1968

The effect of nonstressful exercise on whole blood clotting time

Thomas R. Whiddon
The University of Montana

THE EFFECT OF NONSTRESSFUL EXERCISE ON WHOLE BLOOD CLOTTING TIME

By

Thomas Roney Whiddon, Jr.

B. A. Nebraska Wesleyan University, 1963

Presented in partial fulfillment of the requirements for the degree of

Master of Science

UNIVERSITY OF MONTANA

1968

Approved by:

Chairman, Board of Examiners

Dean, Graduate School

AUG 2 1968

Date

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

ACKNOWLEDGMENTS

The author wishes to express his gratitude and appreciation to Dr. Brian Sharkey, Dr. John Dayries, Dr. John Lawther, Dr. Robert Curry, Dr. James Templeton, and Dr. Larry Duff for their contributions during the completion of this study.

The author is indebted to his wife, Judy, and his son, Derek, for their understanding and sacrifice which made this paper possible.

T.R.W.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

THE PROBLEM

I. INTRODUCTION

Interpretations of the term stress have undergone considerable change during the last two decades. Presently any specific definition of stress depends upon the viewpoint of the researcher, the conditions in which observations were made and the methods employed by the investigator.

According to Selye (34) stress may be induced by environmental, social, physical, or psychological factors or by a combination of these factors. More specifically, Selye labeled muscular exercise as one of the physical factors which is a stressor. He further indicated that the body adjusts to this phenomenon by the discharge of certain chemicals from the adrenal glands. These chemicals, known as corticotropins, appear to affect virtually every part of the body. If these physiological reactions are successful in meeting the stress that occurs, then adaptation to the distressing forces serve to protect the body against damage. Several men have supported this hypothesis. Young, Price, Elder, and Adachi (44) ran dogs to exhaustion and concluded that the rise in adrenal cortical hormones was due to the running. A similar conclusion was expressed by Frenkl and Csalay (15) when rats were forced to swim in water at 29°C to exhaustion. When these experiments were conducted, other factors might have affected the emotions of the animals, thus causing an increase in adrenal cortical

excretion.

Reynolds, Quigley, Kennard, and Thorn (30) studied the eosinophil count of oarsmen, coaches, and coxwains before and after a race and concluded that stress was present as an emotional factor either by itself or in combination with muscular activity. Hill and co-workers (22) repeated this experiment, using the more valid 17-hydroxycorticoid steroid level as a measurement of stress and concluded that the emotional factor was much more effective as a stressor than the muscular activity. They further indicated that muscular activity seemed to cause little if any stress. Cohen and Rubenstein (8) emphasized the complexity of the stress mechanism and the difficulty of distinguishing one cause from another.

Authors of several studies have also concluded that exercise does not produce an increase in adrenal cortical activity. Miller and Mason (28) found no substantial increase in adrenal cortical activity when monkeys were put through climbing exercises. Steadman (35), after running human subjects on a treadmill, concluded that exercise in itself does not cause stress. Connell, Cooper, and Redfearn (9) subjected humans to running on a treadmill, to marching, and to physical training, which included swimming, cross-country running, and classical physical training. They concluded that the increase in adrenal cortical hormones was due only to emotional stress.

Muscular activity has also been reported to be related to blood clotting time. In work on humans, Schneider and Zangari (33) reported that exercise shortened the clotting time of blood. More recent works

by Iatridis and Ferguson (24) and Tinkel and Cumming (36) reported findings in agreement with the preceding investigation. Rizza (31), on the other hand, reported an increase in the antihaemophilic globulins in human blood after exercise. Iatridis and Ferguson (24) reported data indicating an increase in heparin tolerance, Plasma Factor VIII, and Hageman Factor due to exercise. In all of these studies blood coagulation time was reported to be shortened only if the activity was strenuous to the subject.

In an early study Vosburgh and Richards (41) concluded that adrenalin injected into the blood decreased blood clotting time. However, Cannon and Gray (3) failed to find shortening of blood clotting time when adrenalin was injected into the blood of a jugular vein which was tied off. From their study, Cannon and Mendenhall (7) reported that such emotions as fear and rage are capable of greatly shortening the coagulation time of blood. Schneider and Zangari (33) reported that anxiety, fear, and tension decreased blood clotting time. Cosgriff, Diefenbach, and Vogt (12) reported that patients treated with ACTH and cortisone showed faster venous blood coagulation time.

The validity of the various indexes of stress has posed many questions. Many of the early investigators and even some of the more recent ones have used the eosinophil count or the 17-ketosteroid level as a measurement of stress. Yet epinephrine can decrease the eosinophil count in the absence of adrenal cortical activity, whereas the secretion of androgens by testicular activity can influence the level of 17-ketosteroids. Therefore the index which appears to serve as a

good indicator of the adrenal cortex is the measured level of 17-ketogenic steroids (23).

Several studies indicate that if the emotional factor is removed from muscular exercise, an increase in the adrenocortical hormones will not occur (9, 28, 35, 39). Such studies raise the question whether muscular exercise or the emotional factor associated with the exercise causes the shortening of the clotting time.

In some of the previous research (15, 25, 44), exercise has been indicated to be a stressor; therefore one can reasonably hypothesize that the reported change in the whole blood clotting was a result of exercise. In the light of more recent research which contradicts the hypothesis that muscular exercise is a stressor, there is a need to define more definitely whether muscular exercise or the emotional factor associated with exercise preparation is the factor which shortens the blood clotting time.

II. THE PROBLEM

Statement of the Problem

The problem of this investigation was to determine the effects of a prolonged exercise program on the clotting of whole blood. More specifically, the problem attempted to determine the effects of non-stressful exercise on the whole blood clotting time. The sub-problems are the determination of:

1. the length and type of exercise to be used
2. the type of urine analysis that would most accurately

measure the 17-ketogenic steroid level

3. the method for measuring whole blood clotting time.

Significance of the Problem

Several significant factors appear in a study of this nature. Exercise is often used as a prescribed therapy for some ischemic vascular diseases and the prevention of these diseases. A knowledge of the effects of different types of exercises upon blood clotting time could be useful in determining an exercise program for individuals with occluded arteries or thrombogenic tendencies. Also, various experimenters report contradictory effects of exercise on blood clotting time. Hopefully, this study will aid in clarifying such problems.

Limitations and Weaknesses of the Study

This study was limited to five subjects due to the time involved in the analysis of 17-ketogenic steroids. Possible weaknesses of the study include the validity and reliability of the measures of whole blood clotting time and of the 17-ketogenic steroid level. Whole blood clotting time, as measured by the Lee-White Method (37), is not an absolute measurement of the clotting of blood within the body. Moreover the 17-ketogenic steroid level as measured in the urine reflects not only the influence of exercise, but the steroid level may reflect the outside activity patterns of the subjects for a twenty-four hour period after exercising.

Definitions

For purposes of clarification and understanding, the following

terms and their definitions were used in this study.

1. Adrenal Cortical Steroids (Corticosteroids)--A generic name including all C-21 steroids formed in the adrenal cortex and their C-21 metabolites (23).
2. Eosinophil--Weak phagocytes in the blood in which the number is affected by allergic reaction, foreign particles, epinephrine and corticosteroids (16).
3. 17-Ketogenic Steroids--17-Hydroxycorticosteroids whose side chains have been removed and in which 17-hydroxyl has been oxidized to a ketone. They represent about 80 per cent of all excreted C-21 steroids and serve as a good index of adrenal cortical activity (23).
4. 17-Ketosteroids--C-19 steroids with a ketone group at C-17. These represent the androgens metabolic products of adrenal cortical and testicular activity (23).
5. Colorimeter--An apparatus which measures the quantity of a material in solution by measuring the intensity of its color (40).
6. Stress--Any stimulus which causes an increase in the secretion of the andrenocorticotrophic hormone (ACTH) (17).
7. Whole Blood Clotting Time--The time that it takes whole blood to clot at 37°C in glass test tubes.

CHAPTER II

REVIEW OF RELATED LITERATURE

Although many workers have been interested in the effects of exercise on blood clotting, they have done little to determine whether stressful or nonstressful exercise is a factor which shortens the clotting time of blood. The following account not only reviews most of the works concerning the effects of exercise on blood clotting time, but also represents most of the research which attempts to answer the question as to whether exercise is stressful or nonstressful.

I. RELATIONSHIP BETWEEN MUSCULAR EXERCISE AND STRESS

Authors of earlier studies on muscular exercise frequently used a count of the circulating eosinophils or the excretion of 17-ketosteroids in the urine as an index of stress. However, one should approach these studies with some skepticism. For example we now know that the eosinophil count can be decreased by allergic reactions and by epinephrine in the absence of functioning adrenal cortical tissue, both of which might be interpreted as an increase in ACTH excretion (16). The level of 17-ketosteroids in the urine is now known to reflect not only the adrenal cortical activity but also the testicular activity of the individuals (32).

Difficulties arise in the interpretation of muscular experiments because exercise is often associated with emotional factors. Cohen and Rubenstein (8) pointed this out by emphasizing the complexity

of the psychological implications in the realm of physiological stress. They further showed that distinguishing one type of stress from another type is difficult because manifestations of both are similar.

Muscular exercise is traditionally viewed as a stressor. Hartman, Waite, and McCordock (20) found that cats forced to run on a treadmill excreted large amounts of epinephrine which greatly dilated the denervated pupil. During vigorous exercise, the time from the onset of running to the dilation of the pupil was from one and one-half to three minutes. In mild exercises the adrenalin increased much later. If the adrenal nerves were destroyed or the adrenal glands removed, exercise did not cause the dilation of the pupil. They concluded that exercise will upset the homeostasis of the body.

Selye (34), who developed the General Adaptation Syndrone Theory, supported the view that exercise was a stressor. Rats, forced to run, showed the triphasic response associated with stress. At first the animals would increase the adrenal cortical secretion. If the stress persisted, the secretion of adrenal cortical hormones would decrease and the animals would enter the exhaustive state.

If rats are forced to swim in water at 29°C periodically for three weeks, their adrenal cortices increased in weight and secretion (15). However after six weeks, although the weight remained the same, the amount of 17-ketosteroids secreted returned to normal. Frenkl and Csalay (15) concluded that the animals were in Selye's "exhaustive state" and, therefore, that exercise was a stressor.

Young, Price, Elder, and Adachi (44) ran dogs on a treadmill

at various times after eating. They found the level of 17-hydroxycorticoid steroids greatly elevated during a work period ranging from 88 to 600 minutes. They concluded that exercise was a stressor, and that 50 per cent of the increase in the level of 17-hydroxycorticoid steroids was associated directly with work energy.

Prokop (29) subjected forty-two gold hamsters to carefully standardized training loads under exactly comparable conditions when swimming and running. The training loads were repeated three times daily for fifteen consecutive days. They found that the adrenal gland enlarged due to an increase in the cortex. The adrenal cortex-medulla ratio increased from 2.5:1 to 5.1:1.

Ucha and Portes (38) measured the blood level of adrenalin in a soccer team before and after a match. From a group base level of 20 to 60 mg of noradrenalin and adrenalin, the players increased their level of noradrenalin 35 per cent; however, the coach and the goalkeeper increased their level of adrenalin 40 per cent with little change in noradrenalin. They concluded that situations of expectations, not action, increase adrenalin secretion.

Reynolds, Quigley, Kennard, and Thorn (30) counted eosinophils of coxswains, coaches, and oarsmen before and after a race. They observed that the eosinophil count fell as much for the coaches and coxswains as it did for the oarsmen during the period of the race. They concluded that the physical exertion of rowing was not the major stress involved in evoking the changing eosinopenia observed in all members of the crew.

Hill and co-workers (22) found that the pattern of 17-hydroxycorticoid steroids in the urine of the 1953 and 1954 Harvard rowing teams on the practice days was similar to the diurnal pattern on the days without practice. In contrast urinary 17-hydroxycorticoid steroids significantly increased during the time trial as well as during the actual varsity race. The physical exertion in these two types of competition compared to that experienced on the practice days, although the psychological setting was entirely different.

Ulrich (39) studied stress in fourteen women with varsity basketball experience and fourteen women with no varsity basketball experience. She used eosinophil count, pulse rate, and respiration rate as indexes of stress. She measured the stress level of both groups in an activity class, an intramural game, and an interscholastic game. The inexperienced women showed a higher degree of stress; yet the experienced women seemed to have a higher threshold of stress. She also concluded that psychological factors played a major role in the adjustment during stress.

Connell, Cooper, and Redfearn (9) contrasted the effects of emotional tension and physical exercise on men. In the emotional stress of an important examination, the level of 17-ketogenic steroids increased significantly at the .01 level. Short periods of intense physical activity did not produce any alteration in the excretion of 17-ketogenic steroids, and only a modest decrease in the 17-ketosteroid level. Because physical stress caused no convincing trends in the excretion of 17-ketogenic steroids, they concluded that the emotional

stress caused the increase in the formation of adrenal cortical hormones and this increase was anticipatory to the physical stress.

Miller and Mason (28) used adult male monkeys to see if the 17-hydroxycorticoid steroid level changed when the muscular workload was increased. When monkeys were forced to obtain their food by lifting a steel pail which varied in weight, those who did the most work did not have the highest hormone excretion. Furthermore, animals who did many kilogram-meters of work did not always have a rise in 17-hydroxycorticosteroids excretion. Monkeys who worked for food by climbing also did not always have a rise in 17-hydroxycorticoid steroids. They concluded that an increased workload, imposed over several days, does not necessarily result in a markedly increase excretion of 17-hydroxycorticoid, but that the psychological reactions of the animal to the workload usually resulted in a rise in the level of 17-hydroxycorticoid steroids.

Steadman (35) used a Modified Balke Treadmill Test to train five male subjects for four weeks. He found that the greatest increase in 17-ketogenic steroids occurred the first week of training. Yet during the remaining period of training the level of 17-ketogenic steroids in the subjects approached the control level. He postulated that, since the physical exertion was adjusted upward throughout the study, the latter weeks should reflect the adrenal cortical response to the existing physical exercise. From his results he concluded that exercise, by itself, was not the major factor responsible for the change in adrenal corticosteroid excretion.

II. EFFECTS OF EXERCISE AND EMOTIONS ON BLOOD CLOTTING TIME

Hartman (19) published one of the first studies which showed that exercise affected the clotting time of blood. He measured the whole blood clotting time of twenty-one cats, before the cats ran 1,150 meters on a treadmill, immediately after the run, and an hour or more later. Of the twenty-one cats exercised, thirteen exhibited a decrease in blood clotting time, five an increase, and three an increase followed by a decrease. The total change in blood clotting time for the cats immediately after the run on the treadmill was 347 seconds with a mean decrease in clotting time of 17.3 seconds. One hour or more later the total decrease in clotting time for the cats was 490 seconds with a mean decrease for each cat of 24.5 seconds. Hartman concluded that the decrease in clotting time was probably due to the increased output of epinephrine.

Adrenalin was first suspected of being a contributing factor in the shortening of whole blood clotting time when Vosburgh and Richards (41) noted that if they painted the pancreas of dogs with adrenalin chloride, or if they injected adrenalin chloride into the dog's abdominal cavity, that, concomitant with an increase in blood sugar, the blood coagulation time shortened. They concluded that the blood coagulation phenomenon was due to the application of adrenalin to the pancreas.

Cannon and associates (4) used a graphic method to determine the effect of adrenalin on blood coagulation time. When Cannon and Gray (3) injected adrenalin into cats, the whole blood clotting time of

the cats shortened under normal conditions. In one case Cannon and Gray destroyed the brain and cord of the cat. After injecting 1 ml of adrenalin (1:100,000) into this cat, the clotting time shortened from a control level of 3.9 minutes to an average of 2.3 minutes for the next hour and forty minutes. They also separated the anterior part of the cat's circulatory system from the rest by ligatures tied at the aorta and inferior vena cava. A shorter blood coagulation time was not recorded after injecting an adrenalin dose (0.5 ml, 1:100,000). In a normal cat they removed the gastrointestinal canal and the liver. After injecting adrenalin (1:100,000) into the cat with the gastrointestinal canal and the liver removed, they found no change in blood clotting time in the cat. From these experiments they concluded that the adrenalin produced its effects not through changes in the extensive neuromuscular, bony, or surface tissue of the body and not directly on the blood, but probably upon the liver or intestines to produce a factor which aided the clotting process.

Cannon and Mendenhall (5) separated the splanchnic nerve from the spinal cord of an etherized cat and stimulated it with a small tetanizing current, barely perceptible on the experimenters' tongues. A coagulation time of 4.0 minutes was recorded before the splanchnic nerve was stimulated; ten minutes later the clotting time was 1.0 minute and averaged 2.1 minutes for a twenty minute period. In another case, the blood coagulation time of a cat with its brain pithed was 6.0 minutes before stimulation of the splanchnic nerve; eight minutes later, it dropped to 3.0 minutes. They also found that if the adrenal

gland was removed, or if the liver and intestines were removed, no change was recorded in the coagulation time due to the stimulation of their respective nerves.

Cannon and Mendenhall (6) stimulated the sciatic and crural nerves of a cat and shortened clotting time. Other factors shortening clotting time in cats were fear and rage. For example, a very vigorous cat was placed on a holder. The cat became stormy, it snarled, hissed, bit, and lashed its tail. For twenty-four hours the clotting time of the blood of the cat was from 0.5 minutes to 1.0 minute, a time well below that of a normal cat.

Gray and Lunt (18) noted a shorter clotting time when a cat had hemorrhaged 13 per cent of its blood. When the abdominal circulation anterior to the diaphragm was tied off, the clotting time was faster. Upon removal of the adrenal glands, faster clotting time also resulted. They concluded that the liver, the intestines, and the adrenal glands were responsible for the faster coagulation time, but that neither was the sole factor.

Macht (26) observed that the clotting of blood of rabbits and cats, as measured by the Lee-White Method, was shortened after rage and profound terror. The sera of these animals exhibited a phytotoxicity which remained for two and three days. He felt that the prolonged phytotoxicity indicated that increased secretion of epinephrine was not completely responsible for shorter clotting time. In a related study, he classified approximately 400 subjects into three groups: (1) persons apparently normal and composed, (2) persons apprehensive or

mildly nervous, and (3) persons highly nervous and worried. The average clotting time for the apparently normal group was 8.3 minutes, the apprehensive group was 3.4 minutes, and the highly nervous group had an average of 2.2 minutes. Yet phytotoxicity did not differ between the groups.

Henriques, Henriques, and Selye (21) studied the effects of unilaterally nephrectomy on normal female, piebald rats and adrenolectomized female, piebald rats. The blood-fibrinogen increased following unilateral nephrectomy; however, the blood-fibrinogen increase was prolonged in bilateral adrenolectomized rats. Also, total adrenolectomy greatly decreased, but did not abolish, the fibrinogen response to injury. They concluded that the adrenals played an important role in fibrinogen production.

Cosgriff, Diefenbach, and Vogt (12) measured the venous blood coagulation time of ten patients with various diseases under treatment with ACTH or cortisone. Eight patients showed shortened venous blood coagulation time. They concluded that ACTH and cortisone produce hypercoagulability of the blood.

Exposure to extreme cold is followed by an increased production of certain hormones such as corticotrophin, corticoids, thyroid hormones and epinephrine. Of these, corticotrophin and epinephrine caused a marked increase in fibrinogen. Tinkel and Cumming (36) studied the effect of exercise on whole blood clotting time in cold temperatures. The subjects, ten normal males, ranged in age from eight to thirty-five. The subjects exercised on a bicycle ergometer at 25°C. The

clotting time was shortened from 674 ± 180 seconds before exercise to 465 ± 127 seconds after exercise, which was a significant difference at the .01 level. At -20°C the change was from 622 ± 112 seconds to 591 ± 112 seconds, which is not significant. They concluded that proof was lacking that shoveling of snow in the cold precipitated sudden death or coronary thrombosis.

Vuori (42) assumed that the state of the autonomic nervous system influenced blood clotting time and concluded that the clotting time would not be of the same length in the different departments of a hospital. Of seventy-six mental patients tested the mean blood clotting time was 388 ± 10.2 seconds; of fifty-three surgical patients tested the mean blood clotting time was 392 ± 8.4 seconds; and of 116 patients in the medical department tested, the mean blood clotting time was 440 ± 12.6 seconds. To study the effect of muscular work on blood clotting, he took sixty-nine patients from various departments in the hospital and ten healthy recruits. In using muscular work as an irritant, he found that a shorter clotting time occurred after exercise in the patients and healthy persons. He concluded that bodily exertion and mental irritation, which shorten the blood coagulation time, were also factors in causing sudden death from heart failure.

Schneider and Zangari (33) measured the variations in clotting time, relative viscosity, and other physiochemical properties of the blood accompanying physical and emotional stress. When fifteen healthy human subjects ascended and descended two nine-inch steps for a five minute period and a ten minute period, the clotting time was shortened

between 18 and 52 per cent with an average of 30 per cent. After exercising the blood viscosity increased an average of 8 per cent in four of the fifteen healthy subjects. The hematocrit of all persons rose slightly from 0.5 to 4.5 per cent with an average increase of 2.1 per cent. In two healthy human subjects 1.0 ml of epinephrine (1:1,000) was injected. In both subjects the clotting time shortened 41 and 31 per cent; the blood viscosity rose 3.5 and 6.5 per cent; and the hematocrit changed similarly to those seen after exercise. In two hypertensive subjects examined, the clotting time was shortened 11.5 and 28 per cent; the hematocrit rose 3.3 and 2.6 per cent; and the relative viscosity increased 7.5 and 7.0 per cent.

Warnock, Clarkson, and Stevenson (43) walked white Leghorn cockerels, which were being kept on a high cholesterol diet, five days a week for one hour each day. The blood coagulation after seven weeks was 163 ± 48.2 seconds as compared to the control cockerels' clotting time of 108 ± 30.7 seconds. At fourteen weeks the control value was 150 ± 50.6 seconds as compared to the exercise value of 242 ± 60.0 seconds. They concluded that exercising cholesterol-fed cockerels significantly lengthened blood coagulation times.

Iatridis and Ferguson (24) exercised fifty-nine people with normal blood clotting times and one Hageman deficient subject. The mean post-exercise clotting times were significantly shorter than the corresponding pre-exercise times ($P < .001$). For the normal subjects, the clotting time of the blood in glass test tubes averaged 16.5 minutes before exercise but dropped to a mean of 13.0 minutes. In

silicone test tubes the range for the fifty-nine normal subjects was 20 to 44 minutes, with a mean of 32 minutes. After exercising the clotting time for the subjects ranged from 15.5 to 37 minutes, with a mean of 25.5 minutes. After stressful exercise the antihaemophilic factor (Factor VIII) rose 188 per cent, the Hageman Factor (Factor XII) increased 318 per cent and heparin became more tolerated. The level of fibrinogen (Factor I), prothrombin (Factor II), the labile factor (Factor V), the stable factor (Factor VII), and the plasma thromboplastin antecedent (Factor X) did not change significantly.

Rizza (31) performed eighteen experiments on fifteen normal subjects. After the subjects had run as fast as they could for three-fourths of a mile, they showed a post-exercise increase of antihaemophilic factor (Factor VIII) of 250 per cent. The whole blood clotting time tested in either glass or silicone test tubes did not rise in five of the subjects studied.

Keeney and Loramie (25) questioned whether muscular exercise accelerated the clotting time of blood because athletes were not known to be prone to blood coagulation. To test this they administered four different types of exercise programs to thirty-two male college students, twenty-five moderately active students and seven varsity lettermen of various sports. They were: (1) a 15-minute walk on a motor-driven treadmill at 2.5 miles per hour; (2) a 2-hour walk on a motor-driven treadmill at 2.5 miles per hour; (3) a run to near exhaustion on a treadmill at 6.25 miles per hour; (4) a stepping exercise on a 12-inch bench, 30 steps per minute for fifteen minutes. The clotting time

for the subjects before the 15-minute walk averaged 16.5 minutes; one minute later the clotting time averaged 16.9 minutes; and fifteen minutes after exercise it averaged 17.0 minutes. The mean clotting time before the 2-hour walk was 18.3 minutes; one minute after the walk the mean clotting time was 18.8 minutes; and fifteen minutes later it was 18.7 minutes. An analysis of variance revealed no significant difference in whole blood clotting time before or after any of the exercises. Two to four hours after exercising by the step test, the eosinophil count of twelve subjects dropped from 29 to 82 per cent from the control number. They concluded that this study did not support the hypothesis that accelerated blood coagulation is a stress phenomenon since exercise intensities severe enough to elicit eosinopenia failed to affect blood coagulation.

III. SUMMARY

Literature has labeled exercise as a stressor; however, recent published works by Ulrich (39), Connell, Cooper, and Redfearn (9), Miller and Mason (28), and Steadman (35) suggest that the physical aspect of exercise is stressful only if accompanied by emotional stress. However, the experimenters of blood clotting generally support the hypothesis of Cannon that faster blood coagulation is beneficial to the organism in protecting it against the hazard of excessive bleeding from injury encountered in violent activity. Yet great emotional stress accompanies this situation and the question remains unanswered whether or not exercise relatively void of emotional stress will

enhance the clotting of blood. Keeney and Loramie (25) found that vigorous exercise did not change blood clotting time, but it did affect the eosinophil count. Even so, eosinopenia is a questionable index of stress.

CHAPTER III

PROCEDURE

I. THE SUBJECTS

Five subjects were selected Fall Quarter from a general physical education swimming class at the University of Montana. The criteria used in the selection were that the subjects must have

1. been of moderate body weight for their height and build
2. expressed a willingness to undergo a rigorous fitness program
3. expressed a willingness to collect twenty-four hour urine samples and a willingness to have blood extracted from the arm
4. no history of abnormal bleeding time
5. been approved for participation in the study by a physician.

The subjects selected were given an orientation prior to the start of the program. Each subject was informed of the procedure which would be followed upon arrival at the Human Performance Laboratory. He was also informed of the expected diet, behavior, and the manner of collecting and keeping the urine. During this time each subject was informed of the purpose and nature of the study. They were also told that academic credit would be given to them, if they satisfactorily completed the study. This procedure was followed to assure some control over the subjects. The subjects' physical characteristics appear in Table I.

TABLE I

PHYSICAL CHARACTERISTICS OF THE TRAINING SUBJECTS

Subjects	Height (inches)	Weight (pounds)	Age (years)
R.H.	68	158	18
B.L.	70	144	20
T.M.	71	156	25
J.S.	71	188	18
B.S.	74	180	19
Means	70.8	165.2	20.0

II. EQUIPMENT

Treadmill

The subjects were trained on a motor driven treadmill located in the Human Performance Laboratory at the University of Montana. It has a continuous rubber belt for walking or running. The speed may be adjusted from one-half to over ten miles per hour by varying the drive belt tension. Any grade level between 0 and 50 per cent could be precisely set in either direction while the treadmill was operating.

Radio-Electrocardiograph

A Telemedics RKG of 100 Radio-Electrocardiograph system was used to indicate the heart rate levels of the subjects. The system contained disposable electrodes, a small battery-operated transmitter, and a portable radio receiver which forwarded the EKG signal to the

recording equipment.

The electrodes. Small German silver electrodes were held to the skin of each subject by an adhesive moleskin patch. Electrode paste was placed on the skin to assure conductance of the signal to the electrodes. The electrodes were placed over the right and the left fifth rib, slightly forward of the mid-axillary region. Flexible wires carried the EKG signals from the electrodes to the transmitter.

The radio transmitter. The radio receiver operated from a standard 115 watt, 60 cycle power line. The EKG signal was channeled through a channel selector to a recording instrument.

The recording instrument. A Telemedics Cardiotac 400 R Electrocardiograph was equipped with a meter that computed and indicated the average heart rate in beats per minute. Each "QRS complex" of the amplified EKG signal was translated into a distinct "beep."

The Spectronic 20 Colorimeter

The 17-ketogenic steroids were measured with a Spectronic 20 Colorimeter. Light possessing a wavelength of 540 millimicrons was passed through the sample in a calibrated test tube. The waves which were not absorbed struck a photo-sensitive vacuum tube. The resulting electronic signal was then amplified and recorded on a meter indicating percentage transmittance or absorbance of the sample.

The Heating Block

A constant temperature, needed for the Modified Lee-White

Method, was maintained with the use of a Temp-Blok. The unit, located in the Health Service Laboratory, was set at 37°C in the morning and that temperature was maintained throughout the day.

Blood-Drawing Equipment

The sterilized needles had a bore of twenty gauge and a bevel of 1.5 ml. The needles were attached to a 5 ml sterilized Plastipak syringe. Both units were disposed after they were once used.

III. TEST AND MEASUREMENTS

The Modified Balke Treadmill Test

The cardiovascular and cardiorespiratory systems suffer some limitations when the heart rate reaches 180 beats per minute. Therefore, the exercises performed on the treadmill by the subjects were stopped when the heart rate reached 180 beats per minute. The Modified Balke Treadmill Test was given to each subject in the following manner (10):

1. The subject walked on the treadmill at the rate of 3.5 ± 0.1 miles per hour beginning at zero grade level and continued at this same rate as the grade was increased.
2. At the end of the first minute the grade was raised one and one-third per cent and at each succeeding minute thereafter until the test was terminated.
3. The test was concluded when the heart rate reached 180 beats per minute.
4. The time of the test was recorded in minutes with any

fraction rounded to the nearest 0.5 minutes.

Microtechnic for the Estimation of Urinary 17-Ketogenic Steroids

The 17-ketogenic steroids were estimated by the microtechnic proposed by Enriori (14). Both the quantity of the "preformed 17-ketosteroids" and the "total 17-ketogenic steroids" were separately measured. The 17-ketogenic steroids were then computed by subtracting the "preformed 17-ketosteroids" from the "total 17-ketogenic steroids."

The "preformed 17-ketosteroids" are C-19 steroids characterized by a ketone group at the number 17 carbon. These steroids represent the androgens which are the metabolic products of the adrenal cortical and testicular activity. The 17-ketosteroids are excreted in free form and as conjugates of glucuronates and sulphates.

The "preformed 17-ketosteroids" were removed from the conjugates by hydrolysis with hydrochloric acid. The C-19 steroids were then extracted from the aqueous phase with ethyl ether. The extraneous chromagens were removed by washing the ether extract with sodium hydroxide. The ether was evaporated and the remaining residue was mixed with meta-dinitrobenzene and potassium hydroxide. After the completion of the Zimmerman reaction, methanol was added to the tube. The tubes were then read in a colorimeter to determine the concentration of the "preformed 17-ketosteroids."

The "total 17-ketogenic steroids" were treated prior to hydrolysis with sodium bismuthate and glacial acetic acid to oxidized the 17-hydroxyl group of the 17-hydroxycorticosteroids to a ketone. The

"total 17-ketogenic steroids" were then analyzed and measured according to the process used for the "preformed 17-ketosteroids."

Whole Blood Clotting Time

Three sterilized and unused 5 ml glass test tubes were placed in a heating block set at 37°C. Five ml of blood were withdrawn from either the Basilic vein or the Cephalic vein of each subject. From the 5 ml of blood extracted from each subject's arm, 1 ml was discarded and 1 ml was put in each of the three tubes. The remaining blood was discarded along with the syringe and needle.

A Modified Lee-White Method (37) was used to determine whole blood clotting time. The following procedure was used in the determination:

1. The stop watch was started at the first sign of blood in the syringe.
2. One ml of blood was put into each of the three tubes in the heating block.
3. One and one-half minutes elapsed before the first tube was removed from the heating block and checked by tilting to see if the blood had clotted.
4. The tube was replaced into the heating block.
5. At intervals of 30 seconds the first tube was checked until clotting occurred.
6. After the first tube clotted, 30 seconds elapsed before the second tube was checked.
7. At intervals of 30 seconds the second tube was checked

until clotting occurred.

8. The third tube was checked the same as the second.
9. The whole blood clotting time was the total elapsed time from when the blood entered the syringe until the blood clotted in the third tube.

IV. TESTING PROCEDURE

The testing of the subjects was conducted during the 1968 Winter Quarter at the University of Montana. The Modified Balke Treadmill Test was given to the subjects at the Human Performance Laboratory. The urine was analyzed for the quantity of 17-ketogenic steroids in the Chemistry Laboratory. The whole blood clotting time was measured in the Health Service Laboratory.

The Modified Balke Treadmill Test

Before the control week, the subjects selected a forty-five minute time period in which they could be accessible to the investigator on Monday and Wednesday. On these days the subjects were given the Modified Balke Treadmill Test. The procedure was carried out from February 12, 1968 to March 6, 1968.

The Twenty-Four Hour Urine Collections

On Monday of each of the four training weeks plus the control week and Wednesday of the last week, the subjects were given a labeled container in which to collect their urine for the next twenty-four hours. The subjects were told the importance of keeping the urine

TABLE 2

T_{180} TREADMILL TIMES

(Minutes and decimal portions thereof)

Subjects	Feb. 12	14	19	21	26	28	March 4	6
R.H.	10.00*	11.50	10.50	12.00	10.50	11.50	9.50	10.50
B.L.	9.00	9.50	8.00	7.50	7.50	8.00	7.50	9.50
T.M.	10.00	12.00	10.50	10.50	11.00	10.00	11.50	11.00
J.S.	10.00	9.50	10.50	9.50	11.00	10.50	10.00	11.50
B.S.	10.50	9.50	11.00	10.00	12.00	13.50	10.50	13.00
Means	9.90	10.40	10.10	9.90	10.40	10.70	9.80	11.10

*All T_{180} times continued to the nearest one-half minute.

containers in a cool atmosphere. They were instructed to bring the labeled containers to the investigator as soon as possible after the twenty-four hour period ended. The urine samples were stored at 0°C (13) until they were analyzed at the Chemistry Laboratory.

The Daily Corticosteroid Estimate

The daily urine volume was measured in a 1,000 ml graduated cylinder. The average urine volume was 1.08 liters. A 1.5 ml sample of urine was withdrawn with a 2 ml measuring pipette. On Monday of each of the four training weeks plus the control week and the Wednesday of the last week, the subjects went to Health Service for extraction of blood samples.

The Training Procedure

The subjects were told that during the coming weeks they should drink some liquid and urinate before coming to the Human Performance Laboratory. They were also told to maintain an adequate diet and a proper fluid intake. After coming to the laboratory the subjects were weighed and asked questions regarding their outside activities, sleep, and diet. The room temperature and barometric pressure were recorded while each subject was in the Human Performance Laboratory. The subjects were then wired to transmit their EKG signal to the electrocardiograph. The subjects stepped on the treadmill and the Modified Balke Test was administered.

CHAPTER IV

RESULTS, DISCUSSION, AND SUGGESTIONS

I. AVERAGE DAILY EXCRETION OF CORTICOSTEROIDS

The study required a valid and reliable index of the adrenocortical activity. Although several earlier studies had used the 17-ketosteroids and eosinophil count to determine adrenal cortical activity, it was known that these indexes were subjected to factors not related to the adrenal cortex and would not give an exact index of stress. The available evidence indicated that the measurement of 17-ketogenic steroids served as a good indirect index of adrenal cortical activity (23). The 17-ketogenic steroids include all 17-hydroxycorticosteroids having ketol, glycerol, or glycol side chains. These steroids represent about 80 per cent of all excreted C-21 steroids.

The urinary level of 17-ketogenic steroids is influenced by age and weight (23). The 17-ketogenic steroid level varies diurnally reaching a maximum during the morning hours and a minimum at midnight (27) and normally ranges from 4 to 16 mg for twenty-four hours. Table 3 indicates that the control range for the subjects was 6.3 to 14.3 mg. However the 17-ketogenic steroid excretion for male subjects similar to the age that was used in the study averages slightly less than 10 mg (35). The average twenty-four hour excretion of 17-ketogenic steroids in the present study was 9.9 mg.

TABLE 3

AVERAGE DAILY EXCRETION OF 17-KGS

Subjects	Control	1st Monday	2nd Monday	3rd Monday	4th Monday	4th Wednesday
R.H.	9.9*	15.4	15.8	11.3	10.2	9.7
B.L.	7.6	7.8	10.1	9.1	7.5	8.9
T.M.	6.3	9.1	10.7	10.4	9.9	8.0
J.S.	14.3	17.4	15.9	13.0	12.2	11.8
B.S.	11.5	14.9	13.9	13.9	12.2	10.9
Group Average	9.92	12.92	13.28	11.54	10.40	9.86

*milligrams

II. EFFECTS OF STRENUOUS PHYSICAL EXERCISE ON ADRENAL CORTICAL RESPONSE

The results of the study are found in Table 3. The five subjects' average excretions of 17-ketogenic steroids for the control period was 9.92 mg. After the first Monday exercise session the average excretion of 17-ketogenic steroids of the subjects rose to 12.92 mg. The greatest individual gain was 5.5 mg and the smallest gain was 0.2 mg. The mean group excretion rose 0.36 mg after the second Monday session to 13.28 mg. However, only three of the five subjects recorded gains between the two periods. The average excretion of 17-ketogenic steroids fell 1.74 mg after the third Monday to 11.54 mg and continued to fall through the final training session. The final level of 17-ketogenic steroids was 9.86 mg or .06 mg below the control level.

Figure 1 illustrates the group trend in the excretion of 17-ketogenic steroids after each training session. As this graph illustrates the level of 17-ketogenic steroids rose during the early training sessions; then during the later training sessions, it continued to approach the control level. During the same period the mean group exercise time rose from 9.90 minutes to 11.10 minutes (Table II). This indicates that the initial rise in the level of 17-ketogenic steroids reflects the emotional stress associated with exercise. Individual graphs are presented in Figures 3, 5, 7, 9, and 11.

III. THE WHOLE BLOOD CLOTTING TIME

The whole blood clotting time as determined by the Modified

Lee-White Method will vary according to the volume of blood, the size of tubes, and the time interval which elapses between tilting the tubes. The group ranged from 10.0 to 13.0 minutes and the average of 11.0 minutes was considered to be normal for this particular test.

IV. THE EFFECTS OF STRENUOUS EXERCISE ON THE WHOLE BLOOD CLOTTING TIME

The average whole blood clotting time for the subjects dropped from a control level of 11.0 minutes to 9.3 minutes after the first exercise period (Table 4). Individual values ranged from no change to a drop of 3.5 minutes. The clotting time rose slightly to a mean of 9.90 minutes on the second Monday. Of the five subjects, three recorded slower clotting times the second Monday than the first Monday and two experienced no changes. On the third Monday the mean clotting time fell 0.1 minutes to 9.80 minutes. Yet three of the subjects had slower clotting times; one had no change, and only one had a faster clotting time the third Monday. The mean time for whole blood clotting rose the fourth Monday, and each individual also recorded a slower clotting time. The whole blood clotting time for the fourth Wednesday, when compared to whole blood clotting times for the fourth Monday, was slower. Each individual had a slower whole blood clotting time when these periods were compared. The whole blood clotting time for the last training session was 0.6 minutes above the control level.

Figure 2 illustrates the mean whole blood clotting time for the subjects for each recorded training session. A faster clotting time

TABLE 4

INDIVIDUAL WHOLE BLOOD CLOTTING TIME

Subjects	Control	1st Monday	2nd Monday	3rd Monday	4th Monday	4th Wednesday
R.H.	13.00*	9.50	9.50	10.50	12.00	12.50
B.L.	10.00	10.00	11.00	9.50	10.00	11.00
T.M.	11.00	8.50	10.00	9.50	10.50	11.50
J.S.	10.00	8.50	9.00	9.00	11.00	11.00
B.S.	11.00	10.00	10.00	10.50	11.50	12.00
Group Average	11.00	9.30	9.90	9.80	11.00	11.60

*All whole blood clotting times continued to the nearest one-half minute.

was noted during the early training sessions; however, during the later training sessions the whole blood clotting time returned to the control level. This suggests that stress may have produced a faster whole blood clotting time. Individual graphs are presented in Figures 4, 6, 8, 10, and 12.

V. CORRELATION OF WHOLE BLOOD CLOTTING TIME TO STRESS

In most of the previous studies exercise was cited as a factor which would shorten the blood clotting time. In the present study exercise which caused an increase in 17-ketogenic steroids produced a faster whole blood clotting time; however, as the individual became accustomed to the treadmill and increased his fitness, the 17-ketogenic steroid level and the whole blood clotting time returned to the control level even though the amount of exercise increased. This suggests that it was stress and not exercise which caused the faster whole blood clotting time. As can be seen in Figures 1 and 2, a rise in the stress index (17-ketogenic steroids) generally resulted in a faster clotting time; and a fall in the stress level (17-ketogenic steroids) generally resulted in a slower clotting time. Three of the five individual correlations between the stress index and the whole blood clotting time indicate a high negative relationship. The three subjects have coefficients of - .91, - .87, and - .82, which are significant at the .05 level. A fourth subject had a lower negative coefficient of - .52. The fifth subject had a positive coefficient. A possible explanation for this positive coefficient is that the stress index represents

twenty-four hours and only a small part of this time was spent at the laboratory. The correlations for the five subjects are found in Table 5.

TABLE 5

CORRELATIONS AND t RATIOS
OF KETOGENIC STEROIDS AND BLOOD CLOTTING

Subjects	Pearson r	t Value	Significance
R.H.	- .87	3.56	.05
B.L.	+ .53	.62	No
T.M.	- .52	.62	No
J.S.	- .82	2.88	.05
B.S.	- .91	4.40	.01

VI. DISCUSSION

In this study the amount of physical exercise was adjusted upward during the four weeks of training on a treadmill. Whereas the 17-ketogenic steroid level increased during the early part of the training sessions, the level of 17-ketogenic steroids approached the control level during the later sessions. The rise and fall in the level of 17-ketogenic steroids did not reflect the fact that the physical exercise load was increased as the training program progressed. Several studies (9, 28, 35, 39) have suggested that the change in adrenal cortical activity may be due to emotional stress. If emotional stress was the factor which caused the increase in 17-ketogenic

steroids, then a prolonged exercise program on the treadmill should have a greater amount of emotional stress at the beginning of the training, when the subjects are apprehensive, than at the end when they become familiar with the program. Since the exercise load was increased, the 17-ketogenic excretion pattern seems to reflect emotional stress. This supports Steadman's hypothesis, "...exercise, by itself, is not a stressor and does not play a predominant role by inciting a change in adrenal corticosteroid excretion (35)."

If the emotional factor caused an increase in 17-ketogenic steroids, it is quite possible that such a factor may have also caused the faster whole blood clotting time. A faster whole blood clotting time was recorded during the early part of the program and during the later part of the program the whole blood clotting time returned to near the control level. In comparing the change in the whole blood clotting time, it can be seen that a negative correlation seems to exist between the whole blood clotting time and the stress factor. These results strongly support the belief that exercise does not cause a faster whole blood clotting time but that emotional stress, associated with exercise, caused the faster whole blood clotting time.

VII. SUGGESTIONS

Exercise has been utilized as a therapy for reducing the crust found on the inner walls of arteries in arteriosclerosis (11). However, because the endothelial rough spots associated with arteriosclerosis may increase the tendency toward thrombosis, it is necessary that

an exercise program be set up which will not advance the tendency toward blood clotting in the vessels. The present study suggests that nonstressful exercise may be used as a means of reducing the crust in the arteries without enhancing the tendency toward thrombosis, but exercise which is highly competitive, exhaustive or frightening may be harmful to the "coronary prone" individuals.

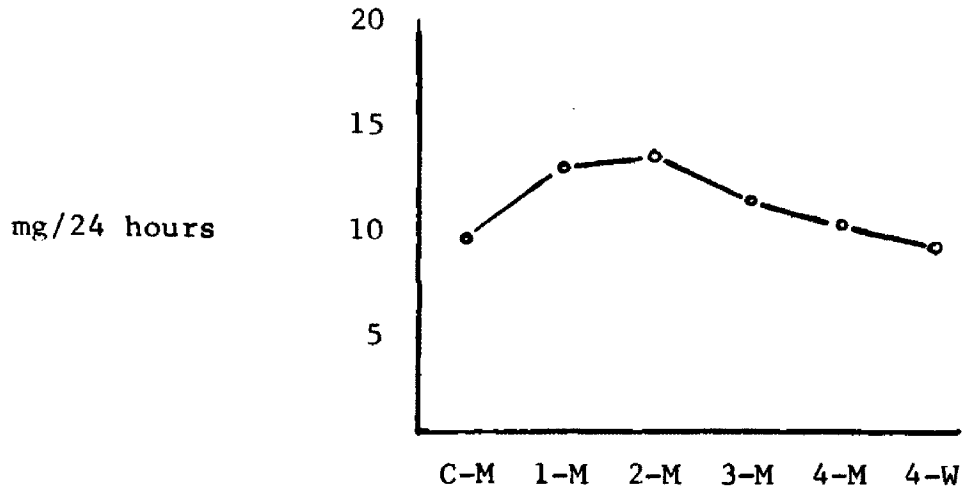

FIGURE 1

THE MEAN EXCRETION OF 17-KETOGENIC STEROIDS

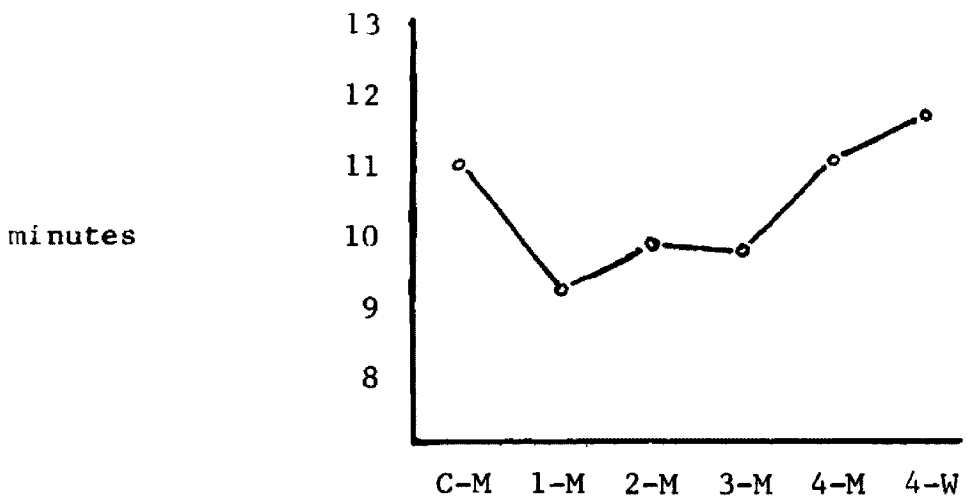

FIGURE 2

THE MEAN WHOLE BLOOD CLOTTING TIMES

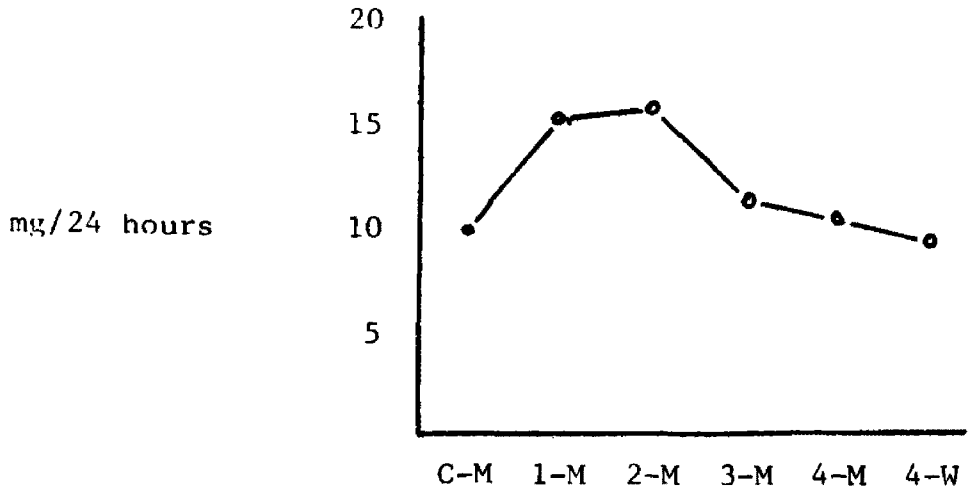

FIGURE 3

THE DAILY EXCRETION OF 17-KETOGENIC STEROIDS (R.H.)

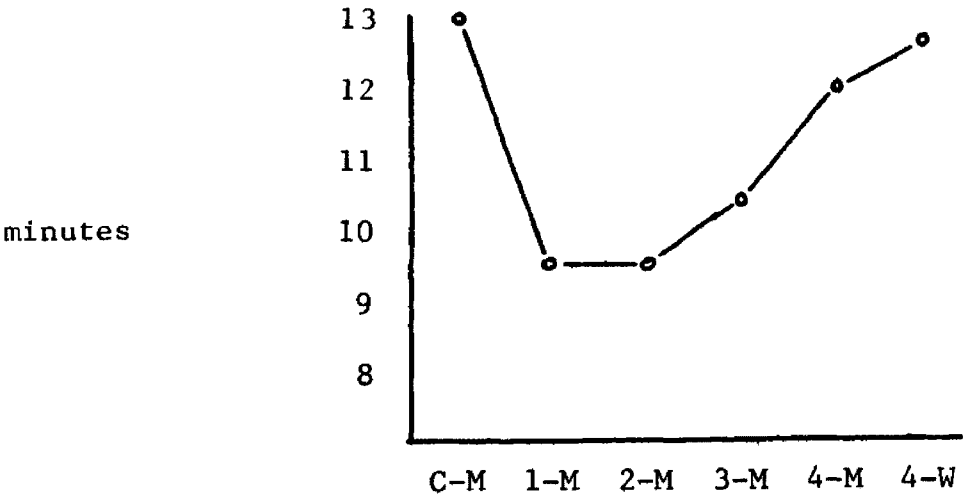

FIGURE 4

THE DAILY WHOLE BLOOD CLOTTING TIMES (R.H.)

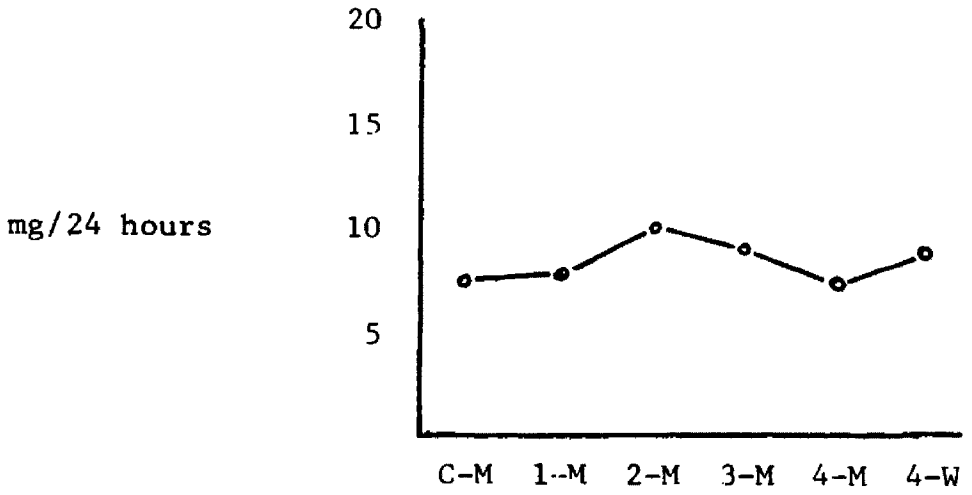

FIGURE 5

THE DAILY EXCRETION OF 17-KETOGENIC STEROIDS (B.L.)

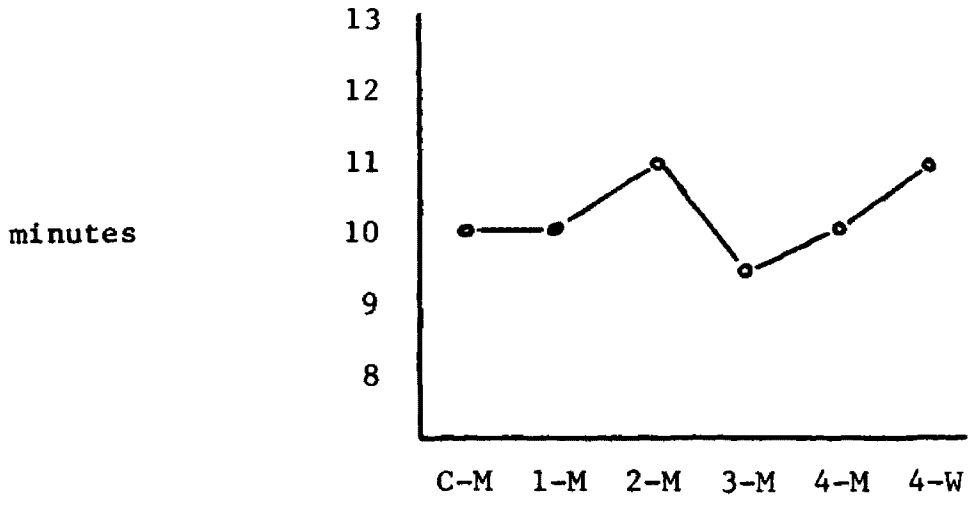

FIGURE 6

THE DAILY WHOLE BLOOD CLOTTING TIMES (B.L.)

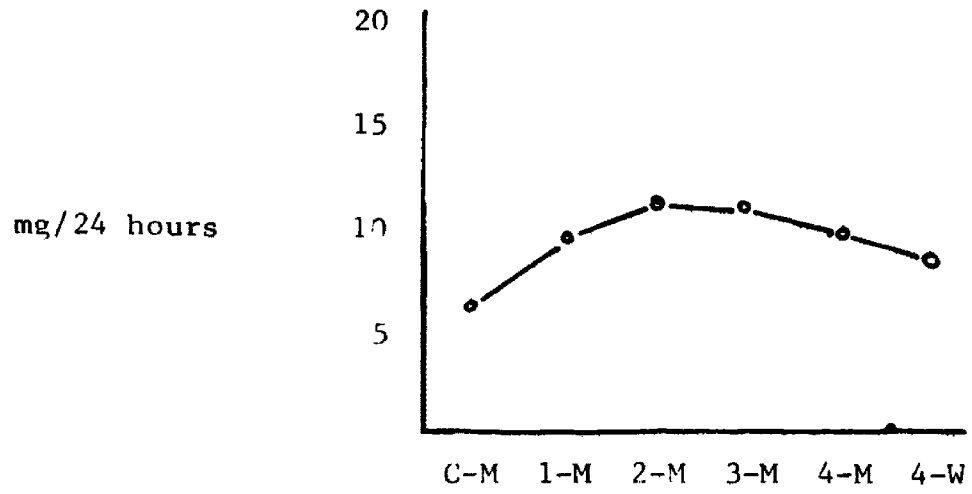

FIGURE 7

THE DAILY EXCRETION OF 17-KETOGENIC STEROIDS (T.M.)

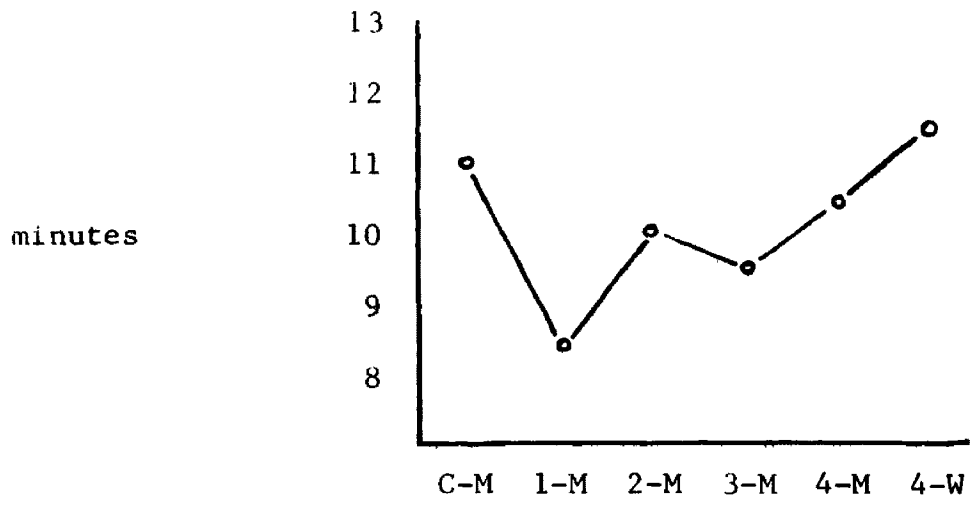

FIGURE 8

THE DAILY WHOLE BLOOD CLOTTING TIMES (T.M.)

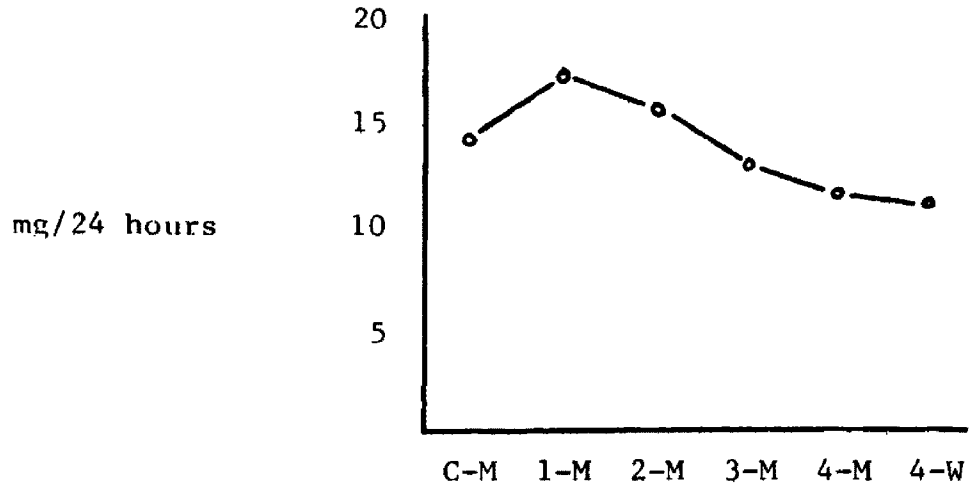

FIGURE 9

THE DAILY EXCRETION OF 17-KETOGENIC STEROIDS (J.S.)

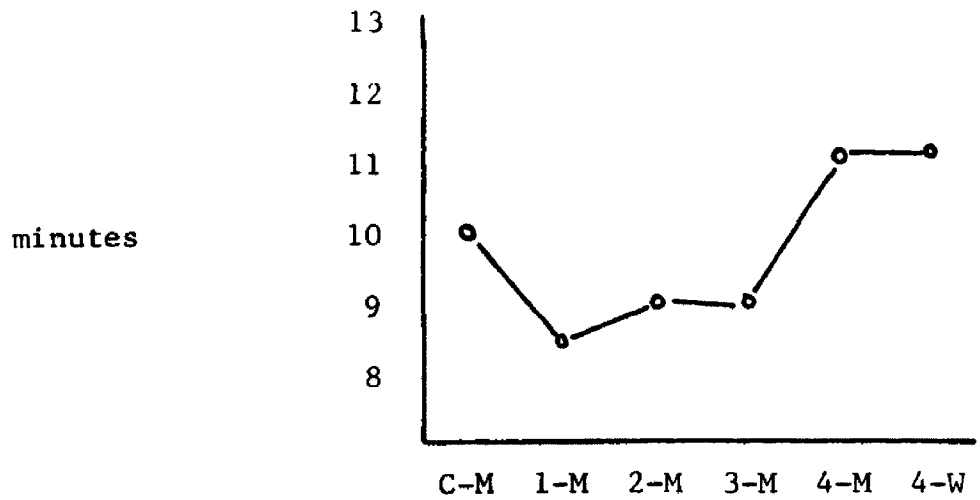

FIGURE 10

THE DAILY WHOLE BLOOD CLOTTING TIMES (J.S.)

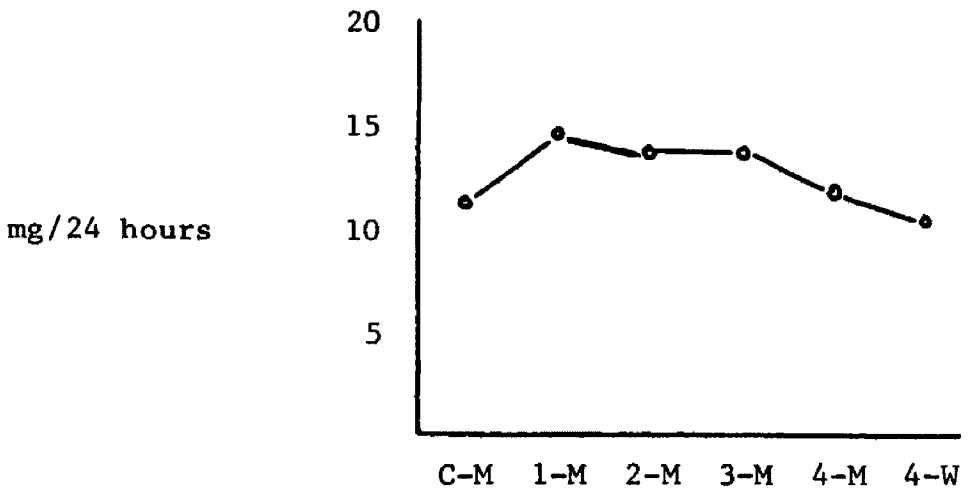

FIGURE 11

THE DAILY EXCRETION OF 17-KETOGENIC STEROIDS (B.S.)

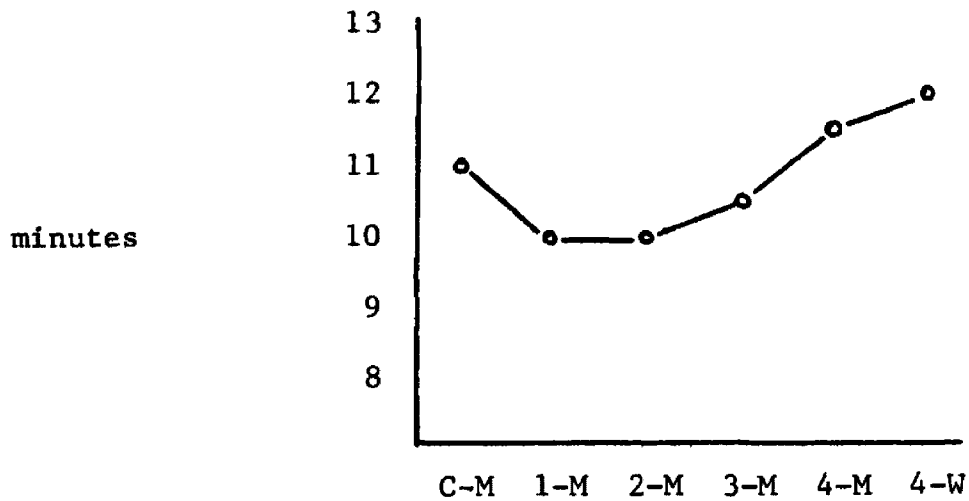

FIGURE 12

THE DAILY WHOLE BLOOD CLOTTING TIMES (B.S.)

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

I. SUMMARY

The purpose of this study was to determine the effect of non-stressful exercise on the whole blood clotting time. The subjects did not perform any physical exercise the control week, but they came to the Human Performance Laboratory their selected time. At this time the control whole blood clotting time was determined for the subjects. Also, for the next twenty-four hours the subjects collected urine in the labeled containers. After the control week the Modified Balke Treadmill Test was administered to the five subjects every Monday and Wednesday. The test was terminated when the heart rate reached 180 beats per minute. On Monday of each of the four training weeks and the fourth Wednesday after the test, the whole blood clotting time was determined and the subjects were given labeled containers to collect their urine for the next twenty-four hours.

The control 17-ketogenic steroid level and whole blood clotting times approached values typical for this age group. During the first two weeks of training, a general rise in the 17-ketogenic steroids was concomitant with a general faster whole blood clotting time. The last weeks of the training program, a reverse trend was noticed in the excretion of 17-ketogenic steroids and in the whole blood clotting times. This downward trend in 17-ketogenic steroids and slower whole blood clotting times continued to the conclusion of the training. The

whole blood clotting times and the 17-ketogenic steroid excretion approximately returned to the control levels at the end of the training period.

Although the evidence of this study suggests that stress plays a major role in causing faster blood clotting time, it is not possible to say whether ACTH directly or indirectly, through some other hormone, triggers the clotting mechanism. The platelets (1) may be the site which the hormone acts upon, or the hormone may act upon the vessel walls (2) resulting in the release of an enzyme which starts a cascade of protein activation necessary for thromboplastin formation.

II. CONCLUSIONS

On the basis of the results found in this study several conclusions can be made.

1. The excretion of 17-ketogenic steroids occurs as a result of the psychological factor assoicated with exercise rather than the physical activity.
2. The faster whole blood clotting time noticed in individuals after exercise is due to stressful exercise.
3. A faster whole blood clotting time appears to be triggered by either a higher level of ACTH hormone or adrenocortical hormone in the blood.

III. RECOMMENDATIONS

In view of the findings of this study the following

recommendations have been made. Further study should be done:

1. to determine the stress level which triggers the faster whole blood clotting time
2. to study precisely which hormones alter the whole blood clotting times
3. to see if different age groups are affected differently by stressful exercise.

SELECTED BIBLIOGRAPHY

SELECTED BIBLIOGRAPHY

1. Ackroyd, J. F. "Role of Platelets in Coagulation, Thrombosis, and Haemostasis, With Some Observations on Platelet Dysfunction, Including Thrombasthenia," British Medical Bulletin, 2(1): 21-27, 1955.

2. Brinkhous, K. M. "Clotting Defect in Hemophilia. Deficiency in a Plasma Factor Required for Platelet Utilization," Society for Experimental Biology and Medicine Proceedings, 66:117-120, 1947.

3. Cannon, W. B., and Horace Gray. "Factors Affecting the Coagulation Time of Blood: (II) The Hastening or Retarding of Coagulation by Adrenalin Injections," American Journal of Physiology, 34: 232-242, 1914.

4. _____, and W. L. Mendenhall. "Factors Affecting the Coagulation Time of Blood: (I) The Graphic Method of Recording Coagulation Used in These Experiments," American Journal of Physiology, 34: 225-232, 1914.

5. _____. "Factors Affecting the Coagulation Time of Blood: (III) The Hastening of Coagulation by Stimulating the Splanchnic Nerves," American Journal of Physiology, 34:243-249, 1914.

6. _____. "Factors Affecting the Coagulation Time of Blood: (IV) The Hastening of Coagulation in Pain and Emotional Excitement," American Journal of Physiology, 34:251-261, 1914.

7. _____. "Some Physiological Factors Affecting the Speed of Coagulation of the Blood," American Journal of Physiology, 33: 38-39, 1914.

8. Cohen, J. B., and J. Rubinstein. "An Experimental Approach to Psychological Stress," American Journal of Psychiatry, 3:276-282, 1954.

9. Connell, A. M., J. Cooper, and J. W. Redfearn. "The Contrasting Effects of Emotional Tension and Physical Exercise on the Excretion of 17-Ketogenic Steroids and 17-Ketosteroids," Acta Endocrinologica, 27:179-194, 1958.

10. Consolazio, Frank C., Robert E. Johnson, and Louis J. Pecora. Physiological Measurements of Metabolic Functions in Man. New York: McGraw-Hill Book Company, Inc., 1961.

11. Cooper, Kenneth H. Aerobics. New York: M. Evans and Company, 1968.

12. Cosgriff, S. W., A. F. Diefenbach, and W. Vogt. "Hypercoagulability of the Blood Associated with ACTH and Cortisone Therapy," American Journal of Medicine, 9(6):752-756, 1950.

13. Engle, L. A. Methods of Biochemical Analysis. Edited by D. Glick. New York: Interscience, 1954.

14. Enriori, C. L. "Microtechnic for Estimation of Urinary Neutral 17-Ketosteroid and 17-Ketogenic Steroids," Metabolism, 14:873-880, 1965.

15. Frenkl, R., and L. Csalay. "Effect of Regular Muscular Activity on Adrenocortical Function in Rats," Journal of Sports Medicine, 2:207-211, 1962.

16. Ganong, William F. Review of Medical Physiology. Los Altos: Lange Medical Publications, 1967.

17. _____. "Secretion and Release of ACTH," Symposium on Neuroendocrinology. Advances in Neuroendocrinology, p. 107, 1961.

18. Gray, H., and L. K. Lunt. "Factors Affecting the Coagulation Time of Blood: (V) The Effects of Hemorrhage Before and After Exclusion of Abdominal Circulation, Adrenals, or Intestines," American Journal of Physiology, 34:332-351, 1914.

19. Hartman, J. A. "Changes in the Clotting Time of the Blood of Cats as a Result of Exercise," American Journal of Physiology, 80: 716-718, 1927.

20. _____, R. H. Waite, and H. A. McCordock. "The Liberation of Epinephrine During Muscular Exercise," American Journal of Physiology, 62:225-241, 1922.

21. Henriques, Olga B., S. B. Henriques, and H. Selye. "Influence of Cold, Fasting and Adrenalectomy on the Blood-Fibrinogen Response to Trauma," Society for Experimental Biology and Medicine Proceedings, 73(4):611-613, 1950.

22. Hill, S. R. Jr., F. C. Goetz, H. M. Fox, B. J. Murawski, L. J. Krakauer, R. W. Reinfenstein, S. J. Gray, W. J. Reddy, S. E. Hedberg, J. R. St. Marc, and G. W. Thorn. "Studies on the Adrenocortical and Psychological Response to Stress in Man," A.M.A. Archives of Internal Medicine, 97:269-298, 1956.

23. Hycel Publication. 17-KS and 17-KGS Cortiset Determinations. Houston: Hycel, Inc., 1963.

24. Iatridis, Sotirios G., and John H. Ferguson. "Effect of Physical Exercise on Blood Clotting and Fibrinolysis," Journal of Applied Physiology, 18(2):337-344, 1963.

25. Keeney, Clifford E., and David W. Loramie. "Effect of Exercise on Blood Coagulation," Circulation Research, 10:691-695, 1962.

26. Macht, David I. "Influence of Acute Emotions on Blood Clotting," Federation Proceedings, 10(1):88, 1951.

27. Mason, John W. "Psychological Influences on the Pituitary-Adrenal Cortical System," Proceedings of the Laurentian Hormone Conference, 1958. Recent Progress in Hormone Research, 15: 345-376, 1959.

28. Miller, R. E., and J. W. Mason. "Changes in 17-Hydroxycorticosteroid Excretion Related to Increased Muscular Work," Symposium on Medical Aspects of Stress in the Military Climate. Walter Reed Army Institute of Research and Walter Reed Medical Center, p. 137-151, April 22-24, 1964.

29. Prokop, Ludwig. "Adrenals and Sports," Journal of Sports Medicine, 3:115-121, 1963.

30. Reynold, A. E., R. B. Quigley, and H. E. Kennard, and G. W. Thorn. "Reaction of the Adrenal Cortex to Physical and Emotional Stress in College Oarsmen," New England Journal of Medicine, 244(20):754-757, 1951.

31. Rizza, C. R. "Effect of Exercise on the Level of Antihaemophilic Globulin in Human Blood," Journal of Physiology, 156(1):128-135, 1961.

32. Sandberg, Avery A., Don H. Nelson, E. Myles Glenn, Frank H. Tyler, and Leo T. Samuels. "17-Hydroxycorticosteroid and 17-Ketosteroids in Urine of Human Subjects," Journal of Clinical Endocrinology and Metabolism, 13(12):1,445-1,464, 1953.

33. Schneider, R. A., and Violet M. Zangari. "Variations in Clotting Time, Relative Viscosity and Other Physiochemical Properties of Blood Accompanying Physical and Emotional Stress in the Normotensive and Hypertensive Subject," Psychosomatic Medicine, 13:289-303, 1951.

34. Selye, Hans. The Stress of Life. New York: McGraw-Hill Book Company, Inc., 1956.

35. Steadman, Richard Thomas. "The Effects of a Strenuous Physical Exercise Program on the Circulating Eosinophils Level and on the Urinary Excretion of 17-Ketogenic Steroids and 17-Ketosteroids," Unpublished Master's thesis, The University of of Montana, 1967.

36. Tinkel, Annette, and G. R. Cumming. "Effect of Exercise in the Cold on Blood Clotting and Platelets," Journal of Applied Physiology, 20(3):423-424, 1965.

37. Tocantins, Leandro M., and Louis A. Kazal. Blood Coagulation, Hemorrhage, and Thrombosis. New York: Grune and Stratton, 1964.

38. Ucha, Ronaldo, and Nora Portes. "Excrecion De Neurohormonas En Actividades Deportivas," Psicologia Dello Sport. Edited by Terruccio Antonelli, Roma: 1965.

39. Ulrich, Celeste. "Measurement of Stress Evidenced by College Women in Situations Involving Competition," The Research Quarterly, 28(2):160-172, 1957.

40. Van Norman, Richard W. Experimental Biology. Englewood Cliffs: Prentice-Hall, Inc., 1963.

41. Vosburgh, Charles H., and A. N. Richards. "An Experimental Study of the Sugar Content and Extravascular Coagulation of the Blood After Administration of Adrenalin," American Journal of Physiology, 9:35-51, 1903.

42. Vuori, A. K. "On Changes in the Blood Coagulation Time Immediately After Mental Irritation and Physical Exercise," Acta Medica Scandinavica, 138:296-300, 1950.

43. Warnock, Nelta H., Thomas B. Clarkson, and Richard Stevenson. "Effect of Exercise on Blood Coagulation Time and Atherosclerosis of Cholesterol-Fed Cockerels," Circulation Research, 5: 478-480, 1957.

44. Young, D. R., R. Price, N. E. Elder, and R. R. Adachi. "Energy and Electrolyte Metabolism and Adrenal Response During Work in Dogs," Journal of Applied Physiology, 17:669-674, 1962.

APPENDIX

APPENDIX A

STATISTICAL ANALYSIS

I. Formula for Computing Correlation--Pearson r

Pearson r-

$$r = \frac{N\Sigma XY - (\Sigma X)(\Sigma Y)}{\sqrt{[N\Sigma X^2 - (\Sigma X)^2][N\Sigma Y^2 - (\Sigma Y)^2]}}$$

II. Formula for Testing Significance of r

t Test for Significance-

$$t = \frac{r}{\sqrt{1 - r^2}} \sqrt{N - 2}$$

$N < 30$

r = Pearson r