THE EFFECT OF PHYSICAL EXERCISE AND ELECTRICAL STRESS ON THE PRODUCTION OF EXPERIMENTAL MYOCARDIAL NECROSES

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This is to certify that the

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ABSTRACT

THE EFFECT OF PHYSICAL EXERCISE AND ELECTRICAL SHOCK ON THE PRODUCTION OF EXPERIMENTAL MYOCARDIAL NECROSIS

By

Richard D. Bell

Cardiovascular disorders are largely responsible for the high premature mortality rates associated with modern day life. More specifically, coronary heart disease is being increasingly recognized as a predominant threat to individuals in highly industrialized societies such as exist in North America. Among the explanations offered for this phenomenon are lack of physical activity and cholesterol rich diets. Occupational stresses unique to these industrialized societies also are thought to play a significant role in the increasing incidence of coronary heart disease. Emotional, sensory, and many other stresses are accompanied by an augmented secretion of adreno-corticoid hormones, and the sensitizing action of these hormones may play an important role in the production of myocardial damage. The danger of increased serum catecholamine concentrations is

due mainly to the development of a discrepancy between the oxygen supply and the oxygen requirement of the cardiac muscle cells.

Physical activity was designed to play a dual role in the present experiment. It was hypothesized that physical activity might have a prophylactic effect on the production of myocardial damage. But when physical activity and anxiety treatments were administered concurrently, a double stressor situation was simulated. It was postulated that such a situation might result in severe myocardial damage. Thus, it was thought that a study which incorporated the effects of physical exercise, the effects of stress, and their cumulative effects on the production of myocardial damage would provide important information concerning the etiology of coronary heart disease.

One-hundred-six male albino Sprague-Dawley rats 60 days of age were randomly assigned to five treatment groups. These groups were: control, anxiety with no exercise, exercise during anxiety, exercise before anxiety, and exercise before and during anxiety.

The exercise treatment consisted of two one-half-hour swim periods seven days a week. A weight equal to two per cent of the body weight was attached to the tip of the rats tail during each exercise period.

The exercise during anxiety animals were subjected to a two-week exercise program, the exercise before anxiety

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animals received a five-week exercise program, and the exercise before and during anxiety animals were administered a seven-week exercise program.

The anxiety treatment consisted of a 0.36 second D.C. electrical shock at 1.5 milliamperes five times per minute nine hours a day for two weeks. The electrical shocks were administered randomly within each minute. All anxiety treatments began when the animals were 100 days of age.

Serum lactate dehydrogenase (LDH) levels were determined twice for each animal during the experiment since serum LDH levels have been frequently used as indicators of myocardial damage. Serum LDH levels were used to reduce the original sample size to 69 animals before sacrifice.

All animals were sacrificed at 116 days of age by an overdose of ether. Immediately after sacrifice, the heart was excised, trimmed, and cut in three equal sections. The apical and basal sections were quick frozen in isopentane cooled with liquid nitrogen while the middle section was fixed in ten per cent formalin for 48 hours. At least two serial cross sections from the inner surface of each frozen block of myocardial tissue were taken and stained with a hematoxylin-eosin (H and E) stain and the histochemical enzymes mono-amine oxidase (MAO), succinic acid dehydrogenase (SDH), and beta-hydroxy butyrate dehydrogenase

(B-OHD). The formalin-fixed tissue block was stained via a paraffin-embedded H and E stain. All histologic slides were subjectively analyzed for myocardial damage on the basis of an arbitrary one to five scale. A rating of one meant no myocardial damage while a rating of five indicated severe myocardial damage.

A Chi-square contingency statistical analysis was employed to determine differences in heart damage for the various treatment groups as well as for the relationship between serum LDH levels and the degree of myocardial damage. No statistically significant differences were found for any of the above-mentioned statistical analyse. It was concluded that neither physical exercise nor electrical shock, as administered in this experiment, had any significant effect on the production of experimental myocardial necroses.

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DEDICATION

To my wife Caryl and son Richard. Also to Dr. V. Reggie Edgerton for his interest and guidance during my graduate program.

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CHAPTER I

THE PROBLEM

Introduction

Cardiovascular disorders have increasingly dominated the mortality statistics of modern day life. In 1962 54.5 per cent of all deaths in North America were due to cardiovascular accidents (193). Furthermore, the per cent of cardiovascular deaths, compared to other causes, has been increasing since 1900. Within this picture, coronary heart disease is regarded as a major threat to the life of most adults in highly industrialized societies. Many explanations have been offered regarding this phenomena, but low levels of physical activity (123, 124, 125), socio-economic stresses (54, 106, 135, 137, 141, 148), and cholesterol-rich diets (93, 94) are among the more prominent ones. As long as man had to use his muscles to provide for his daily needs in primitive surroundings, a minimum level of physical fitness was automatically guaranteed by his mode of life. This condition has, of course, changed markedly in our mechanized, overstimulated, and sedentary society. Often such modern modes of life do not provide enough physical exercise to keep the muscles strong and flexible and to keep the cardiovascular system

sufficiently exercised to maintain a minimum degree of physical fitness (102).

Many authorities believe that increased levels of physical activity promise to be a major preventative for coronary heart disease. The role of exercise in preventing or modifying the course of coronary heart disease has been, and still is, the subject of much scientific discussion; but, the evidence accumulated to date is still inadequate to warrant unreserved support.

Exercise also may act as a specific stressor agent in the production of myocardial damage (176). In fact, the present experiment was designed to take advantage of this situation. It was thought that physical exercise and conditions of anxiety acting concurrently might effectively simulate a double-stressor situation which, theoretically, should produce severe myocardial damage in those animals receiving such treatment.

Ever increasing occupational stresses also play a dominant role in the high incidence of coronary heart disease. Western societies in particular are fraught with stresses which are unique in industrialized societies (164).

Emotional, sensory, and many other stresses are accompanied by an augmented secretion of adreno-corticoid hormones, and the sensitizing action of these hormones may play an important role in the production of myocardial damage (138). The danger of increased serum catechola-

mine concentrations probably is due mainly to the development of a discrepancy between the oxygen supply and the oxygen requirement of the cardiac muscle cells.

Any study of heart disease also must mention the effects of serum cholesterol and high fat diets on the production of myocardial damage. Severe atherosclerosis with its effect on heart disease is closely related to the mean serum cholesterol concentration (70, 94, 95), since high fat diets and increased serum cholesterol concentrations are thought to sensitize the heart for the subsequent production of myocardial damage (176). However, neither diet nor serum cholesterol concentrations were part of the present study.

Purpose

This study was undertaken to determine the effects of various combinations of physical exercise and electrical stress on the production of experimental myocardial damage in male albino rats.

Electrical shock was utilized to simulate conditions of socio-economic stress. When administered without prior conditioning it was thought that the stress of electrical shock might result in moderate to severe myocardial damage. Physical exercise (swimming) was included in the present experiment for two reasons. It was postulated that the main effect of forced physical exercise might be prophylactic in that it would condition the heart against the

production of myocardial damage. But, when electrical shock and forced physical exercise were introduced concurrently, without prior conditioning, it was thought that they would act as a double stressor. It was hypothesized that this situation might result in severe myocardial damage.

Limitations of the Study

1. Exercise programs which involve swimming white rats are limited by lack of control of the intensity of muscular activity. The chief factor in the exercise program which can be controlled is the duration of activity.
2. The exercise results of this investigation are based on sixty minutes per day of swimming and cannot be extrapolated to other forms or durations of muscular exercise.
3. The histochemical methods used for determining specific levels of myocardial damage are quantitatively limited.
4. There was no quantitative control over diet as the animals received food and water ad libitum.
5. The results of this experiment cannot be interpreted as being directly applicable to the human species.

Definition of Terms

1. Ischemic Heart Disease

Ischemic heart disease refers to a group of myocardial disorders characterized by the presence of angina pectoris and/or myocardial infarction. This term is often used to mean coronary heart disease as well, although some authors (78) say coronary heart disease should be used only in the strict anatomical sense and ischemic heart disease to specify a level of functional impairment (78).

2. Cardiomyopathy

Cardiomyopathies are an obscure heterogeneous group of cardiac disorders whose only common feature is that the myocardium is in a state of disorder. They may be pathologically classified as genetic, inflammatory, metabolic, or vascular, or clinically classified as destructive, constrictive, or congestive (1).

3. Stress

Stress, in a biological sense, is the state manifested by a specific syndrome which consists of all non-specifically induced changes within a biological system. Stress is a condition, a state, which manifests itself by measurable changes in the body's organs. Another simple definition of stress states that stress is the sum of all non-specific changes caused by function or damage (184).

4. Stressor

A stressor is that which causes stress. It is a stress-inducing agent.

5. Necrosis

A necrotic area of myocardial tissue is characterized by a depletion or lack of enzyme activity. Should the conditions causing this necrosis (eg. obstruction of a coronary artery) persist, the necrotic area may then be further characterized by the irreversible development of scar tissue.

6. Infarct

An infarct refers to tissue necrosis induced by interference with the blood supply of a particular tissue such as the myocardium. It is usually caused by acute and complete occlusion of an artery (176).

CHAPTER II

REVIEW OF THE RELATED LITERATURE

Introduction

The causes of myocardial damage have been of interest to medical researches for a great many years. Recently, due to the alarming increase in mortality as a result of "cardiovascular accidents," other related fields, such as physical education, have instigated pertinent studies in the field of cardiovascular disease from their own point of view. This has served the purpose of introducing other possible causal factors into the total picture of cardiovascular disease. At present, when discussing cardiovascular disease, it is necessary to consider such diverse factors as circulating catecholamine concentrations, serum cholesterol and triglyceride levels, degree of nervous tension, amount of physical activity, and genetic background of the individual concerned. In order to give the reader an adequate background of the present study, some of the above factors will each be reviewed separately.

Catecholamine Concentrations

Early investigators have described myocardial necrosis in the absence of coronary artery disease. Reference is

made to an article by Josué (87) who demonstrated myocardial lesions produced by catecholamines as early as 1907. Throughout the succeeding years, others have produced similar experimental evidence to confirm this phenomena (7, 160, 176). Starcich (194) outlined several elementary facts concerning the involvement of neuro-hormonal activity in the pathogenesis of acute coronary insufficiency and resulting myocardial hypoxia. First, sympathetic stimulation and the resulting liberated adrenosympathogenic catecholamines such as sympathetic neurogenic norepinephrine and adrenomedullary epinephrine cause a marked augmentation of myocardial oxygen consumption. Secondly, the availability of adequate amounts of oxygen to the myocardial tissue depends simultaneously on the vascular oxygen supply which is unevenly distributed in different areas of the cardiac muscle and on the degree of myocardial oxygen consumption which varies with the degree of sympathetic tone and neuro-hormonal activity (146). Thirdly, the myocardial hypoxia-producing effects of sympathetic stimulation and catecholamine action are greatly intensified when the compensatory coronary dilation which normally accompanies augmented cardiac oxygen consumption is impaired by experimental coronary restriction or by atherosclerosis (3, 51). Finally, no clear relationship exists between the degree of existing atherosclerotic coronary vascular lesions on the one hand and the occurrence of clinical manifestations of myocardial ischemia on the

other except in cases of severe coronary stenosis or occlusion. It is no longer possible to ascribe myocardial ischemic structural changes to vascular factors alone without consideration of those contributory non-vascular mechanisms which interfere in myocardial oxygen metabolism (127). Raab (138) states that some forms of human recrotizing cardiomyopathies may be directly attributable to the reflex liberation of catecholamines which, under certain circumstances, exert a contributory noxious effect on cardiac metabolism. The potential danger of catecholamine overactivity to the functional and structural integrity of the heart muscle may be attributed largely to the development of a discrepancy between the vascular oxygen supply and the oxygen requirement of the cardiac muscle cells. For many years nothing definite was known concerning the precise mechanism through which the catecholamines produced myocardial damage, except for the hypoxic effect of these amines on the cardiac muscle. However, recent comparative histochemical studies, such as the one by Bajusz and Jasmin (10), show that early declines in myocardial phosphorylase activity and in the amount of stainable, labile fraction of glycogen are sensitive indices of anoxic myocardial damage and that the behavior of phosphorylase and glycogen during the development of anoxic cardiac lesions is different from that seen during the development of the "metabolic" type of lesion. Differences were also established in the

reaction of other enzymes such as cytochrome oxidase (CyT.O) and succinic dehydrogenase (SDH) which implies that different histochemical techniques might be applicable for purposes of differentiating between anoxic and metabolic (produced by dietary means or by administration of metabolic inhibitors and other cardio-toxic compounds) cardiomyopathies (10).

Bajusz and Raab (8), using white rats, injected epinephrine subcutaneously in a single dose of 450 ug/100 grams of body weight. The animals were sacrificed at various intervals ranging from five minutes to ninety-six hours after injection. In the hearts affected by epinephrine, a decline or even a complete loss of phosphorylase activity was observed in some endocardial regions as early as ten to fifteen minutes following the injection of epinephrine. Furthermore, a depletion of the stainable glycogen reserves and a disturbance in the normal distribution of potassium also were distinguishable ten to fifteen minutes following injection. The foci showing loss of phosphorylase activity and a parallel depletion of glycogen reserves and potassium content were spotty and mainly located in the sub-endocardium and the apex. A second portion of the experimental series showed that a brief sensitization with fluorocortisol proved extremely potent in enhancing the susceptibility of the heart muscle to the potentially cardiotoxic action of subsequently injected epinephrine. This

combination of agents resulted in the development of large areas completely devoid of phosphorylase, potassium, and glycogen in one hundred per cent of the hearts studied. The greatly enhanced potassium depleting action of epinephrine, after brief sensitization with a corticoid hormone such as fluorocortisol, may be of clinical significance. Emotional, sensory, and many other stresses are accompanied by an augmented secretion of adrenocorticoid hormones (138), and the sensitizing action of these hormones may play an important contributory role in catecholamine-induced myocardial injury.

In a similar type of experiment, Shimamoto (172) injected twenty-six male and female rabbits with one ug/kg. of body weight of epinephrine. Myocardial samples were taken at three, five, fifteen, thirty, and sixty minutes post injection. Specimens sampled thirty minutes after the injection of epinephrine exhibited a definite change in myocardial structure, showing an intracellular edema of the cardiac muscle cells. More specifically, these changes consisted of a definite expansion of the vesicles of the longitudinal system of the sarcoplasmic reticulum, an increase of the intracellular clear spaces around the mitochondria and between the myofibrils, and an appearance of dense bodies in the mitochondria. An analogous expansion of the longitudinal system, an increase of the clear spaces around the mitochondria and myofibrils of the cardiac muscle

cells, and an alteration of mitochondria have also been observed in the early stages of anoxia induced by coronary ligation (28). Vendenyeyeva (205) has also reported producing myocardial necroses by stimulating sympathetic nerve trunks as well as by the injection of epinephrine and norepinephrine.

The catecholamine isoproterenol can also produce early myocardial pathology without detectable alterations of the mitochondria (57). However, during conditions of ischemia the mitochondria undergo severe changes in a comparatively short time. A basic principle postulated by Raab (139), concerning the effects of released catecholamines on the myocardial muscle, is that central stimulation of the sympathetic nervous system exposes the heart to an intensified, potentially hypoxiating action of epinephrine and norepinephrine which can produce necroses in the myocardium. It is possible that the cardiotoxicity of various agents depends upon the sudden liberation in the heart of some metabolite that is toxic only in the presence of conditioning factors and of specifically cardiotoxic agents (154). If this is true, cardiac damage could be prevented by the stress-induced discharge of this metabolite prior to exposure to sensitizing agents. In fact, Raab (140) has shown that antiadrenergic drugs protect rats from stress-induced myocardial necroses.

Regan et al.(156) infused l-epinephrine into the left coronary artery of male mongrel dogs and observed the metabolic and hemodynamic effects of sympathetic stimulation. The fact that the infused epinephrine stimulated glycogenolysis was evidenced by a twenty per cent decrease in the left ventricular glycogen content after fifteen minutes of infusion. There was also a simultaneous decrease in the extraction of oxygen by the myocardium. Coronary blood flow was not significantly altered, but the extraction of oxygen by the myocardium was reduced. In addition, the extraction of potassium and phosphate was significantly altered by epinephrine infusion. This pattern of ion and enzyme loss from the myocardium was the same as occurs after destruction of the left coronary artery.

Since total coronary blood flow was not significantly decreased, an increase in cardiac activity which was not met by an adequate oxygen supply increase may have been a possible cause of the necrosis observed. In fact, in this particular experiment (156), all measured parameters of left ventricular activity such as stroke output and minute work showed increases of approximately sixty per cent while coronary blood flow increased only fifteen per cent, and oxygen extraction decreased.

Richardson (158) agrees that the potentially pathogenic, hypoxiating and cardiotoxic properties of the catecholamines have been known for a long time. Furthermore,

it was recently discovered that even small doses of catecholamines and electrical or reflectory stimulations of the cardiac sympathetic nerves are apt to elicit severe hypoxic and necrotizing manifestations in the heart muscle under certain accessory conditions (158). Examples of such conditions would be atherosclerotic restrictions of the normal compensatory coronary dilatability or the metabolically sensitizing influence of administered adrenal mineralocorticoids. Richardson further suggests that metabolically and structurally deranged hearts lose their ability for normal production and/or accumulation of neurogenic and blood-borne norepinephrine. He concludes that there is a tendency of patients suffering from angina pectoris to discharge abnormally large quantities of catecholamines into the circulation during physical effort and under emotional stress. Furthermore, individuals with coronary atherosclerosis exhibit an exaggerated sensitivity to the myocardial hypoxiating and pain-producing effect of administered and reflex-discharged catecholamines.

Animal experiments (141, 148) suggest that intensive emotional stimuli per se are capable of eliciting myocardial necroses. Both emotional tensions and habitual inactivity share, as their common denominator, a demonstrable augmentation of sympathetic adrenergic activity (149). In the case of emotional tensions, this is presumably due to exaggerated hypothalamic stimulation, in the case of physical

inactivity, to a deterioration of sympathoinhibitory inotropic and vagal chronotropic antiadrenergic counterregulatory mechanisms at rest. It is also pointed out (149) that another contributory element in hypoxic (ischemic) heart disease, tobacco smoking, is likewise associated with adrenergic overactivity caused by nicotine-induced ganglionic stimulation. The long-known myocardial oxygen "wasting" and potentially anoxiating cardiotoxic properties of the adrenosympathogenic catecholamines are being increasingly appreciated with respect to their clinical pathogenic significance (200, 170).

Schimert and Schwalb (173) agree that the concept, proposed by those investigators (138, 156, 194) who have stressed an unduly augmented oxygen demand in the myocardium as a possible accessory functional factor in ischemic heart disease, is being increasingly appreciated. The oxygen requirements of the heart depend on the amount of work performed by the heart muscle and on the specific neurohumoral metabolic influences under which this work takes place. Physical stress, by way of neurohumoral influences which cause a potentially uneconomical mode of action of the heart, also reduces the "coronary reserve quotient" (where a quotient below one is equivalent with myocardial ischemia) unless coronary dilation compensates for the increased oxygen demand of the myocardium. An augmentation of myocardial oxygen consumption is caused by adrenergic

neurohumoral influences even without alterations of the workload and the aortic pressure. As long as the coronary arteries are intact and normally dilatable, this fact is not of any major pathogenic importance, since any increase in myocardial oxygen consumption is promptly followed by an adequate dilation of the coronary arteries. Nevertheless, it has been shown by numerous investigators (137, 156, 139) that repeated injections of epinephrine, along with stress-induced intensive stimulations of the sympathetic nervous system and the hypothalamus, produce myocardial necroses even in healthy animals. If the coronary vessels are narrow, a rising sympathetic tone will endanger particularly the subendocardium by causing a lack of local oxygenation. In contrast to the effect of the sympathogenic catecholamines, sympathetic inhibition and vagal stimulation exert an opposite oxygen preserving effect which enables the heart to work with a lesser oxygen consumption. Poor vascularization of the muscles, inadequate coordination of muscular action and of blood distribution during exercise, and a reduced effectiveness of muscular effort cause an augmented oxygen requirement of the body as a whole and thus impose an additional strain on the heart to which it responds primarily with an acceleration of the heart rate. The most impressive, and clinically most important, augmentation of coronary reserve is achieved by physiological means, namely by physical training. It is known that

systematic physical training, or any form of persistent and vigorous muscular activity, reduces the cardiac sympathetic tone and excitability and raises the vagal tone. This is manifested by a slow heart rate, a decrease of cardiac output, and a lowering of the systolic blood pressure at rest. Other experimental data (52) suggest that, in the presence of stenotic coronary arteries, the development and dilation of preformed collaterals is favored by physical exercise.

Serum Cholesterol, Serum Triglycerides, and Free Fatty Acids

In recent years, experiments have shown the important role of fats as a source of energy for skeletal muscle (99). The previous concept that carboyhydrates were the main source of energy has been changed; and we know now that fats, in the form of free fatty acids, are released from the triglycerides of depot fat and then transported through the circulatory system to the muscles where they are split via beta oxidation to meet the energy requirements of the organism (99).

One of the significant findings of Konttinen's experiment on twenty-six healthy army recruits was the marked rise of plasma free fatty acids during the course of exercise: the resting level was found to be 390 u Eq./L. while at the end of three hours of exercise the plasma free fatty acid concentration was found to be 1230 u Eq./L. (100). This

pronounced rise of plasma free fatty acids occurred without exception in all men who underwent exercise, whether they had fasted or eaten meals consisting of fats or carbohyrates.

It is thought that one of the mechanisms by which free fatty acids are released is mediated through the catecholamines, as both epinephrine and norepinephrine are released from the tissues during exercise and both cause a prompt increase in plasma free fatty acids. This increase enhances the hydrolysis of neutral fats within the body tissues. The triglyceridemia evoked by the ingestion of fats was cleared from the circulation of active men more rapidly than in the case of sedentary men. It was concluded that, during prolonged exercise, energy transport in the form of plasma free fatty acids greatly exceeded the utilization of these compounds (35, 36).

Nikkilä and Torsti (129) agree with Carlson (34) that the energy needs of working muscles--both myocardial and skeletal--are mainly covered by fatty acids. Because the immediately available mass of body nonesterified fatty acids is quite small, these must be cleaved and mobilized from the different triglyceride or phospholipid pools. While some of the fatty acids may be derived from the muscles' own lipid esters, from the adjacent tissue triglycerides or from plasma triglycerides, it is apparent that the ultimate main source must be the triglyceride stores of distant tissue (129). But what about the mechanism of fatty acid

mobilization? It might be assumed that the steady state existing during rest is disturbed by the contracting muscle increasing liberation of transcellular and intracellular free fatty acid carriers. This changed free fatty acid gradient across the muscle cell membrane, together with the increased blood flow, leads to an accelerated efflux of free fatty acids from the plasma. When the systemic arterial plasma free fatty acid level is adequately decreased, a similar mechanism begins to operate in the opposite direction at and within the adipose tissue fat cell; and ultimately this results in an increased rate of triglyceride breakdown and of free fatty acid influx into the blood. This concept is supported by the experiments of Havel et al. (80). However, on continued but quantitatively constant muscular work, the free fatty acids do not reach a steady state level. The initial fall in the plasma free fatty acid concentration is followed by a continuous increase up to values above the resting level. The rate of entry of free fatty acids into plasma constantly exceeds their removal rate. This would seem to indicate that the "muscular pump" of free fatty acid flow during exercise must induce in man some additional stimulatory mechanism(s) for free fatty acid mobilization in excess of actual needs. An elevated blood catecholamine level is thought to play an important role.

Anitchkow (6) has expressed the opinion that myocardial infarctions are practically nonexistent without atherosclerosis of the coronary arteries. It is true that, according to statistical data, myocardial infarctions have occurred in the overwhelming majority of instances in the presence of advanced coronary atherosclerosis. However, the exclusive role of atherosclerosis in the origin of myocardial infarction is being increasingly questioned according to recent investigations. In fact, a growing number of investigators have attributed at least equal pathogenic significance to functional disturbances of the coronary circulation and/or to neurogenic and hormonal influences on myocardial and electrolyte metabolism. Necrotic foci of different sizes were observed in the myocardium of animals with normal coronary vessels as a result of central nervous and peripheral sympathetic stimulation, emotional stress, and injection of the oxygen-wasting sympathogenic catecholamines, epinephrine and norepinephrine (41).

Grande (70) states that it has been known for more than fifty years that atherosclerotic lesions can be produced in animals by feeding them on diets rich in fat and cholesterol. The frequencies of severe atherosclerosis and coronary heart disease among various populations are closely related to the mean serum cholesterol levels of the populations. Keys (93, 94) has shown that the incidence

rate of myocardial infarction is an exponential function of the "effective cholesterol level." This would seem to indicate a close relationship between serum cholesterol levels and the development of the atherosclerotic process. Thus, it would seem reasonable that reduction of the serum cholesterol concentration could result in a degree of prevention or delay in the development of the atheroslerotic process.

Present day opinion seems to make it clear that neither coronary thrombosis nor atheroslerosis per se are obligatory prerequisites for the development of myocardial infarctions. Coronary atherosclerosis must be regarded merely as an extremely important predisposing and contributory factor, but not as the exclusive pathogenic factor involved in the origin of myocardial infarction.

Kipshidze (96) concluded that, in the presence of predisposing coronary atherosclerosis, physical stress can serve as an important contributory factor in the pathogenesis of myocardial infarction and that in certain cases both coronary atherosclerosis and superimposed functional over-strain of the heart are jointly responsible for the occurrence of destructive myocardial lesions.

Lapiccirella (105) also agrees that views of a supposedly purely atherosclerotic origin of ischemic degenerative heart disease are no longer tenable. Myocardial necrotic foci and infarctions occur frequently in the complete absence of vascular occlusions, and coronary athero-

sclerosis is frequently found at autopsy in clinically and structurally normal hearts. It has become obvious that some accessory nonvascular factors must be involved. The super-imposition of these factors over widely varying degrees of coronary atherosclerosis would create the ultimately decisive pathogenic background for acute myocardial injury. Common experience has, for some time, suggested a causal role of emotional tensions, anxieties, and excitements in the origin of anginal pain as well as death.

Stamler et al.(191) are of the opinion that a coronary prevention program may be undertaken on a scientific basis due to the advances in knowledge concerning the etiology and pathogenesis of atherosclerotic coronary heart disease. Some of these advances pertinent to this study may be summarized as follows:

1. Severe atherosclerosis is the underlying pathogenic process in most cases of clinical atherosclerotic ischemic heart disease.

2. A several-fold increase in cholesterol--particularly esterfied cholesterol--is the biochemical hallmark of the atherosclerotic plaque.

3. The excess cholesterol in the plaque is derived from the cholesterol-bearing lipoproteins of the circulating plasma.

4. Sustained hypercholesterolemic hyperlipidemia is associated with frequent, premature, severe atherosclerotic coronary heart disease.

5. In groups of middle-aged patients with clinical coronary heart disease, higher mean serum cholesterol-lipid-B-lipoprotein levels are found than in matched control groups.

6. Sustained ingestion of diets containing increased quantities of cholesterol and fat is a virtual prerequisite for the production of significant atherosclerosis in a wide range of experimental animals.

7. The marked international differences in occurrence rates of premature coronary heart disease are due largely to socioeconomic factors (differences in living habits, principally dietary habits) and not to racial, ethnic, climatic, or geographic factors.

8. Where the mean serum cholesterol levels of populations are low, clinical coronary heart disease and severe coronary atherosclerosis at postmorten are rare, particularly in middle age.

9. High serum cholesterol levels in populations and high rates of middle-age clinical coronary heart

disease occur only where the habitual diets are high in calories, total fat, saturated fat, and cholesterol.

10. In populations studied prospectively, risk of premature atherosclerotic disease is increased in the presence of hypercholesterolemia-hyperlipidemia.

11. In populations with the nutritional-metabolic prerequisites for severe premature atherosclerotic disease, risk is also increased by hypertension, diabetes, overweight, cigarette smoking, and a positive family history of premature vascular disease. Physical inactivity and psychological stress are in all likelihood additional significant risk factors.

12. Atherosclerosis is, at least in part, a reversible disease.

13. The other major coronary risk factors--hypertension, diabetes, overweight, cigarette smoking, and physical inactivity--are amenable to control and correction by nutritional-hygienic-pharmacologic means.

The foregoing compel the conclusion, at least according to these authors (191), that diet is a key factor in the

etiology and pathogenesis of atherosclerotic coronary heart disease.

Skinner et al. (190) subjected fifteen middle-aged men to a six-times-per-week program of running and rhythmical calisthenics. During the latter weeks of the program, the exercises were considered to be quite intense. The results showed that exercise capacity, as expected, was significantly increased. Mean serum cholesterol and phospholipid levels did not change significantly with training, and individual changes in serum cholesterol appeared to be related to changes in diet and/or body weight. Serum triglycerides, however, decreased significantly from a pre-training level of 208 mg./100 ml to 125 mg./100 ml at the completion of six months of training. This reduction appeared to be an acute, short-term effect occurring within two to three hours post exercise and lasting only two days.

According to Carlson (34), it is possible that the inhibitory effect of nicotinic acid on the mobilization of free fatty acids from adipose tissue is the immediate cause of a plasma cholesterol-lowering effect. Approximately one-third to one-fourth of the free fatty acids mobilized from the adipose tissue are taken up in the liver. Normally, the major part of these free fatty acids taken up in the liver is oxidized, and the minor part is recirculated into the plasma coupled mainly to the triglyceride fatty acids of the plasma lipoproteins. These

lipoproteins also contain the plasma cholesterol. Some lines of evidence suggest that inhibition of the rate of mobilization of free fatty acids will decrease the amount of free fatty acids taken up in the liver. This, in turn, would reduce the recirculation of free fatty acids as plasma lipoproteins and hence decrease the concentration of cholesterol and triglycerides in the plasma.

Some investigators (95) have concluded that the serum triglyceride level is a more reliable indication of future susceptibility to ischemic heart disease than is the serum cholesterol level. However, in most major prospective epidemiological studies on ischemic heart disease, more importance has been attached to serum cholesterol levels (95). Rosenblatt (163) found that serum cholesterol was significantly higher in patients with coronary heart disease than in normal subjects, but the fasting serum triglyceride level was not altered. Carlson (37) concluded that elevated serum triglyceride levels are characteristic of ischemic heart disease in men under fifty years of age, but after this age elevation of the serum cholesterol levels is more prominent. Conversely, Katz et al. (90) have reported that hypercholesterolaemia is associated more with ischemic heart disease in men under fifty years of age than in men over fifty. Obviously, there is no clear agreement among investigators as to the relative importance of serum cholesterol or serum triglycerides in relation to ischemic heart disease.

McCabe et al. (116) concluded that the increasing occupational stresses unique to industrialized societies play a dominate role in the high incidence of coronary heart disease. However, it should always be kept in mind that the increase in coronary heart disease probably has other causes as well. A good example is a high standard of living involving too much rich food and too little physical activity. The fact that high-fat, high-carbohydrate diets sensitize the myocardium for the production of acute necroses by stress has been experimentally demonstrated by Selye (176).

Stress

It is well established (106) that conditions of stress are accompanied by reactions from the sympathoadrenomedullary system. If the stress if often repeated or long lasting, it may result in permanent and structural changes of pathogenic significance. The sympathoadrenomedullary system can be activated by a wide variety of stimuli (106, 54). More specifically, (138), a gross disturbance of the central nervous system causes a massive secretion of epinephrine from the adrenal glands which is absorbed by the heart muscle from the blood. It is probable that the centrally-induced accumulation of large concentrations of epinephrine in the heart stimulates augmented oxidative metabolic processes in the myocardium and that these hypoxiating metabolic changes in the myocardium,

alongside disorders in the coronary blood flow within the heart, give rise to dystrophic lesions of the myocardium. Present opinion indicates there are at least two mechanical factors which are common and important for the development of myocardial infarctions. These factors are atherosclerosis of the coronary arteries and coronary thromobosis.

Raab et al. (141) elicited myocardial necroses in sixty-nine per cent of a series of wild rats exposed to frightening noises such as a tape recording of a cat-rat fight. Myocardial necroses were also demonstrated in fifty per cent of flurocortisol pretreated white rats which had been submitted to prolonged frustrating situations. Civilized competitive living with its socioeconomic emotional tensions and stresses, sensory overstimulation, lack of physical activity, and abuse of nicotine combines several factors which increase sympathetic neurohormonal activity. The result is interference in myocardial metabolism both in a sustained fashion and with acute exacerbations (63, 114, 107).

Raab and Krzywanek (148) among others, state that many studies (59, 202, 207, 208) have demonstrated the significant contributory roles of socioeconomic emotional factors and lack of physical exercise in the high mortality rate from multicausal ischemic heart disease in industrialized nations.

Psychic stress is said (135) to be caused by rational

conflict of the "drive discharge" in which the "acting out" of problems is prevented. The suggested pathogenetic link occurs via neurohormonal pathways. Sympathetic over-activity with increased production of catecholamines appears to be a major factor causing myocardial oxygen wastage. It has also been shown that the cardiotoxicity of the adreno-sympathogenic catecholamines is greatly potentiated by adrenal corticoids. One might conclude that the civilized pattern of human behavior leads to conflict and stress. Because of rational control, situations of "emotion without action" occur frequently. The neurohormonal repercus-sions adversely affect the cardiovascular system and eventually cause or contribute to ischemic heart disease (135). Kraus (102) is of the same viewpoint. He argues that the lack of physical activity weakens the heart and skeletal muscles by disuse, but the overstimulation which is inherent in urbanized living keeps man in a state of almost constant alert from which there are few direct or even vicarious outlets. This inbalance in our lives--namely excesive stimulation combined with lack of exercise--is built into mechanized society. As a result, many indi-viduals live in a potentially pathogenic environment. Lack of physical exercise and overstimulation are combined in a constant suppression of the "fight or flight" response (32). This constant suppression of an otherwise normal reaction is an additional source of stress and, therefore,

in the long run a probable cause of disease. To make matters even worse, this source of stress is compounded by conditioned responses which make mere signals of irritation stressful.

Rosenman and Friedman (164) are among those investigators who are convinced that clinical coronary heart disease results from the interaction of multiple causal factors operating within the framework of time. Rosenman's original hypothesis was based on the question of whether the rising incidence of coronary heart disease among middle-aged American males might stem from some emotional interplay induced by the stresses imposed by our industrialized civilization acting in conjunction with high fat diets, diminished physical activity, relatively high serum lipids, and so forth. Stress, it is pointed out, has always been an integral part of life, but Western societies in particular are fraught with stresses which are not only restricted to industrialized groups but are uniquely new. To illustrate his point Rosenman (164) characterized a particular personality structure as Behavior Pattern Type A. Pattern A is characterized by certain personality traits including aggressiveness, ambition, drive, competitiveness, and a profound sense of time urgency. An individual with this particular behavior pattern possesses the above mentioned characteristics to an excessive degree. It was subsequently found that a population of male and female subjects

exhibiting Behavior Pattern A also exhibited a higher than normal prevalence of clinical coronary heart disease. Furthermore, the various physiological and biochemical mechanisms concerned with coronary atherogenesis are significantly altered by Behavior Pattern A in such a fashion that permanent alterations of the coronary vasculature can ensue.

According to Wolffe (212), situational stresses which the individual is unable to cope with are common environmental causes of various functional and organic forms of cardiovascular disease. In fact they constitute the predominating factor in many patients of the younger age groups suffering from ischemic heart disease. Wolffe refers to this as the "Nutcracker Syndrome." This term is used to denote a chain of events resulting from suppressed, crushing environmental circumstances from which the patient cannot extricate himself; and, the person with a vulnerable cardiovascular system may develop ischemic myocardial disease if the situational stresses are not relieved.

Russek (169) lists several factors in decreasing order of importance in the production of coronary heart disease. These factors are: occupational stress, diet, tobacco, heredity, obesity, and lack of physical exercise. Rosenman (167) states a similar case when he says that the occupational stresses unique to industrialized society play a significant role in the high incidence of clinical coronary

heart disease in such societies. However, he also agrees that other factors are of importance and should not be neglected. For example, habitual inactivity could also be a potent predisposing agent for myocardial necroses. Thus, the current trend toward an increasingly sedentary existence may be just as noxious as the occupational stresses just mentioned.

Selye's demonstration (176) of stress-induced myocardial necroses, without coronary artery lesions, provided an impetus for the study of nonvascular hormonal and neurohormonal metabolic elements in myocardial pathology. The well-known participation of potentially cardiotoxic adrenomedullary and sympathogenic catecholamines in the response of the autonomic nervous system to all stresses, makes the appearance of severe cardiac disturbances under emotional and other stresses intelligible. This applies particularly to the frequently occurring coincidence of stress-induced hypothalamic and sympathetic stimulations with pre-existing atherosclerotic limitations. Animal experimentation has, in fact, provided indirect evidence that the endocrine and autonomic nervous systems, especially through exaggerated liberation of cardiotoxic adrenomedullary and sympathogenic catecholamines, are fundamentally involved in the development of emotion-induced lesions of the cardiac muscle.

The relationship between occupation and the frequency and course of ischemic heart disease has received increasing

attention during the last several years (46, 27, 123, 203, 124, 125). Epidemiological studies have received a fair degree of attention; but, at best, they have provided suggestive, ambiguous results. In some instances the results have been contradictory.

Studies conducted on people living in Israeli kibbitzim have added to the growing volume of literature which agrees that ischemic heart disease is a multietiological disease and physical activity is only one important facet of the disease. Brunner (27) did find that the incidence of anginal pain, myocardial infarction, and mortality due to ischemic heart disease was two and one-half to four times higher in sedentary workers than non-sedentary workers.

Selye (175) says that the belief is common, not only among physicians but also among laymen, that sudden exposure to a particularly stressful experience may elicit a cardiac infarct, at least in predisposed individuals. Yet for various reasons, many cardiologists seriously doubt that stress plays any role in the pathogenesis of cardiovascular disease in general and acute cardiac necroses in particular (78). First of all, it is often impossible to identify a particularly stressful experience in the immediate past of a patient who died of cardiac infarction. Secondly, until quite recently, it has not been possible to produce any parallel of a cardiac infarct in experimental

animals by exposure to even lethal stress. Thirdly, there is considerable evidence in support of the view that certain stressful experiences such as exercise or cold baths for example can actually protect the heart against infarction (175). Under normal conditions, exposure to stress produces no serious cardiac damage in healthy young people. However, in unconditioned animals treated with certain electrolytes and certain steroids, such as corticoids in doses ineffective by themselves, subsequent exposure to stress invariably elicits massive infarct-like cardiac necroses. This electrolyte-steroid-induced cardiopathy, characterized by necrosis, fails to occur if the animals are exposed to stress prior to the electrolyte-steroid treatment. For example, rats pretreated with sodium acetate plus fluorocortisol for a few days, and then exposed to the stress of forced muscular exercise in a revolving drum, all died with massive infarctoid myocardial necroses within twenty-four hours after the exercise period. Another group of animals survived when similarly treated with sodium acetate and fluorocortisol but were forced to exercise both before and after the conditioning treatment was given (175). Many observations have been cited in support of the view that stress can elicit myocardial infarcts in man (5, 169, 208).

In an excellent publication, Selye (176) approaches the subject of cardiac disease from a different point of view. As the title The Pluricausal Cardiopathies suggests,

he has concerned himself with the experimental production of different types of cardiac lesions by various types of experimental procedures. According to Selye (176, p. 315) "many structurally distinct cardiopathies can be produced or prevented at will by varying combinations of electrolytes, steroids, and stress." Furthermore, "stress, depending upon circumstances, can both produce and prevent the same cardiopathy." However, as a rule electrolytes, corticoids, or stressors produce no consistent cardiac changes by themselves as only certain combinations of them are cardiotoxic. For example, necroses associated with simple stress-cardiopathy only develop after very severe exposure to stress alone and then occur only under exceptional circumstances.

One of the major experimental cardiopathies classified by Selye is the Electrolyte-Steroid-Cardiopathy with Hyalinization (ESCH). This cardiopathy is produced by combined treatment with the mineralocorticoids and certain sodium salts. Stress, apparently, is not necessary for production of cardiac lesions of this type. The ESCH cardiopath is characterized by the formation of hyaline deposits within the myocardium and the coronary arteries. The hyaline material appears partly in the disintegrating muscle fibers and partly in the stroma around the muscles. As this lesion becomes more chronic, the hyaline material is replaced by connective tissue.

Another major experimental cardiopath is the Electrolyte-Steroid-Cardiopathy with Necrosis (ESCH). This lesion is produced by the administration of sensitizing electrolytes (NaCl or Na_2HPO_4) and corticoids with or without subsequent exposure to stressors. The ESCN is characterized by large infarctoid necroses. Histologically, the first detectable change is a necrosis of the muscle fibers. The coronary arteries are not affected. A notable subdivision of the ESCN cardiopathy is the Ardenergic Cardiopathy produced by heavy overdosage with adrenaline, noradrenalin or other catecholamines such as isoproterenol. Histologically, these lesions are recognizable in the form of a spotty myolysis especially in the subendocardial layers near the apex of the heart. Another subdivision of the ESCN is directly related to the work in this investigation. The Simple Stress-Cardiopathy is produced by exposure to sudden intense stress without any special conditioning. The resulting cardiac lesions manifest themselves histologically as scattered necroses of individual muscle fibers or small fiber groups. However, these individual lesions are so small that they do not tend to terminate in permanent scar formation and thus are difficult to detect. Microscopic foci of necrosis tend to heal within a few days without leaving a trace. During exposure to a stressor there is an increase in the cardiac lipid content and a decrease in the quantities of essential metabolities of oxidative phosphorylation.

Selye also attempts to point out a relationship between clinical and experimental necrotizing cardiopathies (176). Possibly, microscopic chronic coronary lesions often act merely as conditioning factors that predispose the myocardium to the induction of massive necroses by metabolic derangements. Even the development of a thrombus within a vessel damaged by chronic atheromatosis may depend upon metabolic changes. Thus, thrombus formation may be of secondary importance in the precipitation of acute myocardial ischemia. Flame-photometric studies indicate that, in clinical cardiac insufficiency and especially in myocardial infarction, the fall in potassium and the rise in sodium concentration of the heart are of sufficient magnitude to account for a derangement in cardiac energy production and utilization that could be the responsible pathogenic factor.

Whether a stressful situation produces or prevents a cardiac infarct depends on the circumstances that condition the body's reactivity. For example, forced muscular exercise can, if applied before the animal is humorally conditioned for the development of a cardiac necrosis, act as a reliable preventative agent against the same necrotizing cardiac lesions which are elicited, when a similar exercise stressor is applied after humoral conditioning.

Another major experimental cardiopathy is the Electrolyte-Steroid-Cardiopathy with Calcification or ESCC.

It is characterized by extensive calcification of the myocardium and the coronary arteries. This calcification occurs in patches which are irregularly distributed throughout the heart. The ESCC is produced by combined treatment with certain steroids and calcium salts. Concurrent exposure to stress greatly facilitates the production of the ESCC type of lesion.

In general, the stress-related myocardial necroses in corticoid-pretreated rats are thought to be produced by catecholamine discharges which accumulate in the myocardium under stressful conditions (154). Raab (153) is also of the opinion that there is a fundamental causal involvement of metabolic catecholamine action in the origin of stress-induced myocardial damage. In contrast, the protective action of stress is thought to be due to increased glucocorticoid activity (153).

Even though he did not feel justified in presenting a unified hypothesis of the pathogenesis of the pluricausal cardiopathies, Selye (176) did advance some plausible theories. According to his experiments, Selye is convinced that potassium is the decisive pathogenic factor in the electrolyte steroid cardiopathies because:

1. Mineralocorticoids, which cause the loss of potassium, are indispensable for the production of both ESCH and ESCN.

2. In experimental animals, the ESCN can be duplicated by feeding a potassium deficient diet without the treatment of corticoids, sodium salts, or stressors.

3. The classical ESCN can be prevented by oral administration of potassium salts.

4. Acute stress causes a pronounced sudden loss of potassium and can precipitate electrolyte-steroid cardiopathies.

Other observations suggest that a "potassium servomechanism" regulates cardiac work and that potassium efflux from the heart is increased when the cardiac work load and heart rate are increased. For example, in coronary disease myocardial intracellular pottassium loss is accentuated by ischemia and extracellular potassium is elevated. Not only myocardial ischemia, but various stressors such as muscular work and adrenalin deplete myocardial potassium and increase coronary venous potassium. Thus, it seems that potassium is a pivotal factor in the pathology and normal physiology of the myocardium (164).

If there were to be a unified interpretation of his experiments, Selye would classify it as changes in ionic equilibrium. This concept states that the:

> precipitous influx of sodium and/or efflux of potassium renders the cell vulnerable to various potentially pathogenic agents that can produce

> the diverse structural lesions previously discussed. Both mineralocorticoids and stressors enhance the replacement of potassium by sodium in the cell so the precipitation of lesions could be due to the effect of these agents upon ionic equilibrium. Conversely, the protective action of antimineralocorticoids may depend on their ability to prevent such an electrolyte shift. The prophylactic effect of pretreatment with stressors or with mineralocorticoids may find its explanation in the fact that they induce potentially pathogenic changes in ionic equilibrium at a time when the cell is not yet exposed to a potential pathogen and, thereby, diminish the gradient with which such a sensitizing shift could occur during a subsequent critical exposure (176, p. 345).

Physical Activity

The benefits of physical activity in preventing heart disease and in improving cardiac function in patients with heart disease have been accepted for many years even though proof of such benefits was difficult to find. The values of physical activity in coronary heart disease might result from: (a) possible prevention or delay in the development of atherosclerosis, (b) changes in clotting tendency with the possible prevention of thrombotic complications of coronary atherosclerosis, or (c) conditioning the body of stress via exercise which, in turn, could reduce the possibility of ionic imbalance due to the action of either mineralocorticoids or other stressors. Furthermore, improved cardiac function and/or coronary collaterol circulation may result from increased physical activity so that impaired coronary flow may be better tolerated (47).

The biological principle of Roux that all organs are maintained and developed by function also applies to the heart. Lack of function and movement leads to atrophy and disease, especially hypokinetic disease (103). Owing to lack of movement, physical work, and exercise in mechanized civilization, coronary insufficiency has become one of the most common diseases. The observation is born out by the fact that those who regularly engage in physical exercise suffer less frequently from coronary insufficiency (61, 83, 118).

Hernberg (81) was concerned with the correlation between physical working capacity and serum cholesterol in business men. The results seem to indicate that persons with a high physical working capacity have lower serum cholesterol values in the age group of 30 to 49 years. However, other factors such as mode of activity, dietary differences, and degree of business stress also play an important role (40).

Many studies have been carried out comparing the incidence of myocardial disease as well as death due to myocardial disease in physically active and sedentary occupations. The same general conclusions have been reached in most of these studies, and there is general agreement that men and women in physically active occupations appear to have lower rates of fatal coronary disease than those in sedentary occupations. Habitual physical inactivity is associated

with a progressing deficiency of the sympathoinhibitory and vagal mechanisms which normally influence cardiac sympathetic chronotropic and inotropic activities at rest and during exercise (26, 149). According to Raab (155), the characteristics of a "loafer's heart" such as high pulse rate, short isometric contraction period, proneness to develop hypoxic ECG changes during exercise, and low overall efficiency are opposite to those characteristics of the well-trained athlete's heart with its reduced sympathetic tone and high overall efficiency. Physical training reduces the excess oxygen utilization of the myocardium by decreasing the sympathetic activity of the heart (146).

Morris' work on employees of the London transport system (123, 125) showed that sedentary London bus drivers had twice as many fatal heart attacks as did the conductors who were required to climb stairs many times per day. Sedentary postal clerks also showed a higher rate of heart attacks when compared to postmen who were physically active most of the day (124). Similarly, two and one-half million skilled, semi-skilled, and unskilled workers were studied, and higher incidences of fatal heart attacks were observed in those occupations requiring little physical activity. Furthermore, the proportion of men who survived a first heart attack was twice as great among heavy duty laborers. Autopsies on four thousand coronary deaths revealed more extensive cardiac damage in those who had been engaged in

sedentary occupations (125). A study of the relationship between mortality, resulting from an initial myocardial infarction, and physical activity revealed that death for those who pursued a sedentary existence characterized by habitual lack of activity was almost three times that of those individuals who were classified as most physically active (60).

Histochemistry of Myocardial Disease

The heart muscle is extremely rich in a number of oxidative and hydrolytic enzymes; and it is reasonable to assume that alterations in the normal metabolic scheme, which lead to irreversible myocardial damage, can be recognized early via studies on enzyme activities. Histochemical studies are, in fact, of value in demonstrating the onset of damage to the myocardial fibers. Histochemical methods for demonstrating the activity of certain enzymes reveal the presence of myocardial infarctions long before conventional staining methods show convincing structural changes in the damaged fibers (8). The decline in most enzyme activities is probably due to leakage of the enzyme in question from the dying cell (1). However, the decline in monoamine oxidase action "may possibly be a homeostatic response to prevent oxidation of the scanty catecholamines that remain in the infarcted myocardium" (1, p. 234).

According to Niles et al. (131), the use of precise histochemical techniques helps elucidate the biochemical

reactions involved in myocardial lesions. Histochemical techniques can achieve this because they show not only the amount of activity, as disclosed by the intensity of the stain, but also the location of that activity within the tissue and cellular components. Myocardial fibers from hearts treated with isoproterenol or subjected to conditions of hypoxia show predominantly a granular type of staining rather than a characteristic fibrillar staining (130). As early as two hours after the onset of hypoxic conditions, small foci of abnormal granularity are evident with SDH. By fourteen hours, this condition is prevalent; but, a standard hematoxylin-eosin stain shows evidence of scar tissue formation only after approximately eighteen hours.

Following experimental coronary artery ligature, the reactions for the oxidative enzymes start to decline in two to three hours (8). At an early stage of ischemia, the activity of succinic dehydrogenase (SDH) declines more readily than that of Cyt.O. The normal myocardial fibers surrounding the necrotic area retain normal SDH and Cyt.O. activities. Monamine oxidase activity is somewhat different. It becomes rapidly depleted in the affected areas but is also depleted or absent in the remaining parts of the coronary artery ligated heart.

In the case of metabolically induced necroses (i.e. injection of plasmocid) the activity and distribution of the

various oxidative enzymes remain normal for about eight hours following injection. Later, as could be expected, a decrease in reaction intensity is noted but only in those fibers that are obviously degenerating. Again the reaction for SDH declines more readily than that for Cyt.O. The myocardial fibers surrounding the necrotic area are found to be hyperactive as far as histochemical demonstration of the enzymes under consideration are concerned. The earliest histochemically demonstrable alteration than can be detected in plasmocid treated animals is the initial decrease in 5-nucleotidase activity in the capillary walls. This is evident as early as ten minutes following the injection of plasmocid (8).

Schnitka (174) reported a reduced mitochondrial Cyt.O. activity six hours following induced experimental lesions. On the other hand, the activity of LDH was sometimes maintained for a while in the middle of infarcts (110). It is now known (30) from biochemical subcellular fractionation studies that the mitochondria contain Kreb cycle enzymes such as SDH and most of the cells Cyt.O. activity.

Lushnikov (110) reported that B-OHBD and iso-citric dehydrogenase are the first enzymes to decline in activity. Reduced activity has been reported as early as one and one-half to four hours after experimental cardiac infarction of the rat myocardium. In fact, B-OHBD is one of the most sensitive histochemical indicators of early human myocardial infarction (110).

Fine et al. (58) agree that the various enzyme changes are obvious before there is histologic evidence of cardiac infarction; and, twenty-four hours following myocardial infarction, histochemical reactions for SDH and nucleotide-tetrazolium reductases may be nearly absent from experimental lesions. These histochemically demonstrable reductions in enzyme activity in early necrotic myocardial fibers are an indication of an enzyme leakage that occurs from the infarcted myocardium. This, then, would be the reason for the elevated plasma levels of the enzymes in question soon after the onset of the necrotic condition.

According to Müller and Pearse (126), MAO activity is indicative of adrenergic function. MAO deaminates primary aliphatic amines to aldehydes as shown in the following reaction:

$$RCH_2NH_2 + O_2 \xrightarrow{MAO} RCHO + NH_3 + H_2O_2$$

However, the exact function of MAO in the animal body is not known. Bajusz and Jasmin (16) report that MAO activity also disappears early and is also considered to be a sensitive indicator of early myocardial infarction. MAO activity has been identified in the sarcoplasm, especially in the mitochondria of myocardial fibers in biopsies of the human right ventricle (134). It has also been observed that MAO activity is strongly active in the rat heart particularly in the

region of the sino-atrial and atrio-ventricular nodes (126). In fact, in the prenecrotic stages of the development of experimental cardiac lesions, MAO activity declines substantially while the activities of SDH and Cyt.O. remain normal until the actual onset of cellular degeneration. In another article Bajusz (7) reiterates the same facts when he says that during the early stages of myocardial damage, the reactions for such oxidative enzymes as SDH and Cyt.O. remain normal or are even somewhat elevated within the areas devoid of phosphorylase, glycogen, and potassium. When a conventional HPS stain is used, morphologic changes do not occur in the myocardium until five to eight hours following injection with epinephrine, and the HPS stain is found to be informative only when dealing with lesions of at least twenty-four hours duration.

In the case of an obstructive cardiomyopathy, there is a marked increase in SDH in the mitochondria of the affected myocardial fibers. In a similar manner, the activity of MAO is also increased. Possibly, "the proliferation of mitochondria in the cardiomyopathic fibers reflects the increased production of MAO in the tissue as a homeostatic response to the focally high concentration of catecholamines" (1, p. 243).

In cardiac infarcts produced by coronary ligature in the rat, the SDH activity begins to diminish about four hours after the operation and disappears completely within

forty-eight hours. Cyt.O. activity disappears at a slower rate according to comparable histochemical observations (91). However, according to Kaufman et al. (91) the most extensive studies on myocardial changes that follow coronary ligature have been made on dogs. In this species, the evidence has been accumulating that chemical and histochemical alterations (such as glycogen, lactic acid, PAS-tingible material, electrolytes, and enzyme changes) occur from the first few minutes to hours after vascular occlusion. Loss of SDH activity from infarcted myocardial fibers has been detected histochemically as early as two hours after coronary occlusion in man and as early as four to six hours after coronary ligation in rats (174).

LDH is present in the cardiac muscle and its activity is dependent upon the co-enzyme nicotonamide-adenine-dinucleotide (NAD). Under anerobic conditions, the interconversion of pyruvate and lactate shifts toward lactate which necessarily decreases the relative amounts of NADH available to the cell.

The increase of LDH activity in both normal and degenerating cardiac fibers following the injection of plasmocid may reflect an adoptive alteration in enzyme pathways (8). Both Vessels (206) and Wrobewski (213) say that cases of myocardial infarction are accompanied by an increased concentration of LDH. Similarly, Stuart (198) found that LDH concentrations rose immediately after myocardial in-

farction and remained elevated for seven to ten days. Garbus (65) found that untrained male albino rats showed a marked rise in serum LDH concentrating following sixteen hours of exercise. However, in a trained group of rats, the LDH level remained normal. Blatt et al. (22) found no change in the LDH concentration of the liver or skeletal muscles after three to four weeks of exposure to a cold environment. After the first week, the LDH level in the heart increased significantly; but, after the animal adopted to the stress of the cold environment, the LDH concentration in the heart returned to normal. Presumably a similar reaction to other forms of stress could be expected.

Figure 1.--A morphologic lesion in the heart muscle of a rat following injection of epinephrine. The lesion is shown at 48 hours following injection and is fully developed and characterized by more or less confluent sub-endocardial areas of myolysis with inflammatory infiltration (Hematoxylin-phloxime-saffron (HPS) stain). (7)

Figure 2.--The activity and distribution of SDH in a normal control heart. (7)

Figure 3.--Partial decrease and loss of SDH activity in myocardial fibers that are already in various stages of degeneration. Note the increased SDH activity in fibers surrounding the necrotic area. (7)

Figure 4.--Confluent areas of necrosis and development of granulated tissue in the rat myocardial tissue of the right ventricle. (185)

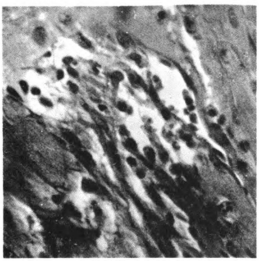


Figure 1

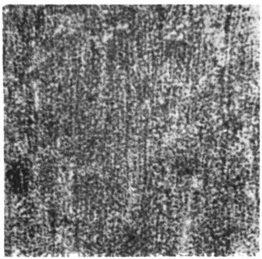
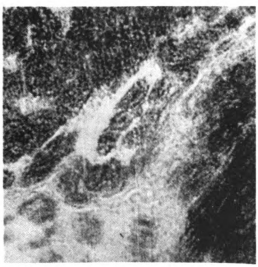


Figure 2



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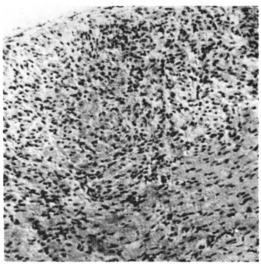

Figure 4

Figure 5.--Degenerating and necrotic foci in the myocardium of rats which had been exposed to frustrating (obstacles in access to food) and frightening (tape recorded cat-rat fights) situations (141).

Figure 6.--Subendocardial infarctoid necrosis in left septum and wall of right ventricle in two rats conditioned by Na_2HPO_4 and Me-CL-Col followed by exposure to cold baths. (176 p. 64)

Figure 7.--Nearly complete loss of succinic dehydrogenase from necrotic myocardial fibers of a 36-hour old human myocardial infarct. Note preservation of SDH activity in normal fibers at left. SDH x 160. (1 p. 228)

Figure 8.--Simple stress-cardiopathy in rat restrained for 17 hours. Focus with edema, necrotic muscle fibers, and round cell infiltration under endocardium. Fuchson x 125. (176 p. 125)

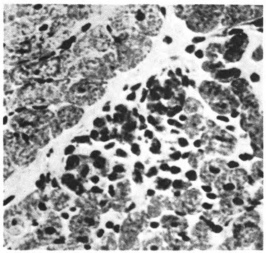

Figure 5

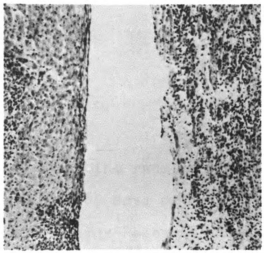

Figure 6

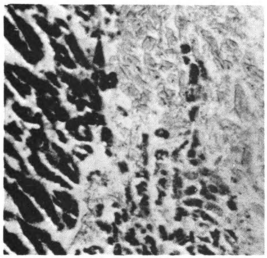

Figure 7

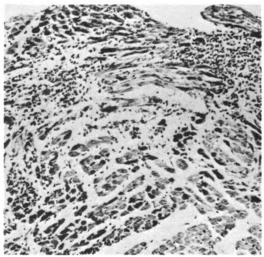

Figure 8

CHAPTER III

RESEARCH METHODS

Sample

The sample consisted of 106 male albino rats of the Sprague-Dawley strain. The animals were 60 days of age when they were delivered to the Human Energy Research Laboratory.

Treatments

Two experimental treatments were used during the course of this investigation: an anxiety treatment and an exercise treatment. These treatments were used separately and in various combinations.

The anxiety treatment consisted of seven shock periods each day for two weeks. During each shock period, the animals received a disturbing, but noninjurious, 36 second electrical shock of 1.5 milliamperes five times per minute. In order to present a random pattern of shock to the animals, the time between shocks ranged from seven to sixteen seconds within each shock period, and the duration of the shock periods themselves ranged from twenty-eight minutes to two hours and eight minutes.

The exercise treatment consisted of a one-half hour swim, twice daily, seven days a week for two, five, or seven weeks. For the first three days of the experiment, each animal completed the exercise program without the attachment of additional weight. Thereafter, each animal had a weight equal to two per cent of his body weight attached to the tip of his tail for all exercise periods. The weights were attached near the tip of the rat's rail with miniature plastic clothespins. White adhesive tape was inserted between the prongs of the clothespins to reduce trauma. The animals were swum in individual cylindrical metal tanks measuring 28 cm. in diameter and having a depth of 75 cm. Immediately after each animals was placed in its individual swimming cylinder, it was lifted out and the air stripped from its fur. It was then replaced in its respective cylinder. If an animal was obviously having difficulty during the exercise period, it was removed from the water and given a brief rest before being returned to the water to complete the one-half hour swim without its tail weight.

Treatment Groups

The animals were randomly assigned to five experimental treatment groups. The five treatment groups used in this experiment were as follows:

1. Controls (Con)

   Ten animals were housed in sedentary cages throughout the duration of the experiment. They

received neither the exercise nor the anxiety treatment and were removed from their cages only once weekly for body weight determinations. It was expected that these animals would show little or no myocardial damage.

2. Anxiety with no Exercise (ANE)

Twenty-four animals were housed in sedentary cages until they were moved to the anxiety cages for the two-week anxiety treatment. This group received no exercise treatment and, on completion of the two-week anxiety treatment, they were returned to their sedentary cages. It was expected that this group of animals would show moderate to severe myocardial damage.

3. Exercise during Anxiety (EDA)

Twenty-four animals were housed in sedentary cages until they were moved to the anxiety cages for the anxiety treatment. They were also subjected to a two-week exercise program concurrently with the anxiety treatment. After completion of the exercise-anxiety treatment, the animals were returned to their sedentary cages until sacrifice. It was expected that this group of animals would show severe myocardial damage.

4. Exercise before Anxiety (EBA)

Twenty-four animals were housed in sedentary cages for one week prior to the start of the exercise treatment and in voluntary cages throughout the five-week exercise period. The exercise treatment was terminated at the start of the anxiety treatment at which time the animals were moved to the anxiety cages for the anxiety treatment. It was expected that this group of animals would show no myocardial damage due to the hypothesized prophylactic effect of exercise.

5. Exercise before and during Anxiety (EBD)

Twenty-four animals were housed in voluntary cages during the five-week pre-anxiety exercise period. Following the five-week exercise period, the animals were moved to the anxiety cages for the anxiety treatment. They also received an additional two-week exercise treatment concurrently with the anxiety treatment. At the completion of the anxiety-exercise treatment, the animals were returned to the involuntary cages until sacrifice. It was expected that this group of animals would show minimal myocardial damage.

Body Weight

All animals were weighed every Saturday at 12:00 noon, and the weights used during the exercise periods were adjusted weekly according to the animal's most recent body weight.

Serum Lactate Dehydrogenase Determinations

Serum lactate dehydrogenase (LDH) concentrations were determined using the standard spectrophotometer technique described by Cabaud and Wroblewski (31) and by the Sigma Chemical Company (189) which supplied an assay kit for serum LDH determinations. The selected wave length on the spectrophotometer was 540 mu. Basically, the serum LDH level was used in an attempt to detect heart damage before sacrifice. Animals were chosen for study, which according to their LDH level, showed some evidence of myocardial damage. The original sample size of 106 was thus reduced to only 69 animals. The four groups of animals receiving the exercise and/or anxiety treatments were reduced to 15 animals per group while the control group was reduced to nine animals due to the accidental death of one control animal prior to sacrifice. Serum LDH determinations were not made prior to the start of the anxiety treatment nor were they made on the control animals as previous studies in the Human Energy Research Laboratory had established serum LDH levels for control animals. Serum LDH determinations were made only on those animals receiving the

anxiety treatment and only five and twelve days after the start of the anxiety treatment.

Sacrifice Procedure

Each animal was sacrificed at 116 days of age with an overdose of ether. Next, a midabdominal incision was made and continued cranially through the sternum. Loose fascia was removed and the heart exposed and quickly excised. The heart was trimmed at both the apex and the base. Three approximately equal blocks of myocardial tissue were obtained by a transverse ablation of the atria and ventricles. The outer two of these sections were mounted on metal chucks with five per cent gum tragacanth and quick forzen immediately in isopentane cooled with liquid nitrogen. This method allowed the tissue to be cooled to approximately -160° C within ten seconds. The middle section of myocardial tissue was placed in ten per cent formalin solution for 48 hours prior to staining with a paraffin embedded H and E stain. Serial tissue sections were obtained from the inner surfaces of the frozen blocks of myocardial tissue.

Histochemical Procedures

At least two fresh-frozen serial cross sections approximately 10 microns thick, were cut from each frozen block of myocardial tissue on an Ames-Lab Tek cryostat. The tissue sections were briefly fan dried immediately after

they were thawed at room temperature and then they were placed on 22 mm. sq. Corning cover glasses. One tissue section from each block of myocardial tissue was stained with a standard H and E stain. Other sections from each tissue block were subjected to specific histochemical procedures--SDH (17), MAO (133), and B-OHBD (133). Tissue sections from all animals of each sacrifice were processed simultaneously in order to ensure the use of identical histochemical techniques and to ensure equivalent staining reactions within the cell tissues being processed.

Succinic dehydrogenase (SDH) activity was studied using NBT (2,2'-di-p-nitrophenyl -5,5'-diphenyl -3,3'-(3,3'-dimethoxy -4,4'-diphenylene) ditetrazolium chloride) as an election acceptor, as described by Barka and Anderson (17). Monamine oxidase (MAO) activity was demonstrated by the method of Glenner et al. (133) with NBT again used as an election acceptor. Beta-hydroxybutyrate (B-OH) activity was studied according to the method as described by Pearse (133).

Incubation times varied slightly according to the histochemical procedure being followed. For example, SDH was allowed to incubate for only seven minutes while MAO was incubated for at least 40 minutes. B-OHBD also was incubated for 40 minutes. Glycerin jelly was used as a mounting medium for all histochemical enzyme procedures. Permount was the mounting medium used for the H and E procedure.

Method of Tissue Analysis

After the histological slides from each sacrifice had been prepared, each slide was individually examined for evidence of myocardial damage. The method of evaluation was subjective and myocardial damage was rated on an arbitrary one to five scale in a manner similar to that of Niles et al. (130).

Within this arbitrary scale, a rating of one was used to indicate no myocardial damage. A rating of two indicated questionable myocardial damage, while a rating of three indicated the presence of a slight degree of myocardial damage. A rating of four indicated moderate myocardial damage, and a rating of five indicated severe myocardial damage.

Both the atria ("a" tissue sections) and ventricles ("b" tissue sections) were examined, but most attention was focused on the left and right ventricles as little myocardial damage was evident in the atrial muscle.

With the histochemical enzymes, granulomatous tissue areas produced by deposition of diformazan granules were considered to indicate sites of decreasing enzyme activity and thus of myocardial damage (Figure 10). The pattern and intensity of enzyme activity were also studied. These variables are indicators of anoxic damage. With H and E slides, small eosinophilic areas characterized by darkened foci indicated areas of myocardial necrosis (Figures 11, 15).

Figure 9.--Right ventricular region of the myocardium showing decreased SDH activity. Animal is from the exercise before and during anxiety treatment group. (X 400).

Figure 10.--Same region as shown in Figure 9 but showing the relatively complete loss of B-OHBD activity. The diformazan granules are plainly visible. (X 400).

Figure 11.--Hematoxylin and eosin stain of the same region as shown in Figures 9 and 10. Necrotic voci and scar tissue formation are evident. (Hand E, X 400).

Figure 12.--Sub-endocardial region of rat from exercise during anxiety group showing a decreased B-OHBD activity, expecially on the right edge of the photograph. (X 400).

Figure 13.--Same area shown in Figure 12. Scar tissue formation is evident as are the normal cardiac fibers to the left. (Hand E, X 400).

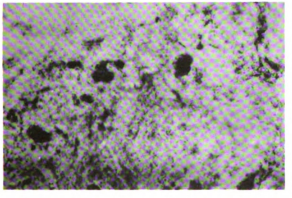

Figure 9

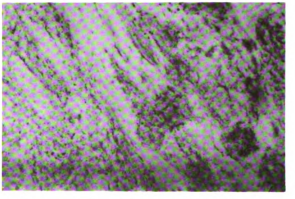

Figure 10

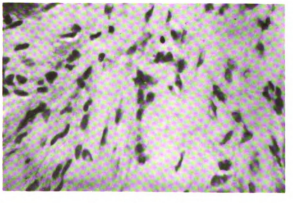

Figure 11

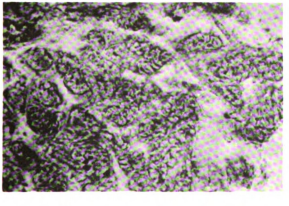

Figure 12

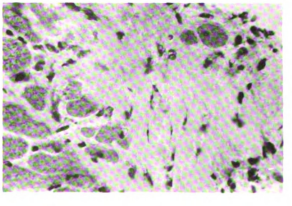

Figure 13

Figure 14.--Epicardial region of the left ventricle of animal receiving the exercise before anxiety treatment. The almost complete loss of MAO activity is evident. Note the degenerating fibers on the left. (X 400).

Figure 15.--Same region as shown in Figure 14. Scar tissue formation is obvious. (Hand E, X400).

Figure 16.--Mid-ventricular region of control animal. (H and E stain, X 400).

Figure 17.--Small endocardial region of left ventricle showing loss of SDH activity for animal receiving the anxiety with no exercise treatment. (X 400).

Figure 18.--Same region as depicted in Figure 17. Note the fibers on the right are in a degenerative state. (Hand E, X 40o).

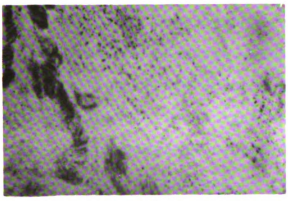

Figure 14

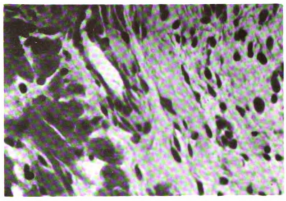

Figure 15

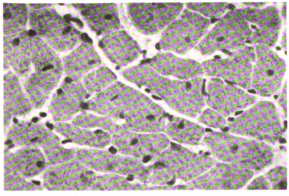

Figure 16

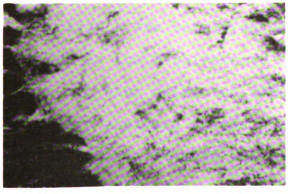

Figure 17

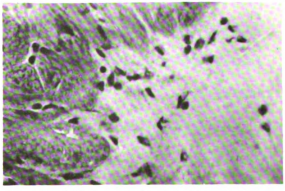

Figure 18

Thus, myocardial damage was easily defined if, in fact, it was present.

The first demonstrable change with the H and E stain is the presence of edema which usually is exaggerated by the formation of ice-crystal artifacts. This was avoided somewhat in this investigation by the quick-freeze technique. Approximately twenty-four hours later, the edematous fibers degenerate to form necrotic areas (1).

Each slide was evaluated at least twice in order to increase the reliability of the subjective evaluations. All ratings were made without prior knowledge of the treatment groups.

Statistical Methods

Because of the nature of the data, statistical analysis was limited to a non-parametric Chi-square test. A contingency Chi-square was calculated to test the hypothesis of no difference in the various treatment groups as far as the production of myocardial damage was concerned. This analysis was completed only on the ventricular tissue sections as the requirements for the Chi-square test could not be met when using the atrial tissue sections. A second Chi-square analysis tested the hypothesis that there was no relationship between the serum LDH levels and the degree of myocardial damage. This analysis was completed for both atrial and ventricular tissue sections.

The .05 level of probability was chosen to determine statistical significance for the contingency Chi-square analyses.

Because the incidence of myocardial necrosis was subjectively determined, some variability naturally existed. In this particular analysis, apparent evidence of myocardial damage was ignored if it occurred on the tissue perimeter or in the immediate vicinity of a large blood vessel, since in these areas it was often difficult to differentiate damage from technique artifact.

CHAPTER IV

RESULTS AND DISCUSSION

Results

The purpose of this study was to determine the effects of a specific exercise program and/or electric shock on the production of myocardial damage in male albino rats. Myocardial damage was determined subjectively by using both histochemical stains and a standard hematoxylin-eosin stain.

Table 1 gives the subjective ratings for myocardial damage for all five treatment groups based on ventricular tissue sections only. Ratings were based on a scale of one to five, with a rating of one meaning no myocardial damage and a rating of five indicating severe myocardial damage. This rating procedure is similar to the one used by Niles et al. (130).

Evidence of myocardial damage was determined largely with the H and E stain. The histochemical stains B-OHD, MAO, and SDH were used to corroborate the evidence of myocardial damage as indicated by the H and E stain. The H and E stain and the histochemical stains had to show agreement, as to the degree of myocardial damage, before the final subjective rating was assigned to each animal.

TABLE 1.--Frequency distribution of subjective rating of myocardial damage for ventricular tissue sections of the myocardium.

Rating	Group					
	Con.	ANE	EDA	EBA	EBD	n
1	2	0	0	0	0	2
2	2	6	6	5	5	24
3	4	7	8	8	8	35
4	1	1	1	2	1	6
5	0	1	0	0	1	2
n	9	15	15	15	15	69

Table 2 gives the subjective ratings for myocardial damage for atrial tissue sections.

Statistical analysis, be means of a contingency Chi-square, showed no significant differences between the five treatment groups as far as degree of myocardial damage for ventricular tissue sections was concerned (Table 3). During this particular analysis, it was necessary to group the data into fewer than five rankings in order to meet the requirements of the contingency Chi-square test.

It seemed logical to group the animals with the ranks of one or two together as these ranks indicated no myocardial damage or questionable myocardial damage. The animals ranked three, four, or five were grouped together as these groups indicated at least some definite degree of

TABLE 2.--Frequency distribution of subjective rating of myocardial damage for atrial tissue sections of the myocardium.

	Group					
Rating	Con.	ANE	EDA	EBA	EBD	n
1	3	7	7	2	0	19
2	4	3	6	13	11	37
3	2	5	2	0	3	12
4	0	0	0	0	0	0
5	0	0	0	0	0	0
n	9	15	15	15	14	68

TABLE 3.--Chi-square test for treatment effects on the degree of myocardial damage for ventricular tissue sections.

Myocardial Damage	Group					
Rank	Control	ANE	EDA	EBA	EBD	n
1-2	4	6	6	5	5	26
3-5	5	9	9	10	10	43
n	9	15	15	15	15	69

Note: Chi-square of .489 (N.S. at the .05 level).

myocardial damage. However, one should not be misled by the apparent high incidence of heart damage rated three to five, as most of these cases had a rank of only three indicating slight myocardial damage (see Table 1). Table 4 gives the results of the contingency Chi-square test for the relationship between the degree of myocardial damage and the serum LDH levels. The degree of myocardial damage in Table 4 was grouped differently from that in Table 3, as the requirements of the Chi-square test could now be met with three groupings. It should be noted that the total number of animals used in this statistical analysis was only 60, as no serum LDH determinations were made on the control animals.

Table 5 gives the results of the contingency Chi-square test for the relationship between the degree of myocardial damage and the level of serum lactate dehydrogenase for the atrial tissue sections.

Thus, statistical analysis indicates that electric shock and/or forced muscular exercise, as administered in this experiment, were not of sufficient duration or intensity to significantly affect myocardial damage.

According to the rationale of this study, it had been expected that the exercise during anxiety group of animals would show the most severe heart damage due to the application of a double stressor. Logically, the anxiety with no exercise group should have shown at least moderate to

TABLE 4.--Chi-square test for relationship between the degree of myocardial damage for ventricular tissue sections and serum LDH level.

Myocardial Damage	LDH Level		n
Rank	0 - .39	.40 - .69	
1-2	8	15	23
3	9	21	30
4-5	3	4	7
n	20	40	60

Note: Chi-square of .5101 (N.S. at the .05 level).

TABLE 5.--Chi-square test for relationship between the degree of myocardial damage and serum LDH levels for atrial tissue sections.

Myocardial Damage	LDH Level		n
Rank	0 - .39	.40 - .69	
1-2	17	33	50
3-5	3	7	10
n	20	40	60

Note: Chi-square of .4595 (N.S. at the .05 level).

severe myocardial damage. The group of animals subjected to the exercise before and during the anxiety treatment should have shown some minimal amounts of myocardial damage, while both the controls and the exercise before anxiety animals should have shown no myocardial damage.

There are several possible explanations for the myocardial damage which was observed in this study. The cardiac myopathies might be accounted for by one or a combination of contributory factors.

The anxiety treatment probably caused, at least in the beginning stages, some increased catecholamine release from the adrenal glands. The harmful effects of large amounts of catecholamines on the cardiac muscle have been well documented in previous studies (7, 57, 137, 138). Thus, if the anxiety treatment was of sufficient intensity to cause massive secretion of catecholamines, the resulting myocardial infarcts could, in part, be attributable to the direct effect of catecholamines on the heart. It is doubtful, however, that sufficient quantities of catecholamines were released in this study to produce such effects. A subjective evaluation of adrenal glad weights showed no apparent differences between the various treatment-groups means.

Even if the secretion of catecholamines elicited in this study was small, it might have been sufficient to cause cardiac necroses if certain accessory conditions were

met. The most obvious of these would be an advanced degree of atherosclerosis in the coronary arteries (94, 127, 138, 191). However, the degree of atherosclerosis in the animals involved in this experiment was not measured, so there was no way of determining whether or not atherosclerosis was a contributing factor.

In contrast, the relative lack of consistent and extensive myocardial damage also could be attributed to one or several factors. If coronary artery atherosclerosis was non-existant or minimal, then the increased myocardial oxygen demand caused by any moderate elevation of catecholamines would be of little pathologic significance. Under normal circumstances, moderate stress produces no serious cardiac damage in healthy animals (175). In fact, electrolytes, corticoids, or stressors, as a rule, produce no consistent cardiac change in themselves. The myocardial necroses of the simple stress cardiopathy only develop after severe exposure to stress alone and occur only under exceptional circumstances. Periodic examination of the animals receiving the anxiety treatment revealed that, except for the first day or two, the amount of electric shock received by the animals provided little stress. Many, in fact, slept through much of the anxiety treatment.

The simple stress cardiopathy categorized by Selye (176) is produced by exposure to sudden intense stress without any special conditioning. The resulting cardiac

lesions manifest themselves histologically as scattered necroses of individual muscle fibers or fiber groups. However, these lesions are so small that they do not tend to terminate in permanent scar tissue formation. In addition, such microscopic foci tend to heal within a few days without leaving a trace. Thus, it is possible the anxiety treatment did, in fact, produce small foci of necrosis, but any trace of these had vanished by the time the animals were sacrificed.

Finally, the effects of the five-week or a seven-week training program may have been sufficient to condition the hearts of some of the animals against the production of myocardial damage. Physical training improves general cardiac efficiency which results in several possible benefits. It could cause improved coronary collateral circulation (97) so that impaired coronary artery flow due to atherosclerosis is better tolerated. Physical training reduces cardiac sympathetic tone and increases cardiac parasympathetic tone which is manifested by a decreased heart rate and decreased cardiac output for a given level of work (77). This, of course, results in a reduced excess oxygen utilization of the myocardium, and conditions of myocardial hypoxia or anoxia are less likely to develop. It should be noted, however, that if these possible benefits of physical activity had been fully operative in this study, significant groups differences should have been observed.

In summary then, myocardial damage could have been elicited by the combined actions of the catecholamines, coronary artery atherosclerosis, and electrical stress. Some transient myocardial damage may have been missed by the techniques used. Finally, myocardial damage could have been prevented by the effects of the physical exercise program and a lack of coronary artery atherosclerosis.

The scarcity of a large number of high LDH readings, a supposed indicator of conditions of myocardial ischemia, provided additional evidence that, in general, the animals had adapted to the anxiety treatment. In any case, no significant results were obtained.

CHAPTER V

SUMMARY, CONCLUSIONS, RECOMMENDATIONS

Summary

The purpose of this study was to determine the effects of physical exercise and/or electrical stress on the production of experimental myocardial damage.

One hundred and six male albino Sprague-Dawley rats 60 days of age were randomly assigned to five treatment groups. These groups were control, anxiety with no exercise, exercise during anxiety, exercise before anxiety, and exercise before and during anxiety. The last three treatment groups were subjected to two thirty-minute swim periods per day, seven days a week, with a weight equal to two per cent of the body weight attached to the tip of the tail. All animals were fed ad libitum with commercial laboratory blockfeed. Ambient air temperatures in the animal quarters were kept between 21 to 25 C.

Those animals subjected to the anxiety treatment received a .36 sec D.C. electrical shock of 1.5 M.A. five times a minute, nine hours a day, for two weeks. The electrical shocks were administered in a random pattern. All anxiety animals began their treatments at one hundred days of age.

All animals were sacrificed at 116 days of age with an overdose of ether. The heart was excised immediately, trimmed and cut in three equal sections. The apical ventricular section and the basal auricular section were immediately quick-forzen in isopentave cooled with liquid nitrogen. The middle section was fixed in a ten per cent formalin solution for forty-eight hours. At least two fresh-frozen serial cross sections approximately ten micra thick were cut and stained with a standard H and E stain as well as with the histochemical enzymes SDH, MAO, and B-OHBD. Formalin-fixed tissue sections were stained with a paraffin embedded H and E stain.

Heart damage was evaluated subjectively using a one to five rating scale. A rating of one indicated no myocardial damage, while a rating of five indicated severe myocardial damage. All ratings were made without knowledge of the treatment groups.

Serum LDH levels were determined by a standard photometric method, and LDH levels were used to select those animals for sacrifice which, according to the LDH level, showed some evidence of myocardial damage.

The data were evaluated statistically using non-parametric contingency Chi-square analyses.

It was found that a chronic swimming program and/or electrical shock did not significantly affect heart damage. Because of the lack of severe heart damage, serum LDH levels

were not useful indicators of myocardial cardiopathies. The heart damage which was elicited by the experimental treatments and detected with the H and E stain was confirmed in each case by decreased histochemical enzyme activity in a similar section of cardiac tissue.

Conclusions

The results of this study have led to the following conclusions:

1. A .36 sec. D.C. electrical shock of 1.5 M.A. lasting 9 hours a day for 2 weeks is not a sufficient stressor to induce extensive myocardial damage.

2. Exercise treatments, as used in this study, did not significantly affect myocardial damage.

3. Serum LDH did not accurately predict the minimal amounts of myocardial damage found in this experiment.

Recommendations

1. A similar study should be done which involves greater exercise intensities as well as different types of exercise.

2. A more systematic guideline for the rating of myocardial damage should be established. This

would be of benefit to future investigators.

3. Animals participating in such a study should be "sensitized" during the study by the use of either a high cholesterol or a high fat diet.

4. Although MAO is a good early indicator of myocardial damage, more work needs to be done with this particular enzyme as uniform results were difficult to obtain.

5. Serum LDH determinations should be made more frequently.

6. Serum catecholamine concentrations should be determined before, during, and after stress.

7. Cross sections of the aorta should be made, and histochemical analysis of the extent of atherosclerosis determined. This should also be carried out on the coronary arteries.

8. A sequence of sacrifice times should be considered in order to take advantage of the predictive qualities of the various histochemical enzymes.

9. A quicker, more administrable method of stressing the animals would be advisable. An example would be 15 to 20 hours of forced restraint which has been proven to yield excellent results (176).

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APPENDIX

APPENDIX A

HEART DAMAGE RATINGS AND SERUM LDH CONCENTRATIONS

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HEART DAMAGE RATINGS AND SERUM LDH CONCENTRATIONS

Animal Number	Treatment Group	Heart Damage (Ventricles)	Heart Damage (Atria)	LDH (early)	LDH (later)
1	Control	2	1	No LDH	No LDH
2	Control	2	1	No LDH	No LDH
3	Control	Not Analyzed	Not Analyzed	No LDH	No LDH
4	ANE	2	1	.43	.44
5	ANE	Not Analyzed	Not Analyzed	.58	.55
6	ANE	Not Analyzed	Not Analyzed	No LDH	No LDH
7	ANE	3	1	.46	.53
8	ANE	Not Analyzed	Not Analyzed	.49	.50
9	ANE	4	1	.55	.46
10	ANE	2	2	.45	.42
11	ANE	3	1	.52	.51
12	ANE	3	3	.53	.44
13	EDA	Not Analyzed	Not Analyzed	.56	.58
14	EDA	2	2	.55	.43
15	EDA	3	1	.39	.43
16	EDA	3	1	.48	.40
17	EDA	Not Analyzed	Not Analyzed	.41	.58
18	EDA	3	3	.44	.50
19	EDA	3	1	.55	.52
20	EDA	Not Analyzed	Not Analyzed	.55	.57
21	EDA	3	1	.53	.49
22	Control	3	2	No LDH	No LDH
23	Control	1	1	No LDH	No LDH
24	Control	Not Analyzed	Not Analyzed	No LDH	No LDH
25	ANE	3	3	.58	.56
26	ANE	2	2	.51	.59
27	ANE	3	1	.63	.53
28	ANE	3	3	.56	.57
29	ANE	2	1	.53	.59
30	ANE	2	1	.37	.62
31	ANE	Not Analyzed	Not Analyzed	.50	.63
32	ANE	Not Analyzed	Not Analyzed	.56	.62
33	ANE	Not Analyzed	Not Analyzed	.53	.63
34	EDA	2	1	.47	.55
35	EDA	2	2	.58	.51
36	EDA	3	2	.63	.50
37	EDA	2	1	.60	.56
38	EDA	Not Analyzed	Not Analyzed	No LDH	No LDH
39	EDA	2	1	.53	.53
40	EDA	Not Analyzed	Not Analyzed	.53	.61
41	EDA	Not Analyzed	Not Analyzed	.54	.64
42	EDA	3	2	.52	.48
43	Control	2	2	No LDH	No LDH
44	Control	3	2	No LDH	No LDH
45	Control	Not Analyzed	Not Analyzed	No LDH	No LDH
46	EBA	3	2	.59	.51
47	EBA	2	2	.40	.23
48	EBA	3	2	.51	.29
49	EBA	Not Analyzed	Not Analyzed	.40	.56
50	EBA	Not Analyzed	Not Analyzed	.50	.60
51	EBA	2	2	.45	.28
52	EBA	Not Analyzed	Not Analyzed	.52	.52
53	EBA	3	2	.24	.31
54	EBA	3	2	.30	.53
55	EDB	Not Analyzed	Not Analyzed	.44	.47

HEART DAMAGE RATINGS AND SERUM LDH CONCENTRATIONS

Animal Number	Treatment Group	Heart Damage (Ventricles)	Heart Damage (Atria)	LDH (early)	LDH (later)
56	EDB	3	2	.41	.33
57	EDB	Not Analyzed	Not Analyzed	.33	.39
58	EDB	3	2	.58	.35
59	EDB	3	2	.54	.29
60	EDB	3	2	.67	.34
61	EDB	2	2	.48	.37
62	EDB	3	2	No LDH	.41
63	EDB	Not Analyzed	Not Analyzed	.46	.41
64	Control	4	3	No LDH	No LDH
65	Control	3	2	No LDH	No LDH
66	Control	Not Analyzed	Not Analyzed	No LDH	No LDH
67	EBA	4	2	.54	.12
68	EBA	3	1	.24	.53
69	EBA	4	1	.62	.26
70	EBA	Not Analyzed	Not Analyzed	.52	.58
71	EBA	3	2	.63	.08
72	EBA	2	2	.61	.18
73	EBA	Not Analyzed	Not Analyzed	.32	.58
74	EBA	Not Analyzed	Not Analyzed	.61	.60
75	EBA	3	2	.49	.55
76	EDB	4	2	.41	.41
77	EDB	3	2	.54	.38
78	EDB	Not Analyzed	Not Analyzed	.57	.60
79	EDB	5	3	.59	.55
80	EDB	3	3	.60	.38
81	EDB	3	3	.57	.20
82	EDB	Not Analyzed	Not Analyzed	.56	No LDH
83	EDB	2	2	.55	.43
84	EDB	Not Analyzed	Not Analyzed	.59	.55
85	Control	3	3	No LDH	No LDH
86	Control	Not Analyzed	Not Analyzed	No LDH	No LDH
87	Control	Not Analyzed	Not Analyzed	No LDH	No LDH
88	ANE	3	3	.33	.45
89	ANE	Not Analyzed	Not Analyzed	.46	.58
90	ANE	5	3	.33	.29
91	ANE	2	2	.64	.28
92	EDA	Not Analyzed	Not Analyzed	No LDH	No LDH
93	EDA	3	2	.56	.25
94	EDA	4	3	.41	.53
95	EDA	2	2	.57	.38
96	EDA	Not Analyzed	Not Analyzed	No LDH	No LDH
97	EBA	Not Analyzed	Not Analyzed	.60	.51
98	EBA	2	2	.57	.46
99	EBA	2	2	.76	.58
100	EBA	3	2	.53	.47
101	EBD	2	2	.63	.04
102	EBD	2	2	.47	.40
103	EBD	Not Analyzed	Not Analyzed	.58	.58
104	EBD	Not Analyzed	Not Analyzed	.51	.60
105	EBD	3	2	.28	.57