THE UNIVERSITY OF MANITOBA

# THE EFFECTS OF EXERCISE STRESS ON THE RAT HEART

BY

BRENDA LOVERIDGE

A Thesis

Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science.

Department of Physiology

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To My Family

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ABSTRACT

Although it is generally believed that exercise is beneficial for an individual, experimental data in support of this view is less than conclusive. In this regard, we have studied two important aspects of the effects of chronic exercise on the heart 1) Is exercise good for the peart per se? 2) Does chronic exercise improve the capability of the heart to withstand a subsequent stress? For this purpose rats were subjected to an exhaustive four week swimming programme,twice daily, five days a week at a water temperature of 22$^{o}$ C. The sedentary rats were maintained at normal cage activities. At the end of four weeks most of the exercise stressed as well as sedentary rats were injected with different concentrations of isoproterenol (20,40 or 80 mg/kg) and the remainder of the animals not injected with isoproterenol served as controls. Electrocardiography (EKG), serum enzyme analysis and heart histology were performed on all rats to assess the beneficial and/or detrimental effects of chronic exercise.

At sacrifice, the exercise stressed control animals had significantly lower body weights, increased wet heart weights, and increased heart weight/body weight ratios as compared to the sedentary rats. Furthermore, these untreated control rats exhibited a significant increase in the amplitude of the R wave. Although heart rate (HR) was slightly decreased in the exercised rats, this was not significant. Serum glutamic oxaloacetic transaminase (SGOT) was elevated in the exercise control group in the absence of any alteration in lactate dehydrogenase (LDH) and creatine phosphokinase (CPK). Focal necroses and increased collagen formation was apparent in hearts from exercise

stressed controls on light microscopic examination.

Following injection with isoproterenol, the exercise stressed rats showed no increase in heart weight/body weight ratios in contrast to significant increases in the sedentary animals treated with isoproterenol. The exercised treated animals exhibited a significantly greater tachycardia response to isoproterenol within 15 minutes of the drug administration. Subsequently, the EKG's showed progressive slowing of heart rate and conduction defects in exercised rats. In contrast, some of the sedentary treated rats demonstrated ventricular arrhythmias progressing to ventricular fibrillation. Mortality was significantly greater in the exercise stressed group but appeared to be associated with symptoms of pulmonary edema. Serum CPK, LDH and SGOT were all significantly elevated in sedentary and exercise stressed rats injected with 80 mg/kg isoproterenol. However, SGOT was significantly higher at all doses of isoproterenol in exercise stressed rats. At all doses of isoproterenol, hearts from sedentary treated rats demonstrated greater histological damage than did hearts from exercise stressed animals.

These data indicated that the stressful exercise protocol induced myocardial necrosis in the control rats which appears to have produced an adaptation that protected the myocardium from further damage when the exercised rats were exposed to another stress (isoproterenol). Thus, exercise appears to be detrimental as well as beneficial to the heart. Further research into the intensity and type of exercise is required to define the limits which will result in only beneficial effects.

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# I INTRODUCTION

Acute as well as chronic exercise protocols are a form of stress. Acute exercise induces many of the same cardiovascular reponses attributed to other forms of stress such as heat, cold or psychological factors. Chronic exercise,on the other hand, induces cardiovascular compensatory adaptations which may include increased sympathetic tone and heart hypertrophy. Whether these adaptive changes in response to chronic exercise are detrimental or beneficial remains to be elucidated. Furthermore, how these adaptive effects in response to chronic exercise can be modified if this stress is combined with some other stress is also not known.

The effects of chronic exercise have been the subject of a great many studies in both human and animal models. The beneficial effects are generally attributed to an improved pumping efficiency of the heart through an increased stroke volume and a lower resting heart rate, and through peripheral cardiovascular adaptations. These include enhancement of skeletal muscle metabolism and performance and an enhanced skeletal muscle oxygen extraction.

In animal models, both beneficial and detrimental effects of exercise on the heart have been observed. Generally, the detrimental effects have been observed in exhaustive exercise programmes or in programmes where the exercise was associated with other stresses, such as intense heat or cold. In exercise protocols where other forms of stress are minimized, the adaptation in cardiac performance has been considered beneficial.

There has been a paucity of work examining the combined effects of exercise and other stressors. The literature that is available suggests that exercise combined with other stressful influences, in contrast to exercise alone, may be detrimental to cardiac performance.

The purpose of this investigation was to examine the effects of exercise stress on cardiac performance.

An exhaustive swimming exercise protocol was employed. The cardiovascular response of the exercised rats in comparison to sedentary animals was monitored by employing electrocardiography, serum enzyme analysis and histological examination. The response of the exercise stressed rats to various doses of isoproterenol was also studied.

# II LITERATURE REVIEW

## THE EFFECTS OF CHRONIC EXERCISE ON THE HEART

### A) GENERAL

Exercise training produces profound biochemical, morphological and functional changes in the cardiovascular system. The effects of chronic exercise on the autonomic nervous system, on myocardial structure and function, and the responses to hypoxia and ischemia will be reviewed.

The animal models which have been most extensively employed to study these effects are the rat and the dog. Exercise studies utilize two major modalities, treadmill running (Krames et al,1976; Barnard et al, 1971; Maher et al, 1972; Sordahl et al,1977; Dowell, et al, 1977; Carew et al, 1978; Schaikle et al, 1979) or swimming, (Baker et al, 1964; Dawson et al, 1968; Penpargkul et al, 1970; Wilkerson et al, 1971; Bhan et al, 1972; Scheuer et al,1974; Bhan et al, 1975; Holloszy et al, 1976; Bershon et al, 1977; Penpargkul et al, 1977; Penpargkul et al, 1978). There are several problems in comparing the results obtained in different species and different exercise models where duration, intensity and the frequency of training protocols vary. The effects of swimming protocols will be reviewed. Reference to treadmill findings will be presented to further support or to refute the effects found in swim trained animals.

### B) EFFECTS ON AUTONOMIC NERVOUS SYSTEM

Miyagi et al (1979) observed that the stress of exercise and the stress of hypoxemia both equally induced sympathetic nervous system excitation. They measured catecholamine levels in the coronary

sinus and arterial plasma in man and found no significant differences between either stressor. Scheuer and Tipton (1977) referred to exercise as a "disruption of a homeostatic relationship that has been caused by bodily movement". The cardiovascular effects resulting from a stress activated sympthetic response are the same regardless of the type of stress but the metabolic effects are specific to the stress situation (Leblanc, 1969).

Increased central nervous system (CNS) resting norepinephrine levels have been observed in exercise trained rats (Brown and Van Huss, 1973). If norepinephrine modulates central sympathetic tone this may represent a higher maintained level of sympathetic output in conditioned animals as compared to sedentary animals.

Brown et al (1973) further observed that exercise training evoked a greater depletion of brain norepinephrine than shock stress to sedentary rats and suggested that exercise evoked a greater sympathetic discharge than shock stress.

DeSchryver et al (1972) reported that chronic exercise induces a decrease in the sympathetic neurotransmitter, norepinephrine, in cardiac tissue. They also observed a close relationship between the intensity of exercise and cardiac catecholamine depletion and found that there was no difference in myocardial norepinephrine levels in animals trained by either spontaneous or forced running (DeSchryver et al, 1972). In other studies, DeSchryver et al (1968) observed that the catecholamine response is reversible and that on cessation of training, normal levels are restored within six days.

Crews et al (1967) and Maher et al (1972) found that cardiac endogenous catecholamine stores were unchanged following

exercise. Crews et al (1967) swim trained rats and then studied cardiac function in an open chest in vivo preparation. Intravenous injection of 3 mg/kg of epinephrine elicited a decrease in isometric systolic tension development of the right ventricle. Similarly, Maher et al (1972) observed a negative inotropic effect when isolated trabeculae muscle from treadmill exercised rats was exposed to a bath containing 0.3 mg/ml norepinephrine. Crews et al (1967) suggested this hyposensitivity may reflect a relative refractoriness of the myocardium to increased circulating catecholamines during muscular exercise and that this decreased sensitivity to catecholamines deprives the heart of an important compensatory mechanism which the sympathetic nervous system usually provides.

In contrast to the report of Crews and co-workers (1967) Wyatt et al (1978) observed that training enhances the sensitivity of cardiac muscle to catecholamines.They suggest the results of Crews et al (1967) may have been affected by the anesthesia and thoracotomy for open chest in vivo observations. Wyatt et al (1978) found an increased adenylate cyclase activity following isoproterenol administration in trained cats. He suggested the affinity of adenylate cyclase for isoproterenol was unchanged by training but that the maximum velocity of interaction was greater. Dowell and Tipton (1970) found that hearts from trained rats beat faster after infusion or injection of isoproterenol than hearts from non-trained animals.

Salzman et al (1970) exercise trained mice and found that the ventricles of these mice were more sensitive to high doses of exogenous epinephrine than sedentary controls and that males were

more sensitive than females. However, the uptake of catecholamines by the sympathetic nerve endings in the myocardium was decreased. The decreased uptake of catecholamines suggests that more of the catecholamines may be free to act on other receptor sites and exert their influence on the heart (Salzman et al,1970).

Resting bradycardia is a widely accepted result of physical training programmes in animal models (Lin and Horvath,1972;Carey and Tipton,1976; Hughson et al,1977; Dowell et al,1977;Schaible et al 1979). The exact mechanism for the development of bradycardia in conditioned animals is unclear. However, current evidence clearly indicates that an alteration in autonomic nervous system activity plays a significant role.

Hughson et al (1977) found that rats maintained on treadmill exercise exhibited decreased intrinsic sino-atrial rate and that this phenomenon occurred independently of any increased parasympathetic activity. Atropine did not block the decreased intrinsic activity in exercised rats but rather enhanced it. These authors further noted that the chronotropic effects of norepinephrine on isolated atria removed from exercised rats were significantly attenuated although the response to acetylcholine was unaltered.

Lin and Horvath (1972) swim trained rats to produce a bradycardia response. They found a greater decrease in sympathetic tone than parasympathetic tone following training, but also observed that both sympathetic and parasympathetic activity was decreased in exercised animals as compared to sedentary rats. Bolter et al (1973) in swim trained rats found a marked subsensitivity to acetylcholine and concluded that this represented an increased tonic vagal cardiac inhibitory activity. He also noted that with total autonomic blockade

with atropine and propranolol, isolated atrial preparations removed from trained animals had significantly lower spontaneous rates than atria removed from untrained animals.

C) MYOCARDIAL HYPERTROPHY

Myocardial hypertrophy is another frequently observed result of an exercise training programme (Krames, et al, 1967; Leon and Bloor, 1968; Penpargkul et al, 1970; Wilkerson et al, 1971; Bhan et al,1972; Scheuer et al, 1974; Carew and Covell, 1978; Hickson et al,1979). However, some authors have found no increase in ventricular mass in dogs trained by treadmill running (Sordhal et al, 1977; Dowell et al, 1977). They further reported that in the absence of ventricular hypertrophy the maximal rate of ventricular pressure development was increased. Tibbits et al, (1978), also found no significant myocardial hypertrophy in rats trained on a treadmill. They further suggested that swimming produces hypertrophy because it places a more significant stress on the heart.

In contrast, Carew et al (1978) observed significant left ventricular hypertrophy in exercise trained greyhounds, but no change in resting contractility or in contractility indices at increased end-diastolic pressures as compared to normal dogs. Rats trained on the treadmill have also been shown to develop myocardial hypertrophy (Krames et al, 1967). However, they measured isometric pressure development of the left ventricle in vivo and did not find any differences in maximum pressures between exercised and control animals. Scheuer and Tipton (1977) stated that forced running resulted in a greater increase in heart weight than spontaneous running. Increased heart weights have been observed repeatedly in swim trained

rats (Bhan et al, 1972; Scheuer et al, 1974; Scheuer and Tipton, 1977).

Leon and Bloor (1968) found that myocardial hypertrophy occurred in continuously exercised rats, but not in animals on an intermittent training programme.

Hypertrophy is considered to be a compensatory mechanism resulting from a sustained period of hyperfunction (Meerson,1974).It has been suggested that exercise protocols of moderate intensity do not produce myocardial hypertrophy whereas more stressful exercise programmes do induce hypertrophy (Bershon et al, 1977).

In view of the contradictory reports regarding the development of myocardial hypertrophy induced by exercise training, an increase in muscle mass should not be considered a sensitive index of training responses in animals.

When myocardial hypertrophy is observed in animal models, it involves an increase in collagen formation as well as an enlargement of myofibrils (Grove et al,1969;Bartosova et al, 1969;Skosey et al, 1972; Zak, 1974; Morkin et al, 1974; Meerson et al, 1974). The contribution of each to the increase in mass is variable depending partially at least on the type of stimulus inducing hypertrophy, the length of its action (Bartosova et al, 1959) and the age of the animal (Bartosova et al, 1969; Zak,1974). Bartosova et al (1969) observed a marked increase in wet heart weight and in collagen formation in two and a half month old rats subjected to a six week treadmill exercise programme. However, they found no increase in either wet heart weight or collagen formation in eight and a half month old rats trained on the same programme. Hypoxia is a powerful stimulus to fibroblast activity and

increased collagen formation in the heart (Bartosova et al, 1969). Zak (1974) reported that in adult rats under work overload only hyperplasia of the connective tissue cells occurred and that in growing animals hyperplasia of both myocytes and connective tissue cells occurred. In adult rats existing myocytes increased in volume not in number (Grove et al, 1969; Carew et al, 1978).

Meerson et al (1974) (1978) stated that hypertrophy of the myocardium as an adaptive mechanism is relative, and that a long period of extensive hypertrophy is usually followed by a more rapid aging of the myocardium.

D) SUBCELLULAR CHANGES

Leon and Bloor (1968) exercise trained rats by swimming in 28-32$^{o}$ C water. They observed a significant increase in coronary artery diameter with training. As well, they observed focal necroses in the myocardium of animals subjected to a six day a week programme, whereas, intermittently exercised rats did not exhibit myocardial lesions. They suggested that a relative hypoxia due to the increased metabolic demands during exercise served as the stimulus for the developed vascular changes and that the focal necroses resulted from a relative coronary insufficiency. The lesions consisted of hyalization of muscle fibers, loss of myofibril bundles, and an extensive mononuclear cellular infiltrate indicating that the lesion was in the later phases of healing.

It is generally accepted that cardiac mitochondria have the reserve capacity to meet the energy demands of most exercise training protocols without compensatory adaptations. No changes in mitochondrial cytochrome C oxidase, ATPase activity or calcium content

has been observed in swim trained rats (Penpargkul et al,1978). Dohnm et al (1972) also found no difference in heart mitochondrial oxygen uptake in treadmill trained rats. Sordhal et al (1977) and Dowell et al (1977) reported that unlike skeletal muscle, the heart does not undergo an adpative increase in respiratory capacity as a result of exercise training. They found the activities of mitochondrial enzymes, the concentration of cytochrome C oxidase and the protein expressed per gram of heart were unchanged following training.

In contrast to more moderate exercise training, exhaustive exercise has demonstrated swelling and disruption of cardiac mitochondria (King et al, 1970; Banister et al,1971; Dohm... et al, 1972). The adverse effects disappear approximately twenty-four hours after the exhaustive exercise session (Banister et al, 1971). In contradiction to these findings, Terjung et al (1973) observed that a single bout of exhaustive exercise employing both swimming and treadmill modalities, resulted in no change in the respiratory capacities or citrate synthase activity of the heart mitochondria. He also found normal mitochondrial yields and normal appearance of mitochondria on electon microscopic examination.

E) EXERCISE TRAINING AND RESPONSES TO HYPOXIA AND ISCHEMIA

Carey and Tipton (1976) found that treadmill trained rats were able to maintain a higher level of cardiac performance (dP/dt max) following hypoxia. They suggested that training may increase receptor sensitivity to released and/or circulating catecholamines, and that this may be related to the improved performance of trained animals during hypoxia. Scheuer et al (1972) found that hearts from rats conditioned by swimming have enhanced pumping ability when subjected to hypoxia.

They observed that conditioned hearts develop the same systolic pressure from a lower end diastolic pressure than hearts from sedentary rats. However, under ischemic conditions trained animals demonstrated a more rapid decline in myocardial performance than sedentary animals (Carey and Tipton,1976).

F) SWIMMING EXERCISE

1) Swimming Exercise at Thermoneutrality

The swimming protocols designed to investigate only exercise responses involved swimming rats at water temperatures between 33-37$^{o}$ C. Extensive research has been conducted utilizing this exercise model. Bershon and Scheuer (1977) swim trained rats for eight weeks and then measured left ventricular and aortic pressures, and cardiac output in an *in vitro* isolated working heart model. From these measurements, they approximated values for external work. They demonstrated an increased cardiac performance in exercised rats as compared to sedentary animals at the same end diastolic pressure and volume, and at all atrial pressures recorded between 5 and 20 cm $H_2O$. They further recorded an increased ejection fraction, peak systolic pressure, peak aortic flow, cardiac output and stroke work in conditioned hearts. It was concluded that the enhanced pumping performance was due to a change in ventricular muscle function. Faster relaxation was a prominent effect of physical training (Bershon et al, 1977; Penpargkul et al, 1978). This improved relaxation may be related to the greater calcium uptake and binding in the sarcoplasmic reticulum (SR) of exercise trained rats (Penpargkul et al,1977). Penpargkul et al, (1977) observed that cardiac

microsomes from conditioned hearts transport calcium to a greater extent than microsomes from sedentary rats. In contrast, Sordhal et al (1977) found no differences in rates of calcium uptake or binding by the SR in treadmill trained dogs. They also observed a decreased rate of release of bound calcium from the SR .

Exhaustive exercise in rats has been found to depress calcium uptake by the SR by approximately 50 percent and to lower calcium stimulated ATPase activities (Sembrowich et al, 1977; and Hashimoto et al, 1978). Maher et al (1972) also found exhaustive treadmill exercise in rats produced a marked depression of myocardial performance.

Interestingly, Sordhal et al (1977) observed no significant difference in mitochondrial calcium uptake in trained dogs. However, he found that the mitochondria from trained dogs failed to take up all the calcium present, and released calcium more rapidly than did control animals.

Other investigations by Bhan and Scheuer (1972 and 1975) and Wilkerson et al (1971) have found increased actomyosin and myosin adenosine triphosphatase (ATPase) activities in conditioned hearts. The improved contractility of the left ventricle may be related to alterations in calcium transport mechanisms or to improved intrinsic functioning of the contractile proteins (Bahn et al, 1972). Crews et al (1967) also found increased myocardial contractility in exercise trained rats. Tibbits et al (1978) in Lanthanum studies on isolated papillary muscle from treadmill trained rats suggest

an enhanced extracellular calcium availability in trained animals and suggested that this increased calcium may play a role in the improved myocardial performance.

Schaible et al (1979) found that the increased rates of relaxation and the increased actomyosin ATPase activities which occurred in swim trained rats did not occur in treadmill trained animals. Dowell et al (1977) also found normal ATPase activities in treadmill trained dogs who demonstrated an increased myocardial contractile performance. This suggests possible differences in the biochemical adaptations between swimming and treadmill training protocols.

It appears indesputable that alterations in calcium transport occur as a result of exercise training. However, the precise mechanism or mechanisms and the contribution of each to enhanced or diminished performance remains unclear.

Furthermore, a greater increase in oxygen consumption, cardiac output and cardiac work has been osberved in conditioned hearts subjected to increasing atrial filling pressures compared to hearts from sedentary rats (Penpargkul et al,1970). The improved dynamic responses were considered to be due, at least in part, to the improved coronary blood flow in trained hearts allowing enhanced mechanisms of oxygen delivery.

2) Swimming Exercise and Hypothermic Stress

Water temperature is critical when employing swimming as the exercise modality. The temperature must be maintained between $33^{o}$ C and $37^{o}$ C to obtain a thermoneutral environment

for the rat (Hart et al, 1963; Dawson et al, 1970; Harri et al, 1975). The maximum duration a rat is able to swim is directly related to water temperature at levels below 30° C as the body temperature of the rat falls and hypothermia results (Tan et al, 1954; Dawson et al, 1968; Dawson et al, 1970; Harri et al, 1975). Rectal temperatures of rats swum in water below 30° C are consistently approximately 26° C at the point of exhaustion (Baker et al, 1964; Dawson et al, 1968). Rectal temperatures do not vary in rats swum at temperatures between 33-37° C (Baker et al, 1964), and the end point of exercise is governed by muscle fatigue (Tan et al, 1954). Body temperatures of rats decrease immediately on immersion in cold water and the limiting factor of exercise is not muscle fatigue but rather hypothermia. No differences in rate of temperature drop was reported between naive rats and previously exercised trained rats when exposed to a cold swim (Dawson et al, 1968). Dawson et al (1968) further observed that at 22° C the maximum swim time of rats was eighteen minutes and that this time was very reproducible on repeated swims.

Exercise and hypothermic stress is considerably more severe than exercise stress alone at 37° C. Cardiac output increases initially and then falls progressively to a level approximately 20 percent below resting cardiac output values as core temperature drops near exhaustion (Dawson et al, 1968). Heart rate also decreases linearly with decreasing rectal temperatures. Stroke volume increases slightly but not

sufficiently to maintain cardiac output. Blood pressure remains constant as a progressive increase in total peripheral resistance occurs. No increased stroke volume was observed in immobilized cold stressed rats, rather only in combined exercise and cold stressed animals (Dawson et al, 1968).

Yang and Lissak (1960) found a significantly higher blood lactate concentration in rats swum to exhaustion in 18$^{o}$ C and 24$^{o}$ C water as compared to animals swum to exhaustion in water at thermoneutrality. They suggested that the increased lactate in the hypothermic exercise model was a result of a relative hypoxia. Leon and Bloor (1968) further suggested that swimming exercise may induce hypoxia through influences on body temperature. Adolph (1950) and Baker et al (1964) observed that cold swum rats showed an initial increase in oxygen consumption followed by a steady decline as body temperatures dropped. Animals swimming in cold water were exhausted before their oxygen uptakes stabilized (1964).

Dawson et al (1968) suggested an inadequate cardiac response occurred in animals subjected to the combined stresses of hypothermia and exercise. As cardiac output falls with decreasing body temperature, the heart as a pump may become a factor limiting swim performance in water below thermoneutrality.

## RELATIONSHIP OF EXERCISE TO OTHER FORMS OF STRESS

### A) GENERAL

It has been suggested that there may be other chronic stress reactions involved in exercise protocols, especially swimming, as it is

considered a life threatening situation (McArdle, 1967; Leon and Bloor 1968; Dawson et al, 1970; Scheuer et al, 1977). However, no conclusive evidence has been established to prove or to refute that some of the effects of exercise programmes might be due to a chronic psychological stress reaction. Exercise, cold and footshock stress have been shown to illicit similar sympathetic responses. Corticosterone is the major glucocorticoid secreted in response to all of these stresses (Gigee, 1961; Raab et al, 1964; Baldwin et al, 1972; Chin et al, 1973; Bassett and Cairncross, 1976). Increased plasma corticosterone has been implicated in the enhanced myocardial sensitivity to norepinephrine observed in rats subjected to irregular foot shock stress ( Bassett and Cairncross, 1976) and in rats subjected to isolation stress (Raab et al, 1964). Catecholamine secretion is also increased in stress (Gigee, 1961; Chin et al, 1973). Three mechanisms for the effects of corticosterone have been suggested by Bassett and Cairncross (1976):

1) Inhibition of catecholamine catabolizing enzymes, specifically catechol-O-methyltransferase.
2) Inhibition of active uptake of catecholamines into storage sites.
3) A direct change in the sensitivity of the effector cell itself.

There is a significant decrease of endogenous catecholamine stores in the myocardium following exercise training (Bescei et al, 1969; Salzman et al, 1970; DeSchryver et al, 1972; Wyatt et al,1978), cold acclimation (Hsieh et al, 1957; Hsieh et al, 1971; Leblanc, 1971), and irregular footshock stress (Bassett and Cairncross,1976).

-- Stressed rats demonstrate a greatly increased catecholamine cardiotoxicity as manifested by a considerable decrease in the lethal dose of isoproterenol (Raab et al, 1964) and norepinephrine (Hsieh et al, 1971).

B) COLD STRESS

Animals subjected to prolonged cold stress and acclimated to cold exhibit some of the same cardiovascular changes as exercise trained animals.

Cold acclimated animals gain weight less rapidly than controls (Stromme et al, 1967; Harri et al, 1975). Animals exposed to combined exercise and cold maintain the same growth curves as do animals exercised in a neutral environment (Stromme et al, 1967). Resting bradycardia is exhibited after eight weeks of cold acclimitization (Stromme et al, 1967).

Gordon et al (1966) observed that the stress of exercise and the stress of cold both produced an increased synthesis of norepinephrine and epinephrine. As in exercise stress, cold stress increases the sensitivity to catecholamines ( Depocas, 1960; Leblanc,1971; Hsieh, 1971).

Hsieh et al (1971) observed that rats exposed to $60^{o}$ C for two months were highly sensitive to infused norepinephrine. During the first three days following this cold stress programme rats were continuously infused with 1 mg/kg norepinephrine and all animals developed massive pulmonary edema and died. Hsieh et al (1957) suggests norepinephrine is an important mediator in the chemical regulation of heat production. Leblanc (1971) found no change in norepinephrine sensitivity with intermittent severe cold adapted rats but increased sensitivity in continuous moderately cold adapted rats.

Dawson et al (1968) found prior cold acclimation increased the duration of performance in rats swum to exhaustion. Conversely, Chin et al (1973) found physical training enhanced cold tolerance in rats.

Cold acclimation improves resistance to cold (Sellers et al, 1951; Hart et al, 1963; Leblanc, 1971). Cold adapted rats can continue to produce heat at a greater rate, whereas non adapted rats initially increase heat production but then it declines as they become hypothermic (Sellers et al, 1951). Cold acclimation is temporary and is lost when the animal is returned to a warm environment for as little as four days (Sellers et al, 1951).

C) HISTOLOGICAL CHANGES

Irregular footshock stress in rats has been shown to produce morphological changes confined largely to the microcirculation (Bassett and Cairncross,1977). Congestion and dilation of large venules, collecting venules and veins was noted.Accumulation of Periodic Acid Schiff staining positive material consistent with platelet aggregation was present on the endothelium of small veins. Coronary arterioles showed some edema of the intima and media and the presence of micro-vacuoles. Marked mast cell infiltration into the perivascular regions was also evident.

Bassett and Cairncross (1977) found glucocorticoids protected against stress induced myocardial pathology in rats by inhibiting the permeability response to inflammatory substances. When adaptation of the steroid response occurred myocardial pathology was observed.

Fifteen hours of restraint stress followed by a six hour rest prior to sacrifice produced focal areas of necrosis and an intense

cellular inflammatory reaction in the myocardium of rats (Gigee, 1961). Fragmentation and the disappearance of many muscle cells were evident. Balazs (1972) also observed that isolation stress and cold stress increased the toxicity to isoproterenol and resulted in higher mortality. However, Balazs found that with repeated injections of 5 mg/kg isoproterenol that cardiac lesions occurred initially but that an adaptive change took place and subsequent injections did not elicit an acute infarct-like lesion.

The histological changes found by Leon and Bloor (1968) in animals exercised in water at 28-32$^{o}$ C may also reflect a stress induced pathology as these temperatures are considered to induce cold stress (Tan et al,1954; Dawson et al,1968; Harri et al,1975; Carew et al, 1978).

# III METHODS

## ANIMALS

Male Sprague-Dawley rats weighing 180-220 grams and 400-470 grams were used. All animals were housed three to a cage in the same room for the duration of the study, and were provided with water and food *ad libitum*. The rats were divided into two groups:

Group I - Exercise Stressed

Group II - Sedentary.

## EXERCISE SPECIFICATION AND PROTOCOL

Group I consisted of thirty-two rats from each weight range. The animals were exercised by swimming in a plexiglass tank 2'x4'x14" deep. Rats were swum in a group to ensure sufficient interaction to prevent animals from floating (Bershon and Scheuer, 1977), for twenty-five minutes twice daily, five days a week for a period of four weeks. The water temperature was maintained at $22^{o}$ C. Fresh tap water was used for each swim session. Rats were rested for four hours between each daily swim. All animals were dried completely immediately after each swim and returned to their cages.

## SEDENTARY

Group II consisting of thirty-two rats from each weight range served as a matched sedentary group. These animals were kept at normal cage activities for the period of the study. Except for the swimming protocol the two groups were handled identically.

At the end of the four weeks, exercise stressed (group I and sedentary (group II) rats were further divided as follows:

A) Controls

Two batches each containing eight rats were separated from stressed (group I) and sedentary (group II) groups. These

two sub-groups of rats did not receive any isoproterenol injection and served as exercise stress and sedentary controls. Each of the control rats was injected with 0.5 ml of normal saline (vehicle).

B) Isoproterenol Injected

The remaining forty-eight animals in group I and II were injected with 20,40 and 80 mg/kg of isoproterenol. At each dose of isoproterenol, eight sedentary (four from each weight range) and eight exercised (four from each weight range) were employed.

Exercise stressed rats were allowed to rest 48 hours following their last exercise session prior to injection with isoproterenol (Brown et al, 1973). Matched sedentary rats were also injected. All rats were weighed and a resting electrocardiogram (EKG) was recorded prior to injection. For isoproterenol injection the drug was dissolved in 0.5 ml of normal saline and injected subcutaneously into the lower abdominal region of each rat.

ELECTROCARDIOGRAM RECORDINGS

Electrocardiograms (EKG's) were recorded from all control as well as all treated unanesthetized animals. For this purpose, rats were placed in a plexiglass holder designed to maintain the animal in the ventral position with free access to front and hind limbs for electrode application (Figure 1). Surface electrodes were applied to both hind paws and to the right forepaw, and the lead II EKG was recorded on a Beckmann R511A Dynograph Recorder. After being placed in the holder, rats were allowed to settle down and then three EKG readings were made five minutes apart. This procedure was repeated

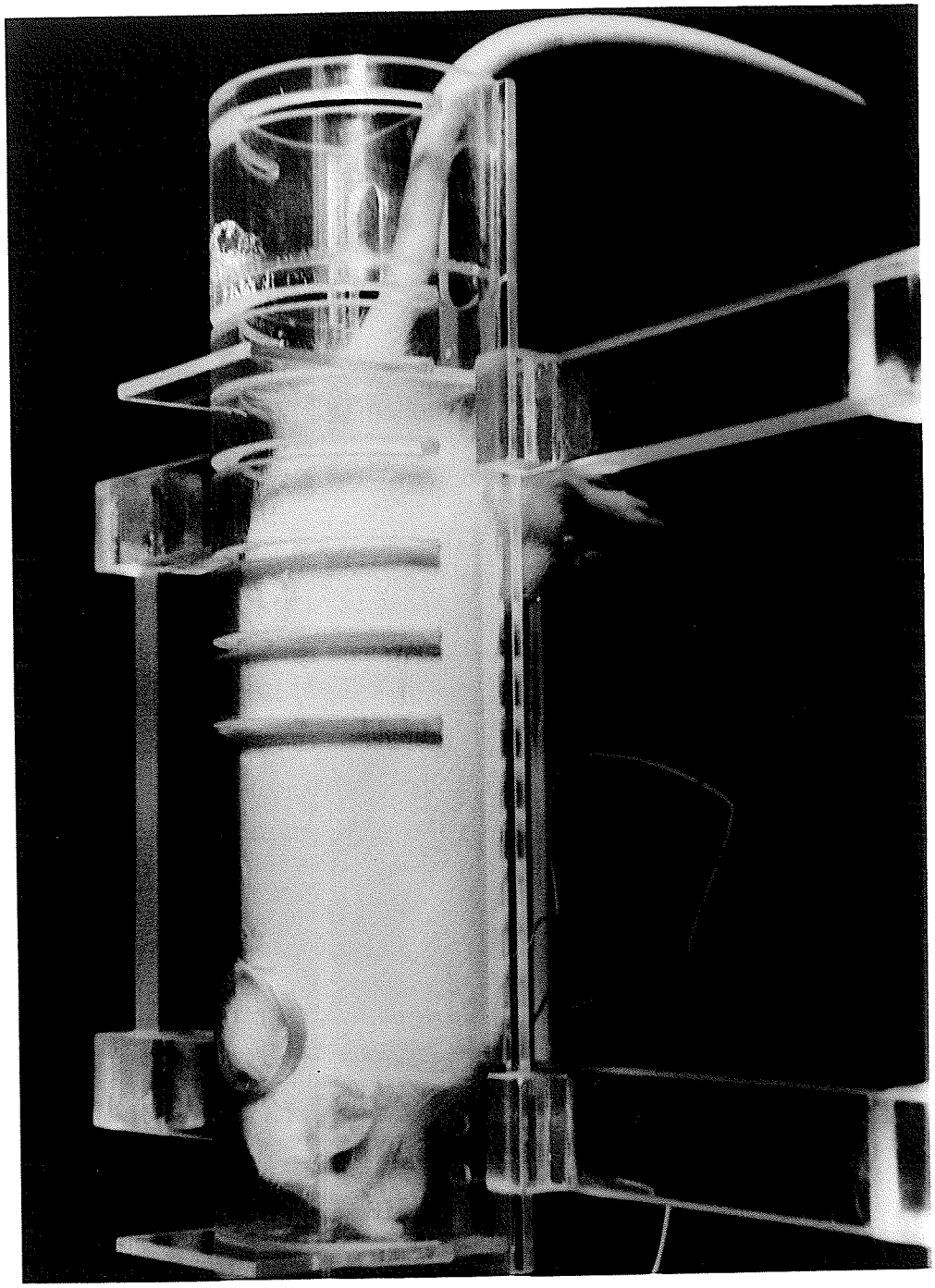

Figure 1: Recording of EKG from an unanesthetized rat.

on three consecutive days and the lowest heart rate recorded was employed as the resting heart rate for that animal.

For a study of the short term effects of isoproterenol on the EKG, immediately following the drug injection rats were placed into the plexiglass holder. Electrocardiograms were recorded every fifteen minutes for two hours or until the animal became unresponsive to blink for a minimum of thirty minutes, or until the rat developed ventricular fibrillation on the EKG.

The responsiveness of the isoproterenol treated animal to blink, the color of eyes, the amount, color and consistency of salivation, and the degree of pulmonary congestion assessed by palpation of the chest were recorded every fifteen minutes following the EKG.

SERUM ENZYMES

At the time of sacrifice, a sufficient amount of blood was collected from the neck veins to yield 0.5-2.0 ml serum. The concentrations of lactate dehydrogenase, creatine phosphokinase and serum glutamate oxaloacetic transaminase were analyzed according to the Worthington Statzyme method for quantitative enzyme determination.

HISTOLOGY

The hearts were excised and excess fat and the stump of the aorta were removed. These hearts were then immersed in a pre-weighted bottle containing a formalin solution supplemented with ten percent calcium chloride. The samples were weighed again to determine the wet weight of each heart. Following two hours of immersion fixation the atria and right ventricle were removed and the left ventricle was fixed for an additional twenty-four hours in the same fixative. The

samples were processed for light microscopy examination. Essentially the hearts were dehydrated in an alcohol series, cleared in benzene and embedded in paraffin.

The whole left ventricular tissue was sectioned from base to apex at a thickness of seven microns. Slides were processed to examine the ventricle at four different levels approximately fifteen hundred microns apart. Starting from the apex to the base of each ventricle the slides were labelled as A,B,C, and D respectively, as shown in figure 2. The regions A to D were selected arbitrarily with a purpose of assessing the histological damage in the whole ventricle without going through each slide.

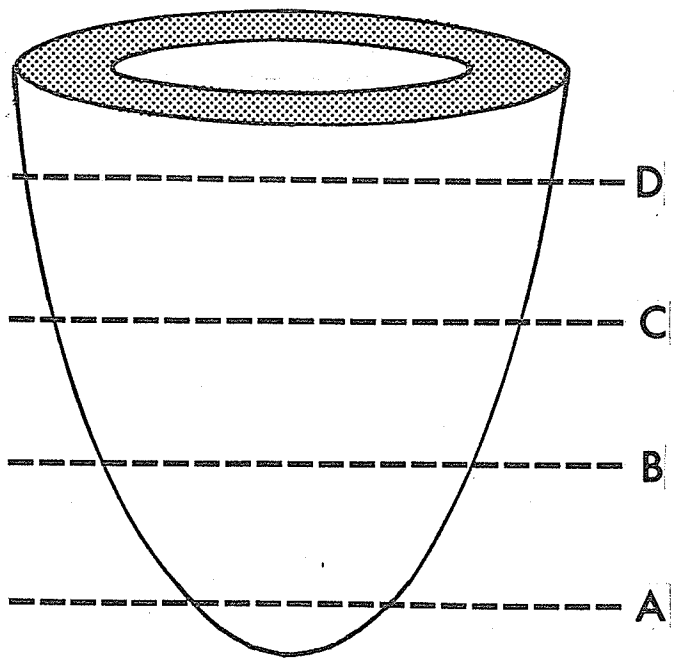


Figure 2: Diagramatic respresentation of regions of the left ventricle that were examined histologically.

The sections from all levels were stained with Delafield's hematoxylin/eosin for gross analysis and with Mallory triple stain to analyze the presence and assess the amount of collagen.

The procedure for Mallory staining as described below was employed:

1) Deparaffinize
   - $60^{o}$ oven for 5 minutes
   - 2 Xylene washes of 5 minutes each
2) Hydrate
   - Absolute Alcohol - 10 minutes
   - Alcohol to water series
   - 2 Distilled $H_2O$ washes - 5 minutes each
3) Mordant saturated aqueous $HgCl_2$ and 5% glacial acetic acid for 10 minutes
4) Rinse in distilled $H_2O$ - 5 minutes
5) Treat with Lugol's solution - 5 minutes
6) Clear in sodium thiosulfate - 5 minutes
7) Rinse in distilled $H_2O$ - 5 minutes
8) Stain in Mallory I - 20 seconds
9) Rinse in distilled $H_2O$ to differentiate red ($\pm$ 10 seconds)
10) Treat with phosphomolybdic acid - 3 minutes
11) Rinse in distilled $H_2O$
12) Stain in Mallory II - 2 minutes, 15 seconds
13) Rinse in distilled $H_2O$
14) Differentiate aniline blue in 70% alcohoh ($\pm$ 10 seconds)

15) Dehydrate

- 80 $\longrightarrow$ 90% alcohol - $\pm$ 15 seconds
- absolute alcohol - 2 washes, 3 minutes each

16) Clear in Zylene - 2 washes, 5 minutes each

17) Mount with permount adhesive

STATISTICAL ANALYSIS

Student's "t" test was employed to determine statistical differences between control and treatment groups. $p < 0.05$ was taken as the significant level of probability.

# IV RESULTS

## GENERAL

In a pilot study twenty-five minutes duration of each exercise session was determined as the maximum which the animal could tolerate without drowning due to fatigue. Furthermore, none of the animals in Group I and Group II were lost prior to the injection with isoproterenol. The description of the results given below is applicable to both weight classes vis a vis 180-220 and 400-470 gram rats employed in this study. Since there were no differences between the two weight classes with regard to the parameters measured in this study, the data from both weight classes was pooled.

## VISUAL RESPONSES OF THE ANIMALS TO ISOPROTERENOL INJECTION

The visual responses of all animals injected with isoproterenol were recorded every fifteen minutes for two hours.

### Sedentary Rats

Sedentary rats injected with 20,40 and 80 mg/kg isoproterenol did not demonstrate pulmonary congestion. Occasional rapid and irregular respirations were observed. Animals remained alert and responsive at all times.

All animals injected with 80 mg/kg of isoproterenol, and three rats at 20 and 40 mg/kg isoproterenol developed marked agitation and convulsive-like movements as well as loss of color of the eyes. Coincident with these responses was the recording of ventricular fibrillation on the electrocardiogram.

### Exercise Stressed Rats

Following 20 mg/kg isoproterenol injection only three of the eight animals injected developed symptoms leading to

unresponsiveness. The other five rats demonstrated minimal pulmonary congestion on palpation, minimal or no discharge at the nose and mouth and remained responsive longer than the two hour monitoring period.

After 40 and 80 mg/kg isoproterenol injection all except one rat in the 40 mg/kg group exercise stressed rats demonstrated a clear watery or frothy white discharge at the nose and mouth, rapid, shallow and at times irregular respiration and marked palpable congestion of the chest. These symptoms increased in severity as the responsiveness of the animal deteriorated. Once the congestion was well established the limbs of the animals became cool to touch, the eyes appeared dark red and the rats became unresponsive to blink. When the rats demonstrated these severe symptoms and were unresponsive to blink for a minimum of thirty minutes, they were sacrificed. The development of severe pulmonary congestion corresponded with the marked slowing and irregular conduction patterns monitored on the electrocardiogram. One animal in the 40 mg/kg group developed less severe pulmonary congestion and this corresponded with lack of rhythm changes in the electrocardiogram.

MORTALITY

The data on mortality in sedentary and exercised rats following the isoproterenol injections has been summarized in Table 1.

None of the sedentary animals injected with 20 mg/kg isoproterenol died within the forty-eight hour post injection period. However 50 percent of the exercise stressed animals died within twenty-four hours of injection.

TABLE 1

Body weight, heart weight/body weight ratio
and mortality of sedentary and exercise stressed control rats
before and after treatment with 20,40 and 80 mg/kg of isoproterenol.

| Body Weight (gms). | | | Heart Weight/Body Weight Ratios (mg/g) | | Percent Mortality 24 Hours | |
|---|---|---|---|---|---|---|
| CONTROLS | Sed. (8) | Ex. (8) | Sed. (4) | Ex. (4) | Sed. (8) | Ex. (8) |
| 203 ± 2.4 | 477 ± 12** | 412 ± 6.0 | 2.80 ± 0.07** | 4.02 ± 0.04 | 0% | 0% |
| 434 ± 3.2 | 493 ± 7.6** | 441 ± 5.5 | 2.65 ± 0.18* | 3.75 ± 0.18 | 0% | 0% |
| Doses of Isoproterenol (mg) | | | | | | |
| 20 | 478 ± 8.3 | 427 ± 14 | 3.82 ± 0.23** | 4.04 ± 0.09 | 0% | 50% |
| 40 | 480 ± 13 | 437 ± 11 | 3.74 ± 0.09** | 3.92 ± 0.25 | 50% | 75% |
| 80 | 488 ± 10 | 453 ± 11 | 4.11 ± 0.30** | 3.58 ± 0.20 | 100% | 100% |

Values represent means ± S.E. Figures in parenthesis indicate the number of animals.
* $p < 0.01$; ** $p < 0.001$ as compared to respective exercised controls.

Following 40 mg/kg isoproterenol injection the mortality of the exercise stressed rats was higher than the sedentary at all time periods. Within twenty-four hours, 75 percent of the exercise stressed rats died compared to 50 percent of the sedentary animals.

Following 80 mg/kg isoproterenol injection there was no difference in mortality between exercise stressed and sedentary rats. They all died within twenty-four hours following injection.

SERUM ENZYMES

Three serum enzymes listed below were measured in the sedentary and exercise stressed rats before and after the injection of different concentrations of isoproterenol.

1) Creatinine Phosphokinase (CPK)

The serum CPK levels shown in figure 3 were slightly higher in the exercise stressed rats, but this increase was not significantly different from the sedentary control values. However, CPK values were significantly elevated ($p < 0.05$) in both exercise stressed and sedentary rats injected with 80 mg/kg of isoproterenol as compared to control levels. Lower concentrations of isoproterenol (20 and 40mg/kg) did not have any significant effect in either group.

2) Lactate Dehydrogenase (LDH)

Exercise alone did not have any significant effect on the serum LDH levels (figure 4). LDH was significantly decreased ($p < 0.02$) from control values in sedentary rats injected with 20 mg/kg isoproterenol. In both 20 and 40 mg/kg isoproterenol injected groups the LDH levels were higher in the exercise stressed rats as compared to the

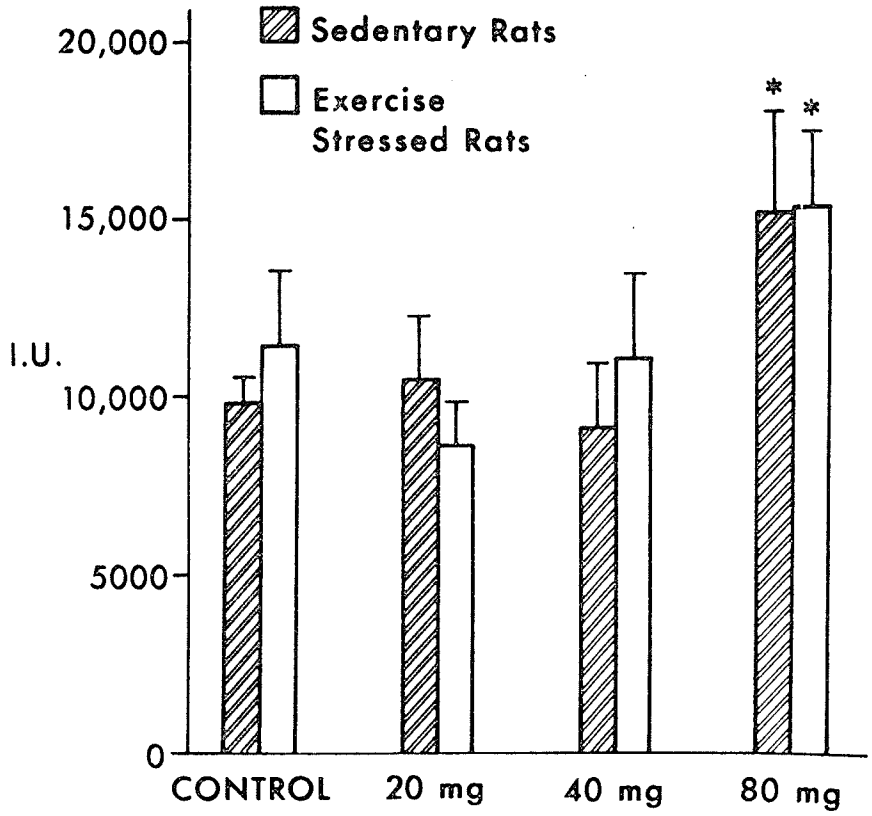


Figure 3: Plasma creatine phosphokinase concentrations in sedentary and exercise stressed untreated (controls) and isoproterenol treated rats. Each value represents the mean $\pm$ S.E. from 8 rats. * $p < 0.05$ as compared to respective controls.

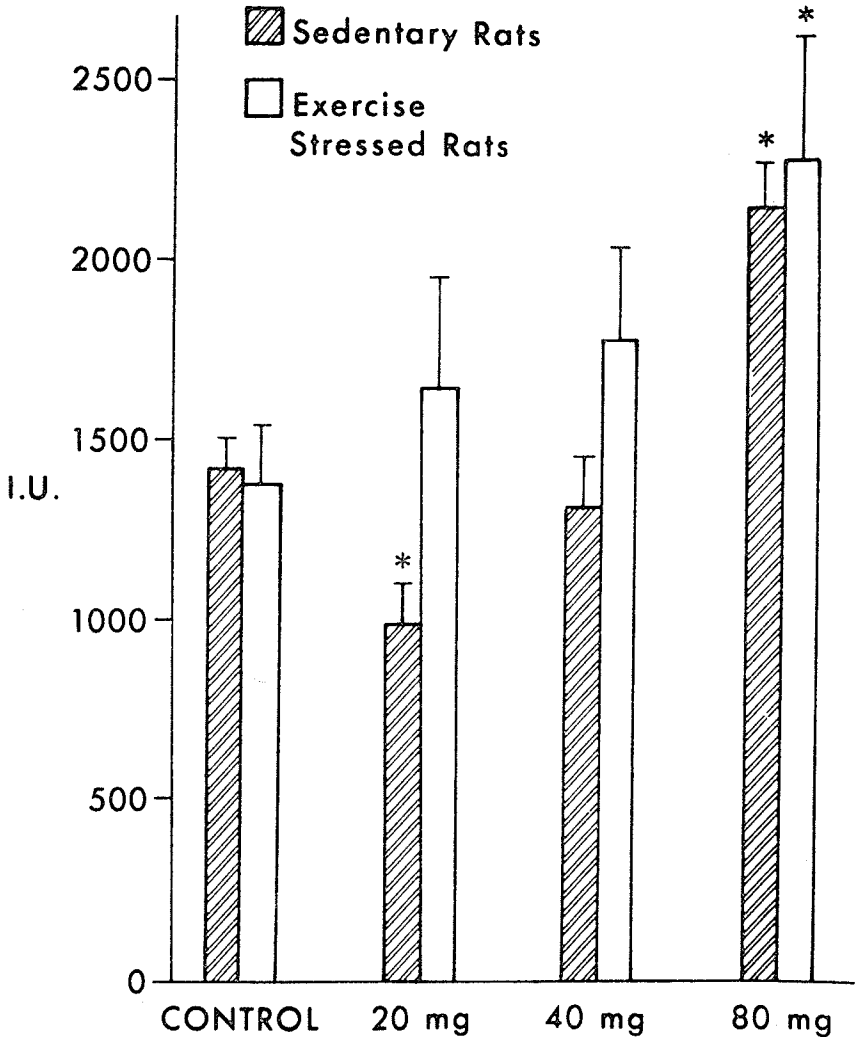


Figure 4: Plasma lactate dehydrogenase concentrations in sedentary and exercise stressed untreated (controls) and isoproterenol treated rats. Each value represents the mean ± S.E. from 8 rats. * $P < 0.025$, ** $p < 0.001$ as compared to respective controls.

sedentary rats injected with the same dose of isoproterenol. Serum LDH was significantly elevated in both exercise stressed ($P < 0.025$) and sedentary rats ($P < 0.001$) injected with 80 mg/kg isoproterenol as compared to the treated controls.

3) Serum Glutamate Oxaloacetic Transaminase (SGOT)

Serum SGOT was significantly elevated in exercise stressed ($p < 0.01$) as compared to sedentary control rats. (Figure 5). At 40 and 80 mg/kg isoproterenol there was a significant increase ($p < 0.005$) in SGOT levels of the exercise stressed rats as compared to the sedentary control animals treated with similar doses of isoproterenol. This increase was also significantly higher when compared with exercise stressed rats. At lower concentrations of isoproterenol (20 mg/kg) the SGOT level was unchanged from untreated exercise stressed rats. In the sedentary group, on the other hand, only 80 mg/kg of isoproterenol caused some elevation of SGOT and the response was markedly less as compared to exercise stressed rats.

ELECTROCARDIOGRAPHY

The mean resting heart rate of the exercise stressed rats was slightly depressed as compared to the sedentary rats at the end of the four week programme (Table 2). However, this difference was not statistically significant. The Q-T interval was significantly increased in the exercise stressed rats (Table 2) whereas P-R and QRS intervals remained unchanged. The R wave amplitude was significantly elevated in the exercised rats.

All isoproterenol treated animals developed sinus trachycardia immediately following the injection of 20,40 and 80 mg/kg

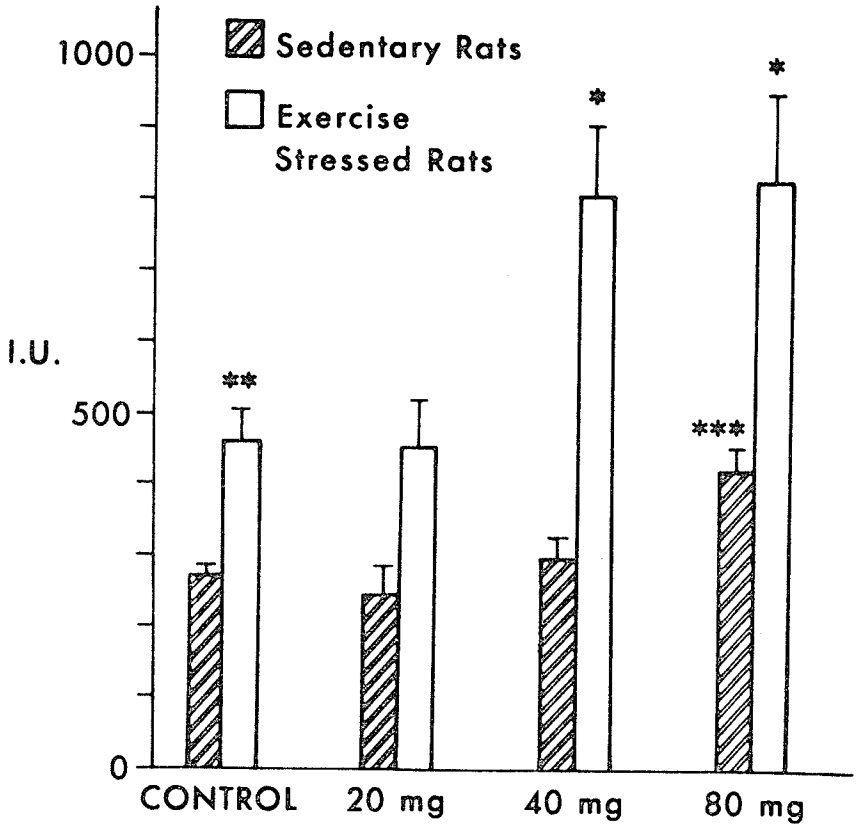


Figure 5: Plasma glutamic oxaloacetic transaminase concentrations in sedentary and exercise stressed untreated (controls) and isoproterenol treated rats. Each value represents the mean ± S.E. from 8 rats. * $p < 0.01$; ** $p < 0.005$; *** $p < 0.001$ as compared to respective controls.

TABLE 2

Electrocardiogram parameters of the sedentary and exercise stressed rats prior to treatment with isoproterenol.

| CONTROLS | H.R. | INTERVALS (msec) | | | R WAVE |
|---|---|---|---|---|---|
| | | P-R | QRS | Q-T | AMPLITUDE (m.v.) |
| Sedentary (8) | 410 ± 8 | 48 ± 0.6 | 28 ± 0.4 | 68 ± 0.2 | .40 ± 0.2 |
| Exercised (8) | 394 ± 10 | 48 ± 1.5 | 31 ± 0.6 | 77 ± 2.5* | .52 ± 0.4* |

Values represent the mean ± S.E.

Figures in parentheses indicate the number of animals

* $p < 0.005$ as compared to sedentary values

TABLE 3

Heart rate response of sedentary (SED) and exercise stressed (EX.) rats during the first 45 minutes following injection with 20, 40 and 80 mg/kg of Isoproterenol.

| TIME AFTER INJECTION (min) | ISOPROTERENOL | | | | | |
|---|---|---|---|---|---|---|
| | 20 mg/kg | | 40 mg/kg | | 80 mg/kg | |
| | SED (8) | EX (8) | SED (8) | EX (8) | SED (8) | EX (8) |
| 0 | 437 ± 9 | 402 ± 3 | 402 ± 10 | 386 ± 12 | 407 ± 9 | 394 ± 4 |
| 15 | 487 ± 7 | 511 ± 10 | 463 ± 16 | 527 ± 6*** | 485 ± 8 | 510 ± 9* |
| 30 | 477 ± 3 | 521 ± 9** | 480 ± 8 | 528 ± 7*** | 478 ± 7 | 520 ± 9*** |
| 45 | 477 ± 3 | 523 ± 15 | 475 ± 15 | 528 ± 7*** | 472 ± 12 | 535 ± 11*** |

Values represent means ± S.E.

Figures in parenteses indicate the number of animals

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.005$ as compared to sedentary values.

of isoproterenol However, the heart rate response of the exercise stressed rats was significantly more as compared to the sedentary treated animals (Table 3).

The electrocardiograms were recorded every fifteen minutes for two hours or until sacrifice. Sedentary as well as exercise stressed animals showed a varied response to the isoproterenol (20,40 and 80 mg/kg) treatment. At all doses of isoproterenol most of the sedentary animals developed premature ventricular contractions (Figures 6,7, and 8). All animals sacrificed within the two hour monitoring period had also developed ventricular fibrillation. In contrast, at all doses of isoproterenol, none of the exercise stressed rats demonstrated premature ventricular contractions (Figures 6,7, and 8). The electrocardiograms prior to sacrifice showed conduction defects, the heart rate slowed and became irregular in the exercise stressed animals.

BODY WEIGHT AND MYOCARDIAL HYPERTROPHY

There was a significant increase ($p < 0.001$) in body weight in the sedentary rats as compared to the exercised rats at the end of the study (Table 1). However, isoproterenol injections (20,40 and 80 mg/kg) did not have any significant effect on the body weight of sedentary or exercised rats.

The wet heart weight of the sedentary control rats ($1.37 \pm 0.3$ gms) was significantly less ($p < 0.01$) than the hearts from exercise stressed animals ($1.57 \pm 0.08$ gms). However, there were significant differences in heart weight/body weight ratios between the sedentary and exercised rats (Table 1) in as much as the exercise stressed control animals exhibited a significant increase

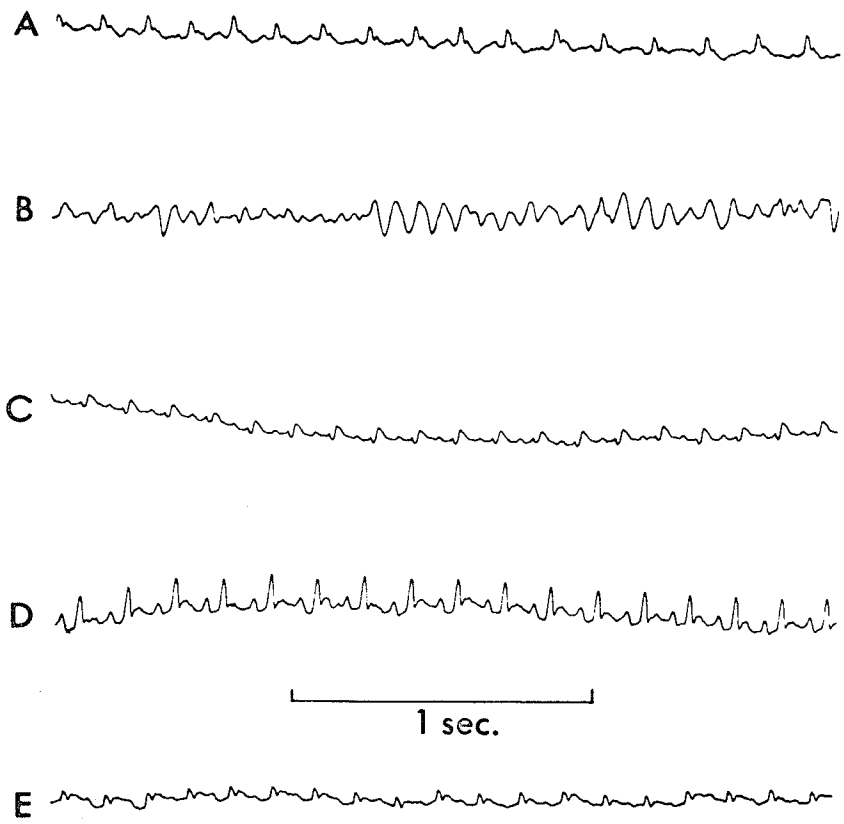


Figure 6: Representative electrocardiogram recordings from 20 mg/kg isoproterenol treated sedentary and exercise stressed rats. A - sedentary pre-injection; B - sedentary 53 minutes post-injection; C - sedentary 2 hours post-injection; D - exercise stressed pre-injection; E - exercise stressed 2 hours post-injection.

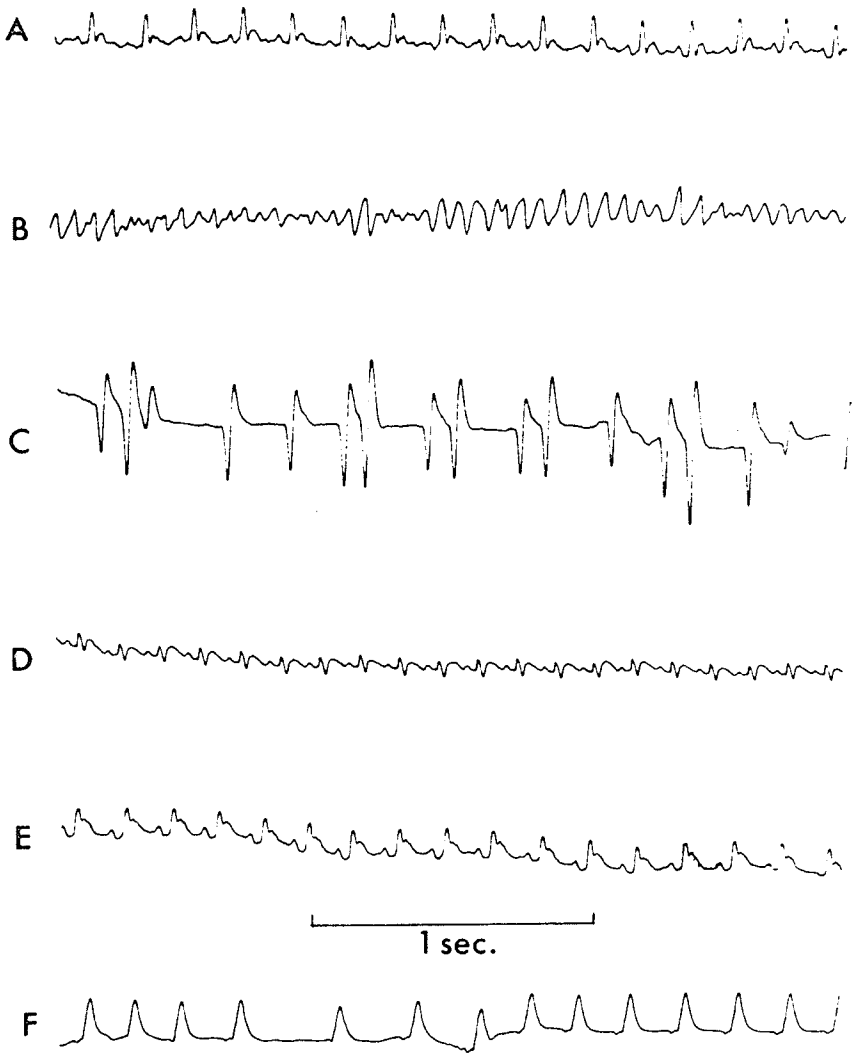


Figure 7: Representative electrocardiogram recordings from 40 mg/kg isoproterenol treated sedentary and exercise stressed rats. A - sedentary pre-injection; B - sedentary 50 minutes post-injection; C - sedentary 52 minutes post-injection; D - sedentary 2 hours post-injection; E - exercise stressed pre-injection; F - exercise stressed post-injection and prior to sacrifice.

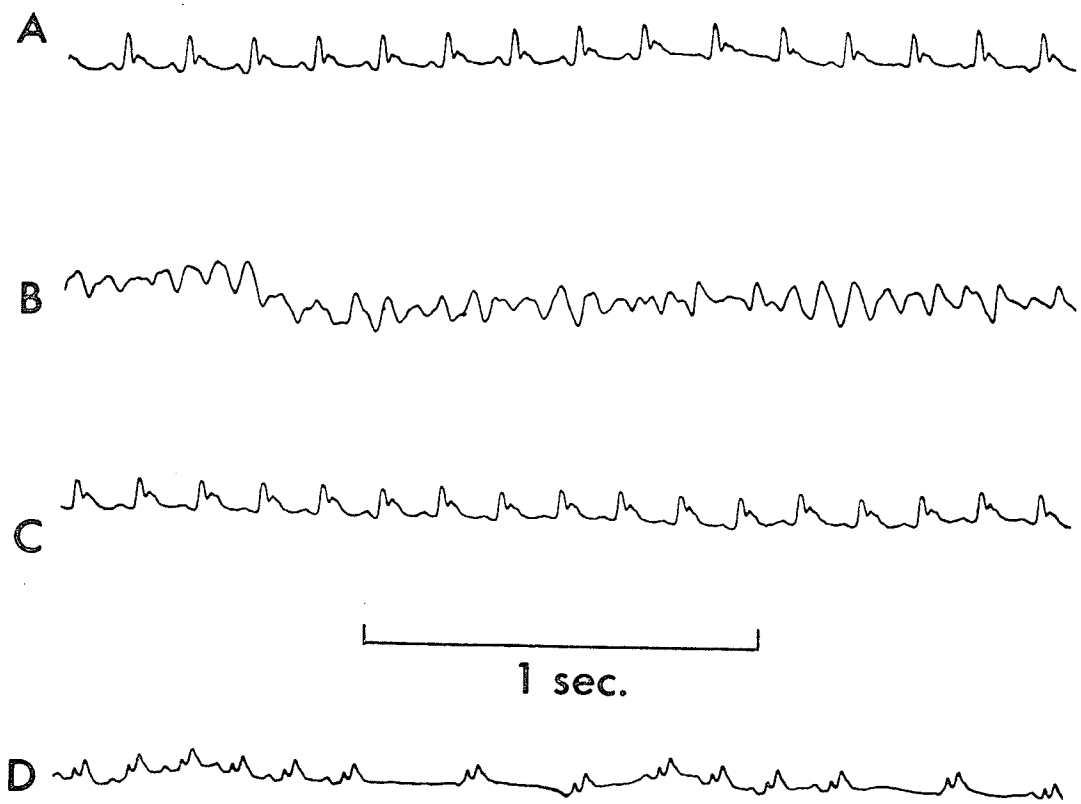


Figure 8: Representative electrocardiogram recordings from 80 mg/kg isoproterenol treated sedentary and exercise stressed rats. A - sedentary pre-injection; B - sedentary post-injection and prior to sacrifice; C - exercise stressed pre-injection; D - exercise stressed post-injection and prior to sacrifice.

($p < 0.005$) in this ratio as compared to the sedentary controls. The sedentary rats exhibited a significant increase in heart weight/ body weight ratios when injected with isoproterenol ($p < 0.001$). On the other hand, no significant change in the heart to body weight ratios was noticed in exercised rats in response to isoproterenol injection.

HISTOLOGY

Sections from the left ventricle of hearts from all sedentary and exercise stressed rats were stained with hematoxylin/eosin (H/E), as well as the Mallory's triple stain for the analysis of histological and connective tissue changes. Paraffin sections of the whole left ventricle were arbitrarily divided into four groups (A,B,C,D), depending upon the area of the heart as described in the Methods (Figure 2). Collagen was graded on an 0 to 4 point scale as follows:

0 - none or only traces of collagen.

1 - collagen present between myofibers only in focal areas

2 - collagen present between myofibers consistently throughout the myocardium and around the arteries and arterioles.

3 - when focal lesions accompanied the collagen distribution described as grade 2.

4 - when gross scarring accompanied the collagen distribution described as grade 2.

1) Controls

A) Sedentary Controls

The normal histology of sedentary controls at levels A,B,C, and D, is shown in Figure 9. In a typical H/E preparation the muscle fibers appeared normal with no signs of

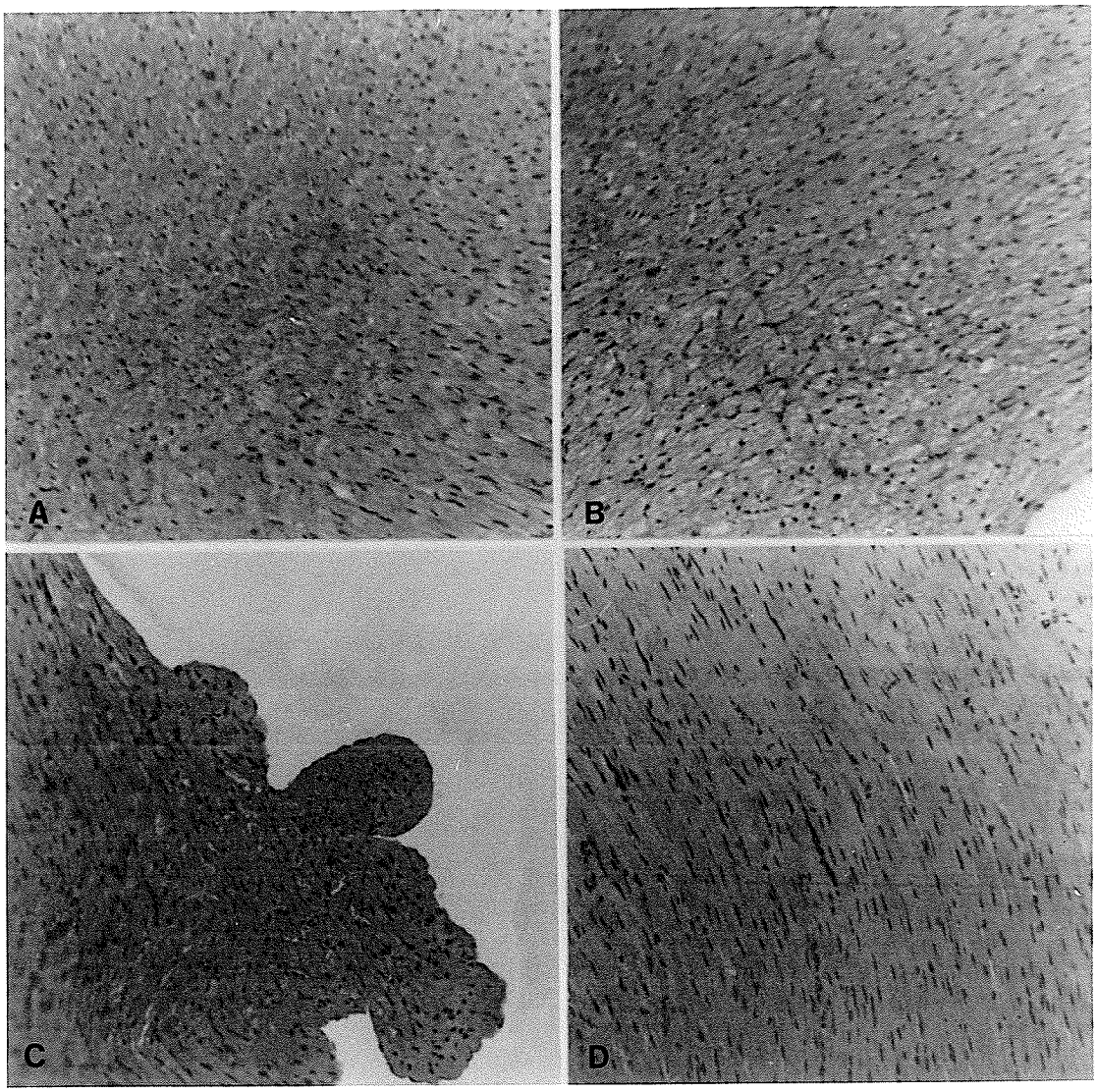


Figure 9: Representative sections of hearts from sedentary control rats depicting normal histological appearance of the heart at levels A,B,C and D (see figure 2). A - apex; B - subendocardium to subepicardium; C - subendocardium; D - mid myocardium. (magnification in all X 120).

edema, hemorrhage or structural disruptions. Collagen in the triple stain was quite sparse and graded as follows: 0 at levels A and B, grade 1 at levels C and D.

B) Exercise Stressed Controls

The histological appearance of the hearts from rats exercised for four weeks is depicted in Figure 10. Focal lesions exhibiting interstitial edema, vacuolization and infiltration with fibroblasts were seen throughout the muscle mass at all levels (A-D) of the left ventricle. Lesions were observed in the subendocardium, subepicardium and mid-myocardial regions, but were more frequently observed in close proximity to larger blood vessels. Focal areas of eosinophilia of the cytoplasm and frank hemorrhage into the interstitial spaces was evident in several of the exercise stressed hearts. However, dark (A) and light (I) bands typical of a normal muscle cell were apparent in the myofibers surrounding these zones of damaged tissue. The amount of collagen varied between grade 2 and 4 in all sections as compared to grade 0 and 1 in the sedentary controls. The maximum necrosis observed in the exercise stressed hearts was at the apex (level A) and included an infarct-like necrosis with extensive collagen formation around the coronary artery which extended deep into the myocardium. Such lesions occasionally extended into level B.

2) Isoproterenol Treated Rats

Sedentary as well as exercise stressed rats were injected with 20,40 and 80 mg/kg of isoproterenol and the

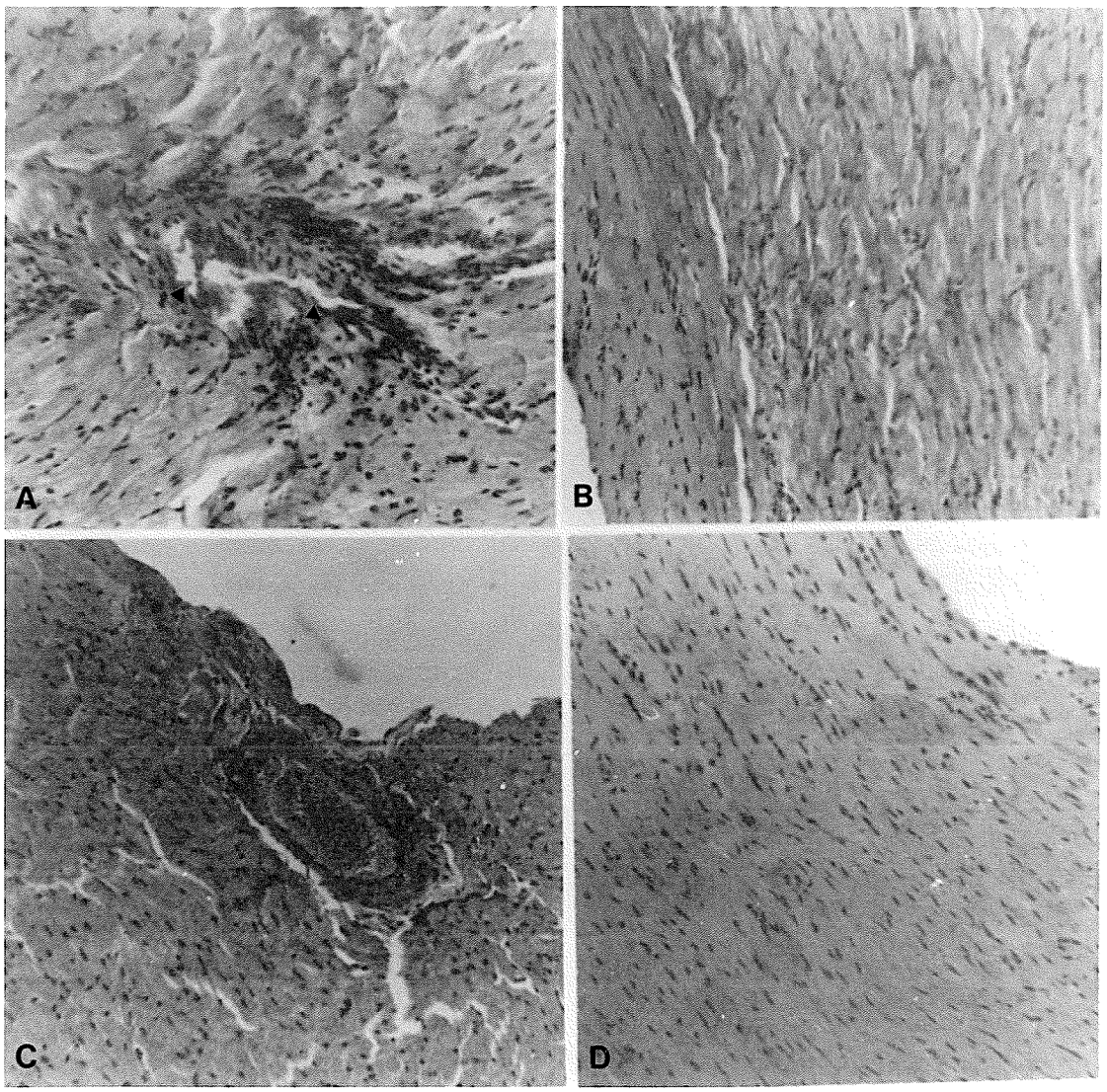


Figure 10: Representative sections of hearts from exercise stressed control rats at levels A,B,C, and D (see figure 2). A - maximal necrosis observed at the apex. Extensive fibrosis surrounding a coronary artery seen here in a grazing view (arrows) and extending deeply into the myocardium; B - mid myocardium increased collagen and fibroblasts. Mild interstitial and intracellular edema; C - typical subepicardial artery with thickened wall and proliferation of fibroblasts in and around the arterial wall; D - subendocardium to mid myocardium. Normal histological appearance. (magnification in all X 120).

histology of their hearts was compared with each other as well as with their respective controls.

A) Sedentary Treated Rats

The histological appearance of the hearts from sedentary rats injected with 20,40 and 80 mg/kg isoproterenol is depicted in Figures 11,12 and 13 respectively. Widespread disruption of the integrity of the myocardium was observed at level A. Interstitial and intracellular edema and vacuolization were prominent. However, there were a few differences of a minor nature. At 20 mg of isoproterenol the extent of the edema was not as severe. Furthermore, the interstitial edema and myofiber disruption was confined to level A and only the subendocardial regions of levels B,C and D were involved at 20 mg of isoproterenol. It is pertinent to note that all the sedentary rats treated with 20 mg isoproterenol showed a marked infiltration of fibroblasts. No such fibroblastic activity was noticed in the hearts of 40 and 80 mg sedentary treated rats.

The pattern of damage described below was true for both 40 and 80 mg treated groups. The normal configuration of the myofibers with alternating dark and light bands was lost in the damaged tissue. There was some sparing of the myofibers throughout the edematous regions in Level A. The marked edema and myofiber disruption was extensive in all areas of Level B except for sparing of some subepicardial regions. However, in some hearts full thickness myocardial damage was also observed at Level B. Edema in levels C and D was more confined to the interstitial regions of the subendocardium

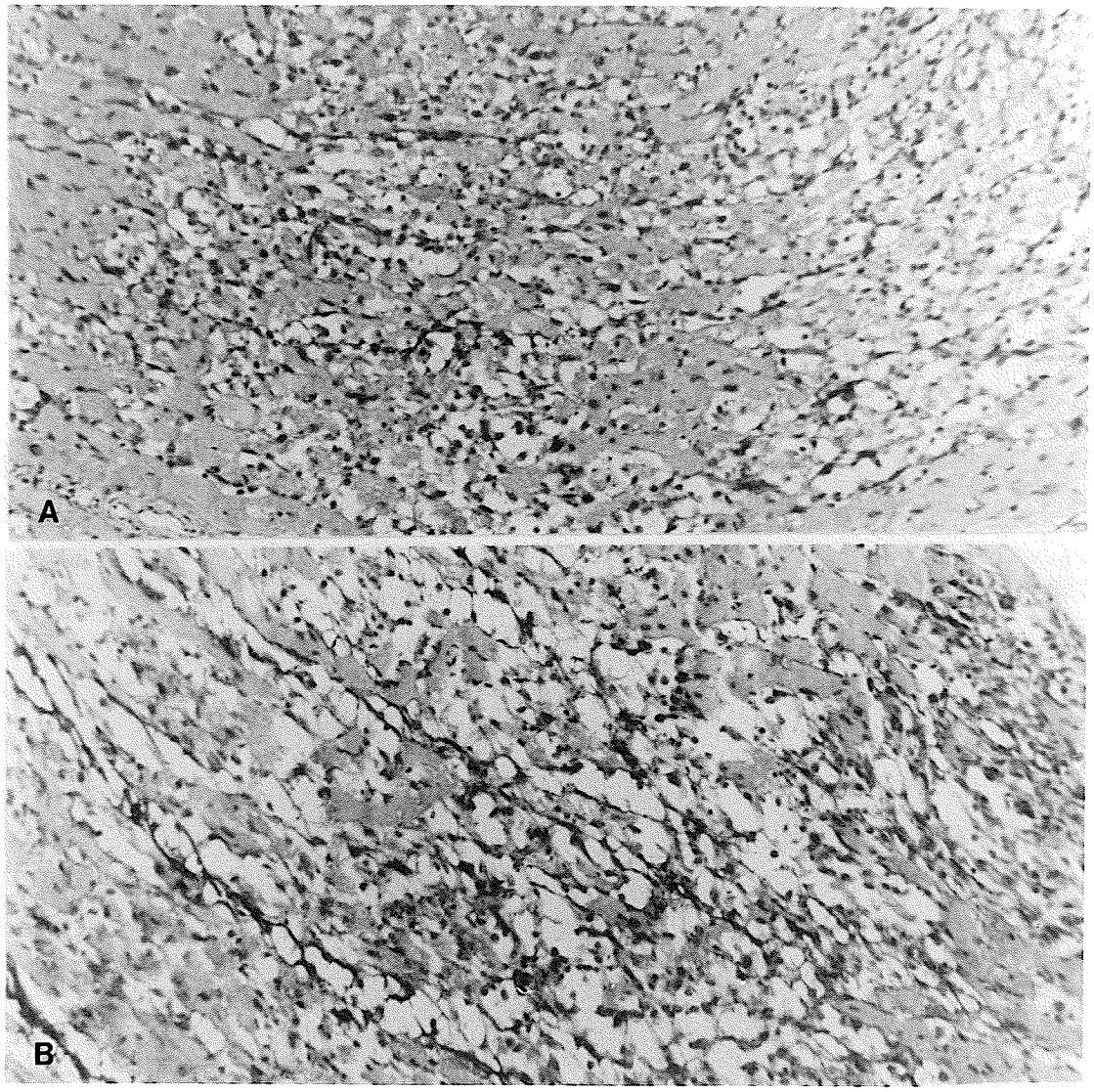


Figure 11: Representative sections of hearts at levels A and B from sedentary rats injected with 20 mg/kg isoproterenol. A - extensive interstitial edema and vacuolization of myofibers: B - extensive interstitial edema and vacuolization of myofibers extending from the subendocardium tc deep into the myocardium. Marked infiltration of fibroblasts is apparent at both levels A and B. (magnification in all X 180).

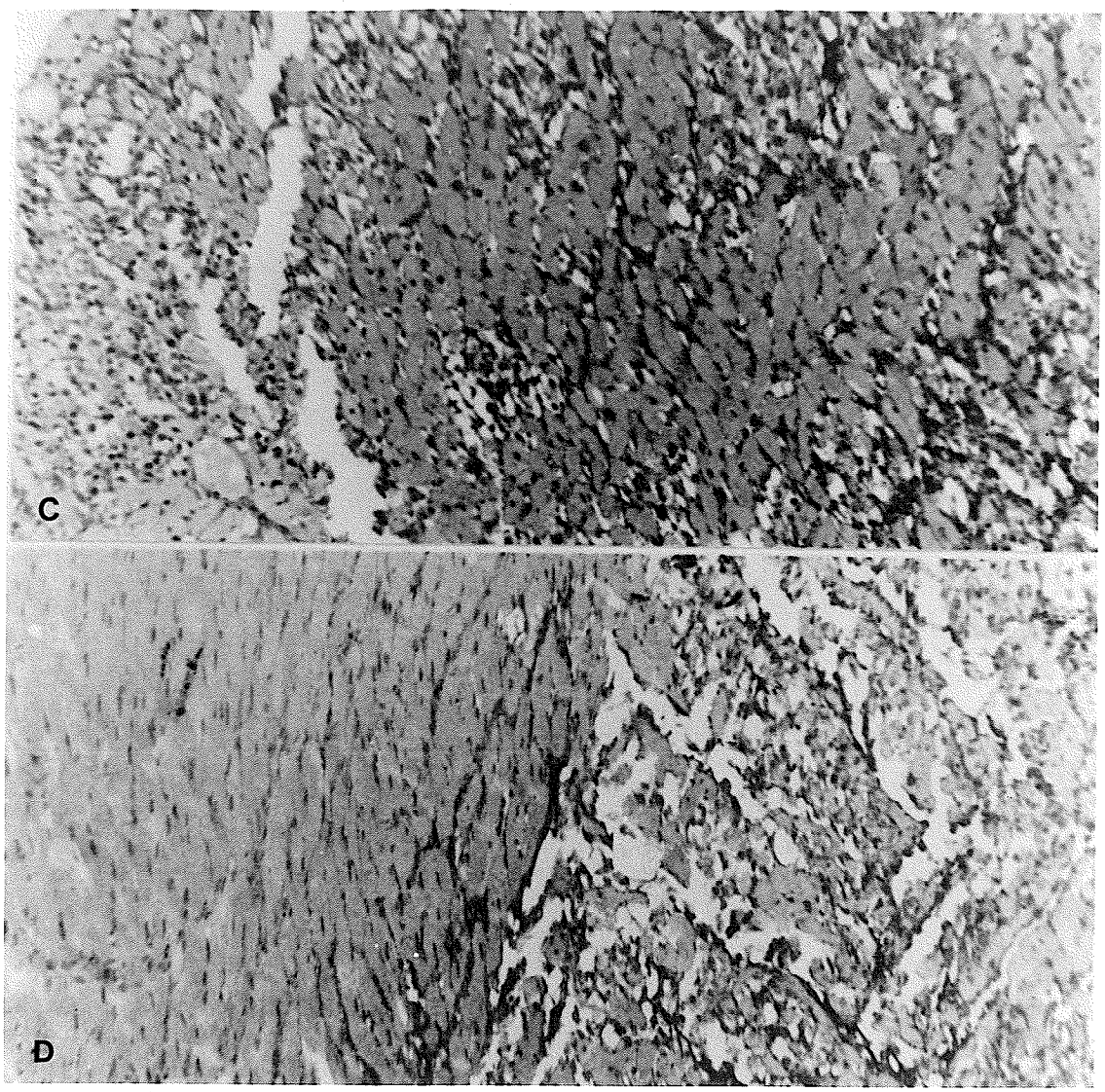


Figure 11 (continued): Representative sections of hearts at levels C and D from sedentary rats injected with 20 mg/kg isoproterenol. Both levels demonstrate subendocardial edema, vacuolization of myofibers and infiltration of fibroblasts. At level D the damage is localized specifically to the subendocardial regions with the remaining myocardium being normal in appearance. (magnification in all X 180).

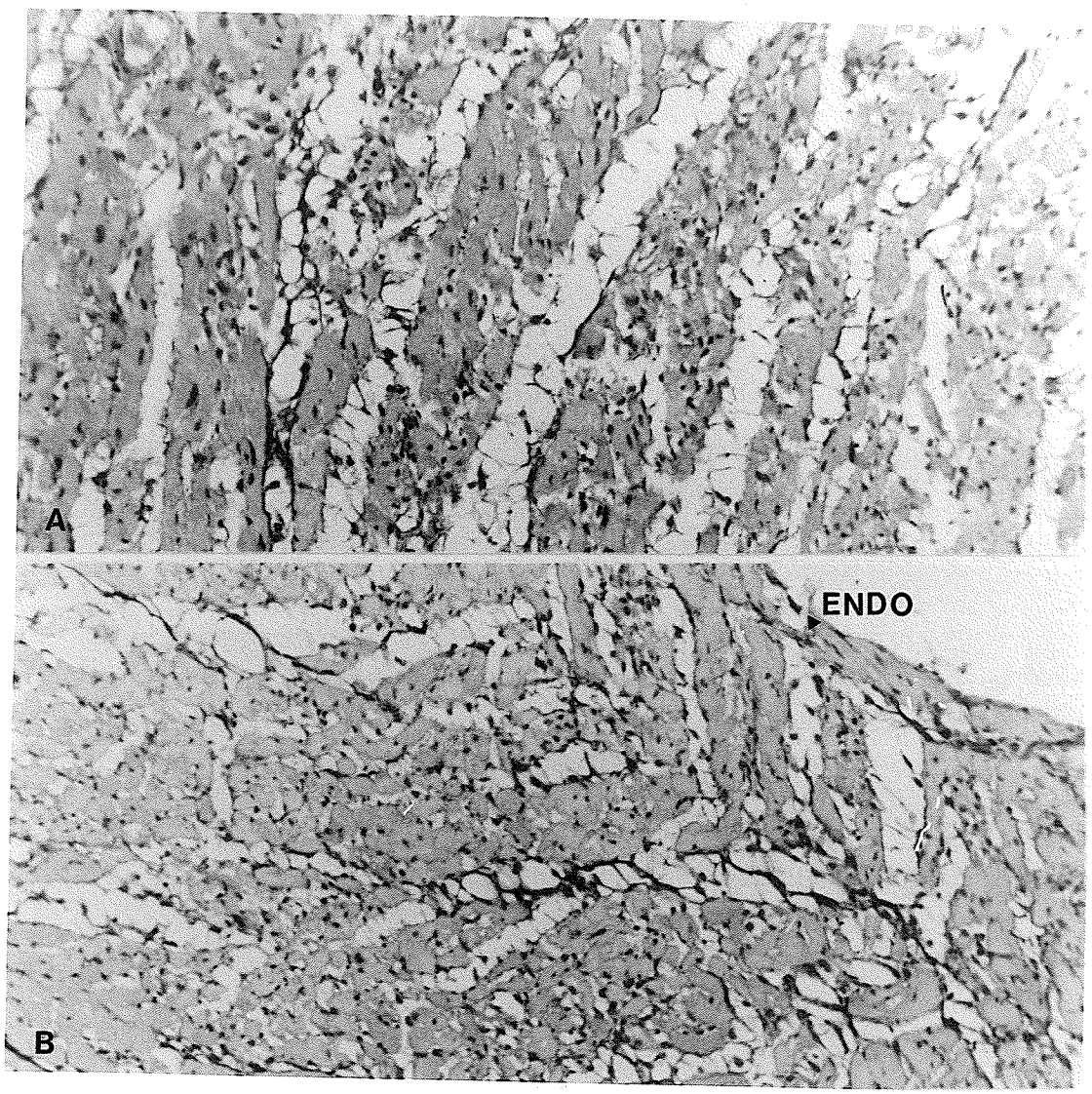


Figure 12: Representative sections of hearts at levels A and B from sedentary rats injected with 40 mg/kg isoproterenol. A - apex - massive interstitial and intracellular edema and vacuolization of myofibers; B - extensive interstitial and intracellular edema and vacuolization extending from the subendocardium (ENDO) deep into the myocardium. (magnification in all X 180).

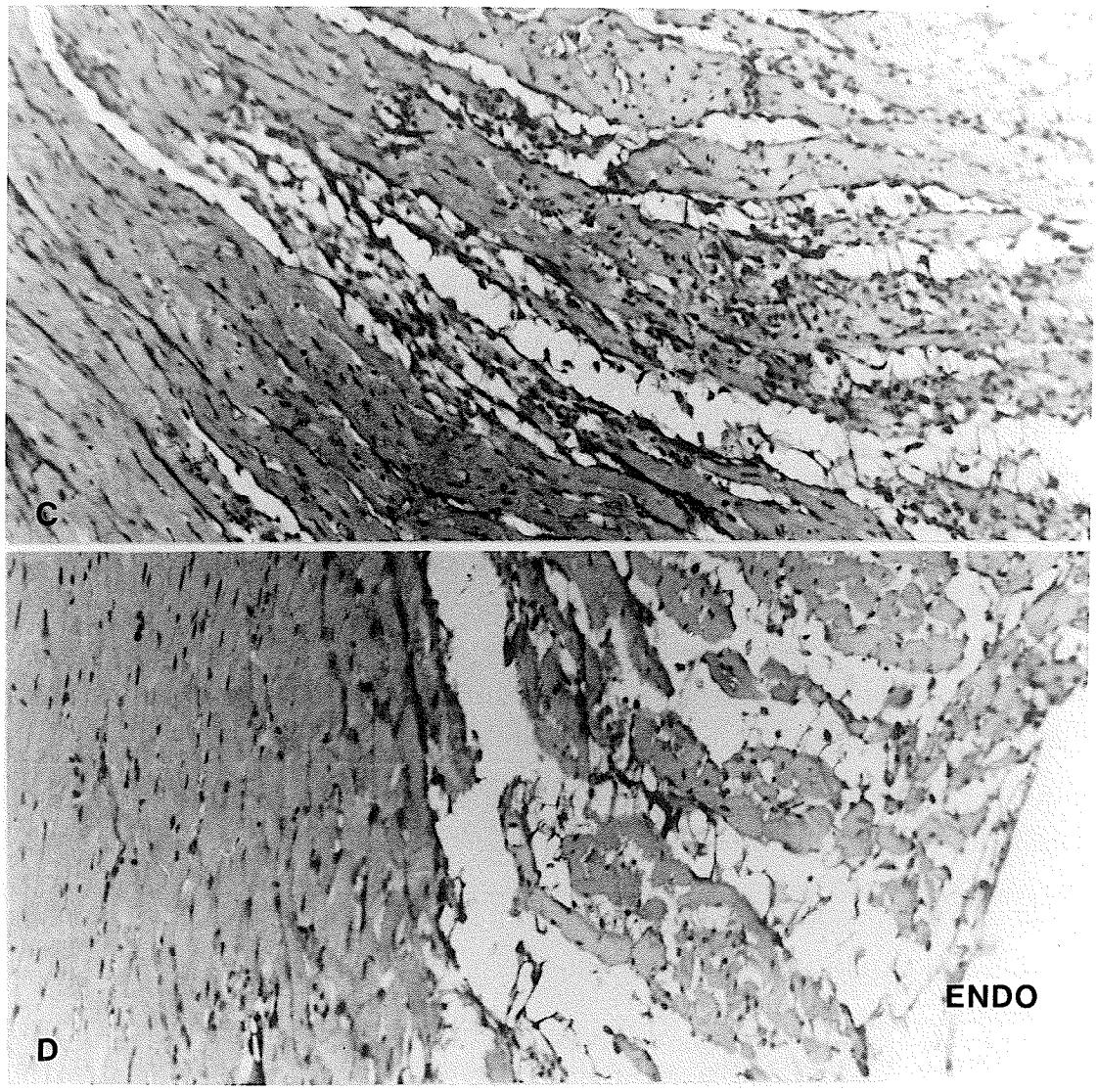


Figure 12 (continued): Representative sections of hearts at levels C and D from sedentary rats injected with 40 mg/kg isoproterenol. C - swelling and myofiber disruption extends from the subendocardium to the mid myocardium; D - swelling and myofiber disruption is confined to the subendocardial (ENDO) regions with the remaining myocardium being normal in appearance. (magnification in all X 180).

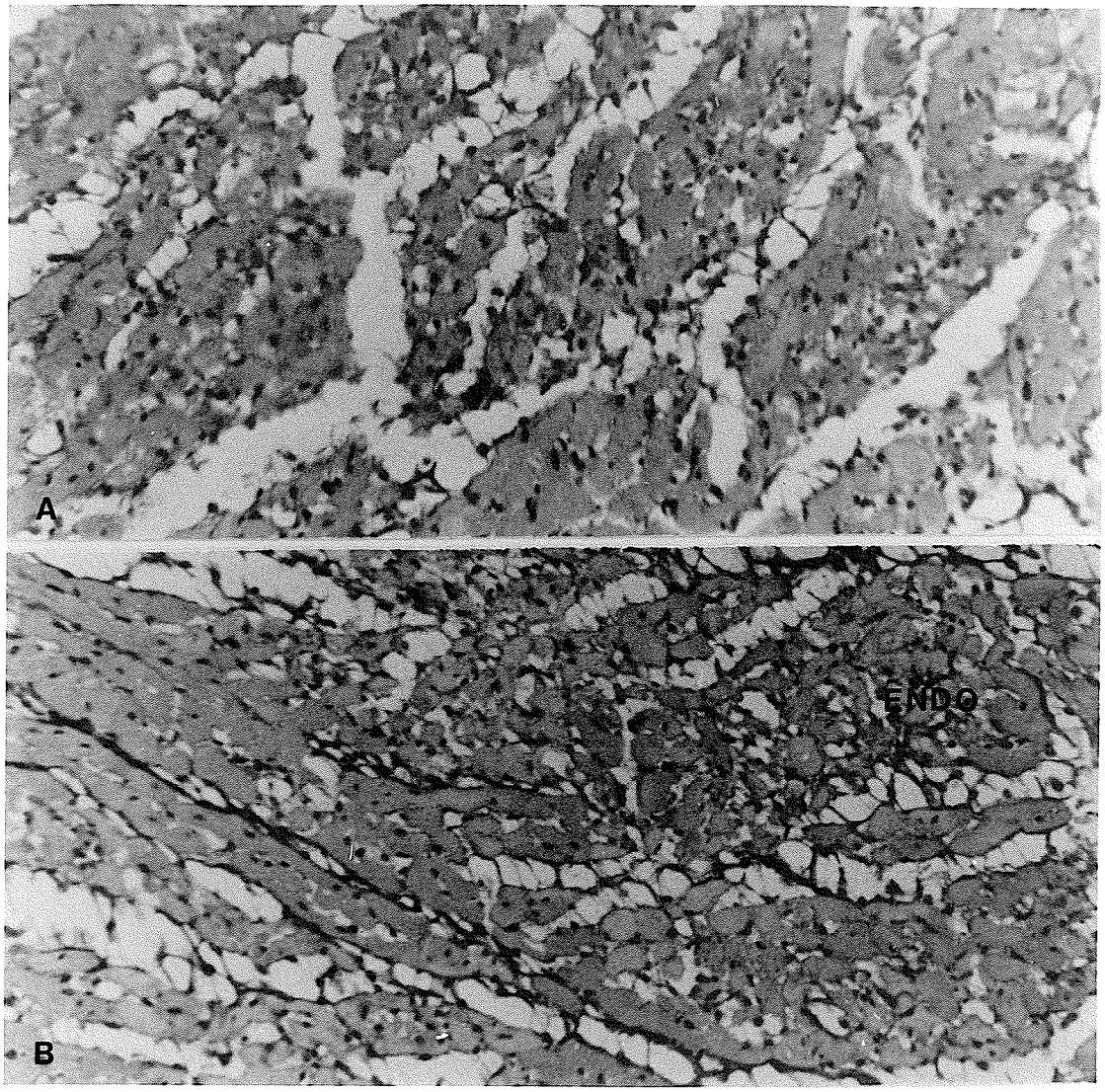


Figure 13: Representative sections of hearts at levels A and B from sedentary rats injected with 80 mg/kg isoproterenol. A - apex - massive interstitial and intracellular edema and vacuolization of myofibers; B - extensive interstitial and intracellular edema and vacuolization extending from the subendocardium (ENDO) deep into the myocardium. (magnification in all X 240).

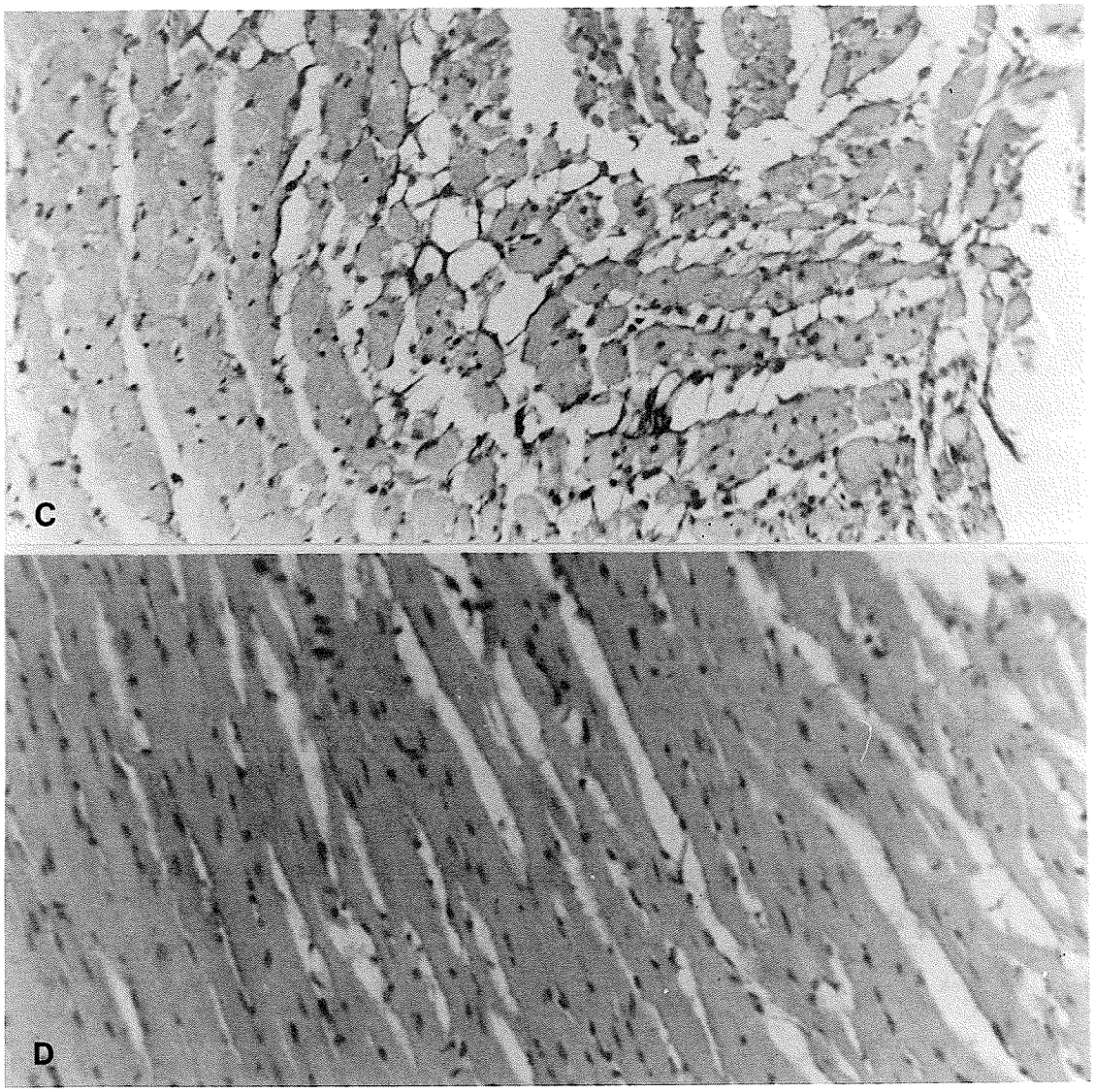


Figure 13 (continued): Representative sections of hearts at levels C and D from sedentary rats injected with 80 mg/kg isoproterenol. C - extensive interstitial and intracellular edema and vacuolization of the subendocardium; D - less severe interstitial edema of the subendocardium. Myofibers normal in appearance can be seen in the damaged zones. (magnification in all X 240).

which in some cases also extended into the mid myocardium. The remaining myocardium appeared relatively normal at levels C and D.

B) Exercise Treated Rats

As compared to the sedentary treated animals, the myocardial damage in the exercise treated rats was less extensive. This difference was apparent at all levels of the heart. The histological appearance of the hearts from exercise treated rats injected with 20,40 and 80 mg/kg isoproterenol is depicted in Figures 14,15 and 16 respectively. A relatively lesser degree of interstitial edema as well as vacuolization was apparent at all levels of the hearts from exercise treated animals. However, it is noteworthy that this edema was restricted to the subendocardial regions except at the levels C and D where some focal lesions were also apparent in the outer layers of the muscle. Marked fibroblastic activity was prominent in these regions. Hemorrhage into the interstitial spaces was evident in many sections. Although no quantitative morphometric study was attempted, the total damaged area of the muscle in the exercise treated rats appeared to be considerably less than sedentary treated rats.

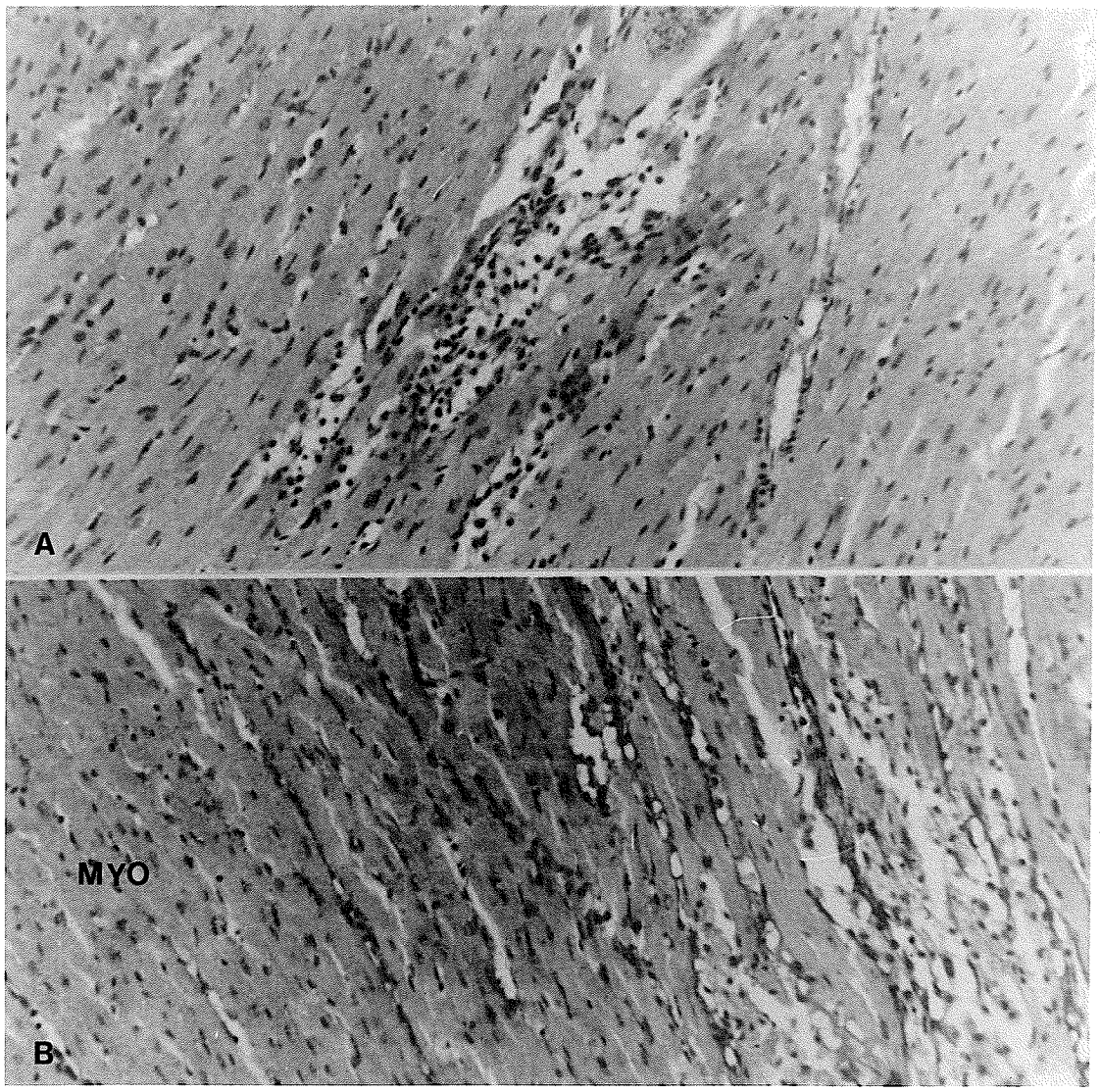


Figure 14: Representative sections of hearts at levels A and B from exercise stressed rats injected with 20 mg/kg isoproterenol. A - apex - focal necrotic lesions infiltrated with fibroblasts. Minimal interstitial and intracellular edema and myofiber disruption; B - subendocardial edema and vacuolization of myofibers. Increased collagen apparent between myofibers in the damaged zones and extending into the mid myocardial (MYO) regions. (magnification in all X 180).

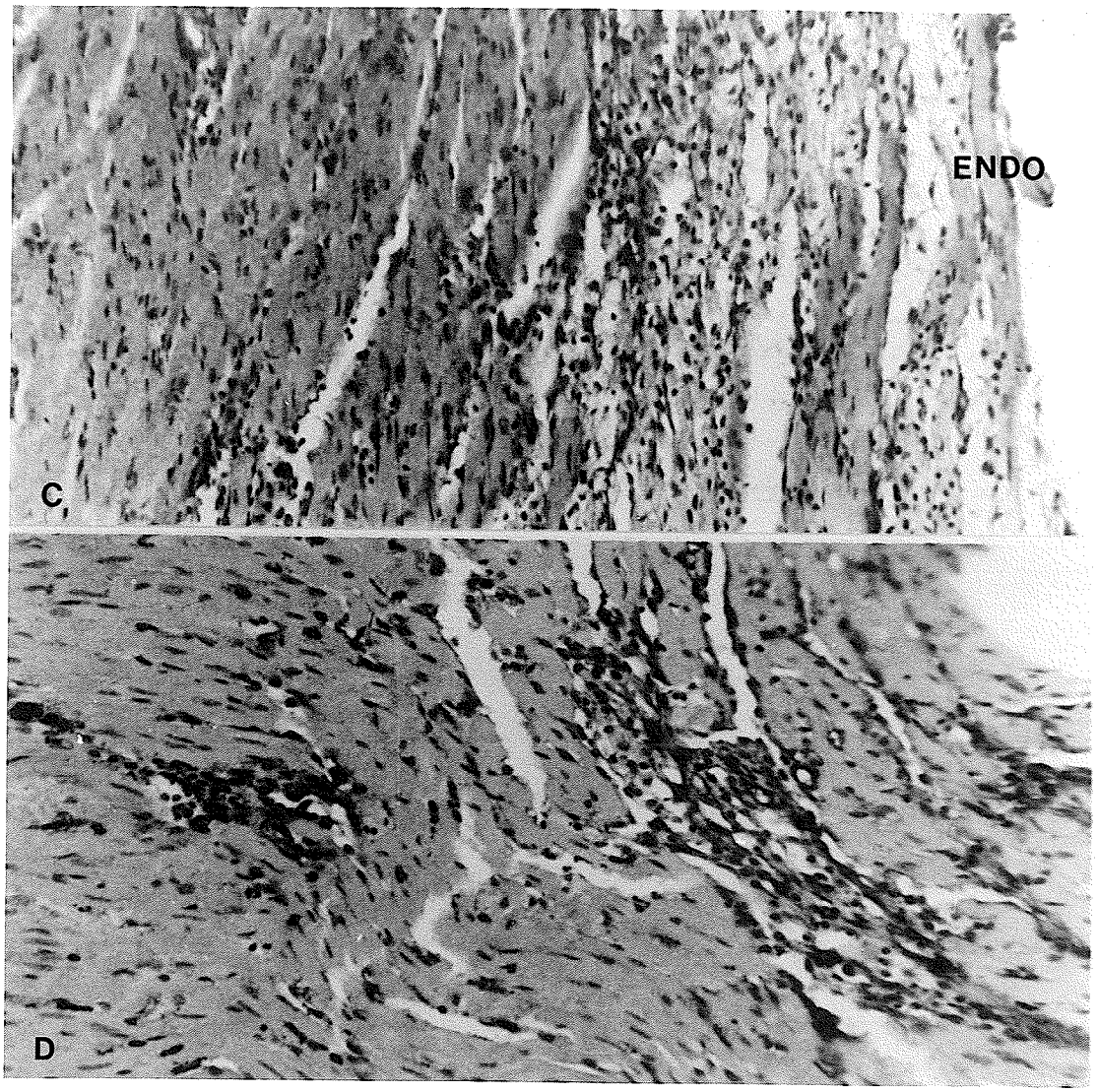


Figure 14 (continued): Representative sections of hearts at levels C and D from exercise stressed rats injected with 20 mg/kg isoproterenol. C - subendocardial (ENDO) edema and vacuolization. Marked infiltration with fibroblasts. Focal necroses rich in fibroblasts extend into the mid myocardial regions. (X 180). D - focal subendocardial necroses infiltrated with fibroblasts. Increased collagen between normal appearing myofibers. (X 240).

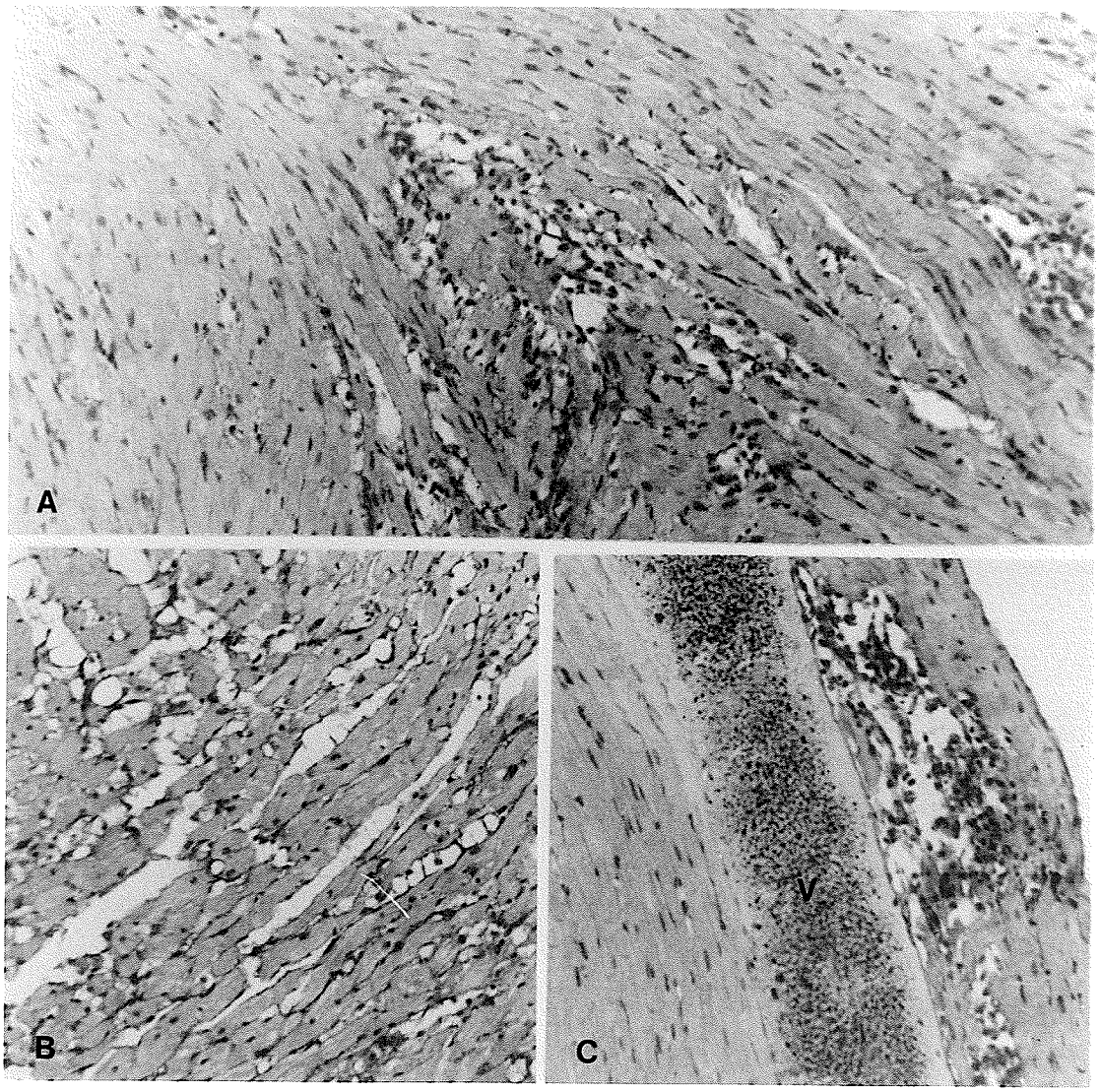


Figure 15: Representative sections of hearts at levels A,B and C from exercise stressed rats injected with 40 mg/kg isoproterenol. A - apex - focal necrotic lesions infiltrated with fibroblasts. Minimal interstitial and intracellular edema and myofiber disruption; B - subendocardial interstitial edema and focal vacuolization of myofibers; C - subepicardial necrotic lesion infiltrated with fibroblasts with the remaining myocardium normal in appearance. (V) blood vessel. (magnification in all X 180).

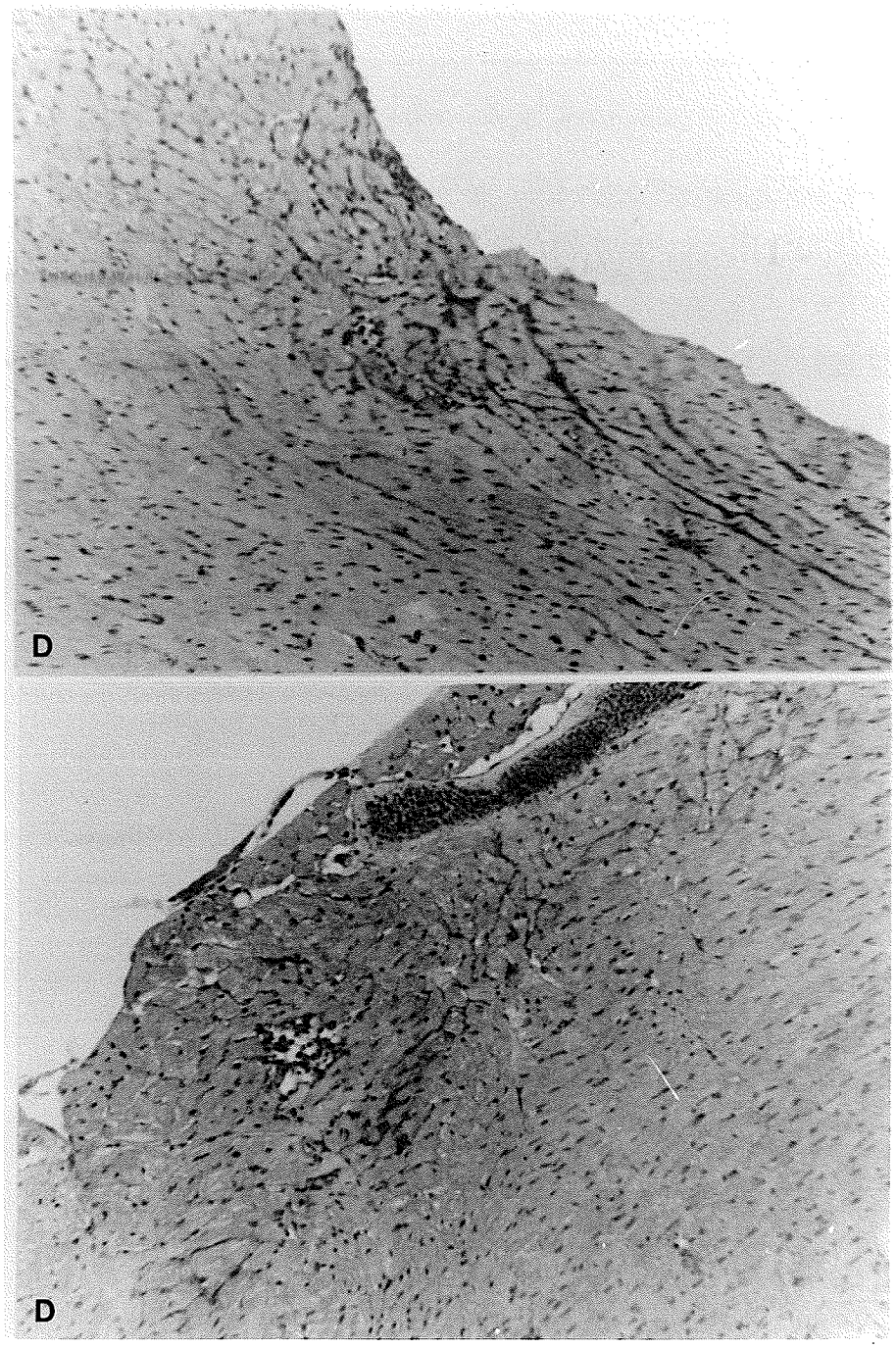


Figure 15 (continued): Representative sections of hearts at level D from exercise stressed rats injected with 40 mg/kg isoproterenol. Upper panel - subendocardial focal necrosis and scarring; Lower panel - subepicardial focal necrosis. The remaining myocardium is normal in appearance. (magnification in all X 120).

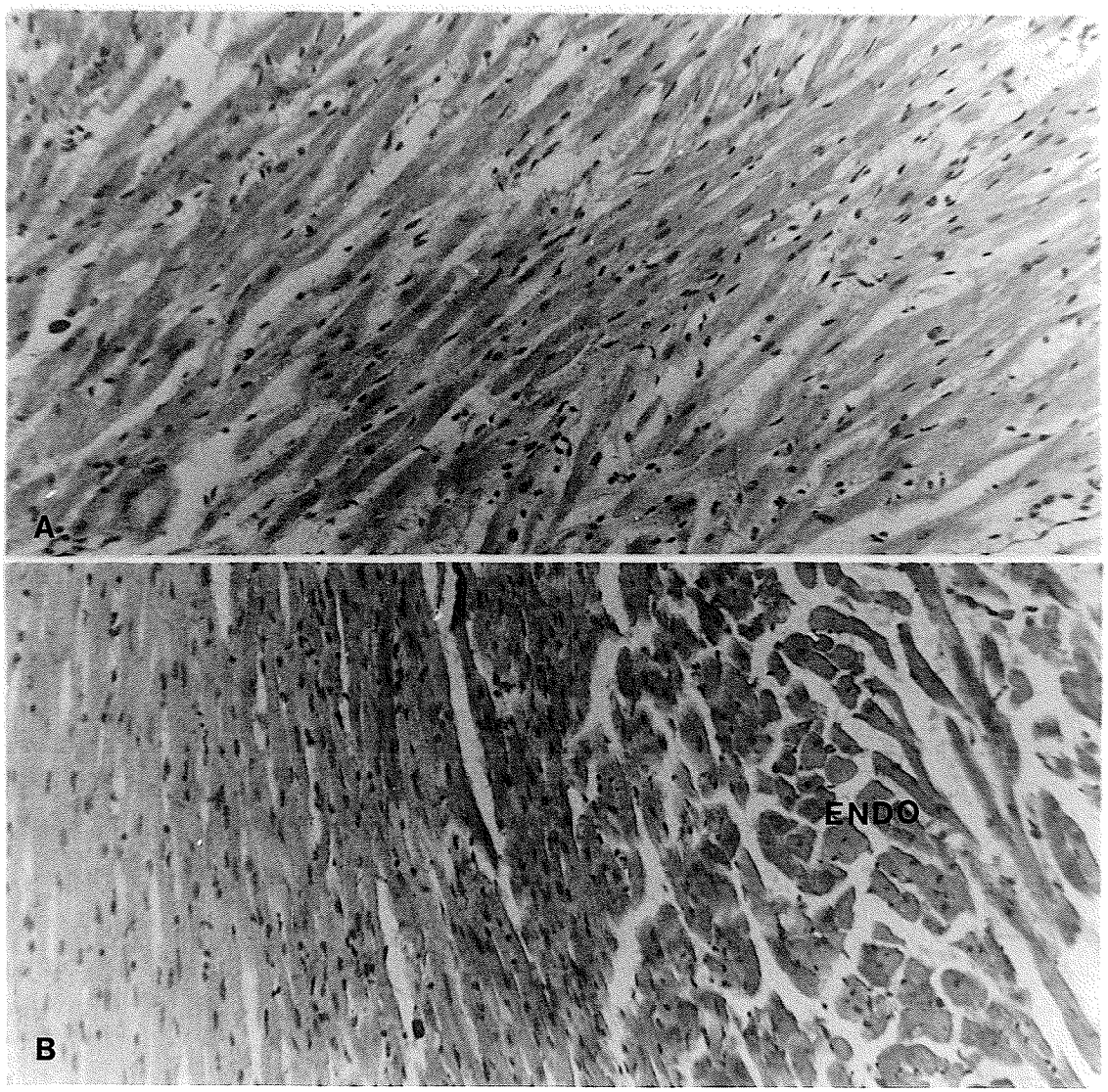


Figure 16: Representative sections of hearts at levels A and B from exercise stressed rats injected with 80 mg/kg isoproterenol. A - apex - interstitial edema and hemorrhage. Myofibers appear relatively normal within the damaged zones; B - interstitial edema is confined to the subendocardial (ENDO) regions. Myofibers maintain a relatively normal appearance within the damaged zones and in the remaining myocardium. (magnification in all X 180).

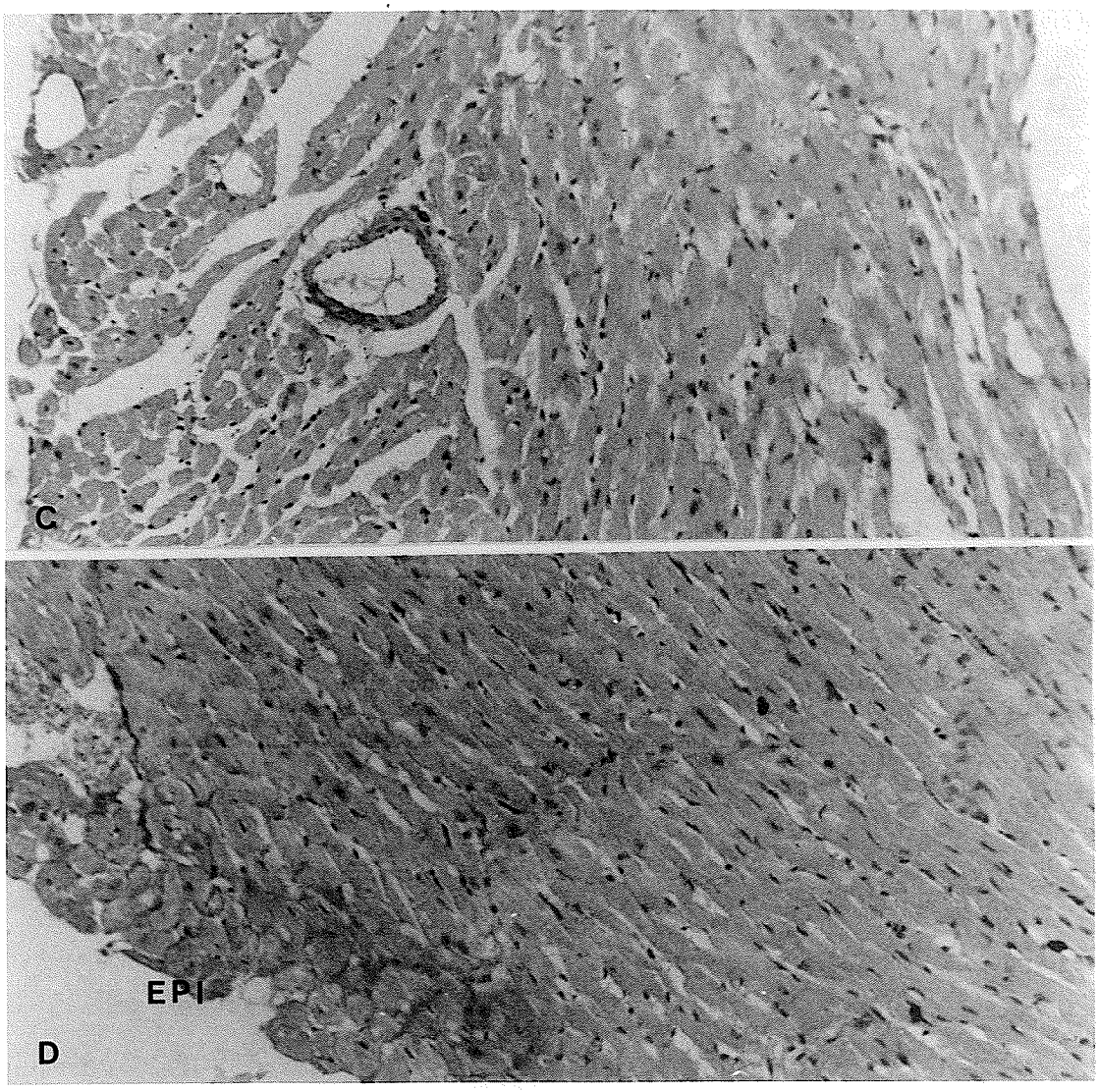


Figure 16 (continued): Representative sections of hearts at levels C and D from exercise stressed rats injected with 80 mg/kg -isoproterenol. C - full thickness myocardial interstitial and intracellular edema and some vacuolization of myofibers; D - minor interstitial edema and vacuolization subepicardial (EPI) region, with the remaining myocardium being normal in appearance. (magnification in all X 180).

# V DISCUSSION

The data presented here demonstrates that exercise may have detrimental as well as beneficial influences on the heart. The detrimental effects are manifest by the presence of myocardial necrosis observed in the hearts from exercise stressed control rats. The protective effects are expressed by the presence of less severe myocardial necrosis in hearts from exercise stressed animals than in sedentary rats following isoproterenol injection.

Hughson et al (1977), and Lin and Horvath (1972), have demonstrated a significant bradycardia in rats subjected to treadmill and swimming exercise programmes of eight to ten weeks duration. Although a decrease in heart rate was observed in the exercise stressed rats in our study, the drop in rate was not significant. This may be partly due to the fact that the exercise programme was only four weeks in duration.

The exercise stressed rats failed to gain weight as rapidly as their sedentary counterparts. The difference may have been due to a loss of weight in the increased production of energy during exercise. As well, there may have been some reduction in the food intake by the exercised animals. A similar explanation has been given by Schaible et al (1979), Penpargkul et al (1977), and Leon and Bloor (1968), to explain the decreased weight gain in male rats subjected to swimming exercise. In contrast, Oscai et al (1971), observed that female exercised rats increased their food intake and maintained growth patterns similar to female controls and no explanation has been offered for the observed phenomenon.

Myocardial hypertrophy was apparent as evidenced by the increased wet heart weight and increased heart/body weight ratio in the exercise stressed rats. As well, the significantly increased R wave amplitude in the EKG tracings of the exercise stressed rats is suggestive of hypertrophy. Histological examination confirmed the presence of myocardial hypertrophy in these animals. There was considerably more collagen present between the myofibrils in hearts from exercise stressed animals as compared to sedentary control rats. Myocardial hypertrophy is a widely observed phenomenon in work overloaded hearts. Exercise induced cardiac hypertrophy has been generally considered to be beneficial as it has been associated with an increased rate of left ventricular pressure development and increased contractility of the myocardium (Bershon et al,1977; Penpargkul et al,1978). In contrast, the myocardial hypertrophy associated with pathological processes is considered a compensatory mechanism of the heart to try to maintain cardiac output at levels required to meet the basal needs, (Wikman-Cofflet et al, 1979). The structural basis of exercise induced hypertrophy, stress induced or pathology induced hypertrophy are similar. In adult rats, there is an increase in the formation of collagen and enlargement of existing myofibrils,(Meerson et al,1974; Skosey et al,1972; Zak,1974; Grove et al,1969). It should, however, be noted that the enhanced cardiac performance associated with exercise training has been observed in treadmill trained dogs (Sordahl et al,1977; Dowell et al,1977) and rats (Tibbits et al,1978), in the absence of myocardial hypertrophy. This suggests that myocardial hypertrophy is not the necessary adaptation associated with exercise

training. In this regard, myocardial hypertrophy is known to occur most frequently in the more stressful exercise protocols, such as swimming (Bhan et al,1972; Wilkerson et al,1971; Scheuer et al,1974) or exhaustive exercise training (Banister et al,1971). Perhaps in these exercise stress protocols as the one adopted in our study, the stimulus for myocardial hypertrophy is a relative hypoxia. Hypoxia could result under conditions in which the oxygen needs of the myocardium exceed the maximum supply such as may occur during exhaustive exercise or in situations in which a combination of stresses are prevalent (Yang et al,1960; Leon et al,1968). Although myocardial hypertrophy starts as a compensatory mechanism, thus representing a physiological response, the continued presence of the stimulus may change it to a pathological state,(Wikman-Cofflet et al,1979). The latter, in the present study may have been the case in exercise stressed rats, therefore, explaining the tissue damage noticed in these animals.

The observation of a significant increase in serum creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and glutamic oxaloacetic transaminase (SGOT) following injection with 80 mg/kg isoproterenol in sedentary rats is consistent with the observations of Wexler (1978) and Rona et al (1959), as opposed to our serum enzyme data on isoproterenol treated exercise stressed rats. Wexler and Greenberg found no differences in CPK,SGOT and LDH enzyme concentrations between sedentary rats and rats exercised for thirty minutes daily for two weeks in water at $32^{o}$ C upon injection of 500 mg/kg of isoproterenol. The reason Wexler and Greenberg did not observe any enzyme changes in their study may be because their protocol was of a very low exercise intensity and of short duration.

The elevated SGOT observed in the exercise stressed control rats prior to treating with isoproterenol has not been previously reported in the literature, and the finding may represent increased oxidation phosphorylation processes within the mitochondria of the heart and other tissues. The increased energy demands placed on the rat by the intense intermittent exercise stress protocol employed in this study may exert sufficient demands on the heart to induce biochemical adaptations. The elevated SGOT in the exercise stressed control animals supports such an hypothesis. Furthermore, it is unlikely that the elevated SGOT represents some non selective leakage as it occurs in the absence of any alterations in LDH and CPK. SGOT also increased significantly from control levels in the exercise stressed rats injected with 40 and 80 mg/kg isoproterenol, whereas LDH and CPK increased only in response to injection with 80 mg/kg isoproterenol.

There was a significantly increased tachycardia in the exercise stressed rats following injection with isoproterenol as compared to the sedentary isoproterenol injected animals. This is consistent with the concept of enhanced myocardial sensitivity to catecholamines in animals exposed to other forms of prolonged stress (Oscai et al,1971; Terjung et al,1973). Carey et al (1976) observed a significantly greater increase in heart rate in treadmill trained rats as compared to sedentary rats when either group was exposed to hypoxic conditions or to coronary artery ligation induced myocardial ischemia. Dowell and Tipton (1970) also found that hearts from exercised trained rats beat faster after infusion or injection of isoproterenol than hearts from non-trained animals. This further

supports the hypothesis that exercise as well as other forms of stress increases the sensitivity of the heart to catecholamines. However, the histological data from the present investigation showed only focal necroses in the myocardium of the exercise stressed control animals and the damage was far less severe than that observed in sedentary rats. Similar focal type of lesions have also been observed by Leon and Bloor (1968) in swim-trained rats in water at 28-32$^{o}$ C. The influx of fibroblasts in most of the damaged zones may suggest that the lesions were in the later stages of healing (Fishbein et al,1978; Fishbein et al, 1978).

At any rate, these results indicate that pacemaker tissue of the heart in animals exhibits an increased sensitivity to catecholamines, whereas the myocardial tissue may have developed some tolerance to catecholamines due to a consistently elevated sympathetic activity during exercise as discussed below.

If the primary lesions observed in the exercise stressed control hearts were catecholamine induced then this may also explain the lack of a significant increase in myocardial damage in exercise stressed animals injected with isoproterenol. It has been shown by Balazs et al (1972) that on repeatedly injecting low doses of isoproterenol into rats the sensitivity of the myocardium to catecholamines is altered. They observed that focal necroses occur following the initial isoproterenol injection but then the myocardium becomes refractory to isoproterenol and there is no further necroses apparent after continued injections. Perhaps in this study, the increased circulating levels of catecholamines released during the intermittent exercise stress sessions produced a similar protection to the exercise

stressed rats when they were subsequently exposed to exogenous isoproterenol injections. Increased plasma (Adolph,1950) and central nervous system (Brown et al, 1973) norepinephrine concentrations have been observed during exposure to stress and to exercise. This contention is also supported by the fact that exercise stressed rats showed focal necrosis even prior to the injection of isoproterenol.

In other forms of stress such as unaccompanied by physical exercise, the cardiotoxicity to isoproterenol in rats is enhanced (Raab et al, 1968; Hsieh et al, 1957; Hsieh et al, 1971). In the exercise stress programme employed in this study, this does not appear to be the case. Perhaps the sympathetic response to all forms of stress is the same, but the heart adapts as a result of programmed training in a manner that protects the heart from additional stresses such as the isoproterenol injections.

The development of ventricular arrhythmias following injection of isoproterenol in sedentary rats is consistent with the findings of Wexler and Greenberg (1974). However, there were marked differences in the elctrocardiograph responses between the sedentary and exercise stressed rats following injection of isoproterenol. The EKG's of the exercise stressed rats exhibited conduction defects and a marked slowing of heart rate coincident with the development of severe pulmonary symptoms. Mortality in the exercise stressed rats was significantly greater than in the sedentary rats injected with isoproterenol. It appears that the deaths in the exercise stressed rats may have been precipitated by the development of pulmonary edema in these animals. Hsieh et al (1971) found that rats developed a marked increased sensitivity to infused norepinephrine following cold stress. Continuous

infusion of 1 mg/kg norepinephrine in these previously cold stressed animals resulted in all animals developing massive pulmonary edema with subsequent death. In this regard,it is important to note that reduced myocardial necrosis, increased heart rate and death rate in exercised rats in response to isoproterenol injection may not be related.

This contention is further supported by visual symptoms recorded in the exercise stressed rats. The marked palpaple congestion of the chest, frothy discharge at nose and mouth, rapid, shallow irregular respiration and perspiration are consistent with the clinical manifestations observed in man in pulmonary edema (Cherniak et al,1972; West,1978). The dark red appearance of the rat's eyes which was observed with the severe pulmonary symptoms suggests that hypoxemia may be a significant factor.

In conclusion, exercise training in combination with the cold stress (temperature of the water 22$^{o}$ C) imposed on animals produced responses in common with those observed in exercise alone, or in other forms of stress without exercise. The enhanced myocardial sensitivity to catecholamines in terms of heart or death rate in exercise stressed rats is apparent. However, the occurrence of necrotic lesions in the myocardium in response to exogenous catecholamine injection was reduced following a four week exercise stress protocol. Interestingly, the exercise stress in this study appeared to produce alterations in the sensitivity of the lungs to increased exogenous catecholamines in such a manner that the rats quickly developed an apparent massive pulmonary edema following injection with isoproterenol. Combination of stresses results in modifications of the responses previously reported in experiments in which a stress was examined in isolation.

Exercise stress induces myocardial damage but also appears to protect the heart from subsequent damage. It is tempting to speculate that the stress component of the protocol was responsible for the detrimental effects observed. Perhaps in the absence of other significant stresses the amount of controlled exercise, low enough not to cause damage to the heart, as well as high enough to build up resistance against subsequent stress, will be beneficial and ultimately protect the heart. However, further research is required to define these two limits of exercise intensity.

# VI REFERENCES


Adolph, E.F. (1950). Oxygen consumptions of hypothermic rats and acclimatization to cold. Am. J. Physiol. 161 (3): 359-373.

Baker, M.A., and Horvath, S.M. (1964). Influence of water temperature on oxygen uptake by swimming rats. J.Appl.Physiol. 19: 1215-1218.

Balazs, T. (1972). Cardiotoxicity of isoproterenol in experimental animals. Influence of stress, obesity and repeated dosing in: Myocardiobiology: Recent Advances in Studies on Cardiac Structure and Metabolism. Edited by E. Balazs, and G. Rona. 1: 770-778, Baltimore, Univ. Park Press.

Baldwin, K.M., Kinkerfuss, C.H., Terjing, R.L., Mole, P.A., Holloszy, J.O. (1972). Respiratory capacity of white, red and intermediate muscle: adaptive response to exercise. Am. J. Physiol. 222: 373-378.

Banister, E.W., Tomanek, R.J., and Cvorkov, N. (1971). Ultrastructural modifications in rat heart: responses to exercise and training. Am.J. Physiol. 220 (6): 1935-1940.

Barnard, R.J., and Peter, J.B. (1971). Effect of exercise on skeletal muscle III. Cyctochrome changes. J.Appl. Physiol.31 (6): 904-907.

Bartosova, D., Chvapil, M., Korechy, B., Poupa, O., Rakusan, K., Turek, Z., and Vitez, M. (1969). Growth of the muscular and collagenous parts of the rat heart in various forms of cardiomegaly. J. Physiol. 200: 285-295.

Bassett, J.R., and Cairncross, K.D. (1976). Endogenous levels of catecholamines in the rat myocardium following exposure to stress. Pharmac. Biochem. Behav. 4 (1): 35-38.

Bassett, J.R., and Cairncross, K.D. (1976). Effect of stress on the uptake of $^{3}$H norepinephrine into rat myocardium. Pharmac. Biochem. Behav. 4: 39-44.

Bassett, J.R., and Cairncross, K.D. (1976). Myocardial sensitivity to catecholamines following exposure of rats to irregular signalled footshock. Pharmacol. Biochem. Behav. 4: 27-33.

Bassett, J.R., and Cairncross, K.D. (1977). Ultrastructural changes in the coronary vascular system following prolonged emotional stress. An experimental model for the study of coronary vascular disease. Br. J. Pharmacol. 59: 471-472.

Bassett, J.R., and Cairncross, K.D. (1977). Changes in the coronary vascular system following prolonged exposure to stress. Pharmacol. Biochem. Behav. 6: 311-318.

Bhan, A.K., and Scheuer, J. (1972). Effects of physical training on cardiac actomyosin adenosine triphosphatase activity. Am. J. Physiol. 223: 1468-1490.

Bhan, A.K., and Scheuer, J. (1975). Effect of physical training on cardiac myosin ATPase activity.Am. J. Physiol. 288 (4): 1178-1182.

Bershon, M.M., and Scheuer, J. (1977). Effects of physical training on end-diastolic volume and myocardial performance of isolated rat hearts. Circ. Res. 40 (5): 510-516.

Bescei, I., and Lammerant, J. (1969). Effect of training on heart and skeletal muscle catecholamine concentration in rats. Am. J. Physiol. 217 (6): 1589-1592.

Bolter, C.P., Hughson, R.L., and Critz, J.B. (1973). Intrinsic rate and cholinergic sensitivity of isolated atria from trained and sedentary rats. Proc. Soc. Exp. Biol. Med. 144: 364-367.

Brown, B.S., and Van Huss, W. (1973). Exercise and rat brain catecholamines. J. Appl. Physiol. 34 (5): 664-669.

Carew, T., and Covell, J.W. (1978). Left ventricular function in exercise-induced hypertrophy in dogs. Am. J. Cardiol. 42: 82-87.

Carey, R.A., Tipton, C.M., and Lund, D.R. (1976). Influence of training on myocardial responses of rats subjected to conditions of ischaemia and hypoxia. Cardiovasc. Res. 10: 359-367.

Cherinak, R.M., Cherniak, L., and Naimark, A. (1972). Respiration in Health and Disease. W.B.Saunders Co., Toronto, 386-391.

Chin, A.K., Seaman, R., and Kapileshwarker, M. (1973). Plasma catecholamine response to exercise and cold adaptation. J. Appl. Physiol. 34: 409-412.

Crews, J., and Aldinger, E.E. (1967). Effect of chronic exercise on myocardial function. Am. Heart J. 74: 536-542.

Dawson, C.A., Nadel, E.R., and Horvath, S.M. (1968). Cardiac output in the cold-stressed swimming rat. Am. J. Physiol. 213 (2): 320-325.

Dawson, C.A., Roemer, R.B., and Horvath, S.M. (1970). Body temperature and oxygen uptake in warm and cold-adapted rats during swimming. J. Appl. Physiol. 29: 150-154.

Depocas, F. (1960). The calorigenic response of cold-acclimated white rats to infused noradrenaline. Can. J. Biochem. Physiol. 38: 107-114.

DeSchryver, C., Mertens-Strythagen, J., Bescei, I., and Lammerant, J. (1969). Effect of training on heart and skeletal muscle catecholamine concentration in rats. Am. J. Physiol. 217 (6): 1589-1592.

DeSchryver, C., and Mertens-Strythagen, J. (1972). Intensity of exercise and heart tissue catecholamine content. Pflugers Arch. 336: 345-354.

Dohm, L.G., Huston, R.L., Askew, E.W., Weiser, P.C. (1972). Effects of exercise on activity of heart and muscle mitochondria. Am. J. Physiol. 223 (4): 783-787.

Dowell, R.T., and Tipton, C.M. (1970). Influence of training on heart rate responses of rats to isoproterenol and propranolol. The Physiologist. 13: 182.

Dowell, R.T., Stone, H.L., Sordhal, L.A., and Asimakis, C.R.(1977). Contractile function and myofibrillar ATPase activity in the exercise-trained dog heart. J. Appl. Physiol: Respirat. Environ. Exercise Physiol. 43 (6): 977-982.

Fishbein, M.C., MacLean,D., and Maroko, P. (1978). The Histopathologic evolution of myocardioal infarction. Chest. 73 (6): 843-849.

Fishbein, M.C., MacLean, D., Maroko, P.R. (1978). Experimental myocardial infarction in the rat. Am. J. Patho-ogy. 90 (1): 57-65.

Gigee, W.R. (1961). Sympathogenic origin and anti-adrenergic prevention of stress induced myocardial lesions. Am. J. Cardiology 8: 203-211.

Gordon, R., Spector, S., Sjoerdsma, A., and Udenfriend, S. (1966). Increased synthesis of norepinephrine and epinephrine in the intact rat during exercise and exposure to cold. J. Pharmac. Exper. Ther. 153 (3): 440-447.

Grove, D., Zak, B.S., Nair, K.C., Aschenbrenner, V. (1969). Biochemical correlates of cardiac hypertrophy IV. Observations on the cellular organization of growth during myocardial hypertrophy in the rat. Circ. Res. 25: 473-485.

Hart, J.S., and Jansky, L. (1963). Thermogenesis due to exercise and cold in warm - and cold - acclimated rats. Can. J. Biochem. Physiol. 41: 629-634.

Harri, M.N., and Valtola, J. (1975). Comparison of the effects of physical exercise, cold acclimation and repeated injections of isoprenaline on rat muscle enzymes. Acta. Physiol. Scand. 95: 391-399.

Hashimoto, I., Sembrowich, W.L., and Gollnick, P.D. (1978). Calcium uptake by isolated sarcoplasmic reticulum and homogenates in different fiber types following exhaustive exercise. Med. Sci. Sport. 10 (1): 42. (Abstract).

Hickson, R.C., Hammons, G.T., and Holloszy, J.O. (1979). Development and regression of exercise-induced cardiac hypertrophy in rats. Am. J. Physiol. 236 (2): H268-H272.

Holloszy, J.O., and Booth, F.W. (1976). Biochemical Adaptations to endurance exercise in muscle. Ann. Rev. Physiol. 38: 273-291.

Hsieh, A.C.L., and Carlson, L.D. (1957). Role of adrenaline and noradrenaline in chemical regulation of heat production. Am. J. Physiol. 100 (2): 243-246.

Hsieh, A.C.L., and Wang, J.C.C. (1971). Calorigenic responses to cold of rats after prolonged infusion of norepinephrine. Am. J. Physiol. 221: 335-337.

Hughson, R.L., Sutton, J.R., Fitjgerald, J.D., and Jones, N.L. (1977). Reduction of intrinsic sinoatrial frequency and norepinephrine response of the exercised rat. Can. J. Physiol. Pharmacol. 55: 813-820.

Humanson, G.L. (1967). Animal Tissue Techniques. 2nd edition, W.H. Freeman & Co. San Francisco.

King, E.W., and Gollnick, P.D. (1970). Ultrastructure of rat heart and liver after exhaustive exercise. Am. J. Physiol. 218: 1150-1155.

Krames, B.B., Northup, D.W., and Van, E.J. (1967). Pressure development in the hypertrophied heart. Proc. Society Experimental Biology & Medicine. 124: 150-152.

Leblanc, J. (1969). Stress and interstress adaptation. Fed. Proc. 28 (3): 996-1000.

Leblanc, J., Roberge, C., Valliere, J., and Oakson, G. (1971). The sympathetic nervous system in short-term adaptation to cold. Can. J. Physiol. Pharmacol. 49 (2): 96-101.

Leon, A.S., and Bloor, C.M. (1968). Effects of exercise and its cessation on the heart and its blood supply. J. Appl. Physiol. 38 (4): 485-490.

Lin, Y.C., and Horvath, S.M. (1972). Autonomic nervous control of cardiac frequency in the exercise-trained rat. J. Appl. Physiol. 33 (6): 796-799.

Maher, J.T., Goodman, A.L., Francesconi, R., Bowers, W.D., Hartley, L.H., and Angelakos, E.T. (1972). Responses of rat myocardium to exhaustive exercise. Am. J. Physiol. 222: 207-212.

McArdle, W.D. (1967). Metabolic stress of endurance swimming in the laboratory rat. J. Appl. Physiol. 22 (1): 50-54.

Meerson, F.Z. (1974). Development of modern components of the mechanism of cardiac hypertrophy. Circ. Res. Suppl.II. 34: 58-63.

Meerson, F.Z., Javich, M.O., and Lerman, M.I. (1978). Decrease in the rate of RNA and protein synthesis and degradation in the myocardium under long-term compensatory hyperfunction and on aging. J. Molec. Cell. Cardiol. 10: 145-159.

Miyagi, T., Kishima, N., Kondo, K., Hameda, H., Ishihara, T., K Kishimoto, T., Imaoka, A., Sano, I., Terachi, A., Akeika, H., Seki, I. (1979). The effect of exercise and hypoxemia stress on the catecholamines in coronary sinus and arterial plasma in man. J. Molec. Cell. Cardiol. II, Suppl. III: 38. (Abstract)

Morkin, E. (1974). Activation of synthetic processes in cardiac hypertrophy. Circ. Res. 34: Suppl II. 37-48.

Oscai, L.B., Mole, P.A., Brei, B., and Holloszy, J.O. (1971). Cardiac growth and respiratory enzyme levels in male rats subjected to a running programme. Am. J. Physiol. 220: 1238-1241.

Oscai, L.B., Mole, P.A., and Holloszy, J. O. (1971). Effects of exercise on cardiac weight and mitochondria in male and female rats. Am. J. Physiol. 220: 1944-1948.

Penpargkul, S., and Scheuer, J. (1970). The effect of physical training upon the mechanical and metabolic performance of the rat heart. J. Clin. Invest. 49: 1859-1868.

Penpargkul, S., Repke, D.I., Katz, A.M. and Scheuer, J. (1977). Effect of physical training on calcium transport by rat cardiac sarcoplasmic reticulum. Circ. Res. 40 (2): 134-138.

Penpargkul, S., Schwartz, A., and Scheuer, J. (1978). Effect of physical conditioning on cardiac mitochondrial function. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 45 (6): 978-986.

Raab, W., Bajusz, E., Kimura, H., and Herrlich, H.C. (1968). Isolation, Stress, myocardial electrolytes, and epinephrine cardiotoxicity in rats. Proc. Soc. Exp. Bio. Med. 127: 142-147.

Rona, G., Chappel, C., Balazs, T., and Gaudry, R. (1959). An infarct like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. A.M.A. Archives of Pathology, 67: 443-455.

Salzman, S.H., Hirsch, E.Z., Hellerstein, H.K., Bruell, J.H. (1970). Adaptation to muscular exercise: myocardial epinephrine-$^{3}$H uptake. J. Appl. Physiol. 29 (1): 92-95.

Schaible, T.F., and Scheuer, J. (1979). Function of hearts conditioned by running and by swimming. Bull. N.Y. Acad. Med. 55 (4): 428.

Scheuer, J., and Stezoski, S.W. (1972). Effect of physical training on the mechanical and metabolic responses of the rat heart to hypoxia. Circ. Res. 30: 418-429.

Scheuer, J., Penpargkul, S., and Bhan, A.K. (1974). Experimental observations on the effects of physical training upon intrinsic cardiac physiology and biochemistry. Am. J. Cardiol. 44: 744-751.

Scheuer, J., and Tipton, C.M. (1977). Cardiovascular adaptations to physical training. Ann. Rev. Physiol. 39: 221-251.

Sellers, E.A., Reichman, S., and Thomas, N. (1951). Acclimatization to cold: natural and artificial. Am. J. Physiol. 167: 644-650.

Sembrowich, W.L., and Gollnick, P.D. (1977). Calcium uptake by heart and skeletal muscle sarcoplasmic reticulum from exercised rats. Med. & Sci. Sports. 9 (1): 64, (Abstract).

Skosey, J.L., Zak, R., Martin, R.F., Aschenbrenner, V., and Rabinowitz, M. (1972). Biochemical correlates of cardiac hypertrophy. Circ. Res. 31: 145-155.

Sordahl, L.A., Asimakis, C.K., Dowell, R.T., and Stone, H.L. (1977). Functions of selected biochemical systems from the exercised-trained dog heart. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 43 (3): 426-431.

Stromme, S.B., and Hemmel, H.T. (1967). Effects of physical training on tolerance to cold in rats. J. Appl. Physiol. 23 (6): 815-824.

Tan, Eng. Meng. Hanson, M.E., and Richter, C.P. (1954). Swimming time of rats with relation to water temperature. Federation Proceedings 13: 150 (Abstract).

Terjung, R.L. Klinkerfuss, C.H., Baldwin, K.M., Winder, and Holloszy, J.O. (1973). Effect of exhausting exercise on rat heart mitochondria. Am. J. Physiol. 225 (2): 300-305.

Tibbits, C., Koziol, B.J., Roberts, N.K., Baldwin, K.M. and Barnard, R.J. (1978). Adaptation of the rat myocardium to endurance training. J. Appl. Physiol: Respirat. Environ. Exercise Physiol. 44 (1): 85-89.

West, J. (1978). Pulmonary Pathology - the essentials. Williams & Williams Co., Balitmore, pp 112-124.

Wexler, B., and Greenberg, B. (1974). Effect of exercise on myocardial infarction in young vs old male rats: Electrocardiograph changes. Am. Heart J. 88 (3): 343-350.

Wexler, B., (1978). Myocardial infarction in young vs old male rats; Pathophysiologic Changes. Am. Heart J. 96 (1): 70-80.

Wikman-Coffelt, J., Parmley, W.W., and Mason, D.T. (1979). The cardiac hypertrophy process. Analyses of factors determining pathological vs physiological development. Circ. Res. 45 (6): 697-707.

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

Wyatt, H.L., Chuck, L., Rabinowitz, B., Tyberg, J.V., and Parmley, W.W. (1978). Enhanced cardiac response to catecholamines in physically trained cats. Am. J. Physiol. 234 (5): H608-H613.

Yang, T.L., and Lissak, K. (1960). Influence of environmental temperature on physical performance, oxygen consumption, blood lactate level and rectal temperature. Acta Physiol. Acad. Sci. 17: 63-68.

Zak, R. (1974). Development and proliferative capacity of cardiac muscle cells. Circ. Res. Suppl II. 34: 17-26.