



127
937
THS

THE EFFECTS OF FORCED - EXERCISE AND ANXIETY -
PRODUCING STRESS ON MYOCARDIAL DAMAGE
IN YOUNG MALE ADULT ALBINO RATS

Thesis for the Degree of M. A.
MICHIGAN STATE UNIVERSITY
D. PAUL THOMAS

1970

LIBRARY
Michigan State
University

ABSTRACT

THE EFFECTS OF FORCED-EXERCISE AND ANXIETY- PRODUCING STRESS ON MYOCARDIAL DAMAGE IN YOUNG MALE ADULT ALBINO RATS

By

D. Paul Thomas

The purpose of this study was to investigate the effects of forced-exercise and anxiety-producing electrical stress on the incidence of myocardial damage in young adult male albino rats.

Twenty animals were randomly assigned to a single housing condition and four experimental groups. The treatments comprised forced-exercise in controlled-running wheels for 35 minutes, and anxiety-producing stress in the form of an electrical shock received every 15 seconds on average, for two hours. Both treatments were administered singly to two of the groups and in combination to a third group. The fourth group received neither treatment and acted as the controls. Treatments were administered seven days a week for a period of 18 days.

At the termination of the study, all animals were sacrificed by decapitation. The heart was then removed from each animal. The muscle was quick-frozen, sectioned

and stained, prior to examination for heart-damage under a light microscope.

The Fisher exact probability test for small samples was used to statistically analyze the data. Significance was found between the heart-damage ratings of the control and combined-treatment animals ($P \leq .10$). The incidence of heart-damage in the other two groups was not found to be significant.

THE EFFECTS OF FORCED-EXERCISE AND ANXIETY-
PRODUCING STRESS ON MYOCARDIAL DAMAGE
IN YOUNG MALE ADULT ALBINO RATS

By

Dr. Paul Thomas

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF ARTS

Department of Health, Physical Education
and Recreation

1970

G-6412-11
12-11-11

DEDICATION

To my father for giving me every opportunity in life.

To Katie for her enduring friendship.

ACKNOWLEDGMENTS

The author wishes to thank Dr. William Heusner, a source of inspiration at all times, for his advice and guidance throughout this study.

Thanks are also due to Dr. Rexford Carrow for use of the Cytology Laboratory and to Barbara Wheaton and Patricia Lamb for assistance with sectioning and staining techniques.

The writer acknowledges the help of Dr. Steven Sleight in rating the slides.

TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
LIST OF PLATES	vii
Chapter	
I. INTRODUCTION	1
Statement of Problem	3
Need for the Study.	3
Definition of Myocardial Damage	3
Limitations of the Study.	4
II. REVIEW OF LITERATURE.	5
Classification of Cardiomyopathies	5
Experimental Cardiomyopathies	6
The Role of Catecholamines in Cardiomyo- pathies.	9
The Role of Exercise in Ischemic Heart Disease.	13
Human Cardiomyopathies	15
III. METHOD	19
Testing Procedure	22
Rating Scale.	23
Analysis of Data	23
IV. RESULTS AND PRESENTATION OF DATA.	25
Introduction.	25
Treatment and Presentation of Data	25
Discussion of Results.	26

Chapter	Page
V. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS . .	29
Summary.	29
Conclusions	30
Recommendations	31
BIBLIOGRAPHY	32
APPENDICES	
Appendix	
A. Body and Organ Weight Data	38

LIST OF TABLES

Table	Page
1. Heart-damage Ratings Taken at Five Different Levels in the Lower Half of the Ventricles of Control and Experimental Animals . . .	27
A-1. Body and Organ Weight Data (in Grams) . . .	38

LIST OF PLATES

Plate	Page
1. Heart-damage Rating Scale	24

CHAPTER I

INTRODUCTION

Despite the advances made in the treatment of many illnesses within the last decade, heart and circulatory disorders continue to rise in number. This increase in degenerative cardiovascular disease in modern man has been blamed in part on lack of physical exercise concomitant with a multiplicity of anxiety-producing situations. An understanding of the heart and its adaptation, or failure to adapt, to these various anxiety-producing situations is necessary if practical programs for the prevention of cardiomyopathies are to be developed. Specifically, more information is needed regarding the effects of physical and emotional stresses on myocardial damage. The effects of these stresses must be studied singly, and perhaps more realistically in combination, if results are to have any application to present-day society.

Unfortunately, the voluminous amount of literature available on the subject has led to inconsistency of terminology; confusion has arisen over terms such as cardiopathy, cardiomyopathy, coronary atherosclerosis, and

ischemic heart disease. Therefore, the term "metabolic cardiomyopathy" has been proposed to cover all disorders of the heart whatever their nature or origin (52). Two broad classifications of cardiomyopathies have been recognized: (a) "primary cardiomyopathies" referring to isolated idiopathic diseases of the myocardium, and (b) "secondary cardiomyopathies" or those cardiac disorders which have their origin outside the heart muscle itself (19).

Recently, attention has been focused on primary cardiomyopathies caused by neurogenic and humoral mechanisms. These mechanisms upset the electrolyte metabolism in the myocardium, thereby increasing its vulnerability to disease, or in some instances producing heart damage themselves. Myocardial damage is known to be augmented by sympathetic interference of catecholamines which impair oxygen economy (3, 37, 45, 46). The role played by stress-induced production of these adrenal hormones in degenerative cardiovascular pathology has now been established beyond doubt (23, 24, 26, 37).

However, the link between stress and heart disease is twofold. Animal studies have shown that previous adaptation to a single stress, such as exercise, may provide a cardioprotective resistance against later unaccustomed stresses (16, 45, 46). In man, epidemiological comparisons of heart disease rates in sedentary and physically active individuals support this concept of

the prophylactic role of exercise (22). In addition, graded exercise programs are increasingly used in the rehabilitation of cardiac patients (6, 9, 10, 28, 54).

Statement of Problem

The purpose of this investigation was to study the effects of physical activity and anxiety-producing stress on the incidence and severity of myocardial damage in young male albino rats. In particular, the effects of forced exercise and electrical shock were evaluated both singly and in combination.

Need for the Study

Knowledge of the heart's reaction to different types of stresses is as yet very limited. There is a need to study both the harmful and beneficial effects of physical and emotional stresses on cardiac function when imposed singly and simultaneously. It is hoped that exposure of rats to treatments of electrical shock and forced exercise will contribute to a greater understanding of the roles these stresses play in the production of cardiomyopathies.

Definition of Myocardial Damage

The term "myocardial damage" is used in this study to describe any cardiomyopathy discernible under the light microscope, whether its etiology be primary, secondary, or a combination of both.

Limitations of the Study

1. The availability of sedentary metabolism cages restricted the number of animals which could be used in the experiment.
2. The short duration of the study may have prevented the full effects of the various treatments from taking place.
3. No attempt was made to determine the exact nature of the myocardial damage elicited by the various treatments.
4. Direct application of the results of this animal study to humans is not possible.

CHAPTER II

REVIEW OF LITERATURE

Classification of Cardiomyopathies

Cardiovascular disorders cause more deaths in the United States than all other diseases combined (23). They are also the best documented of all illnesses. However, consistency of terminology has not prevailed. The confusion created by variations in classification and interpretation has led prominent researchers in the field to contribute articles on terminology alone (19, 51, 52). The term "cardiomyopathy" as introduced by Brigden (5) has lost its original meaning of "isolated non-coronary, myocardial disease." Therefore Wartman (52) has suggested the use of the phrase "metabolic cardiopathy" to refer to any abnormality of the myocardium, endocardium, or epicardium or all three. The two main groups are primary and secondary.

In primary (idiopathic) cardiopathy, there is no coronal, arterial or valvular abnormality, no hypertension and no vascular shunt inside or outside the heart. . . . Secondary cardiomyopathies comprise a vastly greater group in which the heart is involved in a well-known disease process, congenital or acquired, or damaged by known trauma, drugs or other noxious agents (19).

Unfortunately, this distinction is not as clear as it might seem. Selye (46) points out, "It appears that the heart is capable of responding only with a limited number of reactions to the innumerable agents and combinations of agents that can act upon it." These reactions may be listed as inflammation, hypertrophy, degeneration, necrosis, and fibrosis (52). In addition, cardiomyopathies very often are multicausal in nature. Ischemic necrosis may develop not only as the secondary consequence of coronary artery occlusion, but also through metabolic influences on myocardial supply and need (3, 35, 45). The term "pleuricausal cardiopathy" is used by Selye (56), emphasizing the multiplicity of causes in most pathological conditions of the heart. Bajusz (3) also points out that at the level of light microscopy, no clear distinction can be made between a primary heart muscle degeneration and that resulting from coronary artery occlusion.

Experimental Cardiomyopathies

In an attempt to gain a greater understanding of myocardial disfunction in man, much animal research has been carried out in the laboratory with artificially created or "experimental cardiomyopathies" (7, 8, 53). Early experiments of this nature included ligation of the coronary arteries or veins and assessment of the nature of myocardial damage under electron microscope (44). Caulfield and Klionsky (8) noted "Definite changes can be

demonstrated by electron microscope in the early stages of acute myocardial infarction, before lesions become apparent by light microscopy." Using this technique Bryant, Thomas, and O'Neal (7) studied autolysis of the myocardium after ligation, and found the changes similar to those of myocardial infarcts, but with a greater uniformity of damage and less rapid development of structural changes over the first five hours. These results were similar to those reported by Caulfield (8).

The next development came with the use of histochemical staining techniques (2, 31, 53). Using rats for subjects, Bajusz and Jasmin (2) showed that by standardizing the point of ligature, it was possible to obtain not only an "homogenous infarct of predictable size and location without any mortality," but also that its development was remarkably constant. This placed the emphasis on the enzyme histogenesis of cardiac infarcts and, especially, the time relationship between the changes in enzyme activity and histologically detectable structural alterations.

Using a staining technique for succinic dehydrogenase (SDH) on small biopsy specimens, Niles et al. (31) were able to show interference with the metabolic process of oxidative phosphorylation. The decrease in staining-power of the myofibrils as the experiment proceeded, indicated the progressive inability of the mitochondria to oxidise succinate as part of the Krebs cycle, with a

corresponding liberation of hydrogen and thus electrons for transport between the sarcosomes (mitochondria) and the fibrils. Niles, Zarvin, and Morikado (32) also found that interference with hydrogen transportation and electrolyte balance could be produced by subjecting rats to an atmosphere of 6 per cent oxygen or by injection of the catecholamine derivative isoproterenol.

Merules and Fine (12, 27) made histochemical comparisons of experimentally induced cardiomyopathies in rats with human heart muscle 6 to 36 hours after death from infarction. This again indicated the break-down of glycogen metabolism in the pre-necrotic stages of myocardial infarction. In a biopsy study of five cardiac patients, Pierce (33) noted this concentration of glycogen storage in the mitochondria (mitochondriosis). Using a silver stain, he also found abnormally high sympathetic innervation of the muscle with corresponding noradrenaline content. Pierce described this condition with the term "noradrenosis."

Selye and Bajusz, who were convinced of the role played by hormones and electrolytes in cardiomyopathies, induced myocardial necrosis in rats fed on a continuous potassium-deficient diet. They noted, however, that simultaneous withdrawal of sodium protected the heart from these same lesions (42, 43, 45). Potassium depletion was detected as early as ten minutes after subcutaneous injection of epinephrine in rats by Raab and Bajusz (37).

In the same study, further sensitization with fluorocortisole greatly enhanced the susceptibility of the muscle; similar potassium depletions occurred with merely a half-dose injection of epinephrine.

These studies discredited the earlier belief that ion alterations, such as loss of potassium and magnesium from the myocardial cells and uptake of sodium and calcium, were merely secondary consequences of myocardial necrosis. Bajusz (4) sums up the opinion of cardiologists at the present time: "Distortions of intracellular electrolyte gradients seem to be important components of many, if not all, types of disturbances in cardiac metabolism resulting in fatal conduction defects, myocardial degeneration or heart failure."

The Role of Catecholamines in Cardiomyopathies

For a long time, ischemic heart disease was thought to be concerned merely with vascular oxygen supply. Little attention was paid to utilization of oxygen by the myocardium. This is dependent on not only oxygenated blood flowing through the heart, but also on metabolic oxygen consumption by the myocardial tissue (39). Schimert (41) pointed out that the maintenance of metabolic and structural integrity of the heart depends on:

$$\frac{\text{Vascular O}_2 \text{ Supply}}{\text{Myocardial O}_2 \text{ Utilization}} = 1.0$$

This quotient may fall below unity either as a result of a decrease in blood flow or by an uneconomical metabolic use of oxygen by the myocardium. The former may be due to coronary stenosis, while the latter is affected by neuro-humoral influences as in times of stress (24, 26, 41).

Stress conditions provoked by a variety of disturbing or straining factors, either physical or mental, are accompanied by reactions from the sympatho-adrenomedullary system. Stressing factors involving the catecholamine-producing systems may either " . . . induce a reaction from the adrenal medulla, chiefly provoking an increased release of epinephrine, or activate the norepinephrine-producing nerves, or both" (11). However, the harmful role that these hormones play in degenerative cardiovascular pathology has only recently been accepted. Raab (35), one of the early proponents of this theory, has outlined their potentiating effects as follows:

1. Intensification of O₂ consumption by myocardial tissue.
2. Diminution of cardiac energetic efficiency (= percentage wise conversion of oxidative energy to mechanical work).
3. Production of local myocardial hypoxia (due to waste of oxygen plus presumable compression of subendocardial coronary vessels by increased intraventricular pressure) resulting in an accumulation of lactic acid.

4. Tendency to favor the development of myocardial hypertrophy and focal degeneration (possibly by way of alterations of intra-extra cellular electrolyte transfer).
5. Vascular constriction and elevation of blood pressure (probably due to effect on trans-membrane cationic gradient), and
6. Tendency to cause hypoxia of arterial intima and to accelerate and augment intimal lipid deposition.

For this reason, study of catecholamines and their role in cardiac metabolism has intensified considerably. Levels of these hormones and their metabolites have been examined in blood plasma, body tissues, and in the urine (11). Their effects on heart metabolism have been studied in man under stressful working conditions, in clinical cases of essential hypertension, during repeated attacks of angina pectoris, and in post-infarct patients (24, 26, 48, 49, 50). In addition, animal experiments have been carried out which elicit myocardial damage by injection of these adrenal hormones (1, 37, 45).

Comparisons of plasma catecholamine levels in normal and cardiac subjects, under emotionally or physically stressful situations, revealed the "adrenergic drive" of the latter (40, 48). Exercise-induced levels of norepinephrine were increased an average of 119.3 per cent in the

cardiac subjects as against 4.1 per cent in the normals (40).

Valori (49) found urinary levels of noradrenaline and adrenaline to be elevated 800 and 350 per cent respectively above the mean normal levels in his post-infarct patients. These raised levels decreased slowly after the infarct according to Wallace (50), who stressed the relationship between the autonomic nervous system and the incidence of cardiomyopathies.

Studies in man also have implicated norepinephrine metabolism at the vascular receptors and neurovascular storage sites of the heart in essential hypertension (27).

The causative role of these hormones in many types of cardiomyopathies has been confirmed by further studies with animals. Prenecrotic disturbances of myocardial potassium, glycogen, and phosphorylase were noted by Raab and Bajusz (37) who injected rats with epinephrine. Similar disturbances in the electrolyte balance of rat myocardium following isoproterenol injection were found by Niles, Zarvin, and Morikado (32).

Degenerative heart disease thus may be attributed not only to coronary atherosclerosis but also to the hypoxiating effect of sympatho-adrenomedullary activity which results in necrotizing structural changes of the myocardium.

The Role of Exercise in Ischemic
Heart Disease

The link between exercise and heart disease is now thought to be twofold in nature. The physical stress of muscular activity may result in elevated levels of potentially harmful catecholamines (11). However, habitual physical activity in man also has been shown to lower cardiac sympathetic tone (20, 36). Coronary vascular dilatability normally occurs in response to catecholamine-induced alterations of myocardial oxidative metabolism.

The risk of potentially hypoxiating and potassium-depleting effects of acute intensive sympathetic stimulation during physical effort is largely offset by the simultaneous massive mobilization of potassium from the contracting skeletal muscles into the circulating blood, and its transfer into the myocardial tissue (39).

Where coronary dilatability is impaired by vascular rigidity, this compensation cannot take place. Under these conditions, augmentation of adrenergic activity may produce local hypoxia and necrotic cell destruction.

Animal experiments have illustrated the dual role of exercise-produced stress in cardiac metabolism (16, 21, 46). Mice, forced to exercise for a period of ten weeks, showed myocardial hypertrophy, interstitial fibrosis, infiltration of inflammatory cells, and small foci of bleeding (21). Extensive irreversible fibrosis of the myocardium and changes in the nuclei of the atrophied fibers were still visible under the microscope six weeks later. A combination of forced-exercise and treatment with sodium

acetate plus fluorocortisole produced similar results within 24 hours (45). However, one group of rats forced to run both before and after the treatment survived, and no myocardial damage could be detected under the microscope. Selye termed this phenomenon "simple resistance." Further experimentation showed that cardiac necrosis could be prevented even by pretreatment with a stressor different from that used as an elicitor. "The cardiac-necrosis-producing effect of muscular exercise could be prevented by treatment with cold, that of cold by muscular exercise, that of noradrenaline by restraint, that of restraint by noradrenaline, and that of bone-fracture by exercise" (1).

Mature rats exercised during the prepubertal and early postpubertal period were shown to withstand the combined effects of electrical shock and further physical stress far better than those animals which did not receive the previous exercise treatment (16).

Exercise, consisting of daily strolling for a period of four weeks, was carried out in five dogs following experimental infarction by Kitamura (21). Microscopic comparison of myocardial tissue, from the experimental animals and a group of postinfarct sedentary controls, indicated that moderate exercise following myocardial infarction has possibilities of beneficial clinical application.

Human Cardiomyopathies

In man, epidemiological studies have attempted to determine the exact role which exercise plays in cardiovascular disease (6, 10, 54). Comparisons of heart disease rates and those parameters associated with cardiac malfunction have been made in both sedentary and active individuals (6, 10, 23, 38). In a longitudinal study, Dawber (10) demonstrated that age, sex, serum lipids, blood pressure, cigarette smoking, obesity, electrocardiograph abnormalities, and vital capacity were all related to the risk of developing heart damage.

Incidence of cardiac disorders in kibutz workers over a 15-year period was examined by Brunner (6). Sedentary workers were shown to be 2.5 to 4 times more vulnerable than their active contemporaries. Mortality rates in the same subjects six years after the investigation were 59 and 23 per cent respectively.

A comparative study of athletic and inactive elderly individuals showed lower blood cholesterol and lipid levels, greater vital capacity, greater working capacity, and a lower incidence of electrocardiograph abnormalities in the active group (23). Accelerated heart-rate, shorter isometric period, and a relatively prolonged systole, all signs of adrenergic overstimulation, were noted in sedentary individuals exposed to various stresses (38).

Kraus supported this concept of lack of physical exercise leading to disorders of the heart (22). He placed emphasis on the link between overstimulation of the "fight or flight" response and weakness of the myocardium in producing "hypokinetic disease."

Alterations of a beneficial nature also have been shown to take place following a period of conditioning (25, 29). Using 15 sedentary professional men, ranging in age from 35 to 55 years, Skinner, Holloszy, and Cureton (47) were able to show significantly increased exercise capacity and specific gravity following a six-month program of conditioning exercises. Serum tryglycerides in the same subjects fell from a mean pretraining level of 208 ± 127 to 125 ± 78 mg per cent at the end of the study (18). Ballistocardiograph measurements were also recorded, and significant increases occurred in the mean I, J, GI, HI, and IJ forces. Four of the five men with initially abnormal ballistocardiograms had normal records at the end of the program (17).

Properly supervised, graded exercise, even in patients with severe cardiac disease is now known to be less hazardous than was formerly suspected (15).

Physical activity has a dual role in the case of cardiac patients; one therapeutic, directed towards the restoration of physical work capacity lost through illness, and the other, preventive, aimed at the promotion of increased coronary collateral circulation, with a concomitant decrease of sympathetic inotropic and increase of parasympathetic chronotropic tone (30).

Cardiac patients subjected to work-capacity tests for a period of 23 weeks responded to training in exactly the same manner as normal healthy individuals (28). Conditioning was accompanied by a reduction in the levels of systolic and diastolic blood pressure, pulse rate, minute ventilation, and serum cholesterol levels at rest (29).

Gottheiner (15) found incidence of subsequent infarcts was reduced following a period of intense training. He emphasized three important elements involved in the cardio-protective role of exercise training:

1. Improvement of oxygen economy of the myocardium.
2. Augmentation of the coronary collateral network.
3. Transfer of potassium from the contracting striated muscles into myocardial tissue, where the electrolyte balance may be critically threatened by adrenergic hypoxia due to coronary atherosclerosis.

Cureton (9) found that "Rhythmical activities lasting thirty to sixty minutes per day, five or six times a week, combined with emphasis on adequate breathing," were the most beneficial in achieving this effect. He warned of the potentially hypoxiating effect of high intensity weight-lifting or training programs. Mellerowicz (25), in a summary of recent research in the field of preventive cardiology, points out that "Even though training can be considered as only one among several preventive factors, it is a very important one."

Information concerning the persistence of changes produced by physical activity is meager. Proof of the therapeutic value of activity programs must come from longitudinal observation of significant numbers of high-risk subjects (13). Pyorala et al. (35) found that former athletes demonstrated larger heart volumes and lower heart rates on exertion, than age-matched controls. However, the correlation between heart-rate and past physical activity was not significant. Frick (14) pointed out that relative bradycardia may vanish rapidly during periods of non-training.

It is clear that the long-term effect of physical training needs more study, but the available data suggests that continued activity maintains the hemodynamic changes and that the effects of previous activity, if sufficiently vigorous, is still discernible years, and perhaps decades later (14).

There is evidence for both beneficial and harmful effects of various emotional and physical stresses on the heart. Research into the combined effects of such stresses awaits future investigation. It is hoped that this study will shed further light upon the effects of exercise and anxiety-producing stress on the heart.

CHAPTER III

METHOD

The purpose of this study was to investigate the effects of physical activity and anxiety-producing stress on the incidence and severity of myocardial damage in young adult male albino rats. The study was designed with a single housing condition and four experimental treatments. Myocardial damage was assessed at the termination of the experiment. The number of animals used in the study was based solely on the availability of sedentary metabolism cages. Twenty 80-day-old male Sprague Dawley rats were purchased from Hormone Assay Laboratories in Chicago, Illinois. The animals were housed in 22 x 11 1/2 x 11 1/2 cm metabolism cages with an attached spontaneous activity drum for four weeks prior to the experimental treatments. During this feet-conditioning and general adaptation period, all rats were allowed free access to the activity wheels. At the commencement of the experimental period, the animals were denied access to the attached running wheels so that data (urine samples) not used in the present study might be collected. All rats were maintained under sedentary

conditions for the remainder of the study. The animal quarters were lighted 24 hours a day. Room temperature, in both animal quarters and treatment rooms, was held between 70 and 72 degrees Fahrenheit and between 60 and 70 per cent relative humidity. During the study, every animal was handled daily. All were fed Wayne Lab ground feed ad libitum, and had access to water at all times.

The animals were divided randomly into four groups of six, and the groups were randomly assigned to the four experimental regimens:

1. A Control Group. These animals received no experimental treatment, but were placed in "holding" cages for a period of four hours each day, seven days a week, in order to eliminate all environmental variables.
2. An Anxiety Group. These animals received sporadic electrical shock for a period of two hours each day, seven days a week.
3. A Forced-exercise Group. These animals were forced to run a medium-endurance type program in controlled-running wheels for 35 minutes each day, seven days a week.
4. An Anxiety plus Forced-exercise Group. Each day, seven days a week, these animals received sporadic electrical shock for a period of two hours and then were forced to run a medium-endurance type program in controlled-running

wheels for 35 minutes immediately following the shock treatment.

All animals were transferred to "holding" cages in the treatment room during the experimental period which lasted approximately four hours. During the first two hours, electrical shocking was given to groups two and four. Groups three and four then were forced to run in the wheels for a period of 35 minutes. During the final 90 minutes, all animals were kept in a "holding phase," while urine samples were collected. The four groups then were returned to their "home environment."

The anxiety-producing stress consisted of electrical shocks (60 volts, 15ma), preceded by a pleasant tone audible at conversation level. The tone, lasting .5 seconds, was given .5 seconds before each electrical shock. The shock, which lasted .5 seconds, was administered randomly on a 100-second time basis. Ninety-five per cent of the stimulations were from 5 to 20 seconds apart; 5 per cent of the stimulations were from 20 to 80 seconds apart. The average number of shocks on a random basis was four per minute. The duration of the treatment each day was two hours. The stimulations were received through a stainless-steel grid, which served as the floor of plastic-walled cages 17 cm long by 17 cm wide by 17 cm tall.

The training wheels used for the forced-exercise treatment employed avoidance electrical stimulus (1.2 ma) operant conditioning, utilizing a light (60 volts, 120

watts) as the conditioned stimulus. The animals were forced to run at a speed of two feet per second for 10 seconds. They repeated this run 30 times with a rest interval of 10 seconds between repetitions. The program consisted of three bouts with a rest interval of 5 minutes between each bout. The total running time was 35 minutes. All animals were able to satisfactorily complete this exercise program.

After completion of the treatment period which lasted 18 days, each animal was weighed and then sacrificed by decapitation.

Testing Procedure

The heart was removed from each animal and flushed with Ringer's solution to remove as much blood as possible from the chambers. The great vessels then were carefully trimmed. The heart was further washed in distilled water to remove any remaining blood from the chambers, and then dried prior to weighing. The muscle was then mounted (atria downwards) with 5 per cent gum tragacanth, care being taken to leave the entire length of the ventricles exposed. The heart was quick-frozen to -110°C in isopentane previously cooled in liquid nitrogen. Serial sections 8 microns thick then were cut from the block in a cryostat at -20°C . Five sets of four sections were taken from the heart-muscle at equal intervals in the lower half of the ventricles. Immediately after

7

sectioning, the tissues were placed on cover slips, dried, and stained with hematoxylin and eosin (H & E). They were then mounted in groups of four on 50 x 75 mm slides for examination under a light microscope.

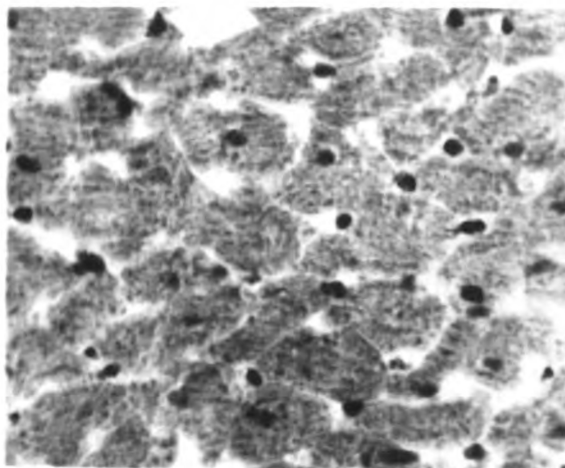
Rating Scale

The degree of myocardial damage was evaluated subjectively on a scale similar to that used by Niles, Zavín, and Morikado (32) and Heusner (16). Any two of the four heart sections, at each of five levels in the ventricles were used to rate myocardial damage. The rating scale used on each section was as follows: 1 = no damage, 2 = slight damage, 3 = moderate damage, 4 = considerable damage, or 5 = severe damage (see Plate 1).

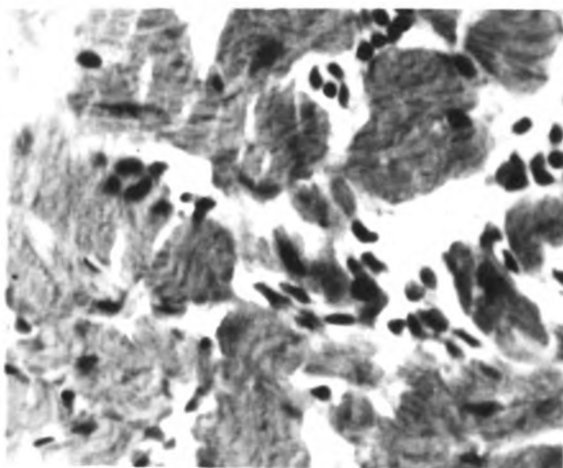
Analysis of Data

Initial ratings were given on ten sections taken from the ventricles of each animal. An overall rating was calculated as the modal value of the five highest ratings from the 10 sections. All sections were then recoded, using random animal identification numbers, and rerated until identical overall ratings were obtained. The Fisher exact probability test for small samples was used to further analyze the data.

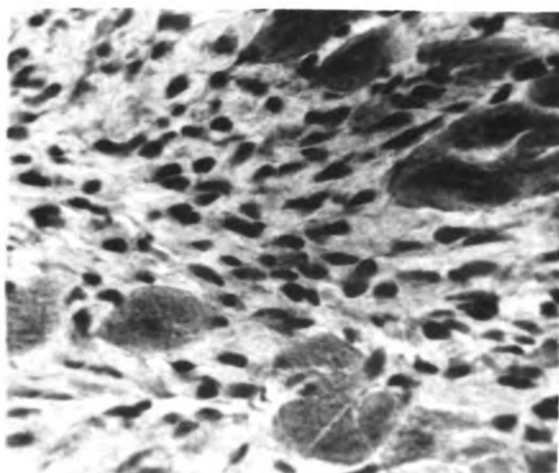
H & E (x500)



NORMAL
HEART
RATING
1



MODERATE
DAMAGE
RATING
3



SEVERE
DAMAGE
RATING
5

Plate 1, Heart-damage Rating Scale

CHAPTER IV

RESULTS AND PRESENTATION OF DATA

Introduction

The purpose of this investigation was to study the single and combined effects of forced-exercise and anxiety-producing electrical stress on the incidence of myocardial damage in young male adult albino rats. Twenty animals were randomly assigned to a single housing condition and four experimental treatments. The rats received treatments hour hours each day, seven days a week, for a period of 18 days.

At the termination of the study, the animals were sacrificed by decapitation and the hearts were removed for examination under a light microscope.

Treatment and Presentation of Data

The degree of heart damage was evaluated subjectively on a similar scale to that used by Niles, Zavin, and Morikado (32) and Heusner (16). Any two of the four heart sections, at each of five levels in the lower half of the ventricles, were used to rate myocardial damage. An overall rating was calculated as the modal value of the five

highest ratings from the 10 sections. All sections were then recoded, and rerated until identical overall ratings were obtained (see Table 1).

The small size of the samples used in this investigation precluded any extensive statistical analysis. Using the Fisher exact probability test, the difference in heart-damage ratings between the controls and the combined-treatment animals was found to be statistically significant ($P \leq .10$). Slight damage was also detected in the "exercise" and "anxiety" animals (see Table 1), but the difference was not significant in these two groups.

Discussion of Results

The results showed that the treatments used in the present study were able to produce slight alterations of the myocardium in young male adult albino rats. Slight accumulations of lymphocytic nuclei associated with small areas of possible muscle necrosis were seen in several of the sections and given a subjective rating of 2. However no extensive lesions or scars were found, and there was no evidence of any cell calcification in the myocardium. The incidence of damage was higher in the group of animals which received both the electrical shock and forced-exercise programs. This indicated the possible "double stressor" effect of the combination of treatments and tends to support Sely's theory of the pleuricausal nature of many cardiomyopathies. The study confirmed the work of Kitamura

TABLE 1.--Heart-damage ratings taken at five different levels in the lower half of the ventricles of control and experimental animals.

Group	Level a	Level b	Level c	Level d	Level e	5 Highest Ratings	Mode
GROUP I Control Group	1	1	1	1	1	1,1,1,1,1	1
	2	1	1	1	1	2,2,2,1,1	2
	1	1	1	1	1	1,1,1,1,1	1
	1	1	1	1	1	1,1,1,1,1	1
	1	1	2	1	1	2,1,1,1,1	1
GROUP II Anxiety Group	1	1	1	1	1	1,1,1,1,1	1
	1	1	1	1	1	1,1,1,1,1	1
	1	2	1	2	1	2,2,2,2,1	2
	1	1	1	1	1	2,1,1,1,1	1
	1	1	3	3	2	2,2,2,2,2	2
GROUP III Exercise Group	1	1	1	1	1	1,1,1,1,1	1
	1	1	2	2	2	2,2,2,2,2	2
	1	1	1	1	2	2,2,2,1,1	2
	1	1	2	3	3	2,2,2,2,2	2
	1	1	2	1	1	2,1,1,1,1	1
GROUP IV Anxiety plus Exercise Group	1	2	2	2	2	2,2,2,2,2	2
	1	1	1	2	2	2,2,2,2,2	2
	1	2	1	2	1	2,2,2,2,1	2
	1	1	1	1	2	2,2,1,1,1	1
	1	2	2	2	2	2,2,2,2,2	2

Heart-damage Rating Scale: 1 = Normal Heart; 2 = Slight Damage

(21) and Heusner (16) who found extensive heart damage in animals forced to exercise for a period of 10 weeks.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This study investigated the single and combined effects of forced-exercise and anxiety-producing electrical stress on the incidence and severity of myocardial damage in young adult male albino rats. The forced-exercise program was designed as mild exercise with only 35 minutes of medium-duration, moderate-intensity activity. The anxiety-producing electrical stress consisted of electrical shocks (60 volts, 15ma) received every 15 seconds on average, over a two-hour period.

Twenty 110-day-old male albino Sprague Dawley rats were randomly assigned to a single housing condition and one of four treatments. The animals in the control group received no experimental treatment but were placed in the same housing as those of the other groups prior to and during treatment. The anxiety group received sporadic electrical shock for two hours each day. The animals in the forced-exercise group were required to run a medium-endurance type program in controlled-running wheels for 35 minutes each day. The anxiety plus forced-exercise

group received the sporadic electrical shock for two hours and then were forced to exercise in the controlled-running wheels for 35 minutes each day.

The animals were housed in sedentary metabolism cages with the animal quarters lighted 24 hours a day. The treatment program was conducted for four hours each day, seven days a week for a period of 18 days.

At the termination of the study, the animals were sacrificed by decapitation and the hearts were removed for later examination. The muscle was quick-frozen, sectioned and stained, prior to being rated subjectively for heart-damage under a light-microscope.

The Fisher exact probability test for small samples was used to statistically analyze the data. Significance was found between the heart-damage ratings of the control and combined-treatment animals ($P \leq .10$). The incidence of heart-damage in the other two groups was not found to be significant.

Conclusions

The results show that the single and combined effects of forced-exercise and anxiety-producing stress, as used in this study, may be of a sufficiently stressful nature to produce histologically detectable damage in the myocardium of young male adult albino rats. More specific conclusions cannot be drawn from this investigation since so few animals

were used in each group and the experimental period was of such short duration.

Recommendations

Further study should be carried out over a longer period of time, with more animals in each group.

A histochemical technique such as the demonstration of Succinic Dehydrogenase (SDH) activity should be used to help distinguish myocardial damage from connective tissue.

BIBLIOGRAPHY

BIBLIOGRAPHY

Articles

1. Bajusz, E., and H. Selye. Adaptation to the cardiac necrosis-eleciting effect of stress. Am. J. Physiol., 199:453, 1960.
2. Bajusz, E., and G. Jasmin. Histochemical studies on the myocardium following experimental interference with coronary circulation in the rat. Acta Histochem., 18:222, 1964.
3. Bajusz, E. Opening remarks, conference on experimental "metabolite" cardiopathies and their relationship to human heart disease. Ann. N.Y. Acad. Sci., 156, 1969.
4. Bajusz, E. Concluding remarks, conference on experimental "metabolic" cardiopathies and their relationship to human heart disease. Ann. N.Y. Acad. Sci., 156:620, 1969.
5. Brigden, W. Uncommon myocardial diseases. The non-coronary cardiomyopathies. Lancet, ii:1179, 1957.
6. Brunner, D. The influence of physical activity on incidence and prognosis of ischemic heart disease. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
7. Bryant, R. E., W. A. Thomas, and R. M. O'Neal. An electron microscopic study of myocardial ischemia in the rat. Circulation Res., 6:699, 1958.
8. Caulfield, J., and B. Klionsky. Myocardial ischemia and early infarction: An electron microscopic study. Amer. J. Path., 35:489, 1959.
9. Cureton, T. K. The relative value of various exercise programs to protect adult human subjects from degenerative heart disease. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.

10. Dawber, T. R., W. B. Kannel, and G. D. Friedman. Vital capacity, physical activity and coronary heart disease. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
11. Euler, U. S. V. Quantitation of stress by catecholamine analysis. Clin. Pharmacol. Ther., 5:398, 1964.
12. Fine, G., A. Merules, and J. R. Scarpella. Experimental myocardial infarction. Archives. Path., 82:4, 1966.
13. Fox, S. M., and J. S. Skinner. Physical activity and cardiovascular health. Am. J. Cardiol., 14:731, 1964.
14. Frick, M. D. Coronary implications of hemodynamic changes caused by physical training. Am. J. Cardiol., 22:417, 1968.
15. Gottheiner, V. Long-range strenuous sports training for cardiac reconditioning and rehabilitation. Am. J. Cardiol., 22:426, 1968.
16. Heusner, W. W. Anxiety, activity and genesis of heart disease. N.I.H. Project (in preparation).
17. Holloszy, J. O., A. J. Barry, J. S. Skinner, and T. K. Cureton. Effects of physical conditioning on cardiovascular function. A ballistographic study. Am. J. Cardiol. 14:761, 1964.
18. Holloszy, J. O., J. S. Skinner, G. Toro, and T. K. Cureton. Effects of a six-month program of endurance exercise on serum lipids of middle-aged men. Am. J. Cardiol., 14:753, 1964.
19. Hudson, R. E. B. The cardiopathies: Order from chaos. Am. J. Cardiol., 25:70, 1970.
20. Jogle, E., and J. B. Wells. Exercise, training and cardiac stroke force. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
21. Kitamura, K. The role of sports activities in the prevention of cardiovascular malfunction. In K. Kato (ed.), Proc. Int. Cong. Sports Science. Tokyo, Japan: Union of Sports Sciences, 1964.

22. Kraus, H. Preventive aspects of physical fitness. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
23. Letunov, S. P., and R. E. Motylyanskaya. Preventive significance of exercise training in elderly adults. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
24. Levi, L. Life stress and urinary excretion of adrenaline and noradrenaline. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
25. Mellerowicz, H. The effect of training on heart and its importance in preventive cardiology. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
26. Mendlowitz, M., R. C. Wolfe, and S. E. Gitlow. Catecholamine metabolism in essential hypertension. Am. Heart. J., 79(3):401, 1970.
27. Merules, A. R., and G. Fine. Early human myocardial infarction. Arch. of Path., 82:9, 1966.
28. Naughton, J., B. Balke, and F. Nagle. Refinements in method of evaluation and physical conditioning before and after myocardial infarction. Am. J. Cardiol., 14:837, 1964.
29. Naughton, J. Peak oxygen intake during physical fitness programs for middle-aged men. J.A.M.A., 196:899, 1965.
30. Naughton, J. Cardiopulmonary responses during physical training in patients who have recovered from myocardial infarction. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
31. Niles, N. R., L. Bitensky, J. Chayen, M. V. Brainbridge, and G. J. Cunningham. The value of histochemistry in the analysis of myocardial dysfunction. Lancet, 1:963, 1964.
32. Niles, N. R., J. D. Zarvin, and R. N. Morikado. Histochemical study of effects of hypoxia and isoproterenol on rat myocardium. Am. J. Cardiol., 22:381, 1968.

33. Pearse, A. G. E. In G. E. Wolstenholme and M. O'Connor (eds.), Cardiomyopathies. London: Churchill Co., 1964.
34. Pyorala, K., M. J. Karvonen, P. Taskinen, J. Takkunen, J. Kyronsezzsa, and P. Peltokallio. Cardiovascular studies on former athletes. Am. J. Cardiol., 20:191, 1967.
35. Raab, W. Key position of catecholamines in functional and degenerative cardiovascular pathology. Am. J. Cardiol., 5:571, 1960.
36. Raab, W., Pde Paula e Silva, H. Marchet, E. Kimura, and Y. K. Starcheska. Cardiac adrenergic preponderance due to lack of physical exercise and its pathological implications. Am. J. Cardiol., 5:300, 1960.
37. Raab, W., and E. Bajusz. Epinephrine-induced, early pre-necrotic disturbances of myocardial potassium, glycogen, and phosphorylase. Circulation, 32: (suppl. II) 174, 1965.
38. Raab, W., and H. J. Krzyanek. Cardiac sympathetic tone and stress response related to personality patterns and exercise habits. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
39. Raab, W. Pathophysiological fundamentals of the origin and prevention of degenerative heart idesease. Ann. N.Y. Acad. Sci., 156:281, 1969.
40. Richardson, J. A. Plasma catecholamines in angina pectoris and myocardial infarction. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas, 1966.
41. Schimert, G. C., and H. Schwalb. Functional and metabolic factors in the origin and prevention of myocardial ischemia. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
42. Selye, H., and E. Bajusz. Stress and cardiac infarcts--effects of sodium deficiency upon necrotizing cardiopathies produced by various agents. Angiology, 10:412, 1959.

43. Selye, H., and E. Bajusz. Sensitization by potassium deficiency for the production of myocardial necrosis by stress. Am. J. Path., 35:525, 1959.
44. Selye, H., E. Bajusz, S. Grasso, and P. Mendell. Simple techniques for the occlusion of coronary vessels in the rat. Angiology, 11:398, 1960.
45. Selye, H. The role of stress in the production of experimental cardiopathies. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
46. Selye, H. The pleuricausal cardiopathies. Ann. N.Y. Acad. Sci., 156:195, 1969.
47. Skinner, J. S., J. O. Holloszy, and T. K. Cureton. Effects of a program of endurance exercise on physical work capacity and anthropometric measurements on fifteen middle-aged men. Am. J. Cardiol., 14:747, 1964.
48. Starcich, R. Plasma catecholamine and urinary vanillylmandelic acid in clinical ischemic heart disease. In W. Raab (ed.), Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
49. Valori, C., M. Thomas, and J. P. Shillingford. Urinary excretion of free noradrenaline and adrenaline following acute myocardial infarction. Lancet, 1:127, 1967.
50. Wallace, A. G. Catecholamine metabolism in patients with acute myocardial infarction. In Julian and Oliver (eds.), Acute Myocardial Infarction. Baltimore: The Williams and Wilkins Company, 1968.
51. Wartman, W. B. The definition of myocardial infarction. In James and Keyes (eds.), Henry Ford Symposium, The Etiology of Myocardial Infarction. Boston: Little, Brown and Co., 1963.
52. Wartman, W. B. Cardiomyopathies and myocardial degeneration: Problems in experimental and clinical pathology. Ann. N.Y. Acad. Sci., 156:7, 1969.
53. Yokoyama, H. O., R. B. Jennings, G. F. Clabaugh, and W. B. Wartman. Histochemical studies of early experimental myocardial infarction. A.M.A. Arch. Path., 59:347, 1955.

Books

54. Karvonen, M. J., and Barry, A. J. Physical Activity and the Heart. Springfield: Charles C. Thomas Co., 1967.
55. Raab, W., ed. Prevention of Ischemic Heart Disease. Springfield: Charles C. Thomas Co., 1966.
56. Selye, H. The Pleuricausal Cardiopathies. Springfield: Charles C. Thomas Co., 1961.

APPENDICES

APPENDIX A

BODY AND ORGAN WEIGHT DATA

TABLE A-1.--Body and organ weight data (in grams).

Group	Body Weight		Adrenals Weight	Spleen Weight	Heart Weight	Kidney Weight	Testes Weight	Kidney Weight
	Before	After						
GROUP I Controls	324	332	.0535	.6571	1.0420	2.6040	3.2368	12.3733
	330	346	.0469	.6210	1.0311	2.2721	2.7549	10.8328
	384	402	.0508	.6453	1.1390	2.7478	3.5907	13.1625
	354	376	.0537	.6938	1.2405	2.8079	3.5658	12.1961
	350	356	.0556	.6450	1.1341	2.6333	3.0740	11.5952
GROUP II Anxiety Group	368	346	.0563	.7767	1.1900	2.8630	4.0912	12.6227
	374	352	.0492	.6018	1.2500	2.7076	3.6816	12.5883
	366	370	.0675	.6957	1.1748	2.8815	3.9040	12.3737
	370	350	.0505	.5633	1.1394	2.5695	2.9624	11.8352
	324	304	.0486	.5552	0.9941	2.3409	3.4154	9.5453
GROUP III Forced- exercise Group	332	322	.0614	.5700	1.2189	2.4760	3.4816	11.6570
	336	316	.0520	.6081	1.0900	2.4325	3.0794	10.5000
	378	350	.0787	.6799	1.2050	2.6384	3.6067	12.7177
	310	306	.0535	.5270	1.0714	2.4331	2.9830	10.3194
	312	296	.0584	.4787	1.0760	2.4278	3.1380	10.7754
GROUP IV Forced- exercise plus Anxiety Group	334	326	.0578	.6122	1.2018	2.6227	3.6600	12.0265
	366	358	.0779	.7300	1.3371	2.9750	4.3969	13.9673
	334	308	.0625	.4945	1.1063	2.5345	3.6220	11.1623
	350	338	.0761	.7430	1.4130	2.7439	3.7914	11.9478
	358	368	.0826	.6938	1.3839	3.2217	3.3778	12.5839



MICHIGAN STATE UNIVERSITY LIBRARIES



3 1293 03177 3447