THE EFFECTS OF DIANABOL AND ANAEROBIC ENDURANCE EXERCISE ON MYOCARDIAL DAMAGE IN THE ADULT MALE ALBINO RAT

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ABSTRACT

THE EFFECTS OF DIANABOL AND ANAEROBIC ENDURANCE EXERCISE ON MYOCARDIAL DAMAGE IN THE ADULT MALE ALBINO RAT

By

Darlene Ann Jones

The purpose of this study was to observe the effects of the anabolic steroid, Dianabol, and an anaerobic program of endurance running on the heart muscle of male albino rats. Some animals were maintained in a sedentary condition while others were trained on a high-intensity, short-duration Controlled Running Wheel (CRW) program established in this laboratory. Myocardial damage was determined through a histologic technique of staining, examining, and rating different levels of the heart. Anatomical measures were taken on body weight, heart weight, ventricle weight, and ventricle length.

Forty-two normal, male, albino rats of the Sprague-Dawley strain were used for the study. All animals were received in the laboratory on the same day. However, they were in three different age groups at the time of their arrival. The differences in the ages of the animals represented a staggering procedure set up to accommodate other concurrent studies using the same facilities. Within his own age level, each animal was randomly assigned to a training-drug treatment group. All animals started treatment at 100 days of age.

Dianabol and a placebo were administered subcutaneously at a 1 mg/day dose. The animals received the training and drug treatments

Monday through Friday for eight weeks. All animals were given food and water *ad libitum*.

The exercise animals were selected for sacrifice on the basis of having the highest percent of expected revolution (PER) within their own drug groups. The final sample consisted of 36 animals (six per cell).

At sacrifice, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital. The heart was removed, trimmed, and weighed. After flushing and removal of the atria, the ventricular length was measured. The apical sections were dehydrated in graded concentrations of alcohol and then embedded in paraffin blocks. Serial cross sections, seven microns thick, were cut at eight levels spaced equally through the blocks. A Gomori trichrome stain was administered to the serial heart sections. Myocardial damage was determined by a subjective rating scale of one-to-five. A rating of one indicated no myocardial damage while a rating of five meant severe myocardial damage.

The results indicated there was a significant drug effect in terms of heart damage. The severity of heart damage was greater within the Dianabol group than in either the placebo or the control groups. Furthermore, training tended to decrease the severity and incidence of heart damage. Exercised animals showed a lesser degree of heart damage than did sedentary animals. Training also appeared to decrease the Dianabol necrotizing effect in that the exercised animals receiving Dianabol had lesser severity of heart damage than did the sedentary animals receiving Dianabol.

The exercised animals had smaller body weights than did the sedentary animals. Greater relative heart weights and ventricular weights were observed in the exercised group than in the sedentary group. There were significant training effects on both absolute and

relative ventricular lengths. The exercised animals had smaller absolute lengths and greater relative lengths than did the sedentary animals.

The body weights of the Dianabol and placebo groups were both greater than that of the control group but not different from each other. The relative ventricular lengths of the Dianabol and placebo groups were less than that of the control group but were not different from each other.

Brigham Young University

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DEDICATION

To my Mom and Dad for enduring with me

and instilling confidence in me

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CHAPTER I

INTRODUCTION

Heart disease has become one of the major causes of premature fatality. The increased incidence of myocardial disease has been attributed to industrialized society. With the help of mechanical inventions, man does not need to physically exert himself as much as he did in the past. Furthermore, research has indicated that habitual physical inactivity can be a causal factor in myocardial abnormalities (18,20,38,50,53,57,71,72,73,74,75,76,78,82). Stress is another potential necrotizing agent which is prevalent in our society. Stress can either be beneficial or detrimental to the myocardium, depending on how it is coupled with other factors (7,8,10,55,78,84). Since the myocardium plays such an important role, care should be taken to maintain proper myocardial function. It would seem that athletes, by means of their training, should enhance the myocardial process, but this may not always be the case.

In our competitive society, athletes endeavor to excel using all types of training procedures. Winning, due to social pressure and/or financial betterment, has become the key factor in most sports. In order to achieve success, many athletes involved in strength and power activities have adopted steroid usage as a means of extending human potential (14,42,43). Because steroids increase muscle growth (14,15, 42,43,49,86), the "anaerobic" competitor is attracted to their use with little concern toward the other side of the spectrum--possible harmful

effects. Since the myocardium is muscle tissue, it may be affected by the administration of anabolic steroids. However, muscle hypertrophy, the major benefit claimed for anabolic steroids, may not be advantageous in the myocardium. This certainly would be the case if the hypertrophy were not accompanied by a compensatory increase in capillarization. The result might well be incipient ischemic heart disease. Specifically, the results of steroid usage on the myocardium have not been investigated adequately at this time (15,37,46,47,62).

Statement of the Problem

The purpose of this study was to observe the effects of the anabolic steroid , Dianabol, and an anaerobic program of endurance running on the myocardium of male albino rats.

Overview of Methods

Some animals were maintained in a sedentary condition while others were trained on a high-intensity, short-duration Controlled Running Wheel (CRW) program established in this laboratory (88). Histochemical tissue analyses of the myocardium at different levels were performed as a means of rating heart damage. The heart weight, ventricular weight, and ventricular length also were measured.

Rationale

The Controlled Running Wheel (CRW) SHORT program was used because it was the program judged to be most appropriate for this study of those currently available at the Human Energy Research Laboratory, Michigan State University.

A review of the literature did not reveal extensive research on the effects of anabolic steroids on the heart. Since the use of steroids

is widespread, possible pathological effects need to be pursued. With regard to other anabolic steroids, Dianabol's anabolic-to-androgenic ratio is high, though its anabolic activity is relatively low. The use of Dianabol in this study reflects its wide acceptance by athletes.

The rat was selected because this laboratory had the facilities for housing and training small animals as opposed to larger ones. Furthermore, most of the research on exercise and the heart has been done on the rat.

Significance of the Problem

The findings of this study will add to the information on the effects of steroid usage on the heart. Comprehensive knowledge of the effects of steroid usage will help the different sport agencies in defining guidelines for athletic competition.

Limitations of the Study

1. The steroid dosage (1 mg/rat/day), chosen upon recommendation by Dr. J. J. Chart of the Department of Endocrinology at CIBA Pharmaceutical Co., may not have been sufficient to induce maximum anabolic effects in the rat (12).

2. The short-duration, high-intensity exercise program represents one form of "anaerobic" exercise. Of the training programs available at the Human Energy Research Laboratory, Michigan State University, it was the most appropriate for the study. Another anaerobic program accentuating a higher expenditure of strength might have produced different results.

3. Only forty-two animals were used in the study due to limitations of laboratory facilities and the number of Controlled Running Wheels (CRW) available.

4. The staining technique used for determining specific levels of myocardial damage was quantitatively limited and may not have been representative of a complete pathological analysis.

5. The results of this experiment cannot be extrapolated directly to humans.

6. The experimental period was only eight weeks long. It is not known if this duration is optimal for maximizing the exercise and drug effects.

7. The animals were motivated to run by a shock stimulus. There was no control for the shock in this study. However, unpublished data from this laboratory have shown that the motivational stimuli used are insufficient to produce noticeable myocardial damage.

Definition of Terms

Steroid Hormones. Those hormones possessing the cyclopentanoperhydrophenanthrene ring system (steroid nucleus) in their molecules. They include the androgens, estrogens, and corticoids.

Androgen. A generic term for an agent (usually a hormone, e.g., testosterone) that stimulates the activity of the accessory sex organs of the male, encourages the development of the male sex characteristics, or in special cases prevents the latter.

Anabolic Steroid. A compound which relates to or promotes the process of assimilation of nutritive matter and its conversion into living substance.

Testosterone. A male steroid hormone with both androgenic and anabolic effects. It is produced by the Leydig cells of the testes under normal conditions.

Dianabol. A synthetic anabolic steroid derivative of testosterone, produced by CIBA Pharmaceutical Co. The pharmacological name of Dianabol

is methandrostenolone. The structural name is 17-methyl-17-hydroxyandrosta-1, 4-dien-3-one.

Necrosis. The death of an area of tissue. If the condition causing the necrosis persists, the necrotic area may be characterized by irreversible development of scar tissue.

Infarct. An area of tissue in an organ, such as the myocardium, which undergoes necrosis following cessation or interference of the blood supply.

Ischemia. Local and temporary anemia due to obstruction of the circulation. If the obstruction continues, an infarct develops.

CHAPTER II

REVIEW OF RELATED LITERATURE

The effects of exercise upon the heart and the effects of exercise in conjunction with anabolic steroids are reviewed separately. Subsequently, the effects of anabolic steroids on the myocardium are reviewed. Because the literature on anabolic steroids and the heart is scarce, some of the studies included involve exercised hearts while others do not.

Effects of Exercise as Specifically Related to the Heart

Catecholamines

Contrary to the traditional view emphasizing vascular oxygen supply, myocardial oxygen consumption has been demonstrated to be of equal importance in the myocardial oxygen economy (78). The term "coronary reserve" refers to the ratio of oxygen supply to oxygen demand:

$$\text{Coronary Reserve} = \frac{\text{Blood (or Oxygen) supply}}{\text{Blood (or Oxygen) demand}}$$

When this ratio is maintained at one or above, the myocardium receives ample oxygen. Myocardial ischemia can be noted if the ratio falls below one. Although traditional views have focused mainly on diminution of vascular flow and oxygen supply as causal factors in myocardial

degeneration, researchers are now realizing the concomitant effects of neurohormonal influences upon oxygen consumption.

Regulation of oxygen consumption takes place by way of the autonomic nervous system in the form of sympathogenic catecholamines. These catecholamines (especially epinephrine—which when liberated from the adrenal medulla infiltrates the heart via the blood stream--and norepinephrine--which is intramyocardially liberated at the postganglionic sympathetic nerve terminals) have been shown to play an important role in the myocardial metabolism.

Catecholamines have been demonstrated to be tissue-necrotizing agents and have the potentiality of inducing morphological changes in the heart muscle (71,72,75,76,77,80). Although large doses are necessary, administration of epinephrine, norepinephrine, and isoproterenol produce myocardial lesions (72,76). Shimamoto and Hiramoto (80), in their study with albino rabbits, found epinephrine responsible for structural changes in the myocardium. Regan *et al.* (77) created myocardial lesions in healthy male mongrel dogs through infusion of l-epinephrine.

Conditions provoking the pathological potentialities of the adrenergic catecholamines have been reviewed by Raab (71,72):

Excessive Catecholamine Action. Through comparative histochemical analyses, Bajusz and Raab (7) studied the cardiac enzymes phosphorylase, cytochrome oxidase, and succinic dehydrogenase and the metabolites glycogen and potassium in one hundred twenty female rats (Sprague-Dawley strain). The rats were divided into two groups, with Group I receiving epinephrine injections and Group II serving as controls. Subcutaneously injected epinephrine was given in a single dose of 450

mg/100 g body weight in 0.2 ml of physiologic saline. An injection of 0.2 ml of physiologic saline served as the control treatment. Following injection, sacrifices were conducted at intervals which ranged from 5 minutes to 96 hours. While the control animals did not show abnormal enzyme or metabolite activity, spotty decreases of phosphorylase, depletion of stainable glycogen reserves, and a disturbance of potassium distribution followed in some of the experimental rats. Most of the above activity was noted in the necrotically susceptible subendocardium apex. These prenecrotic changes could be used as possible indications of myocardial abnormalities before the morphological signs are apparent.

Bajusz and Jasmin (6) also suggested the decrease or loss of phosphorylase activity as a possible indication of early anoxic myocardial damage. Cardiomyopathies were defined as either primary (nonocclusive or metabolic) or secondary (occlusive or anoxic). With three hundred sixty female rats, three primary necrotizing cardiomyopathy groups were produced by: (a) plasmocid (a metabolic inhibitor), (b) dihydrolachysterol (DHT) [a steroid of the vitamin D group], and (c) a K-deficient diet. Myocardial lesions were induced in three secondary groups by: (a) ligature of the left main coronary artery, (b) methoxamine (a synthetic vasopressor amine), and (c) metaraminol (a strong vasopressor). With respect to the primary cardiomyopathies, phosphorylase activity not only both increased and decreased but often was still present in the fibers after degeneration had started. An inverse relationship between phosphorylase activity and glycogen reserves was found in the plasmocid and DHT groups. Rats on the K-deficient diet demonstrated decreased glycogen content with complete loss before degenerative myocardial structures appeared. Secondary cardiomyopathies were accompanied by loss of phosphorylase and glycogen within 1 to 5

minutes after coronary ligature and as soon as 15 to 30 minutes after administration of the vasopressors. Although Bajusz and Jasmin admit phosphorylase activity to be different between the two groups, they suggested phosphorylase to be an early index of anoxic myocardial damage.

Nickerson (64) mentioned epinephrine potentialities with regard to phosphorylase but did not deal with phosphorylase activity as an early index of myocardial abnormalities.

Upon injecting albino male and female rabbits with epinephrine, Shimamoto and Hiramoto (80) observed the response of the heart muscle. Noted aberrations were intracellular edema, an increase of the clear spaces around the mitochondria and myofibrils of the cardiac muscle cells, dense bodies in the mitochondria, an expansion of the longitudinal sarcoplasmic reticulum, and fat droplet increases in the cytoplasm. Although Bryant (16) dealt with rats that had their coronary arteries ligated, he observed similar responses in the myocardial ischemic areas.

Excretion of urinary catecholamines reveals their liberation in the body. An increase in both epinephrine and norepinephrine occurs during muscular work, suggesting the increased secretion is from the adrenal medulla and the adrenergic nervous system. Catecholamine secretion appears to be related to the intensity of the work and to stresses. In a ski-run competition, the most successful skiers exhibited the highest excretion values (23,24).

Raab (71) argued that urinary catecholamine excretion does not reflect the action of circulating catecholamines on the heart and therefore is of little use. Instead, he suggested using as a more accurate measure of catecholamine turnover the excretion of vanillin

mandelic acid (VMA) or free fatty acids (FFA) which have been liberated in the blood by catecholamines. Raab based his rationale for using these measures on the fact that the myocardium absorbs circulating catecholamines, thus more valid conclusions could be drawn from abnormal occurrences of VMA or FFA.

Raab (71,72) summarized the potentially dangerous pathway of excessive catecholamines. By augmenting oxygen consumption, local hypoxia proceeds in cellular structures with depleted oxygen availability. Impaired oxygen availability eventually leads to anaerobic energy production where glycogen depletion occurs, followed by pyruvate accumulation. Lactate, formed from the pyruvate, becomes excessive. When the glycogen stores are depleted, ATP formation becomes inhibited, thereby hindering myocardial contractility. From this, depressed actin-myosin interaction causes inefficient external cardiac work performance. Uneconomic work performance and unbalanced internal metabolism result from excessive catecholamines.

Coronary Vascular Constriction. Excessive catecholamine action, along with sympathetic over-stimulation, results in focal necrotizing hypoxia due to cellular oxygen utilization surpassing oxygen availability. However, upon increased myocardial oxygen consumption, coronary vascular dilatation operates as the hypoxia avoidance mechanism (71, 72,78). When dilatation becomes impaired, hypoxia follows due to coronary vascular constriction.

Impaired Vagal Counterregulation. When Raab and Krzywanek (74) submitted two hundred normal male subjects averaging 44 years of age to varied sensory and mental stresses, increased heart rate and a shortening of the left ventricular isometric period ensued, representing

augmented cardiac sympathetic tone and adrenergic reaction to stresses. Several researchers have confirmed these responses (71,72,75,78). These investigators also have noted that the same responses occur with habitual physical inactivity. Training promotes the opposite effects: a prolonged isometric period and elevated cardiac vagal tone leading to a slower heart rate. In conjunction with an increased vagal tone, training lowers the sympathetic tone. Evidence suggesting sympatho-inhibitory and antiadrenergic mechanisms has been observed through sympathetic preponderance brought about by sedentary living. Physical training promotes the opposite.

Following a training regimen of 90-minute sessions three times a week for three months, De Schryver *et al.* (20) obtained significant decreases in both mean and total heart catecholamine concentrations in exercised rats. Although the concentrations remained low after three days of rest, on the sixth day a slight recovery was observed. After 21 days of rest, the catecholamines had increased almost to control levels. This indicates a reversible phenomenon. De Schryver's study showed that training must be maintained in order to sustain the catecholamine inhibitory effect of exercise. In addition, it demonstrated that the ordinary individual concerned with cardiovascular health may be able to maintain low myocardial catecholamine levels through a regular program of intermittent exercise. This is an important consideration for the "time-pressed" man of today.

In attempts to elicit the potentialities of antiadrenergic agents as protective mechanisms in catecholamine cardiotoxicity, Raab *et al.* (76) experimented with female rats. Before producing stress (restraint on a board, cold water immersion, or nicotine), pretreatment consisted of hormone injections of flurocortisol, dihydrotachysterol or

thyroxine. Neither stress nor pretreatment separately promoted myocardial lesions. However, when stress and pretreatment were combined, cardiac necroses did occur. With the administration of the antiadrenergic factors, diminished myocardial lesions were noted. Of the varied types of antiadrenergic agents used (catecholamine-depleting, adrenergic-blocking, ganglionic-blocking, and centrally-inhibiting), the best results occurred with the ganglionic blockers and the catecholamine-depleting agents. The others failed to inhibit both the circulating epinephrine and the intramyocardial norepinephrine.

From these results, the importance of physical exercise begins to become apparent. Exercise, especially when considered as a form of stress, does not cause excess cortisol activity as occurs with other types of exaggerated stresses. Instead exercise stimulates the adrenergic agents (72).

Nickerson (64) briefly reviewed the functions of the adrenergic receptors (α and β). For antiadrenergic purposes, it is of importance to be aware that catecholamines affect the heart by way of the β receptors. Therefore, β receptor blocking agents may be useful as myocardial-catecholamine inhibitory agents.

Opie (68) points out the fact that antiadrenergic drugs are contraindicated in heart failure because of the existing catecholamine depletion in the heart muscle. Conversely, a normal heart absorbs and stores circulating catecholamines while also storing intramyocardial catecholamines (68,71,75,76). Raab (72) and Nickerson (64) have both discussed the ability of the normal sedentary heart to function in the total absence of catecholamines. However, the concept probably would not hold true under exercise conditions.

Electrolyte Imbalance. As has been shown, catecholamines stimulate myocardial necrosis when certain conditions prevail. One condition not yet mentioned is the cardiotoxicity of adrenocortical overactivity. The corticoids act as sensitizing agents for the potentially dangerous catecholamine activity of epinephrine (7,71,73,76). Becker and Kreuzer (9) pointed out that both the adrenocorticoid system and the sympatho-adrenal medullary system function under stress conditions. In a study by Raab *et al.* (76), rats received corticoid hormone treatments and/or stress. Histologic analyses showed that neither the corticoid-treated animals nor the stress-treated animals suffered structural lesions. However, when animals were treated with corticoids prior to stress, extreme cardiac necrosis was demonstrated. A second study by Bajusz and Raab (7) yielded the same results. The corticoid pretreatment sensitized the myocardium to the cardiotoxicity of epinephrine.

When the "ionic pump", which is needed to maintain the electrolyte balance, is inactivated by focal hypoxia, myocardial structural damage paralleled by cell necrosis results. Intracellular potassium is displaced to the extracellular space and there is a depletion of glycogen and magnesium. Excessive sodium is housed within the myocardial cells (72).

Regan *et al.* (77), Shimamoto and Hiramoto (80), and Bajusz and Raab (7) found a decreased glycogen content and dislocation of potassium after epinephrine injection. Despite the myocardial structural changes in Shimamoto's and Hiramoto's study with rabbits, the electrolyte changes disappeared within one hour after epinephrine injection. Because the amount of displaced potassium was equivalent to the gain of sodium, intercellular replacement was postulated (68,72).

Factors known to be responsible for electrolyte derangement are:

1. Emotional and Sensory Stresses. Investigators agree that stress situations cause catecholamines to be elicited. Becker and Kreuzer (9) attempted to show that although both the adrenal medulla and the sympathetic nervous system discharge catecholamines, one can prevail over the other depending on the nature of the stress factors involved. In one experiment with a well-trained man who had been adapted to treadmill running, norepinephrine excretion increased significantly whereas epinephrine excretion did not. Becker and Kreuzer attributed their results to the fact that the individual was primarily under physical stress and not emotional stress, since his previous experience with treadmill running eliminated mental tension. Further studies on hypoxia (9) were conducted on an expedition to Monte Rosa (4,560 m). The urinary norepinephrine excretion increased to almost twice the amount at sea level, while epinephrine excretion varied little. Attributing the adrenergic nerve ending excretion to low oxygen tension, the experiment was simulated in a barometric chamber. This time, the result was an increased epinephrine excretion. To explain the varied catecholamine excretion pattern under what seemed similar conditions, the researchers concluded that the Monte Rosa expedition dealt primarily with physical activity as the stress factor. In the chamber experiment, the subjects were not exposed to physical work. In both situations, emotional stress factors existed but of different types. Aggressive exhilaration prevailed in the mountain climbing situation; whereas individuals involved in the chamber situation experienced passiveness, anxiety, and unpleasant feelings of confinement. This led Becker and Kreuzer to conclude that the person's mood may be partly responsible for the mode of catecholamine excretion.

Catecholamine increases correlate more closely with the intensity of work than with the quantity of work. Nowacki, Schmid, and Weist (67) showed the catecholamine metabolization with training by measuring the MHMA (vanilmandelic acid) excretion in basketball players. Although MHMA levels increased significantly during training, as compared to pretraining levels, the levels practically doubled following competition.

2. Lack of Physical Activity. As already mentioned, the vagal and sympathoinhibitory mechanisms are hindered by sedentary living. Adrenergic preponderance can be overcome by these counterregulatory mechanisms.

3. Nicotine. Cigarette smoking augments myocardial oxygen consumption, causes cardiac acceleration, and induces shortening of the left ventricular isometric period (71,72,75). Raab *et al.* (75) found that although nicotine stimulates the sympathetic ganglia and the adrenal medulla, the effects are not lasting.

Hypertrophy

In 1956, Whitehorn and Grimmenga (89) chronically exercised rats through swimming. They found bradycardia but no hypertrophy. On the other hand, according to De Schryver *et al.* (20), adaptation to continuous exercise consists of bradycardia and hypertrophy--the so-called "athletic heart." Many researchers support this line of thought (3,4,17,52,54,85).

Though knowledge of cardiomegaly has existed for years, researchers still do not agree on whether it is useful (physiologic) or detrimental (pathologic). In terms of general cardiomegaly, two schools of thought have evolved. The traditional view is that the fiber thickening is an adaptation to increased work load or to a weakened chamber. Thus hypertrophy may be looked upon as a useful physiologic mechanism. The

deleterious school of thought views hypertrophy as essentially pathologic in the sense that the increased muscle size is in response to actual injury. Upon exhaustion of the energy reserves, hypertrophy occurs.

Grant (35) pointed out deleterious aspects of hypertrophy to stimulate clinicians to investigate and possibly revise their attitudes about hypertrophy as a natural consequence of increased work. In terms of strength of contraction, the superiority of the experimentally hypertrophied heart, in the sedentary animal, has not been shown over the normal heart. However, Crews (17), by direct measurement with a strain-gauge lever system, showed that myocardial contractility is increased when hypertrophied hearts are produced in rats through swimming. Crews suggested that hearts hypertrophied by other means might not react in the same manner.

Badeer (3) proposed that the two views on hypertrophy, physiologic versus pathologic need not necessarily be in opposition. Though physiologic adaptation increases work performance by improving contractile force, early stages of pathologic hypertrophy may also be useful in that increased energy expenditure per unit mass of myocardial tissue is not needed. However, in advanced stages of pathologic cardiomegaly, the wall thickens to a point where nutrient and metabolite diffusion are restricted and the result is a deleterious cardiomegaly.

The quantitative aspects of physiologic and pathologic hypertrophy were reviewed by Linzbach (54). Physiologic hypertrophy develops by increasing the work of the heart. The normal human heart weight of 300 gm may be increased to as high as 500 gm, the critical heart weight. The muscle fibers increase in size, become thicker and longer, but do not increase in number. The number of capillaries is increased, but

the nuclei remain unchanged. With pathologic hypertrophy, the heart is continually at work and there is an increase in the number of fibers and nuclei as well as capillaries. In such an instance, the weight of the heart not only exceeds the critical heart weight but can reach 1,000 gm or more.

The mechanisms responsible for the onset of hypertrophy have not yet been determined (4,17,35). Badeer (4) postulated a "common stimulus" could be responsible. In attempting to pin point the stimulus, he reviewed some of the possibilities. Though nutritional deficiency might be a factor in pathologic myocardial hypertrophy, other conditions offer a better chance of explaining the phenomenon. Hormones such as those produced by the anterior pituitary, the thyroid, and the adrenal cortex have been shown to influence hypertrophy of the heart. Increased work has been suggested as a simple mechanical stimulus for physiologic hypertrophy, but a major fault with the theory lies in the fact that the mechanical work and the hypertrophy are not proportional. The "acute-dilatation-injury" hypothesis is not supported by recent studies. Chronic dilatation, or increased tension in the cardiac chamber walls during systole, does seem to be highly plausible as the stimulus in many clinical and experimental myocardial hypertrophies. Increased metabolic rate per beat, sustained over a long period, may constitute an effective stimulus.

Lutenov (52) discussed the development of hypertrophy as a result of anaerobic ATP resynthesis, which causes an increased phosphorylation oxidation in the sarcosomes and, thus, increases in nucleic acid and protein synthesis. Also detecting protein synthesis in hypertrophied hearts, Fanburg (27) attributed it to increased work loads. It should be noted that RNA synthesis was one of the first biochemical changes to

be found with an increased overload (27). Another source of protein synthesis within the hypertrophied heart exists in the increased ribosomes.

Following the swimming of one hundred twenty-five young male rats, Leon (51) noted hypertrophy in animals exercised daily but not in an intermittent group trained twice a week. In accord with previous literature (4,85), cardiac hypertrophy regressed upon cessation of training. Both Kitamura (44) and Bloor (11) have discussed the occurrence of atrophy when excessive training has been discontinued.

Myocardial Infarction

Bryant (16) studied myocardial ischemia in 12 adult male rats and found that gross changes in the infarcted areas could not be detected in less than 5 hours. He stated that the ischemic changes, regardless of how soon they occurred, were probably nonreversible. Enlarged mitochondria, swelled sarcoplasm and sarcoplasm reticulum, and increased lipid bodies were the earliest alterations.

Bajusz and Homburger (5) described the sarcoplasmic reticulum, the nucleus, and the mitochondria as being especially sensitive to injury which substantiates Bryant's findings. In Fine's (29) work with rats and Morales' (59) study with humans, myocardial infarcted areas showed irregularly increased lipid bodies.

Klionsky (45) reported that one of the earliest changes in the ischemic rabbit heart could be seen as a rapid decrease in glycogen. Diminution of glycogen in ischemic hearts also appears to be an early alteration in other animals (6,29). Some researchers have shown the demonstration of decreased enzyme activity to be superior to histologic methods in determining early myocardial damage (29,33,59).

Stress in Relation to the Myocardium

Stress has been shown to have the quality of being either a provocative or a preventive factor in cardiac damage. Selye's (78) concept of "simple resistance" is that insensitivity to a stressor can be achieved through pretreatment with the same agent. "Cross resistance", using a pretreatment stressor different from the one eliciting the damage, also has been shown to provide effective protection against a necrotic inducing stressor.

Fifty female rats, sensitized with Na-acetate and 9α-fluorohydrochlorocortisol (F-COL), developed severe cardiac necroses upon sudden exposure to muscular exercise or forced restraint (8). Under the same circumstances, few, if any, cardiac lesions could be seen in animals gradually exposed to exercise or restraint. In a second experiment, 100 female rats received combined Na-acetate and F-COL pretreatments. After the animals had become adapted to one stressor, a second necrotic inducing stressor was administered. Those groups not receiving the pretreatment showed marked cardiac necrosis while the groups adapted to one stressor and then subjected to a different stressor showed little or no incidence of cardiac damage. In both simple resistance and cross resistance, adaptation to a pretreatment proved to be an effective protective mechanism against cardiac necrosis.

Litwhiler (55) summarized the early research undertaken on the effects of selected stressors on the heart at the Human Energy Research Laboratory, Michigan State University. In one study of the relationships between anxiety, activity and the genesis of heart disease, eight anxiety-activity treatment groups were used. The exercise was a 30-minute swim per day, five days per week, with two percent of the rat's body weight attached to his tail. Anxiety consisted of electrical

shock administered every 15 seconds, 30 minutes per day, five days per week. The results from the study revealed no myocardial damage beyond that considered as routine variations. Because the findings were in contradiction to similar investigations, it was believed that the duration and/or intensity of the electrical stress was not sufficient to produce pathological changes. Furthermore, the detection of heart damage was limited to histologic procedures. To overcome these two problems, a series of pilot studies evolved. By converting the stress cages to "live-in" cages, nine hours per day of the shock treatment were found to produce sufficient anxiety to stimulate mild to moderate heart damage within a two-week period. The accuracy of the histochemical techniques was checked by injecting 30 animals with epinephrine to elicit myocardial necrosis. Histochemical procedures included analyses with hematoxylin and eosin, succinic dehydrogenase, mitochondrial α-glycerophosphate, cytochrome oxidase, monoamine oxidase, and β-hydroxybuterate dehydrogenase. Findings corresponded to those obtained by Bajusz and Raab (7).

Bell (10) attempted to demonstrate the role of physical activity by using exercise and anxiety in various combinations. The exercise treatment consisted of two one-half-hour swim periods seven days a week. Two percent of the animal's body weight was attached to his tail during each swimming period. The anxiety treatment consisted of a 0.36-second D.C. electrical shock of 1.5 milliamperes, five times per minute, nine hours a day for two weeks. Sacrifice took place at 116 days of age. Serial heart sections were stained with hemaxotylin-eosin, succinic dehydrogenase, monoamine oxidase, and beta-hydroxybuterate. Using a Chi-square contingency analysis, no significant differences between five treatment groups were detected. Bell concluded that

neither the exercise nor the electrical shock were of adequate duration or intensity to produce myocardial damage. However, later reanalysis of Bell's data showed that the sample size was inadequate for the statistical technique used. In a follow-up study, Thomas (84) did find significant differences in heart-damage ratings between control animals and rats receiving both the exercise and the electrical-stress treatments. More recent unpublished data, from this laboratory, have shown that aerobic exercise has a protective effect on the myocardium when it is administered for a period of eight weeks prior to the anxiety period; but that the same exercise routine is detrimental when initiated with the anxiety treatment.

Preventive Aspects

Many researchers believe exercise and training to be an effective mechanism in preventive myocardiology (18,38,50,53,57,82). In reviewing the related research on exercise and the heart, Mellerowicz (51) summarized the major factors supporting training as a preventive mechanism. He reported these to be: a physiological increase in the size of the heart, an economical oxygen-saving volume output, increased capillarization, a decrease in the number of heart beats per minute at rest, a reduction in the sympathetic tone with a simultaneous lengthening of the isometric period, a decreased cardiac output at rest, a reduction of the arterial mean pressure, and a reduction in the lipid level of the blood.

Morris *et al.* (61) studied physical activity and the incidence of coronary heart-disease in London Transport System employees. In comparing active conductors and sedentary drivers, it was found that coronary fatality occurred much more frequently and at an earlier age

in the drivers than in the conductors. To further study physical activity as a potential factor for decreasing early mortality, Morris *et al.* (61) studied Postal workers and Civil servants. It was found that active postmen had a lesser incidence of coronary heart-disease and an equivalently decreased fatality rate.

Dawber, Kannel, and Friedman (19) conducted an investigation on vital capacity and coronary heart disease (CHD). An inverse relationship appeared between the two. Though the researchers did not conceive of low vital capacity in itself as pathogenic, they felt it might reveal the mechanism causing CHD. To try and identify the detrimental factor, they studied: body height, body weight, presence of pulmonary disease, cigarette smoking, pre-existing heart disease, and level of physical activity. None of these variables seemed to be responsible for the vital capacity-CHD relationship. Lowered vital capacity and decreased physical activity were both associated with a high incidence of CHD, but the two were not significantly correlated. Despite failing to solve their original problem, the researchers concluded that high energy expenditure activities continued over the years could possibly prevent early CHD. Enselberg (22) and Wolffe (92) both corroborate the view that habitual exercise may have a preventive effect.

Raab *et al.* (75), on the basis of other investigations, stated that strenuous physical exercise is not injurious to the heart. According to Letunov (52), positive health results from a regular regime of sports training. However, in a few instances, overstrain of the left ventricle occurred from incorrect training regimes, thus the possibility of damage through poor training techniques should be recognized.

Though admitting that exercise has many benefits, Kitamura (44) reported hard training can be dangerous, especially in the very young. In 200 twenty-five-day-old mice divided into control and exercised groups, Kitamura found irreversible fibrosis, differences in nuclei, and even atrophy in some animals that had completed a 14-week exercise program six weeks before. Interstitial fibrosis, infiltration of inflammatory cells, and small areas of bleeding developed in a mouse trained for 10 weeks. In other animals trained for eight weeks, muscle fibers were disarranged and the mitochondria became coarse.

The effects of training definitely differ with age. Nocker (66) observed that endurance training performances in humans gradually decrease with age after the 40th year. However, physical training, even in middle-aged and elderly adults, may provide innumerable preventive benefits (53). When older sportsmen, who had trained consistently for years, were compared to people who had not begun training before the age of 50, the number of clinical abnormalities in the sportsmen was found to be substantially lower. Within two to five years of training, the previously inactive subjects began to take on the characteristics of the physically active sportsmen. Although Letunov (53) did not mention longevity in his comparative study, he did view physical training as a preventive measure against "premature aging."

Studying 1655 former Japanese university athletes and 3069 other university graduates, Kitamura (44) found significantly greater longevity among the former athletes. The health records of 355 ex-football players showed that the coronary heart disease victims had participated in less physical activity than had those who died of other causes. Individuals who had engaged in a continued exercise program did not suffer as high an incidence of coronary heart disease as those

who became sedentary following their competitive experience (70).

There appears to be a relationship between coronary heart disease and serum concentrations of cholesterol, phospholipids, and lipoproteins (19). Lower levels have been found to result from physical training (11,51,52,53,58,92).

Increased collateral circulation is a cardioprotective mechanism often attributed to physical training (57,58,72). Leon (51) used three groups of rats to determine how long capillary adjustments persist following cessation of exercise. Rats in Group I swam one hour daily for 10 weeks, while Group II animals swam one hour twice weekly for 10 weeks. The animals in Group III served as controls. Although the intermittently exercised animals did not have hypertrophied hearts, as did the daily exercised group, both groups developed increased myocardial vascularization. In the intermittently trained group, the extracoronary collateral circulation was increased significantly more than in the daily group, and the collateral circulation was maintained longer than in the daily group. In the daily exercised animals, coronary circulation regressed simultaneously with hypertrophy.

Wolffe (92) described increased capillarization as being necessary for sustaining high cardiovascular efficiency. He further claimed that maintaining capillarization at its greatest level requires physical activity involving all muscles of the body.

Although a number of epidemiological studies have been cited which show physical activity to have a prophylactic effect with regard to myocardial disease in later life, there have been few experimental investigations of this phenomenon. One such study was conducted in the Human Energy Research Laboratory, Michigan State University, to determine if exercise during the prepubertal period would alter the response to

suddenly imposed exercise later in life. The design of the study is shown in Table 1.

Table 1. Design of the prepubertal exercise study

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

Animals were assigned to one of three groups of 50 animals and given the first experimental treatment. At the end of that period, all animals were placed in spontaneous exercise cages for 125 days. Finally, the animals were subjected to a second experimental treatment period. Ten of the animals in each original group were retained as controls for the second treatment period, while the other 40 lived in spontaneous exercise cages and swam 30 minutes per day. Myocardial lesions of different degrees and locations were common in the adult-trained animals from all three original groups. Although most of the damage occurred in the endocardium, some was noted in the myocardium.

Wilson (91) ran a companion study of the same design as the Prepubertal Exercise Study. In an attempt to ascertain whether early exercise would act to prevent cardiac pathology in later life, one hundred fifty male albino rats and forty-two female albino rats were used. Myocardial lesions ranged from slight perivascular lymphocytic infiltration to advanced necrosis and polymorphonuclear leukocytic infiltration. No significant differences existed between the three original groups. Wilson concluded that prepubertal forced exercise does not protect the myocardium from damage induced by postpubertal forced exercise.

Therapeutic Aspects

Therapeutic benefits of physical training have been recognized for a long time, but supervision and great care must be considered (34,38,63,81,87). Seven male patients, who had suffered myocardial infarctions two to four months previously, trained three times a week on an ergometer. Frick and Katila (31) studied the effects of the endurance program on the coronary patients using cardiac catheterization before and after the training period. Common training responses observed in normal subjects were achieved with the cardiac patients: reduced heart rate and increased stroke volume during training. From results obtained in their study, Frick and Katila recommended physical activity for coronary patients.

Using ballistocardiographic records, Holloszy *et al.* (40) suggested an improved cardiovascular state might be reached through physical training. Fifteen men, five of which had abnormal initial ballistocardiographs, were studied for six months. Following a conditioning program of running, four of the five men with abnormal records had normalized ballistocardiographs.

By movement restriction and excessive overfeeding, Wolffe (92) produced atherosclerosis in a flock of geese. After a gradual exercise program of six weeks, the diseased state had been markedly reduced and was almost nullified in some of the birds.

Ligation of the coronary artery evokes myocardial infarcts in dogs (44). Control animals exhibit fibrosis while exercised animals do not. As seen in most of the investigations and as advocated by Enselberg (22), endurance programs are more beneficial for the coronary patient than are strength-type activities.

Effects of Dianabol and Other Anabolic Steroids with Exercise

Myotropic Activity

The myotropic effects of steroids were determined first by Wainman and Shipounoff (86). The perineal muscles (levator ani, bulbocavernosus and ischiocavernosus) of castrated rats responded more to testosterone propionate treatment than did other striated muscles. The increase in the muscle bulk was noticed by the increased width of the muscle fibers.

Papanioclaou and Falk (69) found hypertrophy of the temporal muscle in guinea pigs treated with testosterone propionate. The experiment was conducted on immature males and spayed and normal adult females.

While comparing castrated guinea pigs to normals, Kochakian, Humm, and Bartlett (48) noticed a decrease in the temporal muscle weights of the castrated animals. Partial restoration of weight occurred with steroid treatments. The steroid treatments also produced androgenic effects of seminal vesicle and prostate stimulation. Body weight of the castrated animals was observed to be lower than that of normals. Subcutaneous implantation of steroid pellets increased the body weight

gain, but a maximal response was attained whereby further dosage did not increase weight.

Korner and Young (49) found that anabolic treatment with methyl-androstenediol (MAD) led to a significantly greater weight gain in experimental rats than in control rats. Both female and male rats were used, with optimum results achieved at 3-1/2 months of age.

Brown and Pilch (15) noted a significantly larger weight gain in exercised rats treated with Dianabol than in sedentary untreated animals. Human studies with Dianabol by Johnson *et al.* (42) and Bowers and Reardon (14) have yielded similar results. However, the data of Murphy and Eagan (62) on rats is contradictory. They found the mean body weight of a control group to be greater than that of a steroid group and the body weight of an exercised group to be greater than that of an exercise-steroid group.

As indicated by Kochakian (46) and Wainman and Shipounoff (86), most researchers attribute the decreased muscle size after castration and the restoration following steroid treatment to changes in muscle fiber diameter. Contrary to this, Lloyd and Anthony (56) found that pigs exposed to rations containing Dianabol had an increased number of muscle fibers of small diameter.

By subjecting eighteen growth retarded children to oral doses of Dianabol for three to four months, Hochman and Laron (39) stimulated an increase in the growth rate.

Twelve matched pairs of subjects were trained on a weight lifting regime by Johnson and O'Shea (43). One subject of each pair received 5 milligrams of Dianabol twice a day while the second subject served as a control. Diets were supplemented with a high protein powder. The subjects under anabolic steroid treatment had greater final muscular

strength, both dynamic and static, as measured by the bench press, squat, and cable tensiometry. A second study by Johnson *et al.* (42) substantiated the first.

Observing the effects of Dianabol on 18 experienced weight lifters matched into pairs according to size, strength, and age, Bowers and Reardon (14) found increases in bench press, squat, and body weights in the steroid group. Girths of the biceps and forearms also were noted to be greater in the treated subjects.

Contrary to the previous studies, Fowler, Gardner and Egstrom (30) failed to show increases in strength, physical performance, or physical working capacity in men receiving 1-methyl-Δ^1-androstenolone acetate (Nibal) separately or in combination with exercise. In another study (26), where subjects underwent a weight lifting exercise program and the treatment consisted of oral doses of nandrolone decanoate (deca-Durabolin), strength was not enhanced. Male albino rats, trained by high jumping or treadmill running while under Dianabol administration, did not increase performance (15).

Johnson *et al.* (42) attributed the differences in research findings to an age factor. However, the ages of Johnson's subjects were equivalent to those of Fowler's group, who also attributed the conflicting findings to age. According to Fahey and Brown (26) and Boris, Stevenson, and Trmal (13), the diversified effects could have been due to drug type or dosage, treatment duration, training intensity, or a psychological "positive placebo" effect.

Castration of 28-day-old mice terminated tissue growth in the perineal complex (seminal vesicles, prostate, levator ani and bulbocavernosus) (46). Wainman and Shipounoff (86) substantiated this response of the perineal muscles (levator ani, bulbocavernosus, and

ischiocavernosus) to castration and showed that the same muscles increase in size when treated with testosterone propionate. Levator ani weight increases occurred in trained rats that were treated with a high Dianabol dosage in the study of Brown and Pilch (15). Concurrently, testes weight was decreased. In comparing androgenic-myotropic activities of different anabolic steroids, Boris, Stevenson, and Trmal (12,13) showed that all of the steroids decrease testes weight and increase the weights of the seminal vesicles, ventral prostate, and levator ani.

Properties of Dianabol and Its Relation with Other Anabolic Steroids

Izzo and Glasser (41) pointed out Dianabol's failure to inhibit protein catabolism in fasting rats. When Lloyd and Anthony (56) added Dianabol to the rations of pigs, nitrogen retention and the digestibility of protein and fat were not affected.

The metabolic effects of methandrostenolone and testosterone propionate were noted by Almqvist, Ikkos, and Luft (1) on three subjects who had been exposed previously to steroid treatments. Methandrostenolone was given in single, graded, oral doses (5, 10 and 25 mg/day). Administration of testosterone propionate was by intramuscular injection. Five milligrams of Dianabol produced nitrogen and calcium retentions beyond those achieved by 25 mg of testosterone propionate. Further Dianabol dosages up to 25 mg did not increase the nitrogen retention beyond that of the 5-mg dosage.

Dorfman and Kincl (21) studied the potency and androgenic-anabolic activity of various steroids on rats castrated at 21 to 23 days of age. Animals that had received Dianabol had decreased seminal vesicles as compared to those of rats that had been given 17 alpha-

methyltestosterone. No differences were observed in levator ani weights.

Arnold, Potts and Beyler (2), using urinary nitrogen excretion, found methandrostenolone to be 1.2 $\pm$ 0.14 times as effective in nitrogen retention as methyltestosterone. Dianabol was 0.35 $\pm$ 0.045 as androgenic as methyltestosterone. Thus, the methandrostenolone anabolic-androgenic ratio was 3.4. Excluding methyltestosterone, the steroid used as the standard, methandrostenolone yielded the lowest nitrogen retention values, the highest androgenic activity, and the lowest nitrogen retention-androgenic ratio of the six steroids studied.

The effects of daily subcutaneous injections of steroids, including Dianabol, were noted by Boris, Stevenson, and Trmal (12). Decreased testes weight, increased seminal vesicle weight and increased ventral prostate weight occurred with all of the injected steroids. Dianabol was not as effective anabolically or androgenically as the other steroids tested.

Using equivalent experimental conditions, Boris, Stevenson, and Trmal (13) subcutaneously injected steroids for seven consecutive days into rats that had been castrated at 24 to 25 days of age. Again, increases in the weights of the seminal vesicles, ventral prostate, and levator ani muscle were produced by all of the compounds tested. Potency evaluations were conducted in terms of the dosages required to double the weights of the respective target organs. Dianabol ranked last in all cases.

Steroids in Relation to the Heart

A search of the literature revealed few studies on steroids and the heart. Specifically, little was found on Dianabol and the heart.

Kochakian (46,47) showed that the heart weight of castrated animals decreases slightly but is restored to normal by injecting various steroids. In another study (15) on normal rats, significantly increased heart weights were reported following a low dose of Dianabol.

Murphy and Eagan (62) trained 20 male rats on a treadmill and then divided them into the following four groups: control (C), steroid (S), exercise (E), and exercise-steroid (ES). The S and ES groups received daily oral doses of stanozolol (Winstral) and methandrostenolone (Dianabol) and weekly intraperitoneal doses of nandrolone (Durabolin). There were no significant heart weight differences between the groups.

Gudbjarnason *et al.* (37) suggested that Dianabol might have a reparative effect on experimental myocardial infarctions in mongrel dogs. Dianabol was chosen for its protein-synthesis characteristics. Based upon their findings and those of others, these researchers claimed that protein synthesis is not expected to occur in the necrotic myocardium. Methandrostenolone (Dianabol) treatments yielded a 233% increase in the incorporation of glycine-2 ^{14}C into protein in the peripheral infarcted area and a 249.1% increase in incorporation in the central infarcted area. Both increases were statistically significant. Following ten weeks of treatment with methandrostenolone, determinations of scar-tissue thickness and presence or frequency of left ventricular aneurysms again revealed the healing aspects of Dianabol. Control animals had scars of 5.0 ± 0.6 mm mean thickness with two of six animals having aneurysms. Dianabol-treated animals had scars of 3.4 ± 0.6 mm mean thickness and no aneurysms. Other reparative effects of methandrostenolone were reported to be increased fibroblastic repair and a decrease in time of phagocytosis and removal of necrotic myocardial tissue.

CHAPTER III

RESEARCH METHODS

Sampling Procedures

Forty-two normal, male, albino rats of the Sprague-Dawley strain were used for the study. All animals were received in the laboratory on the same day. However, they were in three different age groups at the time of their arrival. Age-Level 1 consisted of fifteen animals 90 days old; Age-Level 2 consisted of twelve animals 76 days old; and Age-Level 3 consisted of fifteen animals 62 days old. The experimentally undesirable differences in the ages of the animals represented a staggering procedure set up to accommodate other concurrent studies using the same facilities.

Within his own age level, each animal was randomly assigned to a training-drug treatment group as shown in Table 2. All animals started treatment at 100 days of age, with the three age levels beginning at two-week intervals. Thus, prior to the study Level 1 animals had 10 days to adjust to laboratory conditions; whereas Level 2 and 3 animals had 24 and 38 days, respectively.

Research Design

The study was organized as a 2 x 3 factorial design. Factor A, Training, consisted of two treatment groups: (E) an exercise group which was subjected to an anaerobic endurance training program, and (S) a sedentary group. Factor B, Drug, consisted of three treatment

Table 2. Staggering procedure for random assignment into training-drug experimental conditions

	Factor A: Training					
	Level 1 (n=15)		Level 2 (n=12)		Level 3 (n=15)	
	Exercise	Sedentary	Exercise	Sedentary	Exercise	Sedentary
Factor B:						
Drug						
Dianabol	4	1	0	4	4	1
Placebo	4	1	0	4	4	1
Control	4	1	0	4	4	1

groups: (D) a Dianabol group, (P) a placebo group, and (C) a control group.

The original plan was to eliminate one of the four exercised animals within each of the Dianabol, placebo, and control groups of Age-Levels 1 and 3. The animal with the poorest training performance, over the entire eight-week treatment period, was to be eliminated. However, due to a possible infectious leg injury, one animal in the exercise-control group of Age-Level 1 was automatically eliminated from the study. A representation of the experimental design with final cell frequencies can be seen in Table 3.

Training Groups

The two training groups in the study were as follows:

Exercise (E)

The exercise treatment was the SHORT program, which is a high-intensity, short-duration Controlled Running Wheel (CRW) program developed at the Human Energy Research Laboratory, Michigan State University. The CRW apparatus can be described as, ". . . a unique animal-powered wheel

Table 3. Experimental design with final cell frequency

	Factor A: Training	
	Exercise	Sedentary
Factor B:		
Drug		
Dianabol	n = 6	n = 6
Placebo	n = 6	n = 6
Control	n = 6	n = 6

which is capable of inducing small laboratory animals to participate in highly specific programs of controlled, reproducible exercise" (88). The animals learn to run by avoidance-response operant conditioning. A low-intensity controlled shock current provides motivation for the animals to run.

Following body weight recordings and drug injections at the start of each treatment period, the animals were placed in individually braked running wheels. A light above the running wheel signaled the start of each work interval. If the animal responded to the light by running at or faster than a preset speed, the light was extinguished and shock was avoided. The time during which the light was on is termed the "acceleration period." If the animal was not running at a predetermined speed by the end of the acceleration period, the light was turned off and a current was applied to the grid which serves as the running surface. If the animal attained the prescribed speed while being shocked, the shock was immediately discontinued. If the animal slowed down below the prescribed speed, the light and shock sequence was repeated. A typical running program consisted of alternate work and

rest periods. During the work periods, the wheel was free to turn; while during the rest periods, the wheel was braked automatically to prevent spontaneous activity. A specified number of alternate work and rest periods (repetitions) constituted one bout of exercise. A single training period would include several such bouts separated by a relatively long time between bouts.

The exercise program was progressive in nature. That is, the intensity of the program was gradually increased until on the thirty-seventh day of training, and thereafter, the animals were expected to complete eight bouts of exercise with 2.5 minutes of inactivity between bouts. Each bout consisted of six repetitions of 10 seconds of work alternated with 40 seconds of rest. During the work intervals, these animals were required to run at the relatively fast speed of 5.5 ft/sec. For a complete day-by-day description of the training program see Appendix A.

Sedentary (S)

These animals did not receive any type of forced exercise. To compensate for the handling of the exercised animals, the sedentary animals were weighed during each treatment period.

Drug Groups

The three drug groups used in this study were as follows:

Dianabol (D)

The animals were given Dianabol five times a week, prior to each exercise period, throughout the eight-week program. The concentration level was 10 milligrams (mg) per cubic centimeter (cc) and the dosage level was 1 mg/rat/day or 0.1 cc/day. The Dianabol was dissolved in

Mazzola corn oil (the solvent was chosen upon consultation with Dr. J. J. Chart, CIBA Pharmaceutical Co.).

Placebo (P)

These animals were given Mazzola corn oil, 0.1 cc/day, prior to each exercise period throughout the eight-week program. The corn oil corresponds to the solvent that was used with the Dianabol group. The placebo was given to counteract any effects which the injection procedure might have had on the Dianabol rats.

Control (C)

These animals did not receive an injection of any kind.

Experimental Procedures

The animals received the training and drug treatments once a day, Monday through Friday, for eight weeks. Body weights of the trained animals were recorded before and after each exercise period. Dianabol and the placebo were injected subcutaneously into the lower back (lumbar) region of the rat. The drug administration took place following initial body weight recordings.

The performance data for each trained animal were recorded daily. Total revolutions run (TRR) and total expected revolutions (TER) were used to calculate percent of expected revolutions (PER): PER = TRR/TER x 100. PER values were used to evaluate performance and to eliminate animals from Age-Levels 1 and 3.

Animal Care

All of the animals were housed in standard, individual, sedentary cages (24 cm x 18 cm x 18 cm) throughout the entire investigation. Since rats are normally more active at night than during daylight hours,

the light sequence in the animal quarters was automatically timed to reverse the rat's active period by having the lights off between 1:00 p.m. and 1:00 a.m.

A relatively constant environment was maintained for the animals by daily handling, temperature and humidity control, and regular cage cleaning. Throughout the experiment, all animals had access to food (Wayne Laboratory Blox) and water *ad libitum*.

Sacrifice Procedures

Three sacrifices of twelve animals each were conducted forty-eight to seventy-two hours following the end of the treatment period for each age level. On the last day of the treatment period, the animals were placed in sedentary metabolism cages. The housing was changed at this time so that urine volumes could be collected for a companion study.

On the sacrifice day, final body weights were recorded and then each animal was anesthetized by a 6-cc intraperitoneal injection of a 6.48 percent Halatal solution (sodium pentobarbital).

With a small stem of the aorta still attached, the heart was removed and the great vessels were trimmed away. Expelling blood out of the chambers and washing the heart in distilled water served to remove all blood. Each heart was then suspended from the aortic stem and fixed in a 10 percent formaldehyde solution. The surface was flushed and the atria were removed by careful dissection along the atrioventricular groove. Using calipers, the length of the ventricles was measured as the distance from the apex to the entrance of the pulmonary artery. The heart was transversely dissected along a line which was 55 percent of the ventricular length from the apex. The heart sections were blotted dry, and the total heart weight was determined.

By removing the atrium from the balance, total ventricular weight was determined.

The apical sections were dehydrated in graded concentrations of alcohol and then embedded in paraffin blocks. With a rotary microtome, serial cross sections were cut at eight levels spaced equally through the blocks. The slices were seven microns thick. After sectioning, the tissues were placed on cover slips, dried, and then stained with Gomori trichrome.

Method of Tissue Analysis

Each histological slide was examined and rated subjectively for myocardial damage on an arbitrary one-to-five scale similar to that used by Niles, Zavin and Monikado (65). The arbitrary scale was as follows: 1 = no damage, 2 = slight damage, 3 = moderate damage, 4 = considerable damage, and 5 = severe damage (see Figures 1 through 5).

Without prior knowledge of the treatment groups, the slides were rated twice by this investigator and once by a second person to increase the reliability of the subjective evaluations.

Statistical Procedures

The statistical analysis consisted chiefly of a series of contingency Chi-square tests of the hypothesis that there were no differences between the treatment groups in terms of myocardial damage. The .10 level was set for statistical significance of the Chi-square analyses.

The anatomical data were analyzed using the FACREP routine on the Michigan State University Control Data 3600 Computer (CDC 3600). The model for the analysis was a two-way, fixed effects ANOVA. The Tukey Test was used to determine the significance of differences between

Figure 1. Normal heart rating 1. H & E (X40).

Figure 2. Slight damage rating 2. Small area of degeneration and necrosis with infiltration of inflammatory cells. H & E (X40).

Figure 1

Figure 2

Figure 3. Moderate damage rating 3. Moderate area of necrosis with increased inflammatory cell infiltration. H & E (X40).

Figure 4. Considerable damage rating 4. Confluent areas of necrosis and formation of granulated tissue and scar tissue. H & E (X40).

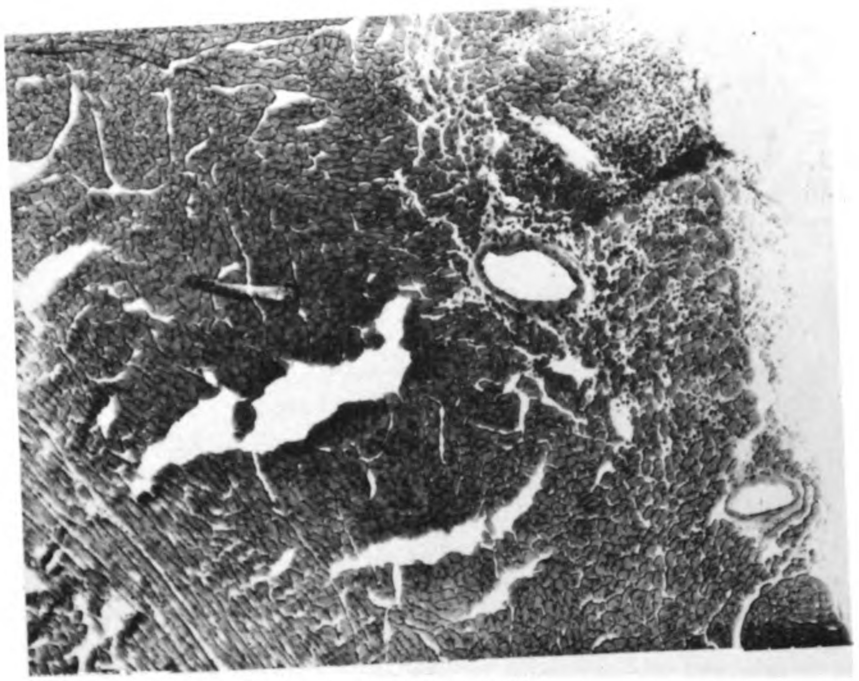

Figure 3

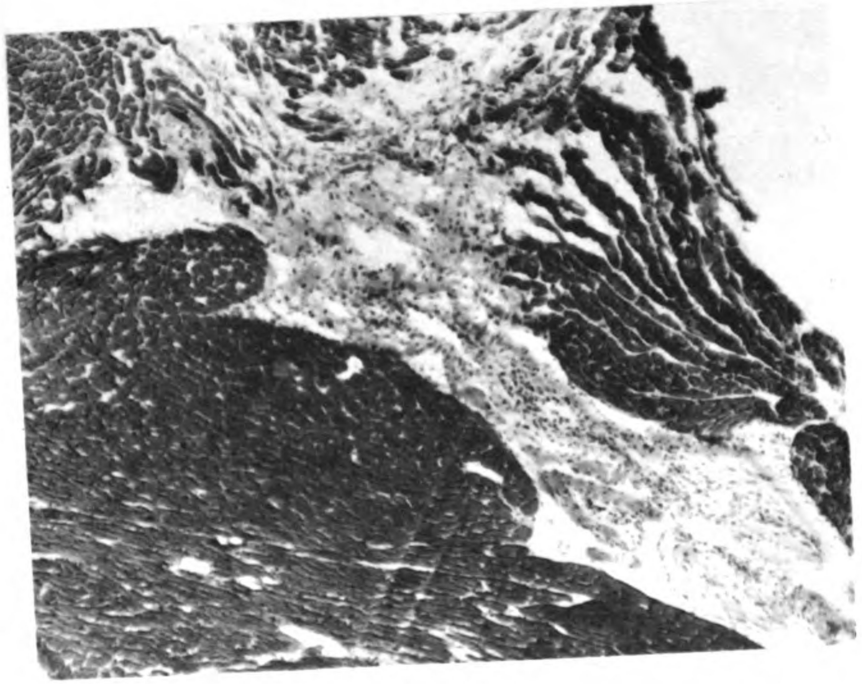

Figure 4

Figure 5. Severe damage rating 5. H & E (X40).

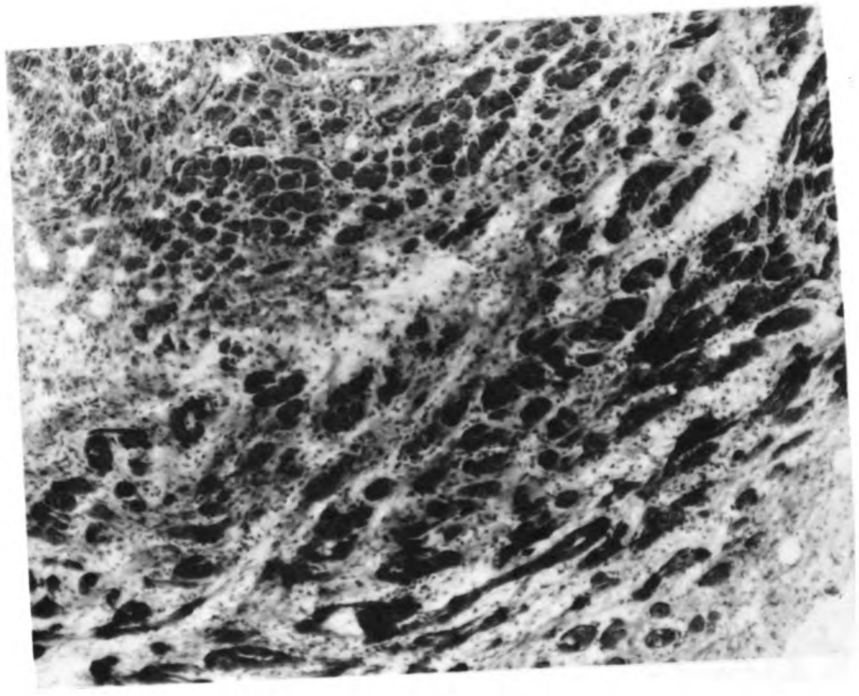

Figure 5

means following significant analysis of variance results for Factor B, Drug, and the Training-Drug interaction. *Post hoc* procedures were not necessary for Factor A, Training, as it contained only two levels. Statistical significance was set at the .05 level for the two-way ANOVA and at the .10 level for the Tukey *post hoc* procedures.

CHAPTER IV

RESULTS AND DISCUSSION

Training Results

Mean daily percent of expected revolutions (PER) was the criterion for determining the performance of the three exercised drug groups on the CRW SHORT program. Figure 6 illustrates the lower PER values of the control group as compared to the higher values of the Dianabol and placebo groups. The differences in PER values were more apparent during the last fourteen days of training when the expected running velocity was increased to 5.0 and 5.5 ft/sec.

Histological Results

Subjective ratings of myocardial damage according to treatments and by section level are listed in Appendix B. Ratings were based on an arbitrarily designated one-to-five scale, with a rating of one indicating no myocardial damage and a rating of five indicating severe myocardial damage.

The requirements of the Chi-square contingency analysis necessitated grouping the data into fewer than five rankings since there was no myocardial damage of a five rating in the original data. The revised rating scale included a rating of one indicating no damage, a rating of two indicating slight damage, and a rating of three indicating definite damage. The Chi-square analysis results are presented in Table 4.

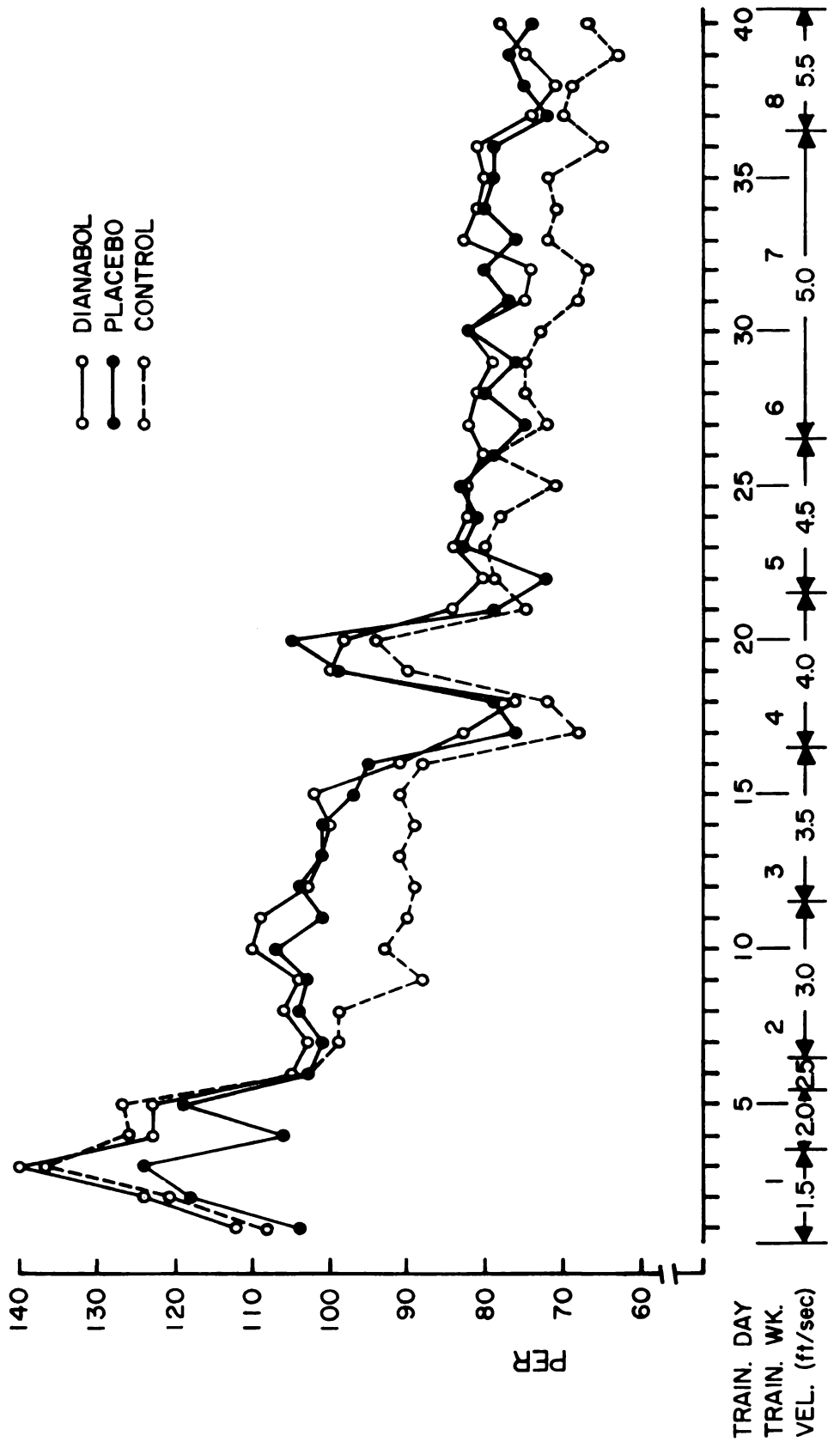

Figure 6. Mean Daily Percent Expected Revolutions (PER) for CRW SHORT Program

Table 4. Chi-square results for treatment effects on myocardial damage

Table 4.1. Training and heart damage

Heart Damage	Training: Sedentary	Training: Exercise
None	111	108
Slight	20	31
Definite	13	5

Chi-square of 5.969

Table 4.2. Drug and heart damage

Heart Damage	Drug: Control	Drug: Placebo	Drug: Dianabol
None	77	82	60
Slight	19	12	20
Definite	0	2	16

Chi-square of 31.212

Table 4.3. Drug and heart damage within the sedentary training group

Heart Damage	Drug: Control	Drug: Placebo	Drug: Dianabol
None	40	41	30
Slight	8	5	7
Definite	0	2	11

Chi-square of 18.546

Table 4.4. Drug and heart damage within the exercise training group

Heart Damage	Drug: Control	Drug: Placebo	Drug: Dianabol
None	37	41	30
Slight	11	7	13
Definite	0	0	5

Chi-square of 13.529

Table 4 (cont'd.)

Table 4.5. Training and heart damage within the control drug group

Heart Damage	Training: Sedentary	Training: Exercise
None	40	37
Slight	8	11
Definite	0	0

Chi-square of 0.591

Table 4.6. Training and heart damage within the placebo drug group

Heart Damage	Training: Sedentary	Training: Exercise
None	41	41
Slight	5	7
Definite	2	0

Chi-square of 2.333

Table 4.7. Training and heart damage within the Dianabol drug group

Heart Damage	Training: Sedentary	Training: Exercise
None	30	30
Slight	7	13
Definite	11	5

Chi-square of 4.050

Heart damage was less severe in the exercised group than in the sedentary group. When looking at the training factor alone, 9.0 percent of the sedentary animals had definite heart damage; whereas, only 3.5 percent of the exercise group showed definite heart damage. In terms of frequency of heart damage, 72.2 percent of the animals with identifiable damage were sedentary animals while only 27.8 percent were exercised animals. The training effect on heart damage could not be explained in terms of the control or placebo drug groups. Though the training effect was not statistically significant, Table 4.7 shows that the difference in the severity of heart damage with the training factor seems to be concentrated in the Dianabol drug group.

The drug factor produced a significant effect on heart damage. The severity of heart damage in the control and placebo animals was less than that in the Dianabol group. Nearly 90 percent of the definite heart damage was found in the Dianabol group. Tables 4.3 and 4.4 show the occurrence of heart damage for the three levels of the drug factor and within specific training groups. There was a nonsignificant tendency for a greater degree of heart damage to occur in the Dianabol-sedentary group than in the Dianabol-exercise group.

Anatomical Myocardial Results

Body weights, absolute and relative total heart weights, total ventricular weights and total ventricular lengths are tabulated in Appendix C. The analysis of variance results and appropriate Tukey Test comparisons are presented in Table 5.

Body weights of the exercised animals were smaller than those of the sedentary animals. There also was a significant effect among the drug groups with respect to body weight. Both the Dianabol and placebo

Table 5. Analysis of variance and Tukey Test results for body weight, absolute and relative heart weight, ventricular weight and ventricular length

	Training		Row	ANOVA	Tukey
	Exercise	Sedentary	Means	Results by Rows	Results by Rows
Table 5.1. Body weight					
Drug					
Dianabol	436	493	464	F=5.69	D > C
Placebo	430	507	468	P=0.008	
Control	411	483	447		P > C
Column Means	426	494	460*		
ANOVA Results by Columns	F=155.38 P<0.0005				
Interaction	F=1.13; P=0.336				
Table 5.2. Absolute heart weight					
Drug					
Dianabol	1.35	1.34	1.35	F=2.96	
Placebo	1.41	1.39	1.40	P=0.067	
Control	1.30	1.37	1.34		
Column Means	1.35	1.37	1.36*		
ANOVA Results by Columns	F=0.46 P=0.502				
Interaction	F=1.29; P=0.289				
Table 5.3. Absolute ventricle weight					
Drug					
Dianabol	1.24	1.22	1.23	F=2.63	
Placebo	1.28	1.28	1.28	P=0.088	
Control	1.19	1.26	1.22		
Column Means	1.23	1.25	1.24*		
ANOVA Results by Columns	F=0.52 P=0.477				
Interaction	F=1.65; P=0.209				

Table 5 (cont'd.)

	Training		Row	ANOVA Results	Tukey Results
	Exercise	Sedentary	Means	by Rows	by Rows
Table 5.4. Absolute ventricle length					
Drug					
Dianabol	0.65	0.67	0.66	F=2.69	
Placebo	0.67	0.69	0.68	P=0.084	
Control	0.67	0.68	0.68		
Column Means	0.67	0.68	0.67*		
ANOVA Results by Columns	F=5.43 P=0.027				
Interaction	F=0.63; P=0.541				
Table 5.5. Relative heart weight ($X10^{-3}$)					
Drug					
Dianabol	3.10	2.72	2.91	F=1.67	
Placebo	3.27	2.75	3.01	P=0.205	
Control	3.17	2.84	3.01		
Column Means	3.18	2.77	2.98*		
ANOVA Results by Columns	F=67.57 P<0.0005				
Interaction	F=1.33; P=0.280				
Table 5.6. Relative ventricle weight ($X10^{-3}$)					
Drug					
Dianabol	2.84	2.47	2.66	F=1.67	
Placebo	2.98	2.52	2.75	P=0.206	
Control	2.89	2.61	2.75		
Column Means	2.90	2.53	2.72*		
ANOVA Results by Columns	F=60.35 P<0.0005				
Interaction	F=1.20; P=0.317				

Table 5 (cont'd.)

	Training		Row Means	ANOVA Results by Rows	Tukey Results by Rows
	Exercise	Sedentary			
Table 5.7. Relative ventricle length ($X10^{-3}$)					
Drug					
Dianabol	1.50	1.37	1.43	F=5.79	
Placebo	1.56	1.36	1.46	P=0.007	
Control	1.64	1.41	1.53		D < C P < C
Column Means	1.57	1.38	1.47*		
ANOVA Results by Columns	F=66.21 P<0.0005				
Interaction	F=1.72; P=0.196				

*Grand mean

groups, though not different from each other, had greater body weights than did the control group. There was no significant difference in absolute heart weight but relative heart weight in the exercised group was larger than that of the sedentary group.

The exercised animals had greater relative ventricular weights than did the sedentary animals. There were no differences in the absolute ventricular weights. There were significant training effects on both absolute and relative ventricular lengths. The exercised group had a smaller absolute ventricular length and a greater relative ventricular length than did the sedentary group. A significant drug effect also occurred in the relative ventricular length. The lengths of the Dianabol and placebo groups were both less than that of the control group.

Discussion

The exercised Dianabol and placebo groups' PER values were similar throughout the study. Thus, a performance response to Dianabol was not evident. The lowest PER values were noted in the control group as the training program progressed in intensity.

The exercised animals were motivated to run by a shock stimulus. Although there was no control for the shock effect in this study, previous research in this laboratory has shown that the shock levels used are not sufficient, by themselves, to cause heart damage.

The significantly smaller body weights of the exercise group are in accord with the results of previous research. The body weights of the Dianabol and placebo groups were greater than that of the control group. Most of the literature on steroids and body weight substantiates these findings (14,15,42,49). One unexplainable finding remains in that the mean body weight of the placebo group was not different from that of the Dianabol group. Possibly this could be due to the solvent used. The Dianabol was dissolved in corn oil, and the placebo contained only corn oil. Although it is not known, the body weight results may be attributable to the corn oil.

The mechanism of the action of Dianabol on the heart needs further investigation. However, certain features are evident. The severity of heart damage was more extensive in the animals receiving Dianabol than in the placebo or control animals. With drug administration, there was a definite effect on the relative ventricular length. The Dianabol and placebo groups had smaller ventricular lengths than did the control group. During training, it is known that hypertrophy develops with myocardial damage; partly from edema and partly from scar tissue formation. Nevertheless, myocardial parameters increase with hypertrophy.

However, it is not known what happens to the trained heart under drug administration. Since the ventricular lengths of the Dianabol group were smaller than those of the control group, it appears that the drug may affect the normal hypertrophy process. The ventricular lengths of the Dianabol and placebo groups were not significantly different from each other. The small sample size may be responsible for this lack of statistical significance.

With regard to training, the sedentary animals had a greater incidence of heart damage than did the exercised animals. This was primarily due to the sedentary-Dianabol group. The training effect on heart damage, between specific drug groups, was not statistically significant. However, the damage which was observed seemed to be concentrated in the Dianabol drug group. It should be noted again that the small sample size may have been responsible for the lack of statistical significance between the exercised drug groups. The fact that the observed damage occurred chiefly in the Dianabol group cannot be ignored.

The results also indicate a tendency for the exercise program to decrease the necrotic drug effect. The severity of damage in all animals receiving Dianabol was smaller in the exercised animals than in the sedentary animals.

While the absolute ventricular lengths were smaller in the exercised group than in the sedentary group, the relative ventricular lengths, ventricular weight, and heart weight in the exercised group were larger than in the sedentary group. These increases support the general opinion that vigorous training over a sufficient period of time produces cardiac hypertrophy.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this study was to observe the effects of the anabolic steroid, Dianabol, and an anaerobic program of endurance running on the heart muscle of male albino rats. Some animals were maintained in a sedentary condition while others were trained on a high-intensity, short-duration Controlled Running Wheel (CRW) program established in this laboratory. Myocardial damage was determined through a histologic technique of staining, examining, and rating different levels of the heart. Anatomical measures were taken on body weight, heart weight, ventricle weight, and ventricle length.

Forty-two normal, male, albino rats of the Sprague-Dawley strain were used for the study. All animals were received in the laboratory on the same day. However, they were in three different age groups at the time of their arrival. The differences in the ages of the animals represented a staggering procedure set up to accommodate other concurrent studies using the same facilities. Within his own age level, each animal was randomly assigned to a training-drug treatment group. All animals started treatment at 100 days of age.

Dianabol and a placebo were administered subcutaneously at a 1 mg/day dose. The animals received the training and drug treatments Monday through Friday for eight weeks. All animals were given food and water *ad libitum*.

The exercise animals were selected for sacrifice on the basis of having the highest percent of expected revolutions (PER) within their own drug groups. The final sample consisted of 36 animals (six per cell).

At sacrifice, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital. The heart was removed, trimmed, and weighed. After flushing and removal of the atria, the ventricular length was measured. The apical sections were dehydrated in graded concentrations of alcohol and then embedded in paraffin blocks. Serial cross sections, seven microns thick, were cut at eight levels spaced equally through the blocks. A Gomori trichrome stain was administered to the serial heart sections. Myocardial damage was determined by a subjective rating scale of one-to-five. A rating of one indicated no myocardial damage while a rating of five meant severe myocardial damage.

The results indicated there was a significant drug effect in terms of heart damage. The severity of heart damage was greater within the Dianabol group than in either the placebo or the control groups. Furthermore, training tended to decrease the severity and incidence of heart damage. Exercised animals showed a lesser degree of heart damage than did sedentary animals. Training also appeared to decrease the Dianabol necrotizing effect in that the exercised animals receiving Dianabol had lesser severity of heart damage than did the sedentary animals receiving Dianabol.

The exercised animals had smaller body weights than did the sedentary animals. Greater relative heart weights and ventricular weights were observed in the exercised group than in the sedentary group. There were significant training effects on both absolute and relative

ventricular lengths. The exercised animals had smaller absolute lengths and greater relative lengths than did the sedentary animals.

The body weights of the Dianabol and placebo groups were both greater than that of the control group but not different from each other. The relative ventricular lengths of the Dianabol and placebo groups were less than that of the control group but were not different from each other.

Conclusions

The results of the study have led to the following conclusions with regard to the albino rat, to the steroid dosage used, to the CRW SHORT program, and to the duration of the experimental period:

1. Dianabol does produce a greater degree of heart damage than either the placebo used or no drug injection.

2. The exercise program does decrease the occurrence of severe heart damage.

3. The exercise appears to decrease the necrotizing effect of Dianabol; however, there were no statistically significant training-drug interaction effects observed in this investigation.

4. Absolute total heart weight and absolute total ventricle weight are not affected by either training or Dianabol.

5. Body weight and absolute ventricle lengths are lower with exercise; however, on a relative basis heart weight, ventricle weight, and ventricle length are all higher. Absolute ventricle length is lower with exercise.

Recommendations

1. A wider variety of histochemical techniques should be used to determine the extent of myocardial damage.

2. The effects of Dianabol should be studied in conjunction with a variety of specific aerobic and anaerobic training regimes.

3. The effects of different doses of Dianabol should be studied.

4. The length of the treatment period should be altered to determine if anabolic steroid action is dependent on duration.

5. Experimentation with anabolic steroids on humans should be withheld until more is known about the actions of these steroids.

6. The effects of Dianabol on the myocardium should be studied in other experimental animals.

REFERENCES

REFERENCES

1. Almqvist, S., D. Ikkos, and R. Luft. Metabolic studies with methandrostenolone (17-hydroxy-17-methyl-androsta-1, 4-dien-3-one) in human subjects. *Acta Endocr.* 38:413-418, 1961.

2. Arnold, A., G. O. Potts, and A. L. Beyler. Evaluation of the protein anabolic properties of certain orally active anabolic agents based on nitrogen balance studies in rats. *Endocrinology* 72:408-417, 1963.

3. Badeer, H. S. Biological significance of cardiac hypertrophy. *Am. J. Cardiol.* 14:133-137, 1964.

4. Badeer, H. S. The stimulus to hypertrophy of the myocardium. *Circulation* 30:128-134, 1964.

5. Bajusz, E., and F. Homburger. Cardiomyopathies. *Science* 156: 1649-1653, 1967.

6. Bajusz, E., and G. Jasmin. Histochemically demonstrable phosphorylase as an early index of anoxic myocardial damage. *Experientia* 20:373-374, 1964.

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

8. Bajusz, E., and H. Selye. Adaptation to the cardiac necrosis-eliciting effect of stress. *Am. J. Physiol.* 199:453-456, 1960.

9. Becker, E. J., and F. Kreuzer. Sympathoadrenal response to hypoxia. In J. R. Poortmans (Ed.), *Biochemistry of Exercise*. Baltimore: University Park Press, 1968.

10. Bell, R. D. The effect of physical exercise and electrical stress on the production of experimental myocardial necroses. Ph.D. Thesis, Michigan State University, 1969.

11. Bloor, C. M., and N. M. Papadopoulos. Plasma lactic dehydrogenase activity and myocardial cellular changes after cessation of training. *J. Appl. Physiol.* 26:371-374, 1969.

12. Boris, A., R. H. Stevenson, and T. Trmal. Comparative androgenic, myotrophic and antigonadotrophic properties of some anabolic steroids. *Steroids* 15:61-71, 1970.

13. Boris, A., R. H. Stevenson, and T. Trmal. A comparison of the androgenic and myotrophic activities of some anabolic steroids in the castrated rat. *J. Steroid Biochem.* 1: 349-354, 1970.

14. Bowers, R. W., and J. P. Reardon. Effects of methandrostenolone (Dianabol) on strength development and anaerobic capacity. *Med. Sci. Sports* 4:54, 1972. (Abstract)

15. Brown, B. S., and A. Pilch. The effects of exercise and Dianabol upon selected performance and physiological parameters in the male rat. *Med. Sci. Sports* 3:1, 1971. (Abstract)

16. Bryant, R.E., W. A. Thomas, and R. M. O'Neal. An electron microscopic study of myocardial ischemia in the rat. *Circulation Res.* 6:699-708, 1958.

17. Crews, J. Effect of chronic exercise on myocardial function. *Am. Heart J.* 74:536-541, 1967.

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

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

20. De Schryver, C., J. Mertens-Strythagen, I. Becsei, and J. Lammerant. Effect of training on heart and skeletal muscle catecholamine concentration in rats. *Am. J. Physiol.* 217: 1589-1591, 1969.

21. Dorfman, R. I., and F. A. Kincl. Relative potency of various steroids in an anabolic-androgenic assay using the castrated rat. *Endocrinology* 72:259-266, 1963.

22. Enselberg, C. D. Physical activity and coronary heart disease. *Am. Heart J.* 80:137-141, 1970.

23. Euler, U. S. von, and S. Hellner. Excretion of noradrenaline and adrenaline in muscular work. *Acta Physiol. Scand.* 26:183-191, 1952.

24. Euler, U. S. von. Sympatho-adrenal activity and physical exercise. In J. R. Poortmans (Ed.), *Biochemistry of Exercise.* Baltimore: University Park Press, 1968.

25. Eyster, J. A. E. Experimental and clinical studies in cardiac hypertrophy. *J. Am. Med. Assoc.* 91:1881-1884, 1928.

26. Fahey, T., and H. Brown. Effects of anabolic steroids plus weight training on normal males--a double-blind study. *Med. Sci. Sports* 4:54, 1972. (Abstract)

27. Fanburg, B. L. Recent studies on cardiac hypertrophy. *Am. Heart J.* 81:447-450, 1971.

28. Fiegel, G. Untersuchungen über die wirkung von anabolen substanzen bei chronisch-degenerativen myokardschädigungen. *Ärztliche Forschung* 1:74-86, 1962.

29. Fine, G., A. Morales, and J. R. Scerpella. Experimental myocardial infarction. *Arch. Path.* 82:4-8, 1966.

30. Fowler, W. M., G. W. Gardner, and G. H. Egstrom. Effect of an anabolic steroid on physical performance of young men. *J. Appl. Physiol.* 20:1038-1040, 1965.

31. Frick, M. H., and M. Katila. Hemodynamic consequences of physical training after myocardial infarction. *Circulation* 37:192-200, 1968.

32. Gordon, E. E., K. Kowalski, and M. Fritts. Adaptations of muscle to various exercises. *J.A.M.A.* 199:139-144, 1967.

33. Gorlin, R. Evaluation of myocardial metabolism in ischemic heart disease. *Circulation* 39 and 40: (Suppl. IV) 155-162, 1969.

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

35. Grant, R. P. Aspects of cardiac hypertrophy. *Am. Heart J.* 46: 154-157, 1953.

36. Grimm, A. F., R. Kubota, and W. V. Whitehorn. Properties of myocardium in cardiomegaly. *Circulation Res.* 12:118-124, 1963.

37. Gudbjarnason, S., I. C. Fenton, P. L. Wolf, and R. J. Bing. Stimulation of reparative processes following experimental myocardial infarction. *Arch. Intern. Med.* 118:33-40, 1966.

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

39. Hochman, I. H., and Z. Laron. The effect of methandrostenolone on pituitary growth hormone secretion. *Horm. Metab. Res.* 2: 260-264, 1970.

40. Holloszy, J. O., J. S. Skinner, A. J. Barry, and T. K. Cureton. Effect of physical conditioning on cardiovascular function. *Am. J. Cardiol.* 14:761-769, 1964.

41. Izzo, J. L., and S. R. Glasser. Lack of anticatabolic effect of anabolic steroids in fasting rats. *Fed. Proc.* 20:197, 1961.

42. Johnson, L. C., G. Fisher, L. J. Silvester, and C. C. Hofheins. Anabolic steroid: effects on strength, body weight, oxygen uptake and spermatogenesis upon mature males. *Med. Sci. Sports* 4:43-45, 1972.

43. Johnson, L. C., and J. P. O'Shea. Anabolic steroid: effects on strength development. *Science* 1964:957-959, 1969.

44. Kitamura, K. The role of sport activities in the prevention of cardiovascular malfunction. In K. Kato (Ed.), *Proceedings of International Congress of Sport Sciences, 1964.* Tokyo: The Japanese Union of Sport Sciences, 1966.

45. Klionsky, B. Myocardial ischemia and early infarction. *Am. J. Path.* 36:575-583, 1960.

46. Kochakian, C. D. Mechanisms of androgen actions. *Lab. Invest.* 8:538-556, 1959.

47. Kochakian, C. D. The protein anabolic effects of steroid hormones. *Vit. and Hor.* 4:255-310, 1946.

48. Kochakian, C. D., J. H. Humm, and M. N. Bartlett. Effect of steroids on the body weight, temporal muscle and organs of the guinea pig. *Am. J. Physiol.* 155:242-250, 1948.

49. Korner, A., and F. G. Young. The influence of methylandrostenediol on the body weight and carcass composition of the rat. *J. Endocrin.* 13:78-83, 1955.

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

51. Leon, A. S., and C. M. Bloor. Effects of exercise and its cessation on the heart and its blood supply. *J. Appl. Physiol.* 24: 485-490, 1968.

52. Letunov, S. P. Effect of many years of sport activities on the cardiovascular system. In K. Kato (Ed.), *Proceedings of International Congress of Sport Sciences, 1964.* Tokyo: The Japanese Union of Sport Sciences, 1966.

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

54. Linzbach, A. J. Heart failure from the point of view of quantitative anatomy. *Am. J. Cardiol.* 5:370-381, 1960.

55. Litwhiler, R. W. A comparative study of the induction of cardiac myopathies in rats by selected stressors. M.A. Thesis, Michigan State University, 1968.

56. Lloyd, L. E., and C. L. Anthony. Methandrostenolone in the rations of young pigs. *J. Anim. Sci.* 24:893-894, 1965. (Abstract)

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

58. Montoye, H. J. Summary of research on the relationship of exercise to heart disease. *J. Sports Med. Phy. Fitness* 2:35-42, 1962.

59. Morales, A. R., and G. Fine. Early human myocardial infarction. *Arch. Path.* 82:9-13, 1966.

60. Morris, J. N., and M. D. Crawford. Coronary heart disease and physical activity of work. *Br. Med. J.* 2:1485-1496, 1958.

61. Morris, J. N., J. A. Heady, P. A. B. Raffle, C. G. Roberts, and J. W. Parks. Coronary heart-disease and physical activity of work. *Lancet* 2:1053-1057, 1953.

62. Murphy, J. D., and C. J. Eagen. Effect of anabolic steroid on endurance and body composition in the rat. *Med. Sci. Sports* 3:1, 1971. (Abstract)

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

64. Nickerson, M. Adrenergic regulation of cardiac performance. *Circulation Res.* 14 and 15: (Suppl. II) 130-136, 1964.

65. Niles, N. R., J. D. Zavin, and R. N. Monikado. Histochemical study of the effects of hypoxia and isoproterenol on rat myocardium. *Am. J. Cardiol.* 22:381-388, 1968.

66. Nocker, J., H. Reindell, and J. Keul. The adaptation of the body to training for endurance. In K. Kato (Ed.), *Proceedings of International Congress of Sport Sciences, 1964.* Tokyo: The Japanese Union of Sport Sciences, 1966.

67. Nowacki, P., E. Schmid, and F. Weist. The turnover of sympathicoadrenal hormones of sportsmen in training, anticipation and during competition, judged by measurements of the urinary excretion of 3-methoxy-4-hydroxy-mandelic acid. In J. R. Poortmans (Ed.), *Biochemistry of Exercise.* Baltimore: University Park Press, 1968.

68. Opie, L. H. Metabolism of the heart in health and disease. Part I. *Am. Heart J.* 76:685-698, 1968. Part II. *Am. Heart J.* 77:100-122, 1969. Part III. *Am. Heart J.* 77:383-410, 1969.

69. Papanicolaou, G. N., and E. A. Falk. General muscular hypertrophy induced by androgenic hormone. *Science* 87:238-239, 1938.

70. Pomroy, W. C., and P. D. White. Coronary heart disease in former football players. *J.A.M.A.* 167:711-714, 1958.

71. Raab, W. The neurogenic metabolic factor in ischemic heart disease. Pathogenesis and prevention. *Dis. Chest* 46:150-157, 1964.

72. Raab, W. *Preventive Myocardiology.* Springfield: Charles C. Thomas, 1970.

73. Raab, W., J. P. Chaplin, and E. Bajusz. Myocardial necroses produced in domesticated rats and in wild rats by sensory and emotional stresses. *Proc. Soc. Exper. Biol. Med.* 116:665-668, 1964.

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

75. Raab, W., P. DePaula e Silva, H. Marchet, E. Kimura, and Y. K. Starcheska. Cardiac adrenergic preponderance due to lack of physical exercise and its pathogenic implications. *Am. J. Cardiol.* 5:300-316, 1960.

76. Raab, W., E. Stark, W. H. Macmillan, and W. R. Gigee. Sympathogenic origin and antiadrenergic prevention of stress-induced myocardial lesions. *Am. J. Cardiol.* 8:203-209, 1961.

77. Regan, T. J., C. B. Moschos, H. A. Oldewurtel, and H. K. Hellems. Metabolic role of catecholamines and the production of myocardial necrosis. In W. Raab (Ed.), *Prevention of Ischemic Heart Disease.* Springfield: Charles C. Thomas, 1966.

78. Schimert, G. C., and H. Schwalb. Functional and metabolic factors in the origin and prevention of myocardial ischemia. In W. Raab (Ed.), *Prevention of Ischemic Heart Disease.* Springfield: Charles C. Thomas, 1966.

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

80. Shimamoto, T., and Y. Hiramoto. Electron microscopic observations on edematous myocardial response to epinephrine. In W. Raab (Ed.), *Prevention of Ischemic Heart Disease*. Springfield: Charles C. Thomas, 1966.

81. Smodlaka, V. Interval training in cardiovascular diseases. In W. Raab (Ed.), *Prevention of Ischemic Heart Disease*. Springfield: Charles C. Thomas, 1966.

82. Stamler, J., H. A. Lindberg, D. M. Berkson, Y. Hall, W. A. Miller, L. Mojonnier, M. B. Epstein, F. Burkey, D. B. Cohen, M. Levinson, and Q. D. Young. Approaches of the coronary prevention evaluation program to the primary prevention of clinical coronary heart disease in middle-aged American men. In W. Raab (Ed.), *Prevention of Ischemic Heart Disease*. Springfield: Charles C. Thomas, 1966.

83. Steinhaus, A. H. Chronic effects of exercise. *Physiol. Rev.* 13: 103-147, 1933.

84. Thomas, D. P. The effects of forced-exercise and anxiety-producing stress on myocardial damage in young male adult albino rats. M.A. Thesis, Michigan State University, 1970.

85. VanLiere, E. J., B. B. Krames, and D. W. Northup. Differences in cardiac hypertrophy in exercise and in hypoxia. *Circulation Res.* 16:244-247, 1965.

86. Wainman, P., and G. C. Shipounoff. The effects of castration and testosterone propionate on the striated perineal musculature in the rat. *Endocrinology* 29:975-978, 1941.

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

88. Wells, R. L., and W. W. Heusner. A controlled-running wheel for small animals. *Lab. Anim. Sci.* 21:904-910, 1971.

89. Whitehorn, W. V., and A. S. Grimmenga. Effects of exercise on properties of the myocardium. *J. Lab. Clin. Med.* 48:959, 1956. (Abstract)

90. Wilkerson, J. E., and E. Evonuk. Changes in cardiac and skeletal muscle myosin ATPase activities after exercise. *J. Appl. Physiol.* 30:328-330, 1971.

91. Wilson, J. L. Forced exercise and cardiac pathology in rats. M.A. Thesis, Michigan State University, 1968.

92. Wolffe, J. B. The role of exercise in the prevention of cardiovascular diseases. In K. Kato (Ed.), *Proceedings of International Congress of Sport Sciences, 1964*. Tokyo: The Japanese Union of Sport Sciences, 1966.

APPENDICES

APPENDIX A

TRAINING PRØGRAM

Table A-1. Standard eight-week, short-duration, high-intensity endurance training program for postpubertal and adult male rats in controlled-running wheels

Wk.	Day of Wk.	Day of Tr.	Acceleration Time (sec)	Work Time (min:sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
	5=F	5	2.0	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
2	1=M	6	1.5	00:10	10	28	4	5.0	1.2	2.5	51:40	700	1120
	2=T	7	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	3=W	8	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	4=T	9	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	5=F	10	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
3	1=M	11	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	2=T	12	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	3=W	13	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	4=T	14	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	5=F	15	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
4	1=M	16	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	2=T	17	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	3=W	18	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	4=T	19	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	5=F	20	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
5	1=M	21	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	2=T	22	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	3=W	23	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	4=T	24	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	5=F	25	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
6	1=M	26	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	2=T	27	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	3=W	28	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	4=T	29	2.0	00:10	35	10	5	5.0	1.0	5.0	54:34	625	500
	5=F	30	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
7	1=M	31	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	2=T	32	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	3=W	33	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	4=T	34	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	5=F	35	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
8	1=M	36	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	2=T	37	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	3=W	38	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	4=T	39	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	5=F	40	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480

APPENDIX B

SUBJECTIVE MYOCARDIAL DAMAGE RATINGS

Table B-1. Subjective ratings of myocardial damage according to treatments and by section level

Animal Number	Treatments		Section Level							
	Training	Drug	1	2	3	4	5	6	7	8
01	E	D	1	1	1	1	1	2	2	1
03	E	D	1	2	2	1	1	1	1	1
04	E	D	3	3	3	3	1	1	3	1
05	E	P	1	1	1	1	1	1	1	1
06	E	P	1	1	1	1	1	1	1	1
07	E	P	1	1	1	1	1	1	1	1
09	E	C	1	1	1	1	1	1	1	1
10	E	C	1	1	1	1	1	1	1	1
11	E	C	1	1	1	1	1	1	1	1
13	S	D	1	1	1	1	1	1	1	1
14	S	P	1	1	1	1	1	1	1	1
15	S	C	1	1	1	1	1	1	1	1
16	S	D	1	2	2	2	1	1	1	2
17	S	D	4	1	1	1	1	1	1	1
18	S	D	3	3	3	3	3	1	1	1
19	S	D	3	3	3	3	3	1	1	1
20	S	P	1	1	1	1	1	1	1	1
21	S	P	1	1	1	1	1	1	1	1
22	S	P	1	1	1	1	1	1	1	1
23	S	P	1	1	1	1	1	1	3	3
24	S	C	1	1	1	1	1	1	1	1
25	S	C	2	2	2	1	1	1	1	1
26	S	C	2	1	2	2	1	1	1	1
27	S	C	2	2	1	1	1	1	1	1
28	E	D	2	1	2	2	2	1	1	1
29	E	D	2	2	1	1	1	1	1	1
31	E	D	2	2	1	1	1	2	1	1
32	E	P	2	2	1	1	1	1	1	1
34	E	P	1	1	1	1	1	1	1	1
35	E	P	2	2	2	2	2	1	1	1
36	E	C	2	2	2	2	2	2	1	1
38	E	C	1	1	1	1	1	1	1	1
39	E	C	1	2	2	2	2	1	2	1
40	S	D	2	2	2	1	1	1	1	1
41	S	P	2	2	2	2	2	1	1	1
42	S	C	1	1	1	1	1	1	1	1

APPENDIX C

ANATOMICAL RAW DATA

Table C-1. Body weight and absolute and relative anatomical myocardial results (gm, cm) presented by animal number, training and drug treatments

Animal Number	Treatments		Body Weight	Heart Weight		Ventricular Weight		Ventricular Length	
	Training	Drug		Absolute	Relative	Absolute	Relative	Absolute	Relative
01	E	D	435	1.3519	0.0031	1.2450	0.0029	0.6590	0.0015
03	E	D	453	1.2689	0.0028	1.1705	0.0026	0.6430	0.0014
04	E	D	446	1.4240	0.0032	1.3119	0.0029	0.6930	0.0016
05	E	P	426	1.3657	0.0032	1.2613	0.0030	0.6580	0.0015
06	E	P	414	1.3268	0.0032	1.2351	0.0030	0.6780	0.0016
07	E	P	456	1.4830	0.0033	1.3439	0.0029	0.6620	0.0015
09	E	C	415	1.3845	0.0033	1.2824	0.0031	0.6590	0.0016
10	E	C	398	1.2493	0.0031	1.1467	0.0029	0.6630	0.0017
11	E	C	410	1.2739	0.0031	1.1704	0.0029	0.6640	0.0016
13	S	D	485	1.3175	0.0027	1.2348	0.0025	0.6480	0.0013
14	S	P	508	1.5116	0.0030	1.4044	0.0028	0.6840	0.0013
15	S	C	502	1.5018	0.0030	1.3972	0.0029	0.6800	0.0014
16	S	D	488	1.3436	0.0028	1.2073	0.0025	0.6570	0.0013
17	S	D	490	1.2592	0.0026	1.1563	0.0024	0.6790	0.0014
18	S	D	500	1.4580	0.0029	1.3088	0.0026	0.6880	0.0014
19	S	D	487	1.3096	0.0027	1.1836	0.0024	0.6770	0.0014
20	S	P	514	1.3782	0.0027	1.2425	0.0024	0.6660	0.0013
21	S	P	483	1.2790	0.0026	1.1702	0.0024	0.6710	0.0014
22	S	P	502	1.4535	0.0029	1.3300	0.0026	0.7200	0.0014
23	S	P	528	1.4036	0.0027	1.2858	0.0024	0.6780	0.0013
24	S	C	482	1.2766	0.0026	1.1823	0.0025	0.6660	0.0014
25	S	C	494	1.3619	0.0028	1.2574	0.0025	0.7090	0.0014
26	S	C	467	1.3073	0.0028	1.1914	0.0026	0.6590	0.0014

Table C-1 (cont'd.)

Animal Number	Treatments		Body Weight	Heart Weight		Ventricular Weight		Ventricular Length	
	Training	Drug		Absolute	Relative	Absolute	Relative	Absolute	Relative
27	S	C	500	1.3230	0.0026	1.1928	0.0024	0.6700	0.0013
28	E	D	419	1.3375	0.0032	1.2072	0.0029	0.6310	0.0015
29	E	D	451	1.3937	0.0031	1.2576	0.0028	0.6430	0.0014
31	E	D	409	1.3240	0.0032	1.2234	0.0030	0.6410	0.0016
32	E	P	451	1.4854	0.0033	1.3328	0.0030	0.6730	0.0015
34	E	P	433	1.4650	0.0034	1.3203	0.0030	0.6490	0.0015
35	E	P	401	1.3215	0.0033	1.1954	0.0030	0.7000	0.0017
36	E	C	435	1.2720	0.0029	1.1410	0.0026	0.6800	0.0016
38	E	C	409	1.3134	0.0032	1.1854	0.0029	0.7020	0.0017
39	E	C	398	1.3114	0.0033	1.1941	0.0030	0.6810	0.0017
40	S	D	506	1.3600	0.0027	1.2164	0.0024	0.6910	0.0014
41	S	P	505	1.3435	0.0027	1.2172	0.0024	0.7090	0.0014
42	S	C	453	1.4543	0.0032	1.3310	0.0029	0.6950	0.0015