32	1.20	1.87	3.15	3.35
Inner	2.53	1.44	0.93	0.48	0.69	2.12	1.35
6 Outer	2.99	1.58	1.85	1.04	5.28	1.96	1.90
Inner	0.75	0.49	0.57	0.35	2.18	0.60	0.54
7 Outer	0.72	2.43	3.00	1.51	1.45	4.11	2.68
Inner	0.39	1.14	1.37	0.66	0.43	2.14	0.99
8 Outer	3.70	1.72	1.66	2.19	5.10	1.59	3.43
Inner	2.20	0.57	0.49	1.00	2.57	0.55	1.68

[a]At a linear magnification of 750X

APPENDIX C--Continued

Animal #		Follicle # 1	2	3	4	5	6	7
Group SA								
1	Outer	1.28	4.77	2.70	1.09	0.94	3.26	1.35
	Inner	0.39	3.45	1.45	0.58	0.15	1.26	0.33
2	Outer	2.28	3.42	2.34	1.00	1.89	3.33	2.01
	Inner	1.13	1.67	0.71	0.17	0.43	1.88	0.73
3	Outer	3.65	2.12	0.99	2.98	3.78	1.15	2.09
	Inner	1.56	0.92	0.18	1.34	1.71	0.25	0.75
4	Outer	2.06	0.76	1.68	2.34	2.50	1.00	3.16
	Inner	0.43	0.05	0.46	0.97	0.92	0.20	0.87
5	Outer	4.28	4.52	1.00	4.40	1.66	5.50	1.68
	Inner	1.67	2.55	0.34	2.56	0.50	2.75	0.52
6	Outer	1.24	1.84	3.25	2.23	2.75	1.19	2.38
	Inner	0.40	0.55	1.13	0.90	0.87	0.24	0.99
7	Outer	0.99	1.94	1.95	2.93	2.67	3.02	1.73
	Inner	0.25	0.95	0.74	1.24	1.09	1.36	0.49
8	Outer	2.09	1.16	3.68	3.26	4.60	1.79	1.82
	Inner	0.70	0.25	1.89	1.67	1.69	0.52	0.63
Group SFA								
1	Outer	2.34	0.55	1.95	1.95	2.13	1.92	0.87
	Inner	1.14	0.14	1.00	0.94	0.96	1.23	0.25
2**	Outer							
	Inner							
3*	Outer	1.60	3.50	1.37	3.51	2.04	4.04	2.18
	Inner	0.74	2.00	0.52	2.20	1.12	1.82	0.84
4	Outer	3.60	1.93	2.40	3.14	1.62	2.87	2.67
	Inner	1.32	0.65	0.92	1.69	0.30	0.83	1.21
5	Outer	2.26	1.92	2.25	1.47	2.30	1.11	2.03
	Inner	0.73	0.30	0.76	0.55	0.97	0.19	0.41
6	Outer	1.97	2.33	2.40	1.42	1.99	2.17	0.93
	Inner	0.89	0.98	1.02	0.57	0.85	0.95	0.28
7	Outer	2.84	1.44	4.45	3.45	2.05	1.95	2.37
	Inner	1.28	0.42	2.20	1.82	0.75	0.75	1.11
8	Outer	5.10	0.95	2.51	2.20	3.07	3.00	2.07
	Inner	3.17	0.46	1.01	0.98	1.73	1.63	0.97

*Animal died during the experiment
**Missing value due to loss of tissue

APPENDIX C--Continued

Animal #		Follicle # 1	2	3	4	5	6	7
Group SAF								
1	Outer	1.15	4.55	0.86	3.48	3.63	2.25	1.23
	Inner	0.37	2.67	0.23	1.72	1.91	1.37	0.70
2**	Outer							
	Inner							
3	Outer	2.22	0.45	2.96	2.54	0.85	2.05	2.51
	Inner	0.93	0.17	1.04	0.80	0.37	0.83	1.17
4	Outer	3.20	2.83	1.30	2.84	3.14	2.35	1.34
	Inner	1.50	0.96	0.40	1.65	0.90	0.84	0.23
5	Outer	3.62	3.26	2.52	0.93	3.26	1.93	0.97
	Inner	2.71	1.23	1.67	0.41	2.09	0.76	0.40
6	Outer	1.25	1.23	1.39	1.98	1.27	1.68	1.97
	Inner	0.89	0.88	0.69	0.97	0.69	0.94	1.04
7	Outer	2.13	1.30	1.31	3.83	3.75	1.60	1.57
	Inner	1.10	0.66	0.61	2.18	1.97	0.65	0.58
8*	Outer	2.69	2.75	2.68	1.28	1.79	3.71	2.50
	Inner	1.27	1.46	1.13	0.40	0.80	1.29	0.95
Group V								
1	Outer	2.41	1.25	1.66	2.54	2.55	1.37	3.77
	Inner	0.96	0.34	0.40	0.73	0.89	0.18	1.78
2	Outer	4.00	3.20	1.65	3.65	1.54	2.73	1.32
	Inner	2.58	1.91	0.67	2.19	0.65	1.62	0.47
3	Outer	2.66	3.43	2.78	0.98	2.58	3.37	2.18
	Inner	1.27	2.05	1.41	0.32	1.29	1.72	0.97
4**	Outer							
	Inner							
5	Outer	2.07	2.42	4.33	1.32	2.68	2.50	3.35
	Inner	0.97	1.23	2.50	0.41	1.33	1.20	1.75
6	Outer	2.33	2.15	3.15	3.96	2.58	4.57	1.45
	Inner	1.02	0.97	1.30	2.26	1.03	2.47	0.32
7	Outer	2.33	3.42	1.55	2.74	2.16	2.67	1.20
	Inner	1.16	1.60	0.45	1.34	0.87	1.28	0.32
8	Outer	1.50	2.58	1.92	2.06	1.60	3.78	2.05
	Inner	0.46	0.89	0.67	0.76	0.34	1.25	0.65

**Missing value due to loss of tissue.
*Animal died during the experiment.

APPENDIX C--Continued

Animal #		Follicle # 1	2	3	4	5	6	7
Group VF								
1	Outer	1.91	0.68	2.31	1.00	3.19	3.36	1.97
	Inner	0.71	0.22	1.16	0.25	1.50	1.78	1.04
2	Outer	2.05	2.54	2.44	1.45	1.47	3.66	4.80
	Inner	0.82	1.03	1.08	0.30	0.36	1.96	2.23
3	Outer	4.63	1.15	1.52	2.61	1.85	3.18	2.10
	Inner	1.98	0.34	0.98	1.05	0.82	1.73	0.45
4	Outer	1.49	3.43	3.69	1.14	2.01	1.37	2.18
	Inner	0.68	1.74	1.62	0.23	0.63	0.37	0.52
5	Outer	1.93	1.08	4.78	3.34	3.63	1.73	1.62
	Inner	0.53	0.33	1.70	1.46	1.50	0.49	0.65
6	Outer	3.33	3.28	2.25	2.63	2.42	3.07	2.38
	Inner	1.33	2.12	0.90	1.30	1.18	1.32	0.87
7	Outer	4.64	2.19	3.74	2.13	2.41	3.53	3.47
	Inner	2.50	0.65	1.82	0.97	1.02	1.40	1.70
8	Outer	1.40	2.14	2.28	1.80	0.92	1.79	1.81
	Inner	0.55	1.15	0.80	0.51	0.23	0.86	0.69
Group VA								
1	Outer	1.00	2.50	1.23	1.90	4.55	2.64	2.67
	Inner	0.09	0.70	0.30	0.73	2.08	1.05	1.25
2	Outer	2.25	3.98	2.75	3.75	1.74	1.68	2.04
	Inner	0.83	2.35	1.35	2.05	0.86	0.72	0.96
3	Outer	1.93	1.85	1.37	1.95	2.74	1.91	2.53
	Inner	0.57	0.68	0.27	0.67	1.48	0.80	1.41
4	Outer	2.00	2.48	2.48	3.70	4.22	1.11	1.64
	Inner	0.79	0.89	0.79	2.35	1.83	0.38	0.39
5	Outer	2.40	2.86	3.52	1.16	1.11	2.27	3.39
	Inner	1.33	1.32	1.63	0.25	0.26	0.80	1.80
6	Outer	4.66	1.53	1.84	1.47	2.13	1.20	3.15
	Inner	2.18	0.35	0.73	0.58	0.73	0.28	1.46
7	Outer	2.35	3.05	2.14	2.14	0.92	1.97	2.19
	Inner	1.33	1.19	1.17	0.93	0.18	1.23	1.14
8	Outer	2.42	3.09	1.69	3.20	2.82	2.38	1.65
	Inner	0.89	1.55	0.69	1.80	1.23	0.84	0.58
Group VFA								
1	Outer	1.20	1.39	2.05	1.06	1.97	3.63	2.77
	Inner	0.69	0.75	1.18	0.44	1.17	2.58	1.67
2*	Outer	2.62	1.35	2.05	3.32	2.70	0.92	6.27
	Inner	0.80	0.40	1.21	1.68	1.57	0.23	3.33
3	Outer	2.64	1.98	2.87	1.82	1.39	1.21	1.73
	Inner	1.92	0.59	1.20	0.40	0.37	0.35	0.53
4**	Outer							
	Inner							

APPENDIX C--Continued

Animal #		Follicle # 1	2	3	4	5	6	7
Group VFA								
5	Outer	2.69	2.23	3.63	0.70	1.15	2.19	2.26
	Inner	1.47	1.47	2.19	0.14	0.60	0.52	0.93
6	Outer	1.84	2.70	1.13	1.84	2.15	1.70	3.01
	Inner	0.76	1.23	0.20	0.80	1.00	0.84	1.42
7	Outer	3.00	1.75	2.51	1.65	2.50	1.54	3.10
	Inner	1.25	0.80	1.30	0.95	1.16	0.53	2.33
8	Outer	2.55	2.39	1.26	3.28	3.19	0.93	2.53
	Inner	0.96	0.67	0.41	1.78	1.72	0.28	1.36
Group VAF								
1	Outer	1.50	1.06	1.74	4.75	2.85	4.19	1.89
	Inner	0.41	0.38	0.57	2.70	1.82	2.48	0.92
2	Outer	2.40	2.54	0.93	1.85	3.85	5.84	3.07
	Inner	1.11	1.19	0.18	0.80	1.68	3.67	1.30
3	Outer	1.35	2.83	1.34	1.94	3.00	2.70	1.98
	Inner	0.58	1.42	0.37	0.87	1.42	0.92	0.96
4	Outer	2.75	2.95	0.92	2.30	1.99	2.12	1.56
	Inner	1.37	1.50	0.28	0.99	0.78	1.10	0.60
5**	Outer							
	Inner							
6	Outer	3.46	1.36	0.90	2.29	3.20	2.77	1.64
	Inner	2.27	0.58	0.40	1.20	2.05	1.71	0.85
7	Outer	1.37	2.53	3.10	1.25	2.18	2.95	1.99
	Inner	0.54	1.52	1.11	0.42	0.90	1.35	1.15
8	Outer	1.87	2.66	1.49	2.40	1.57	1.37	2.89
	Inner	0.50	0.82	0.65	0.73	0.41	0.22	1.00

*Animal died during the experiment.
**Missing value due to loss of tissue.

APPENDIX D

INTER-OBSERVER OBJECTIVITY DATA FOR AREA OF OUTER AND INNER CELL BORDER OF THYROID FOLLICLES (Square inches)

Animal #		Follicle # 1	2	3	4	5	6	7
Observer #1								
1	Outer	1.79	1.15	3.41	1.64	1.74	3.49	1.69
	Inner	0.61	0.45	1.56	0.37	0.30	1.54	0.75
2	Outer	0.84	1.78	3.60	1.75	4.85	1.52	2.85
	Inner	0.15	1.08	2.77	0.92	3.20	0.64	1.75
3	Outer	2.34	0.55	1.95	1.95	2.13	1.92	0.87
	Inner	1.14	0.14	1.00	0.94	0.96	1.23	0.25
4	Outer	1.91	0.68	2.31	1.00	3.19	3.36	1.97
	Inner	0.71	0.22	1.16	0.25	1.50	1.78	1.04
5	Outer	1.50	1.06	1.74	4.75	2.85	4.19	1.89
	Inner	0.41	0.38	0.57	2.70	1.82	2.48	0.92
6	Outer	3.99	3.35	1.20	1.72	2.65	1.35	1.40
	Inner	2.10	2.17	0.50	0.72	1.39	0.63	0.42
7	Outer	2.63	2.05	2.28	3.47	4.59	1.83	0.60
	Inner	1.11	1.05	1.22	1.93	2.73	0.74	0.10
8	Outer	2.25	4.50	2.66	1.99	2.54	1.73	1.77
	Inner	1.10	2.80	1.20	0.96	1.03	1.10	0.75
9	Outer	1.15	4.55	0.86	3.48	3.63	2.25	1.23
	Inner	0.37	2.67	0.23	1.72	1.91	1.37	0.70
10	Outer	1.27	3.65	3.67	2.00	2.92	4.34	2.87
	Inner	0.15	2.14	1.57	1.10	1.44	2.23	0.74
11	Outer	1.57	3.34	2.85	2.67	2.53	4.40	4.65
	Inner	0.56	1.31	1.10	0.69	0.76	1.70	2.20
12	Outer	4.37	2.25	0.95	2.10	2.45	4.16	1.75
	Inner	2.83	1.25	0.54	1.27	1.22	2.93	0.78
13	Outer	2.41	1.25	1.66	2.54	2.55	1.37	3.77
	Inner	0.96	0.34	0.40	0.73	0.89	0.18	1.78
14	Outer	1.72	2.16	1.83	2.28	4.70	2.04	1.93
	Inner	0.70	1.07	0.89	1.20	3.08	0.94	0.93
15	Outer	2.22	0.45	2.96	2.54	0.85	2.05	2.51
	Inner	0.93	0.17	1.04	0.80	0.37	0.83	1.17
16	Outer	2.34	2.18	1.82	1.72	2.40	2.77	2.23
	Inner	0.70	0.83	1.02	0.28	0.84	0.85	0.81

APPENDIX D--Continued

Animal #		Follicle # 1	2	3	4	5	6	7
Observer #2								
1	Outer	7.10	5.46	2.07	1.86	2.28	3.82	2.76
	Inner	5.17	3.01	1.08	0.76	1.09	1.92	1.63
2	Outer	5.74	3.78	1.69	1.74	1.80	3.21	7.79
	Inner	4.54	2.95	0.87	1.14	1.09	2.00	5.72
3	Outer	6.69	1.83	1.73	3.56	4.72	5.42	1.03
	Inner	4.66	1.06	0.68	2.54	3.22	4.26	0.31
4	Outer	8.68	5.68	1.90	2.16	2.07	2.15	3.21
	Inner	6.40	2.99	0.88	0.95	0.99	1.10	1.83
5	Outer	6.92	4.90	2.95	3.28	3.06	3.39	2.07
	Inner	4.70	3.17	1.53	2.04	1.83	1.86	1.02
6	Outer	3.17	7.58	3.83	1.34	1.86	4.12	3.45
	Inner	2.13	5.76	2.32	0.53	0.66	2.55	1.99
7	Outer	2.64	1.63	1.68	4.32	7.24	4.49	3.33
	Inner	1.51	0.83	0.80	2.90	4.68	2.70	1.95
8	Outer	4.31	1.97	3.07	1.97	6.04	3.80	1.93
	Inner	2.97	0.99	1.52	1.00	3.96	2.49	0.92
9	Outer	2.49	4.85	3.61	1.65	8.53	1.58	4.36
	Inner	1.28	2.69	1.88	0.44	6.11	0.84	3.08
10	Outer	2.57	4.20	2.65	1.23	5.59	4.42	2.86
	Inner	1.22	2.44	1.20	0.32	3.65	2.73	1.41
11	Outer	4.20	3.48	1.39	3.34	8.14	3.79	1.34
	Inner	2.08	1.69	0.44	1.61	5.13	1.87	0.43
12	Outer	7.00	4.64	1.71	2.31	2.96	4.40	2.35
	Inner	5.59	3.15	1.03	1.28	1.87	2.92	1.59
13	Outer	6.14	3.80	1.20	2.85	9.64	3.79	0.87
	Inner	3.79	1.85	0.40	1.71	6.07	1.89	0.41
14	Outer	6.77	2.72	1.03	2.95	4.30	3.06	2.10
	Inner	4.46	1.32	0.35	1.46	2.63	1.65	0.90
15	Outer	4.98	2.18	2.96	2.18	7.18	3.71	1.62
	Inner	2.94	1.03	1.87	0.80	5.73	1.90	0.77
16	Outer	1.05	4.93	1.67	2.78	2.69	4.64	2.76
	Inner	0.19	2.50	0.57	1.22	1.08	2.37	0.63

APPENDIX E

HISTOLOGICAL FINDINGS IN THE HEART[1]

Animal #	Anitchkow Cells	Vacuolization of Sarcoplasm	Pleomorphic Nuclei	Granular Sarcoplasm	Fragmentation of Fibers	Differential Eosin Stain of Fibers	Increased number of Sarcolemma Cells
Group S							
1	0	X	0	0	0	0	0
2	0	0	0	0	X	0	X
3	0	0	0	0	0	0	0
4	X	0	0	0	0	0	0
5	X	0	0	0	0	0	0
6	0	0	0	0	0	0	0
7	0	0	0	0	X	0	0
8	X	0	0	0	0	0	X
Group SF							
1	0	0	0	0	0	0	0
2	X	0	0	X	0	0	X
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	X	0	0	0	X	0	X
6	0	0	0	0	0	0	0
7	0	0	X	0	X	0	0
8	0	0	0	0	0	0	0
Group SA							
1	0	0	X	0	0	0	0
2	0	0	0	0	X	0	0
3	0	0	0	0	0	0	0
4	X	0	0	0	X	0	0
5	X	0	X	0	X	0	X
6	X	0	X	0	X	0	0
7	0	0	0	0	0	0	0
8**							

APPENDIX E--Continued

Animal #	Anitchkow Cells	Vacuolization of Sarcoplasm	Pleomorphic Nuclei	Granular Sarcoplasm	Fragmentation of Fibers	Differential Eosin Stain of Fibers	Increased Number of Sarcolemma Cells
Group SFA							
1	0	0	0	0	0	0	0
2*							
3	0	0	0	0	0	0	0
4	X	0	0	0	X	0	0
5	0	0	0	0	0	0	0
6	X	0	0	0	0	0	0
7	X	0	0	0	0	0	0
8	X	0	0	0	0	0	0
Group SAF							
1	0	0	0	0	0	0	0
2	X	X	0	0	0	0	X
3	0	0	0	0	0	0	0
4	0	0	0	0	X	0	0
5*							
6	X	0	0	0	0	X	X
7	0	0	0	0	X	0	0
8	X	0	0	0	0	0	0
Group V							
1	X	X	0	X	0	0	X
2	0	0	0	0	0	0	0
3	X	0	0	0	0	0	0
4	X	0	0	0	0	0	0
5	0	0	0	0	X	0	X
6	X	0	0	0	X	0	0
7	X	0	X	0	0	0	0
8	0	0	0	0	X	0	0
Group VF							
1	0	0	0	0	0	0	0
2	X	0	X	X	X	0	0
3	X	0	0	0	X	0	0
4	X	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	X	0	0	0	0
[illegible]	[illegible]	[illegible]	[illegible]	0	[illegible]	[illegible]	[illegible]
				0			

APPENDIX E--Continued

Animal #	Anitchkow Cells	Vacuolization of Sarcoplasm	Pleomorphic Nuclei	Granular Sarcoplasm	Fragmentation of Fibers	Differential Eosin Stain of Fibers	Increased Number of Sarcolemma Cells
Group VA							
1	X	0	0	0	0	0	0
2	X	0	X	0	0	0	0
3	X	0	0	0	0	0	0
4	X	0	0	0	0	0	0
5	X	0	0	0	0	0	0
6	X	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	X	0	0	0	0	0	0
Group VFA							
1*							
2	0	0	0	0	X	0	X
3	0	0	0	0	0	0	X
4	0	0	0	0	0	0	0
5	X	0	0	0	0	0	0
6	X	0	0	0	0	0	X
7	X	0	0	0	0	0	0
8	X	0	0	0	0	0	X
Group VAF							
1	X	0	X	0	0	0	X
2	X	0	0	0	0	0	X
3	X	0	0	0	0	0	0
4	X	0	0	0	X	0	0
5	0	0	X	0	0	0	0
6	0	0	0	0	X	0	X
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	X

*Animal died during the experiment
**Missing value due to loss of tissue
[1] X-Indicates presence of phenomenon
O-Indicates absence of phenomenon

APPENDIX E--Continued

Animal #	Anitchkow Cells	Vacuolization of Sarcoplasm	Pleomorphic Nuclei	Granular Sarcoplasm	Fragmentation of Fibers	Differential Eosin Stain of Fibers	Increased Number of Sarcolemma Cells
Group VA							
1	X	0	0	0	0	0	0
2	X	0	X	0	0	0	0
3	X	0	0	0	0	0	0
4	X	0	0	0	0	0	0
5	X	0	0	0	0	0	0
6	X	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	X	0	0	0	0	0	0
Group VFA							
1*							
2	0	0	0	0	X	0	X
3	0	0	0	0	0	0	X
4	0	0	0	0	0	0	0
5	X	0	0	0	0	0	0
6	X	0	0	0	0	0	X
7	X	0	0	0	0	0	0
8	X	0	0	0	0	0	X
Group VAF							
1	X	0	X	0	0	0	X
2	X	0	0	0	0	0	X
3	X	0	0	0	0	0	0
4	X	0	0	0	X	0	0
5	0	0	X	0	0	0	0
6	0	0	0	0	X	0	X
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	X

*Animal died during the experiment
**Missing value due to loss of tissue

[1] X-Indicates presence of phenomenon
O-Indicates absence of phenomenon

APPENDIX F

BODY WEIGHT (gms)

Animal #	S	SF	SA	SFA	SAF
			Group		
1	452	358	417	395	458
2	401	434	379	---	437
3	374	359	347	312	376
4	462	364	413	410	410
5	362	498	374	333	---
6	432	465	450	332	404
7	469	456	376	397	368
8	468	411	415	385	411

Animal #	V	VF	VA	VFA	VAF
			Group		
1	402	383	414	---	352
2	351	342	316	316	342
3	427	430	402	264	357
4	354	377	395	374	342
5	365	412	378	437	424
6	442	267	358	344	353
7	392	335	345	346	327
8	361	356	510	346	383

APPENDIX G

THYROID WEIGHT (gms)

Animal #	Group S	SF	SA	SFA	SAF
1	0.0209	0.0212	0.0192	0.0214	0.0255
2	0.0231	0.0296	0.0251	------	0.0275
3	0.0186	0.0274	0.0161	0.0230	0.0227
4	0.0270	0.0266	0.0234	0.0204	0.0214
5	0.0253	0.0341	0.0168	0.0209	------
6	0.0214	0.0303	0.0204	0.0266	0.0257
7	0.0244	0.0300	0.0256	0.0305	0.0218
8	0.0241	0.0206	0.0186	0.0230	0.0220

Animal #	Group V	VF	VA	VFA	VAF
1	0.0196	0.0229	0.0195	------	0.0187
2	0.0237	0.0179	0.0225	0.0233	0.0286
3	0.0174	0.0250	0.0202	0.0129	0.0204
4	0.0178	0.0205	0.0282	0.0229	0.0169
5	0.0176	0.0239	0.0158	0.0235	------
6	0.0276	0.0202	0.0162	0.0250	0.0244
7	0.0213	0.0202	0.0195	0.0214	0.0295
8	0.0189	0.0273	0.0327	0.0194	0.0155