HISTOCHEMICAL OBSERVATIONS ON RAT CARDIAC MUSCLE FOLLOWING CHRONIC EXERCISE

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

ROBERT OTTO RUHLING

1970

This is to certify that the

thesis entitled

HISTOCHEMICAL OBSERVATIONS ON RAT CARDIAC MUSCLE FOLLOWING CHRONIC EXERCISE

presented by

Robert Otto Ruhling

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Health, Physical Education & Recreation

Major professor

Date 8/6/70

O-169

ABSTRACT

HISTOCHEMICAL OBSERVATIONS ON RAT CARDIAC MUSCLE FOLLOWING CHRONIC EXERCISE

By

Robert Otto Ruhling

The purpose of this study was to investigate the effects of seven different levels of chronic physical activity on the metabolic and morphologic characteristics of the left ventricular myocardium of adult male albino rats.

Two hundred and fifty-two, 71-day-old, male, albino rats (Sprague-Dawley strain) were brought into the laboratory and were assigned to one of seven treatments at random. The treatment groups were sedentary control (CON); voluntary running (VOL); short-duration, high-intensity running (SHT); medium-duration, moderate-intensity running (MED); long-duration, low-intensity running (LON); electric stimulus control (ESC); and endurance swimming (SWM). Treatments were administered Monday through Friday. Animals were provided with food and water ad libitum.

The healthiest and best trained animals were selected for sacrifice. Seven selected animals were weighed and

sacrificed on Mondays after 0, 2, 4, 6, 8, and 10 weeks of training. The final sample consisted of 126 animals.

During sacrifice, each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital. The heart was excised and cut transversely at a level just below the atria. The apical portion was placed on a chuck which was immersed in pre-cooled 2-methylbutane. Three hundred micra from the apex a minimum of six 10 μ serial sections were cut.

Five histochemical procedures were utilized to evaluate the relative glycogen, fatty acid, and aerobic enzyme concentrations in the cardiac fibers. A histologic procedure was performed to evaluate the morphology of the hearts. Each histochemical stain was measured objectively using a light transmission meter. The histological sections were rated subjectively on the bases of normalcy and of pathology.

The results indicate that training for eight weeks was sufficient to produce metabolic adaptations in the rats. The eight-week LON group was observed to be significantly lighter than the eight-week CON group ($p < .10$). The fatty acid concentrations were greater in the VOL, LON, and SWM groups at eight weeks than in the CONS group at zero-week ($p < .10$). Also, in the LON group at eight weeks, surprisingly, the glycogen concentrations were greater than in the CONS group at zero-week ($p < .10$).

No pathological changes were observed in any of the seven treatments ($p < .05$). This result is limited to the single section taken approximately 300 μ from the apex.

HISTOCHEMICAL OBSERVATIONS ON RAT CARDIAC MUSCLE FOLLOWING CHRONIC EXERCISE

By

Robert Otto Ruhling

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Health, Physical
Education and Recreation

1970

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DEDICATION

To Holly

ACKNOWLEDGMENTS

Appreciation is extended to the chairman, Dr. W. D. Van Huss and to the individual members, Dr. W. W. Heusner, Dr. R. E. Carrow, and Dr. S. D. Sleight of my guidance committee for their individual and group help that I received over the past four years.

Special thanks go to Dr. R. E. Carrow for allowing me to have unlimited use of the equipment and the supplies of the Cytology Laboratory, Department of Anatomy.

A word of appreciation goes to Mr. James F. Taylor who helped provide a friendly and stimulating atmosphere in which to work.

Thanks also go to Mrs. Barbara Wheaton and to Miss Patricia L. Lamb for their unending technical aid and laboratory assistance.

Special thanks go to Mr. David J. Anderson for drawing the figures.

A thank you is also in order to Mr. Kwok-Wai Ho, Chief Animal Caretaker, and to his assistants for the care and training of the animals in the Human Energy Research Laboratory, Department of Health, Physical Education and Recreation.

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CHAPTER I

THE PROBLEM[1]

Introduction

Investigations reporting general effects of exercise have shown that trained animals tend to weigh less, contain less fat, have shorter but stronger and more dense bones, have larger hearts, and that their skeletal muscle metabolism may be altered (65, 62, 33, 37, 40, 15, 16, 28, 29). The evidence is often conflicting and impossible to interpret because in most instances the training regimen used has not been defined.

The recent evidence suggests that exercise consists of a continuum of specific activities each eliciting a specific response within the organism. Although little is known about specific effects, weight lifting type exercise in the rat produced hypertrophy of the right side of the heart which Krames and Van Liere (36) attributed to hypoxia, whereas endurance type exercise produced hypertrophy of the left side of the heart. Other results (22, 23, 24), from studying skeletal muscle, showed a "sarcoplasmic

[1]This study was supported in part by National Institutes of Health Grant HD 03918.

hypertrophy" or an elevation of the cytoplasmic proteins following training of a high-repetitive, low-resistance nature. The converse was indicated when the work performed was of a low-repetitive, high-resistance type.

A need clearly exists for additional information concerning the specific effects of defined exercise programs. Since three exercise regimens have recently been developed for the rat which simulate specific training programs in man (69), an opportunity existed for contributing to this body of knowledge. Following a search of the literature, no information could be found concerning the specific effects of exercise regimens on heart metabolism and morphology. Thus it was decided to pursue an investigation along these lines.

Statement of the Problem

The purpose of this study was to determine the effects of seven different levels of chronic physical activity on the metabolic and morphologic characteristics of the left ventricular myocardium of adult male albino rats.

Rationale

If exercise is beneficial to the heart, what type of exercise causes the heart to function most efficiently? This study was designed to explore the possibilities of a rational answer to this question.

It was hypothesized that as the type of exercise progressed from the short-duration, high-intensity (SHT) end of the physical activity continuum toward the long-duration, low-intensity (LON) end of the physical activity continuum, metabolic characteristics of the myocardium would progress accordingly from those of anaerobiosis and partial aerobiosis to those of total aerobiosis, respectively.

Significance of the Problem

Specificity of exercise is a reality that investigators are just beginning to comprehend. Previous studies have lacked an exercise apparatus which controls the intensity of various exercise regimens (42, 43). Prior to this study, duration was controllable, intensity was not controllable.

It behooves physical educators to know exactly what is happening to the heart when prescriptions for exercise are given.

Limitations of the Study

1. The results of this study cannot be applied to man or generally to other animals.
2. The morphological analysis was only made on one section taken 300 μ from the apex of the heart.

3. Methods that are employed for the determination of enzyme and substrate levels are quantitatively limited in that actual concentrations are not known.

Definitions and Abbreviations

Certain words and phrases are abbreviated throughout this thesis. It is important that the reader is able to understand the meaning of these symbols. An appropriate list follows.

ATP	Adenosine triphosphate
DPN	Diphosphopyridine nucleotide (oxidized)
DPNH	Diphosphopyridine nucleotide (reduced)
CDS	Cumulative duration of shock (sec) received by both the experimental (SHT, MED, LON), and the control (ESC) animals during all work periods of all bouts of a given training period
CON	Sedentary control
C-RW	Controlled-Running Wheel (11 cm. wide and 38.8 cm. in diameter)
Cy OX	Cytochrome oxidase
Diatex	Synthetic mounting medium
DNA	Deoxyribonucleic Acid
ESC	Electric stimulus control (paired with SHT)
EST	Expected swim time (min)
FA	Fatty acid
H & E	Hematoxylin and Eosin

Histoclad	Synthetic microscopic mounting medium
LDH	Lactate dehydrogenase
LON	Long-duration, low-intensity running exercise (long C-RW program)
MED	Medium-duration, moderate-intensity running exercise (medium C-RW program)
NBT	Nitro Blue Tetrazolium which was developed by Nachlas *et al.* (45) and produced by Sigma[1]
PAS	Periodic Acid-Schiff (indicates presence of glycogen)
PER	Per cent expected revolutions; PER = 100 TRR/TER
PET	Per cent expected time (swimming); PET = 100 STC/EST
PSF	Per cent shock free; PSF = 100-(100 CDS/TWT)
SDH	Succinate dehydrogenase
SHT	Short-duration, high-intensity running exercise (short C-RW program)
STC	Swim time completed (min)
SWM	Swimming exercise
TCA	Trichloroacetic acid
TER	Total expected revolutions that the animal would run, during all work periods of all bouts of a given training period, if he would run at the prescribed speed
TPN	Triphosphopyridine nucleotide (oxidized)
TPNH	Triphosphopyridine nucleotide (reduced)
TRR	Total number of revolutions run by the experimental animal, during all work periods of all bouts of a given training period

[1]Sigma Chemical Company, St. Louis, Missouri.

TWT	Total work time (sec) during all work periods of all bouts of a given training period
VOL	Voluntary running exercise

CHAPTER II

REVIEW OF RELATED LITERATURE

The topical areas reviewed are: myocardial metabolism; related biochemical and histochemical studies including those on glycogen, lactate, fatty acids, and SDH and Cy Ox; and myocardial pathology. These areas were reviewed to give insight into the concept of specificity of exercise as it might apply to the heart.

Myocardial Metabolism

During metabolism of the normal heart, about 35% of the total myocardial oxygen extraction was accounted for by equal amounts of glucose and of lactate. Whereas, 60+% of the energy expenditure of the myocardium was sustained by fatty acids (7). Bing also indicated that as fat intake increased, the free fatty acid content in the myocardium approached 100%. Two mechanisms were proposed. Fatty acid metabolism might have increased or storage of fat in the heart muscle itself had occurred. It is unlikely that phospholipids or cholesterol esters are metabolized by the heart. Data were presented indicating that ketone bodies and amino acids were also metabolized by the heart. The

aerobic extraction of ketone bodies accounted for <5% of the total oxygen extraction and was governed by the quantity of carbohydrates available to the myocardium. The aerobic metabolism of amino acids may account for as much as 40% of the total cardiac oxygen consumption. However, a rather small increase (20%) in arterial blood amino acid content produced a startling disproportionate increase (245%) in the myocardial amino acid content.

The metabolic activities that are present in the heart reflect the function that the myocardium performs (25). Because the heart requires an extraordinary amount of energy for its normal operation, the organization and function of myocardial metabolism are geared to the production of energy on a large scale when compared to other tissues of the body. By the process of oxidative phosphorylation, energy is captured in the high energy bond of ATP. The systems of enzymes and cofactors necessary for oxidative phosphorylation to occur are present in the mitochondrion. Each myocardial cell has an unusually large number of mitochondria. Therefore, the mitochondrion serves a key function in myocardial metabolism. The production of usable energy proceeds in essentially two steps. The first stage is the degradation of certain essential molecules to form acetyl CoA. Carbohydrates become degraded through glycolysis, fats through hydrolysis and fatty acid oxidation and amino acids through transamination and deamination. The second stage is the funneling

of acetyl CoA into the Tricarboxylic Acid Cycle through which carbon dioxide, water and energy are produced.

From another point of view, Opie (48) has stated that glucose is important in the understanding of myocardial metabolism due to the possible use of glycolysis in maintaining anaerobic metabolism. However, glucose usage is limited by the rate of transport across the cell membrane. Anoxia and muscular work increase glucose phosphorylation. Data are presented indicating that glycolysis increases during periods of anoxia. Phosphofructokinase is inhibited by two end products of aerobic glycolysis (ATP and citrate). However, if the pH of the blood increases (by creatine phosphate breakdown), as occurs during exercise, and before lactate can accumulate, the ATP inhibition of phosphofructokinase is relieved. The hexose monophosphate shunt is not usually operative in normal heart tissue. However, it is accelerated when synthetic processes are increased and when TPNH is required. Anoxia causes pyruvate to form lactate rather than enter the Tricarboxylic Acid Cycle. If fatty acid oxidation is occurring the pyruvate dehydrogenase complex activity is further inhibited with the accumulation of acetyl CoA and TPNH. Opie also indicated that glycolysis could be accelerated by a number of factors: hormones, exercise, and work. If any of these factors accelerate glycolysis to a degree which exceeds the capacity of the hydrogen shufflers

(DPN-DPNH), then lactate could form from pyruvate even in the absence of anaerobiosis. He stated that only one-tenth to one-third of the energy needs of the mammalian heart could be met by anaerobic glycolysis. This amount of energy could maintain the normal mechanical function of the dog heart during partial but not total deprivation of oxygen.

There might be practical implications in any manipulations (exercise?) which could be shown to increase the maximal rate of anaerobic glycolysis in the mammalian heart. (Italics are mine). For example, the energy metabolism of the heart might be better sustained during angina pectoris or during myocardial infarction. The present failure of these anaerobic processes to maintain the anoxic heart may possibly indicate the presence of restraints on anaerobic energy production.

Increased glycogen reserves (48) may be observed as a reflection of decreased glycolysis and the sparing of glyocgen associated with increased fatty acid metabolism by the myocardium. Phosphorylase activity may be influenced by exercise but catecholamines appear to be the most important of the known stimuli which convert phosphorylase b to phosphorylase a. Other results indicated that catecholamines have the ability to mobilize free fatty acids in the intact animal, thereby, increasing myocardial free fatty acid uptake with a consequent sparing of glycogen. In rats who had been swum, it was reported

that cardiac glycogen was unchanged (17) or decreased (10). The glycogen usage depended on the availability of alternate substrates and in part on the magnitude of the work load. It was also reported that only in extreme conditions (anoxia) was cardiac glycogen mobilized.

Opie (48) reported that free fatty acid uptake was directly related to the circulating free fatty acid concentration in both the intact heart and in the isolated perfused heart. Indications were such that the heart free fatty acid levels were about twenty times those of the blood. There was no evidence presented to suggest that endogenous lipid was an energy source for the normal heart in situ, except during intense exercise or during prolonged fasting. Evidence was presented indicating that ketone bodies were a minor substrate of the normal human heart. Ketone body uptake accounted for <10% of the total myocardial oxygen during exercise.

In a continuation of his review of myocardial metabolism, Opie (49) indicated the myocardial uptake of phospholipids and cholesterol was insignificant. The nature of the substrate could alter the myocardial oxygen uptake. When fatty acids are added to the isolated, perfused heart preparation, lactate production from glucose increases and more glycogen is formed from the glucose, too. Therefore, the concurrent oxidation of free fatty acids by the myocardium can actually control the intracellular fate of glucose, especially at the level of pyruvate entry into

the Tricarboxylic Acid Cycle. He concluded that lipid (exogenous) was a major source of energy to the heart and that generally, acetate, ketone bodies, short-chain fatty acids, lactate and pyruvate are oxidized in "preference" to glucose.

From studies of skeletal muscle, myocardium could be considered an extreme type of aerobic muscle. Therefore, the heart was metabolically more dependent on the oxidative metabolism of lipids than on glycolysis for its energy requirements. There were no data presented indicating that increased left ventricular work, however produced (exercise?) was a major factor factor in the control of competition between glucose and free fatty acids for oxidative metabolism in the heart.

In the fasting condition, mean oxidation extraction ratios are 60% for free fatty acids, 28% for glucose and 11% for lactate. The increased energy demands of short periods of exercise are met by an increased coronary blood flow with an increased extraction of carbohydrate substrates (lactate), therefore glucose becomes the major source of energy in exercise (short bursts) (49). In the mitochondrion, the inhibition of citrate synthetase by ATP may indicate a regulating mechanism whereby decreased ATP concentrations, such as could be expected after increased myocardial work (exercise?) might accelerate the Tricarboxylic Acid Cycle. Creatine phosphate experiences depletion in the heart prior to ATP depletion. About 70%

of the oxygen uptake is directed toward the contractile process while the other 30+% is used for regulating other cellular activities (ion transport across cell and mitochondrial membranes, other non-phosphorylating cellular activities). The rate of oxidative phosphorylation is coupled to the rate of electron transport in all tissues.

The impulse conducting system of the heart may depend more on glycolysis for its energy supply than does the rest of the myocardium. The oxygen uptake and SDH activity are much lower in the Purkinje fibers, but the glycogen concentration is higher and the capacity to survive anoxia is greater than in other parts of the heart.

The major part of myocardial norepinephrine seems to be synthesized within the heart. Epinephrine cannot be synthesized in the heart, but is taken up from the circulation by the heart. Furthermore, catecholamines may be released into the circulation when the left ventricle develops an increased systolic pressure (exercise?). The rate of glycolysis may either increase or decrease in hearts that have been exposed to epinephrine (49).

Related Biochemical and Histochemical Studies

Glycogen

Evans (17), in 1934, acutely exercised rats by forcing them to swim and reported that the cardiac glycogen content remained unchanged as a result.

An attempt was made to relate the consumption of blood oxygen, glucose, lactic acid and the production of carbon dioxide to the amount and nature of cardiac work by Alella et al. (2). They studied the performance of dogs' hearts in situ under nine sets of conditions of heart work which included three levels of mean cardiac output (852, 1208, and 1665 cc/min.100g. heart weight) at each of three levels of mean aortic blood pressure (79, 97, 122 mm. Hg.). Results indicated that exogenous glucose consumption was not related to the cardiac oxygen consumption.

Two forms of cardiac glycogen have been identified on the basis of solubility in TCA. The soluble glycogen is relatively labile, while the insoluble glycogen is more stable. Using male albino rats (Sprague-Dawley strain), that weighed between 100 and 150 grams, Weisberg and Rodbard (68) attempted to demonstrate the glycogen distribution in the heart. Their results indicated that the total concentration of glycogen was highest in the atria, lowest in the left ventricle, and intermediate in the septum and right ventricle ($p < 0.01$). The distribution of the TCA soluble glycogen was similar to that for the total glycogen in each segment of the heart. While the concentrations of total glycogen in the atria did not differ statistically, the TCA soluble glycogen of the right atrium was significantly greater than that of the

left atrium ($p < 0.05$). The TCA soluble glycogen accounted for 55% of the total glycogen in the rat heart.

Bloom and Russell (9) investigated the effects of injecting norepinephrine and epinephrine (0.2 mg./kg.) on the glycogen content of the rat heart. Using female albino rats (Sprague-Dawley strain), they reported that the glycogen content of the heart had increased at both two and four hours after both epinephrine and norepinephrine injections. These changes were reflected in the TCA soluble glycogen which thus appeared to be more subject to physiological change than was the insoluble glycogen fraction.

In attempts to correlate mechanical events with biochemical events of the heart, researchers have agreed that as the work load was increased on the heart, in vitro, the total glycogen reserve decreased. However, in vivo, the depletion of the concentration of glycogen in cardiac tissues of swimming rats has not been demonstrated (10). Blount and Meyer (10) studied the effects of a swimming exercise on the cardiac glycogen concentration in the left ventricle of male albino rats that weighed between 130 and 250 grams. Their analyses included the determination of the TCA soluble glycogen and the protein bound or residual glycogen. Previous experimental evidence indicated that the free form of glycogen was more readily mobilized under conditions of increased metabolic activity than was the

residual glycogen. Their results indicated that the exercised rats depleted their cardiac glycogen within the first fifteen minutes of swimming (there was no statistically significant difference between the cardiac glycogen of rats that swam for fifteen minutes and those that were forced to swim for sixty minutes). The cardiac glycogen stores appear to be adequate for only the first few minutes of exercise. The conclusion was also reached that during unusually heavy exercise, the heart is anoxic and draws upon its glycogen reserve. The TCA soluble glycogen exhibited greater depletion. Both the free and bound glycogen are rapidly restored to normal levels after extreme depletion and the TCA soluble glycogen even increased significantly above resting levels (supercompensation).

Using the hearts from thirty-three infants, seventeen known or suspected diabetic adults and sixty-three non-diabetic adults, Mowry and Bangle (41) tested for the presence of glycogen using the PAS reaction. The sections were graded as containing from 0 to 5+ glycogen. The results showed that the 5+ presence of glycogen does not definitely relate it to cardiac enlargement in infants. Hearts that were rich in glycogen were observed of all age groups up to eight months. Glycogen concentration was not related to sex. In the adults, they reported that glycogen storage disease was characterized by considerable cardiac enlargement without a known functional cause. Glycogen was found to be diffusely distributed throughout

the myocardium in concentrations exceeding those believed to occur normally.

In an attempt to identify the existence of anaerobic metabolism in the heart, Neill et al. (46), inhibited the heart's oxidative metabolism with the intravascular administration of cyanide to six closed chest dogs (20-25 kg.). They also wanted to demonstrate that the heart could convert anaerobically liberated energy to mechanical work. Their results indicated that during the cyanide effect, cardiac work and heat exceeded the energy available from oxidative metabolism, therefore, the difference represented anaerobic myocardial metabolism. Also, because the energy of mechanical work output was greater than the myocardial aerobic energy source, a portion of the anaerobic energy liberated must have been converted to mechanical work.

Taking twenty-two human hearts from males and nineteen human hearts from females, Wittels and Reiner (71) investigated the relationship between myocardial ischemia and glycogen content. They looked at paraffin embedded PAS tissue sections. Glycogen was quantified in the PAS-stained sections on a 0 to 4 scale. Their results showed that in the left ventricle the glycogen content was no more than 1+ in nine control hearts. However, in hearts with myocardial infarction, 14 of the 19 hearts were found to be rich in glycogen. They concluded that although normal myocardium is apparently rich in glycogen in life, little or no glycogen was detected to be present after death.

Yokoyama et al. (72) proceeded systematically to investigate the PAS changes that occur in early myocardial infarction. Infarcts were produced experimentally by ligating a branch of the left coronary artery. Heart tissue was excised at various time intervals after ligation and samples of both affected and unaffected tissues were taken from the left ventricle. Results showed that glycogen concentration decreased in the affected portions of the hearts. Results also indicated that when healing takes place with granulation tissue, increased quantities of glycogen were observed in those myocardial fibers immediately surrounding the area of the infarct.

Lactate

Studying dogs in situ in a variety of conditions of heart work, Alella et al. (2) reported that the consumption of exogenous lactic acid by the heart showed a positive relationship to its availability, to its arterial blood level, and to the myocardial oxygen consumption. It appeared to be one of the substrates readily used for the work performance of the heart. The relative consumption of exogenous glucose and lactic acid varied with their relative availabilities and are related in an inverse manner.

Warbasse et al. (67) discovered that serum LDH could not be used to differentiate myocardial injury from either skeletal muscle, lung or liver tissue damage.

Research by Meerson and Zayats (39) has given certain clues to the possible metabolic causes of alterations in contractile proteins of failing heart muscle. They observed the hearts of rabbits which had aortic stenosis experimentally induced. Their results indicated that the lactate concentration increased in the myocardium with a concomitant hypertrophy of myocardial fibers. The mitochondrial mass also increased.

Keul et al. (32) presented evidence that exercise altered the lactate/pyruvate ratios which can cause pyruvate output to increase by the heart.

Kaplan and Goodfriend (31) showed that the M-isoenzyme of LDH was present mainly in those muscles (skeletal) that function anaerobically. While the H-isoenzyme of LDH was present in the muscle (cardiac) that functions aerobically. The H-isoenzyme is inhibited by pyruvate and it was suggested that this ensures the pyruvate entry into the Tricarboxylic Acid Cycle during conditions of increased glycolytic flux.

Fritz (19) presented information stating that H-LDH does not act as a regulatory enzyme as M-LDH does. The heart demands a constant supply of energy as produced by oxidation of Tricarboxylic Acid Cycle substrates, but these substrates neither act as feed back regulators nor limit their own concentration.

To further study the effects of physical training and hypertrophy on LDH activity in cardiac muscle, Gollnick and Hearn (20) used male albino rats (Sprague-Dawley strain) with a mean weight of 340 grams. The physical training consisted of forcing the rats to swim thirty minutes daily in 37°C. water for thirty-five consecutive days. Twenty-four hours after the last treatment, the experimental and the control animals (30 pairs) were sacrificed. Their results indicated that the exercised animals gained 38.7% less body weight than did the controls. On a relative basis, the heart ventricles of the exercised group were larger ($p < 0.05$). LDH activities of the heart ventricles of all the exercised animals were significantly increased over the controls. They concluded that these results indicated that enzyme activities could be altered as a consequence of exercise. The increase in enzyme activity for the heart, coupled with the lack of change in the skeletal muscles, would seem to indicate that the exercise, as used in this study, was a greater stress on the heart than on the skeletal muscles.

Gollnick, Struck, and Bogyo (21) investigated the acute effect of exercise on myocardial and skeletal muscle LDH activity of trained and untrained rats. Forty-eight male albino rats (Sprague-Dawley strain) were assigned at random to two groups of twenty-four animals each. The trained group was exercised daily for thirty-five consecutive days by being forced to swim in 35°C. water. The

untrained group served as sedentary controls. The rats were swum in groups of six beginning with thirty minutes duration. The swim time was increased five minutes each day, until a peak of sixty minutes was attained. Both groups of animals were weighed and sacrificed the day after the last day of training. Half of the animals from each group were forced to swim for thirty minutes just before sacrifice. At sacrifice, the cardiac and the gastrocnemius muscles were removed. Their results showed that the training produced a significant increase in heart LDH activity. However, the acute exercise just before sacrifice did not produce any change in LDH activity in the trained or the untrained animals. The increased LDH activity gives the heart a greater capacity for lactate utilization from the blood and complements the oxidative pathways by supplying pyruvate and DPNH.

Fatty Acids

It has been shown that in the postabsorptive state, more than half of the oxygen consumption of the cardiac muscle fiber is due to the oxidation of fatty acids (5, 7, 13, 61). Bing et al. (8) showed that when fatty acid blood levels were increased, due to high fat intake, the myocardial extraction ratio of fatty acids approached 100%. Two mechanisms were proposed: fatty acid metabolism had increased and/or the heart had stored fatty acids.

Shipp (59) demonstrated that the oxidation of free fatty acids by cardiac muscle can actually control the intracellular fate of glucose, especially at the level of pyruvate entry into the Tricarboxylic Acid Cycle.

Holczinger (30) reported that fatty acids may be seen histochemically utilizing a copper acetate incubating medium. The reaction was reported as being quite sensitive, demonstrating both saturated and unsaturated fatty acids in the same manner. The greenish-black inner complex afforded good localization and the sediment was reported to be extraordinarily durable. However, reliable results could only be obtained from fresh frozen unfixed tissue.

SDH and Cy Ox

Padykula (51) indicated that SDH was one of the few dehydrogenases that acts directly with the electron transport system without intervention of a coenzyme. Cardiac muscle showed a higher degree of SDH activity than skeletal muscle. Sections of myocardium presented a monotony of blue diformazan granules which were distributed throughout the tissue in great abundance. The intercalated discs and nuclei were not recognizable in these preparations. She also explained that the specificity of the histochemical reaction may be demonstrated by eliminating succinate from the incubating medium.

To study the exercise effects on SDH activity of both the heart and skeletal muscle, Hearn and Wainio (28) used male albino rats (Wistar Institute strain). The exercise consisted of forcing the animals to swim for thirty minutes daily in 32°C. water for either five weeks (15 pairs), or six, or seven, or eight weeks (10 pairs each). These animals were pair-fed. Their results showed that the actual total activities of SDH were not significantly altered by the training procedure. However, the relative total activities of SDH in the heart ventricles of the exercised animals were increased ($p < .02$). They also reported that the weights of the heart ventricles and adrenals were significantly increased in the exercised animals ($p < .01$). The exercised animals gained 30-40% less body weight than the controls. They suggest that SDH appears to be present in both heart ventricles and skeletal muscle in amounts greater than that needed to cope with the stress offered by moderate exercise.

An attempt was made to investigate the possibility of using the SDH reaction in the gross identification of early myocardial infarcts (44). This technique took advantage of both substrate and enzyme loss from the infarct. Their results showed that the necrotic muscle fibers remained unstained or faintly stained using SDH.

Using fresh frozen tissue, Nachlas et al. (45) demonstrated SDH activity histochemically by incorporating

NBT as the hydrogen acceptor. NBT competes successfully with oxygen for the available electrons. The reaction gave rich blue diformazan particles as indicator sites of SDH activity. The specificity of the reaction was tested by the omission of succinate from the incubating medium. This technique permits the cytochemical visualization of the sites of SDH enzymatic activity in tissue sections. The method is further described in Barka and Anderson (6).

Wachstein and Meisel (66) investigated SDH activity in several diseased states of the myocardium. They reported that the loss of SDH activity in those muscle fibers that were undergoing necrotic changes due to anoxia were striking. In the areas of myocardial necrosis, SDH activity decreased rapidly.

In a related study, Nielson and Klitgaard (47) examined the changes in SDH activity as related to feeding. Using rats, they concluded that daily variations in the activity of SDH were related to periods of eating and fasting. These changes were of such magnitude as to possibly mask other experimental alterations in enzyme activity.

Cytochrome oxidase techniques described by Burstone (11, 12) have been frequently used by investigators interested in the electron transport system. It was indicated that Cy Ox is measured in an indirect manner. Oxidized cytochrome c is reduced by p-aminodiphenylamine. This

reduction results in the formation of reduced cytochrome c and oxidized p-aminodiphenylamine. This latter product combines with 1-hydroxy-2-naphthoic acid and the red-brown to blue-black reaction product is produced. Cy Ox oxidizes reduced cytochrome c, thus replenishing the available oxidized cytochrome c with the formation of water. Cy Ox does not act directly on the reducing substance (64).

Seligman (58) showed that Cy Ox was in the mitochondrion and could be localized specifically by DAB (Diaminebenzadine). In rat myocardium, he used NBT and found SDH to be localized in the same place as Cy Ox. These data confirmed that the localization of SDH was similar to that of Cy Ox. These data also showed that both pigments (DAB for Cy Ox and diformazan for SDH) are appearing on one side of the mitochondrial membrane (the outer surface of the inner mitochondrial membrane).

Myocardial Pathology

Reporting on several pathological conditions found in the heart, Opie (50) said the early occurrence of mitochondrial damage explained the decreased rates of oxidative phosphorylation with a shift from aerobic to anaerobic metabolism in terminal heart failure. The co-existence of the serum LDH pattern, which indicated myocardial damage, with electrocardiographic and radiological abnormalities was strong evidence for myxedema heart disease. In obstructive cardiomyopathy, there was a

tendency to increase lactate production and increase anaerobic metabolism by the heart. Within sixty seconds of infarction, the myocardial cells failed to contract. Opie stated that there was no obvious reason as to why the anoxic heart should stop beating so soon. Creatine phosphate disappears almost immediately and within fifteen minutes ATP is no longer present. The duration of anoxia which is required to produce irreversible damage appears to be greater than fifteen minutes and probably about thirty to sixty minutes. In ischemic heart disease it is not known which factors restrain anaerobic glycolysis from maximal rates which could, perhaps, contribute to the energy supply of the anoxic heart.

Bing (7) indicated that a number of pathologic states may induce anoxia with a consequent shift of cardiac metabolism toward anaerobiosis. With myocardial anoxia, the heart goes into a negative balance of lactate and pyruvate which may be seen in hemorrhagic shock, ventricular tachycardia and fibrillation, atrial fibrillation and in the temporary interruption of coronary flow by embolization of the coronary arteries. The anoxia leads to a disappearance of glycogen with an increase of lactate and glucose-6-phosphate in the myocardium. The lactate accumulates due to the lack of oxygen present; therefore, there is a decreased utilization of lactate. During ventricular tachycardia and fibrillation, the cardiac glycogen decreases

while lactate, pyruvate, glucose-6-phosphate and phosphorylase a increase.

Shnitke and Nachlas (60) observed fresh frozen sections of dog myocardium after they had been incubated for SDH activity. The diformazan pigment, which indicated SDH activity, was restricted to the myocardial cell in the normal heart. Complete loss of SDH activity was noted one day to five days after an infarct in the area of necrosis. Five days after the infarct, DPN diaphorase activity and Cy Ox activity markedly declined, also.

Reichenbach and Benditt (55) described a response of the myocardial cell to injury which was different from coagulation necrosis of ischemic infarction. The major features of the lesion included the following: segmentation of myocardial cell cytoplasm seen as transverse bands between areas of granularity; localization of this cytoplasmic alteration within a short time after injury; frequent localization of damage in the subendocardial cells of the ventricles; a proliferative interstitial cellular reaction; no neutrophils were seen to be present; sparing of the reticulum stroma, blood vessels and nerves; and in some cases, mineralization had occurred in the mitochondria. This lesion may be produced experimentally by several chemical, metabolic and mechanical means (54). The injury affects the structural organization of the myofibrils and is at least partially reversible.

There is a considerable amount of disturbance to the normal metabolism occurring in the region of an infarct. According to Rubel (56), various zones with different levels of enzymatic activity are formed. In the unaffected regions of the heart, metabolism increases presumably to compensate for the losses which may lead to cardiac hypertrophy.

In an attempt to evaluate the susceptibility of domesticated white rats and of wild gray Norwegian rats to myocardial necrosis formation under sensory and emotional stresses, researchers (53) examined H & E stained sections of myocardium from these animals. Upon analysis, the lesions were rated on a scale from 0 to 3. Seventy per cent of the wild rats had myocardial lesions while about 40 per cent of the domesticated rats exhibited myocardial necroses.

Letunov (37) presented data which showed a small percentage of sportsmen differing from the normal in terms of cardiovascular abnormalities. However, in a majority of the cases, the origin of the cardiovascular disease could not be traced to sports activities. He explained that these pathologic conditions usually occurred because of a previous or current infection not related to athletics. He concluded that the adaptation of the individual to muscular activity in the course (years) of athletic training was accompanied by functional changes in middle-aged people that were characteristics of trained athletes.

Investigating the effects of hard physical training on the cardiovascular system of youth, Kitamura (33) reported that excessive training in the very young could be injurious to the heart. After training 200 25-day-old mice for varying periods of time, his data indicated that after six weeks of training there was hypertrophy of the myocardial fibers and of their nuclei. After ten weeks of training, he reported hypertrophy, interstitial fibrosis, cellular infiltration and petechial hemorrhages to be present within the myocardium. He also showed that the capillary to fiber ratio first decreased then increased after terminating the training which was concomitant with atrophy of the muscle fibers.

Investigators (34) performed comparative studies on mitochondria of left hypertrophic heart ventricles of rats. Some of the rats had aortic stenosis experimentally induced, while others were subjected to physical training. Hypertrophy due to both types of experimental treatment showed an increased voluminal mitochondria-to-myofibrillae ratio. The density of the cristae remained approximately normal in the trained animals, whereas the stenosed rats showed a poor internal structure of mitochondria. The mitochondrial protein level of the myocardium was reduced in the stenosed rats, while being unchanged in the swimming rats. Enzyme assays showed a rise of malate dehydrogenase activity amounting to 70% in the stenosed rats and to 170% in the swimming rats.

French (18) described myocardial lesions in potassium-deficient rats. There is a primary degeneration of muscle substance which eventually leads to necrosis and disintegration of myocardial fibers. The muscle debris is removed by phagocytes. Secondary to the fiber degeneration, there are changes in the connective tissue. These changes consist of interstitial edema, proliferation of vascular endothelium and fibroblasts with mobilization of macrophages. He reported no conclusive evidence of effective muscle regeneration. Healing takes place (after potassium is added to the diet) mainly by hypertrophy of the surviving muscle fibers and by condensation of the surrounding connective tissue.

Klionsky (35) reported that the earliest lesion, in evidence of myocardial ischemia is the loss of the labile fraction of glycogen as measured by PAS.

Bajusz and Homburger (3) indicated that myocardial cellular necrosis evolves along a number of different pathways. Ischemia causes two types of necrosis. In the center of the ischemic area, the edematous cardiac cells undergo cytolysis. While in the periphery of the ischemic area, there is an initial contraction of portions of the myofibrils which terminate in coagulation necrosis. The areas of the myocardial cell that are most susceptible to injury include (in order): the sarcoplasmic reticulum, the nucleus, and the mitochondrion.

Bajusz and Raab (4) stated that some forms of human necrotizing cardiomyopathies are attributed to the reflex liberation of catecholamines which exert a contributory noxious effect on cardiac metabolism. Comparative histochemical studies showed that when there was an early decline in myocardial phosphorylase activity with a concomitant decline in the amount of stainable glycogen, anoxic myocardial damage could be deduced. They wanted to study the types of cardiac damage that would be produced with injections of epinephrine. Their results showed that a decrease of histochemically demonstrable potassium occurs within some areas of the myocardium about ten to fifteen minutes after the administration of epinephrine. The involved foci showed a decrease of potassium with a parallel depletion of glycogen reserves, as well as a decrease in phosphorylase activity. These areas were mainly located in the subendocardium and apex where necrotic lesions usually occur. The reactions for oxidative enzymes remained normal or were even somewhat elevated within the respective areas. They reported that these early histochemically demonstrable alterations in the myocardium following injection of epinephrine are identical to those seen in anoxic areas of myocardium following coronary artery ligature.

CHAPTER III

RESEARCH METHODS

Sample

Two hundred and fifty-two, 71-day-old, male, albino rats (Sprague-Dawley strain) were brought into the laboratory in eight shipments. The animals were assigned at random to one of seven treatment groups as shown in Figure 1. Prior to the treatment period, each animal was allowed thirteen days to adjust to the laboratory. The housing for the adjustment period varied according to the treatment group into which an animal was placed. The animals which were placed into the CON group were housed in individual sedentary cages during the adjustment period while the animals that were placed into the remaining six groups were housed in individual voluntary activity cages during the adjustment period. One hundred and twenty-six of the healthiest and best trained animals were selected for the final sample. Table 1 depicts the numerical arrangement of the final sample by treatment and duration.

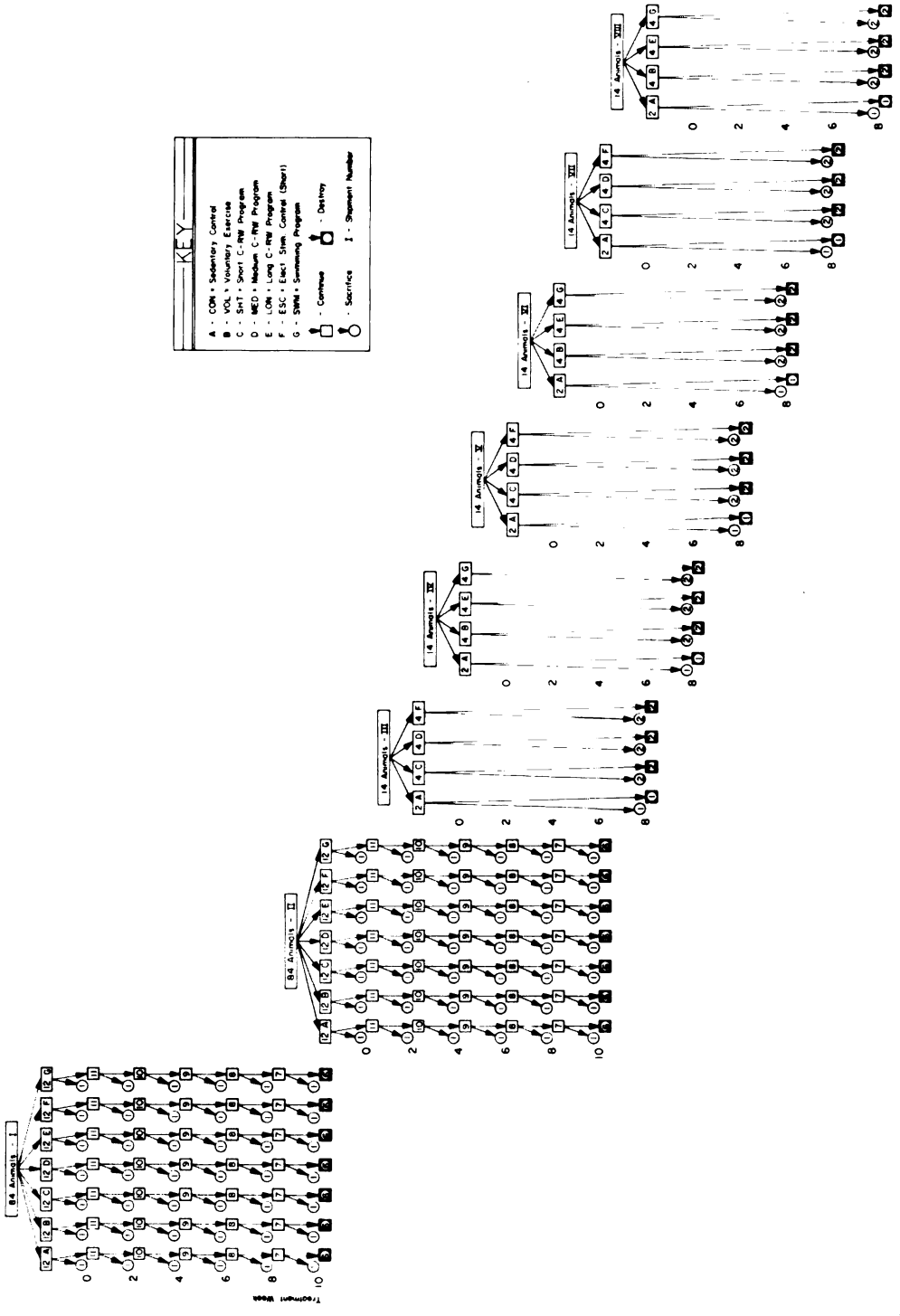

Figure 1 – Flow Sheet of Animal Arrivals, Treatments and Sacrifices

TABLE 1.--The number of animals in each cell by treatment and duration.

Duration	Treatment						
	CON	VOL	SHT	MED	LON	ESC	SWM
0-wk	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]	2[a]
2-wk	2	2	2	2	2	2	2
4-wk	2	2	2	2	2	2	2
6-wk	2	2	2	2	2	2	2
8-wk	8	8	8	8	8	8	8
10-2k	2	2	2	2	2	2	2

[a]Since none of these animals had received any experimental treatment, it was deemed appropriate to group these animals together (N=14) and call them 0-wk CONS.

Treatment Groups

The seven treatment groups used in this study were as follows:

CON

These animals were housed in individual sedentary cages (24 cm. long by 18 cm. wide by 18 cm. tall) during both the adjustment and treatment periods.

VOL

These animals were housed in individual voluntary activity cages (sedentary cage with access to a freely revolving activity wheel) during both the adjustment and the treatment periods. The attached freely revolving

activity wheel had a width of 13 cm. and a diameter of 35 cm. An automatic revolution counter which was attached to the activity wheel recorded each revolution of each wheel. These animals exercised at will.

SHT

These animals were housed in individual voluntary activity cages during the adjustment period and in individual sedentary cages during the treatment period. The exercise treatment that these animals received (short C-RW program) was such that on each of the last four days of the eight-week training program, they were required to run 49 ten-second sprints at six feet per second (69). Those animals that were trained for ten weeks followed the fortieth day SHT program during each of the last ten days (see Appendix A).

MED

These animals were housed in individual voluntary activity cages during the adjustment period and in individual sedentary cages during the treatment period. The exercise treatment that these animals received (medium C-RW program) was such that on each of the last four days of the eight-week training program, they were required to run 40 thirty-second sprints at four feet per second (69). Those animals that were trained for ten weeks followed the fortieth day MED program during each of the last ten days (see Appendix A).

LON

These animals were housed in individual voluntary activity cages during the adjustment period and in individual sedentary cages during the treatment period. The exercise treatment that these animals received (long C-RW program) was such that on each of the last four days of the eight-week training program, they were required to complete 4 twelve-minute-thirty-second runs at two feet per second (69). Those animals that were trained for ten weeks followed the fortieth day LON program during each of the last ten days (see Appendix A).

ESC

These animals were housed in individual voluntary activity cages during the adjustment period and in individual sedentary cages during the treatment period. These animals received no exercise treatment. However, during the treatment period, these animals were paired with animals from the SHT group. When an SHT animal entered the C-RW wheel for his daily exercise, the paired ESC animal was placed into an adjacent stimulus control cage (21.5 cm. long by 14 cm. wide by 10.5 cm. tall) which had a grid floor (69). The ESC animal was exposed to the same light stimulus to which the SHT animal was exposed. The amount of shock that the SHT animal received was, at the same time and with the same intensity, transferred to the ESC animal.

SWM

These animals were housed in individual voluntary activity cages during the adjustment period and in individual sedentary cages during the treatment period. The exercise treatment that these animals received (swimming program) was such that at the conclusion of the eight-week training program, each animal was required to swim one sixty-minute bout with 3 per cent of his body weight attached to his tail. Those animals that were trained for ten weeks followed the fortieth day SWM program during each of the last ten days (see Appendix A).

Treatment Procedures

The treatments began on the thirteenth day that the animal was in the laboratory (84 days of age). Three of the exercise treatments (SHT, MED, LON) and one of the control treatments (ESC) used the C-RW apparatus which " . . . is a unique animal-powered wheel which is capable of inducing small animals to run at a prescribed speed for one or more prescribed durations of time" (69, p. 4). A low-intensity controlled shock current provided motivation for the animal to run. However, the shock was always preceded by a light; therefore, the animal could avoid the shock by responding (running) to the light. The time during which the light was on was termed the acceleration time. This acceleration time began each running period

of exercise. At the end of the acceleration time, the light was turned off and the shock current was applied to the running (grid) surface of the wheel to induce the animal to run at the prescribed speed. In those cases in which the animal had responded to the light and had attained the prescribed speed during the acceleration time, the light was turned off and no shock current was applied. If the animal had attained the prescribed speed while it was being shocked, the shock was immediately discontinued. If the animal slowed down below the prescribed speed, the light and shock (if necessary) sequences were repeated. A typical running program consisted of alternate periods of work and rest. During the work periods (running period of exercise), the wheel was free to turn, while during the rest periods, the wheel was braked automatically to prevent spontaneous activity. A specified number of alternating work and rest periods (repetitions) constituted one bout of exercise. A single training period could include several bouts separated by time between bouts (rest periods). See Appendix A for the individual eight-week training programs.

The animals in the SWM group were swum in individual cylindrical tanks which measured 27.94 cm. in diameter and 76.20 cm in depth. Tail weights, when required, were calculated, using body weight before treatment from the previous Friday. These weights were attached to the tips of the tails by means of miniature plastic clothespins.

If an animal was unable to complete the EST, the animal was removed and his STC for that day was recorded. After each swim, the animals were initially dried with a towel and then put into a carrying cage which was placed under a heat lamp until the animals were completely dry. See Appendix A for the individual eight-week training program.

The animals in the VOL group had revolution counter readings taken at approximately the same time (A.M.) Monday through Friday. Total voluntary activity (revolutions run) for the previous twenty-four hour period, four days per week, Tuesday through Friday, was calculated.

Treatments were given Monday through Friday. The SHT, MED, LON, ESC, and SWM treatments were given in the treatment room in subdued normal room light. The animals in treatment groups SHT, MED, LON, and ESC were weighed daily before and after treatments. SWM group animals were weighed before their daily treatment. TRR and CDS were collected daily for treatment groups SHT, MED, and LON, while ESC used the values for SHT. These data facilitated the calculation of PER and PSF. For the SWM group, STC was collected daily which facilitated the calculation of PET.

Animal Care

Each animal was placed into a clean cage every two weeks. The animals were provided with food (Wayne Laboratory-Blox) and water ad libitum. Ambient air

temperature in both the animal quarters and the treatment room was maintained between 18°C. and 27°C. The relative humidity was maintained between 15% and 44%. The water temperature for the SWM group was maintained between 28°C. and 32°C. The animals were exposed to a sequence of twelve hours of light followed by twelve hours without light daily which was automatically controlled by an electrical timer. When possible, the paper in the animal quarters was changed daily, and usually not less frequently than every other day. The paper under the C-RW apparatus (wheel and stimulus control cage) was changed daily. The C-RW apparatus was washed daily. Each C-RW apparatus was cleaned thoroughly once a week. When possible all animals in all treatments were handled daily, Monday through Friday, during both the adjustment and the treatment period.

Sacrifice Procedures

Animals were selected for sacrifice on the bases of their health and their performance during the treatment programs. Good health was determined subjectively by observing each animal. Adequate performance was determined objectively by observing each animal's graphed values for PER, PSF, PET, and revolutions run, where applicable. The daily mean data (TRR, PER, CDS, PSF) for the SHT, MED, and LON treatments appear graphically in Appendix A. The daily mean body weights for the SHT, MED, LON, ESC, and

SWM treatments also appear graphically in Appendix A as do the daily mean revolutions run by the VOL group.

Animals were selected after their last treatment on Friday for sacrifice on Monday. Selection of animals, in general, was based upon their overall training curve. However, when such animals performed poorly on the last day of training, they were replaced by the next most qualified animals. Mean values for their performance on the last day of training may be seen in Table 2.

TABLE 2.--Mean performance values from the last day of training for the sacrificed animals.

Treatment	Data			
	Revolutions Run	PER	PSF	PET
VOL	921.2	. .	. .	. .
SHT	. .	54.1	60.6	. .
MED	. .	63.2	67.8	. .
LON	. .	72.3	65.9	. .
ESC	. .	. .	60.6	. .
SWM	. .	. .	. .	100

Body weight was taken at sacrifice. Seven animals were sacrificed every two weeks for thirty-six consecutive weeks (see Figure 1).

During the course of this experiment, certain procedures were either added to or deleted from the original

sacrifice sequence. For the sake of clarity to this presentation, only those procedures that were followed the majority of the time will be reported here. A detailed account of the modifications may be read in Appendix B.

Each animal was anesthetized by an intraperitoneal injection with 3.5 cc. of a 6.48% Halatal[1] solution (sodium pentobarbital). A transverse laparotomy was made at about the level of L_4 to L_6. After cutting through the overlying skin, fascia, and connective tissue, the incision was continued through the following musculature: Obliquus Externus Abdominus, Obliquus Internus Abdominus, Transversus Abdominus, and Rectus Abdominus (26). Once into the abdominal cavity, the incision was extended cranially as a parasagittal incision to the right of the xiphoid process, through the costal cartilages to the level of the second rib. The heart was located within the thoracic cavity. The parietal pericardium was removed and with the heart being held over toward the right, the major blood vessels were severed as close to the atria as possible.

Once the heart was removed, it was cut transversely at a level just below the atria. The ventricular portion of the heart, with the apex pointing up, was held in place on an Ames Lab-Tek Cryostat Chuck by 5% gum tragacanth. The prepared check was held with forceps and was lowered,

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[1]From Jensen-Salsbery Laboratories, Division of Richardson-Merrell, Inc., Kansas City, Missouri.

for approximately twenty seconds, into pre-cooled 2-methylbutane (isopentane). The isopentane had been previously cooled to a viscous fluid (-173°C.)[1] by liquid nitrogen. The chuck was placed into an empty 35 mm. film can. The can was put onto a shelf in the cryostat. Within thirty hours, the heart was sectioned at two levels approximately 300 μ and 600 μ from the apex (see Appendix E). Serial sections of 10 μ each were taken at each level at a temperature of -20°C. in the cryostat. The sections were immediately placed onto cover glasses and air-dried for a minimum of one hour and a maximum of two hours. Only the sections cut at the 300 μ level are presented herein.

Histological Procedure

A fresh frozen H & E technique (38) was used to examine the morphology of the left ventricle.

Histochemical Procedures

Presence of glycogen was investigated using the PAS reaction (38). Fatty acids were depicted using Holczinger's technique (30). LDH activity was studied using NBT as the electron acceptor and DPN as the cofactor (52). SDH activity was examined using NBT as the electron acceptor as described by Barka and Anderson (6). The presence of

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[1]As measured by T 2120-3 thermometer which was supplied by Scientific Products, Evanston, Illinois.

Cy Ox activity was studied using the technique of Burstone (52). Incubation times varied according to the individual procedure used.

Slides stained with H & E and PAS were mounted in Histoclad while the other slides were mounted in glycerin jelly. Within three days of sacrifice, all slides were sealed with Diatex.

Previous studies from this laboratory had ascertained the specificity of the enzyme reactions using control sections (16). Control sections were incubated with each of the enzyme procedures during the last three sacrifices so that specificity of the enzyme reaction could be determined. The control incubating media excluded the substrate in some cases (SDH, LDH), and instituted a formalin fixation in the other case (Cy Ox) (64).

At variable time intervals during the course of the study, the slides from the sacrifices were coded by independent members from the laboratory. Therefore, when the analysis was to be performed on the individual slide, there was no information present to indicate either animal number, treatment group, duration, or date of sacrifice.

Tissue Analysis Procedures

Because the heart is a rather homogeneous tissue in terms of its metabolic characteristics (13), the decision was made to choose an area of the left ventricle that could be located consistently in each tissue section.

The constrictor band of muscle (57) which corresponds closely to the cylindrical layer of muscle (63) in the left ventricle was chosen for study.

Histochemical

Each histochemical stain was evaluated objectively with the use of a light transmission meter which used a magnification of 80 X (70). The operator of the light transmission meter was able to isolate the photometric beam on one cardiac muscle fiber (on cross section). The meter was able to determine (on a scale from 0 to 100) the per cent of light that was transmitted through that fiber. The light transmission meter was judged to be accurate to $\pm$ 0.3% (70). Sixty-six fibers were selected at random within the constrictor band of muscle in the left ventricle for light transmission readings from those tissue sections which were taken closest to the apex of the heart. Therefore, sixty-six readings were taken for each stain for each animal. It was felt that more meaning could be gleaned from the histochemical data if the per cent light transmitted values were converted to per cent light absorbed values. This change was instituted by subtracting each of the per cent light transmitted values from one hundred.

Histochemical Reliability.--Reliability of the histochemical data was determined by selecting twenty-five slides at random. Sixty-six light transmission readings

were taken from each slide. The mean values were compared to the mean values from the original data using a rank order correlation coefficient (Rho). Statistical significance was determined with the use of a t-test (1). Rho was +.998 which was significant at the .05 level. The data may be seen in Appendix C.

Histochemical Validity.--Validity of the histochemical data could only be determined by using previously recorded histochemical skeletal muscle staining intensity data from this laboratory. This was necessary because the skeletal muscle (gastrocnemius) showed adjacent cell staining intensity differences, whereas, the heart showed adjacent cell staining intensity homogeneity. Therefore, to subjectively rate the hearts by eye, one would need to assess staining intensity of adjacent hearts. This would be quite difficult. Rho was calculated which compared expert subjective eye ratings (dark: 1 to light: 3) to objective per cent light absorbed values (100% to 0%). This was done on ten fibers from three stains. Calculated Rho's were +.885, +.900, and +.870, for SDH, Sudan Black B, and ATP'ase, respectively. Each was significant at the .05 level. The data may be seen in Appendix C.

The Cy Ox staining intensity faded inconsistently between sacrifice and meter readings. Therefore, these data were not statistically analyzed. However, the individual mean per cent light absorbed values of Cy Ox

are located in Appendix C by animal number, treatment, and duration. No information important to the study was lost (58).

Pathological

The heart sections stained with H & E were evaluated subjectively. They were divided into two groups on the bases of normalcy and pathology exhibited.

Pathological Reliability.--Reliability of the pathological data was determined by selecting ten slides at random and subjectively re-evaluating them. These evaluations were compared to the original data using Rho. The calculated Rho was +.976 which was significant at the .05 level.

Pathological Validity.--Validity of the pathological data was determined after consultation with a pathologist, who specializes in diseases of the cardiovascular system, and after consultation with an anatomist, who specializes in research on cardiac muscle. Each professor subjectively evaluated ten slides. These values were compared to the original observations which were made by the author. Calculated Rho's were +.964 and +.952 for the pathologist, and the anatomist, respectively. Each was significant at the .05 level.

Statistical Procedures

Mean values for each animal for the histochemical data were determined using the UNEQ1 routine by the

Michigan State University Control Data 3600 Computer (CDC 3600). Statistical calculations on these mean values and on body weight at sacrifice, using the LS routine for two-way analysis of variance (treatment and duration), and for adjusted cell means for the interaction were also performed by the CDC 3600. Subsequent statistical calculations on the mean histochemical values and on the mean body weight at sacrifice were performed by the CDC 3600 using the LS routine for one-way analysis of variance (0-wk CONS and 8-2k by treatment), and for another one-way analysis of variance (8-wk by treatment). Scheffé's method for multiple comparisons was employed when the F-ratio was judged to be significant (27). This work was also done by the CDC 3600. When the F-ratio was judged to be not significant, the power of the test was hand calculated (27). The probability of committing a Type I error (α) was set at .05 for the one-way analyses of variance. The probability of committing a Type II error (β) was set at .25 for the one-way analyses of variance. Significance levels for the Scheffé tests were set at $\alpha = .10$.

The pathological data were statistically analyzed using chi-square contingency tables (14). One table was designed to compare the 0-wk CONS data with the 8-wk treatment results. A second table compared the values of the various treatment groups at 8-wk.

CHAPTER IV

RESULTS AND DISCUSSION

Results

Effectiveness of the Training Programs

The initial concern in this study was the effectiveness of the training programs. The programs were relatively new and although considerable pilot testing had been done there was still some mild apprehension present. Since the entire study depended upon the results of these programs, meticulous records of the rats' performances were kept.

The body weight results are shown in Figure 2. When analyzed by one-way analysis of variance and subsequent to obtaining a significant F-ratio, Scheffé comparisons run, the LON group at 8-wk was significantly lighter than the CON group at 8-wk. However, the number of cases were small with eight rats in each group. When one utilizes all of the body weight data in Figure 2, it can be observed that all mean values for the exercise groups, except for the sixth week value for SWM, were lower than the mean body

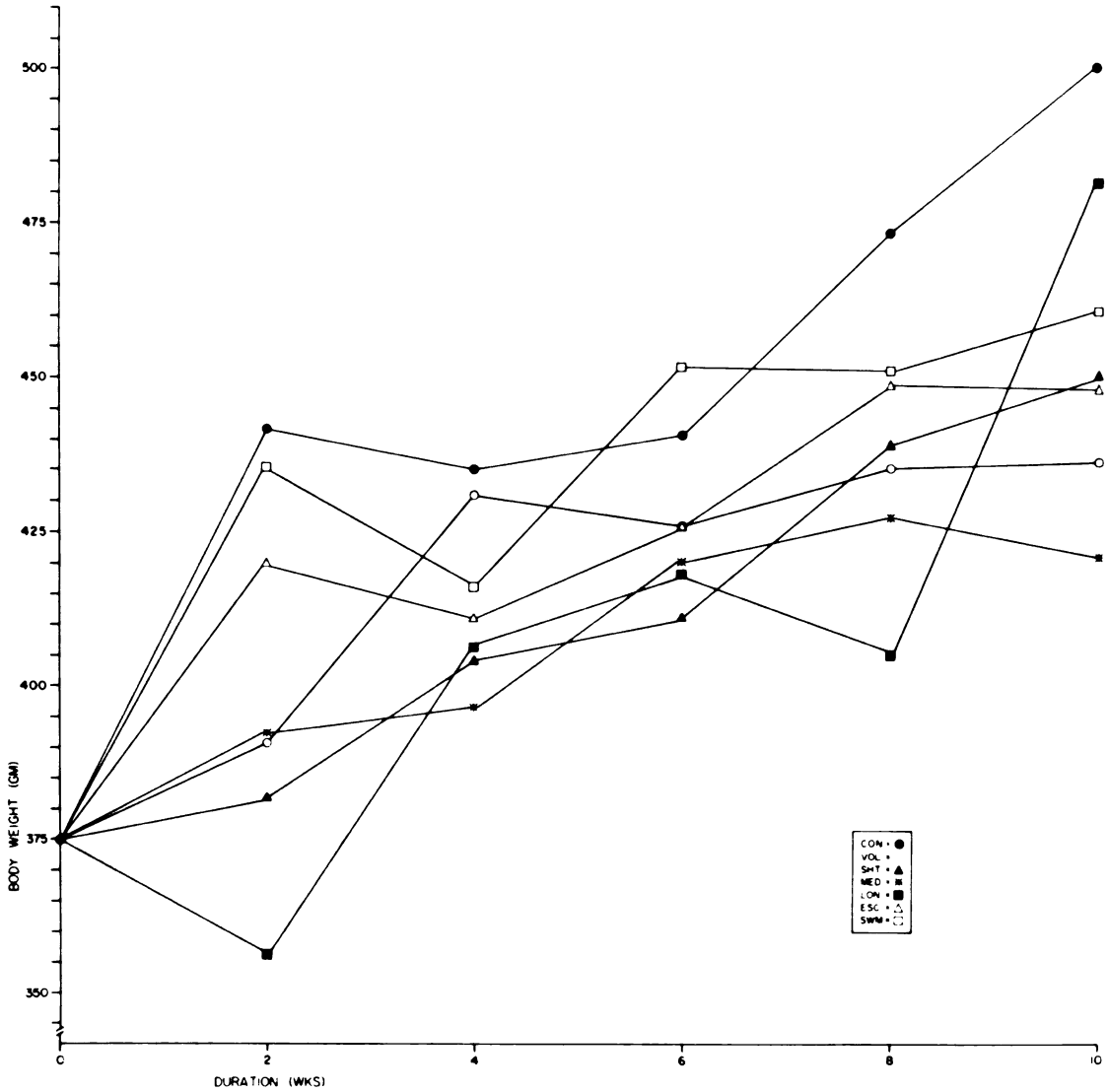

Figure 2 – Mean Body Weight

N = 2 for each group of durations 2, 4, 6, 10 wks
N = 8 for each group of duration 8 wk
N = 4 for 0 wk

weights for the CONS. Therefore, using the sign test, the body weights for VOL, SHT, MED, LON, and ESC were all significantly lower than the body weights for CONS ($p < .05$). The programs utilized were effective in that body weights of the trained animals were lower than the body weights of the control animals. The significant differences shown in Table 3, comparing 8-wk data with 0-wk controls, were expected on the basis of normal growth. The lack of significance in the LON merely reflects the greater effect of that program on the rats in terms of body weight.

TABLE 3.--Scheffé comparisons for body weight between 0-wk CONS and 8-wk treatment.

Scheffé Comparison		Body Weight
		F = 12.09
0-wk	8-wk	p < 0.0005
CONS	- CON	S[a]
CONS	- VOL	S
CONS	- SHT	S
CONS	- MED	S
CONS	- LON	N[b]
CONS	- ESC	S
CONS	- SWM	S

[a]Significant at .10 level.
[b]Not significant.

Considerable insight into the training programs can be obtained from the mean daily TRR, PER, CDS, PSF, and revolutions run for the SHT, MED, LON, and VOL groups as shown in Appendix A. These data indicate that the MED and the LON groups increased their daily TRR from day one

through day forty. The SHT group's TRR tapered off after day twenty-five. Even though the LON group has higher CDS values than either the SHT or the MED group, the PSF values indicate that between day thirty and day forty the LON group ran better than 75% shock free.

TABLE 4.--Scheffé comparisons for body weight between 8-wk treatment.

Scheffé Comparison	Body Weight
	F = 4.07
	p 0.002
CON - VOL	N[b]
CON - SHT	N
CON - MED	N
CON - LON	S[a]
CON - ESC	N
CON - SWM	N
VOL - SHT	N
VOL - MED	N
VOL - LON	N
VOL - ESC	N
VOL - SWM	N
SHT - MED	N
SHT - LON	N
SHT - ESC	N
SHT - SWM	N
MED - LON	N
MED - ESC	N
MED - SWM	N
LON - ESC	N
LON - SWM	N
ESC - SWM	N

[a]Significant at .10 level.

[b]Not significant.

Histochemical

The group mean values of percentage of light absorbed may be seen by treatment and duration in Figures 3 through 6 for LDH, FA, SDH, and PAS, respectively. The individual mean values of percentage of light absorbed may be seen by animal number, treatment, and duration in Appendix C. The one-way analysis of variance tables are located in Appendix D. The Scheffé comparisons between the 0-wk CONS and the various 8-wk treatment groups are indicated in Table 5.

TABLE 5.--Scheffé comparisons for LDH, FA, SDH, and PAS between 0-wk CONS and 8-wk treatment.

Scheffé Comparison		LDH	FA	SDH	PAS
		F = 0.64	4.65	0.98	3.06
0-wk	8-wk	p = 0.723	<0.0005	0.457	<0.008
CONS	CON	. .[c]	N[b]	. .	N
CONS	VOL	. .	S[a]	. .	N
CONS	SHT	. .	N	. .	N
CONS	MED	. .	N	. .	N
CONS	LON	. .	S	. .	S
CONS	ESC	. .	N	. .	N
CONS	SWM	. .	S	. .	N

[a]Significant at .10 level.

[b]Not significant.

[c]Judgment was reserved because the calculated power was <.30.

None of the one-way analyses of variance on the 8-wk treatment groups for LDH, FA, SDH, or PAS was significant. Power was calculated and was <.30 in each case; therefore, judgment was reserved on all comparisons.

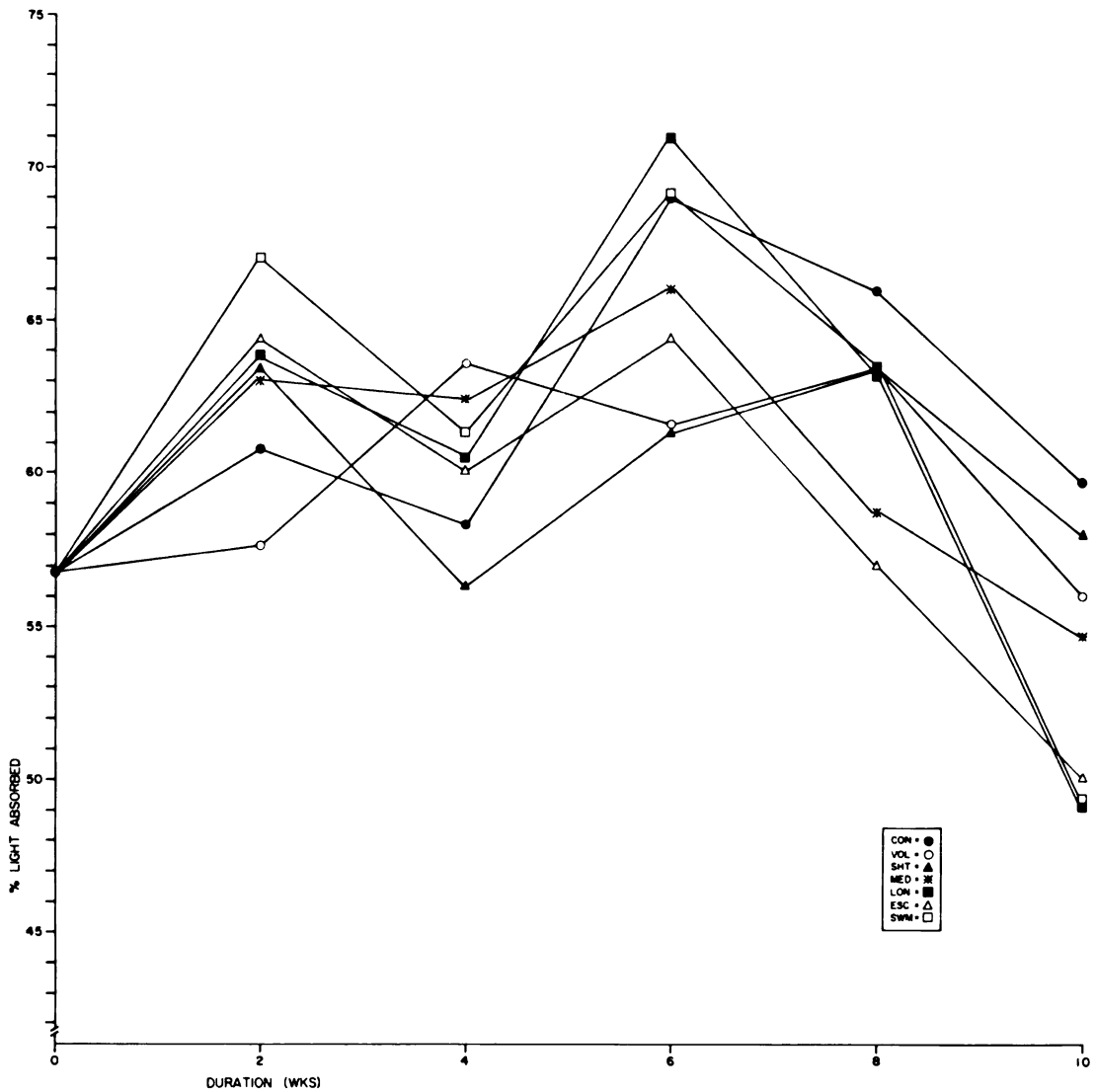

Figure 3 – LDH Mean Values

N = 2 for each group of durations 2, 4, 6, 10 wks.
N = 8 for each group of duration 8 wks.
N = 14 for 0 wk.

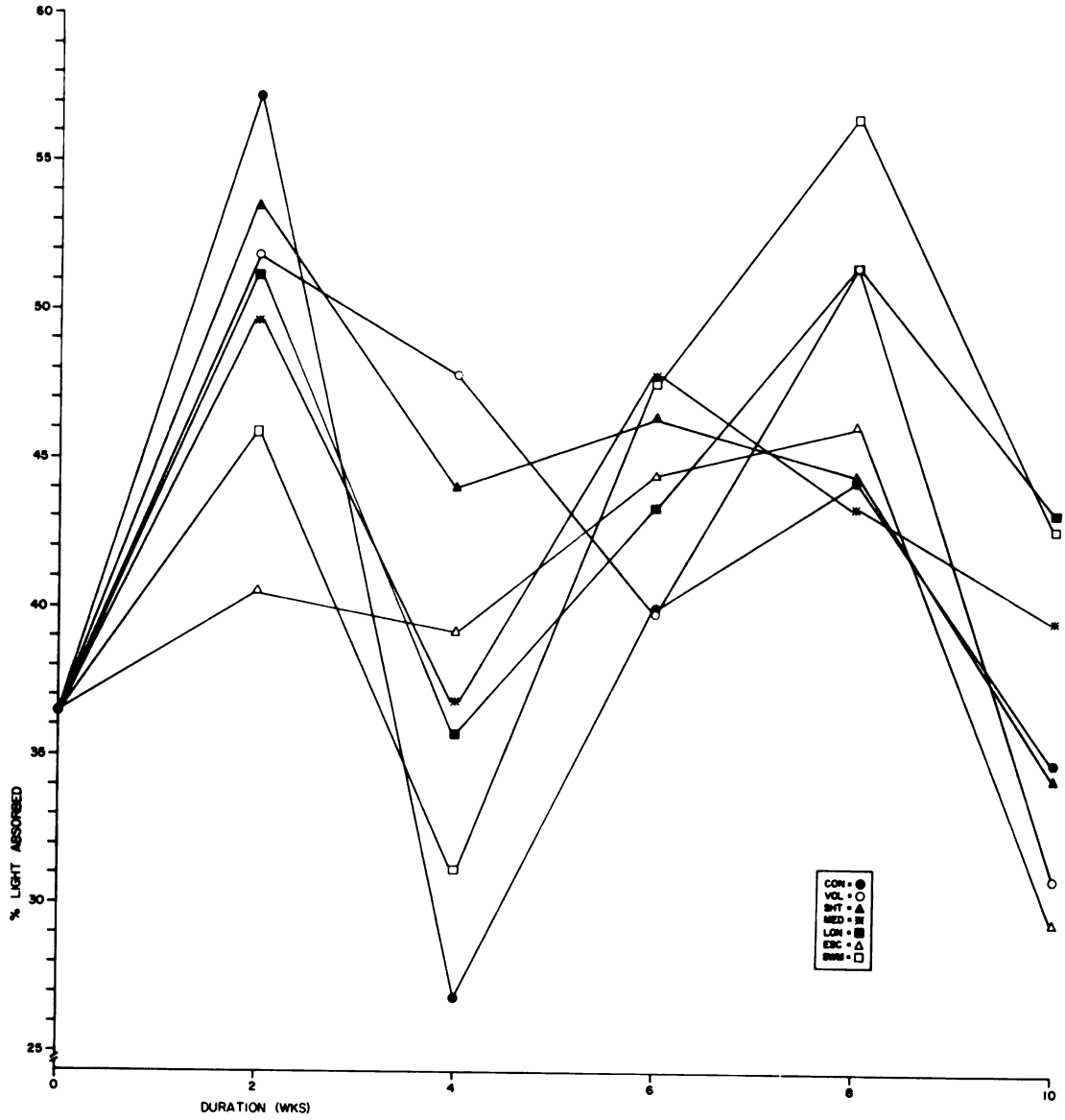

Figure 4 – FA Mean Values

N = 2 for each group of durations 2, 4, 6, 10 wks.
N = 8 for each group of duration 8 wks.
N = 14 for 0 wk.

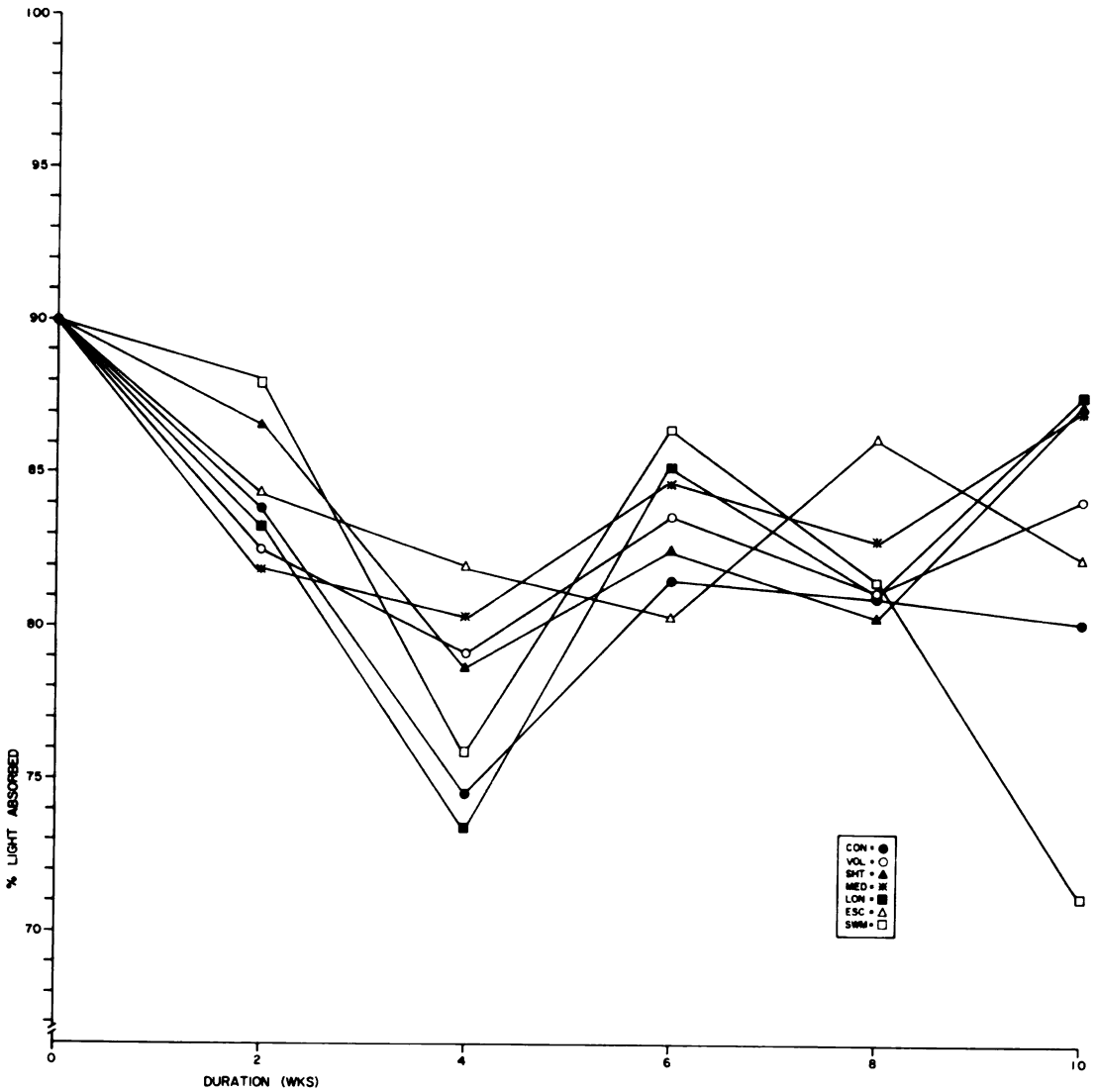

Figure 5 - SDH Mean Values

N = 2 for each group of durations 2, 4, 6, 10 wks.
N = 8 for each group of duration 8 wks.
N = 14 for 0 wk.

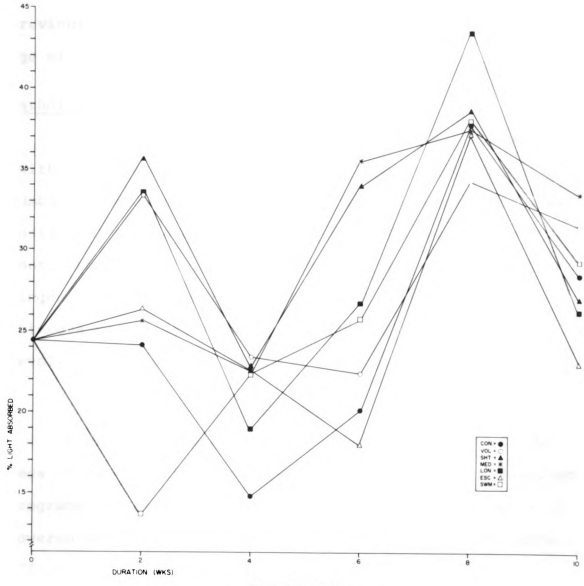

Figure F. - RAS Mean Values

N = 2 for each group of durations 2, 4, 6, 10 wks
N = 8 for each group of duration 8 wks
N = 4 for 1 wk

Control slides of SDH, LDH, and Cy Ox for the last three sacrifices reflected the same information that was previously obtained (16). In this case, the mean percentage of light absorbed was <5% for all slides.

Pathological

The hearts were diagnosed either as being normal (within the range of normality) or as having a minimal number of focal accumulations of lymphocytes present. The individual diagnoses may be seen by animal number, treatment, and duration in Appendix C. The chi-square contingency tables appear in Appendix D. Neither of the chi-squares was statistically significant at the .05 level. Essentially, the hearts were non-pathologic.

Discussion

A primary interest in this study was the effectiveness of the training programs. Were the endurance training programs effective? The results indicate that in terms of lowered body weight, all endurance training programs were effective. Gollnick and Hearn (20) reported that the rats they had swum thirty minutes daily for thirty-five consecutive days gained 38.7% less body weight than the rats they had maintained as sedentary controls. Similar results were reported by Hearn and Wainio (28) in which their exercised animals gained 30-40% less body weight than their sedentary controls. Van Huss, Heusner, and

Mickelsen (65) indicated that at puberty the forced-exercise animals had lower body weights than the sedentary control animals. Effectiveness of a training program is indicated by a decreased body weight gain.

The histochemical and pathological data take on new meaning if in fact a training effect had occurred to the rats. For the purposes of this discussion, the assumption is made that a training effect, as indicated by lowered body weight, had occurred to those rats who were in the endurance training programs.

Histochemically, the data support the original hypothesis in terms of the fatty acid concentrations at eight weeks in the VOL, LON, and SWM groups. It was hypothesized that the LON group would have its ventricular metabolism move more toward that of total aerobiosis after the treatment period. It was stated by Opie (49) that the heart (aerobic muscle fibers) would rather use lipid than carbohydrate as its energy source. When the physical activity becomes one of daily submaximal loads, aerobiosis is enhanced in the myocardium. Although the 8-wk CON, SHT, MED, and ESC groups did not significantly increase their fatty acid values as compared to the 0-wk CONS group, the data do indicate that some type of metabolic adaptation was occurring in the heart which indicated a shift of the metabolism toward more total aerobiosis than was present prior to the treatment period.

It was interesting that the only significant histochemical differences found were in the substrate data in which fatty acid and glycogen concentrations were being measured. The enzyme concentrations of LDH and SDH were not different. These results support the earlier biochemical data of Hearn and Wainio (28). The fact that the LDH and SDH concentrations did not increase as a result of chronic exercise, was surprising. Since SDH and LDH are well recognized enzymes in the aerobic pathway, increased concentrations were expected. Because this did not occur, it would appear that the heart, even of sedentary control animals, contains these enzymes in amounts greater than that needed to cope with the exercise stress afforded by these programs. It is possible that in cardiac metabolism an alternate biochemical pathway exists but at present there is no evidence to support this position.

The histochemical results also indicate that more glycogen is present in the ventricles of the LON group after eight weeks of training than is present in the ventricles of the CONS group at zero-week. Blount and Meyer (10) reported that all rats swimming for varying periods of time depleted their cardiac glycogen within the first fifteen minutes of exercise. Those hearts that experienced severe depletion were shown to undergo supercompensation. This latter statement might very well be the case for the LON group animals, since these animals were clearly the most highly trained as reflected by the

body weight and the training data. Supercompensation of cardiac glycogen might have occurred. Opie (48) stated that the glycogen stores become depleted in the early stages of exercise only to be restored at the completion of the activity. If Opie is correct it would seem that the SHT and the MED groups would also have shown supercompensation. Since this did not occur it would appear from these data that glycogen supercompensation in the heart results from severe depletion as observed in the LON group.

It was not hypothesized as to what the effect of the shock stimulus might be on the SHT, MED, and LON animals. Therefore, the ESC group was paired with the SHT group to measure for those effects. From the results, it is apparent that the ESC group did not differ from either the SHT or the CON group in terms of their metabolic characteristics in the myocardium. Apparently, the shock did not have an adverse effect on those animals that were trained in the C-RW apparatus. However, these results were not statistically significant.

As one observes the histochemical results on Figures 3, 4, 5, and 6, there appear, generally, to be sequential fluctuations of the mean values between zero weeks and ten weeks. LDH, FA, and PAS values generally increase at two weeks and decrease at four weeks. There is another rise at six weeks and a decrease by LDH at eight weeks. FA and

PAS rise at eight weeks over their six week values and all values (LDH, FA, and PAS) drop off at ten weeks. The SDH values generally show a drop at two weeks with a further drop at four weeks. An increase in the values occurs at six weeks followed by a drop at eight weeks. The values rise and fall at ten weeks. However, there is a rhythmicity present. Whether or not this is related to the general adaptation syndrome in any way must await further investigation.

The morphological analyses were included primarily to indicate normalcy in the area of the heart from which the histochemical sections were taken. It was gratifying to discover that no significant pathology was present in any of the hearts from the various treatment groups.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this study was to investigate the effects of seven different levels of chronic physical activity on the metabolic and morphologic characteristics of the left ventricular myocardium of adult, male, albino rats.

Two hundred and fifty-two, 71-day-old, male, albino rats (Sprague-Dawley strain) were brought into the laboratory and were assigned to one of seven treatments at random. The treatment groups were sedentary control (CON); voluntary running (VOL); short-duration, high-intensity running (SHT); medium-duration, moderate-intensity running (MED); long-duration, low-intensity running (LON); electric stimulus control (ESC); and endurance swimming (SWM). Treatments were administered Monday through Friday. Animals were provided with food and water ad libitum.

The healthiest and best trained animals were selected for sacrifice. Seven selected animals were weighed and sacrificed on Mondays after zero, two, four, six, eight,

and ten weeks of training. The final sample consisted of 126 animals.

During sacrifice, each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital. The heart was excised and cut transversely at a level just below the atria. The apical portion was placed on a chuck which was immersed in pre-cooled 2-methylbutane. Three hundred micra from the apex a minimum of six 10 μ serial sections were cut.

Five histochemical procedures were utilized to evaluate the relative glycogen, fatty acid, and aerobic enzyme concentrations in the cardiac fibers. A histologic procedure was performed to evaluate the morphology of the hearts. Each histochemical stain was measured objectively using a light transmission meter. The histological sections were rated subjectively on the bases of normalcy and of pathology.

The results indicate that training for eight weeks was sufficient to produce metabolic adaptations in the rats. The eight-week LON group was observed to be significantly lighter than the eight-week CON group ($p < .10$). The fatty acid concentrations were greater in the VOL, LON, and SWM groups at eight weeks than in the CONS group at zero-week ($p < .10$). Also, in the LON group at eight weeks, surprisingly, the glycogen concentrations were greater than in the CONS group at zero-week ($p < .10$).

No pathological changes were observed in any of the seven treatments ($p < .05$). This result is limited to the single section taken approximately 300 μ from the apex.

Conclusions

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

1. The training programs utilized, were effective in that body weights of the trained animals were lower than the body weights of the control animals.
2. Fatty acid concentrations were greater in the VOL, LON, and SWM groups at eight weeks than in the CONS group at zero-week.
3. Surprisingly, the glycogen concentrations were greater in the LON group at eight weeks than in the CONS group at zero-week.
4. No pathological changes were observed in any of the seven treatments in terms of the single tissue section taken.

Recommendations

1. A similar study should be completed in which the trained animals have mean PER and mean PSF values of nothing less than 90% and 90%, respectively.
2. A quantitative biochemical analysis of the myocardium should accompany the histochemical research.

3. Appropriate hematological data, including serum assays, should be taken at intervals between the 0-wk and the 8-wk durations. The data should include erythrocyte and leucocyte counts, hemoglobin and hematocrit. The assays should include serum lactate dehydrogenase (SLDH) and serum glutamic-oxalacetic transaminase (SGOT).

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APPENDICES

APPENDIX A

TRAINING PROGRAMS AND TRAINING DATA

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

Week	Day of Week	Day of Train.	Acceleration Time (sec)	Work Time (min:sec)	Rest Time (sec)	Repetitions per Bout	Number of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Exp. Revolutions TER	Total Work Time (sec) TWT
1	1=M	1	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	2=T	2	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	3=W	3	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	4=T	4	2.0	00:10	10	40	3	5.0	1.0	2.5	39:45	750	1200
	5=F	5	2.0	00:10	10	40	3	5.0	1.0	2.5	39:45	750	1200
2	1=M	6	2.0	00:10	10	40	3	5.0	1.0	2.5	39:45	750	1200
	2=T	7	1.5	00:10	10	40	3	5.0	1.0	3.0	49:30	900	1200
	3=W	8	1.5	00:10	10	40	3	5.0	1.0	3.0	49:30	900	1200
	4=T	9	1.0	00:10	10	40	3	5.0	1.0	3.0	49:30	900	1200
	5=F	10	1.0	00:10	10	40	3	5.0	1.0	3.0	49:30	900	1200
3	1=M	11	1.0	00:10	10	40	3	5.0	1.0	3.0	49:30	900	1200
	2=M	12	1.5	00:10	15	40	3	5.0	1.0	3.5	59:15	1050	1200
	3=W	13	1.5	00:10	15	40	3	5.0	1.0	3.5	59:15	1050	1200
	4=T	14	1.5	00:10	15	40	3	5.0	1.0	3.5	59:15	1050	1200
	5=F	15	1.5	00:10	15	40	3	5.0	1.0	3.5	59:15	1050	1200
4	1=M	16	1.5	00:10	15	40	3	5.0	1.0	3.5	59:15	1050	1200
	2=T	17	1.5	00:10	20	34	3	5.0	1.0	4.0	60:00	1020	1020
	3=W	18	1.5	00:10	20	34	3	5.0	1.0	4.0	60:00	1020	1020
	4=T	19	1.5	00:10	20	34	3	5.0	1.0	4.0	60:00	1020	1020
	5=F	20	1.5	00:10	20	34	3	5.0	1.0	4.0	60:00	1020	1020
5	1=M	21	1.5	00:10	20	34	3	5.0	1.0	4.0	60:00	1020	1020
	2=T	22	1.5	00:10	25	22	4	2.5	1.0	4.5	57:10	990	880
	3=W	23	1.5	00:10	25	22	4	2.5	1.0	4.5	57:10	990	880
	4=T	24	1.5	00:10	25	22	4	2.5	1.0	4.5	57:10	990	880
	5=F	25	1.5	00:10	25	22	4	2.5	1.0	4.5	57:10	990	880
6	1=M	26	1.5	00:10	25	22	4	2.5	1.0	4.5	57:10	990	880
	2=T	27	1.5	00:10	30	14	5	2.5	0.8	5.0	54:10	875	700
	3=W	28	1.5	00:10	30	14	5	2.5	0.8	5.0	54:10	875	700
	4=T	29	1.5	00:10	30	14	5	2.5	0.8	5.0	54:10	875	700
	5=F	30	1.5	00:10	30	14	5	2.5	0.8	5.0	54:10	875	700
7	1=M	31	1.5	00:10	30	14	5	2.5	0.8	5.0	54:10	875	700
	2=T	32	2.0	00:10	35	10	6	2.5	0.8	5.5	54:00	825	600
	3=W	33	2.0	00:10	35	10	6	2.5	0.8	5.5	54:00	825	600
	4=T	34	2.0	00:10	35	10	6	2.5	0.8	5.5	54:00	825	600
	5=F	35	2.0	00:10	35	10	6	2.5	0.8	5.5	54:00	825	600
8	1=M	36	2.0	00:10	35	10	6	2.5	0.8	5.5	54:00	825	600
	2=T	37	2.0	00:10	40	7	7	2.5	0.8	6.0	51:10	735	490
	3=W	38	2.0	00:10	40	7	7	2.5	0.8	6.0	51:10	735	490
	4=T	39	2.0	00:10	40	7	7	2.5	0.8	6.0	51:10	735	490
	5=F	40	2.0	00:10	40	7	7	2.5	0.8	6.0	51:10	735	490

This standard program was designed using male rats of the Sprague-Dawley Strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have PSF and PER scores of 75 or higher during the final two weeks. Alterations in the rest time, repetitions per bout, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the training program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.

Standard short-duration, high-intensity endurance maintenance program for postpubertal and adult male rats in controlled-running wheels.

Acceleration (Time (sec)	Work Time (min:sec)	Rest Time (sec)	Repetitions per Bout	Number of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Exp. Revolutions TER	Total Work Time (sec) TWT
2.0	00:10	40	5	6	5.0	0.8	6.0	46:00	450	300

TABLE A-2.--Standard eight-week, medium-duration, moderate-intensity endurance training program for postpubertal and adult male rats in controlled-running wheels.

Week	Day of Week	Day of Train.	Acceleration Time (sec)	Work Time (min:sec)	Rest Time (sec)	Repetitions per Bout	Number of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Exp. Revolutions TER	Total Work Time (sec) TWT
1	1=M	1	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	2=T	2	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	3=W	3	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	4=T	4	2.0	00:10	10	28	4	5.0	1.0	2.5	51:40	700	1120
	5=F	5	2.0	00:10	10	28	4	5.0	1.0	2.5	51:40	700	1120
2	1=M	6	2.0	00:10	10	28	4	5.0	1.0	2.5	51:40	700	1120
	2=T	7	1.5	00:10	10	27	4	5.0	1.0	3.0	50:20	810	1080
	3=W	8	1.5	00:10	10	27	4	5.0	1.2	3.0	50:20	810	1080
	4=T	9	1.5	00:10	10	27	4	5.0	1.2	3.0	50:20	810	1080
	5=F	10	1.5	00:10	10	27	4	5.0	1.2	3.0	50:20	810	1080
3	1=M	11	1.0	00:10	10	27	4	5.0	1.2	3.0	50:20	810	1080
	2=T	12	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
	3=W	13	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
	4=T	14	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
	5=F	15	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
4	1=M	16	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
	2=T	17	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
	3=W	18	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
	4=T	19	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
	5=F	20	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
5	1=M	21	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
	2=T	22	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	3=W	23	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	4=T	24	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	5=F	25	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
6	1=M	26	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	2=T	27	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
	3=W	28	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
	4=T	29	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
	5=F	30	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
7	1=M	31	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
	2=T	32	1.5	00:25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
	3=W	33	1.5	00:25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
	4=T	34	1.5	00.25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
	5=F	35	1.5	00:25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
8	1=M	36	1.5	00:25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
	2=T	37	1.5	00:30	30	8	5	5.0	0.8	4.0	57:30	1200	1200
	3=W	38	1.5	00:30	30	8	5	5.0	0.8	4.0	57:30	1200	1200
	4=T	39	1.5	00:30	30	8	5	5.0	0.8	4.0	57:30	1200	1200
	5=F	40	1.5	00:30	30	8	5	5.0	0.8	4.0	57:30	1200	1200

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have PSF and PER scores of 75 or higher during the final two weeks. Alterations in the work time, rest time, repetitions per bout, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.

Standard medium-duration, moderate-intensity endurance maintenance program for postpubertal and adult male rats in controlled-running wheels.

Acceleration Time (sec)	Work Time (min:sec)	Rest Time (sec)	Repetitions per Bout	Number of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Exp. Revolutions TER	Total Work Time (sec) TWT
1.5	00:30	30	8	3	5.0	0.8	4.0	32:30	720	720

TABLE A-3.--Standard eight-week, long-duration, low-intensity endurance training program for postpubertal and adult male rats in controlled-running wheels.

Week	Day of Week	Day of Train.	Acceleration Time (sec)	Work Time (min:sec)	Rest Time (sec)	Repetitions per Bout	Number of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Exp. Revolutions TER	Total Work Time (sec) TWT
1	1=M	1	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	2=T	2	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	3=W	3	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	4=T	4	2.0	00:20	10	30	2	5.0	1.0	2.0	34:40	600	1200
	5=F	5	2.0	00:30	15	20	2	5.0	1.0	2.0	34:30	600	1200
2	1=M	6	2.0	00:40	20	15	2	5.0	1.0	2.0	34:20	600	1200
	2=T	7	1.5	00:50	25	12	2	5.0	1.0	2.0	34:10	600	1200
	3=W	8	1.5	01:00	30	10	2	5.0	1.0	2.0	34:00	600	1200
	4=T	9	1.5	02:30	60	4	2	5.0	1.0	2.0	31:00	600	1200
	5=F	10	1.5	02:30	60	4	2	5.0	1.0	2.0	31:00	600	1200
3	1=M	11	1.5	02:30	60	4	2	5.0	1.0	2.0	31:00	600	1200
	2=T	12	1.0	05:00	0	1	5	2.5	1.0	2.0	35:00	750	1500
	3=W	13	1.0	05:00	0	1	5	2.5	1.0	2.0	35:00	750	1500
	4=T	14	1.0	05:00	0	1	5	2.5	1.0	2.0	35:00	750	1500
	5=F	15	1.0	05:00	0	1	5	2.5	1.0	2.0	35:00	750	1500
4	1=M	16	1.0	05:00	0	1	5	2.5	1.0	2.0	35:00	750	1500
	2=T	17	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	3=W	18	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	4=T	19	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	5=F	20	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
5	1=M	21	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	2=T	22	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	3=W	23	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	4=T	24	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	5=F	25	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
6	1=M	26	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	2=T	27	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	3=W	28	1.0	10:00	0	1	4	2.5	1.0	2.0	47.30	1200	2400
	4=T	29	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	5=F	30	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
7	1=M	31	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	2=T	32	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	3=W	33	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	4=T	34	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	5=F	35	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
8	1=M	36	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	2=T	37	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	3=W	38	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	4=T	39	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	5=F	40	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have PSF and PER scores of 75 or higher during the final two weeks. Alterations in the work time, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.

Standard long-duration, low-intensity endurance maintenance program for postpubertal and adult male rats in controlled running wheels.

	Acceleration Time (sec)	Work Time (min:sec)	Rest Time (sec)	Repetitions per Bout	Number of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Exp. Revolutions TER	Total Work Time (sec) TWT
	0.5	10:00	0	1	3	2.5	0.8	2.0	35:00	900	1800

TABLE A-4.--Standard eight-week, endurance, swimming training program for postpubertal and adult male rats.

Week	Day of Week	Day of Training	Per Cent Tail Weight	Expected Swim Time (min) EST
1	1=M	1	0	30
	2=T	2	0	40
	3=W	3	C*	50
	4=T	4	C	60
	5=F	5	C	60
2	1=M	6	2	40
	2=T	7	2	40
	3=W	8	2	40
	4=T	9	2	45
	5=F	10	2	50
3	1=M	11	3	30
	2=T	12	3	30
	3=W	13	3	30
	4=T	14	3	35
	5=F	15	3	35
4	1=M	16	3	35
	2=T	17	3	40
	3=W	18	3	40
	4=T	19	3	40
	5=F	20	3	40
5	1=M	21	3	40
	2=T	22	3	45
	3=W	23	3	45
	4=T	24	3	45
	5=F	25	3	45
6	1=M	26	3	45
	2=T	27	3	50
	3=W	28	3	50
	4=T	29	3	50
	5=F	30	3	50
7	1=M	31	3	50
	2=T	32	3	55
	3=W	33	3	55
	4=T	34	3	55
	5=F	35	3	55

TABLE A-4.--Continued.

Week	Day of Week	Day of Training	Per Cent Tail Weight	Expected Swim Time (min) EST
8	1=M	36	3	55
	2=T	37	3	60
	3=W	38	3	60
	4=T	39	3	60
	5=F	40	3	60

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 90 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have PET scores of 75 or higher during the final two weeks. Alterations in the percentage of tail weight or expected swim time can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a severe, sudden exercise stress upon the animals and will seriously impair the effectivensss of the training program.

Standard endurance swimming maintenance program for post-pubertal and adult male rats

Per Cent Tail Weight	Expected Swim Time (min) EST
2	40

*C = clothespin only.

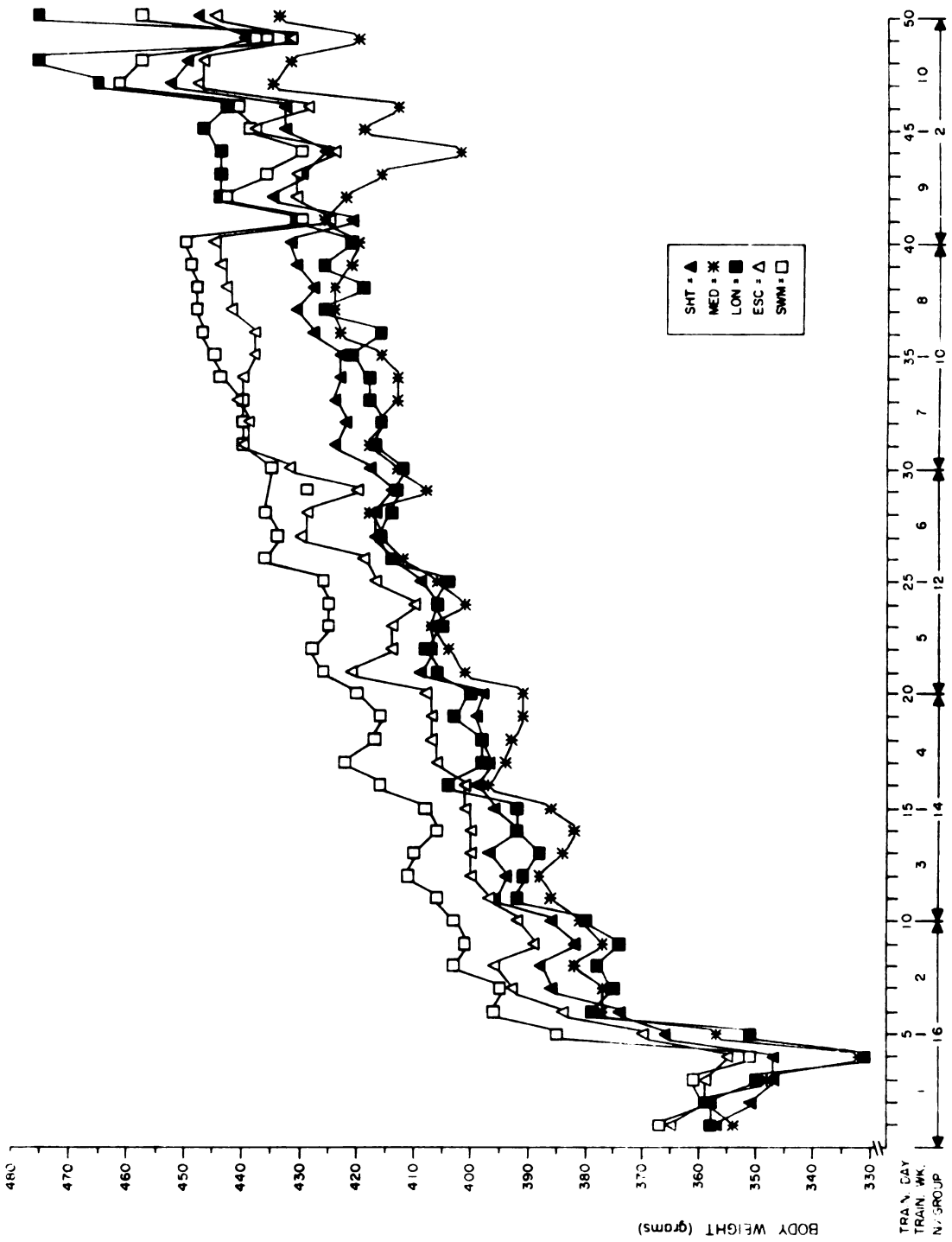

Figure A-1 – Mean Daily Body Weight

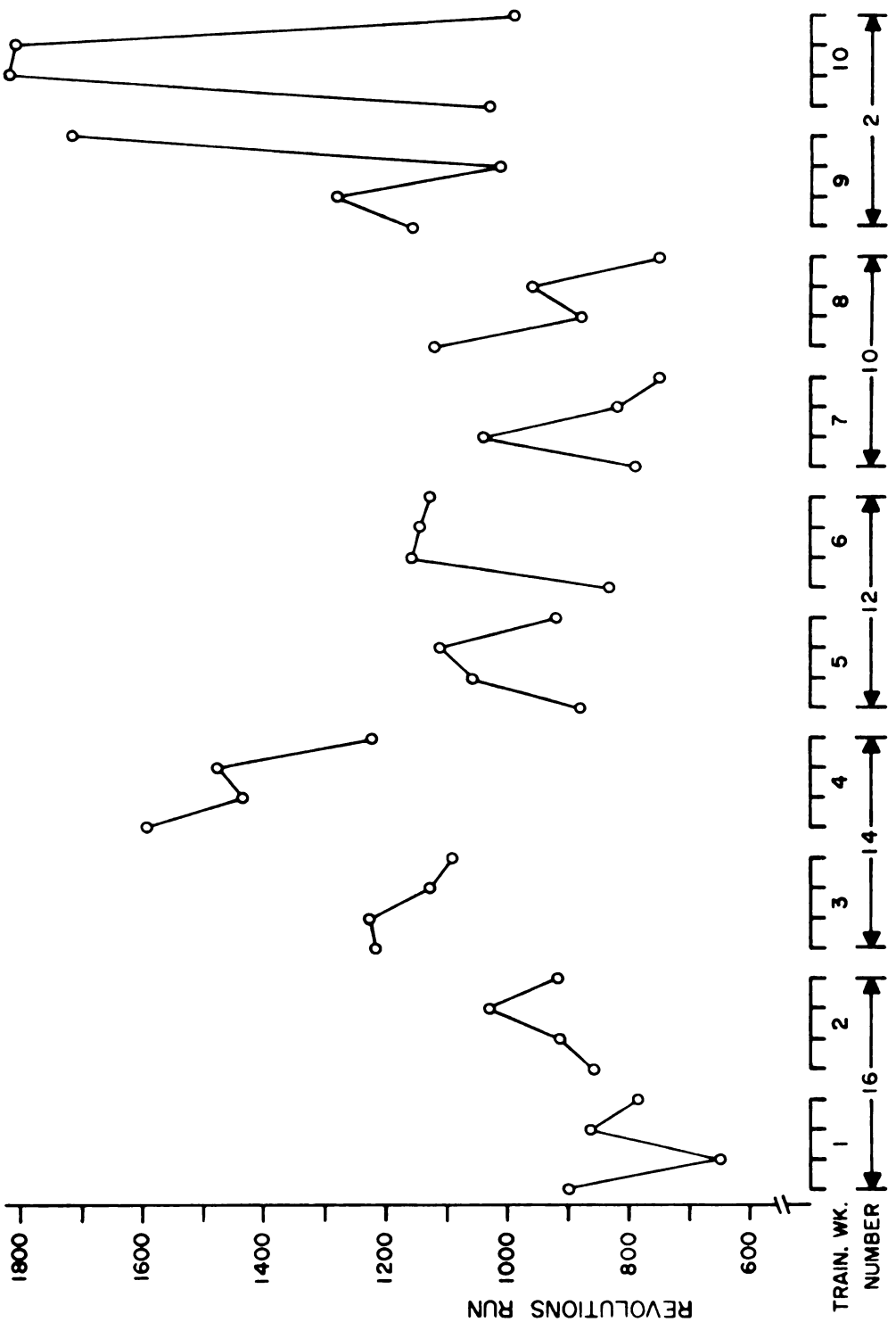

Figure A-2 - Mean Daily Revolutions Run (VOL)

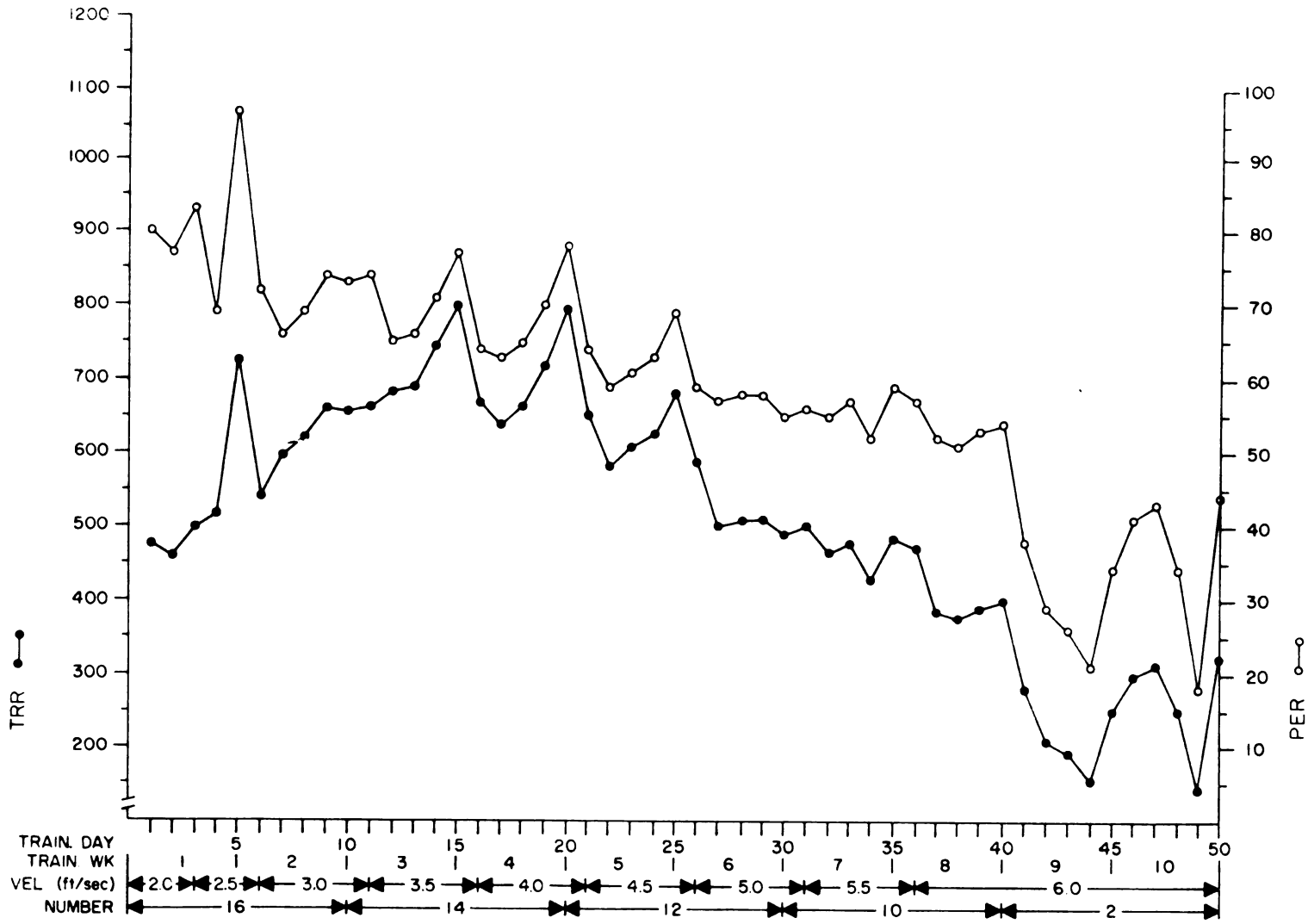

Figure A-3 — Mean Daily Total Revolutions Run (TRR) and Per Cent Expected Revolutions (PER) for SHT

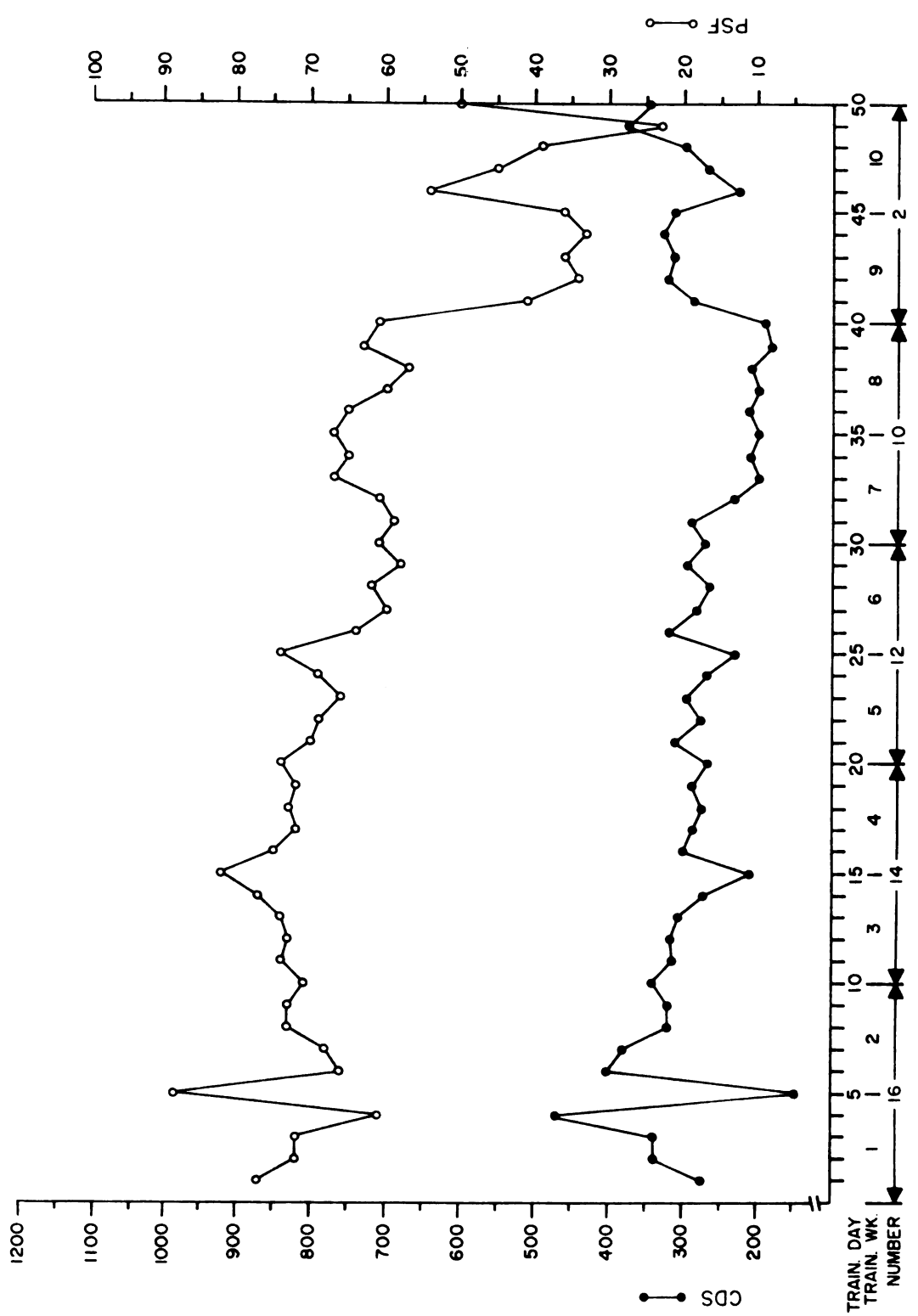

Figure A-4 — Mean Daily Cumulative Duration of Shock (CDS) and Per Cent Shock Free Time (PSF) for SHT

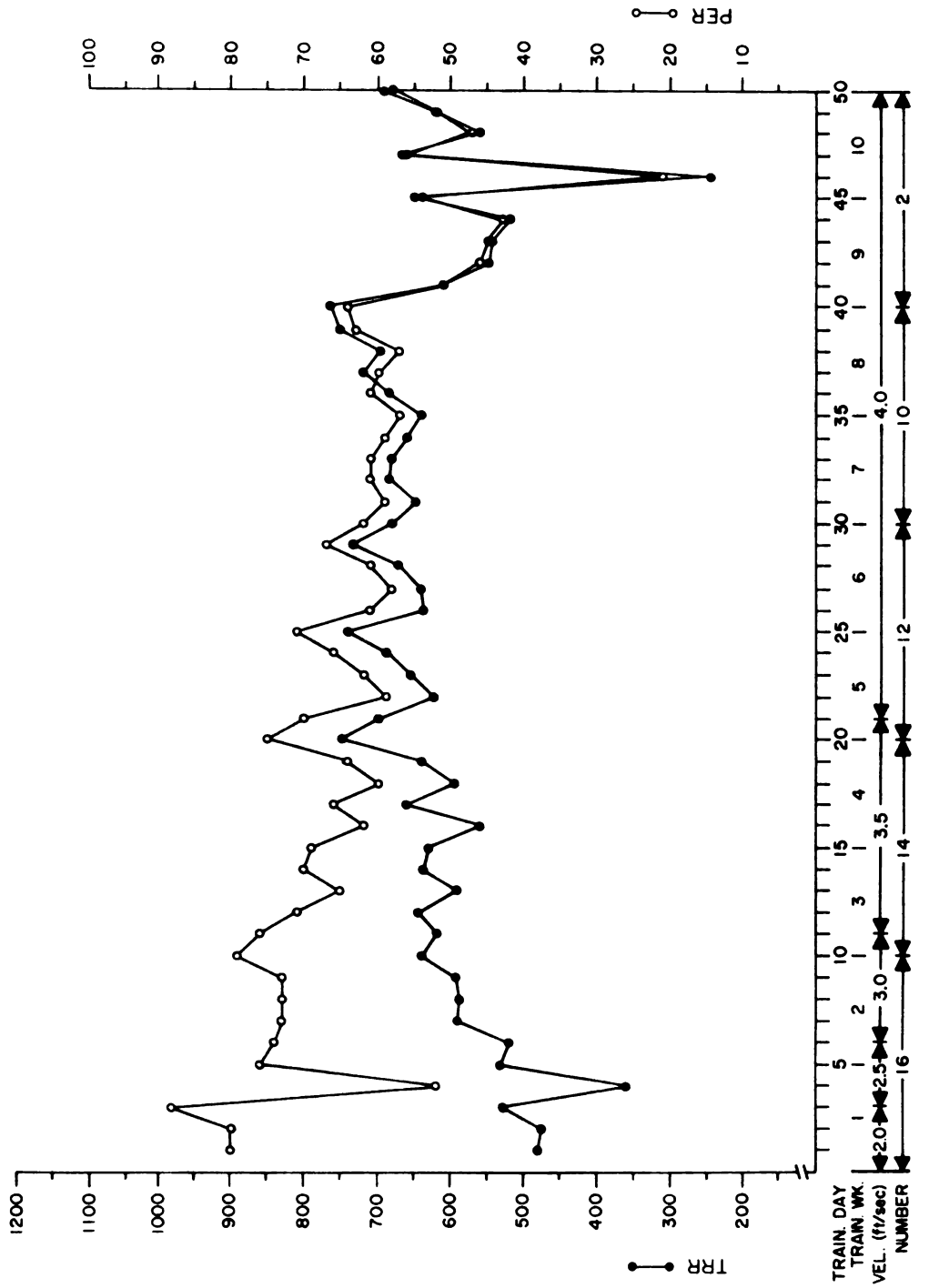

Figure A-5 – Mean Daily Total Revolutions Run (TRR) and Per Cent Expected Revolutions (PER) for MED

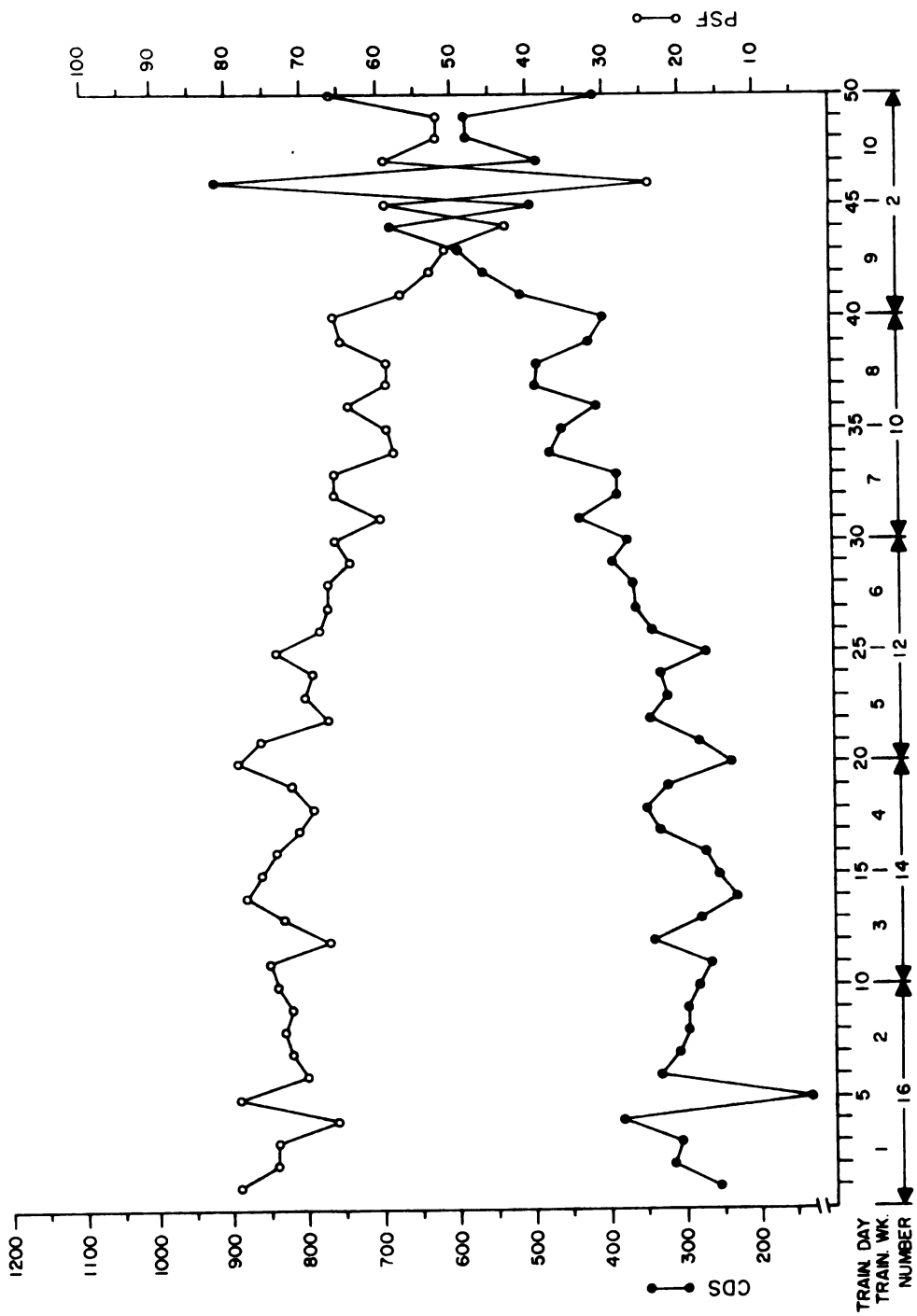

Figure A-6 – Mean Daily Cumulative Duration of Shock (CDS) and Per Cent Shock Free Time (PSF) for MED

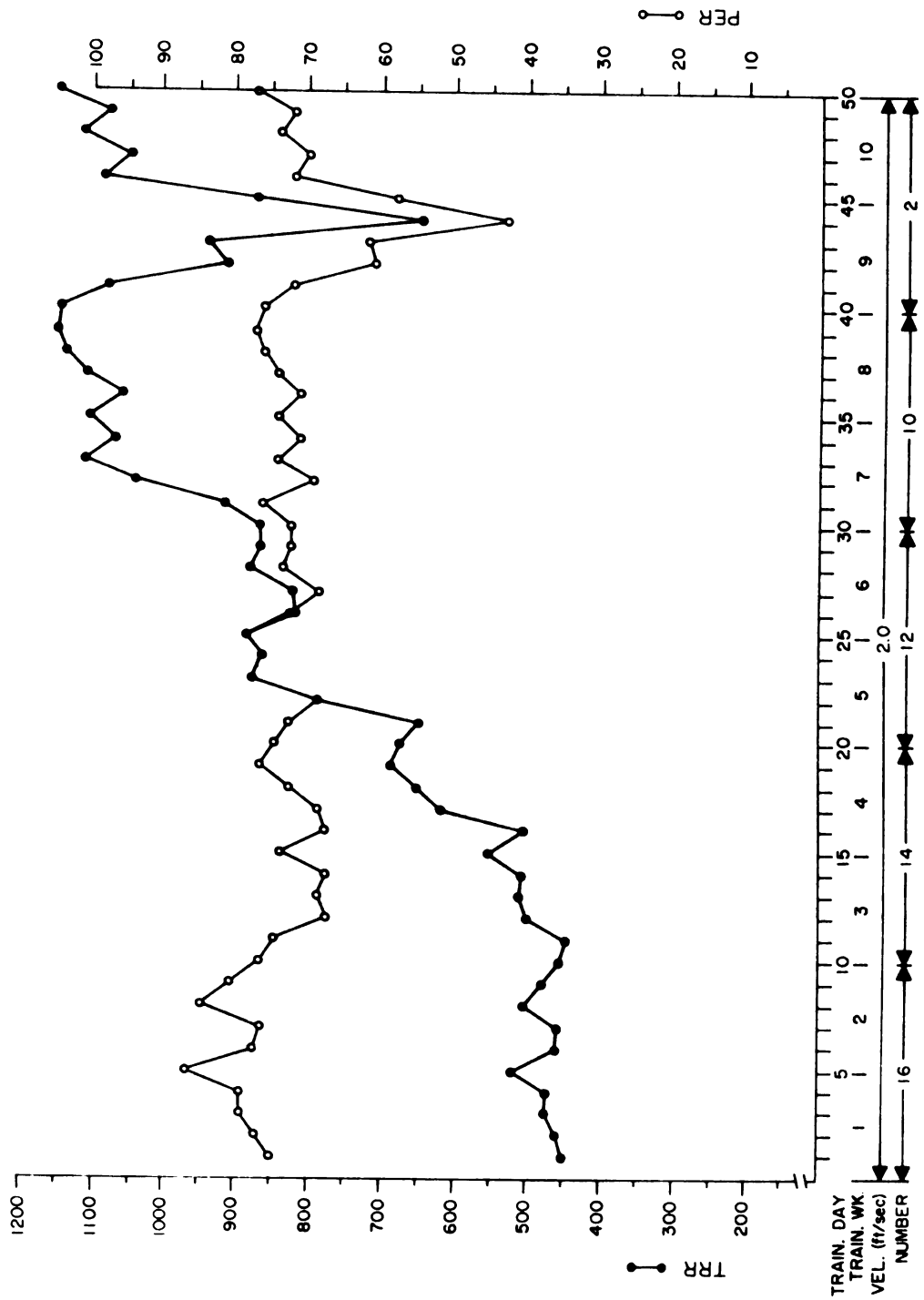

Figure A-7 – Mean Daily Total Revolutions Run (TRR) and Per Cent Expected Revolutions (PER) for LON

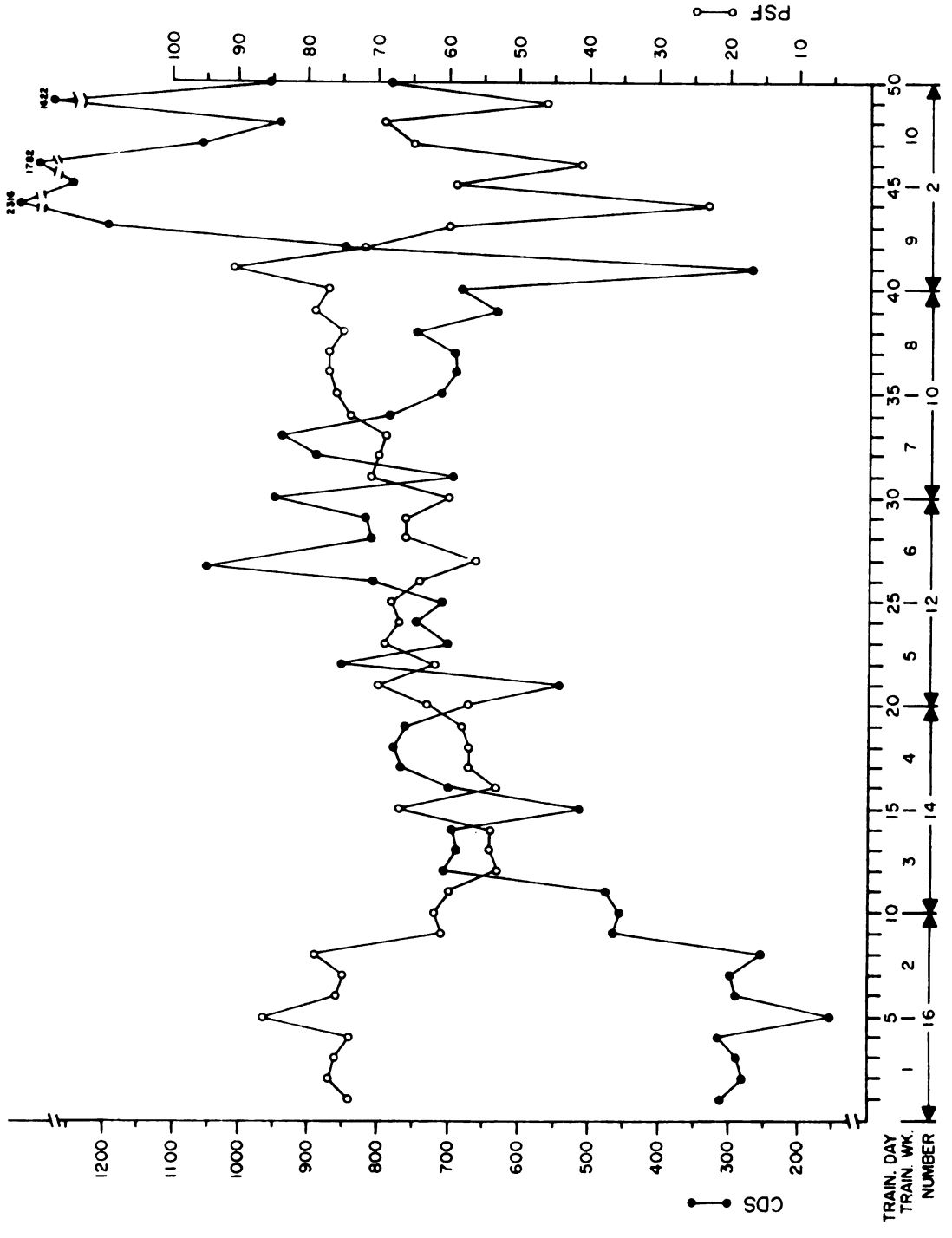

Figure A-8 – Mean Daily Cumulative Duration of Shock (CDS) and Per Cent Shock Free Time (PSF) for LON

APPENDIX B

SACRIFICE MODIFICATIONS

APPENDIX B

SACRIFICE MODIFICATIONS

First Sacrifice: Each animal was anesthetized with 0.35 cc. of the Halatal solution. Once into the abdominal cavity, an *in vivo* injection, of a 2% heparinized solution of black Pelikan ink (10 ml.) into the inferior vena cava between the junction of the common iliac veins and the first set of iliolumbar veins, was performed. The injection was accomplished while the heart was still beating which allowed three to five minutes continued *in vivo* circulation. (This was to be used later as an histologic demonstration of the capillaries of the triceps surae and plantaris muscle groups, which was another part of the study).

Second Sacrifice: Each animal was anesthetized with 0.35 cc. of the Halatal solution. Once into the abdominal cavity, the left common iliac artery and vein were clamped with a taped hemostat. The modifications are the same as in the first sacrifice (above), except for the fact that 5 ml. of Pelikan ink were injected.

Third Sacrifice: Each animal was anesthetized with 2.50 cc. of the Halatal solution. Procedures followed those as reported in Chapter III.

Fourth Sacrifice: Each animal was anesthetized with 2.50-3.00 cc. of the Halatal solution. Procedures followed those as reported in Chapter III.

Fifth Sacrifice through the Twelfth Sacrifice: No modifications from those reported in Chapter III.

Thirteenth Sacrifice through the Fifteenth Sacrifice: Modifications were identical to those reported in the first sacrifice (above), except that 5 ml. of Pelikan ink were injected.

Sixteenth Sacrifice through the Eighteenth Sacrifice: Modifications were identical to those reported in the first sacrifice (above), except that 6 ml. of pelikan ink were injected.

APPENDIX C

HISTOCHEMICAL AND PATHOLOGICAL DATA

TABLE C-1.--Mean per cent light absorbed and body weight presented by animal number, treatment, and duration.

An. No.	Treat.	Dur. (wk)	Bd. Wt. (g)	Mean Per Cent Light Absorbed				
				PAS	LDH	FA	SDH	Cy Ox
4	CON	0	372	33.5	76.0	30.6	90.5	74.7
86	CON	0	401	25.8	48.7	46.1	89.7	66.2
13	VOL	0	378	17.9	73.6	34.3	90.6	80.4
87	VOL	0	400	28.5	48.0	41.6	87.5	63.8
32	SHT	0	339	22.0	74.0	33.1	92.4	75.9
88	SHT	0	393	22.1	31.2	42.2	85.0	57.3
48	MED	0	373	37.2	80.9	34.7	96.8	72.8
89	MED	0	377	19.8	38.9	38.6	85.3	52.5
57	LON	0	375	21.0	84.4	43.1	98.0	74.1
96	LON	0	370	17.2	33.1	26.0	84.0	57.8
68	ESC	0	351	29.5	73.4	34.0	91.8	77.6
101	ESC	0	362	17.7	42.4	34.3	84.8	50.5
74	SWM	0	332	29.3	66.5	36.1	96.6	75.5
162	SWM	0	422	19.5	23.6	36.4	86.7	56.9
8	CON	2	454	31.6	78.0	63.3	91.0	60.8
92	CON	2	432	16.8	43.6	51.3	76.5	37.9
15	VOL	2	392	31.3	78.0	53.2	86.1	58.0
103	VOL	2	390	35.4	37.2	50.3	79.0	44.0
35	SHT	2	393	26.6	81.3	61.6	86.8	70.4
117	SHT	2	371	44.6	45.6	45.7	86.6	46.2
46	MED	2	360	38.0	75.2	65.2	92.6	63.8
126	MED	2	425	13.2	50.8	34.1	71.2	34.1
55	LON	2	373	29.9	78.9	59.7	87.3	63.4
144	LON	2	340	37.1	48.7	42.5	79.3	44.8
71	ESC	2	428	28.8	80.7	42.5	90.0	48.9
153	ESC	2	413	23.8	48.2	38.6	78.6	45.7
76	SWM	2	423	16.8	79.2	44.6	93.7	68.2
164	SWM	2	447	10.6	54.9	47.1	82.1	41.8
12	CON	4	433	12.7	67.4	24.9	73.8	35.6
94	CON	4	437	16.7	49.5	28.6	75.7	11.9
20	VOL	4	422	24.4	79.1	51.5	83.3	46.5
105	VOL	4	440	22.4	48.1	44.0	75.2	8.1
31	SHT	4	388	20.1	71.2	41.0	77.6	45.6
111	SHT	4	419	25.6	41.4	47.0	79.8	18.0
39	MED	4	397	25.1	71.6	41.7	80.8	45.6
130	MED	4	395	20.1	53.4	31.8	79.8	19.9
50	LON	4	425	17.9	75.2	35.0	63.4	41.4
141	LON	4	387	20.0	46.0	36.4	83.5	11.1
67	ESC	4	432	21.7	78.0	42.4	82.0	41.2
147	ESC	4	393	23.4	42.3	36.0	81.9	14.2
84	SWM	4	400	16.9	72.4	12.0	65.5	41.5
158	SWM	4	433	27.7	50.3	50.2	86.3	18.7
2	CON	6	442	20.1	81.5	47.0	85.3	47.7
91	CON	6	440	20.4	56.4	32.9	77.2	35.7
21	VOL	6	428	13.0	79.9	35.9	79.9	41.1
97	VOL	6	423	31.8	43.4	43.8	87.4	41.3
27	SHT	6	414	36.4	83.8	45.5	80.2	48.5
114	SHT	6	408	31.5	39.1	47.4	85.1	31.6
44	MED	6	420	38.4	80.8	52.2	83.5	48.3
127	MED	6	420	32.8	51.1	43.5	85.9	53.7
49	LON	6	418	16.9	81.1	37.5	85.6	38.5
138	LON	6	418	36.6	60.7	49.3	84.9	39.6
63	ESC	6	443	14.7	81.5	50.6	84.5	51.0
150	ESC	6	408	21.1	46.7	38.1	76.1	43.8
79	SWM	6	444	25.1	84.4	54.2	84.9	51.0
165	SWM	6	460	26.5	54.0	41.0	88.1	51.8
7	CON	8	451	14.7	68.5	19.7	53.6	35.1
93	CON	8	457	28.3	64.6	47.0	86.3	10.8
169	CON	8	462	36.9	57.4	40.2	86.0	53.6
184	CON	8	472	43.0	67.8	45.4	81.1	72.2
197	CON	8	546	47.0	77.4	51.1	87.9	8.6
212	CON	8	468	47.4	74.0	41.1	77.3	40.2

TABLE C-1.--continued.

An. No.	Treat.	Dur. (wk)	Bd. Wt. (g)	Mean Per Cent Light Absorbed				
				PAS	LDH	FA	SDH	Cy Ox
225	CON	8	470	48.6	50.5	53.5	90.0	22.1
240	CON	8	455	35.8	67.3	56.5	85.9	92.2
16	VOL	8	435	14.0	78.6	40.0	71.1	67.9
106	VOL	8	454	28.2	67.9	41.2	85.9	3.6
186	VOL	8	438	50.1	68.6	57.0	89.1	78.7
188	VOL	8	446	30.1	61.6	39.9	86.9	71.8
214	VOL	8	407	44.0	55.6	48.0	71.6	51.9
215	VOL	8	427	44.0	55.0	60.2	78.1	53.1
242	VOL	8	447	28.6	56.4	56.7	81.6	92.8
243	VOL	8	428	35.5	63.9	68.8	85.4	93.2
28	SHT	8	448	14.6	88.2	34.9	42.3	63.1
116	SHT	8	434	34.0	57.6	39.2	87.9	10.2
172	SHT	8	433	43.2	52.9	47.6	89.3	65.6
173	SHT	8	464	50.0	53.6	42.7	90.3	66.0
199	SHT	8	434	44.9	73.9	53.3	84.4	10.1
202	SHT	8	479	42.6	76.2	44.4	82.4	8.2
228	SHT	8	423	50.7	55.1	42.8	85.8	13.9
229	SHT	8	394	28.4	49.6	50.6	81.1	9.5
43	MED	8	423	9.5	65.2	23.2	51.1	63.1
132	MED	8	461	38.0	58.8	45.6	85.1	5.8
175	MED	8	470	38.6	49.5	37.1	90.5	59.0
178	MED	8	407	36.0	57.9	41.7	92.4	76.2
203	MED	8	458	47.4	75.2	44.3	86.7	10.9
206	MED	8	398	52.1	71.1	53.9	85.7	10.3
232	MED	8	386	30.1	42.0	41.2	82.3	14.6
234	MED	8	412	46.5	50.7	59.5	89.7	18.5
52	LON	8	389	44.8	72.6	40.0	56.4	71.5
140	LON	8	464	46.7	51.2	55.8	86.7	7.8
190	LON	8	372	44.9	70.5	50.3	91.3	81.3
192	LON	8	424	47.8	64.9	54.6	94.1	82.5
219	LON	8	405	43.9	57.2	47.7	76.0	44.5
220	LON	8	375	28.2	64.4	41.3	74.2	43.6
246	LON	8	392	31.1	61.7	60.3	87.0	91.3
248	LON	8	422	51.4	62.9	62.2	83.3	90.8
64	ESC	8	417	26.0	65.3	42.8	83.7	67.6
152	ESC	8	423	26.7	52.3	44.5	87.9	6.8
180	ESC	8	522	49.4	52.8	43.6	90.3	66.8
181	ESC	8	456	42.0	59.5	42.3	89.4	60.7
207	ESC	8	426	47.8	71.8	54.6	84.3	16.2
210	ESC	8	500	39.8	75.9	41.5	82.7	7.0
236	ESC	8	427	28.4	37.8	47.5	82.3	14.8
237	ESC	8	410	36.9	41.0	52.0	89.2	14.2
83	SWM	8	430	8.4	78.4	25.1	36.6	36.2
160	SWM	8	434	32.9	61.3	55.3	90.2	10.5
193	SWM	8	460	42.5	65.1	57.0	90.2	76.4
195	SWM	8	496	51.0	62.9	63.9	94.4	77.8
221	SWM	8	470	40.5	60.1	53.8	82.8	46.4
224	SWM	8	440	30.8	56.3	60.8	81.2	46.1
249	SWM	8	433	53.3	58.8	71.7	89.9	90.8
251	SWM	8	444	44.6	63.4	64.2	86.5	94.5
3	CON	10	466	24.9	44.5	28.5	70.1	64.1
90	CON	10	533	31.9	74.7	41.2	90.3	90.5
24	VOL	10	462	33.6	47.5	26.9	82.6	65.0
100	VOL	10	409	29.4	64.4	34.9	85.8	78.4
29	SHT	10	444	19.3	52.6	32.1	85.0	70.0
109	SHT	10	457	34.6	63.6	34.8	89.3	93.8
38	MED	10	412	22.4	37.9	35.0	82.3	70.3
125	MED	10	430	44.5	71.8	44.3	91.8	94.1
51	LON	10	434	22.0	39.3	50.4	86.2	63.1
133	LON	10	530	30.5	59.2	36.0	89.0	93.9
65	ESC	10	434	23.2	40.9	18.1	78.0	68.2
145	ESC	10	460	22.8	60.1	40.8	86.6	89.3
82	SWM	10	443	34.3	40.1	50.0	56.9	73.0
157	SWM	10	479	24.2	58.6	35.4	85.6	93.1

TABLE C-2.--Reliability of histochemical data.

An. No.	Treatment	Duration (wk)	Stain	Mean Per Cent Light Ab.	
				Original	2 mos. later
4	CON	0	Cy Ox	74.8	72.0
13	VOL	0	LDH	73.6	75.7
32	SHT	0	FA	33.1	36.1
57	LON	0	LDH	84.4	83.9
15	VOL	2	FA	53.2	54.7
35	SHT	2	PAS	26.6	28.3
71	ESC	2	SDH	90.0	90.0
76	SWM	2	Cy Ox	68.2	73.5
105	VOL	4	SDH	75.2	69.0
31	SHT	4	SDH	77.6	74.5
67	ESC	4	LDH	78.0	75.8
67	ESC	4	SDH	82.0	80.2
44	MED	6	LDH	80.8	79.5
127	MED	6	PAS	32.8	36.4
127	MED	6	FA	43.5	48.2
49	LON	6	Cy Ox	38.5	47.0
138	LON	6	SDH	84.9	83.1
63	ESC	6	PAS	14.7	14.4
79	SWM	6	FA	54.2	60.4
106	VOL	8	FA	41.2	41.9
214	VOL	8	PAS	44.0	44.5
228	SHT	8	LDH	55.1	58.6
229	SHT	8	Cy OX	9.5	14.3
248	LON	8	FA	62.2	55.0
38	MED	10	LDH	37.9	40.2

TABLE C-2a.--Validity of histochemical data.

Stain	Per Cent Light Absorbed	Eye Rating
SDH	70	1
	56	3
	60	3
	74	1
	57	3
	53	3
	72	1
	59	3
	53	3
	66	2
Sudan Black B	16	1
	13	3
	16	2
	19	1
	15	2
	14	3
	18	1
	13	3
	15	3
	20	1
ATP'ase	80	1
	63	3
	52	3
	84	1
	63	3
	59	3
	82	1
	60	3
	60	3
	81	1

TABLE C-3.--Pathologic analysis presented by animal number, treatment, and duration.

An. No.	Treat.	Dur. (wk)	Diagnosis
4	CON	0	Minimal focal accumulations of lymphocytes
86	CON	0	Normal
13	VOL	0	Normal
87	VOL	0	Normal
32	SHT	0	Normal
88	SHT	0	Minimal focal accumulations of lymphocytes
48	MED	0	Normal
89	MED	0	Normal
57	LON	0	Normal
96	LON	0	Normal
68	ESC	0	Normal
101	ESC	0	Normal
74	SWM	0	Normal
162	SWM	0	Normal
8	CON	2	Normal
92	CON	2	Minimal focal accumulations of lymphocytes
15	VOL	2	Normal
103	VOL	2	Normal
35	SHT	2	Normal
117	SHT	2	Normal
46	MED	2	Normal
126	MED	2	Minimal focal accumulations of lymphocytes
55	LON	2	Minimal focal accumulations of lymphocytes
144	LON	2	Minimal focal accumulations of lymphocytes
71	ESC	2	Normal
153	ESC	2	Normal
76	SWM	2	Normal
164	SWM	2	Minimal focal accumulations of lymphocytes
12	CON	4	Normal
94	CON	4	Normal
20	VOL	4	Normal
105	VOL	4	Minimal focal accumulations of lymphocytes
31	SHT	4	Normal
111	SHT	4	Normal
39	MED	4	Minimal focal accumulations of lymphocytes
130	MED	4	Normal
50	LON	4	Normal
141	LON	4	Minimal focal accumulations of lymphocytes
67	ESC	4	Normal
147	ESC	4	Minimal focal accumulations of lymphocytes
84	SWM	4	Normal
158	SWM	4	Minimal focal accumulations of lymphocytes
2	CON	6	Minimal focal accumulations of lymphocytes
91	CON	6	Minimal focal accumulations of lymphocytes
21	VOL	6	Normal
97	VOL	6	Minimal focal accumulations of lymphocytes
27	SHT	6	Normal
114	SHT	6	Minimal focal accumulations of lymphocytes
44	MED	6	Minimal focal accumulations of lymphocytes
127	MED	6	Minimal focal accumulations of lymphocytes
49	LON	6	Minimal focal accumulations of lymphocytes
138	LON	6	Minimal focal accumulations of lymphocytes
63	ESC	6	Minimal focal accumulations of lymphocytes
150	ESC	6	Normal
79	SWM	6	Normal
165	SWM	6	Minimal focal accumulations of lymphocytes
7	CON	8	Normal
93	CON	8	Minimal focal accumulations of lymphocytes
169	CON	8	Normal
184	CON	8	Normal
197	CON	8	Normal
212	CON	8	Minimal focal accumulations of lymphocytes
225	CON	8	Normal

TABLE C-3.--Continued.

An. No.	Treat.	Dur. (wk)	Diagnosis
240	CON	8	Normal
16	VOL	8	Normal
106	VOL	8	Normal
186	VOL	8	Minimal focal accumulations of lymphocytes
188	VOL	8	Minimal focal accumulations of lymphocytes
214	VOL	8	Normal
215	VOL	8	Minimal focal accumulations of lymphocytes
242	VOL	8	Normal
243	VOL	8	Normal
28	SHT	8	Normal
116	SHT	8	Normal
172	SHT	8	Minimal focal accumulations of lymphocytes
173	SHT	8	Normal
199	SHT	8	Minimal focal accumulations of lymphocytes
202	SHT	8	Normal
228	SHT	8	Minimal focal accumulations of lymphocytes
229	SHT	8	Normal
43	MED	8	Normal
132	MED	8	Normal
175	MED	8	Normal
178	MED	8	Normal
203	MED	8	Normal
206	MED	8	Normal
232	MED	8	Normal
234	MED	8	Normal
52	LON	8	Normal
140	LON	8	Minimal focal accumulations of lymphocytes
190	LON	8	Minimal focal accumulations of lymphocytes
192	LON	8	Minimal focal accumulations of lymphocytes
219	LON	8	Minimal focal accumulations of lymphocytes
220	LON	8	Normal
246	LON	8	Normal
248	LON	8	Minimal focal accumulations of lymphocytes
64	ESC	8	Normal
152	ESC	8	Normal
180	ESC	8	Minimal focal accumulations of lymphocytes
181	ESC	8	Normal
207	ESC	8	Normal
210	ESC	8	Normal
236	ESC	8	Normal
237	ESC	8	Minimal focal accumulations of lymphocytes
83	SWM	8	Normal
160	SWM	8	Normal
193	SWM	8	Normal
195	SWM	8	Normal
221	SWM	8	Normal
224	SWM	8	Minimal focal accumulations of lymphocytes
249	SWM	8	Normal
251	SWM	8	Normal
3	CON	10	Normal
90	CON	10	Minimal focal accumulations of lymphocytes
24	VOL	10	Minimal focal accumulations of lymphocytes
100	VOL	10	Minimal focal accumulations of lymphocytes
29	SHT	10	Minimal focal accumulations of lymphocytes
109	SHT	10	Normal
38	MED	10	Normal
125	MED	10	Normal
51	LON	10	Normal
133	LON	10	Minimal focal accumulations of lymphocytes
65	ESC	10	Normal
145	ESC	10	Normal
82	SWM	10	Minimal focal accumulations of lymphocytes
157	SWM	10	Normal

APPENDIX D

STATISTICAL TABLES

TABLE D-1.--Analysis of variance for body weight by 0-wk CONS and 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	68350.62	7	9764.37	12.09[a]	<0.0005
Error	50085.96	62	807.84		
Total	118436.58	69			

[a]Significant at the .05 level.

TABLE D-2.--Analysis of variance for body weight by 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	21082.61	6	3513.77	4.07[a]	0.002
Error	42312.75	49	863.52		
Total	63395.36	55			

[a]Significant at the .05 level.

TABLE D-3.--Analysis of variance for LDH by 0-wk CONS and 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	782.66	7	111.81	0.64[b]	0.723
Error	10876.22	62	175.42		
Total	11658.88	69			

[b]An F-ratio based on 7 and 62 df must be 2.17 or larger to be significant at the .05 level.

TABLE D-4.--Analysis of variance for FA by 0-wk CONS and 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	2722.72	7	388.96	4.65[a]	<0.0005
Error	5188.59	62	83.69		
Total	7911.31	69			

[a]Significant at the .05 level.

TABLE D-5.--Analysis of variance for SDH by 0-wk CONS and 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	892.41	7	127.49	0.98[b]	0.457
Error	8102.32	62	130.68		
Total	8994.73	69			

[b]An F-ratio based on 7 and 62 df must be 2.17 or larger to be significant at the .05 level.

TABLE D-6.--Analysis of variance for PAS by 0-wk CONS and 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	2449.89	7	349.98	3.06[a]	0.008
Error	7078.14	62	114.16		
Total	9528.03	69			

[a]Significant at the .05 level.

TABLE D-7.--Analysis of variance for LDH by 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	457.15	6	76.19	0.71[b]	0.640
Error	5227.19	49	106.68		
Total	5684.34	55			

[b]An F-ratio based on 6 and 49 df must be 2.33 or larger to be significant at the .05 level.

TABLE D-8.--Analysis of variance for FA by 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	1182.52	6	197.09	2.01[b]	0.083
Error	4814.00	49	98.24		
Total	5996.52	55			

[b]An F-ratio based on 6 and 49 df must be 2.33 or larger to be significant at the .05 level.

TABLE D-9.--Analysis of variance for SDH by 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	190.25	6	31.71	0.20[b]	0.98
Error	7811.56	49	159.42		
Total	8001.81	55			

[b]An F-ratio based on 6 and 49 df must be 2.33 or larger to be significant at the .05 level.

TABLE D-10.--Analysis of variance for PAS by 8-wk treatment.

Source	Summary of Analysis of Variance				
	SS	df	MS	F	P
Treatment	351.05	6	58.51	0.44[b]	0.850
Error	6555.97	49	133.80		
Total	6907.02	55			

[b]An F-ratio based on 6 and 49 df must be 2.33 or larger to be significant at the .05 level.

TABLE D-11.--Chi-square[a] contingency table for pathology by 0-wk CONS and 8-wk treatment.

Diagnosis	Treatment and Duration								
	0-wk	8-wk							
	CON	CON	VOL	SHT	MED	LON	ESC	SWM	TOTALS
Normal	12	6	5	5	8	3	6	7	52
Min-fo-ac-lym[c]	2	2	3	3	0	5	2	1	18
Totals	14	8	8	8	8	8	8	8	70

[a]The calculated chi-square was 9.7.[b]

[b]A chi-square based on 7 df must be 14.07 or larger to be significant at the .05 level.

[c]Minimal focal accumulations of lymphocytes.

TABLE D-12.--Chi-square[a] contingency table for pathology by 8-wk treatment.

Diagnosis	Treatment							
	CON	VOL	SHT	MED	LON	ESC	SWM	TOTALS
Normal	6	5	5	8	3	6	7	40
Min-fo-ac-lym[c]	2	3	3	0	5	2	1	16
Totals	8	8	8	8	8	8	8	56

[a]The calculated chi-square was 8.02.[b]

[b]A chi-square based on 6 df must be 12.59 or larger to be significant at the .05 level.

[c]Minimal focal accumulations of lymphocytes.

APPENDIX E

TISSUE SECTION DESCRIPTION

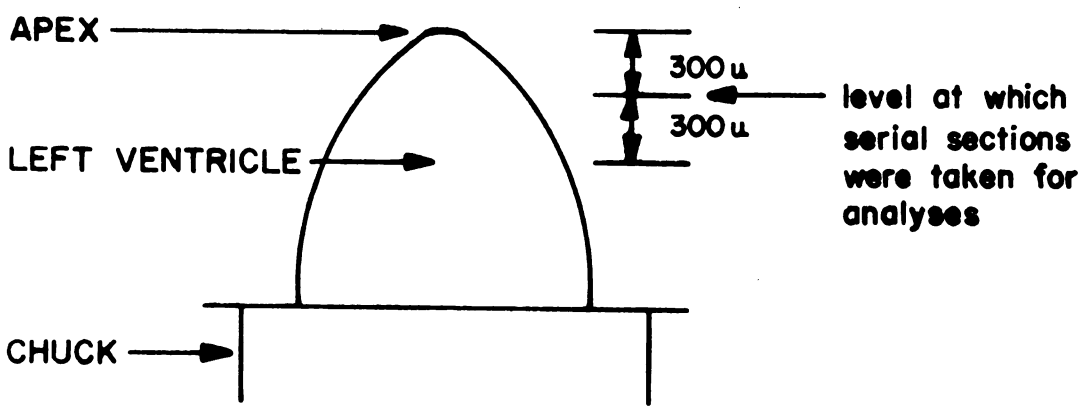

Figure E-1 – Sectioning of the Heart