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The Ohio State University

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THE EFFECTS OF TWO LEVELS OF EXERCISE ON MYOCARDIAL INFARCT SIZE AND SCAR FORMATION IN RATS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Barbara Ann Smith, B.S.N., M.S.N.

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The Ohio State University

1986

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Robert L. Bartels
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TO MY PARENTS CHARLES AND HELEN
and to the memories of three
dynamic ladies
Dr Marilyn Green,
Mrs. Vera Green, and
Miss Rose Piper
who never let me stop
believing in myself.
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To Dorrie Wells for her good humor and her word processor.
To my sisters Beverly and Marilyn for just being themselves; to all my friends; and to all my former teachers both good and bad because they all helped me learn, thanks!

And finally, to Jan Fetters for helping me realize there is more to life, thanks for being my friend.
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Studies in Physiology - Dr. James Grossie
Studies in Cardiac Rehabilitation and Adult Fitness - Dr. Tim Kirby
Studies in Chronic Disease - Dr. Mary MacVicar
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CHAPTER I
INTRODUCTION

In the last 10-15 years physicians have begun to prescribe an increase in physical activity and ambulation earlier during the period of recovery following an acute myocardial infarction (Wenger, Hellerstein, Blackburn, and Castranous, 1982, Wenger, N.K., 1984). In 1982, the Coronary Heart Disease Study Group advocated progressive exercise so that not only could the patient be ambulated by the time of discharge, but also he or she could be exercise tested prior to discharge. Coupled with this trend in prescribing earlier activity is a trend toward earlier hospital discharge after infarction. A recent survey showed a decline of seven days in the average hospital stay from 1970 to 1979 (Wenger et al. 1982).

These practices of earlier physical activity and ambulation have reduced or eliminated many of the deconditioning effects of prolonged inactivity (Convertino Hung, Goldwater, and DeBusk, 1982; Sullivan et al. 1985) and have prevented many of the problems long associated with prolonged bed rest, such as pneumonia, thromboembolism, renal calculus, and decubitus ulcer formation (Harrison 1944; Levine 1944). Additionally, the
practice of predischarge exercise testing has allowed the clinician to prescribe activity more appropriately upon discharge, identify those patients requiring further study or treatment, and reassure the patient that many activities are safe to perform. Finally, several longitudinal studies found little or no significant differences in mortality or reinfarction rates between those discharged early and those discharged later (Hutter, Sidel, Shine, and DeSanctis, 1973; McNeer, et al. 1978; West and Henderson, 1985).

Statement of the Problem

Despite the benefits of early ambulation, predischarge exercise testing, and early discharge a number of clinicians remain concerned about the effects of this increase in activity on myocardial healing during a time when the infarct is not completely healed. Furthermore, these clinicians used the results of a few recent animal studies to substantiate some of their concerns. Hammerman, Schoen, and Kloner, 1983; Kloner and Kloner, 1983; Moskowitz, et al. 1979; These studies found exercise increased infarct size or thinned the infarct scar.

Besides being concerned with a larger infarct some clinicians believed that the thinned scar would lead to a decline in cardiac output as the result of the loss of a portion of each stroke volume into the thinner more
compliant scar. Furthermore, they believed that a more compliant scar may lead to aneurysm formation and possibly myocardial rupture (Eaton, Weiss, Buckley, Garrison, and Weisfeldt, 1979; Hammerman et al. 1983; Kloner and Kloner, 1983).

In these previous studies showing negative effects on infarct size and scar formation, the investigators initiated the exercise within a few hours of the experimentally induced infarction, used swimming as the mode of exercise, and rapidly progressed the intensity and duration of the exercise (See Table 2, Chapter 2). The investigator of the current study believed many of these negative effects on infarction size and scar formation, documented in these animal studies, were related to the above factors; that is, very early initiation of exercise, inappropriate mode of exercise, and a rapid progression of both the intensity and duration of exercise. Therefore the current study uses the rat model of experimentally induced myocardial infarction to determine if two levels of exercise during the phase of incomplete healing will affect infarct size and scar formation. Mild and moderate treadmill exercise was begun 72 hours following infarction and was progressed weekly.

Purpose of the Study

The purpose of this study was to examine the effects mild and moderate treadmill exercise had on myocardial
infarction size and scar formation in rats when exercise was initiated 72 hours after a myocardial infarction was induced by ligation of the left coronary artery. Exercise begun at this time, would occur at a time when the infarct is not totally healed (i.e., the infarction site had not been replaced with a thick fibrous scar) and would correspond to exercise begun at six days post myocardial infarction in human.

Research Questions

1. Does mild treadmill exercise, initiated 72 hours after a myocardial infarction is induced in rats, increase the size of that myocardial infarction?

2. Does moderate treadmill exercise, initiated 72 hours after a myocardial infarction is induced in rats, increase the size of that myocardial infarction?

3. Does mild treadmill exercise, initiated 72 hours after a myocardial infarction is induced in rats, thin the scar that results 21 days later?

4. Does moderate treadmill exercise, initiated 72 hours after a myocardial infarction is induced in rats, thin the scar that results 21 days later?

Research Hypotheses

1. There is no significant difference between a mildly exercised group of rats and a nonexercised control
group of rats in the size of the myocardial infarction measured 21 days post infarction.

2. There is no significant difference between the moderately exercised group of rats and a nonexercised control group in the size of the myocardial infarction measured 21 days post infarction.

3. There is no significant difference between the mildly exercised group of rats and a moderately exercised group in the size of the myocardial infarction measured 21 days post infarction.

4. There is no significant difference between mean infarction size measured by the left ventricular circumference method and mean infarction size measured by the left ventricular area method.

5. There is no significant difference between a mildly exercised group of rats and a nonexercised control group of rats in the mean scar thickness measured 21 days post myocardial infarction.

6. There is no significant difference between a moderately exercised group of rats and a nonexercised group in the mean scar thickness measured 21 days post myocardial infarction.

7. There is no significant difference between a mildly exercised group of rats and a moderately exercised group in the mean scar thickness measured 21 days post myocardial infarction.
8. There is no significant difference between a mildly exercised group of rats and a nonexercised control group of rats in the thinnest portion of scar measured 21 days post myocardial infarction.

9. There is no significant difference between the moderately exercised group of rats and a nonexercised control group in the thinnest portion of scar measured 21 days post myocardial infarction.

10. There is no significant difference between the mildly exercised group of rats and a moderately exercised control group in the thinnest portion of scar measured 21 days post myocardial infarction.

Limitations of the Study

1. The myocardial infarction that results from the surgical ligation of a single vessel (i.e., left coronary artery), its evolution and the effects of activity on the infarction may be different from the myocardial infarction that results from the presence of diffuse coronary artery disease.

2. Despite the histological similarity in the evolution of a myocardial infarction in human and rat models, differences between these models preclude generalizations concerning the effects of activity on myocardial infarct size and scar formation.
Definitions

Mild exercise - Mild exercise for the rat in this study is exercise on a rodent treadmill at a speed of nine meters per minute at zero percent grade. This corresponds to a level of exercise at an intensity of less than 60 percent of the maximal oxygen uptake of a rat (Shepherd and Gollnick, 1976).

Moderate exercise - Moderate exercise for the rat in this study is exercise on a rodent treadmill at a speed of eighteen meters per minute at zero percent grade. This corresponds to a level of exercise at an intensity of greater than 60 percent of the maximal oxygen uptake of a rat (Shepherd and Gollnick, 1976).

No exercise (Control) - No exercise for the rat in this study is no treadmill exercise. The rat is, however, not restricted in movement about his cage.

Myocardial infarct - Death (necrosis) of a portion of the heart muscle usually resulting from loss of a blood supply to that portion of the heart secondary to arteriosclerotic changes in one or more coronary arteries. However, it may be experimentally induced by the use of drugs such as isoproteranol or suture ligation (of a coronary artery). A myocardial infarction is also known as a heart attack, coronary thrombosis, or coronary.

Zeiss Interaction Digital Analysis System (ZIDAS) - The ZIDAS is an image analysis system that derives measurements from images that have been traced on a digitizing tablet.
A coronary artery occlusion procedure is an experimental technique for stopping the blood flow to a portion of the myocardium in laboratory animals. This is usually accomplished by suture ligation but has also been accomplished by electrocautery (Moskowitz et al. 1979).
CHAPTER II
REVIEW OF THE LITERATURE

The literature review consists of the following sections: 1) bedrest and deconditioning, 2) early ambulation of humans, 3) exercise effects on infarct size and scar formation, 4) experimental myocardial infarction in rats, 5) exercise protocols with rats, 6) measurements of infarct size, scar thickness, and septal wall thickness.

Literature Related to Problems of Bedrest and Deconditioning

Herrick (1912) describing the occurrence of cardiac rupture following myocardial infarction probably was largely responsible for beginning the use of bedrest in the treatment of myocardial infarctions that has persisted throughout much of this century.

Several human post mortem studies helped perpetuate the use of bedrest until the mid 1970s. For example, Mallory reported that the infarction was not replaced with a thick fibrous scar until the sixth to eighth week following myocardial infarction (Mallory, White, and Salcedo-Salgar, 1939). Jetter and White (1944), also in a post mortem study involving patients in Massachusetts mental institutions, reported cardiac tamponade secondary to
myocardial rupture in 16 of 22 cases of acute myocardial infarction. They attributed the high incidence of myocardial rupture to the more active lifestyle of mental patients. Another group reported persistent hypertension or excessive effort usually preceded rupture (Wessler, Zoll, and Schlesinger, 1952). Finally, Schlichter, Hellerstein and Katz (1954) advocated four weeks of bedrest after their study of 102 cases of ventricular aneurysm. Conflicting animal studies did not help to clear the way for ambulating the patient. While one study found that artificial restriction of activity in rats lead to greater mortality among the restricted rats (Harrison and Thomas, 1944) Sutton and Davis (1931) had already reported thin scars with aneurysmal bulging in dogs exercised three days after infarction.

However, there were individuals who continued to believe that prolonged bedrest was detrimental (Dock, 1944; Harrison, 1944; Levine, 1944; Irwin and Burges, 1950). In 1944, Harrison documented the abuse of rest as a therapeutic measure in patients following myocardial infarction and identified thromboembolism formation, congestive heart failure and hypostatic pneumonia as disadvantages of prolonged rest. Levine (1944), acknowledging the problems identified by Harrison, also suggested that clinicians actually increased the work of the heart by augmenting venous return to the right side of the heart when placing
patient in a recumbent position and proposed chair rest as a method of avoiding this problem. Irwin and Burges (1950) further undermined the routine use of bedrest when they said there was no statistical evidence to support the use of bedrest in the treatment of a myocardial infarction.

More recently, Fareauddin and Abelman (1969) identified an adverse physiological effect of bedrest. While studying 22 patients convalescing from acute myocardial infarction, they reported a fall in systemic blood pressure of more than 38 mm of mercury in five out of 10 patients treated with strict bedrest when these patients were subjected to 15 minutes of passive upright tilt to 70°. This orthostatic hypotension was not observed in eight patients treated with modified bedrest.

Studies of bedrest in healthy young and middle aged males have identified other negative cardiovascular and hemodynamic effects of prolonged bedrest. Sullivan et al. (1983) in 24 young normal males subjected to three weeks of bedrest reported a decline in maximal oxygen consumption, stroke volume and cardiac output as well as an increase in heart rate and blood lactate response. Further, they identified orthostatic hypotension, tachycardia, and an accentuated renin-aldosterone response in their bedrested subjects. They attributed the latter to the observed fall in blood volume. Convertino et al. (1982) reported a decrease in peak oxygen uptake, a decrease in submaximal
work, and an increase in rate-pressure product (an indicator of myocardial oxygen demand) after 10 days of recumbency in 12 healthy middle aged men. They concluded that the higher rate pressure product at submaximal workloads that they observed in their healthy subjects may increase the risk of angina pectoris and other coronary events in patients with arteriosclerotic heart disease (Convertino et al. 1982).

Literature Related to Early Ambulation

As research identified an increasing number of problems with prolonged bedrest other studies found that there were little or no differences in survival or reinfarction at the six month follow-up between patients discharged early and those discharged late following an uncomplicated myocardial infarction. For example, in one study mortality, complications, and functional status were compared between the groups at six months. There were no significant differences found in mortality, complications, and functional status between 33 patients discharged at one week versus 34 patients discharged later (11 days ± 2 days) (McNeer, et al. 1978). Hutter, et al. (1973) reported no differences in return to work, anxiety or depression, and development of angina congestive heart failure, aneurysm, reinfarction, or death between patients discharged at two
weeks (69 subjects) versus those discharged at three weeks (69 subjects).

Another study involving 742 Welsh patients found no differences in survival at one year, between patients mobilized at 5 days versus 10 days after uncomplicated myocardial infarction. They did report a nonsignificant trend toward less survival of the patients mobilized at five days from the second through the sixth year (West and Henderson, 1985).

Finally, other investigators and clinicians have advocated the use of exercise testing prior to discharge or within 3 weeks of myocardial infarction as a means of identifying those people at higher risk for the development of problems as well as a method of prescribing more appropriate activity levels (Davidson and DeBusk, 1980; DeBusk and Dennis, 1985; DeBusk, et al. 1986; DeBusk, Kraemer, Nash, Berger, and Lev, 1983; Haskell and DeBusk, 1979; Theroux, Waters, Halphen, Debaissieux and Mizgala, 1979; Weiner, 1983; Wenger, 1984; Wenger and Hellerstein, 1984). Another study, found few problems associated with early exercise testing in a population of patients with uncomplicated myocardial infarction (Sivarajan, Bruce, Lindskog, Almes, Belanger and Green, 1982).
Literature Related to Exercise and Effects on Size and Healing of Infarcts:

The results reported from early animal studies on the effects of exercise on experimentally induced myocardial infarctions have conflicted. For example, Sutton and Davis (1931) reported dogs exercised within three days of infarction developed thin scars with aneurysmal bulging. Whereas, Thomas and Harrison (1944) reported greater mortality in rats whose muscular activity was restricted by small cage size.

More recently, the effects of exercise prior to the complete healing of a myocardial infarction on infarct size and scar formation have been examined using the rat model with varying results. Hammerman et al. (1983), Kloner and Kloner (1983), and Moskowitz et al. (1979) found that early exercise following experimentally induced myocardial infarction caused thinning of the scar, or an increase in infarct size.

Hammerman et al. (1983) subjected eight of their rats to daily graded swimming (up to 45 minutes per day) for seven days beginning 24 hours after inducing an infarction by the coronary artery ligation method (discussed in a later section of this chapter). They reported marked thinning of the scar in the rats that swam when they were compared to the rats that did not swim and concluded that
early swimming exercise had long lasting effects on scar formation (Hammerman et al. 1983).

Kloner and Kloner (1983) subjected nine of their 18 rats to a graded swimming protocol (up to 40 minutes per day) for 15 days beginning seven days after inducing an infarction using the coronary artery ligation method. They reported a scar thickness of 1.0 ± 0.2 mm in the rats that swam versus a scar thickness of 1.4 ± 0.3 mm in the rats that did not swim (P<0.05) and concluded that exercise during the phase of incomplete healing thinned the infarct scar (Kloner and Kloner, 1983).

Moskowitz et al. (1979) studying both the effects of exercise and cage size on infarct size in rats subjected one group of his rats (n=10) to mild treadmill exercise (25 feet per minute) for five minutes and another group of his rats (n=11) to moderate graded treadmill exercise (35 to 50 feet per minute) for 5 to 20 minutes after inducing an infarction using a coronary artery cauterization method (discussed in a later section of this chapter). Exercise was conducted over four sessions: session 1, 2, 3, and 4 were held at 4, 26, 30, and 44 hours after surgery, respectively. They reported an infarct size of 18.7 ± 2.44 percent in the mildly exercised group and 40.8 ± 5.09 percent in the moderately exercised group and concluded that moderate treadmill exercise doubled the size of the infarction.
Finally, Hochman and Healy (1986) subjected 90 of their rats to mild treadmill exercise (0.4 to 0.6 miles per hour) for 1.5 hours each day beginning on the day of coronary artery ligation. They reported no difference between the exercised rats and a control group of rats (n=39) with regard to infarct wall thickness, left ventricular diameter, expansion grade, infarct size, or number of animals with aneurysmal shape changes. However, they did report a nonsignificant trend toward higher mortality in the exercised group (Hochman and Healy, 1986).

Thus, as mentioned earlier the results of animal studies have produced conflicting results with regards to the effect of exercise on infarct size and healing and served as a major stimulus for this study.

Literature Related to Techniques Used to Experimentally Induce Myocardial Infarction

Two methods, one pharmacological and the other surgical, have been used extensively to produce experimental myocardial damage and the subsequent development of fibrous tissue in the rat and other animal models. Classic work first described by Rona, Chappel, Balaz and Gaudry (1959) and later by others (Miles, Hunez, and Rapaport, 1978; Wexler, Judd, and Kittinger, 1968; Wexler and Kittinger, 1963) has shown that various doses of isoproterenol, a synthetic catecholamine, given
subcutaneously or intraperitoneally will cause infarct-like lesions in the myocardium. Rona et al. (1959) indicated these myocardial lesions were similar (i.e., found concentrated in the apex of the heart) to those produced by coronary artery ligation but hinted at diffuse distribution of the lesions throughout the heart. Recently, these lesions have been described as single, isolated cells or foci located diffusely throughout the myocardium (Todd, Baroldi, Pieper, Clayton and Eliot, 1985).

Coronary artery ligation as a technique for inducing myocardial infarction in the rat was documented early in the literature by Heimburger (1946) and others, and was further established as a useful research tool in the classic work of Johns and Olson (1954). These researchers sought to find an animal model for the study of myocardial infarction which was less variable, less time consuming, and less expensive than the dog model.

The Johns and Olson model required a left intercostal thoracotomy. Access to the thorax was gained through the third or fourth intercostal space and the lungs were inflated using a positive pressure breathing device and a tight fitting face mask. The heart was delivered to the surface of the incision and partially immobilized with the thumb and index finger of the surgeon. The left coronary artery was then ligated and the heart returned to the
mediastinum. Finally, the chest wall and then the skin were closed (Johns and Olson, 1954).

Selye, Bajasz, Grasso, and Mensell (1960) improved upon the model by introducing a type of purse-string suture which allowed for rapid closure of the skin and muscle following the thoracotomy. Further, he demonstrated that in experienced hands the procedure could be completed rapidly enough not to require ventilation of the animal.

More recently, others have been using modified versions of the original Johns and Olson or Selye techniques to investigate numerous factors which may influence the size and healing process of the myocardial infarction in rats (Evans, Val-Hejias, Kulevich, Fischer, and Mueller, 1985; Hammerman et al. 1983; Hochman and Healy, 1986; Kloner, Fishbein, Lev, and Maroko, 1978; Maclean, Fishbein, Braunwald, and Maroko, 1978; Kloner and Klomer, 1983; Maroko, et al. 1971; McElroy, Gissen, and Fishbein, 1978). Many of these studies looked at the ability of various medications (i.e., those used routinely in humans following an acute myocardial infarction) to preserve ischemic myocardium (Evans et al. 1985; Kloner et al. 1978; Maclean et al. 1978; Maroko et al., 1971), and a few looked at the effects of exercise on infarct size and healing in rats (Hammerman et al. 1983; Hochman and Healy, 1986; Kloner and Klomer, 1983).
A third technique which is an adaptation of the coronary artery ligation model was reported in a study by Moskowitz, et al. (1979). They cauterized the coronary artery rather than ligating it to induce a myocardial infarction before studying the effects of cage size and exercise on infarction size in rats.

When using the coronary artery occlusion method for inducing experimental myocardial infarction, there is less variability in infarct size produced in rats than when the method is used in dogs regardless of the exact location of the occlusion (Johns and Olson, 1954; Fishbein, Maclean, and Maroko, 1978). Variability in size of infarction and subject mortality in studies using rats may be due to the vessel ligated and the exact location selected for occlusion. Table 1 shows the variability in infarct size and mortality of a few of the studies mentioned above. Furthermore, the infarct is more discrete when using the occlusion method than when using catecholamines such as isoproteranol, to induce myocardial necrosis (Todd et al. 1985).

Therefore, because of the discrete lesion produced following coronary artery ligation the coronary artery model rather than the isoproteranol model was deemed more appropriate for use in this study.
### TABLE 1

**Occlusion Location, Size and Variability of Infarction, and Mortality**

<table>
<thead>
<tr>
<th>Author</th>
<th>Occlusion Location</th>
<th>Size &amp; Variability of Infarct</th>
<th>Total Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans et al. 1985</td>
<td>Left Coronary Artery 2 mm Distal To Pulmonary Valve</td>
<td>39.1 ± 6.2 % (Mean ± SD)</td>
<td>43 ± 12%</td>
</tr>
<tr>
<td>Hammerman et al. 1983</td>
<td>Left Coronary Artery Between Pulmonary Outflow Tract and Left Atrium</td>
<td>16.3 ± 1.5% and 13.6 ± 1.5%</td>
<td>36%</td>
</tr>
<tr>
<td>Hochman and Healy, 1986</td>
<td>Left Main Coronary Artery 2 to 3 mm Left of Aorta</td>
<td>21.6 ± 1.1% and 25.1 ± 3.9%</td>
<td>14%</td>
</tr>
<tr>
<td>Maclean et al. 1978</td>
<td>Left Main Coronary Artery, 1-2 mm from its origin</td>
<td>24% to 50%</td>
<td>21%</td>
</tr>
<tr>
<td>McElroy et al. 1978</td>
<td>Left Coronary 3-4 mm from its origin</td>
<td>21.5 ± 1.9% and 31.3 ± 2.6%</td>
<td>—</td>
</tr>
<tr>
<td>Moskowitz et al. 1979</td>
<td>Left Coronary Artery</td>
<td>19% to 41%</td>
<td>52.4%</td>
</tr>
</tbody>
</table>
Literature Related to Exercise Protocols Used with Rats

Over the past several decades acute bouts of exercise as well as exercise training have been used by investigators as the independent (treatment) variable in a large number of studies involving the rat model. Subsequently, these and other investigators have explored the utility of various biochemical and physiological parameters as adequate markers of the rats response to acute or chronic exercise. Holloszy's (1967) classic study provided some of the best data on the biochemical adaptations that occur in the skeletal muscle of rats subjected to a 12 week progressive exercise program. Others have examined the use of certain physiological markers such as maximal and submaximal oxygen consumption pre and post training (Patch and Brooks, 1980) as well as heart weight body weight ratio.

It has been found that certain biochemical and physiological responses to acute exercise as well as adaptations to chronic exercise will occur only when using an appropriate mode, frequency, duration, and intensity of exercise. As a result, investigators have attempted to accurately quantify the intensity of common exercise work loads by measuring submaximal and maximal oxygen consumptions before, during, and after training. McArdle (1966) used closed-circuit spirometry to measure oxygen consumption in resting, free swimming and weighted swimming
male Sprague-Dawley rats. He found that free swimming rats' oxygen consumptions were approximately 2.7 times that of their resting value and that weighting the rats with loads equivalent to two percent of their body weight increased their oxygen consumption to 3.5 times that of their resting value. Baker and Horvath (1965) using an open-circuit technique, measured oxygen consumption in male Wistar rats swimming in thermoneutral water (37°C) and found that those rats had an oxygen uptake approximately three times their basal rate. Shepherd and Gollnich (1976), using the data obtained when running rats, speculated that Baker's and Horvath's (1964) free swimming rats were exercising at approximately 50-65 percent of their maximal oxygen uptake.

Shepherd and Gollnich (1976) looked at the effect of various running speeds (16.0 to 67.0 meters per minute) on oxygen uptake in a group of male Sprague-Dawley rats. They found oxygen uptake increased linearly with increasing running speed up to a speed of approximately 49.5 meters per minute, and thereafter the oxygen uptake decreased. Thus, a running speed of 28.5 meters per minute corresponded to approximately 82 percent of the rats' maximal aerobic capacity. Further, based upon a resting oxygen consumption of 1.2 to 1.5 ml (per 100 grams body weight per minute) their animals increased their oxygen uptake only six to eight times resting. They contrasted
this increase with the 10 to 15 fold increase in humans and speculated that the smaller magnitude of change was due to their method in obtaining resting values, that is, they identified resting oxygen uptake as that value obtained after the rats were placed on the exercise wheel and allowed to rest for 30 minutes (Shepherd and Gollnick, 1976).

Using female Wistar rats Brooks and White (1978) found oxygen consumption correlated well with running speed and that heart rates also correlated reasonably well with running speed. In another study from the same laboratory (Patch and Brooks; 1980), a running speed of 14.3 meters per minute at one percent grade yielded an oxygen consumption of 41.9 ml·kg\(^{-1}\)·min\(^{-1}\) and 44.2 ml·kg\(^{-1}\)·min\(^{-1}\) in untrained and trained rats respectively. Additionally, a speed of 28.7 m·min\(^{-1}\) on a 15 percent grade yielded an oxygen consumption of 65.7 and 60.4 ml·kg\(^{-1}\)·min\(^{-1}\) in untrained and trained rats respectively. These values correspond to 59 and 53 percent maximal oxygen consumption and 93 and 76 percent in these trained and untrained animals respectively.

In the free swim protocol studies (Hammerman et al. 1983, Kloner and Kloner, 1983) which examined the effects of exercise on infarct size and scar formation in rats the speeds (Hammerman et al. 1983; Kloner and Kloner, 1983) would correspond to an intensity of 50-60 percent maximal
aerobic capacity, according to Shepard and Gollnick's (1976) classification. The Moskowitz's protocol would fall between 30 and 59 percent of maximal oxygen consumption when compared to the data obtained from Patch's and Brook's study (1980) of oxygen consumption at rest and at different running intensities. Hockman and Healy's protocol would fall between 30 and 59 percent of maximal oxygen consumption when compared to Patch's and Brook's data. Table 2 summarizes the exercise protocols used in these studies of exercise effects on infarct size and scar formation in rats.

The selection of treadmill speed for the two exercise groups used in this study was based on the data presented in this section and were meant to correspond to an exercise intensity of below 60 percent (mild exercise) and an exercise intensity of above 60 percent (moderate exercise).

**Literature Related to the Measurement of Infarct Size, Scar Thickness, and Septal Wall Thickness**

The need to establish the appropriate clinical management of individuals with myocardial infarction and the need to identify early myocardial infarctions at necropsy led to the post mortem examination of human hearts from individuals who died at varying times following a clinically identified myocardial infarction. Furthermore, it lead to the use of a variety of animal models to study
TABLE 2

Exercise Protocols

<table>
<thead>
<tr>
<th>Author</th>
<th>Exercise Began</th>
<th>Mode &amp; Intensity</th>
<th>Duration</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moskowitz, 1979 (102)</td>
<td>4 hours after surgery</td>
<td>Treadmill @ 25-50 ft.min⁻¹ (7.6 min⁻¹ to 15.24 m.min⁻¹)</td>
<td>5-20 min</td>
<td>2 days</td>
</tr>
<tr>
<td>Hammerman et al., 1983 (62)</td>
<td>24 hours after surgery</td>
<td>Free Swim</td>
<td>15-45 min</td>
<td>7 days</td>
</tr>
<tr>
<td>Kloner and Kloner, 1986 (135)</td>
<td>7 days after surgery</td>
<td>Free Swim</td>
<td>15-40 min</td>
<td>15 days</td>
</tr>
<tr>
<td>Hochman and Healy, 1986 (135)</td>
<td>Day or Surgery</td>
<td>0.6 mph @ 10° Incline 0.4 mph @ 0° Incline (10.72 m.min⁻¹ to 15.08 m.min⁻¹)</td>
<td>30-60 min</td>
<td>7 days</td>
</tr>
</tbody>
</table>
the evolution of the infarct over time. As different methods were developed to induce an infarction in experimental animals and various pharmacological agents were used to reduce the amount of infarcted myocardium, methods to accurately and reliably identify the presence and assess the size and microscopic makeup of infarctions were also developed.

Indirect methods for estimating infarct size in these experimental models include the measurement of serum creatine kinase and other myocardial enzymes over time (Shell, Kjekshus and Sobel, 1971; Shell, Lavelle, Covell and Sobel, 1973; Waldenstrom and Hjalmarson, 1979) and epicardial ST segment and QRS mapping (Hillis, et al. 1976; Haroko et al. 1972).

The indirect methods of assessing infarction size (i.e., serial measurement of serum creatine kinase and ST segment and QRS mapping) were determined to be inappropriate for use in the current experiment for several reasons. First, the methods are indirect methods of measurement, second there is sufficient trauma in rapidly exteriorizing the heart during the ligation procedure to cause the leakage of some myocardial enzymes such as creatine kinase into the serum even without infarction; and finally epicardial ST and QRS mapping would be extremely difficult to accomplish using the rat model both because of
the size of the heart and the normally high heart rate that is seen in this animal.

A number of direct methods for assessing infarct size in animal models have also been popularized. The use of certain oxidation-reduction indicators to identify areas of necrosis in experimentally infarcted dogs was used by Nachlas and Ahnitka (1963) in an effort to develop a method to macroscopically identify myocardial infarctions at necropsy.

Another direct method to quantify the amount of infarcted tissue is the measurement of myocardial creatine kinase activity and was probably first popularized by Kjekshus and Sobel (1970) using homogenized infarcted rabbit hearts. More recently a number of investigators have used this method to quantify the amount of infarcted myocardium in studies testing the efficacy of certain medications and exercise training in reducing infarct size (Brodovicz, Girten, and Merola, 1986; Evans et al. 1985; Maclean et al. 1978) and the effects of exercise and cage size on infarct size (Moskowitz et al. 1979).

Depression of myocardial creatine kinase activity as a measure of infarct size was eliminated from use in the current investigation because the need to homogenize the heart destroys the investigator’s ability to measure scar thickness and noninfarcted septal wall thickness.
Several studies, looking at the evolution of a myocardial infarction, the efficacy of certain medications as well as the effects of exercise have used histochemical techniques as a direct method for describing infarctions. Mallory's classic study of the speed of healing of myocardial infarction in humans involved the use of microscopic histological techniques but these techniques were not well defined (Mallory and White, 1939). Fishbein et al. (1978) used a variety of histochemical stains to expand Mallory and White's original study determine and describe the age of myocardial infarctions in humans at post mortem. Furthermore, Fishbein et al. (1978) using similar techniques described in detail the histological evolution of an experimentally induced infarction in rats and concluded that the healing of the rats' infarction closely paralleled the healing of an infarction in humans except that the process was telescoped in time from the six to eight weeks in humans necessary for complete healing in humans to three weeks in rats.

Evans et al. (1985) and Maclean et al. (1978), used both myocardial creatine kinase activity and histological techniques in measuring infarct size. Evans et al. (1985) using fresh hearts sectioned into five equal slices and stained with triphenyl tetrazolium chloride (TTC) employed a stain-cut-weigh method. This method required the investigator to dissect away the infarcted areas from the
noninfarcted areas and weigh both. Infarct size was expressed as a percent of total left ventricular mass (Evans et al. 1985). Maclean et al. (1978) used a hematoxylin-eosin (H&E) stain for rats sacrificed two days after experimental infarct and Massons' trichrome stain in rats sacrificed 21 days after experimental infarct. A rapid planimetric method, first characterized by Boor, Reynolds and Fishbein (1976) and Weibel (1963), was then used to measure infarct size. This planimetric technique involved measuring cross-sectional areas of the left ventricle from projected 35-mm transparencies of slices of infarcted calf hearts. Other studies, requiring the determination of infarct size, used similar staining methods (Fishbein et al. 1978; Hochman and Buckley, 1982; Hochman and Healy 1986; Roberts, Maclean, Harako, and Kloner, 1984).

Two planimetric methods for measuring and then calculating infarct size have evolved: the area method and the circumference method. The area method involves the measurement of the total left ventricular area and the infarcted left ventricular area of transverse slices of the rat hearts. The proportion of the entire left ventricular area which is infarcted heart is usually calculated by averaging the values obtained from measuring several slices of the entire heart. This method has been used primarily in studies where the rat is sacrificed within two to seven
days following infarction (Fishbein et al. 1978; Hochman and Buckley, 1982; Hockman and Healy, 1986; Maclean et al. 1978, Roberts et al. 1984).

The circumference method for calculating infarct size requires the measurement of the total left ventricular endocardial or epicardial circumference and the portion of the left ventricular endocardial or epicardial circumference that is infarcted. The proportion of the left ventricular circumference that is infarcted is usually calculated by averaging the values obtained from several transverse slices of the heart. This method is primarily used on hearts taken from rats 21 days after infarction (Fishbein et al. 1978; Maclean et al. 1978; Roberts et al. 1984). The use of the area method on hearts taken from rats sacrificed 21 days after infarction is thought to underestimate the size of the infarction because of the reabsorption of necrotic tissue (Fishbein et al. 1978; Maclean et al. 1978; Roberts et al. 1984).

Fishbein et al. (1978) reported that the area of infarct decreased throughout the healing process from almost 46 percent (Day 1) to 26 percent (Day 21). They concluded that the volume of the scar represented only 50 percent of the volume of initially infarcted myocardium. The percentage of the surface area (which is measured when using the circumference method) involved changed insignificantly (Fishbein et al. 1978, p. 64). Maclean et
al. (1978, p. 200) reported using the circumference method due to the "extreme thinning of the infarcted area". Tornling, Carlsson, Unge, and Ljungquist, (1981), believed the number of slices taken from each heart was important in obtaining a more reliable estimate of infarct size in the rat. He found that slices taken from the heart at 0.5 mm intervals correlated better ($r = 0.9971$) with the relative volume of infarcted myocardium (established by sampling the infarcted left ventricle at 100 $u$ distances) than slices taken at 2 mm intervals ($r = 0.9264$). The majority of studies reviewed for this study investigated the heart in 2 to 2.5 mm sections (Evans et al. 1985; Fishbein et al. 1978; Hockman and Buckley, 1982; Hockman and Healy, 1986; Maclean et al. 1978; Roberts et al. 1984). This method was adopted for use in the current study because the correlation between 100 $u$ sections and 2.0 mm sections is reasonably high ($r = 0.9264$) and sectioning the heart at 0.5 mm intervals would dramatically increase the time involved in measuring the variables as well as the cost of measurement.

The studies using histochemical methods for the identification of infarct size were mainly interested in measuring other macroscopic characteristics such as scar thickness, ventricular diameters and noninfarcted septal wall thickness (Fishbein et al. 1978; Hammerman et al.
Direct histological techniques for assessing infarct size, scar thickness and noninfarcted septal wall thickness in rats sacrificed 21 days after infarction were found superior for use in the current study for several reasons. First the use of these techniques and their validity as measures of infarct size have been well established in previous studies (Hammerman et al. 1983; Hochman and Buckley, 1982; Hochman and Healy, 1986; Klone, and Klone, 1983; Roberts et al. 1984). Second these methods although time consuming are relatively easy to learn, and the investigator was able to demonstrate a high degree of reliability when using these techniques.
CHAPTER III

METHODOLOGY

The purpose of this study was to examine the effects of mild and moderate treadmill exercise and this effect on myocardial infarct size and scar formation following coronary artery ligation and subsequent myocardial infarction in the rat model.

Research Design

The study used a three group post test experimental design. Activity level was the independent variable and consisted of no exercise, mild exercise, and moderate exercise discussed in detail in a later section of this Chapter.

The dependent variables included: size of the infarction (measured by the left ventricular endocardial circumference method), mean myocardial scar thickness, thinnest site of the myocardial scar, and mean intraventricular septal wall thickness.

Subjects One hundred twenty-two male Harlan Sprague Dawley rats between 62 and 64 days of age and weighing between 220 and 290 grams were the subjects of this investigation. The rat was chosen for this project for a number of reasons.
First, the value of the rat as a model for studying myocardial infarction has been well established in the literature. (Evans et al. 1985; Maclean, Maroko, 1978; Fishbein et al. 1978; Johns and Olson, 1954; Maclean, Fishbein, Braunvald and Maroko, 1978; Roberts et al. 1984; Selye et al. 1960). Second, the ligation of the left coronary artery in the rat produces myocardial changes that are similar to the changes that occur in man following a myocardial infarct (Fishbein et al. 1978). Third, infection does not appear to be a problem when clean technique is followed (Evans, et al. 1985; Hockman and Healy, 1986; Johns and Olson, 1954; Maclean et al. 1978; Roberts, et al. 1984). Forth, the coronary collaterals of a rat are similar to that of a human. Finally, the time involved in completing a coronary artery ligation procedure in the rat is 2-3 minutes (Evans et al. 1985; Maclean et al. 1978) as compared with the 2-3 hours necessary to complete a similar procedure on a larger animal such as a dog.

Due to the expected mortality of 50 percent, the failure of the ligation procedure to produce a myocardial infarction in some rats (occlusion failure), and the variability in the size of myocardial infarction, 122 rats were used to obtain the 57 rats for random assignment to one of three experimental groups, i.e., no exercise
(control), mild exercise, and moderate exercise group. Random assignment to one of the groups was achieved by use of a table of random numbers.

**Procedures and Conditions of Testing**

**Animal Care and Use Committee:** An Animal Use Protocol was submitted to The Ohio State University Institutional Laboratory Animal Care and Use Committee Appendix A and permission was granted to conduct this research project (See Appendix B).

**General Care and Maintenance of Rats:** All rats were housed and received veterinary care according to the guidelines set forth in The Guide for Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, 1985). Furthermore, these rats were maintained on a 12 hour dark, 12 hour light cycle and provided standard laboratory rat chow (Agway: 22 percent protein, 5 percent fat, 5 percent fiber, 6 percent ash) and water ad libitum.

Upon receipt of the rats, they were placed in cages measuring 8 inches, by 17 inches, by 8 inches or 136 square inches. Four rats were placed to a cage. Following 4 days of cage rest the animals were tattooed at the base of the tail and weighed. Thereafter each rat was weighed weekly on the same day at approximately the same time.
Rodent Treadmill: The rodent treadmill was built by the Purdue University West Lafayette Indiana to accommodate six exercising rats and six control rats.

Treadmill Acclimitization: Prior to the coronary artery ligation each rat was placed on the treadmill belt and exercised for 10 minutes each day for 5 days. This was done to acquaint the rat with treadmill exercise. These 10 minute exercise periods consisted of placing each rat on the treadmill belt and exercising the rat at a speed of 9 meters per minute at 0 percent grade for 5 minutes. At the end of 5 minutes the speed was increased to 18 meters per minute at 0 percent grade for 5 minutes. Mild electrical shock was administered to those rats who lagged via an electric shock grid at the back of the treadmill.

Coronary Artery Occlusion Procedure

Each rat was ether anesthetized in a desiccator by pouring 5 to 10 ml of ether onto 4 by 5 gauze sponges placed under the desiccator plate. Additional ether was added periodically to produce anesthesia in 2 to 3 minutes once the rat was placed in the desiccator.

The rats were then removed from the desiccator, placed on their backs with an elastic strap over their lower abdominal area. The front legs were tied to the sides with umbilical tape to permit easy access for the surgeon to the thorax. A 50 ml beaker stuffed with an ether soaked gauze
sponge was placed over the rat’s mouth and nose to maintain an appropriate level of anesthesia during the operative procedure.

A left coronary artery ligation was performed using the Evan’s method (Evans et al. 1985). A 3 to 4 cm parasternal incision was made through the skin using curved scissors. The muscles overlying the fourth and fifth intercostal space were separated bluntly using hemostats. A purse string using 2-0 Ethicon silk suture attached to a cutting needle was placed through the skin and muscle surrounding the incision for rapid closure of these two layers after the ligation was performed. A curved hemostat was inserted through the fourth intercostal space and opened perpendicular to the sternum. The hemostat was then rotated and opened parallel to the sternum to create an opening large enough to exteriorize the heart. This was accomplished by the surgeon applying pressure with the left index finger on the right side of the thorax dorsal and cranial to the heart. The left thumb of the surgeon was used to stabilize the left side of the thorax. The position of the left index finger was adjusted so that the apex of the heart could be lifted and aimed at the opening created in the left thorax. The heart could then be forced through the opening. The heart was held with the thumb and index finger of the left hand with the apex of the heart
pointing cranially and slightly to the right (Evans et al. 1985, p. 132).

A 5-0 Ethicon silk suture attached to a curved tapered needle was introduced into the wall of the pulmonary conus two millimeters distal to the pulmonic valve and brought out at a point near the middle of a line connecting the pulmonary conus with the insertion of the left auricular appendage (Evans et al. 1985, p. 132). Approximately 5 mm of myocardium is included in the suture to insure the artery was contained within the ligated tissue. This was done because the left coronary artery of the Sprague-Dawley rat, which originates from the aorta and runs cephalocaudally, lies buried in the myocardium and is generally not visible (Evans et al. 1985, p. 133). After the suture was tied with a double knot and cut the surgeon places the heart back into the thorax and the assistant removed the beaker containing the ether soaked sponge from the rat’s mouth and nose. Then lateral pressure was applied to the thorax by an assistant as the surgeon closed the chest by pulling the purse string closed and tying it. That removed the excess air from the chest and allowed rapid closure of the chest wall (Evans et al. 1985, p. 133).

The thoracotomy, ligation of the left coronary artery, and closure of the chest cavity took between 2 to 3 minutes with the chest cavity opened for approximately 30 to 60
seconds. Thus, tracheotomy, intubation, and/or artificial ventilation was not necessary because the thorax was open for such a brief period of time.

Following closure of the chest wall, the animal was immediately transferred to the recovery table where it was placed on a warming pad and its mouth and nose were directed into a cone connected to a Bennett MA-1 model respirator delivering 100 percent oxygen. A single lead electrocardiogram was done on most of the rats to detect the presence of ST segment elevation or arrhythmias. When the rats regained consciousness and became ambulatory (usually within 2 to 10 minutes) they were returned to their cages.

The rats were allowed to recover for 72 hours following surgery. At that time the surviving 57 rats were randomly assigned to one of the three experimental groups. The rats were then subjected to one of three exercise protocols.

**Treatments/Exercise Protocols**

**Mild Exercise Group:** The 19 rats assigned to the mildly exercised group were exercised on the rodent treadmill 5 days each week during the 21 days following surgery beginning 72 hours postoperatively at 9 meters per minute at 0 percent grade. Their time on the treadmill was progressed from 5 to 10 minutes during the 3 weeks
following surgery. Electroshock was not used to encourage these rats to exercise. If the rat attempted to sit on the electric grid at the back of the treadmill the researcher repositioned the rat on the treadmill belt and gave them a gentle nudge with her hand.

**Moderate Exercise Group:** The 19 rats assigned to the moderately exercised group were exercised on the rodent treadmill five times each week during the 21 days following surgery beginning 72 hours postoperatively at 18 meters per minute 0 percent grade. Their time on the treadmill was progressed from 10 minutes to 20 minutes during the 3 weeks following surgery. Electroshock was not used to encourage these rats to walk or run unless they repeatedly attempted to sit on the grid at the back of the treadmill. Initially, the researcher repositioned the rat on the treadmill belt and gave them a gentle nudge with her hand. Following five repeated nudges if the rat continued to refuse to run the shock grid was switched on for a fraction of a second and turned off. This generally resulted in the rat resuming exercise. The above procedure was followed throughout the remainder of that days' exercise period.

**Control (No Exercise) Group:** The 19 rats assigned to the control group were not exercised on the rodent treadmill during the 21 days following surgery. They were, however, placed in specially constructed lanes at the front of the treadmill for 10 to 20 minutes 5 days each week.
beginning 72 hours following surgery to control for the stress of handling experienced by the exercised rats. This was done at the same time the rats in the other group were exercised to expose the control rats to similar noise and vibrations caused by the operation of the rodent treadmill.

**Sacrifice Procedure**

Twenty-one days after surgery the surviving 54 rats were given intraperitoneal sodium pentobarbitol (1/2 grain per kilogram body weight) and sodium heparin 1000 USP units per kilogram body weight (Hammerman et al., 1983; Hochman and Buckley, 1982; Hochman and Healy, 1986). As soon as the rat reached an appropriate plane of anesthesia the rat was weighed. A left thoracotomy was performed, the heart excised, and placed in normal saline for 10 to 20 seconds to clear the ventricles of blood. The heart was then placed in a 30 molar potassium chloride solution to achieve diastolic arrest (Hammerman et al., 1983; Hochman and Buckley, 1982; Hochman and Healy, 1986). Once the heart had ceased beating it was rinsed in normal saline. Using a blunt catheter attached to a 10 cc syringe the ventricles of the heart were perfused through the aortic and pulmonic valves with 10 percent buffered formalin (Hammerman et al., 1983; Kloner and Kloner, 1983). The heart was then placed in individual jars containing 10 percent buffered formalin for a minimum of 72 hours.
The hearts were renumbered at this time and the investigator blinded to group origin of the heart until after all measurements were made including those measurements made for establishing the reliability of the investigator.

The fixed rat hearts were then sectioned into four slices, A, B, C, D (See Figure 1) approximately 2 to 2.5 mm thick from apex to base in a plane parallel to the atrioventricular groove (Fishbein et al. 1978; Hochman and Healy, 1986; Kloner and Kloner, 1983; Maclean et al. 1978). Figure 1 also provides an example of a noninfarcted heart. Those hearts with extensive scaring of the left ventricular free wall were difficult to section as the free wall was thin and tended to collapse following the initial cut (See Figure 2). In order to stabilize the heart and obtain slices of uniform thickness a mold was made from modeling clay and inserted into the left ventricle. Subsequent slices could then be made through the myocardium and clay. The clay was immediately removed and the slice rinsed with 10 percent buffered formalin. The right ventricle of slice B and D were notched with a scalpel blade to aid the investigator in identifying the slices (See Figure 1). Slice A and B were then placed in appropriate tissue cassette. Slice C and D were placed in
Figure 1. Method for Marking Heart Slices and an Example of Noninfarced Heart
Slice A

Slice B

Slice C

Slice D

KEY

LV = Left Ventricle
N = Marking Notch
RV = Right Ventricle
S = Septum

Figure 2: Example of Infarcted Heart
a second tissue cassette. All cassettes were then submerged in 10 percent buffered formalin. After dehydration two slices in each cassette were embedded in paraffin. Five um thick sections were then cut of the two slices, mounted on glass slides and stained with Masson's trichrome (Fishbein et al. 1978; Hammerman et al. 1983; Kloner and Kloner, 1983; Maclean et al. 1978).

**Outcome Measures**

Infarction Size: Infarction size was measured using the left ventricular endocardial circumference method as described in the literature (Fishbein et al. 1978; Maclean et al. 1978). The slides of the hearts were projected onto a sheet of plain white paper and traced. The infarcted area was shaded. A Zeiss Interactive Digital Analysis System (ZIDAS) set in the length program was used to measure noninfarcted and infarcted left ventricular endocardial circumference (mm) on each slice (See Figure 3). Infarct size was calculated by dividing infarcted left ventricular endocardial circumference by total left ventricular endocardial circumference (Fishbein et al. 1978). A mean of the four slices was then calculated and infarct size was expressed as a percent of total left ventricular endocardial circumference. On a small percentage of the hearts one or two slices may have been destroyed during histological preparation. When this
Figure 3. Infarct Size by Circumference Method

Using ZIDAS set in length program:

1. Measure total left ventricular endocardial circumference (mm) by moving cursor along the entire endocardial circumference.

2. Measure infarcted left ventricular endocardial circumference (mm) by moving cursor along the infarcted endocardial circumference.
happened the mean was calculated using the remaining 2 or three slices. The multiple measures were used to give a more precise estimate of the mean of the infarcted circumference not to determine if there were differences between the various slices.

The area method was used to calculate infarction size on a random sample of nine hearts. After the hearts had been projected and traced the ZIDAS set in the area program was used to measure total left ventricular area (mm²) by measuring the left ventricular epicardial surface (See Figure 4). The area (mm²) of the left ventricular cavity was then measured by tracing the left ventricular endocardial circumference. Subtracting the left ventricular cavity area from the total left ventricular area would yield actual left ventricular area. The infarcted area (mm²) of the left ventricle was then traced and divided by the actual area. Infarct size for the slice was then calculated using Hochman and Healy's second formula (1986, p. 127) 100% x (Infarcted left ventricular area/Infarcted and noninfarcted left ventricular area). A mean percent of infarcted area of the four slices was then calculated and used for the whole heart's value.

The mean scar thickness (mm) was calculated by visually determining the half way point of the scar and measuring the thickness of the wall at that point (Point D) using the ZIDAS set in the distance program (See Figure 5). The two
Figure 4. Infarct Size by Area Method

Using ZIDAS set in area program:
1. Measure total left ventricular area ($\text{mm}^2$) by moving cursor along entire left ventricular epicardial circumference (Use --- lines to arbitrarily separate left and right ventricle).

2. Measure left ventricular cavity area ($\text{mm}^2$) by moving cursor along entire left ventricular endocardial circumference.

3. Measure infarcted area ($\text{mm}^2$) by moving cursor around entire infarcted area.

KEY
- LV = Left Ventricle
- N = Marking Notch
- RV = Right Ventricle
- S = Septal
- ** = Infarcted Area
- □ = Noninfarcted Area
- - - - = Boundry Between Right and Left Ventricle
KEY

LV = Left Ventricle
N = Marking Notch
RV = Right Ventricle
S = Septum
A,B,C = Points at which to measure Septal Width (mm)
D,E,F = Points at which to measure Scar Width (mm)

Figure 5. Septal and Scar Thickness with the ZIDAS set in the Distance Program

Measure Septal and Scar Thickness by:

1. Visually dividing the scar on the septum in half
2. Divide the two halves in half again
3. Measure the distance from endocardial to epicardial surface at point A,B,C, to obtain three measures of septal width (mm)
4. Measure the distance from endocardial to epicardial surface at point D,E,F to obtain three measures of scar width (mm)
halves created by this arbitrary division were also divided in half and measurements of wall thickness taken at these two points (Points E and F). The three measurements (measurements taken at point D, E, F) were averaged for each slice. The mean scar thickness for each slice was used to calculate a mean scar thickness for the whole heart.

Thinness: The thinnest portion of the scar in each slice was determined by visual inspection. The thickness of the wall at that point was then measured using the ZIDAS set in the distance program. The values obtained for each of the four slices were then averaged to yield the mean thinnest portion of the scar for the whole heart (mm).

Septal Wall Thickness: The mean noninfarcted septal wall thickness mm was calculated much the same way mean scar thickness was calculated (See Figure 5). The halfway point of the septum was determined visually (Point A) and the thickness of the septum was measured at that point. The two halves created by this arbitrary division were also divided in half and measurements of wall thickness taken at these two points (Points B and C). These three measurements (measurements taken at Points A, B, C) were averaged for each slice. The mean septal thickness for the whole heart was calculated by averaging the mean septal wall thickness for the four individual slices.
Statistical Methods

The differences in means between groups on all variables were examined using a one way analysis of variance. The differences in means, generated when two methods for measuring infarct size were used, were examined using a paired t-test. A Spearman correlation coefficient was calculated to examine the correlation between the two methods. The differences in means between the two subgroups, shocked versus not shocked, of the moderate group were examined using a Student t-test. The reliability of the investigator on a day to day basis was assessed following the calculation of a one way analysis of variance using an intraclass correlation coefficient. Finally, means and standard deviations were used to describe body weight, heart weights and heart weight/body weight ratios.
CHAPTER IV

RESULTS

The report of the results of this study on the effects of a mild or moderate exercise program on myocardial infarction size and scar formation in rats has been divided into eight parts: study groups, mortality and occlusion failures, infarct size, scar thickness, thinness, septal thickness, shocked versus not shocked, and reliability.

Study Groups

One hundred twenty-two male Harlan Spraque-Dawley rats underwent a left coronary artery occlusion procedure. Seventy-two hours after the occlusion procedure the fifty-seven surviving rats of similar origin, sex and of similar age (62-64 days) were randomly assigned to one of three groups: control (no exercise), mild exercise, or moderate exercise. Over the entire project, body weights were measured weekly on the same day at the same time and are summarized in Table 3. Following surgery, all groups showed a change in the pattern of weight gain from the first two weights (i.e., the rats in the control and mild groups declined in weight and the rats in the moderate group showed only a small gain). However mean body
# TABLE 3

Weekly Body Weight by Group

<table>
<thead>
<tr>
<th>GROUP</th>
<th>n</th>
<th>WEIGHT 1 (grams) Mean ± SD</th>
<th>WEIGHT 2 (grams) Mean ± SD</th>
<th>WEIGHT 3 (grams) Mean ± SD</th>
<th>WEIGHT 4 (grams) Mean ± SD</th>
<th>WEIGHT 5 (grams) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>220.93 ± 5.28</td>
<td>249.75 ± 15.04</td>
<td>244.51 ± 17.76</td>
<td>273.60 ± 24.145</td>
<td>290.68 ± 35.66</td>
</tr>
<tr>
<td>Mild</td>
<td>16</td>
<td>227.07 ± 13.63</td>
<td>257.39 ± 19.61</td>
<td>252.36 ± 16.47</td>
<td>280.05 ± 25.44</td>
<td>309.27 ± 23.82</td>
</tr>
<tr>
<td>Moderate</td>
<td>18</td>
<td>225.37 ± 10.64</td>
<td>251.95 ± 16.10</td>
<td>254.05 ± 16.69</td>
<td>278.58 ± 23.14</td>
<td>297.75 ± 35.63</td>
</tr>
</tbody>
</table>

* Weight measured one week after surgery

** Weight at time of sacrifice
weights, and weight gains, were comparable among the
groups. The mean body weights were 244.51 ± 17.7 grams
for the control rats, 252.36 ± 16.47 grams for the mild
rats, and 254.05 ± 16.69 grams for the moderate rats.

At the time of sacrifice the rats ranged in age from 83
to 85 days, the mean body weights remained similar, and the
mean heart weights and heart weight/body weight ratios were
found to be similar also. Table 4 summarizes the three
groups by body weight, heart weight, and heart weight
body/weight ratio at the time of sacrifice. A one way
analysis of variance was used to compare the mean heart
weight/body weight ratios of the three groups. No
significant differences were found among the means of the
groups (F = 0.10; p = 0.90).

Operative Mortality and Occlusion Failures

Immediate operative mortality was approximately 24
percent (29 deaths out of 122 animals). Immediate
operative mortality is defined as those deaths occurring
during surgery or within the 15 minutes following surgery.
These deaths were attributed to overdose of anesthesia,
intraoperative complications, and lethal arrhythmias (i.e.,
complete heart block, ventricular tachycardia, or
ventricular fibrillation). Within the next 24 hours an
additional 29 deaths occurred and between 24 and 72 hours 7
more rats died. The deaths occurring within the first 24
TABLE 4

Body Weight, Heart Weight, Heart Weight/Body Weight Ratio at Time of Sacrifice

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>Body Weight (grams)</th>
<th>Heart Weight (grams)</th>
<th>Heart Weight/Body Weight Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>290.69 ± 35.66</td>
<td>1.84 ± .20</td>
<td>.648 ± .14</td>
</tr>
<tr>
<td>Mild</td>
<td>16</td>
<td>309.27 ± 23.82</td>
<td>1.94 ± .23</td>
<td>.628 ± .07</td>
</tr>
<tr>
<td>Moderate</td>
<td>18</td>
<td>297.75 ± 35.69</td>
<td>1.85 ± .29</td>
<td>.634 ± .14</td>
</tr>
</tbody>
</table>
hours following the immediate operative period were attributed to lethal arrhythmias or heart failure secondary to extensive damage to the left ventricle. The deaths occurring between 24 and 72 hours as well as those occurring during the exercise phase of the program were attributed to heart failure secondary to extensive damage to the left ventricle. During the exercise phase of the program 3 rats died, 2 were from the control group and 1 from the mild group. None of these 3 rats died while running on the treadmill. The total mortality in this study was 56 percent (i.e., 68 deaths out of the original 122 animals).

At the time of sacrifice, 6 rats (3 controls, 2 milks, 1 moderate) were found to have no infarction or only minimal fibrosis at the suture site and were excluded from analysis. Four rats were found to have nontransmural infarctions (2 controls and 2 moderates) and were excluded from analysis of the measures of scar thickness and thinness, but not from other analysis.

**Infarct Size**: Hypothesis 1 stated: There is no significant difference between a mildly exercised group of rats and a nonexercised control group of rats in the size of the myocardial infarction measured 21 days post infarction.
Hypothesis 2 stated: There is no significant difference between the moderately exercised group of rats and a nonexercised control group group in the size of the myocardial infarction measured 21 days post infarction.

Hypothesis 3 stated: There is no significant difference between the mildly exercised group of rats and a moderately exercised control group in the size of the myocardial infarction measured 21 days post infarction. Infarct size for all rats was calculated using circumference method (described in Chapter III). Table 5 summarizes the data regarding infarct size when this method was used. A one way analysis of variance was used to compare the mean infarct sizes of the three groups. No significant differences were found among the means of the groups ($F = 1.00$, $p = 0.38$).

Two methods (area and circumference) have been used to measure infarct size in rats with experimentally induced infarctions. It was important for this study to determine which method was more appropriate.

Therefore, Hypothesis 4 stated: There is no significant difference between mean infarction size measured by the left ventricular circumference method and mean infarction size measured by the left ventricular area method. To address this hypothesis both methods were used to calculate infarct size on 9 randomly selected hearts from this study. The order in which the 9 hearts were
TABLE 5
Infarct Size (Circumference Method) by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>Infarct Size (Percent) (Mean ± SD)</th>
<th>Minimum Value (Percent)</th>
<th>Maximum Value (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>47.92 ± 16.55</td>
<td>5.95</td>
<td>67.41</td>
</tr>
<tr>
<td>Mild</td>
<td>15</td>
<td>50.86 ± 12.02</td>
<td>24.19</td>
<td>67.96</td>
</tr>
<tr>
<td>Moderate</td>
<td>18</td>
<td>41.68 ± 17.77</td>
<td>0.54</td>
<td>63.06</td>
</tr>
</tbody>
</table>
measured were randomized so the investigator would not know which heart she was measuring. The results of the measurements using both methods at each of 3 separate times on the nine hearts were then compared. Table 6 summarizes these data. The mean difference in infarct size (percent infarct by circumference method minus percent infarct by area method) between the two methods was 22.71 ± 7.65 percent. A paired t-test, was used to compare the mean infarct sizes resulting from the two methods. A significant difference was found between the means of the infarct size (t = 8.9, p = .0001). The area method yielded a smaller infarct in each of the nine cases. Even though there was a significant difference in the results using the two methods the methods did correlate (SPEARMAN) = 0.57 (p = .11).

**Scar Thickness and Thinness:** Hypothesis 5 stated: There is no significant difference between a mildly exercised group of rats and a nonexercised control group in the mean scar thickness measured 21 days post myocardial infarction.

Hypothesis 6 stated: There is no significant difference between a mildly exercised group of rats and a moderately exercised group in the mean scar thickness measured 21 days post myocardial infarction.

Hypothesis 7 stated: There is no significant difference between a mildly exercise group of rats and a
TABLE 6

Difference in Infarct Size by Method of Measurement

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Rat ID</th>
<th>Group ID</th>
<th>Infarct Size Circumference (Percent)</th>
<th>Infarct Size Area (Percent)</th>
<th>Difference (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>1</td>
<td>57.25</td>
<td>32.33</td>
<td>24.92</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>1</td>
<td>63.15</td>
<td>36.89</td>
<td>26.26</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>1</td>
<td>60.50</td>
<td>27.07</td>
<td>33.43</td>
</tr>
<tr>
<td>4</td>
<td>107</td>
<td>1</td>
<td>49.00</td>
<td>23.48</td>
<td>25.52</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>2</td>
<td>24.19</td>
<td>15.00</td>
<td>9.19</td>
</tr>
<tr>
<td>6</td>
<td>87</td>
<td>2</td>
<td>43.68</td>
<td>26.02</td>
<td>17.66</td>
</tr>
<tr>
<td>7</td>
<td>115</td>
<td>2</td>
<td>55.22</td>
<td>24.17</td>
<td>31.05</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>3</td>
<td>54.14</td>
<td>33.73</td>
<td>20.41</td>
</tr>
<tr>
<td>9</td>
<td>51</td>
<td>3</td>
<td>48.74</td>
<td>32.76</td>
<td>15.98</td>
</tr>
<tr>
<td>MEAN</td>
<td></td>
<td></td>
<td>50.65</td>
<td>27.94</td>
<td>22.71*</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td>11.46</td>
<td>6.13</td>
<td>7.65</td>
</tr>
</tbody>
</table>

*mean difference significant (t = 8.9, p = .0001)
nonexercised control group of rats in the thinnest portion of scar measured 21 days post myocardial infarction.

Hypothesis 8 stated: There is no significant difference between the moderately exercised group of rats and a nonexercised control group in the thinnest portion of scar measured 21 days post myocardial infarction.

Hypothesis 9 stated: There is no significant difference between the mildly exercised group of rats and a moderately exercised group in the thinnest portion of scar measured 21 days post myocardial infarction.

Hypothesis 10 stated: There is no significant difference between mean infarction size when measured by the left ventricular circumference method and the left ventricular area method.

Therefore, mean scar thickness and thinness were calculated for each group and summarized in Table 7 (See Chapter III for method of calculating these two variables). A one-way analysis of variance was used to compare the mean scar thickness of the groups as well as the mean thinness of the groups. No significant differences were found among the means of the groups on scar thickness ($F = 1.25, p = 0.30$) or on thinness ($F = 0.76, p = 0.47$).

**Septal Wall Thickness:** Mean septal wall thickness was also calculated for each group. The mean septal wall thickness
### TABLE 7

**Scar Thickness and Thinness by Group**

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>Scar Thickness (mm) (Mean ± SD)</th>
<th>Thinness** (mm) (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0.948 ± .15</td>
<td>0.669 ± .23</td>
</tr>
<tr>
<td>Mild</td>
<td>15</td>
<td>0.865 ± .14</td>
<td>0.593 ± .13</td>
</tr>
<tr>
<td>Moderate</td>
<td>16</td>
<td>0.861 ± .18</td>
<td>0.587 ± .21</td>
</tr>
</tbody>
</table>

• Scar Thickness (F = 1.25, p = .3)
** Thinness (F = .76, p = .47)
was 2.13 ± 0.27 mm for the control group, 2.11 ± 0.28 mm for the mild group, and 2.08 ± 0.32 mm for the moderate group. A one-way analysis of variance was used to compare the mean septal wall thicknesses of the groups. No significant differences were found among the means of the groups on noninfarcted septal wall thickness (F = 0.12, p = 0.89).

**Shocked vs Not Shocked:** Ten rats, within the moderate group, received mild electrical shock to encourage them to continue running during their exercise program following coronary artery ligation. Eight rats within the same group did not require electrical shock. No rats within the control or mild group received mild electrical shock.

To determine if there was difference between these two subgroups of the moderate group, mean body weights, heart weights, heart weight body weight ratios, infarct size (circumference method), scar thickness, thinness, and septal wall thickness were compared. Table 8 summarizes these data. A Wilcoxon Rank Sum Test was used to compare the means of the above variables in the rats belonging to these two subgroups. No significant differences were found in the means of the variables between these two subgroups.

**Reliability:** The investigator's ability to reliably measure the various dependent histological variables was tested. Following the use of a one-way analysis of
variance, an intraclass correlation coefficient was calculated for each of the dependent variables (infarct size by circumference measurement, infarct size by area method, scar thickness, thinness, and septal wall thickness) on the random sample of nine hearts on which all measurements were made on each of three separate days. The results, summarized in Table 9, indicated that when using either method to measure infarct size, over 99 percent of the variability was explained by differences in individual hearts and less than one percent of the variability was accounted for by an error component. (One part of this error component would have included the variability of the investigator’s measurements from day to day.) When measuring thinness of the infarct approximately 95 percent of the variability was explained by differences in individual hearts and 5 percent of the variability could be accounted for by error.

Finally, when measuring mean scar thickness and septal wall thickness approximately 89 percent of the variability was explained by differences in individual hearts and 11 percent of the variability could be accounted for by error.
TABLE 8

Difference in Shocked vs Not Shocked Rats
Within the Moderate Group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Shocked (Mean ± SD) (n = 10)</th>
<th>Not Shocked (Mean ± SD) (n = 8)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight</td>
<td>299.73 ± 43 gm</td>
<td>295.28 ± 27 gm</td>
<td>p &gt; .30</td>
</tr>
<tr>
<td>Heart Weight</td>
<td>1.91 ± 25 gm</td>
<td>1.78 ± .33 gm</td>
<td>p &gt; .30</td>
</tr>
<tr>
<td>Heart Weight/Body Weight</td>
<td>0.653 ± .14</td>
<td>0.612 ± .16</td>
<td>p &gt; .30</td>
</tr>
<tr>
<td>Infarct Size (Circumference)</td>
<td>43.27 ± 21.82%</td>
<td>39.80 ± 12.13%</td>
<td>p &gt; .30</td>
</tr>
<tr>
<td>Scar Thickness</td>
<td>0.844 ± 0.14 mm</td>
<td>0.879 ± 0.21 mm</td>
<td>p &gt; .30</td>
</tr>
<tr>
<td>Thinness</td>
<td>0.578 ± 0.21 mm</td>
<td>0.601 ± 0.21 mm</td>
<td>p &gt; .30</td>
</tr>
<tr>
<td>Septal Wall Thickness</td>
<td>2.04 ± 0.34 mm</td>
<td>2.12 ± 0.30 mm</td>
<td>p &gt; .30</td>
</tr>
</tbody>
</table>

*Body weight at time of sacrifice.
<table>
<thead>
<tr>
<th></th>
<th>Infarction Size Circumference (percent)</th>
<th>Infarction Size Area (percent)</th>
<th>Scar Thickness (mm)</th>
<th>Thinness (mm)</th>
<th>Septal Wall Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>50.06</td>
<td>28.00</td>
<td>0.912</td>
<td>0.640</td>
<td>2.15</td>
</tr>
<tr>
<td>SD</td>
<td>1.06</td>
<td>0.39</td>
<td>0.05</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>Variability due to individual variation in heart (percent)</td>
<td>99.15</td>
<td>99.65</td>
<td>89.48</td>
<td>94.89</td>
<td>89.04</td>
</tr>
<tr>
<td>Variability due to error (Percent)</td>
<td>0.85</td>
<td>0.35</td>
<td>10.52</td>
<td>5.11</td>
<td>10.96</td>
</tr>
<tr>
<td>Total variability (Percent)</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>
CHAPTER V
DISCUSSION

The purpose of this study was to determine the effects of a mild or moderate treadmill exercise program on myocardial infarction size and scar formation in rats when the exercise program was initiated 72 hours following experimentally induced myocardial infarction. Fifty-seven male Harlan Sprague-Dawley rats similar in age and body weight were randomly assigned to three groups, control (no exercise) mild, and moderate exercise, and served as the subjects for this study.

Infarct Size: In accordance with this study’s first three hypotheses, the findings of this study demonstrate that a mild or moderate exercise program initiated 72 hours after coronary artery occlusion does not increase the size of the infarct observed at sacrifice 21 days later. This confirms the results of two earlier studies (Hochman and Healy, 1986; Moskovitz et al. 1978). That is, Hochman and Healy (1986) found that mild treadmill exercise of 0.6 mph (approximately 16 meters per minute) did not increase infarct size significantly at sacrifice seven days following occlusion even when the exercise was initiated 24 hours following the occlusion Moskovitz (1978) found
that five minutes of exercise at between seven to eight meters per minute did not increase infarct size when the exercise was initiated as soon as four hours following occlusion. However, he did find an increase in infarct size when exercising rats at between 11 and 15 meters per minute. Additionally, Moskowitz et al. (1979) found that when exercise was initiated after 72 hours of recovery, exercise at even 18 meters per minute did not cause a significant increase in infarct size.

The findings of this study and Hochman and Healy's (1986) may have been able to be predicted from Fishbein's earlier study (Fishbein, Gissen, Spadero, Maclean, and Maroko, 1980). He established that border zone of glycogen depleted but enzymatically active myocardium existed following occlusion for about nine hours and that this zone surrounded a zone of myocardium which although it might be not as severely glycogen depleted had a more severe loss of enzyme activity. This border zone was thought to be a region of potentially salvagable myocardium but the area it surrounded (i.e., the area with severe loss of enzyme activity) was not. What this means is that if steps were taken to reverse the ischemic process within a few hours following the occlusion this region would not become necrotic and the resulting infarct would be smaller. If steps were not taken to reverse the process the resulting infarct would consist of the initially glycogen depleted
zone and the enzymatically inactive zone. Therefore, if exercise is begun following this nine hour period, when the infarcted area was firmly established there should be no further increase in infarct size. That is probably what occurred in this study.

The fourth hypothesis of this study, which addressed the use of two methods for measurement of infarct size was examined next. The investigator felt it was important to establish which method was most appropriate for this study since both methods have been used by a number of investigators. It was believed that the circumference method was more appropriate than the area method because the hearts were not examined until sacrifice at 21 days after occlusion. Fishbein et al. (1978) and Maclean et al. (1986) suggested the area method underestimated the infarct size and believed this was due to a net reabsorption of necrotic myocytes that occurred during the 21 days following occlusion. Both methods were used to calculate infarct size on a sample of nine randomly selected hearts. The values generated using each of the two methods were compared and the area method consistently gave smaller values for infarct size. Even though the area method did appear to underestimate the infarct size it does correlate although poorly with the circumference method. The investigator in this study decided it was more appropriate to use the circumference method in measuring
infarct size on all hearts for two primary reasons: one, the area method underestimated the size of the infarct in all hearts of these rats sacrificed at 21 days following occlusion, and two, using both methods would have increased the amount of time tremendously and the additional time would not have yielded any new information.

**Scar Thickness and Thinness**: It was also hypothesized (Hypotheses 5, 6, 7, 8, 9, 10) in the current study that mild or moderate exercise would not thin the infarct scar and there were no significant differences observed among the three experimental groups with regards to mean scar thickness or a measure of the thinnest part of the scar.

Several of the previous rat exercise studies have shown significant thinning of the infarct scar (Hammerman et al. 1983; Kloner and Kloner, 1983; Moskowitz et al. 1979). The results reported in these studies may be the result of several factors. First, too intense a level of exercise begun within a few hours after the occlusion may have resulted in an increase in infarct size. Second, the mode of exercise used in two of the studies (Hammerman et al. 1983; Kloner and Kloner, 1983) may not have been appropriate. That is, swimming is not the usual mode of locomotion for the rat and swimming may in fact place additional stress on the rats as suggested by Hochman and Healy (1986). Finally, it is difficult to determine if the control rats were handled in the same manner as the
exercising rats in these studies. If the control rats were subjected to only cage rest and not handled like the exercising rats, the experimental rats were subjected both to the stress of exercise and to the stress of handling. Thus, both the stress of exercise and handling may have contributed to the increase in infarct size and not just the stress of exercise.

In the current study, the exercise program was not begun until 72 hours after occlusion and two levels of intensity of exercise were examined. Also the control rats were removed from their cages as often as the exercising rats and placed on specially constructed platforms just above the treadmill belt while the exercising rats were using the treadmill. Thus, besides being subjected to the same handling they also experienced the same noise and vibrations of the moving treadmill. Therefore, the only difference between the control group of rats and two groups of exercising rats was the exercise.

**Septal Wall Thickness as a Measure of Hypertrophy**

The mean thickness of the noninfarcted septal wall was calculated for two reasons. First, because a number of investigators suggested that if there was a proportional amount of compensatory hypertrophy of the noninfarcted myocardium an infarction that was larger may be masked by this hypertrophy (Hochman and Healy, 1986; Maclean et al.
1978, and Roberts et al. 1984). However, Norman and Coers, (1960) did not observe compensatory hypertrophy in their study's rats until six weeks after coronary artery occlusion. Second, hypertrophy may occur because of an exercise induced hypertrophy of individual myocardial cells. Although this study was not designed to produce a training effect (i.e., myocardial hypertrophy) it was important to establish that it had not occurred again because exercise hypertrophy may mask a larger infarct. The results of the septal wall measurements demonstrated no significant difference among the groups in septal wall thickness. These data and the fact that there was no significant difference in heart weight/body weight ratio, (another measure of myocardial hypertrophy), indicate that neither compensatory or training induced hypertrophy occurred. Therefore, infarct size could not have been underestimated due to hypertrophy.

**Reliability:** The validity of the techniques used in this study have been well established by other investigators and the validity of using the ZIDAS for measures of length and distance have been established by the Director of Pathobiology The Ohio State University College of Veterinary Medicine. The reliability of the investigator in this study, however, needed to be established. This was done by randomly selecting nine hearts from the total group of hearts and repeating all
measures (infarct size by both circumference and area methods, mean scar thickness, thinness, and septal wall thickness) on three different days. It was found that the investigator was very consistent from day to day in her measurements. The investigator in this study had an error rate of less than one percent when measuring infarct size. This compared to one study which reported the reliability of the investigator to be less than five percent. Therefore, this study has established that investigators can reduce the amount of error in the measurement of these particular variables to an insignificant level.

**Extrapolation of Results:** The current study attempted to measure the effects of exercise on myocardial infarct size and scar formation in rats. Although there are many similarities between the rat and humans especially in the evolution and healing of a myocardial infarction, one must be careful not to directly extrapolate data from this study involving coronary artery ligated rats who have an otherwise healthy heart to humans with a myocardial infarction and possibly advanced arteriosclerotic changes in other coronary vessels. For example, if one attempted to use this study to say that it is acceptable to exercise humans beginning 72 hours post infarction they would endanger the patient in two ways. First, the evolution of an infarct in a rat is telescoped in time and the status of an infarct in a rat at 72 hours compares more closely to a
6 day old infarct in a human. Also, the rats in this study were assumed to have normal coronary arteries other than the ligated left coronary artery. Humans who have myocardial infarctions due to arteriosclerotic disease, most likely have the disease in other vessels and until their response to different levels of exercise has been evaluated it would be unwise to progress their ambulation based on results of this study.

However, this study can contribute indirectly to humans. First, some clinicians have used the animal studies finding an increase in infarct size and a thinning of the scar as an argument against early ambulation and in particular early exercise evaluation of an individual who is seven to 10 days post infarction. This study, along with that of Hochman and Healy's (1986), establishes in rats that mild treadmill exercise when begun at least 24 hours after experimentally induced infarction does not cause an increase in infarct size or scar thinning. Additionally, this study found that even slightly more intense or moderate exercise does not increase infarct size or thin the scar that is formed. Therefore, these clinicians at the least may have to restate their argument.

Second, this study may be used to stimulate interest in investigating the effects of early ambulation on infarct size and infarct scar formation in humans who have a similar pattern to the evaluation and healing of a
myocardial infarction, especially as noninvasive measures become more exacting with advancing technology.

SUMMARY

The current study demonstrated that a mild or moderate treadmill exercise program, when initiated 72 hours following coronary artery occlusion, did not significantly increase the size of a myocardial infarction nor did it thin the scar which forms 21 days following occlusion in rats. Although several previous studies found both an increase in infarction size as well as thinning of the scar in the rats, this study and a similar study (Hochman and Healy, 1986) did not support those findings. The differences are thought to be related to the mode and intensity of the exercise used in the earlier investigations, as well as when the exercise was initiated. Also, it is felt that these differences may have resulted from differences in the handling of control animals.

Furthermore, it was established that an investigator may reduce the error due to measurement of the variables of infarct size, scar thickness, thinness and septal wall thickness to an insignificant level.
CHAPTER VI
SUMMARY AND CONCLUSIONS

The purpose of this study was to examine the effects of mild and moderate exercise on infarct size, resulting scar thickness, and noninfarcted septal wall thickness in rats. Furthermore, the appropriateness of two methods for measuring infarct size was tested. Finally, the reliability of the investigator in accurately measuring the heart slices from day to day was tested.

One hundred twenty-two male Harlan Sprague Dawley rats underwent coronary artery occlusion seventy-two hours later the surviving rats were randomly assigned to a control (no exercise) group, a mild exercise group, and a moderate exercise group. The rats were then subjected to one of three exercise protocols as described in Chapter III. Twenty-one days following the coronary artery occlusion procedure the rats were sacrificed and their hearts prepared for histological examination as outlined in Chapter III.

Infarct size, scar thickness, thinness, and septal wall thickness were measured. The differences in means between the groups on all variables were examined using a one-way analysis of variance. The differences in means, generated when two methods for measuring infarct size were used, were
examined using a paired t-test. A Spearman's correlation coefficient was calculated to examine the correlation between the two methods. The differences in means between the two subgroups, shocked versus not shocked, of the moderate group were examined using a Student t-test. The reliability of the investigator on a day to day basis was assessed following the calculation of a one way analysis of variance using an intra class correlation coefficient. Finally, means and standard deviations were used to describe body weight, heart weights and heart weight/body weight ratios.

**Results**

The results of this study were:

1. There were no significant differences among the groups in heart weight, body weight, heart weight/body weight ratios, infarct size, scar thickness, thinness, or noninfarcted septal wall thickness.

2. There was a significant difference in infarct size when different methods for measuring infarct size (area vs circumference method) were used ($p = .0001$).

3. Even though there was a significant difference in infarct size using the two methods, the methods were found to correlate but did so poorly ($r$ (SPEARMANS) = .57 ($p = .11$)).

4. There were no significant differences in means of body weight, heart weight, heart weight/body weight ratio,
infarct size, scar thickness, thinness, septal wall thickness between the two subgroups (shocked vs not shocked) of the moderate group.

5. Ninety-nine percent of the variability in infarct size when measured on three different days was due to individual heart differences. Ninety-five percent of the variability in thinness when measured on three different days was due to individual heart differences and, noninfarcted septal wall thickness when measured on three different days was due to individual heart differences.

6. Body weights and heart weights were comparable among the three groups.

Conclusions

The following conclusions were made on the basis of the results of this study:

1. A mild or moderate exercise program initiated 72 hours after a myocardial infarction was experimentally induced in rats does not cause an increase in infarct size or scar thinning.

2. This same exercise program does not induce myocardial hypertrophy in rats as measured by noninfarcted septal wall thickness or heart weight/body weight ratio.

3. There was a significant difference in the size of infarct calculated using two methods, however, these two methods did correlate. The decision to use either method
must be based on past literature and the time that lapses between infarction and sacrifice of the rat.

4. Whether or not a moderately exercised rat is encouraged to run by the use of shock does not influence variables considered in this study.

5. The investigator was very reliable from day to day in measuring the histological variables associated with this study.

Future Research Ideas

Future research ideas were stimulated by the conduct of this study. These ideas include but are not limited to:

1. Studies that explore effects of exercise of different intensities as well as exercise initiated at different times following infarction on the variables measured in this study (i.e., is there a threshold effect).

2. Studies that explore the mechanisms of expansion and scar thinning, i.e., does the level of catecholamines in the circulating blood increase myocardial oxygen consumption and result in an increase in infarct size and scar thinning as was found in other studies.

3. If the above is true, do beta blockers blunt the effect in exercising animals?

4. Studies that answer the question "do humans ambulated early following myocardial infarction show evidence of an increase in infarct size or scar thinning?"
5. Studies that answer the question "does a thinner scar necessarily lead to myocardial rupture?"
APPENDICES
APPENDIX A

ANIMAL USE PROTOCOL

THE OHIO STATE UNIVERSITY
INSTITUTIONAL LABORATORY ANIMAL CARE AND USE COMMITTEE

PRINCIPAL INVESTIGATOR: Robert L. Hamlin, DVM, PhD
(Must be member of OSU faculty)
(Typed name) Signature

Academic Title: Professor Work Phone: 272-9127
Emergency Phone: 265-9242

Department: Vet. Phys. & Pharm. College: Veterinary Medicine
Campus Address: G-50 Session Hall and 1200 Coffey Road

Co-Investigator: Dr. Robert Bartels Barbara Smith
(Typed name) Signature (Typed name) Signature

PROTOCOL TITLE: THE EFFECTS OF TWO LEVELS OF TREADMILL EXERCISE ON MYOCARDIAL HEALING FOLLOWING CORONARY ARTERY LIGATION IN RATS

REVIEW STATUS: Initial Review X Continuation Continuation
No Change Major Change

PERIOD OF PROTOCOL: Dates Protocol will be in effect from 2/1/95 to 1/1/98 (not to exceed 3 years).

DEPARTMENT CHAIRPERSON'S ENDORSEMENT: I have reviewed the proposal which is the basis of this animal use protocol and endorse its submission.

Comments:

Young Lin D.V.M., Ph.D. (Typed name) Signature Date

CONSULTATION WITH COLLEGE ATTENDING VETERINARIAN: I have reviewed this protocol and discussed it with the principal investigator in the context of provisions of the Animal Welfare Act, the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training and other statutes and regulations relating to animals. I have provided him/her with a copy of the Guide for the Care and Use of Laboratory Animals. The principal investigator assures me that personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in these procedures.

Comments:

Vernon Carter D.V.M., Ph.D. (Typed name) Signature Date

ILACCE A-91 (11/85)
Protocol

82
<table>
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<tr>
<th>Year</th>
<th>Species</th>
<th>Number</th>
<th>Age</th>
<th>Sex</th>
<th>Weight</th>
<th>Proposed Housing Location</th>
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<td>1</td>
<td>Harlan Sprague</td>
<td>120</td>
<td>50-54 days</td>
<td>Male</td>
<td>175-200</td>
<td>Central Lab. Animal Resource Station W-11</td>
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<tr>
<td></td>
<td>Dawson Hall</td>
<td></td>
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</table>

**SURGERY** - Will surgery be conducted on animals? Yes ☑ No

If yes, complete this section:
- Non-Recovery Surgery
- Recovery Surgery ☑
- Multiple Surgeries

Surgeon(s) see attached
- R. Gregory Evans, Ph.D., Location of Surgery (Bldg. & Room #) Sisson G-17
- Robert L. Hamlin, D.V.M., Ph.D.

**INFECTIOUS OR TOXIC AGENT** - Will infectious and toxic agents be used in this protocol? Yes ☑ No X

If yes, identify agents.
-3-

RESTRANT - Will prolonged restraint (see page 9 of Guide) be used?

Yes ______ No X

If yes, describe the type and duration of the restraint.

PAIN OR DISTRESS - Does this procedure have the potential to inflict pain or distress?

Yes X No ______

If yes, describe the measures taken to alleviate or minimize pain or distress.

During the coronary artery ligation procedure, anesthesia will be administered by pouring 5ml of ether onto paper towels placed under a bell jar. In the surgeon's past experience, this was sufficient to anesthetize the rats in approximately two minutes. During surgery anesthesia is maintained by use of a 5ml beaker stuffed with an ether-soaked gauze sponge and placed over the rat's

(continued on attached page)

ANESTHESIA, ANALGESICS, TRANQUILIZERS, ETC. - Will anesthetic, analgesic or tranquilizing agents be administered?

Yes X No ______

If yes, complete this section.

<table>
<thead>
<tr>
<th>Species</th>
<th>Agent</th>
<th>Dose</th>
<th>Route</th>
<th>Performed by</th>
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<tbody>
<tr>
<td>Marlan Sprague</td>
<td>Ether</td>
<td>5ml</td>
<td>Inhalation</td>
<td>R.I. Hamlin, D.V.M., Ph.D.</td>
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<tr>
<td>Dulley Rats</td>
<td></td>
<td></td>
<td>under desicator</td>
<td>B.A. Smith, R.N., M.S.N.</td>
</tr>
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</table>

EUTHANASIA - Will euthanasia be carried out? Yes X No ______

If yes, complete this section.

<table>
<thead>
<tr>
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<th>Agent/Procedure</th>
<th>Dose</th>
<th>Route</th>
<th>Performed by</th>
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<tr>
<td>Marlan Sprague</td>
<td>Uthyl</td>
<td>1 ml/sept</td>
<td>Intraperitoneal</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>B.A. Smith, R.N., M.S.N.</td>
</tr>
</tbody>
</table>
FMTOCOL - Describe your protocol involving the use of animals. (See attached) (DO NOT EXCEED THREE PAGES) In addition, answer the following questions:

ALTERNATIVE METHODS - What alternative methods to the use of live animals have been considered and are applicable to this subject?

Currently there are no other methods for conducting this research without the use of live animals.

SPECIES - What is the rationale for using this (these) species of animals?
There are several reasons for the selection of this species of animals (Sprague Dawley Rats)

1. Infarct variation is less pronounced in the rat than in other animals such as the dog, following the methods described by Evans (1985). Infarct size is 39.1 ± 6.2 percent.

2. Immediate operative mortality is low (21 and 26 percent).

3. Infection does not appear to be a problem (Evans, et al., 1985; Johns and Olson, 1954; Maclean, et al., 1970; Roberts, et al., 1984) even when long term survival (Continued on attached page )

ALTERNATIVE SPECIES - What alternative species were considered?

The dog was initially considered for this investigation but was eliminated primarily because the infarct size produced by ligation of the coronary artery in the dog is quite variable; thus requiring large numbers of these animals to detect a statistically significant difference in infarct size between the control and experimental animals. Further, the operative procedure in the dog is more complex requiring the use of a ventilator and takes 1-2 hours to complete. The guinea pig was also considered, however there was a lack of appropriate models using this animal described in the literature.

NUMBERS OF ANIMALS - Justify the number of animals to be used.

Due to overall operative mortality and the failure of the ligation procedure to produce an infarction, 120 rats must be operated (using the Evans's Method) to obtain a minimum of 60 rats (20 per each of the three groups: control, mild, and moderate exercise groups.) A minimum of 20 rats per group are required to detect a 15 percent difference in infarct size based on the previously established variability in infarct size (39.1 percent ± 6.2 percent) using the Evans's method.
Prepare a typed abstract of this project and its significance and rationale in lay terms. (NOTE: This information may be used by OSU in official communications with the public and news media.) DO NOT EXCEED ONE DOUBLE-SPACED, TYPED PAGE.

In recent years, early ambulation and other increases in physical activity have been prescribed for patients with uncomplicated heart attacks. This practice has virtually eliminated many of the deconditioning effects of prolonged inactivity such as a rapid decline in work capacity, decrease of total blood volume, and prevented many of the problems associated with prolonged bed rest such as pneumonia, blood clots, and bed sores.

More recently a few cardiologists have even begun low-intensity exercise testing in patients seven to 10 days after a heart attack in order to more appropriately and safely prescribe activity levels for the patient upon discharge, identify those patients requiring further study or treatment, and reassure the patient that certain activities are safe to perform.

Despite the obvious benefits, a number of cardiologists remain concerned about the effects of early ambulation and predischARGE exercise testing. Specifically, they are concerned that exercise may increase the amount of heart muscle contingent, and the nature of the scar that is formed. Furthermore, some early animal studies have indicated that heart attack size and scar thickness are negatively affected by exercise. However, a number of these studies with rats have used vigorous exercise protocols. Therefore, it is believed that many of the negative effects seen in these early studies were related to the inappropriate level and mode of exercises.

This study proposed to look at mild and moderate treadmill exercise and its effect on heart attack size and scar formation following coronary artery ligation and subsequent heart attack in the rat. (Treadmill exercise, walking and running, is a more appropriate mode of exercise and the mild exercise level is less vigorous than used in most earlier studies.) This study is important because data from it may be used to establish the safety of early ambulation and predischARGE exercise testing in humans who have suffered a heart attack and demonstrate that mild exercise will have little effect on amount of heart damage and scar formation.
PAIN OR DISTRESS (CONT.)

Nose and mouth. Other forms of anesthesia have been tested including Methoxyflurane, Halothane, and Chloroform and were found to be difficult to regulate.

SPECIES (CONT.)

Is the atm.

4. The procedure (coronary artery ligation) in Sprague Dawley rats is very rapid (2 minutes) compared to the procedure in larger animals such as dogs which must be intubated or tracheotomized and ventilated.

SURGEONS (CONT.)

Dr. R. Gregory Evans (St Louis University Medical Center) has completed over 3000 coronary artery ligations in Sprague Dawley rats over the past 4 years and has developed the procedure as described in his article entitled "Evaluation of a Rat Model for Assessing Interventions to Salvage Ischemic Myocardium" in Cardiovascular Research, 1985, 19, 132-138.
One hundred twenty (120) male Sprague Dawley rats weighing 250-270 grams will be acclimatized to the treadmill over a 1-2 week period prior to surgical occlusion of the left coronary artery. These rats will be fed, housed, and receive appropriate veterinary care according to the Guide for Care and Use of Laboratory Animals (1993).

At the end of the acclimatization period, the rats will be anesthetized in a desiccator by pouring 5 ml of ether on paper towels and then placing the towels inside the desiccator. Anesthesia will be maintained during the procedure with ether loaded gauze sponges stuffed into a 50 ml beaker which is then placed over the nose and mouth of the rat. The rat will be restrained on its back using elastic straps over its front legs and over the lower pelvic area (Evans, 1933).

The rats will then undergo a left coronary artery ligation performed by Dr. R. Gregory Evans, Ph.D., and R. L. Hamilton, M.B., Ch.B., Ph.D., according to the procedure described by Evans et al. (1933). This procedure generally takes 3 minutes with the chest cavity opened for no more than 30 seconds. (It is the rapidity of the procedure which is thought to be responsible for the low mortality of the rats.) Single loop ligation will be performed on each rat immediately postoperatively and then electrically fibrillate will be measured 10-24 hours postoperatively.

Seventy-two hours after the surgery the surviving rats will be randomized into a control group, a mildly exercised group, and a moderately exercised group. The rats in the control group will be placed in special cages just above the ventilation system while the other rats are exercised so that the control rats will not be subjected to similar handling, noises and vibrations. The rats in the mildly exercised group will be exercised 5 days each week beginning at eight meters per minute, 10 percent grade for five minutes duration and progressed to 20 meters per minute, 20 percent grade for 20 minutes duration at two and at 21 days. The rats in the moderately exercised group will be exercised 5 days each week beginning at 15 meters per minute, 20 percent grade for five minutes duration and progressed to 30 meters per minute, 30 percent grade for 30 minutes duration at the end of 21 days (Fusco et al., 1977).

At the end of the 21 days the animals were sacrificed for the first time to walk the rats will be lightly anesthetized with ether in the manner described above and a single lead ECG will be performed. On the following day the rats will then be administered intraperitoneally to carry our euthanasia. The heart will be excised, fixed in formalin for 72 hours, and appropriate histological analysis for all: of interst. scar thickness versus noninfarcted left ventricular wall thickness and interstitial thickm will be completeing.
THE OHIO STATE UNIVERSITY

INSTITUTIONAL LABORATORY ANIMAL CARE AND USE COMMITTEE

ACTION OF THE COMMITTEE

- Original Review
- Periodic Review

RESEARCH PROTOCOL:

84AO024 THE EFFECTS OF TWO LEVELS OF TREADMILL EXERCISE ON MYOCARDIAL HEALING FOLLOWING CORONARY ARTERY LIGATION IN RATS, Robert L. Hamlin, Robert Bartela, Barbara Smith, Veterinary Physiology and Pharmacology

The Institutional Laboratory Animal Care and Use Committee took the following action on the protocol cited above:

- Approved
- Disapproved
- Approved with Modifications

The Committee has accepted the requested modifications and, therefore, the protocol is APPROVED.

Approval of the protocol is for the period 02/86 through 02/87.

The Investigator(s) shall immediately bring to the attention of the Institutional Laboratory Animal Care and Use Committee any changes proposed for the approved protocol as they relate to the care or use of laboratory animals. The Committee will decide whether the extent or type of changes proposed warrant formal Committee review. If such a review is deemed necessary, the chairperson shall schedule the review for the earliest feasible time.

The Committee will periodically contact investigators to seek information about approved protocols. Periodic contact for this protocol is next scheduled for ___.

Certification of review and approval will be transmitted to external sponsors, as required, by the Research Foundation. INVESTIGATORS ARE RESPONSIBLE FOR CONVEYING A COPY OF THIS DOCUMENT TO THEIR SPONSORED PROGRAM DEVELOPMENT OFFICER.

Date: January 24, 1986

Signed (Chairperson)

ILACUC A-03 (11/85)

Committee Action
REFERENCE LIST
REFERENCE LIST


