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Chapter I

A large body of research has been formulated which establishes that catecholamine and corticoid secretions are related to conditions of human stress (Bogdonoff, 1960; Elmadjian, 1956; Feller, 1964; Funkenstein, 1956; Kotchen, 1971). Pioneer efforts in this area were made by Walter Cannon who published one of the first such research treatises in 1911. Further interest in the topic was spurred by Hans Selye with the publication of his classic volume of 1947, in which he stated, in part,

> . . . numerous individual manifestations of the alarm reaction in particular, and of the general-adaptation-syndrome as a whole, . . . are only individual manifestations of a coordinated syndrome whose ultimate aim is defense. This syndrome evolves in three distinct stages (the alarm reaction, the stage of resistance and the stage of exhaustion) and its primary biologic purpose is to raise resistance to non-specific stress (p. 840).

With widespread agreement that secretions of the adrenal glands are related to stress (Frankenhaeuser, 1964; Kotchen, 1971; Renold, 1951; von Euler and Hellner, 1952) it is logical to expect that quantitative data regarding secretions of epinephrine and norepinephrine would have been used to explore the validity of Selye's theory. Several workers including Elmadjian, et al., (1956), Zuspan (1970), Feller and Hale (1964) and Bogdonoff, et al., (1960) have found that quantities of catecholamines in the urine correlated positively with conditions of stress. Their opinions differ, however, regarding specific and non-specific stresses, in Selye's sense, and the differential secretions of catecholamines. Epinephrine and norepinephrine elicit different physiological reactions, with epinephrine evoking widespread reactions and norepinephrine being responsible principally for increasing peripheral resistance (Funkenstein, 1956). It has been found, accordingly, that inwardly directed anger (Funkenstein, 1956), acute anxiety (Curtis, 1960; Bogdonoff, 1960; Feller, 1960), maximum physical work (Kotchen, 1971) and both fear and anger (Arnold, 1945) elevate significantly the urinary levels of epinephrine, whereas overt behavior (Funkenstein, 1956; Friedman, 1960), chronic stressors such as long term submaximal exercise (Kotchen, 1971), depression (Curtis, 1960), and cold (Feller, 1964) have been associated with elevated levels of norepinephrine.

Regardless of a possible flaw in Selye's argument for the General Adaptation Syndrome as it proceeds from non-specific stress, it is agreed that stress does affect the autonomic nervous system, and sympathetic and parasympathetic reactions can produce adjustments until homeostasis is regained. Improvement of the functioning of the autonomic nervous system should result, therefore, in more efficient reactions to stress (Michael, 1957). Since long-term physical exercise appears to increase the size and sensitivity of the adrenal glands (Engle, 1953; Richter, 1953; Hoagland, 1955), it is possible that such activity can result in a more efficient response to stress.

The use of exercise as a means of adapting to stress is advantageous in that exercise can affect the adaptive mechanism without increasing also a reaction to an emotion (Persky, 1953; Michael, 1957). The variables of type, frequency and duration of exercise have not, however, been determined.

Statement of the Problem

The investigation entailed the study of 13 adult women volunteers between the ages of 30 and 40 to determine catecholamine secretions before and after a four-week exercise program during the months of June and July, 1973. Upon the basis of the findings the investigator determined whether the four-week training program altered urinary catecholamine levels. To assess the validity of the training program, oxygen consumptions and performances of work were recorded periodically during the four weeks.

Definitions and/or Explanations of Terms

For the purpose of clarification the following definitions and/or explanations of terms were established for use in the study.

Catecholamine (or catechol amine). The investigator accepted the explanation of Best and Taylor (1966, p. 1556) who state that catecholamine is a general term describing the three amines secreted by the adrenal medulla: epinephrine, norepinephrine, and dopamine. This investigation is concerned only with the urinary levels of total catecholamines as determined by the Pisano (1960) method and epinephrine levels as determined by the Zuspan, Nelson and Ahlquist (1967) method. Determinations were made at the onset of the investigation, at the end of the second week of exercise, and at the conclusion of the four-week training program. Throughout the study, epinephrine and norepinephrine are referred to as E and NE, respectively. Determinations made from urine samples taken during 24 hour periods in which subjects did not exercise are referred to as resting levels. Samples taken during 24 hour periods in which subjects performed their 15 minute exercise bouts yielded determinations referred to as exercise levels. It should be noted that adrenaline and noradrenaline are synonymous terms for epinephrine and norepinephrine and are used interchangeably in the literature.

<u>Oxygen consumption</u>. The investigator accepted the definition of Mathews and Fox (1971): "The amount of oxygen consumed per minute is equal to the difference between the amount of oxygen inspired and the amount of oxygen expired (p. 219)." In this investigation, oxygen consumptions were determined during the fourteenth minute of the first, sixth, and last of twelve fifteen minute exercise periods in which the subjects engaged. The exercise periods were conducted on a motor driven treadmill that was programmed to alter its speed to maintain the subjects' heart rates at 150 beats per minute. The formula for the calculation of oxygen consump-

tion was that of Profant, et al., (1972):

$\dot{V}O_2 = V_e \left[KX_2 - (K + 1) \cdot X_1X_2\right] / X_1$ where $\dot{V}O_2$ = oxygen consumption; V_e = minute volume of expired air corrected for STPD conditions; K = ratio of percentage inspired oxygen to percentage expired nitrogen; X_1 = percentage of expired oxygen after the removal of carbon dioxide; and X_2 = percentage of expired oxygen before the removal of carbon dioxide. Throughout the study, oxygen consumption is referred to as $\dot{V}O_2$.

Performance of work. The investigator defined performance of work as the number of revolutions of the treadmill belt that transpired during the 15 minute exercise periods. Performance of work was recorded each exercise day for all subjects.

Delimitations of the Study

The study was subject to the following delimitations:

(a) Thirteen women between the ages of 30 and 40 years who volunteered for the study during the months of June and July of 1973.

(b) Freedom of the subjects from medications, allergies and other health conditions which might affect the urine samples.

(c) The objectivity, reliability, and validity of the selected instruments for the measurement of $\dot{V}O_2$

(d) The objectivity, reliability, and validity of the selected techniques for the urinary analysis.

(e) The degree to which the subjects were representative of the population from which they volunteered.

(f) The degree to which the subjects cooperated in the procurement of the urine samples.

Purpose of the Study

The purpose of the investigation was to determine the relationship between a four-week exercise program and changes in urinary catecholamines in women. The major null hypothesis tested was:

> Urinary catecholamine secretions among women are not altered significantly by participation in a four-week exercise program.

Sub-hypotheses tested were:

(a) There is no significant difference between the amount of work performed at the beginning of the investigation and work performed at the conclusion of the investigation.

(b) There is no significant positive trend in the amount of work performed on each exercise day throughout the four-week exercise program.

(c) There is no significant difference between the oxygen consumption at the beginning of the investigation and the oxygen consumption at the conclusion of the investigation.

(d) There is no significant positive trend in the amount of oxygen consumed as determined during the four-week exercise program.

(e) There is no significant difference between initial and final determinations of resting catecholamine levels.

(f) There is no significant trend in the amounts of resting catecholamines secreted over the four-week exercise program.

(g) There is no significant difference between initial and final determinations of exercise catecholamine levels.

(h) There is no significant trend in the amounts of exercise catecholamines secreted over the four-week exercise period.

(i) There is no significant difference between initial and final determinations of resting epinephrine.

(j) There is no significant trend in the amounts of E secreted during resting conditions over the four-week exercise period.

(k) There is no significant difference between initial and final determinations of exercise epinephrine.

(l) There is no significant trend in the amounts of E secreted during exercise conditions over the four-week exercise period.

(m) There is no significant difference between exercise catecholamine levels and resting catecholamine levels prior to the onset of the four-week exercise program.

(n) There is no significant difference between exercise catecholamine levels and resting catecholamine levels obtained at the midpoint of the four-week exercise program.

(o) There is no significant difference between exercise catecholamine levels and resting catecholamine levels obtained at the conclusion of the four-week exercise program.

The .05 level was accepted as the criterion for significance.

Chapter II presents the review of literature which was found pertinent to this investigation.

Chapter II

Review of Related Literature

The purpose of this investigation was to determine the relationship between a four-week exercise program and changes in urinary catecholamines in women. A review of the literature revealed that a large body of research has been undertaken to determine the role of the endocrine system with specific emphasis allocated to secretions of epinephrine and norepinephrine in various stressful situations. To simplify the review of the related literature, only studies relating to normal urinary levels of catecholamine secretions in man, and studies concerned with differences in catecholamine secretions in situations of stress were selected. Reviews of the studies are presented in chronological order.

In a study concerning a Harvard-Yale four-mile boat race with two associated weeks of intensive, controlled, pre-race training, Renold et al., (1951) investigated the reaction of the adrenal cortex to physical and emotional stress of college oarsmen, coxswains and coaches. Subjects included the Harvard crew members, whose ages ranged from 19 through 24 years and whose weights averaged 177 pounds, and the Yale crew, whose ages ranged from 17 through 22 years and whose weights averaged 175 pounds. Subjects were examined medically and deemed healthy, especially with respect to allergies. Subjects were exposed minimally to extraneous emotional or other forms of stress. On the twelfth, eighth, and sixth days prior to the race, time trials were made during which both crews were evaluated carefully upon the factors of fatigue, stress, and individual effort.

Significant changes were reported in the adrenal cortical response to physical exertion and added emotional stress as indicated by changes in circulating eosinophil levels. The quthors concluded that in "well-trained persons, emotional stress, either alone or in combination with muscular activity, may lead to a highly effective adrenal stimulation and may represent a necessary link in the mechanism of adaptation (p. 757)."

A series of investigations were executed by von Euler and Hellner (1952) in which they scrutinized the relationship between catecholamine excretions and muscular work. Group I_a (N=10) was comprised of subjects whose muscular exercise was controlled by oxygen consumption and Group I_b (N=8) was comprised of subjects performing individual muscular exercise under controlled conditions. Group II (N=19) consisted of subjects who were participating in moderate exercise. Group III (N=13) utilized subjects who were engaged in a ski run competition of ten kilometers in length. Subjects were young healthy male students enrolled at the Gymnastics High School of Stockholm.

Members of Group I participated in individual muscular work for 5 to 70 minutes at which time oxygen consumption was measured and urine was collected. A time reading was taken between the emptying of the bladder prior to the work and again immediately after the work. Resting urine samples were collected during light activity such as office work. The second and third groups engaged in general athletics and the 1,500 meter run respectively. The second group maintained steady activity throughout the time of urine collection, whereas urine from the third group was obtained for a period of one hour and 55 minutes to two and one-half hours after participating in the general athletics. The last group participated in the ten kilometer ski run competition.

Evidence was provided by the investigators that "strong physical exertion is accompanied by an increased excretion of catechol amines in the urine (p. 188)." Urinary output of noradrenaline and adrenaline ranged from 0.03 micrograms (ug) per minute at rest to a maximum of 0.35 ug per minute during heavy muscular work. The investigators reported that a

> correlation appears to exist between the degree of 'stress' involved in the work and the output of catechol amines, moderate work causing an insignificant rise in the catechol amine secretion and maximal work inducing a high output (p. 190).

Hoagland et al., (1955) reported several pursuit meter studies with and without hypoxia and their relationships to changes in adrenaline and noradrenaline. The studies consisted of four teams of five normal men per team. Each subject was tested between eight and ten times, one time a week in runs lasting from two to three hours. Motivation included an hourly pay rate plus a bonus graduated in terms of high scoring ability.

Team 1, with a mean age of 32 years, performed for three hours from one until 4:00 p.m. with five minute rest intervals at two and three o'clock. Control urinary samples were taken in the morning on the test day. Team 2, with a mean age of 26 years, performed for two hours, with no rest interval, from nine until 11:00 a.m. Control urinary samples were obtained on other mornings. Oxygen was controlled at ten and one-half per cent to 15 per cent, with a mean of 13 per cent. Team 3, with a mean age of 18 years, performed for two hours, with no rest interval, from one until 3:00 p.m. Oxygen was controlled at ten and one-half per cent. Control urinary samples were collected on the test day. Team 4, with a mean age of 17 years, performed for two hours, with no rest interval, from one until 3:00 p.m. Control urinary samples were taken on the test day. Team 1 was considered to be the least stressed and Teams 3 and 4 were reported to be extremely stressed and suffered from considerable after-effects. Control and test urine samples were collected from Teams 1, 3, and 4 on three different test days.

The investigators reported mean increases over the controls of urinary adrenaline for Team 1 at 109 per cent following the stress, and, for Team 3 adrenaline increased by 208 per cent on the average over the control levels. Noradrenaline, in Teams 1 and 3, increased by 54 per cent and 32 per cent respectively over pretest levels. The most greatly stressed Team, Team 4, had increases of 170 per cent in adrenaline and 125 per cent in noradrenaline. Caution was suggested by the investigators in interpretation of the noradrenaline results because of the inconsistency and variability in the data. The conclusion proposed by the investigators was that adrenaline "usually" increases after stress.

von Euler, Luft, and Sundin (1955) undertook an investigation to determine whether reflex vasomotor activity which operates during standing is associated with an increase in urinary noradrenaline. Subjects included 15 healthy medical students whose ages ranged from 21 through 28 years. Subjects were confined to beds in the laboratory during the night before the investigation. Bladders were emptied at 5:00 a.m. while the subjects remained in bed. This was followed by the ingestion of a glass of tap water. Subjects were permitted to return to sleep until 8:00 a.m. at which time their bladders were emptied again. This sample represented the baseline sample during the recumbent position. At 8:00 a.m. the subjects were placed on a table which was tilted at 75 degrees; the subjects were maintained at this angle for three to four hours. Pulse rates and blood pressures were taken at regular intervals. Urine samples were collected at nine, ten and eleven o'clock, and, in 7 of the 15 experiments, at twelve o'clock. Diurnal variations were controlled for by having ten healthy subjects remain in the supine position over the same time period. The control subjects emptied their bladders at five, eight, nine, ten, eleven, and twelve o'clock.

Results indicated that the subjects who were placed on the tilted table showed a marked increase in noradrenaline when compared with their controls. The increases in noradrenaline were interpreted as being the result of reflex activation of the vasomotor system. The urinary adrenaline excretions during the tilting test were slight to moderate and were probably the result of the stress factors of muscular activity and the uncomfortable position.

Elmadjian et at., (1956) conducted a study to determine the excretion of adrenaline and noradrenaline in sleep and waking states, after infusion of adrenaline and noradrenaline into an adrenalectomized subject, after psychomotor stress, under normal and hypoxic conditions, and after the administration of ACTH.

Normal and psychotic subjects were studied in sleep and waking states. Night samples of urine were taken from approximately 10:00 p.m. to 6:30 a.m., and day samples were collected from 6:30 a.m. to 12:00 noon. Total noradrenaline and adrenaline was determined in preliminary experiments. Five samples of day urine were extracted in duplicate. The differences between duplicates were compared with the differences reported between night and day samples of two normal subjects. Results indicated a marked increase in noradrenaline and adrenaline in the day samples as compared with the night samples, otherwise, fair to good agreement was obtained in the duplicate samples.

Six normal subjects and one male schizophrenic patient were tested for the distribution of amines in sleep and waking states. An increase in the excretion rate of adrenaline was reported in each case along with a wide range of values. The noradrenaline results were more uniform and percentage wise increases were not as great as those reported for adrenaline.

A comparison was made of adrenaline, noradrenaline, and 17-ketosteroid excretions in night and day samples collected from four normal subjects, two male schizophrenics, and one adrenalectomized female psychotic patient. Diurnal variations were reported in adrenaline, noradrenaline, and 17-ketosteroids in all subjects but the female who had been adrenalectomized. Increases were greatest, in descending order, for adrenaline, noradrenaline, and 17-ketosteroids. The subject who was the exception did not excrete adrenaline, but had a marked increase in noradrenaline during the day compared with the night sample.

The investigators indicated that the increase in adrenaline and noradrenaline excretions during waking as opposed to sleeping did not mean that these differences were of diurnal rhythm independent of the general physical and psychological factors involved during waking. They reported that the waking process might be considered a control. The data further indicated that the percentile increase of adrenaline is greater than the percentile increase of noradrenaline, though the value for the excretion rate of noradrenaline is greater in terms of micrograms per hour. Additional studies on sympathicoadrenal (adrenaline and noradrenaline) and adrenal-pituitary (17-ketosteroids) functions measured from the same urines, revealed that both increased upon waking as compared to sleep.

Four normal subjects, whose ages ranged from 26 to 34 years, operated the Hoadland-Werthessen pursuit meter for three hours to determine psychomotor stress. Rest periods of five minutes at the end of the first and second hours were allowed. Pre-stress urine samples were collected approximately between 8:00 a.m. and 1:00 p.m. and the stress samples were obtained during the operation of the pursuit meter from 1:00 p.m. to 4:00 p.m. The investigators reported that the number of experiments was too small for generalizations but did indicate that consistent increases in noradrenaline and adrenaline, or both, were evidenced in all four subjects.

Six men, aged 17 to 19 years, were subjected to three or four psychomotor-stress tests under hypoxic conditions (ten per cent oxygen) for two hours. Control urine was collected in the forenoon and the experiments were conducted from 1:00 p.m. to 3:00 p.m. Results indicated that as a group there was a significant increase in the levels of adrenaline but not noradrenaline. Individual differences were indicated but could not be explained by the investigators.

During the psychomotor stress great variability was found in the excretion of adrenaline but not noradrenaline. The investigators suggested that the individual variation in the degree of psychomotor stress was because of motivation and effort.

Funkenstein (1956) reviewed and summarized investigations which were conducted over a ten-year period. He reported evidence of excessive secretions of norepinephrine and epinephrine in healthy subjects and in psychotic patients who excreted excessive amounts of the hormones while in the wards without external stressful situations and the healthy subjects who excreted excessive amounts only when under stress. The crucial element, as indicated by Funkenstein, was the ability of the subjects to master or fail to master stress.

The author defined epinephrine as evoking widespread physiological reactions, such as, increased oxygen consumption of the brain, increased pulse rate and cardiac output, increased peripheral resistance, relaxation of the bronchioles, increased blood clotting time, and changes in glucose metabolism. Norepinephrine has primarily only to increase peripheral resistance throughout the body.

Hill et al., (1956) conducted a series of investigations related to adrenocortical and psychological responses to stress in man. Other studies conducted included those which would allow comparisons of a crew race with other forms of exercise, and those which were designed to explore the possibility of altered metabolism of adrenal steroids as part of the exercise response. Only those studies pertinent to this investigation are included in this review, one of which consisted of subjects who were oarsmen on college crews in 1953 and 1954. Both crews, which consisted of eight oarsmen, coxswain, and the 1954 crew manager ranged in age from 19 to 21 years, and both had similar weight ranges of approximately 165 to 195 pounds. All members were reported to be in excellent health.

Both crews experienced the same environmental conditions and similar schedules. Two weeks prior to the race, crew members were isolated, visitors were prohibited, and distractions were minimized. Subjects were provided with ample food and rest.

Practice rowing included two sessions each day except Sunday. Morning practices were conducted from 7:00 to 8:30 a.m. and afternoon practices were conducted from 5:30 to 7:30 p.m. Other vigorous exercise was not allowed. Once each year, replacing a practice period, the four mile course was run, over three time trials, with the culminating trial taking place one week before the varsity race. The physical exertion was reported to be comparable to that expended during the varsity race.

Urine samples were collected from 9:00 a.m. to 1:00

p.m., 1:00 p.m. to 5:00 p.m., 5:00 p.m. to 9:00 p.m., overnight, on a Sunday, on a time-trial day, and on the race day. Determinations of 17-hydroxycorticoid and creatinine were made within two weeks of collection. Similar procedures were executed on urine samples for both crews, except that 17-ketosteroid and uropepsin determinations were made also on the 1954 specimen.

The 1953 data were analyzed by statistical comparisons of the three collections through the Student "t" test. For the 1954 data, a split-plot analysis of variance was done on the 17-hydroxycorticoid, 17-ketosteroid, and uropepsin determinations.

Changes in the urinary 17-ketosteroid excretion were observed for only the 1954 crew. A significant difference ($P < .01$) was reported between the data collected on race day and those on control and practice days. Significant differences ($P < .05$) were reported also in the 1:00 to 5:00 p.m. period just prior to the race. Subjects varied significantly ($P < .01$) from one individual to another in their four hour output.

Michael (1957) reported research studies which support the theory that exercise "conditions" the stress adaptation mechanism. Evidence was provided which indicated that the adjustment to stress is aided through increased sensitivity of the adrenal glands; therefore, less adjustment is necessary because of a more efficient system.

Additional rewards from regular exercise were indicated, also, by Michael who stated that increases in strength and motor skill increase movement efficiency and assist in allaying fatigue caused by physical exertion. This improved tonus augments venous blood return and circulation. The improved circulation assists, in turn, with the slowing of pulse rate during exercise and hastens its return to normal after exercise. He reported further that the euphoric sense resulting from exercise might be of an emotional nature and, therefore, may result partly from an adjustment of the autonomic nervous system. Assuming this to be true, he stated "exercise may prove to be important in man's adjustment to stress (p. 53)."

Michael reported agreement with the literature that stress does affect the autonomic nervous system which is kept equilibrated through adjustments of the sympathetic and parasympathetic reactions; thus, if there were some means of improving the autonomic nervous system, more efficient reactions to stress should result.

Michael (1957) postulated that repeated exercise seems to sensitize the adrenal gland which might result in an increased time increment between shock and countershock and that exercise seems to increase the size of the adrenal glands which would provide greater storage for countershock steroids. A person who is conditioned might expect, therefore, an improved adjustment to the stress. The questions of how much and what kind of exercises should be utilized for the adaptation to stress were raised by the investigator as well as the possibility that exercise may precipitate a breakdown rather than aid in adaptation.

Ulrich (1957) undertook a study to investigate the alarm reaction and resistance stages of emotional stress by measurement of eosinophil count and cardio-respiratory symptoms in college women who were subjected to various competitive experiences. Subjects included 28 women students, between the ages of 17 and 21 years, who attended Madison College in Virginia and who were enrolled in the regular physical education classes in basketball and had voluntarily participated in the intramural basketball program.

The 28 subjects were divided into an "experienced" group (N=14) and an "inexperienced" group (N=14) according to their athletic excellence and in accordance with a random selection. The experimental design comprised three conditions (base, anticipatory, and post-stressor), five situations (class, intramural, interscholastic, written test, and spectator), and two circumstances (participation or non-participation in the anticipated activity). The base condition was established previous to the experimental period by averaging the results of blood eosinophil determinants taken on three different occasions. Pulse rate and respiration were taken, also. The anticipatory condition was established by an ordinary announcement of the approaching situation. The blood determinations were made no longer than 15 minutes before the anticipated event. Post-stressor conditions were determined through blood determinations made one and four hours after the experimantal situation.

Under the situation and circumstance variables, subjects participated alternately in a class basketball game, an intramural basketball game, and an interscholastic basketball game. Finally, subjects completed a written test on basketball and attended a basketball game as a spectator.

Stress scores were subjected to a three-way analysis of variance with repeated measures. Statistically significant evidence supported the presence of anticipatory stress as follows: (a) by the "experienced" group in interscholastic and test situations; (b) by the "inexperienced" group in class, intramural and test situations. Within group scores revealed differential levels of adaptive anticipatory response to stressors of potentially different degrees. For the "experienced" group these were, in descending order, the written test, interscholastic game, the class game, the intramural game, and spectator experience. For the "inexperienced" group, the levels were, in descending order, written test, intramurals, class, and spectator. Cardiorespiratory results verified the eosinophil data.

Statistically significant evidence supported the following with regard to post-stressor stress: post-stressor stress occurred in all of the stressor situations except that class situation in which the "experienced" group participated; the "inexperienced" group had higher stress scores than the "experienced" group in all situations concerning basketball. Again differential levels, when situations were compared within group scores were found for the "experienced" group when actually participating in the anticipated situation. Subjects in the "experienced" group who were not allowed to participate manifested higher stress scores. Higher stress scores were reported, also, for those in the "inexperienced" group who did participate.

Ulrich (1957) concluded that under normal educational experiences of college women, stress may be elicited by anticipation of a stressor situation, participation in, or denial of a stressor situation. The stress of individuals varies from situation to situation and is related more definitely to psychological elements in the situation than components involving physical activity. Stress appears to be related to past experiences which are relevant to the current situation.

Elmadjian, Hope, and Lamson (1957) conducted a study to determine the excretion of epinephrine and norepinephrine of normal and psychiatric patients in a variety of emotional states. Their hypothesis was that active, aggressive emotional displays are related to increased levels of norepinephrine, with or without increased levels of epinephrine, whereas tension and anxiety with passive emotional displays are related to increased excretions of epinephrine with normal excretions of norepinephrine.

Data were collected on subjects in sleeping and waking states, professional hockey players with their coach before and after a game, amateur boxers before and after a bout, neuropsychiatric patients during staff interviews, normal subjects in states of anticipation, psychiatric patients receiving lysergic acid diethylamide (LSD), and psychiatric patients on whom mental status and Malamud-Sauls rating scale records were obtained during collection. The relationship between general exercise and excretions of epinephrine and norepinephrine was examined also.

Results of the study indicated elevations in norepinephrine with normal epinephrine values for those subjects in anticipatory states and in hockey players during a moderately tense game. In the involuntional-depressive subjects receiving the psychotomimetic agent (LSD) and in the neuropsychiatric patients during an interview, there were elevations in epinephrine with little elevation in norepinephrine. Finally, when an intense emotional display was experienced by manic-depressives treated with LSD, amateur boxers, hockey players, and disturbed neuropsychiatric patients the levels of both epinephrine and norepinephrine were elevated. When increased levels of norepinephrine were obtained there was evidence of aggressive, active emotional display. Conversely, when increased levels of epinephrine were obtained, there was evidence of tense, anxious but passive displays.

The question of whether norepinephrine excretion could be a result of motor activity was examined also by the investigators. Results indicated that several subjects with marked emotional disturbances who were lying or sitting had high norepinephrine excretions regardless of epinephrine levels. Additionally, the goal tender in the hockey study, who remains essentially in one place during the game, manifested a great increase in the level of norepinephrine.

To test the effect of generalized exercise, an experiment was conducted on a subject who raked grass for two hours, during which much walking and movement of the muscles in the arms and shoulders was involved. This subject was not emotionally involved and was physically tired upon completion. Results indicated a normal excretion of norepinephrine (2.2 ug per hour) and an elevation of epinephrine (0.35 ug per hour).

An additional inquiry into the effect of generalized exercise included two laborers, one who mixed mortar and carried bricks, and one who unloaded trucks and was a pick-and-shovel man. Excretions of norepinephrine were 3.2 ug per hour on the first subject and 5.7 ug on the second subject. Epinephrine excretions were reported at 0.54 ug per hour and 0.12 ug per hour, respectively. The conclusions drawn from these investigations were that exercise, without marked emotional involvement, is not a major factor in obtaining high norepinephrine levels as manifested by the hockey players or boxers and that the original hypothesis was confirmed regarding a variety of emotional states and catecholamine levels.

In a study conducted by Bogdonoff et al., (1960) 20 fourth-year medical students were tested for metabolic and cardiovascular changes occurring before, during, and after a 15 minute oral examination which was described by the students as "tense . . . anxious . . . doubtful about passing . . . scared." One of the various analyses included during this evaluation was that for adrenaline and noradrenaline in urinary excretions. Procedures for the collection included: a light breakfast, a fast from 9:00 a.m., and, at 11:00 a.m., an hourly ingestion of 200 milliliters of tap water. Approximately two hours prior to the scheduled oral examination the bladder was emptied and the urine discarded. After an hour, an additional sample, labeled "pre-specimen," was collected and saved. One-half hour after the oral examination another urine sample, labeled "during," was taken and kept. The last urine was collected one hour after the "during" sample. All samples were acidified with three milliliters of 1N H_2SO_4 and placed on refrigeration.

An interview was made upon completion of all sample collections. At this time affect arousal was determined by an unbiased member of the faculty. Through content analysis of the interviews, feelings of "anxiety with a fearful quality" prevailed and little or no feeling of aggressivity was reported.

On control, non-test day, measurements were made in the same sequence with the exceptions that there were no interviews or electrocardiographic tracings. Results indicated that the mean increase in adrenaline excretion for the entire group was statistically significant ($P < 0.01$) during the actual test period with no such change in noradrenaline excretion. Posttest excretions of adrenaline returned to the pretest level and there was no consistent pattern of change in the excretions of noradrenaline in the posttest. It was reported also that there was a consistent and significant increment obtained in the urinary excretions of adrenaline which the investigators assumed to reflect increases in medullary secretion. No biochemical substantiation was made with regard to the absence of any consistency in noradrenaline excretions. Pronounced sustained increases of heart rates of 133, 140, or 156 beats per minute provided additional commentary on the fact that individuals can exhibit impressive physiological changes during life stresses which may or may not be measured, as in the case of the oral examination.

Mendelson et al., (1960) undertook an investigation involving ten male volunteers between the ages of 20 and 27, from which catecholamine excretions and behaviors during sensory deprivation were recorded. Subjects were medical students, university students, or medical house officers who were recruited by the offer of pay per hour for the duration of the experiment. All urine was collected 24 hours prior to the beginning of each experiment, but was separated into two 12 hour samples. Experiments were begun on Saturdays for the convenience of the subjects.

to 67 years and a mean age of 31, collected urine for a 24 hour period on a day not anticipated to be stressful. Group II, consisting of 15 females and 5 males, aging from 16 to 69 years and with a mean age of almost 32 years, collected urine similarly except that two consecutive 24 hour samples were collected. Eleven of these subjects were common to both groups. Thirty-seven psychiatric patients formed the third group which was categorized into affective characteristics of (a) mainly depressed, (b) mainly anxious, (c) equally mixed depressed-anxious, and (d) miscellaneous.

Results indicated that secretions of adrenaline were highly related to secretions of noradrenaline and that secretions of adrenaline and 17-hydroxycorticosteroids were significantly related but not as highly as those of adrenaline and noradrenaline. Additional results indicated that during depression, noradrenaline is excreted preferentially as compared to adrenaline and the corticoids, while corticoids are excreted preferentially in states of anxiety. Curtis stated finally that "The findings suggest that various qualities of 'emotional stress' are not non-specific in Selye's sense if one relates the affective state to the balance between hormones rather than to a single hormone (p. 183)."

Friedman et al., (1960) conducted a study to determine possible relevant urinary hormones in groups of men who were free from medication and who exhibited different behavior patterns. Group A was comprised of 12 subjects selected upon the basis of (a) occupations requiring competitive activity

Subjects were given standardized directions and were, in effect, totally isolated in a tank-type respirator, virtually free from any extraneous stimuli. Control conditions were inflicted by regulating and restricting the subject's auditory stimuli, visual stimuli, by restricting physical movement, and by feeding by tube.

All data collected for epinephrine and norepinephrine and urine volume were reported in values of 24 hour periods. The experimental time for each subject varied according to his ability to tolerate the stress, so corrections were made according to "the assumption of a linear rate of clearance of catechol amines under a fixed set of conditions (p. 149)." Increases were reported in epinephrine and norepinephrine during the experiment with a subsequent decrease toward control values during the post-experimental period. A significant difference ($P < .05$) was reported in epinephrine levels between the experimental condition and post-experimental condition and norepinephrine levels were significantly increased ($P < .05$) between the control condition and experimental condition. The Wilcoxon Matched-Pairs Signed-Ranks Test for significant differences was used.

Curtis et al., (1960) designed a study to investigate the relationship between adrenaline, noradrenaline, and 17-hydroxycorticosteroids in the urine of two groups of normal human subjects (Group I = 19; Group II = 20) and one group of psychiatric patients (Group III = 37). The first group, comprised of 12 females and 7 males, with ages ranging from 22 and deadline preoccupations, (b) a self-admitted competitive drive, and (c) observed motor phenomena during the interview. Converse criteria were used to select the twelve subjects for Group B. Subjects were asked to estimate the urgency of voidance during each of the four days and were cautioned to refrain from eating bananas. The samples of two men from Group A and two from Group B were obtained each week. Subjects in the two groups were of approximately the same age, height, weight, and level of fitness. Group A subjects consumed a greater amount of cigarettes and alcohol, but both groups had similar dietary habits.

The urine which was collected from 10:00 a.m. to 12:00 noon and from 2:00 to 5:00 p.m. on Monday through Thursday was pooled. A single, overnight sample was collected immediately upon arising. The urine collected at 9:00 a.m. and after lunch was discarded.

Urinary excretions of the various hormones while at rest for both groups was about the same, as was the excretion of epinephrine during the working period for both groups. However, day time excretions of norepinephrine for Group A were significantly higher than for Group B, which indicated that the characteristic of overtness, a Group A characteristic, manifests itself not during typical situations but during stressful situations. Additional discussion of the potentiality of the relevance of these findings to coronary heart disease was included.

Cohen et al., (1961) reported a hypothesis that there exists a consistent relationship between catecholamine levels and the absence or presence of duodenal ulcers. Subjects included ten male, Caucasian non-ulcer patients selected by unbiased physicians. The control subjects included seven healthy volunteers and three who were hospitalized for reasons other than ulcers. Subjects were matched as closely as possible for age and socio-economic status.

Subjects were tested on two separate days beginning at 7:30 a.m. after fasting overnight. On the first day the subject remained quietly in bed with heads raised forty-five degrees. After one hour the first sample was collected which was followed by the collection of gastric juices, and another urine collection, both of which were referred to as "basal" samples. This same procedure was followed until four urine samples had been obtained. Similar procedures were followed on the second day; however, in addition, an interview was made after the final collection which included nine predetermined questions.

Results indicated that in all instances there was a significantly higher level of noradrenaline in the control subjects than in the ulcer patients on all four samples, also the mean adrenaline levels were higher in the control group than the ulcer group, however, there was greater variability and over-lap than in the case of noradrenaline. The investigators postulated cautiously that "low catechol amine output in the urine of ulcer patients is indicative of low sympathetic activity . . . (p. 115)" and that this may be helpful in expanding an approach to control of duodenal ulceration. An additional verification was that excretions of adrenaline and noradrenaline correlated with the degree of aggressivity and anxiety expressed and that certain psychological characteristics can determine potential ulcer and non-ulcer patients.

Levi (1961) undertook an investigation to determine the efficiency of estimating work capacity through urinary epinephrine and norepinephrine output as an ultimate diagnostic tool for determining reactions to stress. Included in the investigation were 19 "normal" volunteer subjects who were divided into a high stress tolerance group (N=9) and a low stress tolerance group (N=10). Clinical criteria as determined by questionnaires and interviews were used to place the subjects into groups.

The procedure of the investigation required the subjects to sort 200 steel balls of differing sizes while under the stresses of industrial noise, various light intensities, rushed performance, and verbal criticisms. Subjects were evaluated in terms of speed, accuracy, and perseverance with regard to the sorting of the steel balls; their behaviors and subjective feelings were determined by psychological questionnaires and observation, and their hormonal changes were determined by the urinary output of adrenocortical and medullary hormones. Urine samples were collected in three two-hour periods, one preceding, one during, and one following the stress period.

The results indicated a significant increase in epinephrine output in the urine, with a subsequent decrease during the post stress period to values below the base levels. Epinephrine levels were significantly higher in the tolerance group during stress ranging from 17.5 nanograms per minute to 25.8 nanograms per minute. The level of significance was not indicated. The decrease in epinephrine after stress to 12.5 nanograms per minute was more obvious in the low tolerance group than in the high tolerance group (6.7-8.3-4.2 nanograms per minute). Only the low tolerance group showed a significant increase in norepinephrine.

Westfall and Watts (1964) investigated the 24 hour urinary excretion of epinephrine and norepinephrine in nonsmokers and heavy smokers. In addition, smoking influences on catecholamines were investigated at 30 minute intervals before, during, and after smoking among students who ordinarily smoke. Other variables investigated were blood pressure, pulse rate, and metabolic rate.

Subjects were healthy male university students whose ages averaged 25 years. Group I (N=10) was comprised of smokers including only those who inhaled, and Group II (N=11) consisted of nonsmokers. The groups were homogeneous with regard to diet and activity.

Samples were collected for all subjects, for 24 hour periods and placed in glass containers in which four milliliters of concentrated sulphuric acid had been placed for preservation purposes. The analysis revealed significantly greater ($P < 0.001$) excretion of epinephrine in smokers than nonsmokers whereas no significant differences were evidenced in norepinephrine excretions.

Fifteen male students with an average age of 24 years were utilized to determine catecholamine excretions in heavy smokers before, during, and after smoking. Only those who inhaled smoke from approximately 20 cigarettes a day were included. The experiments were conducted while the subjects were sedentary between the hours of 7:00 p.m. and 12:00 midnight. Abstinence from smoking for at least one and one-half hours was required before the test was begun. A control sample was collected which was followed by three consecutive periods during which the subjects smoked one cigarette every ten minutes for 30 minutes. Finally, one 30 minute postsmoking sample was collected. Five drops of sulfuric acid per 100 milliliters was placed in each specimen container for preservation.

The analysis revealed that epinephrine and total catecholamine levels increased progressively above the control levels reaching a maximum in 120 minutes. The increases were statistically significant for the second and third 30 minute smoking period, therefore, heavy smokers did excrete more epinephrine than did nonsmokers. In the short term study, it was revealed that total catecholamine output increased progressively above control levels during heavy smoking, but decreased when smoking subsided.

Feller and Hale (1964) investigated the relationship between climatic factors and catecholamine excretions in residents living in a subtropical climate in autumn, winter, and spring, with emphasis upon whether daily exposures to mild cold might cause long-term changes in sympathoadrenal activity. A secondary purpose was concerned with the effects of cigarette smoking and anxiety (anticipation of unusual event) on climate induced changes in urinary catecholamines.

Subjects were 231 males, whose ages ranged from 17 to 22 years. All were in their first month of military service stationed in San Antonio, Texas, and were homogeneous with respect to state of health, diet, living conditions, sleep and activity schedules. Subjects were studied under standardized laboratory conditions in autumn (November), winter (January-February), and spring (March-April) during which time the maximum temperatures reported were, respectively, 66, 60, and 74 degrees Farenheit. The six subjects who were studied daily, arrived at 8:00 a.m. and rested in a lounge under controlled temperatures of 74 to 76 degrees Farenheit until noon at which time urine samples were collected and analyzed for epinephrine and norepinephrine. Each of the seasons had four treatment groups consisting of control, smokers, anxious, and smokers-plus-anxious. The latter two groups were instructed to expect extremely vigorous physical fitness tests later in the day.

Results indicated a significant variation with seasons ($P < .01$). Pooling of the data provided the following values for epinephrine output: autumn, 0.62 ug; winter, 0.59 ug;

and, spring, 0.44 ug per hour which indicated high epinephrine output during autumn and winter. Epinephrine levels were elevated in the group of smokers ($P < .01$) and anxiety and season interactions were significant ($P < .05$). Pooled data with seasons ignored resulted in the following epinephrine values for control, anxiety, smoking, and smoking-plus-anxiety groups, respectively: 0.45 ug, 0.45 ug, 0.52 ug, and 0.63 ug per hour.

Significant smoking and anxiety results were not established for norepinephrine. Pooling of the norepinephrine data by seasons, ignoring treatments, revealed the following: autumn, 1.26 ug; winter, 1.92 ug; and, spring, 1.59 ug per hour ($P < .01$). The seasonal variation provided evidence of rather high sympathoadrenal activity in winter with some reversal in spring. The investigators reported that norepinephrine was the predominant catecholamine produced in chronic cold as was evidenced when masking factors were eliminated and that norepinephrine levels were highly relative to epinephrine levels.

Frankenhaeuser and Patkai (1964) undertook an investigation to examine the relationship between the urinary excretion of adrenaline and noradrenaline and performance during stress. Included in the study were 110 college students whose urine was collected after a 90 minute period of inactivity and after a 90 minute period of mental work. Baseline values were obtained from the former.

Subjects performed two parallel series of three tests and worked in groups of 10 to 20 persons. The tests were not complex but required concentration. Various stressors were introduced during the second work period, such as, workshop noise and verbal reprimands.

The analysis included the calculation, for each subject, of the difference between scores obtained during the two periods. Coefficients of correlation were calculated between the measures of performance change and the urinary catecholamine excretions. The results indicated that improvement of performance during stress conditions was correlated positively with the amount of noradrenaline secreted during the period of work ($r = 0.409$, $P < .001$) and that improved performance was related to an increase in noradrenaline secretion during work as compared with inactivity ($r = 0.214$, $P < .005$). Those who improved their performances during stress manifested also the greatest amount of noradrenaline excretions. Adrenaline secretions appeared not to be related to performance, and practice effects were not observed to have influenced the subjects.

In a review by von Euler (1964), examples were provided which related various stress conditions to the excretion of catecholamines. Included in the review were studies pertaining to mental stress, gravitational stress, cold, emotional stress, and flying.

Emphasis was directed by von Euler to obtaining an objective measurement of the occurrence and degree of stress in individuals. Accent was placed upon adequate collection procedures and correct presentation of the catecholamine excretion values. von Euler concluded his review by stating that "the present data suggest a definite correlation between the urinary excretion of catecholamines and the various types of stress (p. 403)."

Haskell and Fox (1966), reported an increasing interest in attempts to determine whether participation in physical activity programs might be a deterrent for coronary heart disease. Included in the report were methods through which this prevention might accrue. Those indicated which are pertinent to this study were: beneficial alteration of sympathetic-parasympathetic circumstances, reduction of other elements contributing to hypertension, and enhancement of personal image and thus increased tolerance for stress (decreased strain resulting from psychic and physiologic stress).

The investigators reported that "even though at the present time there is no concise or complete pharmacopeia of physical activity from which an individual prescription can be written, there are certain general suggestions which might be considered . . . (p. 646)." The investigators suggested that: physical activity must involve a substantial and sustained increase in metabolic, cardiovascular and respiratory functions which are manifested by the following symptoms: (a) elevated heart rate according to fitness, age, and anxiety; (b) shortness of breath; (c) perspiration, and (d) sense of muscular fatigue. The duration, intensity, and frequency necessary for physical activity to provide optimum rewards for each individual was reported to be unknown.

Mason (1968) reviewed the literature with regard to psychoendocrine research of the sympathetic-adrenal medullary system. He reported the following generalization:

> Perhaps the predominant urinary-response pattern observed in human subjects exposed to acute moderately stressful situations has been an elevation of epinephrine excretion, with smaller and more variable changes in norepinephrine excretions (p. 642).

He indicated further the need for clarification of the importance of muscular activity as a variable in psychoendocrine studies of catecholamine response.

Mason included several general guidelines which emerged from past research. He reported average urinary epinephrine levels of 5 ug per day under basal conditions in normal adults. Twofold or threefold elevations were reported in some cases in response to stressful situations. The urinary norepinephrine levels averaged about 30 ug per day under similar conditions and tended to show smaller percentage changes with elevations rarely above 90 ug per day.

The author reported that psychoendocrine studies of norepinephrine and epinephrine under stress have been limited almost exclusively to short-term samples, usually of one to three hour durations. Notation was made of the limitations of such studies which included complications caused by diurnal changes and the acute lability in catecholamine levels even within a five-minute time span.

Mason (1968) reported, finally, a need for systematic studies of a variety of activities which could be correlated with performance effectiveness ultimately providing industrial, military, physiological, and medical implications. In addition, he indicated an urgent need to include the psychoendocrine aspect of sympathetic-adrenal medullary regulation into the physiological areas, especially those from the practical work viewpoint in human subjects.

Walker and McTaggart (1969) investigated the physiological changes of 13 trained race car drivers with respect to releases of epinephrine and norepinephrine. Venous blood samples were obtained immediately before and five to ten minutes after the 30 minute sport car competition held in July of 1966 and 1967.

The levels of plasma epinephrine and norepinephrine obtained before and after the competition were in the same range. The concentration of epinephrine before the race was 18.9 nanograms with a $\pm$ 2.14 standard error from the mean, and, after the race was 16.4 with a $\pm$ 2.47 standard error from the mean. The concentration of norepinephrine before the race was 49.1 nanograms with a $\pm$ 6.1 standard error from the mean, and, 49.8 nanograms with a $\pm$ 5.9 standard error from the mean after the race. These differences were not statistically significant but the investigators suggested that if the changes observed during competition were the result of "burst" rather than "sustained" sympathetic activation, increased epinephrine and norepinephrine levels may not have been detectable at the time the samples were drawn. The following suggestion was reported: "The changes might have been detected in urine as accumulated excretory metabolites of epinephrine and norepinephrine if urinary samples had been collected during the total racing period (p. 141)."

Gibby et al., (1969) conducted a study to test the following hypothesis: "The rate of change of heart beat rate does not shift significantly from non-stress conditions but rather that it tends to remain relatively consistent within the individual from one situation to another (p. 463)." Subjects included 80 Caucasian males, 19 to 31 years of age, with a mean age of 20.81 years and a standard deviation of 2.60 years. Subjects were either engineering or science majors categorized by the investigators as being of superior intelligence and free of any significant degree of physical, psychological, or emotional disturbance.

Procedures included obtaining an electrocardiogram on the first day along with a determination of the rate of change index by means of the Townsend Technique (Townsend, p. 464). At the same time, the subjects underwent simultaneous physiological and psychological stress after which the heart measurements were again obtained. Two criteria were necessary to ensure that stress had been accomplished and only subjects who demonstrated change were utilized in the investigation. The first criterion was that each subject was required to show an increase in subjective feelings of distress from day one to day two as determined by means of the "Subjective Stress Scale" (Gibby and Gibby, 1968). The second criterion was met when the subject manifested a change in the activity of the adreno-medullary system as determined by measuring the amount of urinary catecholamine excretions under non-stress and stress conditions.

The Pearson product-moment correlation of 0.64 revealed a statistically significant difference (p .001) between rate of change under stress and non-stress conditions and that this rate of change of heart beat remains essentially consistent from one situation to another. The implication drawn by the investigators was that the change in heart-beat rate obtained under non-stress conditions may be used to predict heart rate performance under stressful conditions.

Frankenhaeuser and Rissler (1970) conducted a study to determine catecholmine levels of 40 normal, healthy university students who were subjected to a circumstance of anticipation and uncertainty and a circumstance of relaxation. Urine samples were collected before and after each session.

The method included the participation in two 90 minute morning sessions, one evoking anxiety and the other allowing relaxation. On the first visit to the laboratory the subjects were isolated in order to evoke the feelings of uncertainty and anticipation. A tape recorder informed the subjects that after the initial 25 minute period, during which base levels for heart rate and blood pressure were obtained, they would receive harmless, but strong, electrical shocks which would be released automatically with each heart rate change. In actuality, 20 shocks, given at predetermined intervals, were administered to all subjects. The shock duration was 0.9 seconds at an alternating current of 50 cycles per second. The intensity varied randomly between two and six times the individual sensation threshold.

The relaxation period started four weeks later and consisted of having the subjects sit alone and read magazines. Subjects were informed that they would not be exposed to any unpleasant conditions. No shock was administered.

Adrenaline excretion was significantly higher ($t = 6.94$, $df = 39$, $P < 0.001$) during anticipation than during relaxation. Diuresis was significantly higher during anticipation, 0.99 milliliters per minute than during relaxation, 0.70 milliliters per minute ($P < .01$). There was a significant positive correlation between adrenaline excretion in the two circumstances and between the two noradrenaline measures. Adrenaline excretion during both states was correlated positively ($P < 0.05$) with heart rate and blood pressure during anticipation; and, diuresis was correlated positively ($P < 0.05$) with adrenaline excretions during anticipation. The anticipation of unpleasant circumstances which the subjects could not influence brought about almost a threefold increase in adrenaline output.

Zuspan (1970) reported an in-depth, longitudinal study of six normal, non-pregnant women of child-bearing age and six pregnant patients who were hospitalized as early as 30 weeks of gestation and lasting through four weeks postpartum. Normal, pregnant subjects were housed in a clinical investigation unit where controlled conditions existed. Urine which was refrigerated and pooled for a 24 hour period was later frozen for subsequent epinephrine and norepinephrine analyses. Samples for three successive days were collected at designated weeks of gestation, such as, 30, 32, and 36 weeks, and comprised the sampling for that time block. The entire first week of postpartum urine was analyzed and in the remaining three weeks, for three successive days each week, urine samples were collected and analyzed.

Results indicated that except for the delivery day and deliver-day-plus-one-day the urinary results collected from pregnant subjects were not significantly different from the non-pregnant 24 hour urinary excretions. There were no significant differences between urinary excretions of the normal non-pregnant women and from the antepartum and postpartum epinephrine and norepinephrine values. There was an increase in norepinephrine excretions during late labor but within a few hours following delivery the level decreased which the investigator attributed to "emotional and physical activity during the delivery process (p. 360)."

Kotchen et al., (1971) undertook a study to describe the renin response in normal human volunteers who exercised at three quantified work loads of increasing intensity, specifically, 40 per cent, 70 per cent, and 100 per cent. Plasma epinephrine and norepinephrine levels were measured also at the three work loads to determine the role of the sympathetic nervous system as a potential stimulus for renin excretion.

Subjects included six healthy male Caucasian military personnel who had just previously completed eight weeks of voluntary physical training which included intense exercise three times a week. The non-athletes were of average build, aged 20 to 24 years, and free from medication. To familiarize the subjects with the laboratory setting, each was exposed to exact simulated conditions several times prior to the investigation.

The projected values of 40 per cent, 70 per cent, and 100 per cent oxygen consumption were reported to have been closely proximated by the results. Mean heart rate, maximum oxygen consumption, and hematocrit increased progressively with the increased work loads. A highly significant correlation ($r = 0.98$; $P < .001$) was obtained between oxygen consumption and the actual work load, thereby indicating that oxygen consumption is a valid measure of the intensity of exercise.

Responses to graded exercise were progressively greater at increasingly intense work loads for renin, epinephrine, and norepinephrine. Comparatively small changes in epinephrine, even after maximum exercise, were reported and both norepinephrine and epinephrine levels returned to normal by 30 minutes after maximum exercise. The mean norepinephrine level was significantly elevated after each work load (p .05) and epinephrine was significantly greater only after maximum work loads (p .05). A significant correlation (r = .52; p .01) was reported between renin and norepinephrine indicating that each was stimulated by exercise and that the amount of stimulation depended upon the exercise intensity.

Okada et al., (1972) conducted a study designed to determine the stress imposed upon championship bobsleigh-tobogganning contestants with regard to their positions on the sleigh. Ages ranged from 19 to 28 years; none of the subjects had participated longer than three years. The investigators suggested that when considering the susceptibility to stress in this case that age and length of time participating should be considered as being negligible in influence.

Urine samples were collected from 36 male, Japanese four-man participants of whom 28 participated, also, in the two-man sport. The samples were collected one-half to one hour before the competition and immediately afterward. Precompetition samples were regarded as the control data. Noradrenaline and adrenaline were extracted and analyzed. The data were subjected to an analysis of variance and t-tests. The analysis of variance revealed no statistically significant differences among the players of either the four-man or two-man sport, however, the mean noradrenaline value of the players in the third position of the four-man teams appeared to be higher than those of the other players. When the t-test was applied to these means, a statistically significant difference was found ($P < .05$) in favor of the third man. The authors postulated that the third man exhibited the higher levels of noradrenaline, and therefore anxiety, since he did not pilot or brake the vehicle, nor did he receive motions of the pilot. It was determined also that noradrenaline and adrenaline urinary values did not change in a parallel fashion and that the players in position III showed strong anxiety during the competition as determined by the increased levels of noradrenaline.

Hartley et al., $(1972)_a$ conducted a study which included seven men between the ages of 20 to 24 years who pedaled an electronically braked bicycle ergometer in the sitting position at 60 revolutions per minute. The purpose of the investigation was to examine the levels of selected hormones in plasma obtained during rest and while working at loads of submaximal and maximal intensity. The specific hormones were selected because of their influence on either the cardiovascular or metabolic systems. Epinephrine and norepinephrine were included in the selection.

The authors prefaced their report by stating that: ". . . the hormones under examination could theoretically be of importance in either the regulation of the physiological adjustments which occur during muscular exercise or the changes which result from physical training (p. 602)." Efforts were taken to minimize emotional factors by allowing the subjects to become familiar with the laboratory, equipment, procedures, and personnel. De-emphasis was placed upon competition, and diurnal changes were eliminated through scheduling. Assessments were made of psychoendocrine responses to the anticipation of exercise by obtaining two blood samples, one 20 minutes before and one immediately prior to the exercise.

On the day of the experiment the subjects ate a normal breakfast, then exercised consecutively at three work levels of 40, 70, and 100 per cent maximum oxygen uptake. Subjects pedaled for eight minutes at the first two levels and for five minutes at the heavy load. Recovery periods of 30 minutes after the mild load and one hour after the moderate load ensued.

Upon completion of the pretraining tests the subjects participated in a seven-week training program which was comprised of three-hour periods of distance running, basketball, and volleyball on three mornings a week. The intensity of training was assumed to be high as determined by heart rates of 145-180 beats per minute. After the training sessions the bicycle exercises were repeated and oxygen and hormone responses were determined.

The results indicated an increase during exercise in concentrations of norepinephrine and epinephrine and a 14 per cent increase in maximum oxygen uptake. Plasma norepinephrine levels were the same at rest and during mild work ($p < .05$) but were greater at moderate ($p < .05$) and heavy ($p < .01$) work. Plasma epinephrine was significantly greater than the resting level only at the heavy work load ($p < .05$). Finally, both plasma and urinary norepinephrine levels during work were observed to be lower at equal loads in trained as compared to untrained subjects.

Hartley et al., $(1972)_b$ proposed the hypothesis that "the improvement in endurance time which occurs after physical training may be related to changes in plasma hormone concentration (p. 602)." To test the hypothesis, seven healthy male volunteers between the ages of 20 and 24 years were subjected to prolonged exhaustive work before and after a physical training program of seven weeks. Plasma levels of epinephrine and norepinephrine were examined.

Protocol for the subjects included a usual breakfast at 7:00 a.m. followed by rest until 11:00 a.m. at which time they pedaled an electrically braked bicycle at 60 revolutions per minute during repeated 20 minute bouts with 10 minute rest intervals. This procedure was continued until the subjects were unable to maintain the pedal rate. The endurance time was defined as the total performance time of the subjects while on the bicycle. Blood samples were obtained 20 minutes before the exercise, just prior to the exercise, after 40 minutes of exercise, and when the subjects appeared to be near exhaustion. These procedures were repeated after the seven-week training program.

The results indicated that pretraining plasma norepinephrine values almost doubled during the first 40 minutes of exercise; no additional change was observed at exhaustion. Similar results were reported following the training period, but the plasma levels during work were only half the increase observed before training ($P < .05$). Plasma epinephrine levels tended to increase during work; however, statistical significance was obtained only between resting levels and exhaustion levels ($P < .05$). The epinephrine response to exercise was unchanged by training.

Chapter III will present the procedures followed in the development of the study.

Chapter III

Procedures Followed in the Development of the Study

The purpose of the investigation was to determine the relationship between a four-week exercise program and changes in urinary catecholamines in women. Thirteen adult women participated in an exercise program which consisted of 15 exercise bouts on a motor driven treadmill which was programmed to maintain each subject's heart rate at 150 beats per minute. Subjects exercised three days a week for four weeks. Both oxygen consumption and performance of work were determined during the first, sixth, and last of the twelve 12 minute exercise periods. Twenty-four hour urine samples were collected during three phases of the study: (a) prior to the beginning of the exercise program, (b) at the mid-point of the exercise program, and (c) after the exercise program had been completed. Catecholamine determinations were assessed through the methods of Pisano (1960) and Zuspan, Nelson, and Ahlquist (1967).

In this chapter the procedures followed in the development of the study are described under the following headings: (a) Sources of Data, (b) Preliminary Procedures, (c) Criteria for Selection of Methods, (d) Selection and Description of Methods, (e) Criteria for Selection of Instruments, (f) Selection and Description of Instruments, (g) Criteria for Selection of Subjects, (h) Selection and Description of Subjects, (i) Procedures Followed in Collection of Data, (j) Organization and Treatment of Data, and (k) Preparation of the Final Written Report.

Sources of Data

The data used in the present study were collected from 13 adult women with respect to their (a) oxygen consumptions, (b) performances of work, (c) urinary secretions of total catecholamines, and (d) urinary secretions of epinephrine. The data were obtained during the summer of the academic year of 1972-1973.

Materials pertinent to the development of the research design through which the data were collected were gleaned from authorities in the fields of psychology, physiology, chemistry, medicine, endocrinology, medical technology, physical education, and health education. Further documentary sources comprised books, periodicals, theses, dissertations, micro-cards, and other reports of research related to aspects of the study.

Preliminary Procedures

Prior to the actual collection of data, certain preliminary procedures were necessary. Permission was secured from the Dean of the College of Health, Physical Education and Recreation at the Texas Woman's University to use the equipment necessary to the proposed investigation. Additional permission was requested of and granted by the Human Research Review Committee of the Texas Woman's University.

The investigator surveyed, studied, and assimilated information pertinent to the study from a large number of available documentary sources of data. The documentary analyses of the literature revealed that no completed study duplicated the specific design of the present investigation. As a result of this finding, the investigator developed and presented a tentative outline for research during a Graduate Seminar of the College of Health, Physical Education and Recreation at the Texas Woman's University in Denton, Texas. The outline was revised in accordance with the suggestions offered by members of the Dissertation Committee and filed as a prospectus of the approved study in the Office of the Dean of Graduate Studies.

Criteria for Selection of Methods

The criteria established for the selection of the methods were as follows: (a) reliability, (b) validity, (c) objectivity, and (d) availability of methods designed to determine performances of work, oxygen consumption levels, total catecholamine levels, and epinephrine levels in urine.

Selection and Description of Methods

A treadmill exercise program devised by Rosentswieg and Burrhus (1973), oxygen consumption and carbon dioxide determinations as demonstrated by the Beckman E_2 Oxygen

Analyzer and Beckman LB-1 Carbon Dioxide Analyzer, total catecholamine levels as determined by the Pisano (1960) method, and epinephrine levels as determined by the Zuspan, Nelson, and Ahlquist (1967) were selected empirically as the methods to be used in the collection of data for the present investigation.

The exercise program was administered on a Quinton, motor-driven treadmill which was programmed to alter its speed in order to maintain the subjects' heart rates at 150 $\pm$ 5 beats per minute. Subjects exercised three days a week for four weeks. Resting heart rates were determined while each subject was standing on the treadmill and were noted on each day of training. The fifteen minute exercise session was begun when the resting heart rate had stabilized. Treadmill revolutions were recorded on the days on which $\dot{V}O_2$ determinations were made and were used as data points representing performance of work. Detailed descriptions of the methods used in this investigation are included in the Appendix.

Criteria for Selection of Instruments

A survey of authoritative sources, Zuspan et al., (1967) von Euler and Floding (1955) and, Drujan et al., (1959) indicated the minimum criteria of validity, reliability, objectivity, and availability that the instruments utilized in the study should meet. The treadmill system, oxygen analyzer, and carbon dioxide analyzer were available at the Human

Performance Laboratory of the College of Health, Physical Education and Recreation at the Texas Woman's University. The instruments necessary for analyzing total catecholamine levels, as required by the Pisano (1960) method, were also available in the Human Performance Laboratory. The facilities and instruments for the final chemical analyses, that of Zuspan, Nelson, and Ahlquist (1967), were made available through the Biology Department at North Texas State University in Denton, Texas. All instruments necessary to this investigation met acceptable standards of reliability, validity, and objectivity.

Selection and Description of Instruments

A brief description of the instruments utilized in the investigation follows under the subheadings of: Exercise Training Program Instruments and Urinary Analyses Instruments.

Exercise training program instruments. The inherent nature of the Quinton model treadmill system provided that the instrument was objective, reliable, and valid both for measuring heart rates at rest and during exercise and for maintaining the speed of the belt of the treadmill at a rate which stabilized the subjects' heart rate at 150 $\pm$ 5 beats per minute. The Beckman E_2 Oxygen Analyzer and the Beckman LB-1 Carbon Dioxide Analyzer were selected because of their demonstrated efficiency and accuracy in the Human Performance Laboratory of the Texas Woman's University. Determinations can be made in less than three minutes with 98 per cent accuracy.

Urinary analyses instruments. The instruments used to determine urinary catecholamine secretions were: a pH meter, a magnetic stirrer, four chromatographic columns, a spectrophotometer, a refrigerated ultracentrifuge, a Mettler weight instrument, and a spectrophotofluorometer. Each of these instruments met the requirements of objectivity, validity, and reliability, the latter of which was determined by replication of samples by the examiner.

Criteria for Selection of Subjects

The following criteria were established for the selection of subjects to participate in the study: (a) women who volunteered and were between the ages of 30 and 40 years, (b) subjects who had completed satisfactorily the health screening questionnaire, (c) subjects who had arranged for and passed satisfactorily a medical examination, (d) subjects who had signed and dated a form releasing the investigator and the University from the possibility of claims of infringement of personal rights or acts of coercion, and (e) subjects who were aware that they could withdraw from the study at any time. Particular emphasis was placed upon compliance with the guidelines established by the United States Department of Health, Education, and Welfare and published in their Publication, Number (NIH) 72-102, December, 1971. These guidelines pertain to the protocol of investigations utilizing human subjects. See Appendix for copies of the forms.

Selection and Description of Subjects

A list of potential subjects was compiled from which telephone contacts were made. The potential subjects were informed of the nature of the investigation and were then asked to participate. Voluntary participation was maintained throughout the investigation

The original group of 20 subjects dwindled to 13 female volunteers between the ages of 30 and 40 years. Table 1 describes the subjects in terms of ages and weights.

Table 1

Description of Subjects by Ages and Weights

	Means	Median	S.D.	S.E.$_{\bar{X}}$	Range
Age (Yrs.)	34.5	34	3.23	.93	8 Years (31-39 Yrs.)
Weight (Lbs.)	132.3	131	17.55	5.07	55 Pounds (105-160 Lbs)

A review of the table reveals that the subjects ranged in age from 39 years through 31 years with a mean age of 34.5 years. The standard deviation of ages is reported at 3.23 years with a standard error of the mean of .93. Weights ranged from 105 pounds to 160 pounds with a mean weight of 132.3 pounds. The standard deviation was 17.55 pounds with a standard error of the mean of 5.07.

Procedures Followed in Collection of Data

Prior to the actual study each subject was oriented to the instruments to be used during the exercise program. Specifically, these included the treadmill, the mouthpiece attached to the apparatus for the subsequent $\dot{V}O_2$ collections, and the electrodes which monitored heart rates. Simple, predetermined instructions were given to each subject and any questions were answered. At this time subjects were requested to schedule regular periods for the four-week training program. Subjects were scheduled on Mondays, Wednesdays, and Fridays throughout the study. There were no exceptions to this schedule.

Two consecutive 24 hour urine samples were collected prior to the exercise training program. Samples were analyzed and the results were averaged for total catecholamine output according to the Pisano (1960) method. Additional samples were frozen with the intention of a later epinephrine analysis using the Zuspan, Nelson, and Ahlquist (1967) method. The frozen samples were inadvertently thawed through a power failure and were rendered unusable.

Oxygen consumption was determined during the first, sixth, and last of twelve 15 minute exercise sessions. The open circuit method was employed through the use of a modified Otis-McKerrow valve and 1500 liter gasometer. Oxygen and carbon dioxide were analyzed immediately and recorded.

Subjects were informed that a total of seven 24 hour urine collections would be required beginning on Saturday, June 16, and Sunday, June 17, 1973. For reference purposes, the initial collections will be referred to as the base-line or beginning samples since they were taken prior to the exercise program. Subsequent collections were made on Tuesday, June 19 (after one day of the exercise program), Thursday, June 28 (24 hours without exercise), and June 29 (24 hours including the exercise session). The final urine collections were made on Thursday, July 12 (24 hours without exercise) and Friday, July 13 (24 hours including the exercise program).

Subjects were given the following directions with regard to the actual collection:

> Day 1
> Discard the first urine in the morning; collect all urine thereafter and add the acid to preserve the urine. (Subjects were cautioned about proper care in using the acid).
>
> Day 2
> Continue collection from Day 1 (do NOT discard first urine). Begin a new bottle after the first urination and add acid to preserve.
>
> Day 3
> Save first urination only.

Additional directions instructed the subjects to abstain from eating bananas (Friedman et al., 1960) during the study and to maintain their regular diets.

As urine samples were completed, the investigator identified, measured, and recorded the volumes in milliliters. Samples were refrigerated or frozen in adequate amounts for the chemical processes which followed.

Organization and Treatment of Data

The procedures which follow include those related to studying the statistical evidence collected during the exercise training program and, specifically, catecholamine levels, epinephrine levels, $\dot{V}O_2$ consumptions, and levels of performance. The statistical techniques employed and treatment of the data reflected the initial purposes of the investigation, that is, to determine the relationship between the four-week exercise program and changes in urinary catecholamines in women.

The appropriate statistical treatment included a one-way analysis of variance with repeated measures on each of the following variables: exercise catecholamine levels, resting catecholamine levels, $\dot{V}O_2$ consumptions, treadmill revolutions, exercise epinephrine levels, and resting epinephrine levels. Subsequent analysis techniques were employed where necessary.

Preparation of Final Written Report

The preparation of the written report of the study entailed the writing of each chapter in accordance with its topical outline, submitting the report to members of the dissertation committee for corrections and/or suggestions and revising each chapter in accordance with the expressed wishes of the committee members. A summary of the research was prepared; the findings were interpreted, discussed, and presented. The final procedures included making recommendations for future studies, developing the Appendix, and compiling a bibliography.

The findings of the investigation appear in Chapter IV of the dissertaion.

Chapter IV

Statistical Treatment of the Data

This chapter will present the statistical treatment of the data on 13 women between the ages of 30 and 40 years to determine the relationship between a four-week exercise program and changes in urinary catecholamines. To determine the relationship, one-way analyses of variance were used with each of the following variables: treadmill revolutions (performances of work), $\dot{V}O_2$ consumptions, resting catecholamines, exercise catecholamines, resting epinephrine, and exercise epinephrine. The Scheffe Test of Mean Comparisons was used where indicated to determine subsequent differences between means. Data points were determined at the beginning, mid-point, and conclusion of the four week experimental period. Orthogonal comparisons were computed to determine linear and non-linear trends of the data.

The interpretation of the data will be reported under these headings: Descriptive Data and Results From One-Way Analyses of Variance. A summary of major fingings concludes the chapter.

Descriptive Data

Table 2 describes the data in terms of the three time periods of the study: (a) the beginning; (b) the mid-point,

Table 2

Means, Standard Deviations, and Ranges for Subjects Comprising the Study

	Treadmill Revolutions in 15 Min.	$\dot{V}O_2$ Consumptions (ml./Kg./Min.)	Resting Catecholamines (mg./24 Hrs.)	Exercise Catecholamines (mg./24 Hrs.)	Resting Epinephrine (mg./24 Hrs.)	Exercise Epinephrine (mg./24 Hrs.)
Means						
Period 1	1366.15	24.71	.224	.256	Lost	16.53
Period 2	1457.00	26.54	.155	.079	8.12	9.17
Period 3	1535.38	27.19	.111	.032	6.92	8.27
S. D.						
Period 1	198.48	4.95	.12	.30	Lost	13.78
Period 2	276.62	5.05	.17	.08	5.18	7.48
Period 3	288.94	6.38	.10	.02	3.64	5.49
Sex						
Period 1	57.30	1.43	.04	.09	Lost	3.98
Period 2	79.85	1.46	.05	.02	1.50	2.16
Period 3	83.40	1.84	.03	.006	1.05	1.59
Ranges						
Period 1	782.00	7.1	.40	1.04	Lost	33.5
High	1897.00	31.6	.48	1.10		37.4
Low	1115.00	14.5	.08	.06		3.9
Period 2	872.00	19.7	.65	.31	15.4	23.7
High	1983.00	34.5	.67	.33	16.7	25.4
Low	1111.00	14.8	.02	.02	1.3	1.7
Period 3	964.00	21.4	.34	.057	11.5	15.1
High	2106.00	37.6	.36	.066	12.6	17.0
Low	1142.00	16.2	.02	.009	1.1	1.9

and (c) the end of the exercise training program. The term "exercise catecholamine" refers to those catecholamine determinations which were made over a 24 hour period which included one 15 minute exercise period on the treadmill. The phrase "resting catecholamine" refers to a 24 hour period during which no treadmill exercise existed. Means, standard deviations, standard errors of the mean, and the ranges are reported for the treadmill revolutions, $\dot{V}O_2$ consumptions, resting catecholamines, exercise catecholamines, resting epinephrine, and exercise epinephrine. The treadmill revolution scores are expressed as the number of belt revolutions which occurred during the 15 minute sessions; $\dot{V}O_2$ consumptions are expressed in terms of milliliters per kilogram of body weight per minute; and resting catecholamine, exercise catecholamine, resting epinephrine, and exercise epinephrine levels are expressed in terms of milligrams per 24 hours.

A review of the table reveals that the mean treadmill revolutions per 15 minutes for the subjects increased from the beginning of the experimental program to the mid-point, and from the mid-point to the end of the study. As the measure for performances of work for the subjects, these increments indicate an improvement in the ability to do greater amounts of work by subjects involved in the investigation.

The mean $\dot{V}O_2$ consumption scores indicate a similar increase from the beginning of the study to the mid-point and from the mid-point to the end of the experimental program. These increments provide data in verifying a trend of improvement in physical fitness levels of the subjects participating in the study.

The mean catecholamine levels during 24 hour periods without the exercise training program decreased from the beginning to the mid-point of the study and from the mid-point to the end of the study. These decreases indicate similar results to those reported by von Euler and Hellner (1952), Kotchen et al., (1971), and Elmajian et al., (1956).

Catecholamine determinations were made at the beginning, mid-point, and at the end of the study during 24 hour collection periods which included the exercise training program. Mean catecholamine levels decreased in a linear fashion from the beginning to the mid-point and from the mid-point to the end of the four-week exercise training program. These steady decreases in total catecholamines provide additional data to support the premise suggested by Michael (1957) that "it is possible then that exercise provides a means of strengthening the adaptive mechanism of the body." Additional implications of the decreases in total catecholamine levels were suggested by Hartley et al., (1972) that ". . . it follows that the improvement in endurance time which occurs after physical training may be related to changes in plasma hormone concentration."

Epinephrine determinations were made on samples which had been frozen. The first samples of epinephrine obtained during the 24 hours without the exercise training program were inadvertently lost due to electrical power failure and subsequent thawing. However, epinephrine levels per milligrams per 24 hours (without the exercise training program) were determined for samples collected at the mid-point and at the end of the study. Mean epinephrine levels from the mid-point to the end of the study decreased, giving further substantiation to the possibility that the endocrine system adjusts to the various levels of physical fitness. The results obtained from this investigation coincided with those reported by Elmadjian et al., (1956) and Kotchen et al., (1971).

Mean epinephrine levels were also determined for 24 hour periods which included the exercise training program. These determinations were made at the beginning of the study, at mid-point, and at the end of the study. Similar decreases were obtained and further substantiations of the previously mentioned investigations might be indicated.

Results from One-Way Analyses of Variance

Table 3 presents the analysis of variance with repeated measures for treadmill revolutions (performance of work) for the 15 minute sessions at the beginning, mid-point, and end of four weeks. The F ratio for time periods was significant at the .01 level indicating that there was significant improvement in fitness levels during the four weeks. To determine the trend of the data, orthogonal comparisons

Table 3

Summary Table of a One-Way Anova with Repeated Measures of Treadmill Revolutions

Source of Variance	df	SS	MS	F*	f
Between Time Periods	2	186490.31	93245.16	11.21	p < .01
Linear	1	186153.85	186153.85	22.37	p < .01
Non-Linear	1	336.46	336.46	.04	n.s.
Between Subjects	12	2193058.41	182754.87		
Residual	24	199716.36	8321.52		

*$F_{2,24}$ (.01) = 5.61

$F_{1,24}$ (.01) + 7.82

were computed with a resultant F ratio for linear trend showing significance at better than the .01 level. Figure 1 shows this trend graphically and incorporates the respective

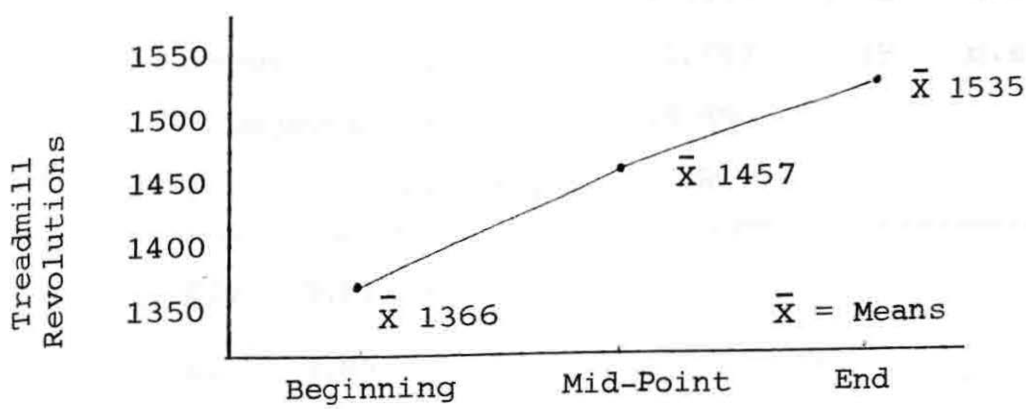

Fig. 1. A Comparison of Average Treadmill Revolutions Obtained at the Beginning, Mid-Point, and End of the Study. N = 13.

means. The F ratio for the between subjects variation is not reported since it is of no real importance to the analysis of the data.

Table 4 presents the analysis of variance with repeated measures on $\dot{V}O_2$ consumptions per milliliter per kilogram per minute. The F ratio of 1.36 for the time periods indicates a lack of significant difference among the three times; the F ratio for trend was, similarly, non-significant. These data are shown graphically in Figure 2.

Table 4

Summary Table of a One-Way Anova with Repeated Measures of $\dot{V}O_2$ Consumptions

Source of Variance	df	SS	MS	F*	f
Between Time Periods	2	43.128	21.56	1.359	n.s.
Linear	1	40.375	40.375	2.55	n.s.
Non-Linear	1	2.753	2.753	.18	n.s.
Between Subjects	12	706.66	58.89		
Residual	24	380.68	15.86		

$^*F_{2,24}$ (.01) = 5.61

$F_{1,24}$ (.01) = 7.82

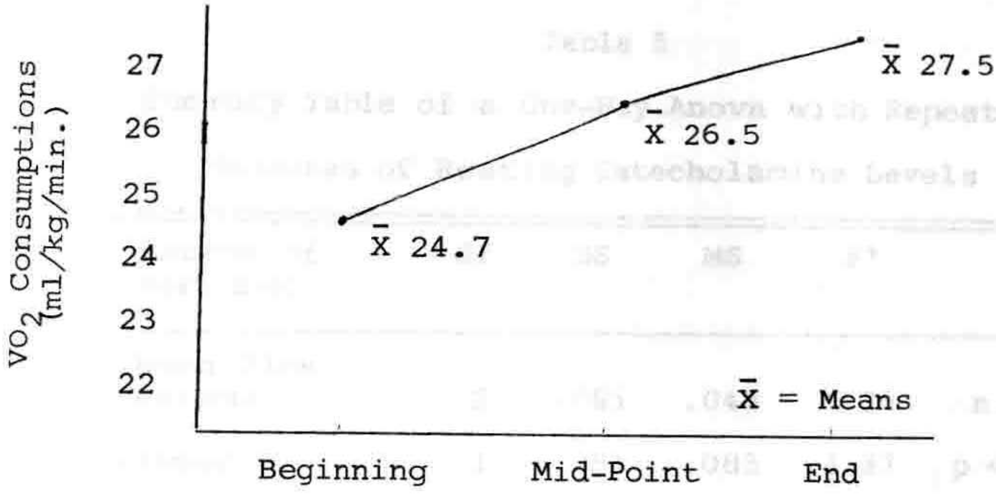

Fig. 2. A Comparison of $\dot{V}O_2$ Consumption Means Obtained at the Beginning, Mid-Point, and End of the Study. N = 13.

Table 5 represents the results of the analysis of variance of resting catecholamines per milligram per 24 hours over the three time points. The F ratio for the time periods was not significant; however, a significant linear trend was noted. These data are presented graphically in Figure 3.

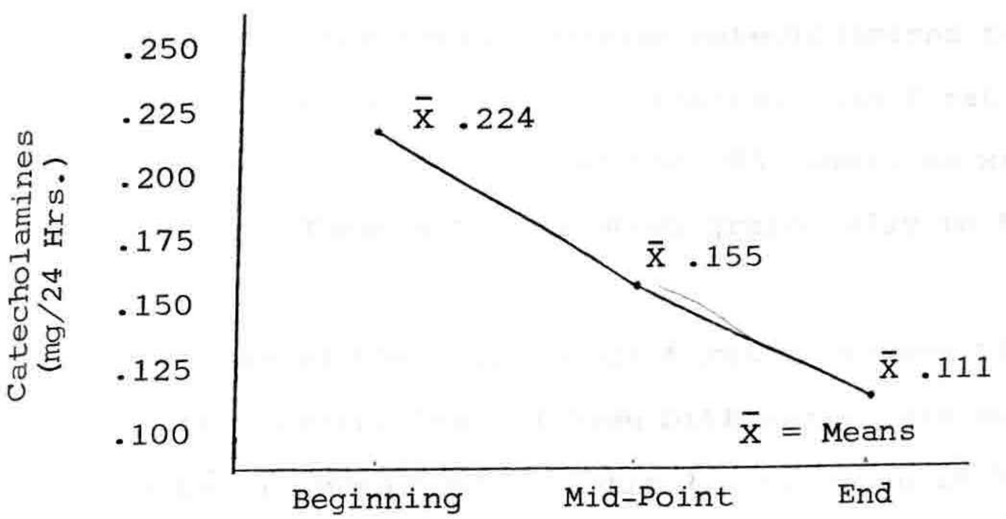

Fig. 3. A Comparison of Mean Resting Catecholamine Levels. N = 13.

Table 5

Summary Table of a One-Way Anova with Repeated Measures of Resting Catecholamine Levels

Source of Variance	df	SS	MS	F*	p
Between Time Periods	2	.085	.043	2.26	n.s.
Linear	1	.083	.083	4.37	$p < .05$
Non-Linear	1	.002	.002	.11	n.s.
Between Subjects	12	.208	.017		
Residual	24	.459	.019		

*$F_{2,24}$ (.01) = 5.61

$F_{1,14}$ (.01) = 7.82

$F_{1,24}$ (.05) = 4.26

Table 6 presents the analysis of variance with repeated measures for total exercise catecholamines per milligram per 24 hours for the 13 subjects. The F ratio for time periods was significant at the .05 level, as was the linear trend. These data are shown graphically in Figure 4.

Because of the significant F ratio between time periods, the Scheffe Test of Mean Differences was computed and results are presented in Table 7. As shown in Table 7, the mean level at the end of the experiment was significantly lower than the mean level at the beginning of the

Table 6

Summary Table for a One-Way Anova of Exercise Catecholamine Levels

Source of Variance	df	SS	MS	F*	p
Between Time Periods	2	.363	.182	5.52	$p < .05$
Linear	1	.327	.327	9.90	$p < .01$
Non-Linear	1	.036	.036	1.09	n.s.
Between Subjects	12	.373	.031		
Residual	24	.802	.333		

$^{*}F_{1,24}$ (.01) = 7.82

$F_{2,24}$ (.05) = 3.40

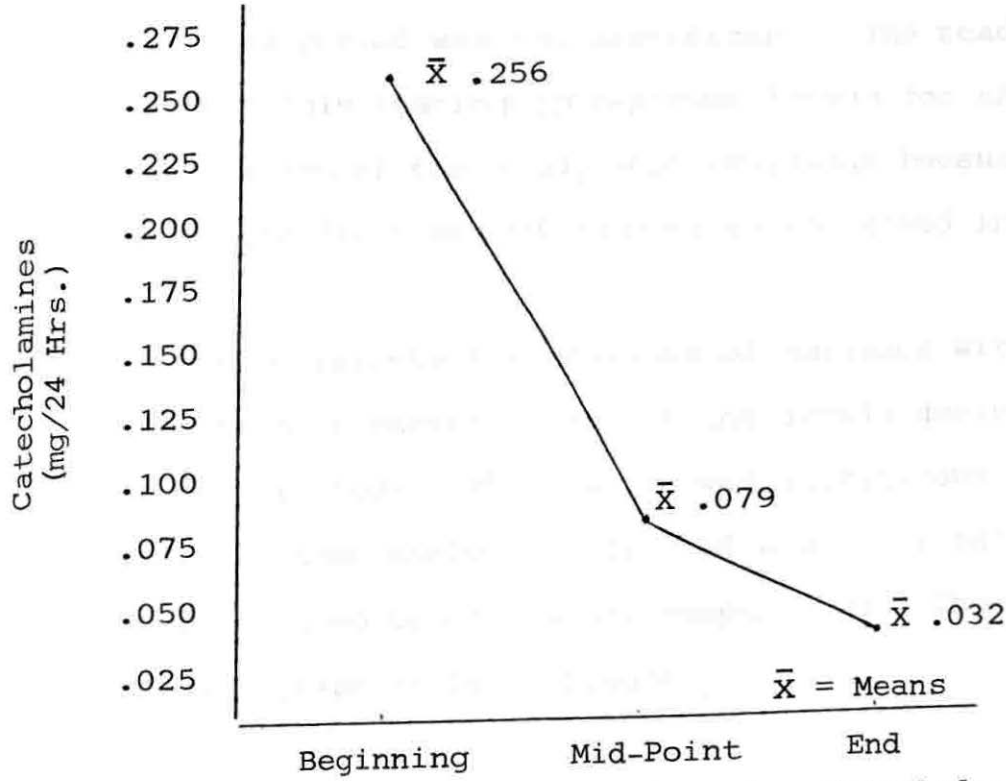

Fig. 4. A Comparison of Mean Exercise Catecholamine Levels.

experiment. No other mean differences were significant.

Table 7

Scheffe Test* on Means of Exercise

Catecholamine Levels

		1 .256	2 .079	3 .032
1	.256	0	.154	2.24*
2	.079			.047
3	.032			

*C $\pm$ .588 (.05)

Table 8 presents the analysis of variance with repeated measures for resting epinephrine excretions obtained over the three 24 hour periods. As shown in Table 8, the F ratio for time period was not significant. The reader is reminded that only resting epinephrine levels for the mid-point and the end of the study were available because of the loss of the first set of samples as explained in Chapter 3.

Table 9 presents the analysis of variance with repeated measures of exercise epinephrine levels during the three 24 hour periods. The F ratio was significant at the .05 level. Further analysis indicated a significant linear trend as determined by orthogonal comparisons. These data can be viewed graphically in Figure 5.

Table 8

Summary Table of a One-Way Anova with Repeated Measures of Resting Epinephrine Levels

Source of Variance	df	SS	MS	F*	p
Between Time Periods	1	10.86	10.86	.88	n.s.
Between Subjects	12	333.16	27.76		
Residual	12	148.15	12.34		

$^*F_{1.24}$ (.05) = 4.75

Table 9

Summary Table of a One-Way Anova of Exercise Epinephrine Levels

Source of Variance	df	SS	MS	F*	p
Between Time Periods	2	534.11	267.05	3.73	$p < .05$
Linear	1	443.6		6.20	$p < .01$
Non-Linear	1	90.5		1.26	
Between Subjects	12	1596.34	133.03		
Residual	24	1717.76	71.57		

$^*F_{2,24}$ (.05) = 3.40

$F_{1,24}$ (.01) = 5.61

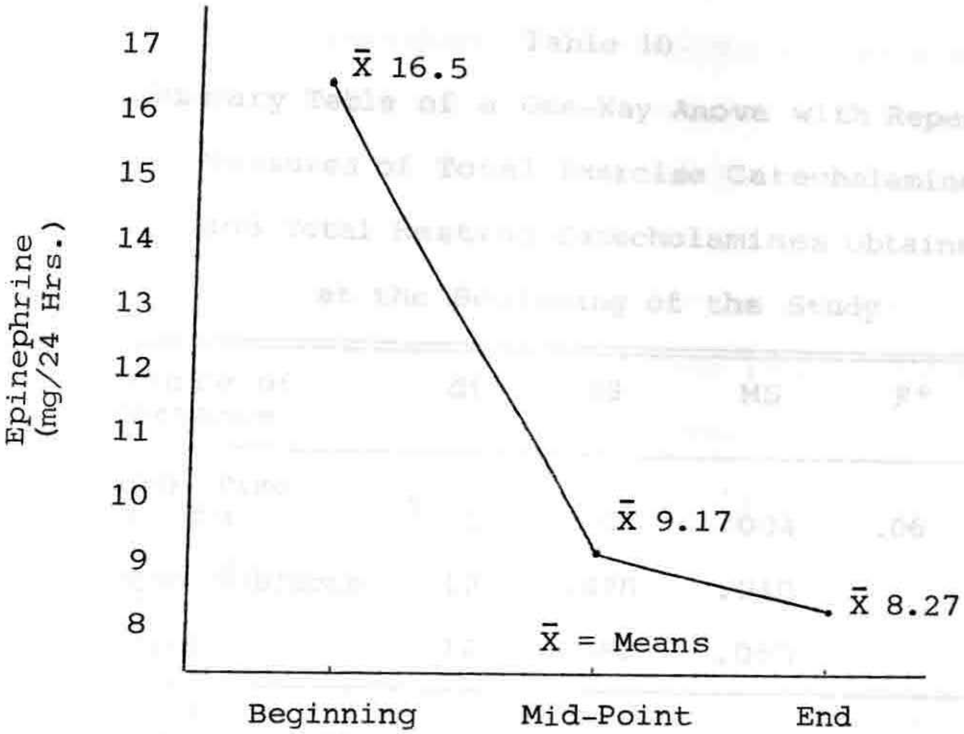

Fig. 5. A Comparison of Exercise Epinephrine Levels. N = 13.

Table 10 represents the one-way analysis of variance with repeated measures comparing exercise with resting total catecholamine levels for the first set of samples. The f ratio for the comparison was not significant, indicating that although the exercise levels were higher than the rest-levels, the difference was not significant.

Table 11 represents the one-way analysis of variance with repeated measures which compares exercise and resting total catecholamine levels at the mid-point of the experimental period. The F ratio of 2.64 was not significant, indicating that exercise and resting catecholamine levels for the subjects were not substantially different after two weeks of exercise.

Table 10

Summary Table of a One-Way Anova with Repeated Measures of Total Exercise Catecholamines and Total Resting Catecholamines Obtained at the Beginning of the Study

Source of Variance	df	SS	MS	F*	p
Between Time Periods	1	.004	.004	.06	n.s.
Between Subjects	12	.478	.040		
Residual	12	.784	.065		

$^{*}F_{1,12}$ (.05) = 4.75

Table 11

Summary Table of a One-Way Anova with Repeated Measures of Total Exercise Catecholamines and Total Resting Catecholamines Obtained at the Mid-Point of the Study

Source of Variance	df	SS	MS	F*	p
Between Time Periods	1	.037	.037	2.64	n.s.
Between Subjects	12	.276	.023		
Residual	12	.170	.014		

$^{*}F_{1,12}$ (.05) = 4.75

Table 12 represents the one-way analysis of variance with repeated measures between exercise and resting total catecholamines at the conclusion of the experimental period. The F ratio of 10.25 was significant at the .01 level and indicates that the catecholamine levels at exercise were significantly lower than those during rest. The mean catecholamine level during exercise at the conclusion of the four weeks was .32 milligrams while the mean catecholamine level during rest was .111 milligrams.

Table 12

Summary Table of a One-Way Anova of Total Exercise Catecholamines and Total Resting Catecholamines Obtained at the End of the Study

Source of Variance	df	SS	MS	F*	p
Between Trials	1	.041	.041	10.25	$p < .01$
Between Subjects	12	.09	.0075		
Residual	12	.045	.004		

*$F_{1,12}$ (.01) = 9.33

Summary of Findings

The statistical procedures applied to the data and presented in this chapter included the computation of the means, standard deviations, and ranges. In addition, one-way analyses of variance with repeated measures were computed on the following variables: treadmill revolutions, $\dot{V}O_2$ consumptions, total resting catecholamine excretions during 24 hours, total exercise catecholamine excretions during 24 hours, resting and exercise epinephrine levels during 24 hours, and total resting and exercise catecholamine levels. These data were obtained at the beginning, mid-point, and conclusion of the four-week experimental period.

Based upon the results from the statistical analysis the following decisions were made.

<u>Major Hypothesis</u>

Urinary catecholamine secretions among women are not altered significantly by participation in a four-week exercise program. REJECTED

<u>Sub-hypotheses</u>

(a) There is no significant difference between the amount of work performed at the beginning of the investigation and work performed at the conclusion of the investigation. REJECTED

(b) There is no significant positive trend in the amount of work performed on each exercise day throughout the four-week exercise program. REJECTED

(c) There is no significant difference between the oxygen consumption at the beginning of the investigation and the oxygen consumption at the conclusion of the investigation. ACCEPTED

(d) There is no significant positive trend in the amount of oxygen consumed as determined during the four-week exercise program. ACCEPTED

(e) There is no significant difference between initial and final determinations of resting catecholamine levels.
ACCEPTED

(f) There is no significant trend in the amounts of resting catecholamines secreted over the four-week exercise program.
REJECTED

(g) There is no significant difference between initial and final determinations of exercise catecholamine levels.
REJECTED

(h) There is no significant trend in the amounts of exercise catecholamines secreted over the four-week exercise period.
REJECTED

(i) There is no significant difference between initial and final determinations of resting epinephrine. ACCEPTED

(j) There is no significant trend in the amounts of E secreted during resting conditions over the four-week exercise period. NOT TESTED -- insufficient data.

(k) There is no significant difference between initial and final determinations of exercise epinephrine. REJECTED

(l) There is no significant trend in the amounts of E secreted during exercise conditions over the four-week exercise period. REJECTED

(m) There is no significant difference between exercise catecholamine levels and resting catecholamine levels prior to the onset of the four-week exercise program. ACCEPTED

(n) There is no significant difference between exercise catecholamine levels and resting catecholamine levels obtained at the mid-point of the four-week exercise program. ACCEPTED

(o) There is no significant difference between exercise catecholamine levels and resting catecholamine levels obtained at the conclusion of the four-week exercise program. REJECTED

A summary of the investigation, the conclusion of the study, and recommendations for future studies are presented in Chapter V.

Chapter V

Summary, Findings, Conclusion, Discussion and Recommendations for Future Studies

In this chapter a summary of the investigation, findings of the investigation, discussion of the findings, and conclusions of the investigation were presented. Recommendations for further studies were made based upon the results of this research.

Summary of the Investigation

During this century there has been an abundance of research which relates catecholamine secretions to various types of stress. It was the purpose of this investigation to inquire specifically into an area heretofore unexamined; that is, catecholamine determinations before, during, and after a four-week physical exercise program utilizing women as subjects.

Indications are that long-term physical exercise appears to increase the size and sensitivity of the adrenal glands (Engle, 1953; Richter, 1953; Hoagland, 1955); there exists, therefore, the possibility that such activity might result in a more efficient response to stress. No information was located, however, with regard to the type, frequency, and duration of exercise that could be considered as optimal for the adult female. It was the purpose of this investigation to determine the relationship of a four-week physical training program to urinary catecholamines in women.

A review of related literature revealed that there is a paucity of data related to urinary catecholamine secretions among women who are involved in exercise training programs. Relatively few studies were located which referred to the relationship of urinary catecholamines in the average healthy male to physical exercise.

Data are bountiful with regard to plasma and urinary catecholamines in physically and emotionally atypical subjects such as those who were hypoglycemic or schizophrenic. Catecholamine data are readily available also on other "stress-inducing" situations such as thermal stresses, electrical shock, and induced pain.

Various methods for the determination of catecholamines are abundant also in the literature; however, little information was located with regard to the procedure currently recommended for making total catecholamine determinations.

The procedures followed in the development of the study were described under the following headings: (a) Sources of Data, (b) Preliminary Procedures, (c) Criteria for Selection of Methods, (d) Selection and Description of Methods, (e) Criteria for Selection of Instruments, (f) Criteria for Selection of Subjects, (g) Selection and Description of Subjects, (h) Procedures Followed in Collection of Data, and (i) Preparation of Final Written Report. The methods for use in the study were the Rosentswieg and Burrhus (1973) exercise training program, the Pisano (1960) method for determining total urinary catecholamines, and the Zuspan, Nelson, and Alhquist (1967) method for determining urinary epinephrine.

The subjects for the study included 13 women between the ages of 30 and forty years who participated voluntarily. The investigation took place during the first summer session of the academic year of 1972-1973 at the Texas Woman's University in Denton, Texas.

Data were collected, treated statistically, and analyzed in order to determine the relationship between a four-week exercise training program and urinary catecholamine changes in women. Data were analyzed by one-way analyses of variance with repeated measures. The variables were treadmill revolutions, $\dot{V}O_2$ consumptions, total resting catecholamine excretions during 24 hours, total exercise catecholamine excretions during 24 hours, resting and exercise epinephrine levels during 24 hours, and total resting and exercise catecholamine levels. These data were obtained at the beginning, mid-point, and conclusion of a four-week exercise period.

Findings of the Investigation

Significant differences were found between the three time points for treadmill revolutions, for exercise catecholamines, and for exercise epinephrine levels. Further analysis of these three variables revealed a significant linear trend over the four-week period. The number of revolutions per 15 minutes increased significantly from the beginning to the end of the exercise program. Exercise catecholamine and exercise epinephrine levels decreased significantly from week one to week four. Non-significant F ratios were found for the remaining variables.

Analyses of variance were computed to determine if there was a difference between resting catecholamine levels and exercise catecholamine levels for each of the three time points. At the beginning and mid-point of the experimental period there were no significant differences as indicated by the F ratios. A significant difference was found, however, between resting and exercise catecholamine levels at the end of the experiment, with the exercise catecholamine levels significantly lower than the resting catecholamine levels.

Discussion of the Findings

Mean treadmill revolutions per 15 minutes for the 13 subjects increased from the beginning of the experimental period to the mid-point, and from the mid-point to the end of the study. Similar increases were not obtained, however, $\dot{V}O_2$ consumption analyses. The lack of significant improvement in $\dot{V}O_2$ is in accordance with findings of Karlsson (1967), Drinkwater (1973), and Andersen (1967), who state that $\dot{V}O_2$

will not be elevated significantly unless a subject works to approximately 80 per cent of maximum aerobic capacity. According to other work by Astrand (1970), a heart rate of 150 represents work levels just over a fifty per cent of maximum aerobic capacity for female subjects between the ages of 30 and 40. Consequently, it would appear the work load imposed on the subjects of this investigation was enough to cause some elevation in ability to perform submaximal work but not enough to elevate maximum aerobic capacity.

Consistent decreases were obtained from the beginning to the mid-point and from the mid-point to the end of the experimental period in both exercise and resting total catecholamine levels. These decreases substantiate the investigation done by Hartley et al., (1972) in which he stated "that improvement in endurance time which is seen to occur after physical training may be related to changes in concentrations of adrenal secretions." Additional validation of the findings of this investigation is found in the premise suggested by Michael (1957) that exercise may provide a means of strengthening the adaptive mechanism of the body. In view of the finding that catecholamine secretions decreased as performance of work increased, it is postulated by this investigator that adaptation occurs in the cardiovascular and cardiorespiratory systems, and as such adaptation occurs, the exercise task required of the subject no longer represents a stressful situation.

Conclusions

Within the limitations of this investigation, it appeared reasonable to conclude that urinary catecholamine secretions among women are altered significantly by participation in a four-week exercise program. Specifically, it was concluded that adrenal secretions of total catecholamines as well as of epinephrine decrease significantly and linearly in response to continued submaximal exercise. Continued submaximal exercise does not, however, tend to alter resting levels of either total catecholamines or epinephrine, and does not appear to give rise to differences between resting and exercise levels of catecholamines until at least two weeks of submaximal exercise has been taken.

It was concluded further that the exercise program selected for this investigation was sufficiently rigorous to allow for an increase in work performance in a given time period, but not sufficiently rigorous to elevate maximum aerobic capacity of female subjects between 30 and 40 years of age.

Recommendations for Further Study

In the development of the present investigation many questions of interest suggesting further persual were noted by the investigator. These are incorporated into the following recommendations for future studies:

(a) Replicate the study including differences of ethnic groups.

(b) Expand the study to include determinations of norepinephrine, dopamine, and 17-ketosteroids.

(c) Expand the study to include stresses in addition to exercise such as respiratory difficulties, pain or fear.

(d) Elongate the time period of the experiment to determine plateaus of catecholamine secretions.

(e) Incorporate psychic stressors as part of the study to determine whether catecholamine secretion is equally reduced in the face of stresses other than exercise.

Appendix A

Description of Procedures Included in the Pisano

Method for the Determination of Catecholamines

Appendixes

Appendix A

Description of Procedures Included in the Pisano Method for the Determination of Catecholamines

Procedures:

1. Add 1/20 of a 24 hour urine sample to a 50 ml tube.
2. Add .1 volume 2N HC1.
3. Dilute up to 20ml with distilled water.
4. Heat to 100 degrees centigrade for 20-30 minutes; allow to cool to room temperature.
5. Using pH meter, adjust pH to 6.0-6.5 with 5N NaOH.
6. Prepare column by adding slurry of resin in distilled water to a 1 cm diameter chromatographic column. When packed the resin should form a column about 5 cm high. This step can be done while the urine is being heated.
7. Add urine sample to column; allow all urine to pass through column then wash with 20ml water (do not allow column to dry out).
8. Elute column with 10 ml of 4N NH_4OH. The metanephrine and normetanephrine are concentrated in the purple band which forms when the ammonia is added to the column. Collect 10 ml of eluate-1,2 ml after purple band has been eluted from column.
9. From the eluate take two 4 ml samples--sample and blank. Prepare a standard by adding 0.02 ml of metanephrine standard to 4 ml of 4N NH_4OH. Use 4 ml of 4N NH_4OH as a blank for this sample.
10. To each sample tube add 0.1 ml of periodate solution. To each blank tube add 0.1 ml water. After 2 minutes add 0.1 ml sodium bisulfite solution to each tube.
11. Zero spectrophotometer at 360 nm. Measure optical density of each sample against the corresponding blank.
12. Calculate the amount of metanephrine + normetanephrine present per 24 hours using the following formula: mg/24 hr. = (OD Sample/OD Standard) x 10

Appendix B

Description of Reagents Necessary for Total Catecholamine Determinations Using the Pisano Method

1. Amberlite CG-50, 200-400 mesh, Ion exchange resin
2. NaH_2PO_4 (sodium dehydrogen phosphate)
3. Con. HCl (concentrated hydrogen chloride)
4. NaOH (sodium hydroxide)
5. Con. NH_4OH (ammonium hydroxide)
6. Sodium meta periodate
7. Sodium meta bisulfite
8. d, 1-normetanephrine-hydrogen chloride (M8625 Sigma)

Appendix C

Description of Procedures Included in the Zuspan, Nelson, and Ahlquist Method for Urinary Epinephrine Determinations

Step 1

Place 10 CC urine plus 200 mg of EDTA plus 1gm. of alumina in 100 ml beaker.

Step 2

Swirl for 2 minutes with constant monitoring . . . adjusting PH to 8.4 with 0.5 N Na OH.

Step 3

Let alumina settle and aspirate and discard urine.

Step 4

Wash twice with 15 ml portions of EDTA water and aspirate and discard supernatant.

Step 5

Add 10 ml of 0.2 N acetic acid and shake for 30 min. at 140 rpm.

Step 6

Centrifuge at 2,500 rpm at 5 degrees C. for 10 min.; remove supernatant and centrifuge again.

CAN STORE IN REFRIGERATOR AND USE SAME DAY OR FREEZE

Step 7

Pipet 1 ml of eluate into fused quartz curvette and add 0.2 ml of 3.5 buffer (400 ml of 5 Normal acetic acid adjusted to Ph 3.5 by addition of 5 Normal sodium acetate).

Step 8

Add 0.1 ml of 0.2 M iodine solution; mix and set stand for 1 minute EXACTLY (use timer); add 0.05 ml of 0.1 M sodium thiosulfate and mix.

IMMEDIATELY add and mix 0.2 ml of alkaline ascorbic mixture (prepared immediately before use by dissolving 10 mg of ascorbic acid in 0.1 ml of glass distilled water and adding 5 ml of 10 Normal sodium hydroxide).

Step 9

Let stand 15 minutes and read in fluorometer with 410 act wavelength and 510 fluorescence.

Appendix D

Description of Reagents Necessary for Urinary Epinephrine Determinations Utilizing the Method of Zuspan, Nelson, and Ahlquist

1. 5 Normal acetic acid (147 ml con. $NC_2H_3O_2$----500 ml water)
2. 0.2 Normal acetic acid (6 ml con. $NC_2H_3O_2$----500 ml water)
3. 10 Normal sodium hydroxide (40 grams----500 ml water)
4. 5 Normal sodium acetate (68 grams----100 ml water)
5. 0.5 Normal sodium hydroxide (10 ml 5N NaOH----100 ml water)
6. .183 Normal acetic acid (4 ml 5N $NC_2H_3O_2$----100 ml water)
7. 0.1 Molar sodium thiosulfate solution (4 grams----25 ml water)
8. 1.345 Molar Potassium Phosphate dibasic (153.5 grams----500 ml water)
9. 0.2 Molar iodine

Appendix E

Voluntary Participation Form Required of All Subjects in the Study

This is to certify that my involvement as a subject in the Dissertation research conducted by Miss Janice Williams and entitled "The Relationship Between A Four-Week Exercise Program and Changes in Urinary Catecholamines in Women" is voluntary, uncoerced, and is not considered by me to be an invasion of my rights as a human being or as a student of Texas Woman's University.

I certify further that I have been informed that I may withdraw from the investigation at any time without penalty.

____

Signature

____

Date

Appendix F

Health Screening Questionnaire

1. Do you wheeze or have to gasp to breath? __Yes __No
2. Are you bothered by coughing spells? __Yes __No
3. Have you ever coughed up blood? __Yes __No
4. Have you ever been told that you had high blood pressure? __Yes __No
5. Do you cough up a lot of phlegm (thick spit)? __Yes __No
6. Do you get chest colds more than once a month? __Yes __No
7. Are you sweating more than usual? __Yes __No
8. Have you been bothered by a thumping or racing heart? __Yes __No
9. Do you ever get pains or tightness in your chest? __Yes __No
10. Do you have trouble with dizziness or light-headedness? __Yes __No
11. Does every little effort leave you short of breath? __Yes __No
12. Do you have trouble with swollen feet or ankles? __Yes __No
13. Are you getting cramps in your legs at night or upon walking? __Yes __No
14. Do you have hot flashes? __Yes __No
15. Have you ever been told that you have a heart murmur? __Yes __No
16. Are you a diabetic? __Yes __No
17. Have you had surgery in the last six months? __Yes __No
18. Have you consulted a doctor within the last six months? __Yes __No
19. Have you been ill within the last six months? __Yes __No

20. Have you ever been told that you should not exercise strenuously? __Yes __No

21. Have you ever been told you have had sny heart complications? __Yes __No

Signature____________________ Date________________

Appendix G

Air Analysis and Calculations--Profant (1972) Method

$$VO_2 = VE\left[K\,X_2 - (K + 1)\ X_1X_2\right] / X_1$$

1. Tissot No. 2
2. Tissot No. 1
3. Tissot
4. Tissot factor
5. Uncorr. V_E (#3 x #4)
6. BP/temp
7. STPD

V_E 8. Corr. V_E (#7 x #5)

9. D.R. room air
10. F O_2 insp.
11. D. R. V_E + CO_2

X_2 12. F O_2 exp. before removal (#11 + 40) of CO_2

13. D.R. V_E - CO_2

X_1 14. F O_2 exp. after removal (#13 + 40) of CO_2

15. F CO_2 exp.
16. F N_2 exp. 1.00-(#12 + #15)

K 17. Corr. Factor (#10 + #16)

18. VO_2 ml/min
19. VO_2 l/min
20. VO_2 ml/Kg/min
21. R.Q. (#15 + #12)

H.R.

Rest ______

1 min ______	11 min ______
2 min ______	12 min ______
3 min ______	13 min ______
4 min ______	14 min ______
5 min ______	15 min ______
6 min ______	16 min ______
7 min ______	17 min ______
8 min ______	18 min ______
9 min ______	19 min ______
10 min ______	20 min ______

$$VO_2 = \frac{\underset{\#8}{\_\_\_\_}\left[\underset{\#17}{\_\_\_\_}\ \underset{\#12}{\_\_\_\_} - (\underset{\#17}{\_\_\_\_} + 1)\ \underset{\#14}{\_\_\_\_}\ \underset{\#12}{\_\_\_\_}\right]}{\#14}$$

Appendix H

Raw Scores for Treadmill Revolutions Obtained at the Beginning, Mid-Point, and End of the Study

Subjects	Beginning Revolutions*	Mid-Point Revolutions	End of Study Revolutions
1	1897	1983	2106
2	1344	1506	1588
3	1115	1143	1142
4	1461	1843	1900
5	1334	1401	1484
6	1275	1345	1464
7	1223	1292	1515
8	1197	1301	1296
9	1222	1111	1215
10	1311	1242	1312
11	1488	1853	1941
12	1382	1433	1515
13	1511	1488	1482

*Treadmill revolutions reported in belt revolutions per 15 min.

Appendix I

Raw Scores for $\dot{V}O_2$ Consumptions at the Beginning, Mid-Point, and End of the Study

Subjects	Beginning $\dot{V}O_2$ Consumptions*	Mid-Point $\dot{V}O_2$ Consumptions	Final $\dot{V}O_2$ Consumptions
1	24.6	34.5	37.6
2	27.4	25.7	26.6
3	14.5	14.8	17.3
4	27.0	27.2	29.0
5	22.0	29.5	32.0
6	33.1	30.9	32.6
7	22.8	29.8	26.7
8	23.4	23.6	21.7
9	23.0	22.3	24.2
10	31.6	22.8	16.2
11	25.1	29.9	35.2
12	27.8	24.5	28.1
13	18.9	29.5	26.3

*$\dot{V}O_2$ consumptions reported in ml/Kg/min

Appendix J

Raw Scores for Total Catecholamine Excretions with and Without Exercise at the Beginning, Mid-Point, and End of the Study

Subjects	Beginning Catecholamines*		Mid-Point		End Catecholamines	
	Without Exercise	With Exercise	Without Exercise	With Exercise	Without Exercise	With Exercise
1	.119	.548	.045	.045	.009	.018
2	.191	1.096	.227	.045	.182	.027
3	.313	.082	.670	.015	.027	.018
4	.096	.164	.227	.14	.045	.0182
5	.147	.140	.082	.131	.164	.066
6	.145	.137	.227	.045	.018	.009
7	.286	.082	.015	.044	.0364	.009
8	.287	.110	.071	.036	.027	.011
9	.075	.550	.167	.067	.091	.036
10	.467	.137	.133	.333	.264	.064
11	.095	.053	.167	.030	.023	.027
12	.097	.082	.500	.033	.138	.046
13	.310	.164	.049	.050	.132	.064

*Catecholamine reported in milligrams/24 hr

Appendix K

Raw Scores for Epinephrine Excretions with and Without Exercise at the Beginning, Mid-Point, and End of the Study

Subjects	Beginning Epinephrine* After One Day of Exercise	Mid-Point Epinephrine Resting	Mid-Point Epinephrine With Exercise	End Epinephrine Resting	End Epinephrine With Exercise
1	1x48	1x40	1x44	1x92.5	1x63
2	1x69	1x30	1x30	1x36	1x59
3	1x36	1x33	1x29	1x55	1x16
4	1x43	1x43	1x48	1x29.5	1x27
5	1x28	1x28	1x25	1x47	1x75
6	1x48.5	1x46	1x35.5	1x35	1x25
7	1x79.5	1x41	1x50	1x24	1x33
8	1x39.5	1x30	1x25	1x21	1x36
9	1x49	1x59	1x43	1x38	1x30
10	1x52	1x45	1x90	1x25	1x46
11	1x38.5	1x19	1x23	3x36	3x39
12	1x44	1x33	1x37	1x74	1x93
13	1x51	1x33	1x43	1x51	1x50

*Epinephrine reported by slit-width and mg/24 hrs

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