Myocardial adaptations to age and exercise in rats: responses to acute swimming and 1-iso-proterenol-induced myocardial ischemia

Ronald Dean Fell
Iowa State University

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Myocardial adaptations to age and exercise in rats: Responses to acute swimming and l-isoproterenol-induced myocardial ischemia

by

Ronald Dean Fell

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

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INTRODUCTION

Heart diseases represent the most serious health problem in today's society (Fletcher and Cantwell, 1974; Braunwald, 1976). Exercise has been established as a valuable aid in diagnosis and evaluation of coronary heart disease (Bruce and Hornsten, 1969; Blomqvist, 1971). However, exercise training in treatment and prevention of coronary heart disease is a controversial issue (Blackburn, 1974; Bruce, 1974).

Programs of graded exercise have beneficial effects in men with coronary heart disease (Kennedy et al., 1976). It has also been established that men with physically active occupations have a lower mortality rate from coronary heart disease than do those engaged in sedentary work (Morris et al., 1948; Taylor et al., 1962).

A common response of an animal which has been subjected to a chronic exercise program is myocardial hypertrophy (Bloor et al., 1970; Banister et al., 1971; Froelicher, 1972). Exercise-induced cardiac hypertrophy was suggested to be a physiological response of the heart rather than a pathological hypertrophy. Experimental or pathological cardiac hypertrophy has been induced by imposing increased work loads on the heart in experimental animals by surgically constricting the aorta, inducing valvular insufficiencies, and administration of thyroxine or catecholamines. The mechanisms of pathological and physiological hypertrophy have
been suggested to consist of different cardiac responses (Rabinowitz and Zak, 1972).

Several biochemical estimations (DNA, RNA, protein, collagen) of cellular components of hypertrophied hearts have been carried out in pathological hypertrophy states. Surprisingly very few studies have used these biochemical determinations to characterize the growth of hypertrophy which has been induced by exercise training. When these determinations have been measured, the investigations involved either very young or very old animals. It was the purpose of Part I of the present investigation to examine the effects of moderate aging and exercise on cardiac growth as characterized by biochemical measurements of DNA, RNA, protein and collagen concentrations.

It was of further interest to examine whether or not the exercise hypertrophied heart could protect itself against an experimentally induced ischemia which would lead to irreversible myocardial necrosis. Wexler and Judd (1970) described a positive fuchsinophilia associated with increased tissue acidity due to injection of the potent synthetic beta catecholamine isoproterenol. That investigation suggested the hearts of isoproterenol-injected rats were changing from aerobic to anaerobic metabolism during the influence of the drug. Isoproterenol injections appeared to be a means of providing a model animal system to test beneficial effects of exercise against experimentally-induced ischemic cardiac
conditions. The isoproterenol model was examined in Part II of the present investigation.
PART I:

EFFECTS OF AGE, EXERCISE AND ACUTE SWIMMING ON RAT HEARTS
LITERATURE REVIEW

Normal Development of Myocardial Tissue

During embryonic development of cardiac tissue, an active proliferation of undifferentiated myogenic cells occurs. These cells begin to synthesize myofibrillar protein but still maintain their ability to divide mitotically. As muscle protein synthesis increases in these cells, the number of dividing cells decreases. These events are described in several morphological investigations (Weinstein and Hay, 1970; Oberpriller and Oberpriller, 1971; Hay and Low, 1972).

In neonatal hearts mitosis of cardiac muscle cells is still evident (Zhinkin and Andreeva, 1963; Overy and Priest, 1966; Neffgen and Korecky, 1972). Chacko (1972) suggested a myocardial cell can not undergo mitosis once myofilaments are organized into well defined myofibrils (Chacko, 1972). However, a recent study by Kelly and Chacko (1976) provided in vitro evidence that myocardial cells can divide, not only in the presence of myofibrils, but also while beating. They pointed out the necessity to maintain normal myocardial cell architecture during division, which minimized disturbances between cells and allowed the young heart to retain forceful contractions. No explanation is available to explain why myocardial cells, shortly after the neonatal period of development, lose their ability to undergo mitosis. Loss of myocyte mitotic ability occurs approximately at 28 days of age in rats and at 3 to 6 months of age in humans (Brown, 1971).
During heart organogenesis, nonmuscle cells (connective and epithelial) invade the myocardium (Manasek, 1968). These cells proliferate rapidly, and in an adult heart, non-muscle cells outnumber myocardial cells by more than 3 to 1 (Zak et al., 1976). When myocardial cells stop dividing during neonatal life, subsequent enlargement can be attributed to hypertrophy of myocardial cells and hyperplasia of connective tissue and epithelial cells (Zak, 1973).

Response of the Heart to Increased Work Loads

Adult hearts have a remarkable capacity to respond to functional demands placed on them by body metabolism (Kohn, 1971; Johnson, 1971). A large number of experimental procedures have been used to produce an increase in cardiac mass (measured by heart weight) in response to an increased work load. These included aortic constriction (ascending or descending), pulmonary artery constriction, renal or steroid induced systemic hypertension, damage to cardiac valves resulting in aortic, pulmonic, or tricuspid insufficiency, chronic hypoxia, anemia, arteriovenous fistula, administration of thyroxine or catecholamines, and severe prolonged exercise with either a treadmill or an enforced swimming program. All these procedures produce either an increased pressure load, increased volume load, or direct heart stimulation.

Crews and Aldinger (1967) reported that clinical findings revealed a difference between physiological cardiac hypertrophy and pathological hypertrophy. Increased myocardial mass in physiological
hypertrophy was a result of enlargement of the myocardial fibers rather than their multiplication. Myocardial mass in pathological hypertrophy was a result of hyperplasia (increased DNA concentrations) rather than an increase in size. Rabinowitz and Zak (1972) suggested differences may exist between volume and pressure overload-induced myocardial hypertrophy and that exercise hypertrophy had different cardiac responses since it was more of a physiological hypertrophy. In an investigation of various cardiac hypertrophy-inducing factors, Bartosova et al. (1969) suggested all possible variations in heart response may occur in the development of cardiac hypertrophy, depending on type and duration of the stimulus causing hypertrophy. They further suggested age and sex of the organism may modify cardiac responses to a hypertrophy stimulus and, therefore, need to be considered in comparing results of cardiac hypertrophy.

Meerson (1965) suggested the maximal work load a heart can adapt to during hypertrophy depends on its distensibility rather than its ability to develop tension in response to that work load. Experimental procedures (i.e. aortic constriction), which induced mainly an isometric hyperfunction in the heart, allow myocardial cells to increase the amount of tension they could develop. Increased development of tension by a hypertrophied heart has been supported by several authors (Beznak, 1958; Grimm et al., 1963; Kerr et al., 1961). The ability of a heart to develop more tension
after an animal had been exercise-trained has also been demonstrated (Whitehorn and Grimmenga, 1956; Crews and Aldinger, 1967; Penpargkul and Scheuer, 1970).

To discuss each experimental procedure mentioned above which induces cardiac hypertrophy is beyond the scope of this dissertation. This literature review will be limited to descriptions of cardiac growth as observed in response to exercise. When appropriate, comparisons from other forms of induced adaptive growth of the heart which apply or add support to observations in exercise-induced hypertrophy will be described.

Adaptive Changes of the Heart to Exercise

A fundamental adaptive mechanism of the heart to an increased functional load is myocardial hypertrophy. Biochemical and cellular responses to an increased work load which result in cardiac hypertrophy are being investigated. However, mechanisms which induce these patterns of change in the heart remain unknown.

Exercise in laboratory rats was shown to decrease their body weight, but does not uniformly affect their heart weight (Scheuer et al., 1970). Some studies reported increased heart weight while others have reported heart weights of exercised rats do not deviate from control heart weights. Shelley et al. (1943) found male rats (60 to 80 g) developed significantly greater heart weight to body weight ratios when swum three hours per day for 60 days. This significance was not attributed to a decreased body weight in exercised animals,
thus a true cardiac hypertrophy had occurred. In another study, Poland and Blount (1968) showed that when male rats (150 to 180 g) were forced to run on a treadmill, their body weights and left ventricle weights were significantly lower than those of corresponding controls. However, the ventricle to body weight ratios of exercise-trained rats were greater than control ratios. Stevenson et al. (1964) found when 215 g male rats were exercised on a treadmill, no increase in absolute heart weight was observed when compared to controls. They found a more rigorous treadmill exercise program caused a lower gain of body weight and a lower coronary bed size to heart weight ratio. They suggested moderate exercise with adequate rest may be more beneficial to an animal than a rigorous frequent exercise. Their data were similar to another report in which swimming and treadmill exercise programs did not increase absolute heart weights of exercised male rats (200 to 240 g) above control weights (Scheuer and Stezoski, 1972). Even when the exercise program had been rigorous, increased heart weight of exercised animals above controls was only borderline (Crews and Aldinger, 1967). The implication has been made that if the exercised rat hearts do not increase their weight above control heart weights, the exercised rat hearts may not be conditioned (Penpargkul and Scheuer, 1970). In their study male rats (180 to 200 g) were exercised by swimming, and the absolute heart weights were similar in control and exercised animals. Cardiac output measurements indicated that an exercised
rat heart was conditioned. Exercised rat hearts had a higher cardiac output, stroke volume, and a greater extent of myocardial fiber shortening (Penpargkul and Scheuer, 1970). Their results supported earlier work which also showed higher tensions could be developed in the hearts of conditioned rats than in sedentary control animals (Crews and Aldinger, 1967; Whitehorn and Grimmenga, 1956).

Crews and Aldinger (1967) exercise-trained female rats (214 to 264 g) by swimming them for progressively longer time periods. Some of their animals were sacrificed after 118 hours of swimming (6 hours per day to a total of 118 hours), while the rest were killed through 250 hours of exercise. They observed a significantly heavier heart in exercised animals compared to controls and the body weight was not depressed by their exercise regimen. Another investigation using female rats did not observe a decreased body weight in treadmill exercised animals (Dowell et al., 1976a). These studies revealed that sex of an animal does play a part in what response is observed with exercise, thereby confirming a suggestion made by Bartosova et al. (1969) that sex of an animal must be considered during an exercise regime. It was reported that treadmill exercised male rats reduced their food intake which resulted in a reduced growth rate (Oscai et al., 1971). Reduction in male exercised rat body weights has been a common report (Stevenson et al., 1964; Poland and Blount, 1968; Sohal et al., 1968; Scheuer et al., 1970; Tomanek et al., 1972; Dowell et al., 1976b). Reduction in body weight by
exercised male rats, and not by female rats, has led to some confusion in interpretation of the results reported in the literature. Beznak (1954) and Dunn et al. (1947) found that total heart weights were significantly correlated with body weights in rats. It would appear from this correlation that even though male rats decrease their body weight in response to exercise, absolute heart weights of these animals parallel the growth of control rat hearts (Stevenson et al., 1964; Crews and Aldinger, 1967; Sohal et al., 1968; Scheuer and Stezoski, 1972; Penpargkul and Scheuer, 1970; Scheuer et al., 1970; Dowell et al., 1976b). Decreased body weights, along with normal heart growth, caused a greater heart weight to body weight ratio in exercised rats in the above-cited investigations. Exercise training was suggested to cause cardiac hypertrophy, in order to keep absolute heart weights similar between control and exercised rats, but exercised rat hearts do not exceed normal control heart weights (Dowell et al., 1976b).

It has been reported that a positive correlation existed between heart size and maximum cardiac output (Grande and Taylor, 1965). This would suggest the increased heart weight to body weight ratios in exercised animals would be correlated with a greater cardiac output. Under hypoxic conditions an exercise-trained rat had a higher cardiac output, more cardiac work, and a higher systolic pressure than sedentary controls (Scheuer and Stezoski, 1972). It was concluded such effects were the result of increased pumping capacity of
conditioned hearts, probably due to more efficient energy utilization.

Since adenosine triphosphatase (ATPase) activity of actomyosin and myosin were increased in exercised rat hearts (Bhan and Scheuer, 1972; Wilkerson and Evonuk, 1971), it was suggested this would play a role in increased myocardial contractility and could serve to supply more blood, hence more oxygen, to exercising muscles (Holloszy, 1976). These factors may play a role in protecting the exercising heart against hypoxia.

Biochemical Changes in Adaptive Growth of the Heart

Adaptive heart growth has been associated with changes in deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein concentrations of heart tissue. A brief explanation of the use of these biochemical estimations is in order.

Increased concentrations of cardiac DNA have been suggested to indicate increases in cell number. In 1962, Enesco and Leblond concluded that the amount of DNA per diploid nucleus in rat brain and muscle was a constant 6.2 picograms per nucleus. It has been documented that a maximum amount of cytoplasmic volume exists that can be controlled by one nucleus (Moss, 1968; Cheek et al., 1971; Goldspink, 1972). It appears from these studies that in mononucleated cells where the degree of ploidy is constant, DNA concentrations are a valid estimate of cell numbers per unit tissue weight. Total DNA concentrations would indicate cell numbers in that organ. DNA concentrations have been used to estimate
cell numbers in many tissues (Hotchkiss, 1955). In cardiac muscle there have been several reports of polyploid amounts of DNA present in myocardial cells. Numbers of polyploid nuclei in human and monkey ventricular muscles may be as high as 80 percent (Kompmann et al., 1966; Sandritter and Adler, 1971). In rat and several other mammalian species, the population of polyploid nuclei was significantly lower, representing 2 to 15 percent of the nuclear population (Grove et al., 1969a). In addition to polyploidy, mitochondrial DNA would contribute to tissue concentrations. However, mitochondrial DNA concentrations as well as polyploidy have been reported to contribute insignificant amounts of DNA to the rat heart, at least while it is undergoing enlargement (Grove et al., 1969a; Grove et al., 1969b).

An associated increase in protein synthesis for structural components of the new cells would accompany DNA changes. The amount of protein per unit DNA may be used to estimate physiological cell size (Enesco and Leblond, 1962; Winick and Noble, 1965).

Changes in RNA would estimate changes in protein synthetic machinery available to the cells, and RNA-protein ratios would express the growth rate or protein synthetic activity of the cells (Gros, 1960). When RNA is expressed per unit DNA, the protein synthesizing capability of the physiological cell may be estimated (Winick and Noble, 1965). A large increase in an RNA-DNA ratio would indicate an increased cellular size (Robinson, 1971).
With the exception of one investigation discussed later, to this author's knowledge no studies have been made where cardiac hypertrophy due to prolonged exercise had been characterized by the biochemical cellular estimations of DNA, RNA and protein. The following investigations briefly describe pathologically induced cardiac hypertrophy using these estimations.

Increased DNA content of experimentally enlarged hearts has been correlated with increased heart weight and suggested to show increased cardiac cell numbers (Meerson et al., 1968; Morkin and Ashford, 1968; Grove et al., 1969a; Grimm et al., 1970).

Grimm et al. (1966) examined DNA and RNA concentrations in subdiaphragmatic aortic constriction and nonconstricted rats. Rat hearts were divided into three phases of growth according to their nucleic acid concentrations. Phase one was characterized by hypertrophy and hyperplasia of cells. Phase two consisted of only hypertrophy, while phase three resembled phase one in that both hypertrophy and hyperplasia were suggested. It would be difficult to rely on these phases of growth for accurate nucleic acid data comparisons with any type of normal control rat heart growth experiments since both normal unoperated rat hearts and experimentally induced hypertrophied hearts were pooled to give the range of heart weights. However, the results of other experimentally induced myocardial hypertrophy studies which have investigated nucleic acid concentrations under conditions of induced
hypertrophy do agree with the results of the above-cited work of Grimm and coworkers.

Several studies utilized radioautographic techniques to follow the incorporation of $^3$H-thymidine, and showed the increased DNA synthesis in the hypertrophied heart was confined to connective tissue cells (Meerson et al., 1968; Morkin and Ashford, 1968; Grove et al., 1969b).

RNA concentrations per mg tissue as well as total RNA concentrations have been shown to increase in the early stages of myocardial hypertrophy (Nair et al., 1968; Koide and Rabinowitz, 1969; Posner and Fanburg, 1968; Meerson, 1969; Kako et al., 1969). This has been attributed to increased activity of DNA-dependent RNA polymerases. When polymerase activity was measured in the presence of Mn$^{2+}$ or Mg$^{2+}$, a biphasic pattern of RNA synthesis occurred in hearts of rats subjected to aortic constriction. An early increase (4 hours after banding the aorta) of polymerase activity measured in the presence of Mg$^{2+}$ followed by a 24 hour postconstriction increase in activity measured in the presence of Mn$^{2+}$ has been noted (Kako et al., 1972). The authors suggested that the 4 hour post-operative rise in RNA was due to increased synthesis of messenger RNA (mRNA) followed by a 24 hour post-operative rise in ribosomal RNA (rRNA). Another group of workers (Schreiber et al., 1971) suggested the increased work load, due to increased perfusion pressure of an isolated heart, caused the myocardial cell nuclei to synthesize mRNA
and to increase protein synthesis by the third hour of increased pressure. As the overload was continued, more ribosomes were synthesized which further augmented protein synthesis. Higher metabolic rates and cellular constituent turnover (i.e. myosin, mitochondria, lysosomes) were suggested to further stimulate protein synthesis which results in myocardial cell hypertrophy. The increased RNA concentrations seen during experimentally induced cardiac hypertrophy coincide with increases in protein synthesis (Zak and Fischman, 1971).

A response to pathologically induced cardiac hypertrophy is increased numbers of connective tissue cells as mentioned previously by autoradiographic studies. It was shown that hydroxyproline was primarily located in the protein, collagen, in constant concentrations (Neuman and Logan, 1950). Because of this, several investigations have utilized measurements of hydroxyproline (Hyp) to determine the extent of connective tissue development in cardiac hypertrophy (Bartosova et al., 1969; Buccino et al., 1969; Spann et al., 1971).

Several investigations studied development of connective tissue in an exercised rat heart (Bartosova et al., 1969; Chvapil et al., 1973). These authors found both absolute increases and relative increases in collagen during cardiac hypertrophy induced by physical exercise in young rats but not in old animals. Tomanek et al. (1972) found no changes in connective tissue in hearts of either young (6 months) or old (22 months) rats that had been exercised.
However, higher concentrations of collagen were seen in older rat hearts when compared to young hearts. It had been shown earlier that collagen in the heart increases with age (Schaub, 1964/65). In a more recent study, connective tissue did not increase with exercise in the hearts of young trained mice (Kiiskinen and Heikkinen, 1976), confirming the results described above. It was suggested that physical training stimulated increases in collagen and noncollagen proteins equally in the young mouse heart.

Collagen content of a heart is an important determinant of myocardial compliancy and may be responsible for loss of compliancy in older rat hearts (Weisfeldt et al., 1971a). Increased stabilization of collagen with increasing age might be responsible for increased myocardial rigidity and perhaps loss of physiological function (Zwolinski et al., 1976).

Since collagen surrounds blood vessels, any increase in coronary vasculature may be responsible for increased collagen concentrations. Development of cardiac vasculature will therefore briefly be reviewed.

Cardiac Coronary Vasculature

During normal heart development, capillary growth occurs in proportion to the increase in muscle cell size which results in a constant density of capillaries per square millimeter of muscle tissue (Shipley et al., 1937; Roberts and Wearn, 1941; Linzbach, 1960). Even in experimental enlargement of the heart by aortic
constriction or induced anemia, capillaries and muscle cells proliferate with no change in capillary density per unit area of tissue (Shipley et al., 1937; Rakusan et al., 1967; Poupa et al., 1964). Rakusan et al. (1967) reported decreased capillary densities as the heart size increased due to aortic constriction in old (26 to 27 months) rats.

Several investigators have examined coronary circulatory volume after exercise training by injecting vinyl acetate into the coronary vessels and allowing it to harden. After the tissue was digested, significantly heavier acetate cast weights were observed in exercised animals (Stevenson et al., 1964; Tepperman and Pearlman, 1961). Leon and Bloor (1970) reported that swimming exercise increased capillary numbers, capillary to myocardial cell ratios, and coronary vessel lumen diameters. In this study rats of three different ages were suggested to correspond to teenagers, 20-40 year old, and 50-70 year old humans. Leon and Bloor did not observe increased coronary circulation measurements in the older rats.

Increased coronary circulation in response to treadmill running has been reported (Tomanek, 1970). Rats of 3 age groups (40, 130, and 515 days of age) were run on a treadmill 40 minutes per day for three months, and an observed increase in capillary to myocardial cell ratio resulted in younger animals. Capillary concentration decreased in older rat hearts. Tomanek suggested the blood supply to the heart muscle was increased in young exercised rats.
In another investigation (Poupa et al., 1970), coronary capillary supplies of wild and tame rats and rabbits were compared. It was demonstrated that wild, more active, animals had greater vascular networks than tame ones. Capillary development was greater in younger animals. This implied that they were more responsive to growth stimuli than the older animals. The above studies suggested that the coronary vessel development in animals may be enhanced if exercise is begun at young ages. Data concerning coronary vascular development and collagen concentration do not agree. Sizes of coronary vessels, age, and intensity of exercise must be accounted for in further investigations.

Little data exists regarding the physiologically induced exercise hypertrophy seen in hearts of experimental animals. To this author's knowledge Dowell et al. (1976b) are the only investigators who have measured DNA, RNA, and protein content in the hearts of an exercised rat.

Dowell et al. exercise-trained rats of two different age groups, 21-25 and 80-90 days of age, on a treadmill. Progressively greater speeds of running were used until 1.5 km/hour for 1 hour per day had been reached by the end of ten weeks. To compare different types of cardiac hypertrophy some animals from the young age group were injected with desoxycorticosterone acetate (DOCA) while others underwent aortic constriction surgery. Body weights were significantly lighter in older exercised rats compared to controls. This
effect was not evident in younger exercised animals. Aortic constriction significantly increased absolute heart weights, whereas exercise had no effect on absolute heart weights in either age group. Due to decreased body weights in older exercised rats, a significantly greater relative heart weight was observed when adjusted for body weight. No differences in DNA, RNA, protein or Hyp concentrations were noted between exercised and control rats of either age. It was concluded that since hearts were larger in the older exercised rats, the biochemical estimations had increased proportionately with the heart weights. Total DNA concentration per ventricle supported this conclusion. Aortic constriction and DOCA administration increased ventricular myocardial cell size, but this did not occur with exercise treatments. These results clearly revealed a distinct difference in the response to pathological and physiological hypertrophy. No explanation of greater total RNA, protein, or Hyp concentrations was discussed in this investigation by Dowell et al (1976b).

It is this author's opinion that there still has not been a description of the biochemical estimations of cell growth parameters in an exercised rat's heart. Furthermore, age studies have not described cardiac hypertrophy measurements over a range of ages at the time of cessation of growth in the rat. The purpose of the following study was to examine cardiac changes which occur before, during, and after the growth rate of a rat slows, and to compare
that normal pattern of development to that of exercise-trained rat hearts.
MATERIALS AND METHODS

Treatment of Animals

Male albino Sprague-Dawley rats of three age groups (100, 200 and 300 days old at the start of the experiment) were used in this study. Animals from each group were divided into four treatments designated exercise (Ex), machine controls (MC), guillotine controls (GC), and control controls (CC). Ex were subjected to a running exercise on a motor driven treadmill (Jette et al., 1969; Auth, 1975) five days per week for ten weeks. Week one served as a training period, with running speed and time gradually being increased throughout it. Running speed and time remained constant, 15 meters per minute at an 8° incline for 30 minutes each day, from week two through week ten. MC animals were placed on the treadmill with identical handling procedures as those in the Ex treatment group. The motor was turned on but disengaged so animals were not exercised. GC and CC rats were maintained under normal animal room conditions and were not subjected to the handling procedures of Ex or MC groups.

Each treatment (Ex, MC, GC, CC) within each age group (100, 200, 300) was further subdivided, at termination of the experimental period, into swim (S) and nonswim (NS) groups. Animals designated swimmers were subjected to a single exhaustive swimming exercise just prior to sacrifice. Nonswim animals were not so exposed.

All animals were maintained in an animal room where lighting
(0800 to 2200 hours) and temperature (25 ± 3°C) remained constant. A standard diet (Teklad) and water were provided ad libitum throughout the investigation.

Swimming Stress

Immediately before sacrifice, swimmers were individually swum in 20 gallon plastic drums filled with water (35 ± 2°C). Prior to swimming, lead weights amounting to 4 percent of the body weight were attached to each rat's tail. Animals were considered exhausted and removed from the water when they were unable to surface within a time period of ten seconds (Dawson and Horvath, 1970). Body weights and swim times (for S rats) were recorded prior to sacrifice between 1600 and 2400 hours.

Sacrifice Procedure

Animals in treatment groups Ex, MC, and CC were anesthetized with an intraperitoneal injection of sodium pentobarbital (25 mg/kg). Blood samples were removed either from the jugular vein (Ex, MC, CC) or from the neck of decapitated (GC) rats. Plasma was frozen for later study. A thoracotomy was performed immediately after removing blood, and hearts were excised while contracting. Atria, major vessels, and fat were trimmed from ventricles. The latter were rinsed with Locke's solution, blotted, and weighed. Two pieces of left ventricle from the apical regions were immediately excised and fixed for histological examination. The remainder of the heart was sealed in a plastic freezer bag and quick-frozen in acetone and dry
ice. Hearts were still contracting when placed in the acetone bath.

Heart Histology

Left ventricular tissue was fixed in 5 percent phosphate buffered glutaraldehyde. This tissue was postfixed in 1 percent phosphate buffered osmium tetroxide, prior to dehydration in ascending concentrations of acetone, followed by embedding in Epon 812. Thin sections were cut on an LKB Ultratome III and stained in uranyl acetate and lead citrate. Sections were examined on a Hitachi HS-8 electron microscope.

A second piece of left ventricle was fixed in 10 percent buffered formalin, embedded in paraffin, and stained for collagen content (Mallory's triple stain).

Nucleic Acid Determinations

Frozen ventricles were dry fat-freed (Appendix A) and total nucleic acids were extracted in hot trichloroacetic acid, according to Schneider's method (1945) as modified by Ferreri (1971, Appendix B). DNA and RNA were estimated from this extract by colorimetric procedures. DNA content was determined by the diphenylamine method of Burton (1956, Appendix E). RNA content was determined by the orcinol procedure of Mejbaum (1939) as described by Schneider (1957, Appendix F). Absorbances measured in nucleic acid extracts of hearts were compared to standard solutions of hydrolyzed DNA and RNA which were prepared according to the method of Webb and Levy (1955, Appendix C). Accuracy of these standards was established by colorimetrically
analyzing phosphorus content of DNA and RNA by the method of Fiske and Subbarow (1925, Appendix D).

Protein Determinations

Samples of dry fat-free tissue were used in determination of cardiac total protein according to the method of Lowry et al. (1951, Appendix G).

Hydroxyproline Determinations

Collagen content of the heart was determined using the modified technique of Kivirikko et al. (1967, Appendix H) for the determination of hydroxyproline concentration. Hydroxyproline concentration is multiplied by 7.46 to give collagen concentration (Neuman and Logan, 1950).

Analysis of Data

Data were analyzed using The Statistical Analysis System (Barr and Goodnight, 1971) in consultation with personnel from the Iowa State University Statistics Laboratory. The ANOVA procedure was used to calculate the analysis of variance and the MEANS procedure was used to calculate the means of all variables in each experimental group. Individual experimental groups were compared using t-tests with unequal variances and unequal numbers (Snedecor and Cochran, 1967).
RESULTS

Since animals were weighed prior to, and sacrificed immediately after, swimming, parameters measured in this study would not be altered by this short term exercise. Statistical analysis verified this, thus animals in nonswim and swim subdivisions of each treatment group were combined. No significant differences existed between any control treatments (MC, GC, CC) within each age group. Therefore, to simplify further discussion, all control animals at each age have been combined in one group, labelled controls (C).

Body Weights

Mean final body weights for exercise-trained rats were significantly lower than control body weights in all three age groups (Figure 1.1). Two hundred and seventy day-old rats had body weights significantly greater than 170 day-old animals in both exercise (P<0.001) and control (P<0.001) treatments, but no significant differences were observed between 270 and 370 day-old rat body weights in either treatment.

Heart Weights

Mean heart weights were significantly heavier between 170 and 270 days of age in both exercised (P<0.001) and control (P<0.001) rats (Figure 1.2). No additional change in heart weight was observed between 270 and 370 day-old rat hearts in either treatment group. There were no significant differences between treatments (Ex, C) at any age.
Figure 1.1. Means ± standard errors of final body weight.

Ex C Ex C Ex C
170-Ex - 386.5 ± 7.3 (N=30) P<0.02 a
170-C - 408.1 ± 4.8 (N=62) P<0.001
270-Ex - 449.8 ± 5.5 (N=36) P<0.01
270-C - 488.6 ± 5.4 (N=57)
370-Ex - 450.5 ± 7.2 (N=21)
370-C - 476.1 ± 6.2 (N=61)

aSignificance between treatments at each age.
Figure 1.2. Means ± standard errors of absolute heart weight (g).

170-Ex - 1.06 ± 0.021 (N=30)  
170-C - 1.02 ± 0.014 (N=61)  
270-Ex - 1.20 ± 0.016 (N=36)  
270-C - 1.17 ± 0.013 (N=57)  
370-Ex - 1.20 ± 0.026 (N=21)  
370-C - 1.14 ± 0.016 (N=61)  

^N.S. = not significant at P<0.05.
When heart weights were expressed as a ratio of heart weight per body weight (mg/g), highly significant differences (P<0.001) were observed between treatments within each age group (Figure 1.3). All Ex rats had greater heart weights per g body weight than C animals. Ex rat hearts per g body weight at 170 days of age were significantly (P<0.05) heavier than Ex rat hearts of 270 day-old animals, but not significantly different from 370 day-old exercised rat hearts. No differences were seen between any control treatment heart ratios.

DNA Concentration

Heart DNA concentration was expressed as ug DNA per mg dry fat-free tissue in Figure 1.4. No significant differences existed between treatment (Ex, C) DNA concentrations except in 270 day-old rat hearts (P<0.02) where Ex hearts had less DNA. DNA concentrations of Ex rat hearts exhibited no significant differences between age groups. DNA concentrations of C animal hearts at 270 days of age were significantly greater than 370 day-old C (P<0.05), but no difference existed between 170 and 270 or 170 and 370 day-old C concentrations.

RNA Concentration

Mean RNA concentrations (ug RNA/mg DFFT) are shown in Figure 1.5. No significant differences in these concentrations were observed between treatments (Ex, C) in 170 and 270 day-old rat hearts. Ex rat heart RNA concentrations in the 370 day-old group were signifi-
Figure 1.3. Means ± standard errors of relative heart weights (mg heart/g body weight).

170-Ex - 2.76 ± 0.03 (N=30)  P<0.001
170-C - 2.47 ± 0.05 (N=62)
270-Ex - 2.66 ± 0.03 (N=36)  P<0.001
270-C - 2.40 ± 0.02 (N=57)
370-Ex - 2.67 ± 0.05 (N=21)  P<0.001
370-C - 2.41 ± 0.02 (N=61)

Significance level between treatments in each age group.
Figure 1.4. Means ± standard errors of DNA concentrations (μg DNA/mg DFFT).

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Treatment</th>
<th>DNA Concentration (μg DNA/mg DFFT)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>Ex</td>
<td>7.12 ± 0.28</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>6.97 ± 0.23</td>
<td>54</td>
</tr>
<tr>
<td>270</td>
<td>Ex</td>
<td>6.66 ± 0.19</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>7.32 ± 0.20</td>
<td>51</td>
</tr>
<tr>
<td>370</td>
<td>Ex</td>
<td>7.07 ± 0.28</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>6.69 ± 0.20</td>
<td>55</td>
</tr>
</tbody>
</table>

N.S. = not significant at P<0.05.

Significance level between treatments within age group.

\[^a\] N.S. = not significant at P<0.05.
\[^b\] Significance level between treatments within age group.
Figure 1.5. Means ± standard errors of RNA concentrations (ug RNA/mg DFFT).

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>170-Ex</td>
<td>16.50 ± 0.33</td>
<td>N=29</td>
<td>N.S. a</td>
</tr>
<tr>
<td>170-C</td>
<td>16.10 ± 0.26</td>
<td>N=54</td>
<td></td>
</tr>
<tr>
<td>270-Ex</td>
<td>18.15 ± 0.25</td>
<td>N=34</td>
<td>N.S.</td>
</tr>
<tr>
<td>270-C</td>
<td>17.87 ± 0.33</td>
<td>N=51</td>
<td></td>
</tr>
<tr>
<td>370-Ex</td>
<td>18.21 ± 0.78</td>
<td>N=19</td>
<td>P&lt;0.01 b</td>
</tr>
<tr>
<td>370-C</td>
<td>15.64 ± 0.27</td>
<td>N=55</td>
<td></td>
</tr>
</tbody>
</table>

a N.S. = not significant at P<0.05.
b Significance level between treatments within that age.
cantly greater than C of the same age. C rat heart RNA concentrations in 270 day-old rats were significantly greater (P<0.001) than 170 and 370 day-old control concentrations. RNA concentrations of Ex rat hearts were significantly greater (P<0.001) between 170 and 270 and between 170 and 370 days of age. No significant difference was observed between 270 and 370 day-old Ex rat heart RNA concentrations.

RNA-DNA Ratio

Mean RNA-DNA ratios (ug RNA/ug DNA) are shown in Figure 1.6. C animals of all three age groups showed no significant differences in their ratios. RNA-DNA ratios of exercised rat hearts at 270 days of age are significantly greater (P<0.001) than Ex rat heart ratios at 170 days of age. There was no significant difference between 270 and 370 or 170 and 370 day-old Ex groups. Exercise caused a significant increase (P<0.05) in the RNA-DNA ratio over controls in the 270 day-old group. No significant differences between Ex and C treatments within 170 and 370 day-old ratios were noted.

Protein Concentration

Protein concentration (mg protein/mg DFFT) differs significantly (P<0.001) between Ex and C treatments at 170 days of age (Figure 1.7). Treatment effects (Ex, C) were not significantly different with respect to heart protein concentration, in 270 or 370 day-old rats. A significant treatment-age interaction occurred with respect to protein concentration. Hearts from Ex rats at 170 days of age contained significantly greater (P<0.01) amounts of protein than
<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>Mean ± SE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>Ex</td>
<td>2.40 ± 0.090</td>
<td>29</td>
</tr>
<tr>
<td>170</td>
<td>C</td>
<td>2.45 ± 0.090</td>
<td>54</td>
</tr>
<tr>
<td>270</td>
<td>Ex</td>
<td>2.80 ± 0.095</td>
<td>34</td>
</tr>
<tr>
<td>270</td>
<td>C</td>
<td>2.53 ± 0.076</td>
<td>51</td>
</tr>
<tr>
<td>370</td>
<td>Ex</td>
<td>2.63 ± 0.118</td>
<td>19</td>
</tr>
<tr>
<td>370</td>
<td>C</td>
<td>2.48 ± 0.098</td>
<td>55</td>
</tr>
</tbody>
</table>

N.S. = not significant at P<0.05.

Significance between treatments within that age group.

Figure 1.6. Means ± standard errors of RNA-DNA ratios (ug RNA/ug DNA).
Figure 1.7. Means ± standard errors for protein concentrations (mg protein/mg DFFT).

170-Ex = 0.937 ± 0.0087 (N=26)  P<0.001\textsuperscript{a}
170-C = 0.871 ± 0.0073 (N=49)

270-Ex = 0.910 ± 0.0120 (N=34)
270-C = 0.883 ± 0.0117 (N=47)  N.S.\textsuperscript{b}

370-Ex = 0.885 ± 0.0156 (N=19)
370-C = 0.912 ± 0.0077 (N=55)  N.S.

\textsuperscript{a}Significance level between treatments in that age group.

\textsuperscript{b}N.S. = not significant at P<0.05.
hearts from Ex rats at 370 days of age. C 170 day-old hearts had significantly (P<0.001) lower concentrations of protein than did hearts at 370 days of age. Two hundred and seventy day-old heart protein concentrations in both Ex and C treatments were intermediate too, but were not significantly different from either 170 or 370 day-old hearts of the same treatment group.

**Protein-DNA Ratio**

Mean protein-DNA ratios (mg protein/ug DNA) are shown in Figure 1.8. No significant treatment effect of exercise on protein-DNA ratios in 170 and 370 day-old hearts when compared to C hearts of the same age existed. A significantly greater ratio (P<0.01) was seen with exercise in the 270 day-old age group. C rat heart protein-DNA ratios from 170 and 270 day-old animals were significantly lower (P<0.001) than 370 day-old C heart ratios. Ex rats had heart protein-DNA ratios which were not significantly different between any ages.

**Protein-RNA Ratio**

Figure 1.9 shows mean protein-RNA ratios (mg protein/ug RNA). C rat hearts showed a significantly greater ratio (P<0.001) than Ex rat hearts in the 370 day-old group. No significance was seen between Ex treatments at any age. C rat hearts were not significantly different between 170 and 270 day-old age groups but ratios of both were significantly lower (P<0.001) than 370 day-old C heart ratios.
Figure 1.8. Means ± standard errors of protein-DNA ratios (mg protein/ug DNA).

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>Mean ± SE (N)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>Ex</td>
<td>0.127 ± 0.010 (N=29)</td>
<td>N.S. a</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.114 ± 0.007 (N=54)</td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>Ex</td>
<td>0.141 ± 0.005 (N=34)</td>
<td>P&lt;0.01 b</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.116 ± 0.006 (N=51)</td>
<td></td>
</tr>
<tr>
<td>370</td>
<td>Ex</td>
<td>0.129 ± 0.006 (N=19)</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.149 ± 0.006 (N=55)</td>
<td></td>
</tr>
</tbody>
</table>

A N.S. = not significant at P<0.05.

b Significance between treatments in that age group.
Figure 1.9. Means ± standard errors of protein-RNA ratios (mg protein/ug RNA ratios).

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>Mean ± SE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>Ex</td>
<td>0.052 ± 0.0035</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.048 ± 0.0027</td>
<td>54</td>
</tr>
<tr>
<td>270</td>
<td>Ex</td>
<td>0.050 ± 0.0011</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.047 ± 0.0022</td>
<td>51</td>
</tr>
<tr>
<td>370</td>
<td>Ex</td>
<td>0.050 ± 0.0019</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.059 ± 0.0011</td>
<td>55</td>
</tr>
</tbody>
</table>

* a N.S. = not significant at P<0.05.

* b Significance level between treatments within that age.
Hydroxyproline Concentration

Hydroxyproline (Hyp) concentration (ug Hyp/mg DFFT) is shown in Figure 1.10. Ex hearts displayed significantly greater amounts of Hyp per mg DFFT than C at 170 (P<0.001) days of age. Two hundred and seventy day-old hearts differed from the younger age group; Ex rat hearts had significantly (P<0.001) less Hyp/mg DFFT than C rat hearts at 270 days of age. No significant difference was observed between treatments at 370 days of age. Hearts from 170 day-old C rats had significantly (P<0.001) less amounts of Hyp/mg DFFT than C hearts at 270 days of age, but did not differ significantly from 370 day-old C rat hearts. Three hundred and seventy day-old C hearts had significantly lower concentrations of Hyp than 270 day-old C hearts. No significant differences in Hyp concentration existed between Ex rat hearts at any age.
Figure 1.10. Means ± standard errors of hydroxyproline concentrations (ug Hyp/mg DFFT).

<table>
<thead>
<tr>
<th></th>
<th>Ex</th>
<th>C</th>
<th></th>
<th>Ex</th>
<th>C</th>
<th></th>
<th>Ex</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>170-Ex</td>
<td>4.40 ± 0.180 (N=8)</td>
<td>3.70 ± 0.116 (N=8)</td>
<td>P&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170-C</td>
<td>4.27 ± 0.116 (N=8)</td>
<td>5.05 ± 0.271 (N=8)</td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>270-Ex</td>
<td>4.33 ± 0.309 (N=8)</td>
<td>3.96 ± 0.101 (N=8)</td>
<td>N.S.&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>270-C</td>
<td>3.70 ± 0.116 (N=8)</td>
<td>5.05 ± 0.271 (N=8)</td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>370-Ex</td>
<td>4.33 ± 0.309 (N=8)</td>
<td>3.96 ± 0.101 (N=8)</td>
<td>N.S.&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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</tr>
<tr>
<td>370-C</td>
<td>3.70 ± 0.116 (N=8)</td>
<td>5.05 ± 0.271 (N=8)</td>
<td>P&lt;0.001</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<sup>a</sup>Level of significance between groups.
<sup>b</sup>N.S. = not significant at 0.05 level.
DISCUSSION

C animals exhibited greater final body weights at 270 and 370 days of age compared to 170 day-old C rats (Figure 1.1). There were no significant differences between 270 and 370 day-old rat final body weights in either treatment. These results showed that age groups selected for this study involved a growing animal (170 day age group), a group of animals in a transition period where growth rate was slowing down (270 day age group), and a mature animal whose rate of growth had reached a plateau (370 day age group). This report of decreased rate of body weight growth with age is in agreement with several other authors (Singh and Kanungo, 1968; Story, 1970).

Exercise significantly decreased final body weights in all three age groups. Lower body weights induced by exercise may result from decreased body fat. Exercise has been reported to decrease epididymal and visceral fat (Jones et al., 1964; Hanson et al., 1967; Crews et al., 1969). A mechanism which would reduce body fat may be the increased capacity of Ex animals to utilize lipids more than carbohydrates for their energy source (Christensen and Hanson, 1939; Hermansen et al., 1967; Borensztajn et al., 1975; Rennie et al., 1976). Another possibility for decreased body weight may be a voluntary reduction in food intake (Whalley et al., 1951; Mayer et al., 1954), especially in exercised male rats (Oscai et al., 1971).

Cardiac ability to respond to increased work loads by increased adaptive growth has been well-recognized (Goss, 1971; Rabinowitz, 1973).
The amount of physical activity an animal undergoes has been shown to correlate positively with an animal's heart weight to body weight ratio (Zak, 1973). Absolute heart weights (Figure 1.2) in this study suggested that if a change was occurring in an Ex rat's heart, it was a small, statistically undetectable one. However, when heart weight was expressed per g body weight (Figure 1.3), significantly larger relative heart weights were observed in Ex animals. This significance may be attributed to a marked decrease in body weight of Ex rats. Although both these weight measurements may not give a reliable index of cardiac hypertrophy, they do demonstrate that the Ex rat has a larger heart per body weight mass. The Ex rat has a heart which corresponds to the size of a C rat heart at that respective age, but due to its decreased body size it has developed a more efficient heart to body weight ratio.

Biochemical assays measured in this study have all been utilized to estimate some phase of cell growth or capability of growth. These determinations were DNA, RNA, protein, and hydroxyproline concentration per mg heart (DFFT).

It is apparent that there is a maximum cytoplasmic volume that can be controlled by one given nucleus (Moss, 1969). DNA concentrations estimate the number of cells per unit tissue volume (Robinson, 1971). This appears to be a valid assumption in homogeneous tissues where only one cell type exists. However, in heterogeneous tissues, interpretation of changes in DNA concentrations must involve
consideration of which cells are contributing to those changes.

Mitosis of mammalian ventricular muscle cells, although apparent at birth, is almost completely absent after several weeks of neonatal life (Zak, 1973). In several studies of experimentally induced cardiac hypertrophy, connective tissue hyperplasia as well as myocardial cell hypertrophy has been suggested to be responsible for increased cardiac size (Morkin and Ashford, 1968; Spann et al., 1971; Zak, 1974). Connective tissue must be measured in studies dealing with cardiac hypertrophy since connective tissue cells outnumber muscle cells three to one (Zak et al., 1976) and undergo mitosis.

RNA concentrations may be used to determine the protein synthesizing capabilities of a tissue. The heart has a remarkable capacity to synthesize and renew its structural components (myofibrillar proteins, mitochondria, membrane proteins and soluble proteins) which are all in a constant state of dynamic equilibrium, continuously being destroyed and synthesized (Rabinowitz and Zak, 1972; Rabinowitz, 1971). Another estimate of tissue synthetic activity in individual cells would be RNA-DNA ratios. This determination must be used with caution if DNA concentrations are subject to variation.

Protein concentrations when expressed per ug DNA give an indication of cell size (Winick and Noble, 1965). Protein per ug RNA estimates the activity of the protein synthesizing machinery. Both of these parameters have been used in past investigations (Kako et al., 1972; Hogberg, 1976).
Hydroxyproline (Hyp) concentration (ug Hyp/mg DFFT) may be used to quantify collagen per unit tissue (Neuman and Logan, 1950). When all of the aforementioned determinations are measured in the same tissue, it allows analysis of cellular changes which may have taken place. Through such analysis, an estimation of the metabolic activity of that tissue can be made.

In order to clarify further discussion of changes which have occurred in cardiac muscle in this investigation, the remainder of this section will be divided into three parts: Control Parameters with Age, Exercise Parameters with Age, and Exercise-Control Comparisons with Age, respectively.

Control Parameters with Age

Control rat hearts between the ages of 170 and 270 days exhibited no significant changes in the amount of DNA per mg heart weight (DFFT) when tested with a t statistic using the variance of each group. When the pooled variance for ug DNA per mg DFFT for all rats in the study was used in a t-test, a highly significant (P<0.001) increase was observed between 170 and 270 day-old C rat DNA concentrations. Animals were not weight-matched between treatments which would contribute to more variability between groups. The author feels this variability was reflected in all assays measured in this study. Therefore, rather than using a pooled estimate of the variance in t-test comparisons, the more conservative method of using individual variances for comparisons was used. This method
decreased the chances of claiming false significance in statistical comparisons. Regardless of the method for comparison between 170 and 270 day-old C rat heart DNA concentrations, the tendency still existed for 270 day-old concentrations to be greater than 170 day-old concentrations. Since there were no significant differences between RNA-DNA ratios per mg tissue in 170 and 270 day-old C rat hearts, and yet there were significantly greater RNA concentrations in 270 day-old C rat hearts, this would suggest increased DNA concentration in the 270 day-old rat heart. Therefore, it is postulated that more cells existed per mg heart in the 270 day-old C rat than in the 170 day-old rat heart, thus hyperplasia was indicated in 270 day-old C rat hearts. Marginal changes in cell number per unit volume of heart would be expected in animals which were not subjected to strenuous increased work loads except the increased work load due to increased body weight.

Protein-DNA ratios (Figure 1.8) suggested cell size remained constant between 170 and 270 day-old C rat hearts. Yet a tendency for increased protein concentrations with increasing age is apparent in C rat hearts (Figure 1.7). Increased Hyp per mg heart in 270 day-old C rats suggested an increased amount of connective tissue present in these hearts. It is difficult to state whether this increased collagen was due to connective tissue hyperplasia or increased collagen fiber synthesis by the fibroblast. Nucleic acid data would suggest hyperplasia since protein-RNA ratios
do not reveal significant differences in synthetic activity between 170 and 270 day-old control groups.

DNA concentrations in Figure 1.4 (ug DNA/mg DFFT) suggested cell numbers per mg tissue decreased in C rat hearts between 270 and 370 days of age. Figure 1.6 showed there were equal amounts of RNA per ug DNA between these same age groups. Thus, significantly decreased RNA per unit tissue volume (Figure 1.5) also suggested decreased cell numbers per mg tissue as well as similar amounts of activity per cell (RNA-DNA ratio) between 270 and 370 day-old rat hearts. These measures would indicate fewer cells were making up the same volume of heart tissue in 370 day-old C rat hearts. Protein-RNA ratios significantly increased between 270 and 370 days of age (Figure 1.8), indicating more protein synthetic activity. Along with this, the amount of protein per ug DNA significantly increased in C rat hearts between 270 and 370 days of age, indicating larger cells in 370 day-old C rat hearts. Hyp concentrations between 270 and 370 day-old C rat hearts were not significantly different, although a decrease at 370 days of age approached significance.

Data therefore suggested that between 270 and 370 days of age a C rat heart decreases the number of cells per unit tissue, but the cells are actively synthesizing more protein in order to increase cell size, thus indicating myocardial cell hypertrophy. It would take fewer of these larger, more active, cells to make up a mg of heart tissue and this would account for fewer cells per unit
tissue, or decreased DNA concentrations observed in 370 day-old C rat hearts.

Hyp concentrations would appear to support myocardial cell hypertrophy between these older age groups since the amount of Hyp per mg heart is lower at 370 days of age than at 270 days of age. This would suggest connective tissue is not increasing in cell number along with the increase in size of myocardial cells, hence connective tissue is making up a lower proportion of unit heart volume. No increased synthesis of collagen by the fibroblast, coupled with increased protein synthesis by myocardial cells, gave a net increase in protein per unit tissue between 270 and 370 days of age.

Data from C rats suggests the following two hypotheses: In a growing rat between the ages of 170 and 270 days of age, increases in heart size are due to connective tissue proliferation. This serves to increase the compliance of the heart and strengthen it to meet the demands of an increased work load due to increasing body size and activity. The larger that animal grows the more work load is placed on the heart. Between the ages of 270 and 370 days, it is no longer beneficial to the heart to increase its connective tissue components. Connective tissue can add to the heart's compliance but it cannot serve to strengthen the force of contraction, and has been suggested to decrease cardiac compliance with increasing age (Weisfeldt et al., 1971a). Once the connective tissue-
myocardial tissue ratio reaches an optimum, collagen synthesis may be turned off and, if further demands are made on the heart, myocardial cells undergo hypertrophy.

Previous investigations have shown greater concentrations of collagen in older hearts (Clausen, 1962; Schaub, 1964-65; Chvapil et al., 1973; Tomanek et al., 1972). This has been demonstrated in our own laboratory where a 10.6 percent increase in collagen density was observed in 400 day-old rat hearts when compared to 300 day-old rats (Einspahr, 1974). It appears that collagen accumulation and parenchymal cell growth in body organs are independent of each other. That is, organ growth and the accumulation of that organ's collagen proceed at independent and variable rates (Harkness, 1955), and collagen accumulation proceeds at a faster average rate than organ parenchymal cells (Harkness and Harkness, 1954). Data from C rat hearts in the present investigation agree with variable rates of growth between collagen and parenchymal myocardial cells. Results show that if shorter time increments of aging rat hearts are analyzed for collagen per mg heart, the pattern of increased collagen concentration with increasing age is not followed. It is suggested that normal growth of cardiac tissue may cycle between periods of collagen synthesis and myocardial cell growth (width and length). Stimulation of one or the other may depend on the ratio of collagen to myocardial cell size.

Another distinct possibility for cyclic cardiac Hyp concentra-
tions would be in development of coronary blood vessels. It is known that collagen is distributed along blood vessels, and would therefore increase in concentration if vascularization increased. As myocardial cells get larger due to normal growth, or increased work load, more coronary vessels develop to meet increased oxygen demand (Schaper, 1971). If further growth or work loads were placed on the heart the process of increased vascularization and greater collagen accumulation would repeat itself. This cyclic process could continue until an age where further vascularization could not take place. It was demonstrated that a constant capillary density is maintained in normal growth of the heart (Shipley et al., 1937). In old rats (26-27 months), the capillary density is reduced as heart size increased (Rakusan and Poupa, 1964). If this process occurs in the growing heart, then concentrations of Hyp would be highly dependent on the age and the amount of activity of the animal.

Regardless of what pattern is followed in development of cardiac collagen concentrations, data in this study suggest significant age changes in DNA, RNA, protein and Hyp concentrations occurred before the animal significantly aged. Studies including comparisons between extremely young and extremely old animals which involve cardiac growth measurements may not reflect accurate aging alterations. Mechanism of aging studies should utilize age groups where age changes are occurring and not extreme ages as used in many past investigations.
Exercise Parameters with Age

DNA concentrations (Figure 1.4) in hearts of Ex rats were not significantly different between any age groups. RNA concentrations (Figure 1.5) of Ex rat hearts were greater at 270 days of age than at 170 days of age, and remained at that high concentration through 370 days of age.

It has been reported that in aortic constriction-induced cardiac hypertrophy (20-50% increase in heart weight), DNA concentration per mg rat heart is not different from sham operated animals (Nair et al., 1971). In the same investigation it was found that total cardiac DNA as well as RNA concentrations significantly increased. Increased RNA concentrations were suggested to be due to increased activity of RNA polymerase within hours after aortic constriction. Nucleic acid data in the present investigation follow the same pattern as the above-mentioned study in that RNA increases in 270 day-old Ex rat hearts compared to 170 day-old Ex animals. Concentrations of RNA implied that older hearts (270 and 370 days of age) were capable of synthesizing more protein. This idea was supported by higher RNA-DNA ratios in older Ex rat hearts (Figure 1.6). It has been shown that increased RNA synthesis occurring in the hearts of aortic banded rats was primarily an increase in ribosomal RNA (Kako et al., 1972). This would suggest that older Ex rats in the present study had myocardial cells with a greater capacity for protein synthesis than 170 day-old Ex
rat hearts. However the translation efficiency of this RNA is apparently lost in these older rats since protein concentrations were decreased with increasing age.

Decreased protein concentrations observed with increasing age in Ex rat hearts were not explained by alterations in amounts of collagen since Hyp concentrations were the same in all three Ex age groups. These data suggested Ex rat hearts have a set requirement for collagen regardless of the age of the heart. This point will be discussed further under Exercise-Control Comparisons with Age.

Another possible explanation may be that protein synthesis was occurring but the protein was being broken down or removed from the cell as rapidly as it was synthesized. If translation was less efficient, the increased RNA concentration in older rat hearts would have decreased the protein-RNA ratios, thus indicating cell activity had decreased in older Ex rat hearts. However, since this ratio remained constant (Figure 1.9), it suggested that protein synthesis was occurring in the older rat heart and that the protein was being lost from the cell, perhaps catabolized in the myocardial cell. If the protein was not being lost or broken down at a comparable rate to synthesis, protein-DNA and protein-RNA ratios would have increased in older rat hearts.

The level of exercise in this study apparently is better tolerated by a younger animal. Although relative heart weight data
suggested adaptive cardiac growth has occurred in the 170 day-old Ex rat heart, this is probably due to an increased proliferation of collagen as discussed later. The young rat (170 days of age) apparently can adapt its heart to the exercise regimen due to more efficient translational machinery allowing it to maintain lower RNA concentrations and yet still increase protein synthesis. Older rat hearts had less efficient RNA and it is suggested that in order for the older rat to adapt to this Ex induced work load it must turn on translation and increase protein synthesis (probably in myocardial cells) only to break down that protein as rapidly as it is made. It would seem reasonable from Ex rat data to suggest the older rat heart may be using its protein as an added energy substrate. Regardless of how the increased RNA and decreased protein concentrations are interpreted, one fact remains clear: the exercise regimen used in this investigation stimulated transcription in older rats and not in a young growing animal.

Exercise-Control Comparisons with Age

It may be recalled from earlier portions of this discussion, significantly greater heart weight to body weight ratios were observed in exercised rats. Ex rats had absolute heart weights which were not significantly different from C weights at any age. It has been shown that an endurance trained rat increases its skeletal muscle respiratory capacity (Baldwin et al., 1972), and an associated increase in the demand for oxygen. This causes an increased work load on the heart to supply a
greater blood flow to skeletal muscles and the heart (Stevens, 1969). This increased work load on the heart increases its adaptive growth (Rabinowitz, 1973). The nucleic acid data along with significantly greater relative heart weights suggest Ex rat hearts grow to the level of C rat hearts and represents a true hypertrophy, not just a continuance of normal growth. This would suggest cardiac hypertrophy probably consists of both an increased myocardial cell size as well as hyperplasia of connective tissue cells but data presented in this study show that the two processes occur at different ages.

At 170 days of age, the only determinations which revealed significant differences between C and Ex rat hearts were Hyp and protein concentrations. In both measurements the Ex rat hearts had significantly greater concentrations than did the C hearts. It is suggested the exercise regimen used in this study caused the young rat heart to stimulate existing connective tissue translational machinery to produce more collagen which is reflected in higher amounts of protein per mg tissue. At 270 days of age Ex rat hearts exhibited significantly lower concentrations of DNA and Hyp and had significantly greater RNA-DNA and protein-DNA ratios than did C rat hearts at that age. This suggested that 270 day-old rats had larger heart cells per mg heart which would result in a dilution of the collagen per mg heart tissue.

It is therefore postulated from this study that exercise, of the intensity used in this investigation, has stimulated collagen
synthesis in a growing rat heart (170 days of age) which does not take place until a slightly older age (270 days of age) in a nonexercised animal. This is possibly a reflection of increased cardiac vascularization to an optimum level due to the increased work load placed on the young heart by exercise. The rat heart of a 270 day-old Ex animal may no longer increase its collagen content, since it is already optimum, and thus triggers transcription and increased myocardial cell hypertrophy (width and length), which normally would not occur until 370 days of age in a nonexercised rat heart.

Three hundred and seventy day-old Ex rat hearts had significantly greater amounts of RNA and Hyp per mg heart than C rat hearts and also greater RNA-DNA and protein-DNA ratios. However, the protein-RNA ratio was greater in the 370 day-old C rat hearts than it was in Ex rat hearts. As suggested earlier, the C rat heart apparently undergoes myocardial cell hypertrophy in this old age group and this is reflected in less collagen per mg heart tissue. These data reveal a more efficient C heart at 370 days of age in that RNA and DNA concentration have decreased from 270 day-old C heart values due to myocardial cell hypertrophy, but the activity of the RNA is high. However, in the 370 day-old Ex rat hearts, RNA concentration was high but the activity was low. Again as suggested by protein-RNA ratios earlier, this heart is breaking down protein and would not be as efficient as the C rat heart.
Although hypertrophy apparently occurs in C rat hearts at 370 days of age, this is probably a normal pattern of development for an older rat heart. When this older heart is subjected to a heavier work load it attempts to compensate but at the sacrifice of efficiency. It would follow that a young exercised animal's heart would be in a better position to withstand further increases in work load than an older rat heart which had been exercise-trained. However this needs to be explored further.
SUMMARY

Biochemical determinations of DNA, RNA, protein, and hydroxyproline concentrations were used to characterize cardiac growth in control and exercise-trained male rats. Rats consisted of three age groups: 100 days, 200 days and 300 days of age. Each age group of animals was divided into four treatment groups: exercise (Ex), machine control (MC), guillotine control (GC), and control control (CC). Ex animals were physically trained 30 minutes per day, five days per week for a period of ten weeks on a motor driven treadmill. The treadmill was operated at an eight degree incline and a speed of 15 meters per minute. MC animals were subjected to identical handling and confinement within the treadmill chambers as Ex rats, but were not subjected to treadmill running. GC and CC rats received no handling associated with treadmill exercise and remained in their cages throughout the experimental period. Since no significant differences existed between any parameters measured in this study between CC, GC, and MC animals, these three groups were combined into one control (C) group.

At the conclusion of the ten week period, all treatment groups were further divided into swim (S) and nonswim (NS) animals. All S rats were subjected to an exhaustive swimming exercise prior to sacrifice. NS rats were sacrificed without having been subjected to a swimming exercise. No significant differences were noted in any of the parameters measured between NS and S rats. These
divisions of each treatment were pooled for further analysis.

Ventricles from all rats were weighed and frozen at the time of sacrifice. Samples of dry fat-free ventricles were used for determining DNA, RNA, protein and hydroxyproline concentrations.

Results of this study were as follows:

1) Exercised rats were not able to swim longer than control animals. Swimming to exhaustion had no effect on the biochemical measurements utilized in this study.

2) Final body weights for exercise-trained rats were significantly lower than control body weights in all three age groups. Two hundred and seventy day-old rats had significantly heavier body weights than 170 day-old rats, but were not significantly different from final body weights of 370 day-old rats in either treatment group.

3) Absolute heart weights were not significantly different between treatments (Ex, C) at any age group. Heart weights of 270 day-old rats were significantly heavier than 170 day-old heart weights, but not different from 370 day-old rat heart weights.

4) Relative heart weights (mg heart per g body weight) of Ex rats were significantly greater than those of C rats at all three age groups. The only age effect on relative heart weights was between 170 Ex and 270 Ex animals, where 270 Ex rats had lighter heart to body weight ratios.

5) DNA concentrations were used to estimate cell numbers per mg
dry fat-free heart tissue (DFFT). C rat heart DNA concentrations were similar between 170 and 270 days of age, but decreased significantly between 270 and 370 days of age. Age had no effect on rat heart DNA concentrations in Ex animals. No significant differences in DNA concentrations existed between treatments (Ex, C), except in 270 day-old rat hearts, where C hearts had more DNA per mg DFFT.

6) RNA concentrations were used to estimate the protein synthetic machinery available to a cell for protein production. No significant differences existed between treatments with respect to RNA concentrations except at 370 days of age where Ex rat hearts had greater RNA concentrations than C hearts. Rat heart RNA concentrations increased significantly at 270 days of age when compared to 170 day-old values. C rat hearts at 370 days of age had significantly lower concentrations of RNA than did 270 C rat hearts.

7) Age had no significant effect on C rat heart RNA-DNA ratios. Exercise caused a significant increase in RNA-DNA ratios over C rat heart ratios at 270 days of age only. A significant increase in the RNA-DNA ratio was observed in 270 day-old Ex rat hearts compared to 170 day-old Ex hearts.

8) Protein concentrations in 170 Ex rat hearts were significantly greater than C hearts at that age. Protein decreased with age in Ex rat hearts, while it increased with age in C hearts. Thus a significant age-exercise interaction was observed with respect to heart protein concentration.
9) Mg protein per ug DNA was used to estimate average cell size. C rat heart protein-DNA ratios of 170 and 270 day-old animals were significantly lower than 370 day-old C rat heart ratios. Exercise caused an increase in the protein-DNA ratio only in 270 day-old rat hearts when compared to the same age controls. Age did not effect Ex rat heart protein-DNA ratios.

10) Mg protein per ug RNA was used to estimate cellular protein synthetic ability or activity. No significance was observed between Ex treatments at any age. C rat hearts at 170 and 270 days of age revealed no difference from Ex rat hearts with respect to their protein to RNA ratios. However, at 370 days of age C rat hearts had a higher ratio than Ex hearts.

11) Hydroxyproline concentrations were used to estimate the amount of collagen per mg heart tissue. Exercise maintained a constant concentration of collagen through all three age groups. C rat hearts increased their collagen content with age between 170 and 270 days of age, but a significant decrease in collagen was observed in hearts of 270 and 370 day-old rats.

12) Data in this study suggested normal growth of a C rat heart from 170 to 370 days of age proceeded by hyperplasia of connective tissue cells between 170 and 270 days of age. From 270 to 370 days of age C heart growth was due to hypertrophy of myocardial cells rather than addition of more cells.

13) Exercise caused the young (170 day-old) rat heart to grow
by adding more connective tissue elements. With increasing age the myocardial cells were producing protein at a faster rate but evidence presented suggested protein was being broken down as fast as it was being made.

14) This study suggested that a young rat heart which has been exercise-trained may be in a better position to adapt to increased work loads than an older exercise-trained heart.
PART II:

EFFECTS OF EXERCISE ON ISOPROTERENOL INDUCED MYOCARDIAL ISCHEMIA IN RATS
LITERATURE REVIEW

Although conflicts exist in the literature between absolute heart weights and relative heart weights (heart weight-body weight ratios), data from many studies confirm the fact that the heart responds to chronic exercise by enlarging (Shelley et al., 1943; Stevenson et al., 1964; Crews and Aldinger, 1967; Penpargkul and Scheuer, 1970; Bloor et al., 1970; Banister et al., 1971; Froelicher, 1972; Tomanek et al., 1972; Dowell et al., 1976a; 1976b).

In 1965, Grande and Taylor reported that heart size correlated with cardiac output. Increased functional capacities of exercise-enlarged hearts were then demonstrated by several investigators. Penpargkul and Scheuer (1970) found that isolated hearts from conditioned rats had a greater cardiac reserve, as measured by cardiac output, stroke volume, and greater extent of myocardial fiber shortening when they were challenged by an increased heart rate or increased atrial filling pressure. Bhan and Scheuer (1972) suggested hearts from exercised rats had improved mechanisms of oxygen delivery to myocardial cells as well as increased cardiac actomyosin adenosinetriphosphatase (ATPase) activity. Increased myosin ATPase activity was also demonstrated in exercised rat hearts (Wilkerson and Evonuk, 1971). Scheuer and Stezoski (1972) examined the cardiac response of exercise-trained rat hearts to hypoxic conditions. In an in vitro perfusion system, they reported that during hypoxia the hearts of conditioned animals maintained enhanced cardiac
output and cardiac work. When these investigators found that oxygen delivery and lactate production during hypoxia was the same between conditioned and control hearts, they concluded exercise-trained rat hearts were more efficient in their energy utilization while maintaining twice as much cardiac output. A later study (Scheuer et al., 1974) demonstrated in vitro the amount of oxygen extracted by cardiac muscle of a non-exercised rat was slightly greater than that of an exercised animal, but exercised rats had increased their oxygen delivery to the heart by increasing coronary blood flow. Control animals had no increased coronary blood flow and thus had a lower oxygen delivery to myocardial cells. They further demonstrated conditioned rat hearts were capable of increased aerobic performance through improved oxygen delivery and more efficient energy utilization.

Increased coronary blood flow in hearts of exercised rats may be due to several factors. Tomanek (1970) exercised rats on a treadmill and demonstrated an increased ratio of coronary vessels to myocardial fibers. Poupa et al. (1970) observed that more active wild animals had a greater increase in coronary capillary development than more sedentary domestic animals. Both of these investigations found the coronary vascular bed to be more extensive in younger animals. It was further demonstrated that lumen diameter of larger coronary arteries increased with exercise training.

Weisfeldt and coworkers (1971b) reported that in older rats, oxygen extraction by myocardial cells was not impaired, but coronary
vascularization did not increase in proportion with the size of the heart, hence a reduced coronary flow in older animals was postulated.

Myocardial ischemia was suggested by one investigator as being a causative factor in increased coronary vascularization (Schaper, 1971). Collateral vessel development mechanisms were referenced by Schaper and Pasyk (1976) in a report where they found 24 hours was needed to induce increased collateral blood flow due to addition of more coronary vessels. This implied collateral developments would have more than enough time to occur in a graded exercise program which is sufficiently intense enough to cause mild oxygen debts to the myocardium. As Schaper and Pasyk pointed out, the potency of any protective measure against an ischemic myocardium may be judged by its ability to delay or ward off irreversible necrosis.

Past investigations showed the synthetic beta-adrenergic catecholamine isoproterenol could induce an infarct-like myocardial lesion in experimental rats (Rona et al., 1959a; 1959b; Chappel et al., 1959a; 1959b). These lesions were found to depend on breed and weight of the animal, which in turn was indirectly influenced by sex and age of the animal. Dose dependent lesions were characterized by cardiac edema, followed by hyaline degeneration of myocardial cells. A tremendous infiltration of leucocytes, and swelling along with an increased mitotic index of fibroblasts, was noted. It was suggested that these lesions were caused by hypoxic conditions of the heart from increased demand for oxygen due to direct cardiac
stimulation, which in turn exceeded the oxygen supply from coronary circulation (Chappel et al., 1959a). This theory was substantiated by several other workers. Handforth (1962) injected India ink into coronary vessels of a rat heart after giving it an injection of isoproterenol. This technique revealed that blood flow to the endocardium was stopped and he concluded isoproterenol caused myocardial ischemia before necrosis. He considered the lesions produced by isoproterenol as true infarcts. Ischemia from isoproterenol was also supported by histochemical and electron microscopic studies of Ferrans et al. (1964).

In further work by Rona et al. (1963) it was found potassium-supplemented diets decreased, and a potassium-deficient diet aggravated isoproterenol induced lesions. These results supplemented earlier data where it had been established that steroids with mineralocorticoid activity aggravated the effects of isoproterenol (Chappel et al., 1959b). These responses were later investigated by Wexler and Kittinger (1963; 1965). They presented evidence that adrenal glands responded to isoproterenol by increasing the secretion of aldosterone, which in turn exerted its sodium and thus water retaining effect on the kidney, leading to congestive heart failure. Wexler and Kittinger (1963; 1965) further demonstrated that isoproterenol injections increased serum enzymes (GOT, GPT, and LDH), indicating cardiac damage. Wexler found in 1970 that increases in SGOT and CPK concentration appeared during the first 24 hours after
one injection of isoproterenol. He reported that it was not necessary for myocardial tissue damage in order to see increased enzyme concentrations, and suggested the permeability of myocardial cells had been altered to facilitate enzyme release.

More recently it was demonstrated that the adrenal gland was responsible for some effects seen in myocardial infarction acutely induced by isoproterenol (Wexler, 1976). When the adrenal function of rats was suppressed by aniline treatment, no development of a previously noted hydrothorax was observed. Hydrothorax had been suggested to be a contributing factor in the death of isoproterenol injected rats. Furthermore, some catabolic responses evident in acute myocardial infarction, i.e. elevated serum glucose, free fatty acids, and cholesterol, were not observed in aniline plus isoproterenol treated rats. Serum GOT, SPT, LDH, and triglycerides were still elevated regardless of the suppressed adrenal function by aniline. Wexler concluded that suppression of normal adrenal function altered the pathophysiologic response to acute myocardial infarction.

Isoproterenol has also been utilized to study myocardial hypertrophy. Bartosova et al. (1969) reported that low dose injections of isoproterenol (0.5 mg/kg body weight) into male rats resulted in no loss of body weight but caused a significant increase in heart weights. They suggested that since total heart nitrogen remained constant between controls and injected rats, and hydroxyproline estimation of
collagenous protein increased, noncollagenous protein was restricted but connective tissue had proliferated. It had been shown hypoxia would cause fibroblast proliferation (Chvapil and Hurych, 1968). Bartosova and his coworkers (1969) deduced that isoproterenol had increased oxygen consumption of injected hearts, which could not be compensated for by existing circulation. This proposal was also supported by Beznak and Hacker (1963).

Further interest in the cardiac hypertrophy effect of isoproterenol was demonstrated in work by Stanton and Schwartz (1967) and Stanton et al. (1969). They induced cardiomegaly in rat hearts with two (80 mg per kg, s.c.) injections of isoproterenol per day for two days. It was reported that RNA, RNA-DNA ratios, and protein concentration of the hearts increased significantly with only a small increase in DNA. It was suggested from the data that cardiac hypertrophy consisted primarily of increased cell size with some addition of new cells. Also the total cardiac catecholamine concentration was determined (Stanton et al., 1969). Total concentration of catecholamines for the heart remained constant but ug catecholamines per g heart tissue decreased. This led them to suggest adrenergic innervation of the heart did not increase even though hearts were adding new tissue to increase their size.

More recently it was shown repeated doses (5 mg/kg, s.c.) of isoproterenol caused an elevation of Type I cyclic AMP-dependent protein kinase in the heart (Byus et al., 1976). They suggested
that Type I kinase may be involved in some way with the hypertrophic response to isoproterenol.

To the author's knowledge, no studies exist in the literature which show the effect of isoproterenol on a rat heart which has adapted to chronic exercise training. Since it was postulated in Part I of this paper that a young rat heart which had adapted to exercise training might be more able to withstand a further stress, the present investigation was undertaken.
MATERIALS AND METHODS

Treatment of Animals

Male rats (Sprague-Dawley Farms, Madison, Wisconsin) were 100 days old at the start of this investigation. Their initial mean body weight was 288.6 ± 4.69 g. Animals were randomly placed in one of two groups, exercise (Ex) or control (C).

Ex rats were subjected to a running-exercise on a motor-driven treadmill (Jette et al., 1969; Auth, 1975) five days per week for 13 weeks. Week 1 served as a training period where time and speed of running increased each day until a rate of 15 meters per minute, an 8° incline, and a time of 30 minutes per day had been reached. This exercise regimen was maintained from week 2 through week 12. C rats were not exercised and maintained normal cage activity.

All animals were maintained in an animal room where lighting (0800 to 2200 hours) and temperature (25 ± 3°C) remained constant. A standard diet (Teklad) and tap water were provided ad libitum throughout the study.

Injection Treatment

After week 13, one-half the rats in each group were randomly selected for one of two treatments. One treatment (Iso) involved two subcutaneous injections of l-isoproterenol (70 mg/kg/injection; Sigma Chemical Co.) 24 hours apart. The first injection was given 24 hours after the last exercise period of week 13. The second treatment (Sal) group received two placebo injections of physiological
saline at the same time injections of 1-isoproterenol were given to iso treated rats. Twenty-four hours after the second injections had been given, all animals were sacrificed by decapitation.

Treatment of Tissue

After decapitation, a thoracotomy was performed and hearts were excised while contracting. Atria, major vessels, and fat were trimmed from ventricles and the latter were rinsed with Locke's solution, blotted, and weighed. While still contracting, hearts were placed in 10 ml of cold buffer solution (Morré et al., 1970) and minced coarsely with scissors. This preparation was homogenized with a Tekmar polytron at a medium speed for ten seconds. Aliquots of homogenate were placed in sealed plastic tubes and frozen for later analysis.

Nucleic Acid Determinations

Total nucleic acids were extracted from an aliquot of the homogenate using Schneider's method (1945; Appendix B). Aliquots of this extract were used in determining DNA concentration (Burton, 1956; Appendix E) and RNA concentration according to Mejbaum's method (1939) as described by Schneider (1957; Appendix F). DNA and RNA standard solutions were prepared according to Webb and Levy (1955; Appendix C). Accuracy of these standards was established by colorimetrically analyzing phosphorus content of DNA and RNA standards (Fiske and Subbarow, 1925; Appendix D).
Protein Determinations

Noncollagenous protein determinations were made on aliquots of the homogenate sample (Lowry et al., 1951; Appendix G).

Glycogen Determinations

Glycogen concentrations were determined on homogenization aliquots using anthrone reagent (Seifter et al., 1950; Appendix I).

Analysis of Data

Data were analyzed using The Statistical Analysis System (Barr and Goodnight, 1971) in consultation with personnel from the Iowa State University Statistics Laboratory. The ANOVA procedure was used to calculate the analysis of variance and the MEANS procedure was used to calculate the means of all variables in each experimental group. Individual experimental groups were compared using t-test with unequal cell variance and unequal cell numbers (Snedecor and Cochran, 1967).
RESULTS

General Observations

In a preliminary investigation one dose of 1-isoproterenol of 100 mg per kg body weight killed 100 percent of the rats injected. When the dose was reduced to 70 mg per kg body weight approximately 50 percent of injected animals survived and all exhibited gross signs of increased sympathetic nervous system activity. Respiratory secretions were increased and respirations became deep and irregular. Animals remained in an anorexic prostrate condition in one corner of the cage for several hours before the effects of 1-isoproterenol diminished and normal activity of the animal resumed. When these animals were sacrificed, a perfuse hydrothorax was noted along with gross signs of massive myocardial necrosis. The 1-isomer was found to be much more potent than the racemic mixture of isoproterenol as also reported by Innes and Nickerson (1970). A dose of 70 mg per kg was used in this study to insure maximum stress on the heart.

Body Weights

Mean body weights for exercise (Ex) and control (C) rats are shown in Figure 2.1. Ex rats have a significantly (P<0.02) lower mean body weight than C rats after three weeks of treadmill exercise. This lower mean body weight was maintained through week 13. After injections of 1-isoproterenol and saline had been given, 48 percent of C-Iso animals died. No animals from the exercised group died after injections had been given. Rats from the C-Iso group that
Figure 2.1. Mean body weights.

Control - rats not exercised on the treadmill.
Exercise - rats exercised on the treadmill.
Exercised rat mean body weight significantly lower at $P<0.02$. 
died had a mean body weight at the end of week 13 of 479.8 ± 12.8 g. C-lso animals which survived the injections had a mean body weight of 407.6 ± 9.33 g at the end of week 13. This difference in mean body weight between C survivors and C animals that died was highly significant (P<0.001).

Figure 2.2 shows mean body weights (week 10 through sacrifice) of C and Ex animals which survived injection treatments. Weights of C-Sal animals decreased an average of 5.33 ± 1.58 g from time of the first saline injection to sacrifice time. Body weight of C-lso rats decreased an average of 24.14 ± 5.4 g from the first injection of l-isoproterenol to sacrifice. A significantly greater (P<0.001) average loss of body weight was observed in C-lso rats compared to C-Sal rats. Similarly, a significantly greater (P<0.02) decrease in body weight was observed in Ex-lso rats (lost 23.2 ± 3.96 g) compared to Ex-Sal rats (lost 4.38 ± 5.27 g) between the time of the first injection to the time of sacrifice.

Heart Weights

Upon sacrifice, C-lso rat hearts had extremely large areas of necrosis and an extremely perfuse hydrothorax (fluid within the thoracic cavity). Necrotic areas varied from 50 to 100 percent coverage of the anterior surface of the heart as observed by gross visual observation. Ex-lso rats at sacrifice contained only minor amounts of fluid in the thoracic cavity. Areas of necrotic heart tissue in the Ex rats were absent in many cases, but when necrosis
Figure 2.2. Mean body weights of injection treated rats.

Body weights of animals that died from isoproterenol injection have been removed from control-iso group.
Control-Sal. - Nonexercised saline injected rats.
Control-Iso. - Nonexercised isoproterenol injected rats.
Exer.-Sal. - Treadmill exercised saline injected rats.
Exer.-Iso. - Treadmill exercised isoproterenol injected rats.
Arrows denote time of injections.
was observed, it occupied only small areas which were localized towards the base of the ventricles. The location of small areas of necrosis found in Ex-Iso rats in this study conflicts with other observations which claimed Iso caused predominantly apical necrosis (Selye, 1970; Wexler and Kittinger, 1963).

Mean absolute heart weights (mg) are found in Figure 2.3. In both groups, Iso treated animals had a significantly greater heart weight ($C = P<0.001$; $Ex = P<0.02$) than Sal treated rats. No significant difference was observed between Ex-Sal and C-Sal heart weights. A significantly lower ($P<0.01$) heart weight was seen in Ex-Iso when compared to C-Iso heart weights.

In order to remove any influence of body weight on heart weight, the latter was expressed as mg heart per g body weight (Figure 2.4). C-Iso and Ex-Iso both had significantly greater ($P<0.001$) mean heart weight to body weight ratios when compared to Sal treatments in their respective groups. Ex-Sal had a significantly greater ($P<0.001$) mean heart weight per g body weight ratio when compared to C-Sal heart ratios. However, Ex-Iso mean heart weight to body weight ratios were significantly lower ($P<0.05$) than ratios of C-Iso rats.

Nucleic Acids

Myocardial mean DNA concentrations (ug DNA per mg heart) are found in Figure 2.5. No significant differences were observed between C and Ex groups with either injection treatment. Highly significant differences ($P<0.001$) existed between Sal and Iso treatments in C
Figure 2.3. Means ± standard errors of absolute heart weights (mg).

C-Sal - 1157.78 ± 24.9 (N=9)  P<0.001\textsuperscript{a}
C-Iso - 1604.28 ± 57.7 (N=7)  
Ex-Sal - 1107.50 ± 41.1 (N=8)  
Ex-Iso - 1309.00 ± 60.1 (N=10)  P<0.02

\textsuperscript{a}Level of significance between treatments.
Figure 2.4. Means ± standard errors of relative heart weights (mg heart/g body weight).

C-Sal - 2.58 ± 0.048 (N=9)  P<0.001
C-Iso - 4.17 ± 0.119 (N=7)
Ex-Sal - 3.01 ± 0.063 (N=8)  P<0.001
Ex-Iso - 3.85 ± 0.088 (N=10)

*Level of significance between treatments.
C-Sal - 1.37 ± 0.037 (N=9)  P<0.001a
C-Iso - 1.12 ± 0.035 (N=7)  
Ex-Sal - 1.34 ± 0.046 (N=8)  
Ex-Iso - 1.15 ± 0.049 (N=10)  P<0.02

aSignificance level between treatments.

Figure 2.5. Means ± standard errors of DNA concentrations (ug DNA/mg heart).
rat heart DNA concentrations. Ex rat heart DNA concentrations were significantly greater (P<0.02) in Sal-treated animals compared to Iso-treated animals. 1-isoproterenol caused a decrease in DNA concentration per mg heart in both Iso groups.

When DNA concentrations were multiplied by total heart weight (mg), an estimate of total DNA (ug) per heart was obtained (Figure 2.6). In C rat hearts, significantly greater (P<0.05) amounts of total DNA per heart were observed in Iso-treated animals compared to Sal-treated rats. Ex-Sal rat hearts were not significantly different from C-Sal hearts with respect to total DNA per heart. However, a significant decrease (P<0.01) in total heart DNA concentrations was observed in Ex-Iso rats when compared to C-Iso heart DNA concentrations. No significant difference existed between Sal and Iso treatments in Ex rat heart total DNA concentrations.

RNA mean concentrations (ug RNA per mg heart) are shown in Figure 2.7. Significant differences were observed between treatments (Sal and Iso) in C rat (P<0.001) and Ex rat (P<0.05) heart RNA concentrations. In both groups Iso treatment caused a reduction in mean RNA concentrations. Significant differences were also observed among treatments between groups. A significantly greater (P<0.05) amount of RNA per mg heart was observed in Ex-Sal rat hearts compared to C-Sal concentrations. Likewise a significantly greater (P<0.02) amount of RNA per mg heart was observed in Ex-Iso rat hearts when
Figure 2.6. Means ± standard errors of total DNA (ug) per heart.

C-Sal - 1579.88 ± 44.95 (N=9)  P<0.05<sup>a</sup>
C-Iso - 1803.27 ± 92.47 (N=7)  
Ex-Sal - 1478.10 ± 34.50 (N=8)  N.S.<sup>b</sup>
Ex-Iso - 1480.28 ± 41.93 (N=10)  

<sup>a</sup>Level of significance between treatments.
<sup>b</sup>N.S. = not significant at P<0.05.
Figure 2.7. Means ± standard errors of RNA concentrations (ug RNA/mg heart).

C
- Sal - 4.54 ± 0.13 (N=9)  
- Iso - 3.25 ± 0.21 (N=7)  
Ex
- Sal - 5.14 ± 0.24 (N=8)  
- Iso - 4.32 ± 0.28 (N=10)  

*Level of significance between treatments.*
compared to C-Iso heart concentrations.

When RNA concentrations per mg heart were multiplied by the weight of the heart (mg) an estimate of total heart RNA was obtained (Figure 2.8). No significant differences were observed between injection treatments in either C or Ex group rat heart total RNA concentrations. A significantly greater (P<0.01) amount of total RNA was seen in Ex-Sal rat hearts compared to C-Sal hearts. An increase in total RNA in Ex-Iso hearts over C-Iso hearts was noted but it was not statistically significant.

When RNA concentrations (ug RNA per mg heart) were expressed as a ratio (ug DNA per mg heart), no significant differences were observed between injection treatments in either C or Ex rat hearts (Figure 2.9). Both treatments in the Ex rat heart RNA-DNA ratios were significantly greater (P<0.01) than their respective treatment groups in C rat heart RNA-DNA ratios.

Protein Concentrations

Figure 2.10 shows mean mg protein per mg heart tissue. C-Sal rat heart protein concentrations were significantly greater (P<0.01) than C-Iso concentrations. No significant difference was observed between Ex-Sal and Ex-Iso heart protein concentrations. Protein concentrations between C-Sal and Ex-Sal rat hearts were not significantly different. However, Ex-Iso heart protein concentrations were significantly greater (P<0.05) than Iso-treated rat hearts in the C groups.
Figure 2.8. Means ± standard errors of total RNA per heart.
Figure 2.9. Means ± standard errors for RNA-DNA ratios.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Sal</td>
<td>3.34 ± 0.11</td>
<td>9</td>
</tr>
<tr>
<td>C-Iso</td>
<td>2.92 ± 0.20</td>
<td>7</td>
</tr>
<tr>
<td>Ex-Sal</td>
<td>3.82 ± 0.09</td>
<td>8</td>
</tr>
<tr>
<td>Ex-Iso</td>
<td>3.76 ± 0.17</td>
<td>10</td>
</tr>
</tbody>
</table>

N.S. - not significant at P<0.05 level.

*N.S.* - not significant at P<0.05 level.
Figure 2.10. Level of significance between treatments.

- C-Sal - 0.017 ± 0.00074 (N=9)
- C-Iso - 0.013 ± 0.00065 (N=7)
- Ex-Sal - 0.016 ± 0.00035 (N=8)
- Ex-Iso - 0.015 ± 0.00045 (N=10)

**a** Level of significance between treatments.

**b** N.S. - not significant at P<0.05 level.

Figure 2.10. Means ± standard errors of protein concentrations (mg protein/mg heart).
When heart protein was expressed per ug DNA, no significant differences were observed between treatments in the C rat hearts (Figure 2.11). However, a significantly greater (P<0.05) protein-DNA ratio was observed in Ex-Iso hearts when compared to Ex-Sal hearts. No significant differences existed between Sal heart protein-DNA ratios in C and Ex groups. A significantly greater (P<0.01) amount of protein per ug DNA was observed in Ex-Iso hearts when compared to C-Iso hearts.

Glycogen Concentrations

Glycogen-DNA ratios are shown in Figure 2.12. No significant differences were observed between Sal or Iso treatments in either C or Ex groups. No significant differences existed in glycogen concentration in Sal-treated rats between C and Ex groups. A significantly greater (P<0.02) glycogen concentration was observed in Ex-Iso when compared to C-Iso animal heart concentrations.

Caution must be used in interpretation of glycogen concentrations since some dextrose was used in the homogenization buffer solution. Dextrose contribution to the optical density could not be removed due to various dilutions of the initial 10 ml of buffer medium by variable heart volumes, some of which were more edematous than others. Relative differences between groups may be used for comparative purposes.
Figure 2.11. Means ± standard errors of protein-DNA ratios (ug protein per ug DNA).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SE (N)</th>
<th>Stat.</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Sal</td>
<td>12.36 ± 0.83 (N=9)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>C-Iso</td>
<td>11.36 ± 0.43 (N=7)</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Ex-Sal</td>
<td>12.09 ± 0.37 (N=8)</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Ex-Iso</td>
<td>13.33 ± 0.37 (N=10)</td>
<td>P&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

aN.S. = not significant at P<0.05 level.
bLevel of significance between treatments.
Figure 2.12. Means ± standard errors of glycogen concentrations (mg glycogen per mg DNA).

\[\begin{align*}
\text{C-Sal} &\quad 35.60 \pm 1.62 \quad (N=9) \quad \text{N.S.}^a \\
\text{C-Iso} &\quad 31.69 \pm 1.93 \quad (N=7) \\
\text{Ex-Sal} &\quad 37.27 \pm 1.58 \quad (N=8) \\
\text{Ex-Iso} &\quad 39.42 \pm 2.22 \quad (N=10) \\
\end{align*}\]

\(^a\text{N.S.} = \text{not significant at } P<0.05\text{ level.}
DISCUSSION

Body weight data presented in this investigation confirm an earlier observation, that the exercise regimen used reduces body weight (Part I). Rate of body weight gain was reduced significantly in Ex rats after three weeks of exercise and remained lower than C weights throughout the remainder of the experimental period. Although voluntary reduction of food intake (Whalley et al., 1951; Mayer et al., 1954; Oscai et al., 1971) and decreased body fat (Jones et al., 1964; Hanson et al., 1967; Crews et al., 1969) have been suggested to be responsible for decreased body weight due to exercise, another possibility exists. Ex rats were exposed to the stress of running throughout this study, whereas C rats were not exposed to this stress. During initial weeks of exercise, several animals lost significant amounts of body weight and gross symptoms of respiratory distress became apparent, resulting in death of some of these animals. This process did not occur in control animals. Ex rats have therefore been subjected to a selection process, whereby animals which were unable to adapt to the exercise were eliminated. C rats have not undergone a similar selection process, therefore this group probably consisted of some animals which would not be able to adapt to a stress situation.

If this selection process did occur, it is possible that the exercise-adapted rats which survived exercise training could also adapt more readily to a second stressor better than C rats. All
animals in this study were exposed to the stress of two subcutaneous injections of isoproterenol (IPR). One-half the C animals died whereas none of the Ex-trained rats died from IPR. This supported the above-mentioned selection hypothesis. IPR doses given to rats in this investigation were proportional to their body weight (70 mg/kg); however, heavier C animals were unable to survive the injections. These results agreed with findings of Wexler and Kittinger (1963), who reported heavier animals were more sensitive to IPR.

Injections of IPR caused a significantly greater average decrease in body weight when compared to Saline-injected animals in both Ex and C groups. This may be explained by periods of anorexia after each injection of IPR in this study, as well as an increased metabolic rate, evidenced in part by hyperglycemia, hyperlipidemia of certain lipid fractions resulting in fatty livers (Wexler et al., 1968), tachycardia, and increased oxygen consumption (Beznak and Hacker, 1963).

Heart weights (Figure 2.3) were not significantly different between C-Sal and Ex-Sal groups. Yet, due to a smaller body weight in Ex-Sal rats, the heart weight per g body weight (Figure 2.4) was significantly larger in Ex-Sal rats. It is suggested that exercised rats have a retarded rate of body growth but the hearts have continued to grow at a control animal's rate. The end result was an endurance-trained rat with more heart per unit body mass, thus more heart
pumping capacity for a smaller vascular system. This suggestion of more efficient hearts in exercise-trained rats was also made in Part I of this dissertation. It appeared from this observation that an exercised rat heart would not have to work as hard during the challenge of a second stress in order to meet body demands as would a control rat heart which has to supply blood to a larger body.

Histologically, IPR has been reported to induce intense edema and leucocytic infiltration of necrotic areas of the heart (Wexler and Kittinger, 1963). This would contribute to a significant increase in heart weight, seen in this study, in Iso rats in both C and Ex groups. Ex-Iso rat heart weights were significantly lighter than C-Iso rat hearts (Figure 2.3), which suggested that since exercise had decreased the areas of necrosis, invasion of leucocytes and edema was decreased, causing a lower heart weight. This was further reflected in heart weight per g body weight data (Figure 2.4).

If death of C-Iso rats was due to a "first stress" selection process as a result of IPR injection, then C rats which survived this stress should be the stronger, more adaptable population of animals. Results of this study present evidence that even though the more adaptable C rat survived IPR-induced myocardial necrosis, an exercise-trained rat was protected against the development of this necrosis. Presumably this protective effect was in some way related to exercise training.
During brief homogenization of cardiac muscle in this study, it was noted connective tissue was rapidly removed and remained attached to the homogenizer blades in small clumps. It was believed the majority of connective tissue cells were removed rapidly and nuclei of these cells would not have been released during the short homogenization time. It was felt connective tissue was not included in DNA, RNA, and protein estimations made from heart homogenates. This assumption will be followed in the remainder of this discussion.

DNA concentrations have been used to estimate cell numbers in a tissue (Robinson, 1971). Total DNA (ug) per heart (Figure 2.6) was not significantly different between C-Sal and Ex-Sal rats. This indicated an equal number of cells per heart between C and Ex rat hearts. Further support for equal cell numbers per heart was given by DNA concentrations (Figure 2.5) as well as results of a previous investigation (Part I). The present exercise regime apparently does not cause a significant myocardial cell hypertrophy which would be reflected in lower amount of DNA per unit heart tissue weight. Total DNA per heart in C-Iso rat hearts was significantly greater than C-Sal total DNA values. This was probably a reflection of a tremendous infiltration of leucocytes which were attracted to the heart by necrotic tissue release of chemotaxic agents. When DNA was expressed per g heart, a highly significant decrease in DNA concentration was observed in
C-ISO rat hearts when compared to C-Sal rat hearts. Necrotic cells would cause an increased osmotic pressure due to cell breakdown and release of cellular constituents to extra-cellular fluid. Increased osmotic pressure would be responsible for edematous dilution of ug DNA per g tissue. In Ex-ISO rat hearts necrosis was limited, thus a smaller inflammatory response with less leucocyte infiltration and less edema. This was reflected in significantly lower total DNA concentrations per heart in Ex-ISO rats compared to total concentrations in C-ISO rat hearts. A smaller inflammatory response in Ex-ISO rat hearts would also account for lack of significance between Ex-ISO and Ex-Sal total DNA concentrations.

It was suggested earlier that there was some edema in Ex-ISO hearts. Although the degeneration process in Ex-ISO hearts was small compared to C-ISO hearts, enough edema would have occurred to dilute the DNA concentration per g heart. This was supported by ug DNA per g heart in Ex-ISO which were lower than Ex-Sal concentrations.

It would appear that more edema had occurred in C-ISO treated rat hearts than Ex-ISO hearts, and the latter have not had as great an inflammatory response since total DNA per heart was not elevated in that group, suggesting not as great an infiltration of white blood cells, whereas DNA concentration per g heart of C-ISO animals would have been lower than Ex-ISO hearts if white blood cell nuclei could have been eliminated from DNA determinations.

Significantly lower concentrations of DNA may not
be totally explained by dilution of the tissue with increased edema. It seems reasonable that an increased work load placed directly on the heart by a strong beta catecholamine such as IPR would tend to induce protein synthesis and cause myocardial cell hypertrophy. An increase in cell size would cause fewer nuclei per g tissue. It could not be determined from data in this study whether a slight increase in cell size was occurring.

RNA concentrations (Figure 2.7) were significantly lower in Iso rat hearts than Sal treated rat hearts in both C and Ex animals. This may have been due to dilution of the RNA by edema. More relevant was the fact that Ex-rat hearts in both Sal and Iso treatments had significantly greater concentrations of RNA than their respective C-group hearts. This suggested Ex rat hearts had more capability to produce protein than a C-rat heart. Total RNA concentrations (Figure 2.8) were not significantly different between treatments (Sal and Iso) within either C or Ex groups. However, greater amounts of total RNA in Ex treatments compared to C treatments added support to an increased capacity for protein synthesis in an endurance-trained animal's heart.

Amount of protein synthesizing ability per cell has been estimated by RNA-DNA ratios (Robinson, 1971). RNA-DNA ratios in this study (Figure 2.9) suggested exercise had increased the cardiac cell's ability to synthesize protein. No differences in RNA-DNA ratios existed between Sal and Iso treatments in either group.
suggesting the IPR did not cause an increase in protein synthesizing ability as seen with exercise.

Protein concentrations (Figure 2.10) were not significantly different between C-Sal and Ex-Sal groups. Since a majority of collagenous protein was not being measured this would support the hypothesis made in the investigation in Part 1 that the increased protein concentration seen in Ex-rat hearts at 170 days of age was due to increased connective tissue synthesis only. Protein concentrations in this study revealed no increased translation by myocardial cells in exercised rat hearts. However, Ex-rat heart cells did have an increased capacity to produce protein if needed, as supported by greater RNA-DNA ratios, total RNA, and RNA concentrations, when compared to C rat hearts in either treatment.

Involvement of greater amounts of RNA in exercised rat hearts in transcription is questionable. Protein concentrations were not different between C-Sal and Ex-Sal groups. This suggested Ex-rat heart RNA was not involved in increased transcription, but may have been acting as a reserve supply if increased protein synthesis was needed. C-Iso rat hearts had significantly lower concentrations of protein per mg heart than C-Sal hearts. This would reflect a dilution of the protein by edema. The amount of edema was suggested to be greater in C-Iso than Ex-Iso hearts, and a greater decrease in C-Iso rat heart protein concentrations would be expected. This was
the case since there was no significant difference in Sal or Iso treatment protein concentrations in Ex rat hearts, and Ex-Iso rat hearts had significantly greater concentrations of protein than C-Iso rat hearts. This suggested that greater amounts of RNA in Ex-Iso rat hearts over C-Iso hearts were more actively involved in translation. Therefore, the following protective measures against IPR-induced myocardial necrosis are suggested.

Exercise maintains higher levels of RNA in readiness for increased protein synthesis if the need arises. When IPR is injected, the cardiac work load is increased by direct stimulation, and it responds in exercised animals by increasing translation to increase protein synthesis which may in turn facilitate oxygen uptake and avoid ischemia and subsequent development of necrosis. C hearts do not have a reserve of RNA and protein synthesizing ability. C rat hearts cannot increase their protein synthesis fast enough to meet demands placed on the heart by IPR, thus necrosis develops more readily in these hearts and protein breakdown is reflected in significantly lower protein concentrations in C-Iso rat hearts compared to C-Sal hearts.

The less efficient C-Iso rat heart may be utilizing cell protein, triglycerides and glycogen stores for energy substrates, whereas Ex-Iso hearts do not have as great a need for energy substrates. This suggestion was supported by significantly greater amounts of cardiac glycogen per mg heart in Ex-Iso hearts when compared to C-Iso heart concentrations
It must be cautioned that although connective tissue is in part removed by homogenization procedures used in this study, some connective tissue may have been included in the biochemical assays used. This could tend to mask certain results. Also the "first stress" selection process postulated in the beginning of this discussion must be considered in other investigations involving exercise. Even with these two cautions in mind it is still apparent that more investigation of the protective effect of exercise against myocardial-induced necrosis needs to be carried out.
SUMMARY

Biochemical determinations of DNA, RNA, protein and glycogen were used to characterize male rat cardiac changes in response to exercise and subcutaneous injections of l-isoproterenol (IPR). One hundred day-old male rats were divided into two groups, exercise (Ex) and control (C). Ex rats ran on a treadmill set on an 8° incline at a speed of 15 meters per minute and were subjected to this exercise 30 minutes per day, five days per week, for a period of 12 weeks. C rats maintained normal cage activity and were only handled during a weekly weighing period which Ex rats also received. Twenty-four hours after the last exercise period, one-half the rats of the Ex group were injected with IPR (70 mg/kg) while rats in the other half of each group received a placebo injection of physiological saline. Twenty-four hours after the first injection, a second injection of isoproterenol was given. Twenty-four hours after the second injection all rats were sacrificed by decapitation.

Hearts were removed from decapitated rats, and ventricles were trimmed, weighed and homogenized in a buffer solution. From this homogenate, aliquots were used to determine DNA, RNA, protein and glycogen concentration of the hearts.

Results of this study were as follows:

1) Exercise has protected rat hearts against myocardial necrosis induced by IPR. Forty-eight percent of the C-lso rats died from IPR injections. Those C rats which survived IPR injection had gross
evidence of massive myocardial necrosis. No animals which underwent exercise-training died from IPR injections, nor did they exhibit gross signs of myocardial necrosis at sacrifice. C rats which died from IPR injections had significantly heavier body weights than those which survived the injections.

2) Body weights were reduced in Ex animals by three weeks of exercise, and remained significantly lower than C animals throughout the remainder of the experimental period. Injections of IPR caused a greater average weight loss than did injections of Sal.

3) Exercise had no effect on absolute heart weights since no significant difference was observed between absolute heart weights of C-Sal and Ex-Sal animals. IPR injections caused C-Iso and Ex-Iso hearts to be significantly heavier than respective Sal-treated hearts. Ex-Iso absolute heart weights were significantly lighter than C-Iso heart weights.

4) Relative heart weights were significantly greater in Iso treatments compared to Sal treatments in both groups (C, Ex). C-Iso relative heart weights were significantly greater than Ex-Iso relative weights. C-Sal heart to body weight ratios were significantly less than Ex-Sal ratios.

5) DNA concentrations were used to estimate cell numbers per unit tissue. Both C and Ex groups revealed significant treatment effects, with iso-treated animals having less DNA per mg heart. This was suggested to reflect an intense edema, or dilution
of DNA per mg heart tissue. No differences were noted between respective treatments in both C and Ex groups.

6) Total DNA concentration showed no significant differences between C-Sal, Ex-Sal, or Ex-Iso concentrations. C-Iso total heart DNA concentrations were significantly greater than all other groups. Higher total DNA seen in C-Iso rat hearts suggested leucocytic infiltration due to large areas of myocardial necrosis observed in these hearts.

7) RNA concentrations were used to estimate the amount of protein synthetic machinery available to cardiac cells. Exercise significantly increased concentrations of RNA in both treatments when compared to respective C treatments. IPR injections were found to significantly decrease RNA concentrations in Iso-treated rats in both C and Ex groups.

8) Total RNA concentration showed no significant differences between injection treatments in either C or Ex group animals. Exercise maintained higher concentrations of total cardiac RNA in both treatments compared to respective nonexercised controls.

9) RNA-DNA ratios were found to be significantly higher in Ex rat hearts in both injection treatments. No differences were noted in RNA-DNA ratios between Sal or Iso treatments in either C or Ex rat hearts.

10) No significant differences were noted in protein concentrations between C and Ex rat hearts. Injections of IPR to C rats
caused a significant decrease in cardiac protein per mg heart tissue. IPR injections to Ex rats did not cause loss of protein as observed in C animals. Exercise was suggested to protect the heart against extensive protein breakdown caused by IPR.

11) When protein content was expressed per ug DNA no significant differences existed between C-Sal and Ex-Sal. No differences existed between injection treatments in C rats, but significantly greater ratios of protein-DNA were found in Ex-Iso rat hearts when compared to Ex-Sal rat hearts. Ex-Iso rat hearts maintained higher amounts of protein per ug DNA than C-Iso hearts.

12) Glycogen concentrations revealed no differences between injection treatments in either C or Ex rat hearts. Significantly greater concentrations of glycogen were observed in hearts of Ex-Iso rats when compared to C-Iso hearts.

13) It was suggested from data in this study that exercise has allowed myocardial cells to maintain higher levels of RNA in readiness for increased protein synthesis if needed by the heart. Added work loads placed on the heart by direct stimulation of IPR has turned on protein synthesis and protected the heart against ischemia and subsequent necrosis. The possibility of increased coronary blood flow or increased oxygen extraction by myocardial cells was suggested as a means of avoiding cardiac damage.
caused a significant decrease in cardiac protein per mg heart tissue. IPR injections to Ex rats did not cause loss of protein as observed in C animals. Exercise was suggested to protect the heart against extensive protein breakdown caused by IPR.

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APPENDIX A

Removal of Lipids from Hearts

1. Mince hearts with a razor blade.

2. Place minced hearts in 20-30 ml (enough to cover the tissue) of a chloroform-methanol solution (2:1 by volume) for 12 hours.

3. Decant solvent and replace with fresh chloroform-methanol solution (20-30 ml) for another 12 hours.

4. Decant this second solution and add ethyl ether (20-30 ml) to the minced tissue for 12 hours.

5. Pour off ether extract and replace with another 20-30 ml aliquot of ether for a final 12 hours.

6. Decant ether and allow the tissue to air dry overnight under a hood.

7. Grind dry fat-free tissue to a fine powder using a Wiley Mill equipped with a number 40 sieve.

Note: Extraction times are not critical and mild agitation may be used if needed.
APPENDIX B

Total Nucleic Acid Extraction

1. Suspend 25 mg samples of dry fat-free tissue in 4.5 to 5.0 ml of 5 percent trichloroacetic acid (TCA) in 15 ml screw-capped tubes.

2. Allow tubes to stand 1-2 hours until tissue is saturated. Gentle tapping of tubes will cause tissue to fall to the bottom when it is saturated.

3. Cap tubes and place in a 90°C water bath for exactly 15 minutes. (Temperature should remain constant.)

4. Remove tubes from water bath and immediately cool in tap water.

5. When cool, centrifuge tubes for 10-15 minutes at 2000-3000 rpm.

6. Decant supernatant into a 10 ml volumetric flask.

7. Resuspend the residue with 4.5 to 5.0 ml of 5 percent TCA and repeat steps 3 through 5.

8. Combine the supernatant from the second digest with the decant of the first extraction in the volumetric flask.

9. Combine extracts and bring to volume with 5 percent TCA.

10. Transfer extracts to new screw-capped tubes for storage under refrigeration until ready for analysis.
APPENDIX C

Preparation of DNA and RNA Standards

Stock solutions of hydrolyzed DNA and RNA are prepared containing a concentration of 1 mg/ml. From these stock solutions, dilutions may be made for standard curve determination.

1. Place approximately 80 ml of 5 percent trichloroacetic acid (TCA) in a 250 ml beaker along with a teflon-coated magnetic stirring bar.
2. Cover the beaker with aluminum foil.
3. Place this beaker on a heated magnetic stirrer and heat the TCA solution to exactly 90°C. This temperature must be constant for 15 minutes.
4. Add 100 mg of DNA (Calf thymus, Calbiochem) or RNA (Nutritional Biochemical Corp.) to the hot TCA. After exactly 15 minutes, remove the beaker and cool the solution in cold tap water.
5. Pour solution into a 100 ml volumetric flask and dilute to volume with 5 percent TCA. This stock can be stored under refrigeration up to six months.
Solutions

1. Standard phosphate solution: Make a stock solution of mono-
   potassium phosphate which contains 80 ug of phosphorus per ml.
   Into a liter volumetric flask dissolve 0.3509 g of monopotassium
   phosphate in distilled water. Add 10 ml of 10 N sulfuric acid,
   and dilute to one liter. At least three dilutions of this stock
   solution should be made (40 ug/ml, 20 ug/ml, 10 ug/ml).

2. Molybdate (Acid) reagent: Dissolve 25 g of ammonium molybdate
   in 200 ml distilled water. Add 500 ml of 10 N sulfuric acid and
   dilute this solution to one liter. Store in a wax-lined bottle
   under refrigeration.

3. Molybdate reagent: Dissolve 25 g of ammonium molybdate in a
   liter of distilled water. Store in a wax-lined bottle under
   refrigeration.

4. Amino-napthol-sulfonic acid reagent (ANS): Purchased from
   Harleco-Leedon Company, item number 52532; prepare as directed.

5. Sulfuric acid: Prepare stock solutions of 5 and 10 N concentra-
   tions.

Phosphorus Standard Curve

Color development of standard phosphate solutions is carried out
in 50 ml volumetric flasks.

1. Add 5 ml standard phosphate solution or 5 ml distilled water (blank)
1. Into a large pyrex tube (200 mm x 25 mm) place 5 ml of stock nucleic acid solution and add 5 ml of 5 N sulfuric acid.

2. Place several glass boiling beads in the tube and boil this solution under a hood. When solution has boiled down to approximately 1 ml volume, a dense fume appears and the solution turns yellow. Continue boiling over a low flame and observe the solution turning black. When there is no further blackening, add one drop of nitric acid down the side of the tube. Color of the solution should return clear (another drop of nitric acid may be added). Continue boiling for about 30 seconds to remove the nitric acid.

3. Remove from heat and allow tube to cool.

4. Empty contents into a 50 ml volumetric flask. Wash the test tube three times with 10 ml of distilled water per washing. Pour
each washing into the volumetric flask.

5. Add 5 ml molybdate reagent and 2 ml ANS reagent.

6. Mix, fill to volume, mix again.

7. Let the flask stand 5 minutes, then immediately measure the absorbance against a blank at 660 nm.

Prepare a blank by mixing the same reagents, replacing 5 ml of nucleic acid solution with 5 ml of 5 percent TCA. Boiling steps may be eliminated.

Comparison of absorbance measured in the nucleic acid solution with the phosphorus standard curve will give the concentration of phosphorus in each nucleic acid stock solution. Concentrations of DNA and RNA can be estimated by assuming phosphorus is 10 percent of the weight of the nucleic acid.
APPENDIX E

Colorimetric Determination of DNA

Solutions

1. Diphenylamine reagent: Prepare this reagent fresh each day.
   Dissolve 1.5 g Diphenylamine (Matheson, Coleman and Bell) in
   100 ml of glacial acetic acid. Add 1.5 ml concentrated sulfuric
   acid. Just before use add 0.5 ml aqueous acetaldehyde (16 mg/
   ml).

2. Standard hydrolyzed DNA solutions: From prepared stock DNA
   solution make at least five different dilutions, the largest
   containing no more than 300 ug of DNA.

3. 1 N perchloric acid: Add 9 ml concentrated (70%) perchloric
   acid to 91 ml glass distilled water.

Procedure

1. Place one ml aliquots of nucleic acid extracts, as well as
   standards, in test tubes. To each tube add 1.0 ml of 1 N
   perchloric acid and 4.0 ml of diphenylamine reagent.

2. Prepare a blank by combining 1.0 ml of 5 percent TCA with 1.0
   ml of 1 N perchloric acid and 4.0 ml of diphenylamine reagent.

3. Develop color at room temperature 16-20 hours.

4. Measure absorbance at 600 nm.

Calculations

Prepare a standard curve by plotting optical densities of the 5
DNA standards against their concentrations. From this, the concen-
tration of DNA in each of the extracts can be determined. Since standards and extracts were treated the same, no dilution corrections are necessary.
APPENDIX F

Colorimetric Determination of RNA

Solutions

1. Orcinol reagent: Prepare a solution of 0.5 g FeCl₃ per 100 ml of concentrated HCl, which can be stored indefinitely. Immediately before use, add 1.0 g of orcinol per 100 ml of solution.

2. Standard dilutions of both the hydrolyzed DNA and RNA stock solutions are required, five dilutions of each, the largest solution having no more than 500 ug DNA or RNA.

Procedure

1. Place 0.5 ml aliquots of nucleic acid extracts, as well as 0.5 ml aliquots of both DNA and RNA standards in test tubes. To each tube add 2.0 ml of 5 percent TCA and 2.5 ml orcinol reagent.

2. Prepare a blank by mixing 3.0 ml of 5 percent TCA with 3.0 ml of orcinol reagent.

3. Place tubes in a hot water bath (95°C) for exactly 20 minutes. Place marbles on top of the tubes to prevent loss from evaporation.

4. Cool tubes in a cold water bath.

5. Measure absorbance at 610 nm.

Calculations

Prepare two standard curves; one for DNA standards and one for RNA standards. Orcinol reacts with DNA as well as RNA, although to a lesser extent. Nevertheless, in each sample a correction must be
made for the fraction of total optical density measured which was due to the presence of DNA in the extract. This is done as follows:

1. Prepare a standard curve of optical densities of the DNA standards versus their concentrations.

2. Having previously determined DNA by the diphenylamine method, the amount of DNA present in each of the 0.5 ml aliquots can be determined. A separate calculation must be made for each sample.

3. From the above standard curve it can be determined how much optical density will result from the amount of DNA in the aliquot.

4. This calculated optical density due to DNA must be subtracted from the total optical density obtained from the spectrophotometer. Remaining optical density is that due to RNA present in the 0.5 ml aliquot ("corrected" optical density).

5. Make a standard curve plotting optical density of RNA standards versus their concentration.

6. Using this standard curve, determine the concentration of RNA in each sample from the "corrected" optical densities.
APPENDIX G

Protein Determination

Solutions

1. 2 N sodium hydroxide.
2. 2% Na$_2$CO$_3$ in 0.1 N NaOH: Dissolve 4.0 g NaOH and 20.0 g Na$_2$CO$_3$ in distilled water and dilute to 1 liter.
3. 1% CuSO$_4$ $\cdot$ 5 H$_2$O: Dissolve 1.0 g and make to 100.0 ml with distilled water.
4. 2% K-Na-tartrate: Dissolve 2.0 g and make to 100.0 ml with distilled water (keep refrigerated).
5. Prepare copper reagent by mixing equal volumes of reagents 3 and 4 just before making reagent 6.
6. Add 1.0 ml copper reagent (reagent 5) to 50.0 ml of reagent 2.
7. Folin-Ciocalteu phenol reagent.

Protein Standards

Prepare a stock solution with 1.0 mg bovine serum albumin per ml distilled water. Make at least four dilutions from this stock solution (30 ug/ml, 20 ug/ml, 10 ug/ml, 5 ug/ml).

Procedure

1. Add 5.0 mg dry fat-free tissue to 5.0 ml 2 N NaOH in 15.0 ml screw-capped tubes. Allow 24 hours for digestion of tissue. During this period occasional shaking will aid in saturation and digestion of tissue.
2. Add 10.0 ml distilled water.
3. Further dilute a 0.1 ml aliquot of this digest to 10.0 ml with distilled water.

4. To 1.0 ml of final dilution, add 5.0 ml alkaline copper (reagent 6). Let stand at room temperature for 10 minutes.

5. Add 0.5 ml Folin-Ciocalteu reagent (reagent 7). Shake immediately.

6. Let stand for 30 minutes at room temperature or 10 minutes at 50°C.

7. Read absorbance at 550 nm against a blank prepared as above except substitute distilled water for the protein solution.
APPENDIX H
Hydroxyproline Determinations

Solutions

1. 6 N hydrochloric acid.

2. Potassium borate buffer: Dissolve 61.84 g boric acid and 225.0 g potassium chloride in approximately 800.0 ml distilled water. Adjust pH to 8.7 with 10 N and 1 N KOH. Adjust final volume to 1 liter.

3. Alanine solution: Dissolve 10.0 g alanine in approximately 90.0 ml distilled water and adjust pH to 8.7 with KOH. Adjust final volume to 100.0 ml.

4. Chloramine T solution (Eastman): Make 0.2 M solution in methyl cellosolve (prepare daily).

5. p-Dimethylaminobenzaldehyde (Ehrlich's reagent): Slowly add 27.4 ml of concentrated sulfuric acid to 200.0 ml absolute alcohol and cool. Add 120.0 g of p-dimethylaminobenzaldehyde to 200.0 ml absolute alcohol and cool. Slowly add acid-alcohol mixture to the second mixture in an ice bath. This solution can be stored under refrigeration several weeks. To redissolve crystals which will form due to refrigeration, place solution in a warm water bath and gently swirl until crystals redissolve.

6. 1% phenolphthalein: Dissolve 1.0 g phenolphthalein in 100 ml 50% ethanol.

7. KOH: 10 N, 1 N, 0.1 N.
Procedure

1. Place 10.0 mg dry fat-free tissue in 10.0 ml 6 N HCl in a screw-capped tube. Hydrolyze tissue 20-24 hours at 110-115°C.

2. Add one drop of phenolphthalein.

3. Adjust to faint pink with KOH (10 N, 1 N, 0.1 N); pH adjustments at this point are not critical since pH adjustments will be corrected later.

4. Place 1.0 ml supernatant in a large screw-capped tube.

5. Add distilled water to 6.0 ml.

6. Add one drop of phenolphthalein.

7. Adjust pH to pale pink with KOH and HCl.

8. Saturate this solution with KCl (3.0 ± 0.5 g).

9. Add 0.5 ml 10% alanine solution.

10. Add 1.0 ml potassium borate buffer and mix.

11. Oxidize with 1.0 ml of 0.2 M chloramine T solution (mix well upon addition).

12. Allow tubes to stand 25 minutes with occasional shaking at room temperature.

13. Add 3.0 ml 3.6 M sodium thiosulfate.

14. Add 5.0 ml toluene and cap tubes tightly.

15. Shake vigorously for 5 minutes, centrifuge to separate layers (if necessary), aspirate toluene layer and discard.

16. Boil tubes 30 minutes in boiling water bath.

17. Cool in tap water.
18. Add 10.0 ml toluene, shake 5 minutes, centrifuge (again if needed).

19. Place 5.0 ml toluene extract in a clean test tube.

20. Add 2.0 ml Erhlich's reagent (mix as adding).

21. Allow tubes to stand at room temperature 30 minutes.

22. Read absorbance at 560 nm against a blank prepared with distilled water in place of sample at step 4.

23. Prepare a standard curve with at least 3 dilutions of a stock solution containing 1.0 mg hydroxyproline per ml distilled water (standards should range from 5 ug/ml to 30 ug/ml).
APPENDIX I

Glycogen Determinations

Solutions
1. 30% potassium hydroxide (KOH).
2. 95% ethyl alcohol.
3. 0.2% anthrone, prepared by adding 100 ml 95% sulfuric acid to 0.2 g anthrone. Must be prepared daily.
4. 20 ug/ml glucose standard in saturated benzoic acid solution.

Procedure
1. Place 0.5 ml of the homogenate in a tube suitable for centrifuging and which contains 2.5 ml 30% KOH.
2. Put tubes in boiling water bath for 20 minutes.
3. Cool tubes and add 2 ml 95% ethanol to precipitate glycogen.
4. Mix thoroughly and return to boiling water bath until solution boils.
5. Cool again and centrifuge 15 minutes.
6. Discard supernatant and redissolve glycogen with 10 ml distilled water.
7. Transfer 0.2 ml of this solution to a larger tube and dilute to 5 ml with water.
8. While cooling in ice, add 10 ml of the anthrone reagent slowly while mixing.
9. Develop the color in boiling water bath for 9-10 minutes.
10. Cool and read absorbance at 620 nm.
11. Prepare a blank by completing steps 9 through 11 with 5 ml water replacing the sample.

12. Prepare a standard by completing steps 9 through 11 with 5 ml glucose standard containing 100 ug glucose.

Calculations

By comparing the absorbance values of heart samples to those of the standard glucose solution, and accounting for appropriate dilution factors, the amount of glucose per mg heart tissue can be calculated. Then by using a conversion factor of 1.11 the amount of glucose (ug) can be converted to glycogen concentration (ug) per mg heart (Morris et al., 1948).