A COMPARATIVE STUDY OF THE INDUCTION OF CARDIAC MYOPATHIES IN RATS BY SELECTED STRESSORS

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ABSTRACT

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Experiments which have been done at Michigan State University with rats on the effects of exercise on the heart have hitherto been considered individually and thus little solid information has come forth. This thesis is an attempt to combine, compare and contrast three such studies which have been completed. In order to gain a better perspective in this area an extensive review of the related literature was needed. The results of this review confirmed the fact that more extensive experimentation is needed in this particular area.

The first experiment considered was "Anxiety, Activity and the Genesis of Heart Disease." This study involves shocking of the animals in an attempt to simulate an anxiety situation, thus causing cardiac damage. The results showed that the duration of the treatment period was important in the production of degenerative heart damage. The second experiment considered was the "Injection

Study." It was shown that animals injected with epinephrine subsequently would develop cardiac necrosis. This experiment was used to check histochemical procedures and was a repeat of work done by Bajusz and Raab. Our results were essentially the same. The third study considered was the "Prepubertal Study" where the animals were force exercised and myocardial lesions were produced.

Cardiac damage in all three studies was found to be strikingly similar thus suggesting a common etiology. Two very interesting results have come from these studies. The first from the "Anxiety, Activity and the Genesis of Heart Disease" suggested the role of anxiety in the pathogenesis of heart disease and the second from the "Prepubertal Study" suggested that the age at which strenuous exercise is attempted (very young or very old) may actually be a causal factor in heart disease.

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By

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Dedication

This thesis is dedicated to my parents, Daniel W. and Dorothy Litwhiler, whose love and understanding is forever appreciated.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

For the past several years, the people in the Cytology Laboratory of the Department of Anatomy and the Human Energy Laboratory at Michigan State University have directed considerable effort to studies on the effects of exercise upon the cardiovascular system in general, and to the heart in particular.

Although several separate investigations have been conducted, very little solid information related to the effects of exercise upon the heart has come forth. However, within the past year, one study indicated that from 25 to 30% of both experimental and control animals exhibited some degree of alteration in heart musculature after a physical exercise program conducted for 35 days in adult rats from 247 to 285 days of age.

These results not only renewed the interest of the investigators but they also made several points very clear:

a) The literature pertinent to coronary heart diseases needed to be reviewed extensively in order to determine where the results obtained in our laboratory fit into the total picture.

b) All previous studies should be reviewed for any evidence which would be helpful in structuring new investigations in this area.

c) A series of pilot studies should be carried out to obtain a firm grasp on the techniques needed to successfully complete any new proposals.

The items mentioned here serve as the basis for the report which follows and will be considered in their proper order.

CHAPTER II

REVIEW OF LITERATURE

Early descriptions of the clinical signs and anatomic findings connected with coronary thrombosis were published by Hammer (42), Leyden, and Huchard as reported by Raab (102). The full importance of these studies was not realized, however, until Herrick (50, 51), and Keefer and Resnik (62), emphasized the role of myocardial oxygen lack in coronary artery disease. For several decades, nearly all degenerative and functional disturbances of heart muscle were attributed to impairments of coronary blood flow.

The presence of coronary artery lesions, in relation to myocardial infarcts certainly justify the designation of coronary artery disease as the causal factor. At the same time, this terminology disregards the "noncoronary" mechanisms which determine the degree of "vulnerability" of the heart muscle to coronary artery problems. This was emphasized by Fulton (34), when at autopsy he found necrotic foci and infarctions without any obstruction of the local coronary arteries. Blumgart and his colleagues (12) observed acute myocardial infarctions without coronary occlusion in 27 per cent of their autopsy material. Ehrlich and Shinohara (28) failed to detect occlusions in more than

one-half of 29 infarcted hearts. Master (75, 76) called attention to endocardial necrotic lesions in the absence of coronary artery occlusion. In 6 out of 25 cases with disseminated and sometimes grossly visible subendocardial necroses, Horn and associates (55) found arterial involvement missing or only minimal. Myocardial lesions similar to those seen in human coronary heart disease have been produced in animals with normal coronary vessels, in large numbers and by a variety of experimental procedures.

From the foregoing information it is evident that myocardial lesions do occur either with or without coronary impairment. Under these circumstances, the routine application of the term "coronary heart disease" is misleading and ignores those pathophysiologic and metabolic factors which are co-responsible for the degree of "vulnerability" of the myocardium to degenerative heart diseases.

In 1966 Raab noted that from information gathered in the past decade "the problem of the pluricausal vulnerability of heart muscle to disease should be given prime consideration." That is, attention should be directed to "neurogenic and hormonal, and functional and metabolic factors which seem to be responsible for heretofore unexplained myocardial necrosis." (104) These factors fall into three categories and will be discussed under their respective headings as: (a) pathophysiological, (b) epidemiologic and (c) preventive measures.

Pathophysiologic

In order that we may better understand the factors involved in heart disease, we should first look at the causes or the pathogenesis of hypoxic (coronary or ischemic) heart disease. The forms of hypoxic heart disease can be divided into two categories of derangement: (a) vascular structure and (b) myocardial metabolic problems. Vascular structural problems refer to coronary atherosclerosis with resultant mechanical interference in oxygen supply to the heart muscle. The factors responsible for such a condition are heredity, diet and hypertension. The second category (myocardial metabolic problems) refers to an increased myocardial vulnerability to coronary atherosclerosis with neurogenic and hormonal biochemical interference in oxygen consumption (102). The etiologic agents of this condition are socio-economic and environmental stress, lack of physical activity (exercise) and smoking (nicotine). The condition resulting from these factors has been described as "myocardial oxygen-wasting adrenosympathetic over-activity" (100, 102, 104, 105, 107).

Physical inactivity and emotion play an important role in the genesis of heart disease and a brief description of the role of each would be helpful in understanding the problem.

Lack of physical activity weakens the heart and muscles by disuse while inherent urbanized overstimulation,

such as is found in Western civilization today, causes emotional disturbances (64).

Habitual physical inactivity is associated with a progressing deficiency of the sympathoinhibitory and vagal mechanisms which normally keep cardiac chronotropic (rate) and inotropic (contractability) levels within certain limits during exercise and at rest (15, 65, 102). The characteristics of the "loafer's heart" (98) with high sympathetic tone and high rate, short isometric contraction periods, proneness to develop hypoxic ECG changes during exercise and low efficiency are the opposite of the characteristics of the well-trained athlete's heart with its low sympathetic tone and high efficiency (65).

One of the main problems involved in heart disease involves sympathetic over-activity in the overstimulated individual. In an overstimulation environment, the individual is almost always in an alert reaction state. When this situation is found where there is no commensurate muscular action, the effects on the heart can be compared with the "racing" of a non-moving car's engine (64, 102).

Thus, man today lives in a potentially pathogenic environment, that is, where he is over-rested, overfed, overstimulated, over-protected, underexercised, and under-released. These conditions result in suppressed release (fight and flight), exaggerated response (conditioned reflex) and stress to the endocrine and central nervous

systems which finally lead to a number of possible physical (endocrine, cardiovascular, metabolic, gastrointestinal and musculo-skeletal)as well as emotional (neurosis, anxiety, depression, compulsion and maladjustment) diseases due to the tension state of the individual (64).

Psychologic stress, tension and the pace of modern life has shown related effects on serum cholesterol and urinary excretion rates of vanilmandelic acid, a metabolite of epinephrine and norepinephrine(46, 64, 74, 96, 97, 101, 113, 122). It has been shown that myocardial necrosis can be produced in domesticated and wild rats by sensory and emotional stress which could be compared to an anxiety state (106).

It is evident that the pathogenesis of heart disease involves many factors which are related in various ways (115). The purpose of epidemiological studies is to try to discover the various risk factors involved in the genesis of heart disease, particularly the factor of activity.

Epidemiologic

One of the main functions of epidemiological research is to determine the relationship between heart disease and physical activity of work. It is now evident that men and women in active occupations requiring greater physical exertion appear to have lower rates of fatal coronary disease than those in sedentary occupations (11, 14, 17,

18, 24, 41, 52, 56, 78, 81, 85-88, 120, 125, 126, 131, 134, 135). A brief review of some of the studies will aid in clarifying the relationship between heart disease and physical activity of work.

Morris, in his studies in England, found that London bus drivers had twice as many fatal heart attacks as conductors who were required to climb stairs many times a day (87, 88) and that postal clerks (classified as sedentary workers) showed a higher rate of attacks when compared with postmen who walked most of the day (88). Morris also observed 2 1/2 million skilled, semi-skilled and unskilled workers and found a greater incidence of fatal heart attacks among the occupations involving little physical activity (88). In another study, autopsies on 4,000 coronary deaths revealed about twice as many scars, indicating more cardiac damage, in those who have been engaged in light occupations (86).

In a fifteen year period the frequency to ischemic heart disease was surveyed in 5,279 men and 5,229 women, aged 40 to 64 living in an Israeli kibbutzim under uniform living conditions. Socioeconomic differentiation did not exist in this situation and the population included many persons of high intelligence. Under such conditions, many of the emotional and mental factors which play an important role in ischemic heart disease were also eliminated. The only remaining factor, which is the differential

factor, is the nature of the individual's work (17). The results of this study showed that the frequency of anginal syndrome, myocardial infarction and fatalities due to ischemic heart disease was 2.5 to 4 times higher in the sedentary than in the nonsedentary workers. Also, women in this society were heavy workers and they showed little incidence of ischemic heart disease. The study concluded that habitual physical activity should be considered a valuable principle in the primary and secondary prevention of ischemic heart diseases (17).

In another study (13) an unusually low death rate from myocardial infarction was found in the Italian-American community of Roseto, Pennsylvania when compared with the surrounding communities. Naturally, question arose as to what was responsible for the lower incidence in Roseto. Of the three possibilities suggested in the study (genetic, ethnic factors or social patterns) the correlations with social patterns seemed to best apply. Apparently, the low incidence of myocardial fatality in Roseta was related to the way of life which is vigorous and fun loving. This again suggests the possible importance of anxiety in the genesis of heart disease.

In the Framingham Study (25, 57) the habitual level of physical activity was compared to the risk of developing coronary heart disease. It was found that the most sedentary observations were particularly liable to fatal

heart attacks, but not to angina pectoris whether physical activity was assessed by ventricular rate, by vital capacity or by history (physical activity index). (Appendix B, table 1).

A summary of these and other such population studies tends to tentatively lead to the following conclusions (30).

1. Those who are in physically active groups have fewer manifestations of coronary heart disease than those who are not (31, 52, 121, 130).

2. The amount of physical activity needed to decrease the risk of coronary heart disease may be acceptable to many.

3. If it is to have a beneficial influence, increased physical activity must be continued throughout life. Past training and adaptation do not have a long term prophylatic effect.

4. Benefits of exercise are associated with a reduction in the incidence and severity of infarcts rather than a marked difference in the degree of atherosclerosis (regular exercise lowers oxygen requirement by decreasing sympathetic activity (99, 101, 105). Dietary changes may improve some aspects of the "high-risk" syndrome, increased physical activity may improve others, and some may respond to joint management of many influences (69, 103).

5. The enigmatic relationship of occupational activity and angina pectoris challenge us to further study (high rate of anginal syndrome even in heavy working populations) (48, 86, 87, 132).

6. Although we have no definite facts concerning the possible prophylaxis in coronary heart disease, there have been many suggestions as to these mechanisms, some of which might only operate through an increase in physical activity (99, 103, 104, 139).

Although population studies have contributed greatly to our understanding of heart disease, two important points should be kept in mind when reviewing epidemiological data. The first point is that more definite information about the factors that influence the selection of occupations and recreation is needed (why did the postman decide to be a postman instead of a clerk?). The second point involves the levels of activity involved in each type of activity. No universal standards have been accepted pertaining to the assessment of different levels of work and certainly the arbitrary designation of "low, medium, and high" levels are not sufficient to permit accurate evaluations (3, 104).

Coronary Risk Factors

One means of determining the importance of various factors in the pathogenesis of heart disease is through the designation of various risk factors. Risk factors

are those abnormalities, demonstrable in persons without clinical coronary heart disease, known to be associated with significantly increased risk of developing the disease in subsequent years (112, 124, 141). Some of these factors are as follows:

1. Persons with hypercholesterolemia, hyperbetolipoproteinemia and hypertriglyceridemia in middle age experience 3 to 4 times as many heart attacks as those with normal cholesterol levels (1, 21, 24, 49, 58, 59, 122, 123).

   a. The amount of cholesterol deposited in the arteries is proportionate to the amount in the blood (29).

2. Hypertension is a second risk factor but this is not a universal phenomenon (21, 84, 122-124).

3. Diabetes mellitus (124).

4. Obesity (21, 124).

5. Cigarette smoking (124).

6. Heredity (35).

7. Diets high in calories, fats, saturated fats, cholesterol, sugars and salt (124). The relationship between this and the first four risk factors is evident and the practical implications of these findings are obvious in terms of prevention. (Appendix B, table 2).

8. Physical inactivity, sedentary living and habitual lack of exercise (32). A population study looked at the mortality experienced in the four weeks following the

onset of the initial clinically documented myocardial infarction in man and found that there was a higher rate of mortality during that time among men classified as "least active" at the time of the infarction. The mortality with initial myocardial infarction in relation to physical activity showed that death among the "least active" was almost 3 times that of the most active (33). (Appendix B, table 3). The same study noted that there seems to be a relationship between physical activity off the job and mortality from first myocardial infarction.

9. Psychological stress, tension, high drive personality, behavior pattern, and the pace of life as a risk factor. As mentioned before, this has a related effect on serum cholesterol levels of urinary excretion rates of catecholamines and vanilmandelic acid (46, 64, 74, 96, 97, 101, 124).

Preventive Measures

In order to understand the possible relationship of physical activity and exercise and heart disease we can look at many studies showing the effects of exercise on the heart.

One interesting study by Selye (116) showed that certain types of acute experimental cardiac necrosis can be prevented by pretreatment with stressors such as physical exercise. He noted that animals conditioned by pretreatment with electrolytes (Na_2HPO , Na_2SO_4) and steroids

(corticoids) in doses ineffective by tehmselves, with subsequent exposure to stress (running in a drum) invariably develop massive infarct-like cardiac necrosis. However, animals which were preconditioned by running in the drum showed no cardiac necrosis after treatment. Selye attributed this to a condition he called simple resistance since the pretreatment was the same as the stressor used. He also noted that animals preconditioned (running in a drum) then given treatment (steriods or electrolytes), then exposed to the stress of bone facture (breaking the legs) showed no damage, however, in those animals which were not preconditioned, cardiac necrosis was again found. Selye stated that this condition was due to cross resistance since the preconditioning was different than the stressor used. (Appendix B, tables 4a and 4b).

Eckstein (26, 27) carried out one of the most interesting studies done on the effects of exercise on blood supply to the heart. He showed that ischemic collateral vascularization of the heart could be developed through exercise after partial and complete ligation of one of the coronary arteries. In his experiment, he noted that dogs restricted to their cages for 6 to 8 weeks after survival of mild constriction of the circumflex coronary arteries showed no increase in vascularization, but, in the animals that were exercised during that period, a much greater collateral circulation developed. He also presented evidence

that a significant pressure differential and decreased myocardial oxygen tension are necessary for collateral growth following arterial narrowing.

The results of this study would tend to encourage middle-aged human persons with possible "silent" or "symtomless" coronary disease, where the sclerotic process has begun to narrow the arteries, to exercise (27). Since Eckstein's work on coronary circulation showed a possible improvement of blood supply to the heart, many other investigators have continued such experiments with interesting results (66, 110, 129, 140).

Some studies have shown that physical training reduces the excess oxygen utilization of the myocardium by decreasing sympathetic activity (83, 101, 113). Cardiac sympathetic adrenergic manifestations (accelerated heart rate, shorter isometric period, relatively prolonged systole) at rest and during exposure to standard sensory and mental stresses (noise, flicker light, mental arithmetic) are more pronounced in emotionally unstable and sedentary individuals than in placid and physically active ones (109).

Several reports have indicated there is a decrease in electrocardiographic abnormalities through physical training (47, 68, 79, 80). Training results in slower cardiac rate with high voltage of QRS complexes T and U waves. The picture is characteristic of right and left

ventricular enlargement without evidence of cardiac disease (10). There is also ischemic RS-T segment depression which may be the result of increased coronary vascularization, improved myocardial efficiency, changes in peripheral distribution of blood and its return or decreased pressure on flow requirements (30). Age increase in pulse wave velocity occurs in trained persons to a lesser degree than untrained ones, suggesting a larger preservation of vascular elasticity (89).

Many investigations into electrocardiograph, metabolic and hemodynamic changes produced by training tend to show that habitual physical exercise increases total efficiency and myocardial efficiency (6, 43, 54, 67, 68, 77, 83, 86, 90, 95, 114, 119, 128, 133, 136, 138).

In one study (33) fourteen sedentary men were put on a program of two months of hard training with the resulting effects:

1. Improved working capacity (also 43).
2. Increased heart volume.
3. Increased stroke volume by 14%.
4. Cardiac output at rest was slightly higher (probably a direct result of increased stroke volume).
5. Heart rate reduced an average of 7 beats per minute.
6. Left heart work was unchanges but there was a smaller oxygen cost.

Other studies have attempted to show the relationship between exercise and serum lipid levels. As stated earlier, the amount of cholesterol deposited in the arteries is proportionate to the amount in the blood (29). The results from numerous investigations in regard to the response of blood fatty acids and cholesterol to acute or prolonged physical exercise are contradictory (36, 37, 53, 64, 71, 91, 111, 117, 124, 132). In addition, it has been shown that exercise (treadmill running) after doubling caloric intake, with resultant rise in cholesterol level, did not lower the cholesterol level (70). Even though the effect of a six month program of endurance exercise on serum lipids showed no significant change in mean serum cholesterol or phospholipids, there was a significant decrease in serum triglyceride from a pretraining level of 208 mg/100 ml to 125 mg/ml at the end of the study (Appendix B, table 5). The experimental group included 15 sedentary, middle-aged men (118). The same study indicated that serum cholesteral levels decreased during a period of weight loss but it always returned to its original level.

One experiment related to the effect of physical activity on postprandial (after meal) serum lipid levels of triglyceride, non-esterified fatty acids and cholesterol after a fatty meal (55 g. milk fat) showed a significant difference in controls and experimentals for serum-triglyceride and fatty acids but not for cholesterol (93).

The exercise consisted of a 16 km. march. Thus, it is felt that the high levels of serum-triglyceride and non-esterified fatty acid, following a high fat meal, can be significantly reduced. The question remains in these studies as to the value of serum triglyceride levels as a predictor of clinical coronary heart disease. There is some evidence that levels of serum cholesterol are valuable in predicting heart disease but not serum triglycerides (131).

Cureton (23) has concentrated on the use of exercise as a protective measure in degenerative heart disease. He has found that the most effective form of exercise is a progressive program, consisting of rhythmic endurance exercises. That is, rhythmic activity 30-60 minutes a day, five to six times a week combined with emphasis on adequate breathing (activities such as swimming, walking, jogging, running, skating and skiing) (22, 23, 38).

One other area of preventive measures which deserves attention is that of"reconditioning" centers (2). Much work has been done in the area of preventive cardiology in reconditioning centers all over Europe. The purpose is to provide systematic physical training, breathing exercises, relaxation periods and the emotionally equilibrating influence of scenic environments. Extensive statistical data is yet lacking in most of these areas, however, in most situations these combined environmental-emotional and physical cardiac preventive programs have a favorable

effect on the autonomic and cardiovascular systems (4, 9, 16, 44, 63, 89, 108, 137). It has been shown that persons who have suffered myocardial infarction can participate successfully in an exercise program (8, 20, 45, 48, 60, 82, 90-92, 136).

From a review of the literature it is obvious that exercise is not the whole answer to preventive cardiology, but effective prevention can be expected from: Limitation of dietary saturate fats and supplementation of poly-unsaturated fats; environmental, emotional and sensory relaxation; abstinence from smoking; and regular vigorous physical activity (104).

CHAPTER III

METHODOLOGY

Under ordinary conditions, this section of a research paper is confined to the methods and procedures pertinent to the experiment at hand. The present situation is unusual, however, in that several projects are being reviewed at the same time. Therefore, the experimental procedures for each study are being recorded separately:

Anxiety, Activity and the Genesis of Heart Disease

This study was designed to attack a variety of questions related to the pathogenesis of ischemic heart disease:

(1) Is physical activity alone capable of producing necrotic heart lesions?

(2) Is it possible to bring about emotion-induced myocardial necroses?

(3) Is the acquisition of cardioprotective resistance against unaccustomed severe stresses by preceding stresses of a lesser degree (e.g., exercise, electrical stimulation) a feasible mechanism for reducing myocardial damage?

The following animal groups were set up to investigate these questions.

S--A sedentary control group (each animal was housed in an individual sedentary cage).

SF--A sedentary forced-exercise group (each animal housed in a sedentary cage but forced to swim for 30 minutes per day, five days per week, with 2 per cent of his body weight attached to the base of his tail).

SA--A sedentary anxiety group (each animal housed in a sedentary cage and subjected to a disturbing but noninjurious and nonpainful shock every 15 seconds for 30 minutes per day, five days per week).

SFA--A sedentary forced-exercise plus anxiety group (each animal housed in a sedentary cage and receiving both swimming and electrical-stress treatments, five days per week).

V--A voluntary control group (each animal housed in an individual voluntary cage).

VF--A voluntary forced-exercise group (each animal housed in a voluntary cage and receiving the swimming treatment only, five days per week).

VA--A voluntary anxiety group (each animal housed in a voluntary cage and receiving electrical-stress treatment, five days per week).

VDA--A voluntary forced-exercise plus anxiety group (each animal housed in a voluntary cage and receiving both swimming and electrical-stress treatments, five days per week).

At the conclusion of the experimental period, the animals were sacrificed, the hearts were excised immediately and prepared for histologic analysis. At autopsy, specimens were obtained by transventricular ablation 10 mm from the apex of the heart. The ablated portion was sliced transversely into two equal portions. Both slices were fixed in 10% buffered formalin, dehydrated, embedded in paraffin and cut at 6 microns. The resulting histologic sections from both slices were stained with hematoxylin and eosin and McFarland's trichome stain for connective tissue. Sections were also stained with Oil Red O and Sudan Black B solutions for fat localization as well as PAS solution for glycogen localization (Appendix A).

Subsequent microscopic examination of the sections provided the opportunity to evaluate the incidence and extent of cardiac damage. All slides were studied without previous knowledge as to which experimental group they belonged. Similar evaluation was performed by a qualified pathologist.

Although the results of this study were reported to be negative, it must be remembered that the histologic procedures used for slide analysis were basically of a gross nature. In addition, it was possible that the duration of the electrical stress was not sufficient to produce myocardial damage.

These two problems were solved by a series of pilot projects with (1) application of more sensitive histochemical

techniques and (2) alterations in electrical-stress procedures (anxiety treatment).

In the early studies (1966), the animals were placed in individual electrical-stress cages for 30 minutes per day, five days per week for ten weeks. During each electrical-stress period, they received a .36 m sec. D. C. electrical shock of 1.5ma every 15 seconds.

In the pilot project, the electrical stress cages were converted to "live-in" cages. The animals were housed in these cages for two consecutive weeks. Food and water were supplied ad libitum. A timing device was constructed which could expose the animals to a variable number of hours of electrical stress per day. Experimentation showed that a total of about nine hours of electrical stress per day, broken into seven intervals of unequal length, induced sufficient anxiety to produce mild to moderate heart damage. In each shock interval, the animals received a .36 m sec. D. C. electrical shock of 1.5 ma five times per minute. The time between shocks ranged from 7 to 16 seconds.

The reader of this thesis may be interested in other studies involving electrical stimulation as a stressor and so I have included a few in my bibliography. Kaye, McDonald and Randall found that systolic hypertension and subendocardial hemorrhages could be produced by stimulation of the stellate ganglion (61), and Manning, Hall and Banting (72)

as well as Groover and Stout (39) found that myocardial damage could be produced through vagus stimulation.

Histological and Histochemical Analyses

It is possible to detect early or marginal heart damage by sensitive histochemical techniques. Therefore, at sacrifice, approximately four minutes after the administration of pentobarbital and before the animal ceased respiration, the heart was removed. Four blocks of tissue were obtained by transventricular ablation. One block was fixed in 10% buffered formalin, dehydrated in graded alcohols and embedded in paraffin. Sections 6 microns thick were cut from the block and stained with hematoxylin and eosin. Three blocks were quick frozen in isopentane cooled with liquid nitrogen. Serial sections 10 microns thick, were cut on a cryostat and fan dried. Four sections were stained with hematoxylin and eosin while additional sections were subjected to the following histochemical procedures:

1. Succinic dehydrogenase (SDH) activity was demonstrated using 2,2'-di-p-nitrophenyl -5,5'-diphenyl 3,3'-(3,3'-dimethoxy--4,4'biphenylene) ditetrozolium chloride (NBT) as described by Barka and Anderson (7).

2. Mitochondrial α-glycerophosphate dehydrogenase (menadione-linked α-GPD) activity was demonstrated by the Pearse technique (94) using menadione with NBT.

3. Cytochrome oxidase (Cyt-O) activity was demonstrated by the method of Burstone (19) which employs p-aminodiphenylamine (Variamine blue RT) and 8-amino--1, 2, 3, 4--tetrahydroquinaline in hydroxymethyl aminomethane (tromethanine or Tris) buffer at a pH of 7.4.

4. Monoamine oxidase (MAO) activity was demonstrated by the method of Glenner, et al. with NBT, as described by Barka and Anderson (7).

5. B-hydroxybuterate dehydrogenase (B-OH DH) activity was demonstrated, as described by Barka and Anderson (7), using NBT with a Tris buffer at a pH of 7.4.

Epinephrine Injections and Myocardial Necrosis

It has been mentioned that studies from this laboratory designed at producing a break down in heart tissue with the application of physical exercise and electrically induced stress have been negative. In this regard, it was also noted that the stressors may not have been strong enough to incur damage. Likewise, the evaluating techniques may not have been sensitive enough to detect minor or marginal damage. Several pilot studies have shown that neither of the factors were true, however, one other possibility did exist. That is, the histochemical techniques being very critical in nature may not have been conducted in such a way as to show any alterations that were present.

The histochemical techniques were checked out in a study where 30 animals were injected with epinephrine to

elicit myocardial necrosis. Experiments of this nature have been reported by several authors, however, the most suitable of these was by Bajusz and Raab in 1966 (5).

Doses of 225, 112, 56 and 36 ugm/100 gm of body weight, in 0.2 ml of physiologic saline, were injected subcutaneously. All animals receiving doses of 225 and 113 µgm/100 gm of body weight were sacrificed 48 hours after injection. Animals receiving 56 and 36 µgm/100 gm body were killed at intervals of 48, 96, and 144 hours after injection. Histologic and histochemical procedures were carried out in the same manner as mentioned earlier.

Prepubertal Forced Exercise and Myocardial Lesions

To this author's knowledge, there are no reports available which indicate that myocardial necrosis is either precipitated or prevented by physical exercise (running or swimming) per se. This information stimulated the design of an experiment which would in part at least get at the problem. The specific objective of the task was to determine if exercise early in life plays a preventive role in the incidence of myocardial lesions.

The design of this experiment, known as the "prepubertal exercise study" is noted on the following page for the convenience of the readers.

One hundred seventy animals arrived in the laboratory as weanlings (23 days old) and were placed in voluntary

Design--Prepubertal Exercise Study

Experimental Treatment I	Post-Experimental Period	Experimental Treatment II
35 days	125 days	35 days
Sedentary (N=50)	All animals put into spontaneous exercise cages	Original Condition (N=10) Forced Exercise (=40)
Voluntary Activity (N=50)		Original Condition (N=10) Forced Exercise (N=40)
Forced Activity (N=50)		Sedentary Condition (N=10) Forced Exercise (N=40)

activity cages for one week. The last 3 days activity was recorded and the animals were matched into 50 trios on the basis of activity. This permitted the elimination of excessively active or inactive animals.

During the first experimental treatment period, 50 animals were housed in sedentary cages and allowed no activity other than that available in this comparatively restrictive setting. Fifty animals in the voluntary activity group were housed in spontaneous exercise cages. A like number of animals were housed in spontaneous exercise cages, but in addition were forced to swim 30 minutes per day with a weight equal to 2 per cent of their body weight attached to their tails.

At the end of the first experiment period, all animals were dispatched to spontaneous exercise cages for an

additional 182 days. At this time, the animals were again separated as indicated by their original three groups of 50 animals each to enter a second experimental treatment period. However, only 10 animals of each group were retained as they were during the first experimental treatment period. The remaining 40 animals of each group lived in spontaneous exercise cages and were forced to swim 30 minutes per day with the 2 per cent body weight added. At sacrifice, paraffin sections of the hearts were prepared and processed by conventional methods as described earlier.

CHAPTER IV

RESULTS AND DISCUSSION

In the previous chapter the methods pertaining to several different studies were presented. The results of these studies will be recorded and discussed in accordance with our present knowledge of the subject.

Anxiety, Activity and the Genesis of Heart Disease

Initial examination of the heart slides revealed no obvious lesions or pathologic changes in individual cardiac fibers. A subsequent pathologists' report was of a similar nature. The apparent absence of lesions as a result of the experimental treatments was in contradiction to investigations of a similar nature and led us to believe that the stress was not sufficient in time and/or degree to elicit the type of changes observed by others. Any changes that were noted are found routinely in autopsies of "normal" animals, and were therefore believed to be due to individual variations in animals or to inadequate histologic techniques.

The problems relating to the time and/or duration of electrical stress and inadequate histologic procedures were solved by a series of pilot studies.

Pilot Studies

1. Nine hour live in (electrical-stress):

Experimentation showed that if rats were housed for two consecutive weeks in cages wired so the animals could be shocked a variable number of hours (in this case, nine hours) per day, sufficient anxiety was created to produce mild to moderate heart damage (figures 1, 2). These results were indeed gratifying and indicated that the duration of subjection to electrical stress was important in the production of degenerative heart changes. In addition, the changes were easily detected by suitable histochemical as well as by less sensitive histologic procedures.

2. Epinephrine injection studies:

Several animals were injected with epinephrine as described by Bajusz and Raab (5), in order to check on our histochemical methods. Our results (figures 3-5) were essentially the same as those obtained by Bajusz and Raab and indicated that our laboratory procedures were not in error. Furthermore, it emphasized the fact that the type and duration of exposure to electrical stress were critical factors and therefore responsible for the negative results in earlier studies.

3. Prepubertal forced exercise and myocardial lesions:

Lesions of varying severity and location were detected in microscopic sections (figures 6-9). In most instances the reactions were localized in the endocardium (figures

6, 8), however a few lesions were present in the myocardium as well (figures 7, 9). The focus shown in figure 6 is minimal when compared to the advanced stage of scar tissue formation seen in figure 9.

If these three studies are looked at separately their results fall into the category of "interesting." However, if they are considered collectively, several important perspectives are evident.

The same basic features with respect to lesion formation are evident in the hematoxylin and eosin sections from each study (figures 1, 3, 6-9). The muscle fiber destruction represented by the epinephrine injection study (figure 3) is not new, since it only repeats the work of others (5). But, the fact that these results are so similar to those from studies which have not been reported before (electrical-stress, figure 1 and prepubertal forced exercise, figures 6-9) is not only striking but highly suggestive.

For example, it seems reasonable to assume that the intermittent electrical shocks over a nine hour period, brought about a reflex liberation of catecholamines which under certain circumstances exert a noxious effect on the metabolism of heart muscle (100). Another possible explanation involves the interplay of corticoid hormones and catecholamines. Adrenocorticoid hormone secretion is increased with emotional and sensory stresses, and, the sensitizing action of these substances greatly enhance the necrotizing action of catecholamines to myocardial injure (100).

If physical exercise can be regarded as just another type of stressor then it could be reasoned that similar pathways might be responsible for the varieties of heart damage noted in this study. Such an explanation does not seem so simple, however, and in fact, to this author's knowledge there are no reports available which indicate the production of heart damage by physical exertion alone. Although the evidence from this study is by no means conclusive (and is being repeated with more controls), in light of other investigations, it does offer some challenging suggestions.

Selye (116) has indicated "that certain stressful experiences (exercise, cold bath, etc.) can actually protect the heart against infarction." He went on to show that rats pretreated with electrolytes and steroids and then exposed to forced exercise, died with massive infarctions within 24 hours. Animals which were similarly treated but forced to exercise both before and after treatment showed no evidence of cardiac necrosis.

By comparison, our study revealed that 37% of the animals which were forced to exercise 35 days before and after a 182 day period of rest, elicited some degree of myocardial involvement (figures 6-9). Although the results of these two experiments are clearly different it must be pointed out that:

a) Although Selye did not record the age of the animal he used it is assumed they were adults. In

contrast our animals started the program at 23 days of age.

b) Likewise the length of Selye's experimental period was not stated but was taken to be of short duration. The animals in this study were carried a total of 252 days.

c) Finally, the conditioning treatment used by Selye was electrolytes and corticoids whereas it was a 182 day period of rest in the present study.

What may be drawn from the similarities and dissimilarities in these two studies is conjectural at this point. Nevertheless, it seems highly probable that the age of the animals, length of the experimental period and types of conditioning treatments used may individually or collectively affect the results.

APPENDIX A

HISTOCHEMICAL STAINING PROCEDURES

I. General procedure for Histochemical Techniques.

A. Obtain liquid nitrogen and isopentane (freezing medium).

B. Remove heart, cut with new raxor into section "A" (apex) and "b" (midheart or left and right ventrical).

Use 7% Gum Tragacanth as mounting medium--set tissue into gum.

Orient on block so that good sections can be obtained.

C. Plunge block (with tissue on it) into isopentane which has been made viscous by the liquied nitrogen. Isopentane has been cooled to -160 degrees C. Tissue should not be left in isopentane more than 10 seconds. Place block into cryostat at temperature of -25 degrees C.

D. Histochemical sections are good for one to three days.

E. To section, raise cryostat temperature to -20 degrees C.

F. The sectioning was done on an Ames Lab-Tek cryostat with an anti-roll plate.

G. Set microtone to cut sections 10 microns thick.

H. Air or fan dry sections 15 to 30 minutes.

II. Histochemical Staining Procedures.

A. Alpha-glycero-phosphate- "Menadione" linked (94).

1. Incubating Medium

0.2M Tris buffer (7.4 pH)	10 ml.
Menadione	4 mg.
NBT 1mg/ml	10mg.
Alpha-G-P	30 mg.

2. Procedure

(a) Incubate 30-60 minutes at 37 degrees C.
(b) Distilled H_2O rinse
(c) Acetones 30%-60%-90%-60%-30%
(d) Distilled H_2O wash
(e) Mount in glycerine jelly

B. Beta-hydroxybuterate dehydrogenase (B-OH DH) (7)

1. Incubating Medium

0.2M Tris buffer (7.4 pH)	10 ml.
DL-OH-butyrate (sodium salt) *pH 7.0-7.2	1.0 ml.
DPN	10 mg.
$MgCl_2$ 0.05M	1.0 ml.
NBT 4mg/ml.	12 mg.
NaA_3 0.1M	0.2 ml.

2. Procedure

(a) Incubate 30 minutes to one hour at 37 degrees C.
(b) Distilled H_2O wash
(c) Mount in glycerine jelly

C. Cytochrome Oxidase (19)

1. Incubating Medium

1-Hydroxy-2 napthoic acid	2 mg.
N-Phenyl-P-phenylene diaine	2 mg.
Reagent ethanol (95%)	0.1 ml.
Distilled H_2O	7 ml.
Tris buffer (.2M, pH 7.4)	3 ml.

Shake and Filter

2. Procedure

(a) Incubate 15 to 60 minutes at room temperature.
(b) Fix for one hour in 1% cobalt acetate in 10% formalin.
(c) Distilled H_2O wash.
(d) Mount in glycerone jelly.

D. Monoamine Oxidase (MAO) (7)

1. Incubating Medium

Tryptamine-HCl	25 mg.
Na_2SO_4	4 mg.

NBT	5 mg.
0.1M Phosphate buffer (pH 7.6)	5 ml.
Distilled H_2O	15 ml.

2. Procedure

(a) Incubate 30 to 45 minutes at 37 degrees C.
(b) Distilled H_2O rinse
(c) 10% Formaline 1 to 2 hours
(d) Mount in glycerine jelly

E. Succinic dehydrogenase (SDH) (7)

1. Incubating Medium

Sodium succinate 0.06M	2.0 ml.(32 mg.)
NBT 0.2% 10mg/5ml.	5 ml.
P buffer 0.2M (7.4 pH)	2 ml.
Ringers solution	1 ml.
Menadione	6.9 mg.

2. Procedure

(a) Incubate 20 minutes at 37 degrees C.
(b) Distilled H_2O wash
(c) Mount in glycerine jelly

III. Histologic Staining Procedures

A. Hematoxylin and eosin (H & E)

1. Allow sections to air dry on coverslips
2. Fix in 10% formalin 10 to 15 minutes
3. Wash in tap water five minutes
4. Harris Hematoxylin (filtered) five minutes
5. Distilled H_2O rinse
6. .25% HCl for 10 seconds
7. Tap water wash for 5 minutes
8. Dehydrate 95%-100% alc.-xylene
9. Mount in permount

B. Oil Red O (94)

1. Allow sections to air dry on coverslips
2. Formalin 10 minutes
3. Dip in 70% ROH - one second
4. Place in Oil Red O; tightly closed container- 10 minutes
5. Wash quickly in 70% ROH
6. Wash in H_2O
7. Harris Hematoxylin - one minute

8. Wash in H_2O
9. Mount in glycerine jelly

C. Periodic Acid Shiff (PAS) (127)

1. Have glassware acid clean
2. Air dry sections
3. .5% aqueous periodic acid - five minutes
4. Distilled H_2O wash
5. Shiff's solution - seven to eight minutes
6. Wash in tap water for 10 minutes
7. Dehydrate
8. Mount in permount

PAS - Diastase modification

Section placed on coverslip prepared with albumin - otherwise sections will fall off. Allow to set overnight.

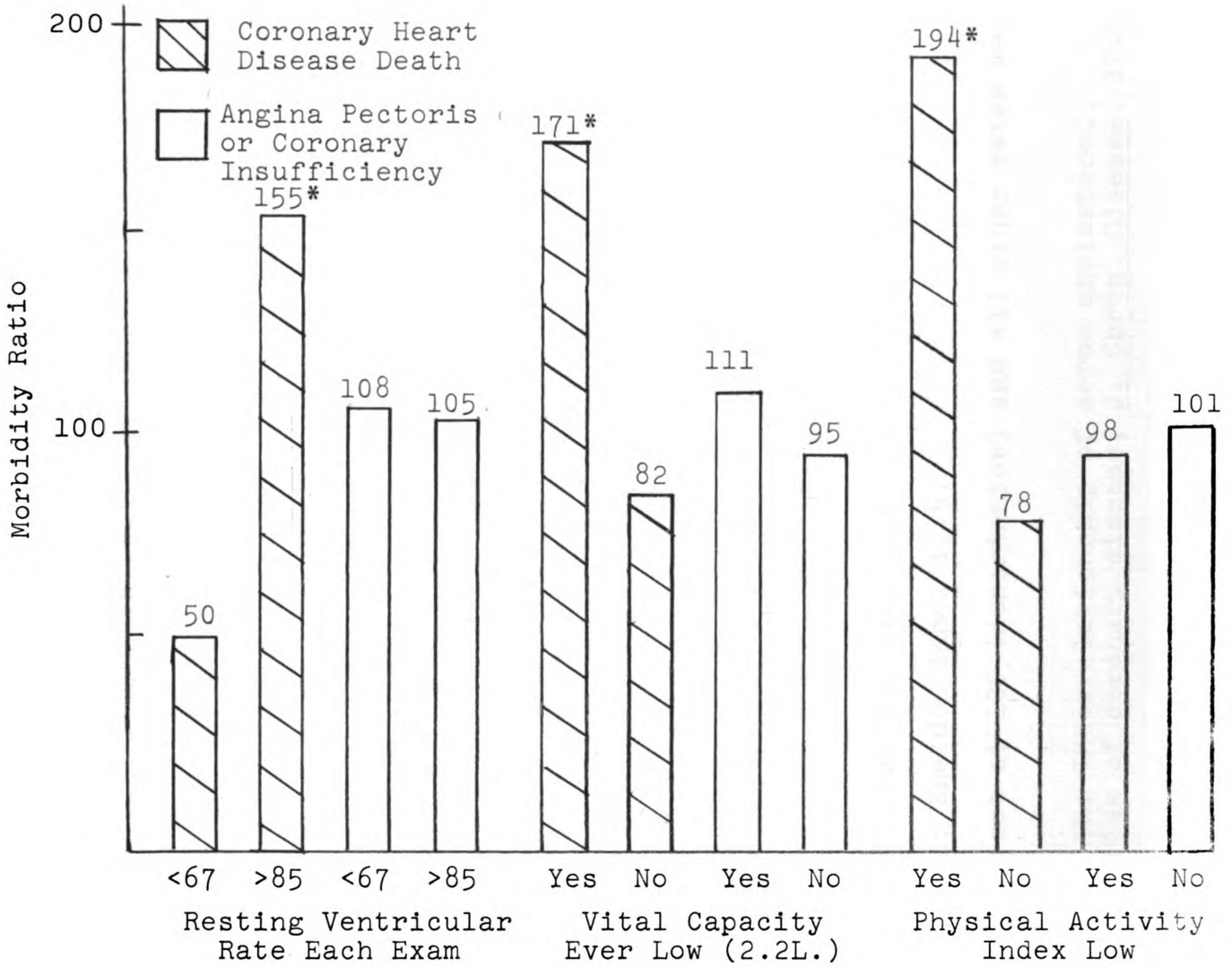
D. Sudan Black B (73)

1. Allow sections to air dry on coverslips as usual
2. Fix in 10% formaline for 15 minutes
3. Wash
4. Absolute propylene glycol - 10 minutes
5. Sudan Black B - 45 minutes
6. 85% Propylene glycol - 3 minutes
7. Distilled H_2O wash
8. Tap H_2O wash
9. Mount in glycerine jelly

E. Trichrome - McFarland (for frozen sections) (40)

1. Harris Hematoxyline - 3 to 4 minutes
2. Tap H_2O - 5 minutes
3. Picro-Acid fuchsin - 3 minutes
4. 2% Acetic acid - rinse
5. Red differentiator - 5 minutes
6. Tap H_2O - 5 minutes
7. Aniline Blue - 2 minutes
8. 2% Acetic acid - rinse
9. Blue differentiator - 5 minutes
10. Acetic acid .2% - 2 minutes
11. Tap H_2O - 10 minutes

APPENDIX B



*Significant different from standard risk $p < .05$.

TABLE 1.--Rise of manifestations of coronary heart disease, classified according to various indices of physical activity. Men and women, aged 30-62 at entry: Framingham Heart Study.[a]

[a]From W. B. Kannel, Habitual level of physical activity and risk of coronary heart disease: The Framingham Study, Canad. Med. Assoc. Journ., 96:811, 1967.

TABLE 2.--Relationship between findings for three factors (blood pressure, serum cholesterol and relative weight) and 11 year incidence of coronary heart disease and myocardial infarction, Los Angeles Civil Servants Study.[a]

Blood Pressure*	Serum Group Cholesterol**	Relative Weight†	No. of Men	CHD Incidence Rate/ 1,000	CHD Incidence Ratio % ††	Myocardial Infarction Incidence Rate/ 1,000	Myocardial Infarction Incidence Ratio %
-	-	-	254	47.2	100	23.6	100
-	-	H	152	52.6	111	32.9	139
-	H	-	342	105.3	223	70.2	297
-	H	H	281	138.8	294	85.4	362
H	-	-	54	129.6	274	37.0	157
H	-	H	97	134.0	284	61.9	262
H	H	-	112	169.6	359	98.2	416
H	H	H	207	207.7	440	106.3	450
Any 1 of 3 high			548	93.1	197	56.6	240
Any 2 of 3 high			490	144.9	307	83.7	355
Any 2 of 3 or all three high			697	163.6	347	90.4	383
All			1.499	118.1	250	66.7	283

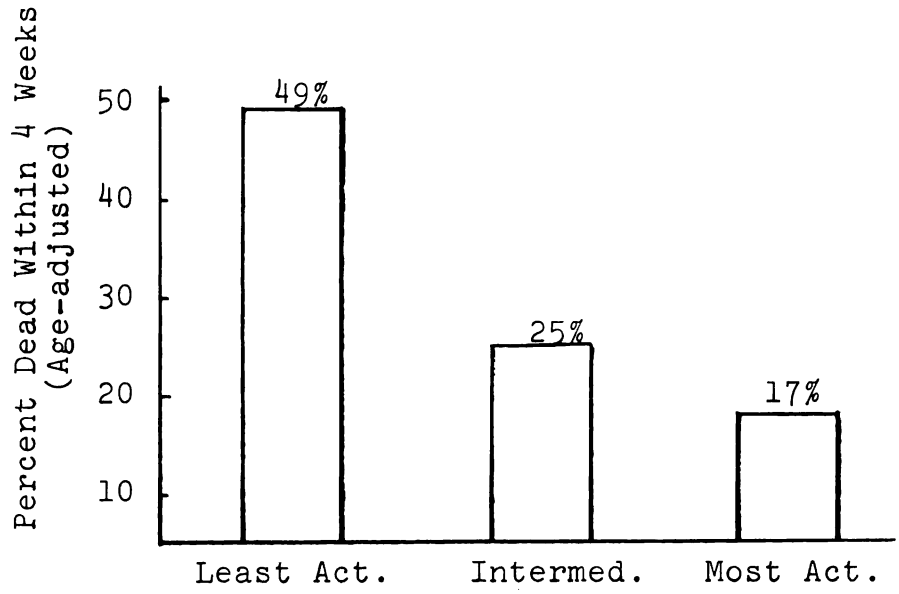
*H in blood pressure is a systolic blood pressure > 140 mm. Hg and diastolic blood pressure > 90 mm. Hg.

**H in serum cholesterol is 270 mg./100 ml.

†H in relative body weight is Sheldon index 12.5.

††Rate for no-risk group was set at 100 (denominator) and all other rates were compared with it.

[a]From J. Chapman and F. Massey, The Interrelationship of serum cholesterol, hypertension, body weight and risk of coronary disease, J. Chron. Disease, 17:933, 1964.



(Difference is significant at the 0.01 level [Least-Most])

TABLE 3.--Physical activity and early mortality among men after first myocardial infarction[a]

[a]C. W. Frank, E. Weinblatt, S. Shapiro, and R. V. Sager, Physical inactivity as a lethal factor in myocardial infarction among men, Circulation, Vol. 34, Dec. 1966, pp. 1022-1033.

TABLE 4a.--Simple resistance in prevention of cardiac necrosis.

	Exercise
Period of Humoral Conditioning	Cardiac Necrosis
Exercise	Exercise
Period of Humoral Conditioning	No Necrosis

TABLE 4b.--Cross resistance in prevention of cardiac necrosis.

	Bone Fracture
Period of Humoral Conditioning	Cardiac Necrosis
Exercise	Bone Fracture
Period of Humoral Conditioning	No Necrosis

Tables 4a and b are from Selye, H. The role of stress in the production and prevention of experimental cardiopathies. In Prevention of Ischemic Heart Disease. Ed. by Raab, Springfield: Thomas, 1966.

TABLE 5.--Mean serum lipid values before and after six months of endurance training.[a] (MG/100ML)

	Experimentals		Controls	
	Oct.	Apr.	Oct.	Apr.
Cholesterol				
1. Bloor, Pelkan and Allen method	272	275	267	279
2. Abell-Kendal method	249	257	249	262
Phospholipids	290	296	289	290
Triglycerides	208	125*	157	146

*$p < 0.01$.

aFrom Skinner and others. Effects of a six-month program of endurance exercise on work tolerance, serum lipids and ULF-ballistocardiograms of fifteen middle-aged men. In Phys. Act. and the Heart, edited by Karvonen and Berry (Springfield: Thomas, 1967).

FIGURES

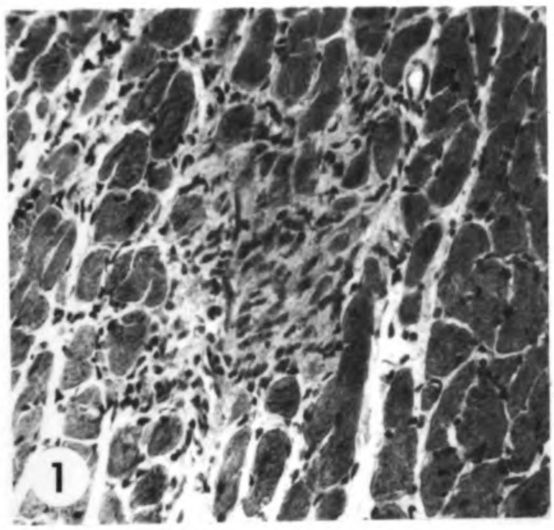
Figure 1. Rat myocardium of nine hour live-in anxiety study. Hematoxylin and eosin. X 165.

Figure 2. Same area as Figure 1, demonstrating lack of succinic dehydrogenase activity. SDH stain. X 180.

Figure 3. Myocardium of rat involved in injection study showing diverse damage. Hematoxylin and eosin. X 205.

Figure 4. Same area as Figure 3, demonstrating lack of beta-hydroxybuterate dehydrogenase. B-OH DH stain. X 180.

Figure 5. Same area as Figures 3 and 4 demonstrating lack of succinic dehydrogenase activity. SDH stain. X 195.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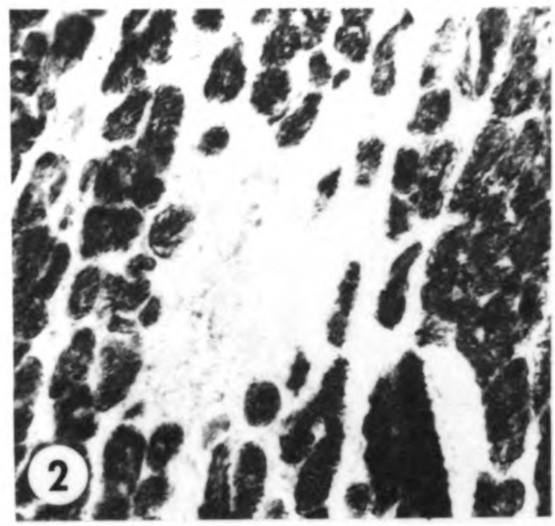
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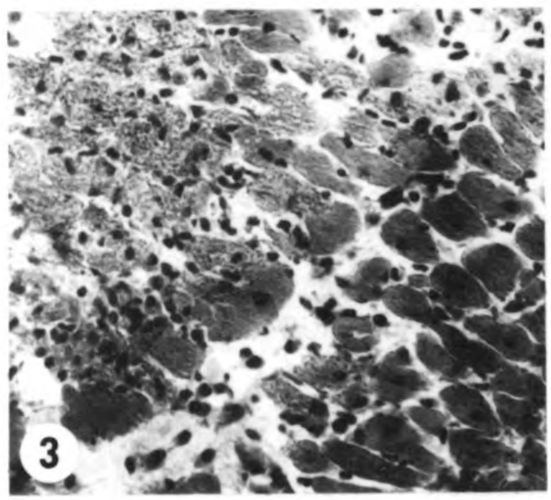
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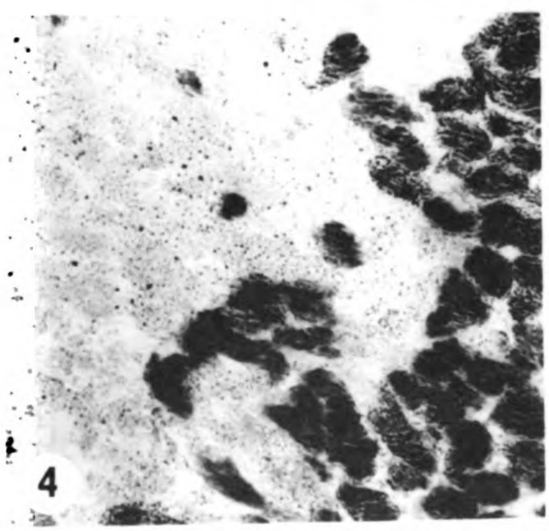
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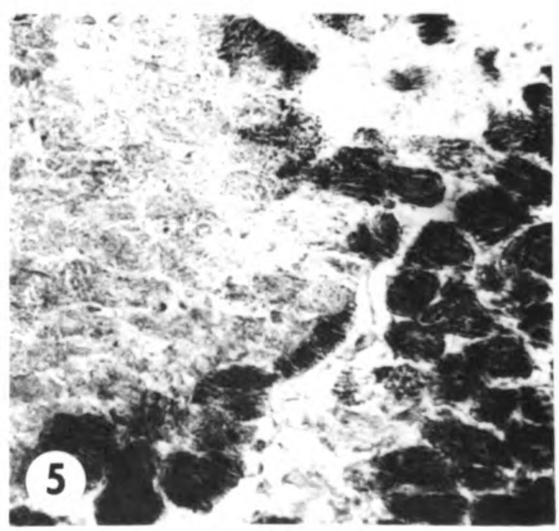
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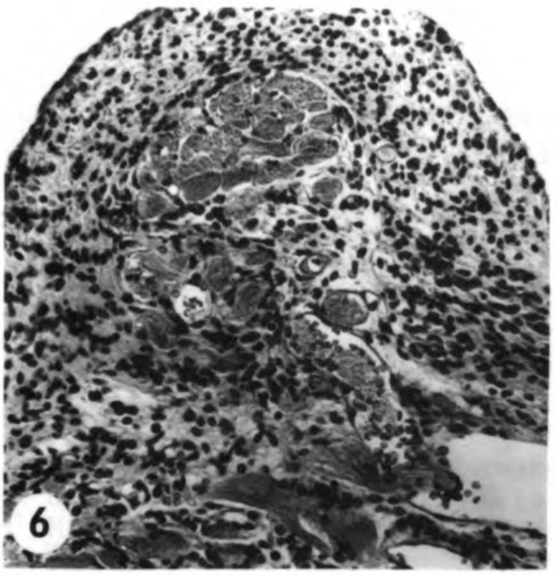
Figure 6. Diverse damage in endocardium (left ventrical) of rat in Wilson's study. Hematoxylin and eosin. X 180.

Figure 7. Recent heart damage in myocardium of rat from Wilson's study. Hematoxylin and eosin. X 180.

Figure 8. Scar tissue in myocardium of rat from Wilson's study. Hematoxylin and eosin. X 165.

Figure 9. Older scar tissue in endocardium of rat from Wilson's study. Hematoxylin and eosin. X 185.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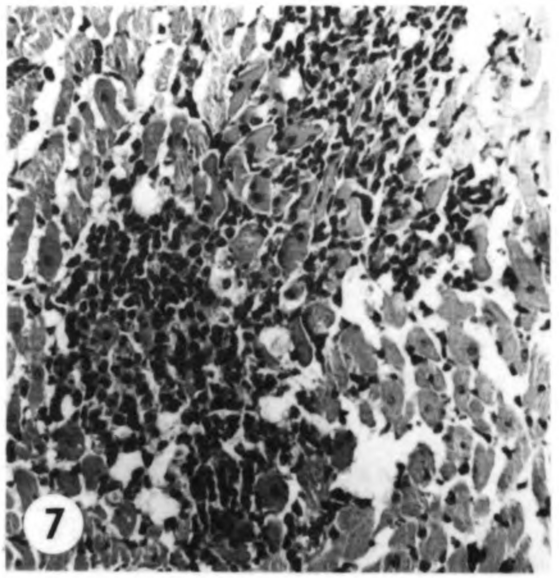
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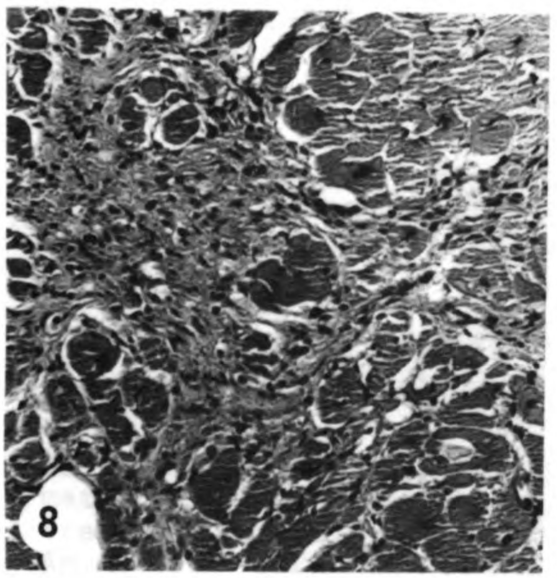
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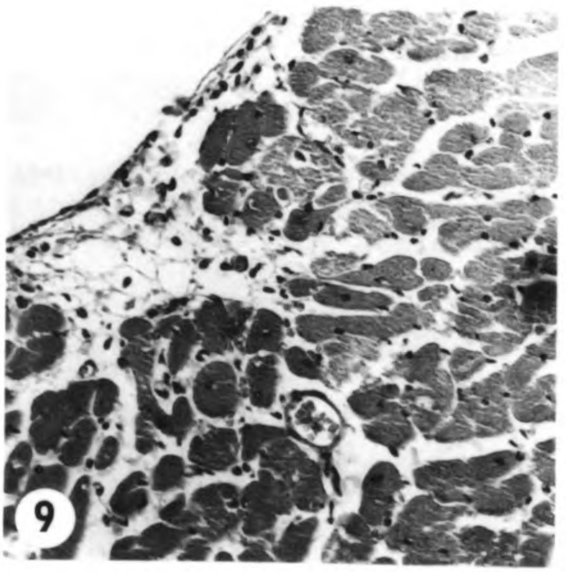
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BIBLIOGRAPHY

1. Albrink, M. Triglycerides, lipoproteins and coronary artery disease. Arch. Int. Med. 109: 345, 1962.

2. American Medical Association. Postwar prosperity, cardiovascular disease, and reconditioning centers. Journ. Am. Med. Asso. 185: 49, 1963.

3. Astrand, P. O. Concluding remarks: Physical activity and cardiovascular health. Canad. Med. Asso. Journ. 96(1): 907, 1967.

4. Badal, J. Preventive measures against cardiovascular diseases in Czechoslovakia. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

5. Bajusz, E. and W. Raab. Early metabolic aberrations through which epinephrine elicits myocardial necrosis. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

6. Balke, B. and R. T. Clark. Cardiopulmonary and metabolic effects of physical training. Health and Fitness in the Modern World. pp. 82-89, Chicago: The Athletic Institute, 1961.

7. Barka, T. and P. J. Anderson. Histochemistry Theory, Practice and Bibliography, New York: Harper and Row, 1963.

8. Barry, A. J., J. W. Daly, E. D. R. Pruett, J. R. Steinmetz, N. C. Birkhead and K. Rodahl. Effects of physical training in patients who have had myocardial infarction. Am. Journ. Cardiol. 17: 1, 1966.

9. Beckmann, P. The combined environmental-emotional and physical cardiac reconditioning programs. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

10. Beckner, G. L. and T. Winsor. Cardiovascular adaptations to prolonged physical effort. Circulation. 9: 835, 1954.

11. Biorck, G., G. Blomquist and J. Sievers. Studies on myocardial infarction in Malmo 1935-1954; II. Infarction rate by occupational group. Acta Med. Scand. 21: 159, 1958.

12. Blumgart, H. L., M. J. Schlesinger and P. M. Zoll. Angina pectoris, coronary failure and acute myocardial infarction. The role of coronary occlusion and collateral circulation. Journ. Am. Med. Asso. 116: 91, 1941.

13. Brandt, E. N., C. Stout, J. W. Hampton, T. N. Lynn and S. Wolf. Coronary heart disease among Italians and non-Italians in Roseto, Pennsylvania, and nearby communities. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

14. Breslow, L. and P. Buell. Mortality from coronary heart disease and physical activity of work in California. Journ. Chronic Disease. 11: 421, 1960.

15. Brouha, L. Effect of work on the heart. In Work and the Heart, F. F. Rosenbaum and E. L. Belknap (eds.), New York: P. B. Hoeber, Inc., 1959.

16. Bruce, R. A., L. B. Rowell, J. R. Blackmon and A. Doan. Cardiovascular function tests. Heart Bulletin 14: 9, 1965.

17. Brunner, D. The influence of physical activity on incidence and prognosis of ischemic heart disease. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

18. Brunner, D. and G. Manelis. Myocardial infarction among members of communal settlements in Israel. Lancet. 2: 1049, 1960.

19. Burstone, M. S. Modifications of histochemical techniques for demonstration of cytochrome oxidase. Journ. Histochem. Cytochem. 9: 59, 1961.

20. Chapman, C. B. and R. S. Fraser. III. Cardiovascular responses to exercise in patients with healed myocardial infarction. Circulation. 9: 347, 1954.

21. Chapman, J. and F. Massey. The interrelationship of serum cholesterol, hypertension, body weight and risk of coronary disease. Journ. Chronic Disease. 17: 933, 1964.

22. Cureton, T. K. The nature of cardiovascular conditioning and protection by exercise fitness programs. Journ. Phys. Educ., 61: 30, 1963.

23. Cureton, T. K. The relative value of various exercise programs to protect adult human subjects from degenerative heart disease. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

24. Dawber, T. R. and W. B. Kannel. Susceptibility to coronary heart disease. Mod. Conc. Cardiov. Dis. 30: 671, 1961.

25. Dawber, T. R., W. B. Kannel and G. D. Friedman. Vital capacity, physical activity and coronary heart disease. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

26. Eckstein, R. W. Effect of exercise and coronary arterial narrowing on growth of interarterial coronary anastomoses. Fed. Proc. 15: 54, 1956.

27. Eckstein, R. W. Effect of exercise and coronary artery narrowing on coronary collateral circulation. Circ. Res. 5: 230, 1957.

28. Ehrlich, J. C. and Y. Shinohara. Low incidence of recent thrombotic coronary occlusion in hearts with acute myocardial infarction studied by serial block technique. Circulation. 26: 710, 1962.

29. Firstbrook, J. B. Factors influencing the atherosclera-tic process. Circulation. 2: 464, 1950.

30. Fox, S. M. and W. L. Haskell. Population studies. Canad. Med. Asso. Journ. 96: 806, 1967.

31. Fox, S. M. and J. S. Skinner. Physical activity and cardiovascular health. Amer. Journ. Cardiology. 14 (6): 731, 1964.

32. Frank, C. W., E. Weinblatt, S. Shapiro and R. V. Sager. Physical inactivity as a lethal factor in myocardial infarction among men. Circulation. 34: 1022, 1966.

33. Frick, M. H., A. Kontinen and H. S. Sarajas. Effects of physical training on circulation at rest and during exercise. Am. Journ. Cardiology. 12: 142, 1963.

34. Fulton, W. F. M. Chronic generalized myocardial ischaemia with advanced coronary artery disease. Brit. Heart Journ. 18: 341, 1956.

35. Gertler, M. M. Ischemic heart disease, heredity and body build as affected by exercise. Canad. Med. Asso. Journ. 96: 728, 1967.

36. Golding, L. Effect of physical training upon total serum cholesterol levels. Res. Quart. 32: 499, 1961.

37. Goode, R. C., J. B. Firstbrook and R. J. Shephard. Effects of exercise and cholesterol-free diet on human serum lipids. Canad. Journ. Physiol. Pharmacol. 44: 575, 1966.

38. Gottheiner, V. Strenuous sports as treatment for degenerative heart disease. In Prevention of Ischemic Heart Disease. W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

39. Groover, M. E. and C. Stout. Neurogenic myocardial necrosis. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

40. Guyer, M. F. Animal Micrology, Chicago: University of Chicago Press, 1953.

41. Hames, C. G., J. R. McDonough, S. C. Stulb, and G. E. Garrison. Physical activity and ischemic heart disease among Negroes and whites in Evans County, Georgia. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

42. Hammer, A. Thrombosis of one of the coronary arteries of the heart diagnosed during life. Canad. Journ. Med. Sc. Toronto, 3: 353, 1878.

43. Hellerstein, H. K. A primary and secondary coronary prevention program--in progress report. In Prevention of Ischemic Heart Disease. W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

44. Hellerstein, H. K. Reconditioning and the prevention of heart disease. Mod. Med. May 11, p. 266, 1964.

45. Hellerstein, H. K. and A. B. Ford. Comprehensive care of the coronary patient. Circulation. 22: 1160, 1960.

46. Hellerstein, H. K. and A. B. Ford. Rehabilitation of the cardiac patient. Journ. Am. Med. Asso. 164(3): 225, 1957.

47. Hellerstein, H. K., A. Burlando, E. Z. Hirsch, F. H. Plotkin, G. H. Feil, O. Winkler, S. Marik and N. Margolis. Active phsyical reconditioning of coronary patients. Circulation. 32(2): 110, 1965.

48. Hellerstein, H. K., T. R. Hornsten, A. Goldbarg, A. G. Burlando, E. H. Friedman, E. Z. Hirsch and S. Marik. The influence of active conditioning upon subjects

with coronary artery disease: A progress report. Canad. Med. Asso. Journ. 96: 901, 1967.

49. Hernberg, S. Correlation between physical working capacity and serum cholesterol in leading businessmen. In Physical Activity and the Heart, M. J. Karvonen and A. J. Barry (eds.), Springfield: Charles C. Thomas, 1967.

50. Herrick, J. B. Angina pectoris. Journ. Am. Med. Asso. 70: 67, 1918.

51. Herrick, J. B. Clinical features of sudden obstruction of coronary arteries. Journ. Am. Med. Asso. 59: 2015, 1912.

52. Holloszy, J. O. The epidemiology of coronary heart disease: National differences and the role of physical activity. Journ. Am. Geriat. Soc. 11:718, 1963.

53. Holloszy, J. O., J. S. Skinner and G. Toro. Effects of a six month program of endurance exercise on the serum lipids of middle-aged men. Am. Journ. Cardiol. 14: 753, 1964.

54. Holloszy, J. O., J. S. Skinner, G. Toro and T. K. Cureton, Jr. Effect of physical conditioning on cardiovascular function. Am. Journ. Cardiol. 14: 761, 1964.

55. Horn, H., L. E. Field, S. Dack and A. M. Master. Acute coronary insufficiency; Pathological and physiological aspects. Am. Heart Journ. 40: 63, 1950.

56. Kahn, H. A. The relationship of reported coronary heart disease mortality to physical activity of work. Am. Journ. Pub. Health. 53: 1058, 1963.

57. Kannel, W. P. Habitual level of physical activity and risk of coronary heart disease: The Framingham Study. Canad. Med. Asso. Journ. 96: 811, 1967.

58. Kannel, W. B., T. R. Dawber, G. D. Friedman, W. E. Glennon and P. M. McNamara. Risk factors in coronary heart disease: An evaluation of several serum lipids as predictors of coronary heart disease. The Framingham Study. Ann. Internal Med. 61: 888, 1964.

59. Kannel, W. B., T. Dawber, A. Kagar, N. Revotskie and J. Stokes. Factors of risk in the development of coronary heart disease - six year follow-up experience. The Framingham Study. Ann. Internal Med. 55: 33, 1961.

60. Kattus, A. A. Exercise conditioning of cardiovascular systems--effects on cardiac patients. Presented June 25, 1966 at the Am. Heart Asso. Meeting Exercise and the Heart, Seattle, Washington.

61. Kaye, M., R. McDonald and W. C. Randall. Systolic hypertension and subendocardial hemorrhages produced by electrical stimulation of the stellate ganglion. Circ. Res. 9: 1164, 1961.

62. Keefer, L. S. and W. H. Resnik. Angina Pectoris: A syndrome caused by anoxemia of the myocardium. Arch. Internal Med. 41: 709, 1928.

63. Kirchhoff, H. W. Cardiovascular function before and after reconditioning periods in a mountainous terrain. In Prevention of Ischemic Heart Disease. W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

64. Kraus, H. Preventive aspects of physical fitness. In Prevention of Ischemic Heart Disease. W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

65. Kraus, H. and W. Raab. Hypokinetic Disease. Springfield: Charles C. Thomas, 1966.

66. Laurie, W. and J. D. Woods. Coronary interarterial anastomoses. Am. Heart Journ. 65: 579, 1963.

67. Leon, A. S. and C. M. Bloor. Effects of exercise and its cessation of cardiac blood supply. Clinical Research. 15(2): 212, 1967.

68. Letunov, S. P. and R. E. Motylyanskaya. Preventive significance of exercise training in elderly adults. In Prevention of Ischemic Heart Disease. W. Raab (ed.). Springfield: Charles C. Thomas, 1966.

69. Levine, S. A. Exercise and heart disease. Atlantic Monthly. 212: 42, 1963.

70. Mann, G. V., K. Teel, O. Hayes, A. McNally and D. Bruno. Exercise in the disposition of dietary calories, regulation of serum lipoproteins and cholesterol levels in human subjects. New Eng. Journ. Med. 253: 349, 1955.

71. Mann, G. V., R. D. Shaffer, R. S. Anderson and H. H. Sandstead. Cardiovascular disease in the Masai. Journ. Atheroscler. Res. 4: 289, 1964.

72. Manning, G. W., G. E. Hall and F. G. Banting. Vagus stimulation and the production of myocardial damage. Canad. Med. Asso. Journ. 37: 314, 1937.

73. Manual of Histologic and Special Staining Techniques, Armed Forces Institute of Pathology, Washington, D. C., 1957.

74. Mascittell-Coriandoli, E., R. Boldrini and C. Citterio. Cardiac damage caused by experimental stress. Nature. 181: 1215, 1958.

75. Master, A. M. Survival and rehabilitation in coronary occlusion. Am. Journ. Cardiol. 7: 340, 1961.

76. Master, A. M., H. L. Jaffe, L. E. Field and E. Donoso. Acute coronary insufficiency: Its differential diagnosis and treatment. Ann. Internal Med. 45: 561, 1956.

77. Mastropaolo, J. A. Exercise conditioning of cardiovascular systems--Active intervention in Chicago, Presentation given June 25, 1966 at the American Heart Asso. meeting Exercise and the Heart. Seattle, Washington.

78. Mastropaolo, J. A., J. Stamler, D. Berkson and N. Jackson. Physical activity of work, physical fitness and coronary heart disease in middle-aged Chicago men. In Physical Activity and the Heart. M. J. Karvonen and A. J. Barry (eds.), Springfield: Charles C. Thomas, 1966.

79. Mazzarella, J. A. and J. O. Evans. Effects of interval training on the exercise electrocardiogram. Circulation. 34(3): 165, 1966.

80. Mazzarella, J. A. and J. W. Jordan, Jr. Effects of physical training on emotional myocardial ischemia in middle-aged men. Circulation. 32(2): 147, 1965.

81. McDonough, J. R., C. G. Hames, S. C. Stulb, and G. E. Garrison. Coronary heart disease among Negroes and whites in Evans County, Georgia. Journ. Chronic Disease. 18: 443, 1965.

82. McPherson, B. D. and A. Paivio. Psychological effects of an exercise program for post infarct and normal adult men. Journ. Sports Med. and Physical Fitness. 7: 61, 1967.

83. Mellerowicz, H. The effect of training on heart and circulation and its importance in preventive cardiology. In Prevention of Ischemic Heart Disease. W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

84. Miall, W. E. and P. D. Oldham. Factors influencing arterial blood pressure in the general population. Clin. Sci. 17: 409, 1958.

85. Morris, J. N. Health and social class. Lancet. 1: 303, 1959.

86. Morris, J. N. and M. D. Crawford. Coronary heart disease and physical activity of work. Brit. Med. Journ. 2: 1485, 1958.

87. Morris, J. N., J. Heady and P. Raffle. Physique of London busmen. Lancet. 2: 569, 1956.

88. Morris, J. N., J. Heady, P. Raffle, C. G. Robert and J. W. Parks. Coronary heart disease and physical activity of work. Lancet. 2: 1053 and 1111, 1953.

89. Myasnikov, A. L. Prevention of hypertensive disease and of ischemic heart disease in the Soviet Union. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

90. Naughton, J. Cardiopulmonary responses during physical training in patients who have recovered from myocardial infarction. In Prevention of Ischemic Heart Disease. W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

91. Naughton, J. and J. F. McCoy. Observations on the relationship of physical activity to the serum cholesterol concentration of healthy men and cardiac patients. Journ. Chronic Disease. 19: 727, 1966.

92. Naughton, J., G. Sevelius and B. Balke. Physiological responses of normal and pathological responses to a modified work capacity test. Journ. Sports Med. 3: 201, 1963.

93. Mikkila, E. A. and A. Konttinen. Effect of physical activity on postprandial levels of fats in serum. Lancet. 1: 1151, 1962.

94. Pearse, A. G. E. Histochemistry: Theoretical and Applied. Boston: Little, Brown and Co., 1961.

95. Pyorala, K., M. J. Karvonen, P. Taskinen, J. Takkunen and H. Kyronseppa. Cardiovascular studies on former endurance athletes. In Physical Activity and the Heart. M. J. Karvonen and A. J. Barry (eds.), Springfield: Charles C. Thomas, 1967.

96. Raab, W. Andrenergic-cholinergic control of cardiac metabolism and function. In Advances in Cardiology, S. Karger (ed.), New York: Basel, 1956.

97. Raab, W. Key position of catecholamines in functional and degenerative cardiovascular pathology. Am. Journ. Cardiol. 5: 571, 1960.

98. Raab, W. Loafer's heart. A. M. A. Arch. Internal Med. 101: 194, 1958.

99. Raab, W. Metabolic protection and reconditioning of the heart muscle through habitual physical exercise. Ann. Internal Med. 53: 87, 1960.

100. Raab, W. Neurogenic multifocal destruction of myocardial tissue. Revue Canadienne de Biologie. 22: 217, 1963.

101. Raab, W. The neurogenic metabolic factor in ischemic heart disease. Pathogenesis and prevention. Dis. Chest. 46: 150, 1964.

102. Raab, W. The nonvascular metabolic myocardial vulnerability factor in coronary heart disease. Am. Heart Journ. 66: 685, 1963.

103. Raab, W. Organized Prevention of Heart Disease, Vermont: Queen City Printers, 1962.

104. Raab, W. Prevention of Ischemic Heart Disease, Springfield: Charles C. Thomas, 1966.

105. Raab, W. The sympathogenic biochemical trigger mechanism of angina pectoris. Its therapeutic suppression and long-range prevention. Am. Journ. Cardiol. 9: 576, 1962.

106. Raab, W., J. P. Chaplin and E. Bajusz. Myocardial necrosis produced in domesticated rats and in wild rats by sensory and emotional stress. Proc. Exp. Biological Med. 116: 665, 1964.

107. Raab, W., P. DePaula, E. Silva, H. Marchet, E. Kimura and Y. K. Starcheska. Cardiac adrenergic preponderence due to lack of physical exercise and its pathogenic implications. Am. Journ. Cardiol. 5: 300, 1960.

108. Raab, W. and L. B. Gilman. Insurance-sponsored preventive cardiac reconditioning centers in West Germany. Am. Journ. Cardiol. 13: 670, 1964.

109. Raab, W. and H. J. Krzywanek. Cardiac sympathetic tone and stress response related to personailty patterns and exercise habits. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

110. Roberts, J. T., J. T. Wearn and J. J. Badal. The capillary-muscle ratio in normal and hypertrophied human hearts. Proc. for Soc. Exp. Biol. and Med. 38: 322, 1938.

111. Rochelle, R. H. Blood plasma cholesterol changes during a physical training program. Res. Quart. 32: 538, 1961.

112. Rosenman, R. H., M. Friedman, R. Straus, M. Wurm, R. Kositchek, W. Hahn and N. T. Werthessen. A predictive study of coronary heart disease. Journ. Am. Med. Asso. 189(1): 15, 1964.

113. Russek, H. I. Emotional stress and the etiology of coronary artery disease. Am. Journ. Cardiol. 2: 129, 1958.

114. Scott, J. C. Physical activity and the coronary circulation. Canad. Med. Assoc. Journ. 97: 853, 1967.

115. Selye, H. Pluricasual Cardiopathies, Springfield: Charles C. Thomas, 1961.

116. Selye, H. The role of stress in the production and prevention of experimental cardiopathies. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

117. Shane, S. R. Relation between serum lipid, and physical conditioning. Am. Journ. Cardiol. 18: 540, 1966.

118. Skinner, J. S., J. O. Holloszy, G. Toro, A. J. Barry and T. K. Cureton. Effects of a six-month program of endurance exercise on work tolerance, serum lipids and ULF-ballistocardiograms of fifteen middle-aged men. In Physical Work and the Heart, M. J. Karvonen and A. J. Barry (eds.), Springfield: Charles C. Thomas, 1967.

119. Smodlaka, V. Interval training in cardiovascular disease. In _Prevention of Ischemic Heart Disease_. W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

120. Spain, D. M. and V. A. Bradess. Occupational physical activity and the degree of coronary atherosclerosis in "normal" men. A post mortem study. _Circulation_. 22: 239, 1960.

121. Spain, D. M. and V. A. Bradess. Relation of sex, age and physical activity to sudden death from coronary arterial occlusion. In _Work and the Heart_, F. F. Rosenbaum and E. L. Belknap (eds.), New York: P. B. Hoeber, 1959.

122. Stamler, J. Athersclerotic coronary heart disease--the major challenge to contemp. public health and preventive medicine. _Conn. Med_. 28: 9, 1964.

123. Stamler, J. The epidemiology of atherosclerotic coronary heart disease. _Postgrad. Med_. 25: 610, 685, 1959.

124. Stamler, J. _Preventive Cardiology_. New York: Grune and Stratton, 1967.

125. Stamler, J., M. Kselsberg, Y. Hall and N. Scotch. Epidemiological studies of cardiovascular-renal diseases: I. Analysis of mortality by age-race-sex-occupation. _Journ. Chronic Disease_. 12: 440, 1960.

126. Stamler, J., H. A. Lindburg, D. M. Berkson, A. Shaffer, W. Miller and A. Poindexter. Prevalence and incidence of coronary heart disease in strata of the labor force of a Chicago industrial corporation. _Journ. Chronic Disease_. 11: 405, 1960.

127. Stein, J. M. and H. A. Padykula. Histochemical classification of individual skeletal muscle fibers of the rat. _Am. Journ. Anat_. 110: 103, 1962.

128. Steinhaus, A. H. Chronic effects of exercise. _Physiol. Rev_. 13: 103, 1933.

129. Stevenson, J., V. Feleki, P. Rechnitzer and J. R. Beaton. Effect of exercise on coronary tree size in the rat. _Circ. Res_. 15: 265, 1964.

130. Taylor, H. Coronary heart disease in physically active and sedentary populations. _Journ. Sports Med. and Phys. Fitness_. 2: 73, 1962.

131. Taylor, H. L. The occupational factors in the study of coronary heart disease and physical activity. Canad. Med. Asso. Journ. 96: 825, 1967.

132. Taylor, H. L., J. Anderson and A. Keys. Physical activity, serum cholesterol and other lipids in man. Proc. Soc. Exper. Bio. and Med. 95: 383, 1957.

133. Taylor, H. L., E. Buskirke and A. Henschell. Maximum oxygen intake as an objective measure of cardio-respiratory performance. Journ. Appl. Physiol. 8: 73, 1955.

134. Taylor, H. L., E. Klepetar, A. Keys, W. Parlin, H. Blackburn and T. Puchner. Death rates among physically active and sedentary employees of the railroad industry. Am. Journ. Public Health. 52: 1697, 1962.

135. Taylor, H. L., E. Klepetar, A. Keys, W. Parlin, H. Blackburn and T. Puchner. Death rates among physically active and sedentary employees of the railway industry. Circulation. 22: 822, 1960.

136. Varnauskas, E., H. Bergman, P. Houk and P. Bjorntorp. Hemodynamic effects of physical training in coronary patients. Lancet. 2: 8, 1966.

137. Venediktov, D. D. Prophylaxis of cardiovascular disease in the Soviet Union. In Prevention of Ischemic Heart Disease, W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

138. Wassermil, M. and M. Toor. The effect of graded work exercise in 100 patients with ischemic heart disease: Two year follow-up. In Prevention of Ischemic Heart Disease. W. Raab (ed.), Springfield: Charles C. Thomas, 1966.

139. Wolffe, J. B. Continual vigorous physical activity as a possible factor in the prevention of atherosclerosis. Circulation. 16: 517, 1957.

140. Zoll, P. M., S. Wessler and J. J. Schlesinger. Interarterial coronary anastomoses in the human heart, with particular reference to anemia and related cardiac anoxia. Circulation. 4(6): 797, 1951.

141. Zukel, W., R. Lewis, P. Enterline, R. Painter, and L. S. Ralston. A short-term community study of the epidemiology of coronary heart disease. Am. Journ. Public Health. 49: 1630, 1959.

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