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THE EFFECTS OF RUNNING ON HEARTS
OF NEONATAL RATS

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THE EFFECTS OF RUNNING ON HEARTS

OF NEONATAL RATS

BY

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ABSTRACT

THE EFFECTS OF RUNNING ON HEARTS OF NEONATAL RATS

By

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The purpose of this study was to determine the effects of physical conditioning on the cardiac muscle of neonatal rats. Forty-two rats were used in the study, twenty-nine animals were put on a daily vigorous treadmill run with periodic increases in speed, incline, and duration. Thirteen animals served as controls. Each week an average of four experimental and two control rats were sacrificed. The heart of each animal was removed, weighed and sectioned. H & E stain was used for detection of degenerative foci and von Kassa's stain for calcification.

Treatment and age had significant effects resulting in increased body and heart weights of control animals. However, heart weight to body weight ratio was inversely related with time. The HW/BW ratio was significantly effected only by age and not treatment.

Histopathalogical observations showed the hearts of the animals, both control and experimental, to be essentially normal.

Dedication

To my family

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Drs. Kwok-Wai Ho, Wayne Van Huss and William W. Heusner for their patience and guidance.

I wish also to thank pathologist Richard Horowitz, M.D. and histologist Elizabeth Binns-Floto of E.W. Sparrow Hospital in Lansing for their assistance.

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CHAPTER I

THE PROBLEM

In 1908, a letter was submitted by a superintendent of gymnastics stating that since "the heart is in the process of development, because it is still an incompletely developed instrument, one dare not in any way overburden it. The danger of an acute dilation of the heart is very great, a defect which the child may retain throughout his life." In response to the letter a school physician replied : " I suggest respectfully that my opinion, which is held by the entire medical profession, be brought to the attention of the teachers of physical education, in order to avert the great harm which will be done if they put this into practice (29)."

Today, more is known about the effects of exercise on the development of a child but unfortunately, there is still some question as to the specific effects of vigorous exercise on the hearts of children.

Forced exercise during the pre-pubertal period has been found to significantly impair the growth of bone length and

cause lower body weight (33).

Exercise is known to have positive cardiovascular responses in adults. Oxygen consumption, cardiac output, heart rate, stroke volume, and oxygen extraction from blood all show improvement with physical activity (6).

In adult rats, coronary flow was found to increase in regular treadmill exercises (5). Controlled exercise has been found to retard the biological aging process (23). This was concluded in a study conducted on 100 male albino adult rats subjected to daily exercise. These rats were found to have higher caloric intake, greater oxygen utilization, and a higher basal metablic rate than non-exercised littermates.

Statement of the Problem

To determine the effects of strenuous training at a young age (10-60 days) on the pathological and morphological development of the hearts of male and female rats. In particular this study was designed to determine the negative effects, if any, of strenuous exercise at an early age.

Rationale for the Study

Today more than ever before young children are exerting themselves physically for the sake of training. Many individuals believe the younger a child begins training in a

specific sport event the more likely he/she is to achieve higher goals in later life.

The purpose of this study is to determine whether or not a strenuous exercise regimen administered to neonatal rats has any histopathological and/or morphological effects on the cardiac muscle. Heart weight and body weight were needed to detect cardiac hypertrophy. Histologically, the heart sections were screened to evaluate histopathology of the myocardium.

Following review of the relevant research on cardiomyopathies in neonatal rat hearts, it was hypothesized that as the intensity of the training program increased body weight and heart weight would decrease but hypertrophy as measured by the heart weight to body weight ratio would increase. The frequency of microscopic degenerative foci observed would increase.

Ultastructural changes such as capillary proliferation and mitochondrial protein content were not investigated but have been shown in studies to be altered as a result of physical activity (17, 19, 30, 32).

Calcification and connective tissue presence is also not expected in hearts of neonatal rats but have been observed in the myocardium of stressed adults rats (8, 7).

Significance of the Problem

Much attention has been focused on the cardiomyopathies of old animals with very little attention on those of the young. Porter, et al. looked at the interaction of age and exercise. Several differences between old and young animals were pointed out :

1) Old animals have a decreased enzyme transit from the intracellular compartment into the vaccular compartment.

2) Old animals may have decreased tissue concentrations of lactate dehydrogenase.

3) There is an impaired cell membrane permeability in old animals.

They concluded that "there are definite age-related differences in the myocardial metabolic adjustments to the stress of exercise (21)."

It is important to determine if strenuous exercise in young animals has negative effect. Such information would provide a basis for the use of exercise in young animals.

Limitations of the Study

1. Results of this animal study may not be directly translated to humans.

2. Only two sections of heart were used for myocardio-histopathological evaluation. Some foci may have been missed.

3. The treadmill is not a good exercise modality for young animals.

4. Temperature in training room varied (75 to 80 degrees Fahrenheit).

CHAPTER II

REVIEW OF LITERATURE

Myocardial Hypertrophy

Cardiac hypertrophy is defined as an increase in mass of the myocardium resulting in an increased weight of the heart and a thickening of the myocardial muscle wall. Hypertrophy must be differentiated from dilation in which an increased volumetric capacity is due to elongation of the muscle fibers. Dilation of the heart may not include an increase in thickness of the myocardial muscle wall. Hypertrophy is also characterized by firmness of cardiac muscle. The papillary muscle and the trabeculae carnae in the ventricles are rounded and enlarged. In atrial hypertrophy, the muscle fasciculi are prominent. In addition to thickening and firmness of the myocardium, the weight of the heart is increased (1). Although heart weight varies in accordance with body size (26), the heart weight to body weight ratio is increased in cardiac hypertrophy.

Microscopically, hypertrophy can be evidenced by an increase in size of individual muscle fibers. The nuclei

often appear variable in shape and enlarged. It is generally believed that hyperplasia, a proliferation of muscle fibers, is not the cause of enlargement of the heart in adults (1). However, several studies have demonstrated evidence of hyperplasia of the myocardium in infants results in an increase of myocardial mass (1).

Ultrastructural studies in hypertrophied hearts have shown that enlargement of cardiac muscle cells are due to an increase in number of myofilaments through formation of new myofibrils and possibly by addition of filaments to pre-existing myofibrils (24). Other investigations support the view that cardiac hypertrophy is the result of an increase in size of cells. This view has been gained using nucleic acid determinations.

Cardiac hypertrophy has also been found to accompany degenerative changes within muscle fibers, including basophilic degeneration and multiple minute foci of myocardial necrosis followed by connective tissue replacement (14). This minute foci of myocardial necrosis is believed to be caused by the ischemia which results from unbalanced proportion of capillary concentration to myocardial fiber diameter (25).

The essential stimulus for cardiac hypertrophy is stretching of muscle fiber caused be stress. This stress which may influence muscle cells to increase in size is

anoxia (1). Myocardial hypertrophy can be induced by anoxia caused by anemia (1) or by overwork brought on by increased cardiac output. This chronic pressure and volume overload is necessary to compensate for the oxygen lack (13).

Physical exercise is associated with an increase of cardiac work and subsequent hypertrophy of myocytes (11). The duration of exercise has a highly significant effect of cardiac wet weight in both young and old rats (12).

The need for hypertrophy of the myocardium in times of stress is to maintain and improve the cardiac function in order to meet the nutritive demand of other tissues as well as the heart muscle. Within limit, hypertrophy is necessary to maintain normal ejection upon increased demand (13). Hypertrophy also results in greater surface area allowing for better nutrition and fiber growth.

The negative effects of myocardial hypertrophy include the subsequent decrease of intrinsic contractility. In other words, when venous return is not excessive, hypertrophy is effective in maintaining pump function although the individual fibers have a reduced intrinsic contractability (13).

Myocardial Fibrosis

Foci of diffuse fibrosis of the myocardium is often observed in patients with chronic myocardial ischemia.

These patients often have a history of angina pectoris or have died suddenly as a result of coronary insufficiency without myocardial infartion. It is believed that with each episode of angina pectoris there is some damage to the heart muscle accounting for development of myocardial fibrosis (1).

Currently, there are two theories for the development of myocardial fibrosis. Some investigations contend that the fibrosis is the result of replacement of degenerative muscle fibers (4). While others contend that "focal myocytolysis" is responsible for the generation of myocardial fibrosis. In the second case muscle fibers disintegrate within small areas and are eventually replaced by the collapse of the remaining stroma (28).

Myocardial fibrosis may be accompanied by hypertrophy or may be found in normal sized hearts. Hickson, et al. (8) using adult female rats that swam daily noticed a slight (8-10%) increase in the degree of connective tissue as measured by the amount of DNA and hydroxyproline in the heart. He also found that connective tissue hyperplasia did not decrease if hypertrophy regressed.

Myocardial Calcification

Pathologic calcification in the heart is mostly the dystrophic type. Dystrophic calcification occurs especially

in hyalinized scars of healed myocardial infarcts. Necrotic foci of the myocardium resulting from a ischemia maybe the sights of calcification (7). In these cases, calcium is deposited within necrotic muscle fibers. If degenerated and necrotic foci are present in the heart muscle, there will be a greater tendency of calcium deposits due to the increased availability of calcium (7).

Myocardial Contractility

Hearts of rats trained in swimming have been found to have a slightly increased myosin calcium ATPase activity and actin-activated ATPase activity. There still remains a great deal of investigation to be dome on this topic. There have been some investigators who have not been able to detect alterations in the ATPase activity of rats trained by running (19). This increased myosin calcium ATPase and actin-activated ATPase actinity could be the result of increased phosphorylation rates after training. These adaptive responses can only be brought about by catecholamine stimulation (22).

Bonner, et al. (3) studied the contractile activity of neonatal heart cells from offspring of exercised pregnant rats. They found that the cells from the offspring of exercised pregnant rats had a slower beating rate, a larger cell size, and increased percentage of contracting cells in the sample.

Myocardial Mitochondrial Changes

Mitochondria from hearts of sedentary adult rats were compared with mitochondria isolated from hearts of physically conditioned rats. Calcium uptake per mg mitochondrial protein was depressed 25% in conditioned rats. However, estimation of mitochondrial content per gram wet weight indicated a 52% increase of mitochondrial protein in conditioned hearts when compared to the sedentary animals. The results indicate that despite diminished oxidative and calcium uptake activity per mg mitochondria, the oxidative phosphorylation as well as calcium uptake per gram tissue was not decreased in conditioned hearts and might even be increased (20).

A study conducted by Holloszy (10) revealed that rats exposed to a strenuous program of treadmill running showed a 60% increase in mitochondrial protein and a two-fold increase in various mitochondrial enzymes. He found that mitochondrial adaptation to vigorous exercise included increased coupling of oxidative phosphorylation and a subsequent rise in capacity to produce ATP.

These findings were not duplicated in other studies using milder forms of exercise.

Myocardial Capillarization

Change in cardiac vascularization as a result of physical exercise is controversial. Parizkova, et al. (18)

found that hypertrophy of the muscle fibers leads to a lower capillary density in the trained heart. These investigators concluded that the capillaries are pushed farther apart and that exercise training does not stimulate the multiplication of cardiac capillaries (18). On the other hand, other studies claim that training prior to pubescent growth spurt (3) or at young age (32) lead to a more pronounced capillary proliferation than in older animals.

CHAPTER III

METHODS AND MATERIALS

The exercise protocol used in this study has been modified slightly from the training program used by MacIntosh and Baldwin for neonatal rats (15).

Histochemical procedures are similar to those used by Ho, et al. (9) in which effects of exercise on myocardium of dystrophic hamsters were studied. The staining techniques were originally obtained from the Manual of Histological Staining Methods of the Armed Forces Institute of Pathology in 1968 (16).

Study Sample

Forty-eight normal, male and female neonatal, albino rats of the Spraque-Dawley strain were obtained from Harlan Research Laboratory in Indiana. They were received in one shipment in separate compartments. Each compartment contained one female rat with six ten-day old offspring.

The animals in each compartment were randomly assigned into either experimental or control groups. They were not,

however, separated from their natural mother until the age of 30 days. Exercise treatment began when the young animals were 11 days of age.

Research Design

The study was organized as a two-way (2x8) factorial design. The first factor (treatment) consisted of two levels of physical activity: (a) Experimental- an exercised group that was subjected to a vigorous forced exercise program, (b) Control-a non-exercised group that remained relatively inactive throughout the experimental period. The second factor (age at sacrifice time) was represented by eight groups of animals: (a) 10-day old_ a group that was sacrificed before exercise program was implemented, (b) 15-day-old_ a group that was sacrificed six days after the experiment started, (c) 22-day-old_ a group that was sacrificed after receiving the treatments for thirteen days, (d) 30-day-old_ a group that was sacrificed after receiving the treatments for twenty-one days, (e) 37-day-old_ a group that was sacrificed after receiving the treatments for twenty-eight days, (f) 45-day-old_ a group sacrificed after receiving the treatment for thirty-six days, (g) 52-day-old_ a group that was sacrificed after receiving treatment for forty-three days, (h) 59-day-old_ a group that was sacrificed after fifty days. A description of the training program is given in Appendix A.

Training Procedures

The exercise treatment was conducted by using a specially designed motor driven treadmill in the Human Energy Laboratory, Michigan State University.

The exercised animals were trained daily for an increasing amount of time, running speed, and percent incline of the treadmill (as shown in Figure 1). Stimulation of running for animals was provided by the technicians using a small poke or push to touch the back and/or rear end of the animals.

Those animals designated as controls remained in each of the compartments and, thus, were relatively inactive throughout the study.

Animal Care

All animals were initially housed and nursed with their natural mothers in sedentary cages. Mothers were given food (Wayne Lab Blocks) and water *ad libitum*.

At thirty days of age, animals were separated from their mothers and were housed randomly in groups (5-6 animals in each group) in cages. Food and water were given *ad libitum*.

A relatively constant environment was maintained in the animals' quarters where temperature and lighting were automatically controlled between 75 to 80 degrees Fahrenheit

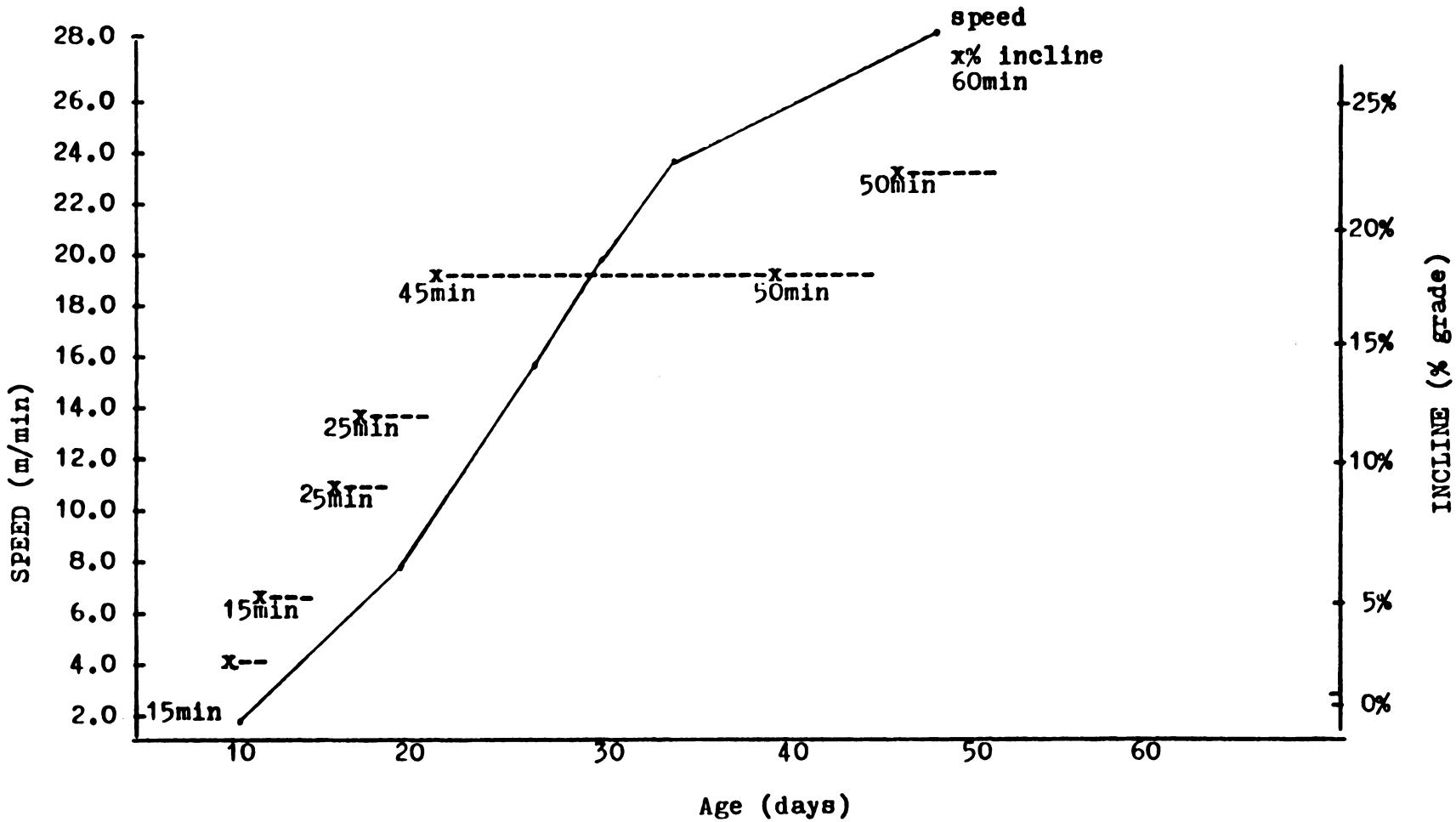


Fig. 1. Training protocol progression as a function of age. Speed (m/min) on left ordinate, incline of treadmill (% grade) on right ordinate, and time (min) of each training session as shown.

and 12 hours on and 12 hours off, respectively. Daily handling of animals was done with large forceps until they were separated from their mothers. After separation, handling was done by hands. The cages were cleaned regularly according to NIH guidelines for animal care.

Sacrifice Procedures

After each animal was weighed, it was sacrificed by an intraperitoneal injected (4 mg/100 g body weight) of a 6.48% sodium pentabarbital solution. The heart was then removed, blot dried, trimmed (only atria and ventricle remain), and weighed.

Animal sacrifices were conducted at each scheduled time. An average of four experimental and two control animals were sacrificed each time.

Tissue Analysis

After the weight of the heart was determined a transverse cut at approximately mid-length of the ventricles was carefully done and the two sections were then placed with a specific orientation in separate numbered cassettes for processing. The diagram in Figure 2 illustrates the area where muscle was cut. The shaded areas on the sections represent areas sectioned for slides.

The cassettes including heart sections were then processed in a vacuum infiltration processor for nine hours, where they were fixed and dehydrated. After being processed they were embedded in paraffin blocks, cooled, then cut with a microtome. (See Appendix B-Heart Muscle Tissue Processing Procedures).

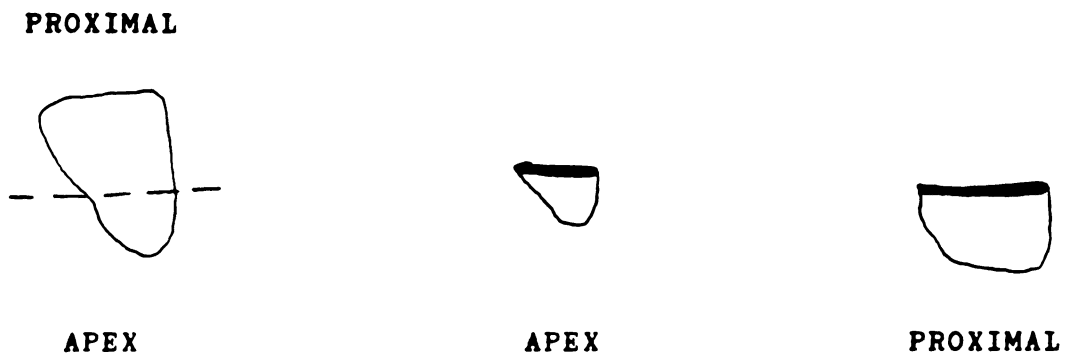


Fig. 2. Heart sectioning

The slides were then stained with hematoxylin-eosin for any general detection of degenerative foci, and von Kassa's stain for calcification. (See Appendix C-Staining Techniques).

Histopathological evaluation of all slides was performed blind by a pathologist. The hearts were ranked as having "slight damage" if loss of cytoplasm or loss of cross striations or mast cell infiltrate was observed. "Extensive

damage" was recorded if areas of muscle leukocytic infiltration was noted.

Statistical Procedures

The data were analyzed using a fixed effects Analysis of Variance (AOV) for a two-way (2x8) factorial design. Whenever a signigicant F-ratio was obtained, Student-Newman-Keuls tests were used to analyze differences between pairs of means. The alpha-level was set at 0.05 for all analyses.

CHAPTER IV

RESULTS AND DISCUSSION

The results of this study are presented in the following order: a histopathological observation of the heart, and the body weight, heart weight, and the heart weight to body weight ratio results.

The pathological raw data are presented in Appendix D and the morphological raw data are presented in Appendix E. All of the F-ratios and P values for age effects on body weight, heart weight and heart weight to body weight ratio are presented in Appendix F.

Histopathological Results

Cross-sections from two separate levels (obtained via transverse cut at approximately mid-length of ventricles) of each heart were evaluated by microscopic examination. Heart damage was rated according to the following subjective

scale:

Normal

1. Slight damage
2. Mild damage
3. Extensive damage

Inconclusive

Only four of the forty-six animals had apparent tissue damage; one animal showed extensive damage; three showed slight damage. The animal with extensive damage was from the sedentary, control group showing large areas of loss of muscle with leucocytic infiltrate. Of the three animals showing slight damage, two from the exercised group showed focal mast cell infiltration. The control, untrained animal with slight damage showed focal loss of cytoplasm and cross-striations.

Although the remainder of the animals were evaluated as being normal, signs of diffuse anomalies were observed. At age 15 days the two control animals appeared normal but the exercised animals showed sub-epicardial dilated capillaries. At age 52 days, one trained animal revealed marked venous congestion.

Since at age 10 days, only three untrained animals were sacrificed without any trained counterparts, these animals were also omitted from data analysis.

Forty-two slides were evaluated: 29 from exercised animals and 13 from controls. The histopathological results were not statistically significant. These observations suggest that overall the hearts of the animals were normal. The results of the pathological evaluation as presented in Appendix D show that of the 42 slides evaluated, four slides showed some form of damage and that three of these were from control animals. Slight damage was noted in one heart section of a 37-day old control rat and in two 59-day old control rat.

Body Weight Results

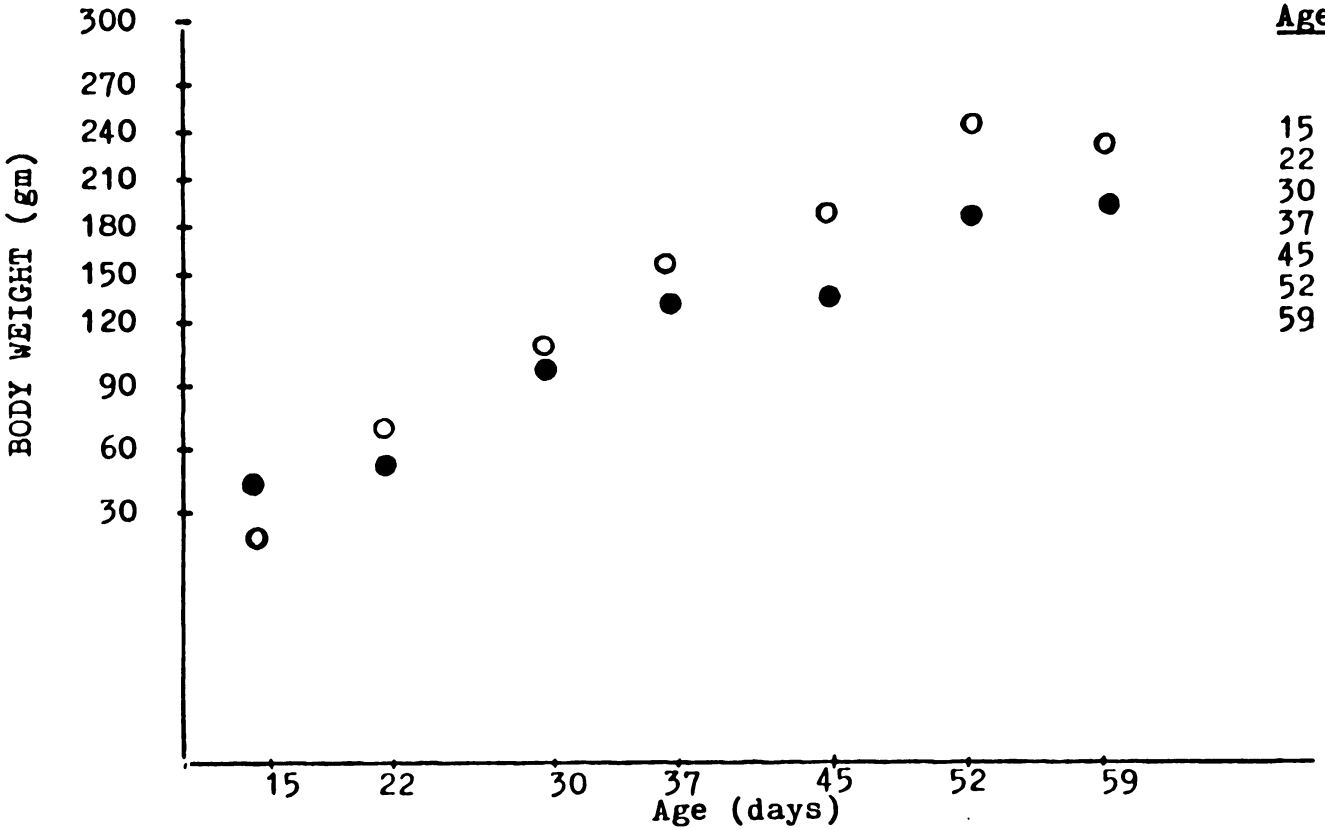
The final results for the means and standard error of the means are presented in Figure 3.

Overall, as seen in Appendix E, the control, untrained animals weighed significantly more than did the exercised animals. Treatment had a significant effect on body weight. Age also had a significant effect on the body weight. The control animals weighed more than the exercised animals at every age except 15 days. The body weight of both groups increased with age with a slight drop in mean body weight of control animals at age 59 days.

Heart Weight Results

The final results for the means and the standard error of the means are presented in Figure 4.

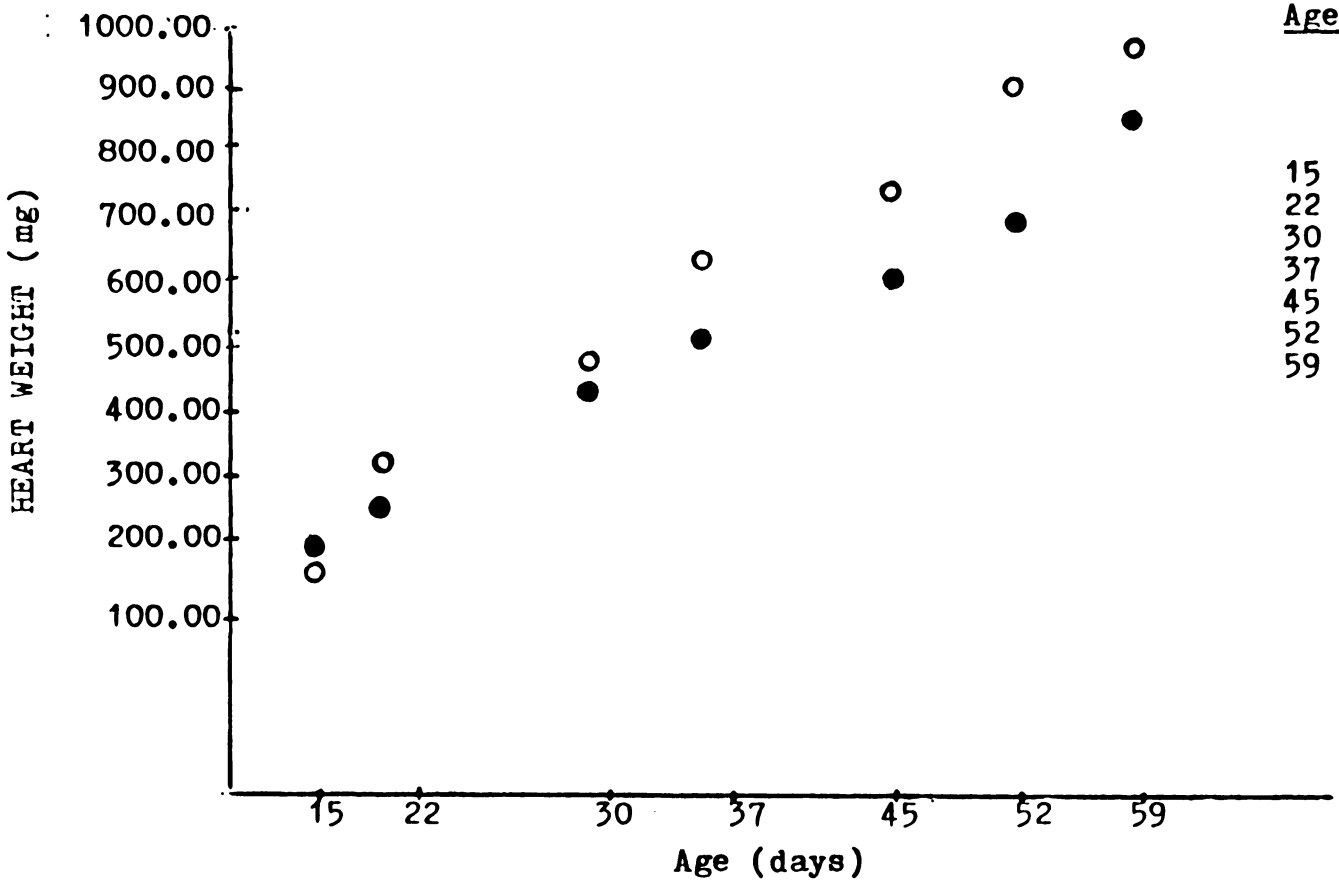
BODY WEIGHT (gm)



Age	Body Weight	
	Con (O)	Ex (●)
15	26.50 n=2	34.75 n=5
22	63.00 n=2	56.60 n=5
30	108.50 n=2	99.50 n=4
37	157.00 n=2	132.75 n=4
45	187.00 n=2	148.00 n=4
52	267.00 n=2	186.50 n=4
59	262.50 n=2	221.50 n=4

Fig. 3. Means and standard errors of the mean of body weight as a function of age.

HEART WEIGHT (mg)



Age	Heart Weight Con (O)	Heart Weight EX (●)
15	153.10 n=2	194.15 n=5
22	316.75 n=2	269.16 n=5
30	498.60 n=2	459.65 n=4
37	622.60 n=2	523.70 n=4
45	700.90 n=2	566.40 n=4
52	939.00 n=2	654.00 n=4
59	942.45 n=2	783.25 n=4

Fig. 4. Means and standard error of the means of heart weight as function of age.

Results show, as indicated in Appendix E, that the heart weights of the control animals were significantly greater than those for the exercised animals. Treatment, therefore, had a significant effect on the heart weight. Age also had a significant effect on the heart weight. The heart weights of the control, untrained animals weighed more than the hearts of the exercised animals at every age except age 15 days. Figure 4 also shows an increasing difference in the heart weights of the control and exercised animals with age. There also seems to be a leveling off of heart weights as animals near puberty (age 60).

Statistics confirm, as seen in Appendix F, the significant effects that treatment, age, and treatment x age have on the heart weight.

Heart Weight to Body Weight Ratio Results

Figure 5 illustrates the means and standard error of the means for the heart weight to body weight ratios for all animals.

Figure 5 shows a negative effect of HW/BW to age, ie. as age increases the HW/BW ratio decreases. This relationship was true for both control and exercised animals with the exception of animals (both control and exercised) at age 59 days. Between the ages of 52 to 59 days the mean HW/BW ratios for both groups slightly increased.

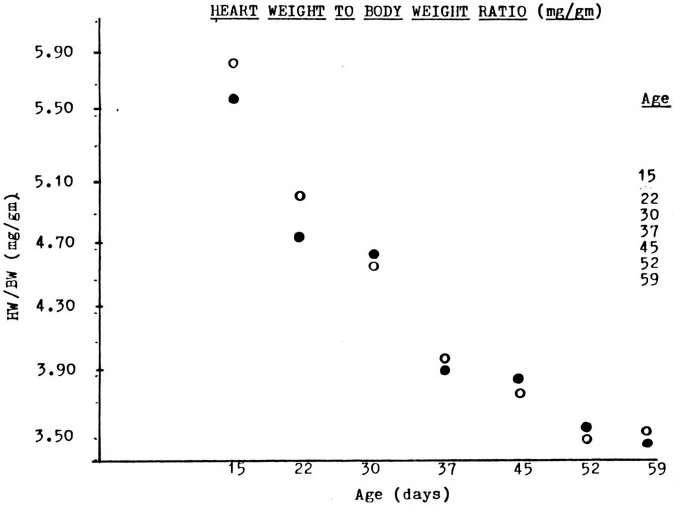


Fig. 5. Means and standard error of the means of heart weight to body weight ratio as function of age.

Statistically, one finds that only age had a significant effect on the HW/BW ratio with a P value of 0.001. While neither treatment nor treatment x age had any significant effect of the ratio.

DISCUSSION

The exercised animals gained significantly less weight than did the sedentary animals (Figure 3). The increase in heart weight of exercised animals was significantly less than that of the sedentary animals (Figure 4). The heart weight (HW) to body weight (BW) ratios were only significantly affected by age (Appendix F). Although treatment had a significant effect on HW and BW, it did not have a significant effect on the HW/BW ratio. The significant increases in the HW/BW ratios can be attributed only to the effect of age, not treatment.

Several studies (2, 31, 33) support the theory that exercised animals gain significantly less body weight than sedentary controls. This is due to the metabolic demand that accompanies exercise. Bloor and Leon (2) attributed this lower body weight to a retarded growth rate as opposed to the exercise. They conducted a study in 1970 affirming that cardiac hypertrophy occurred in trained young rats and not as a result of exercise in old rats. Training old animals has a catabolic effect on the heart and other

organs. This decrease in weight was attributed to a loss of myocardial fibers and an overall decrease in fiber mass. In the young exercised animal, the amount of sarcoplasm in the individual myocardial fibers remained similar to that observed in the control group. However, the mass of myocardial capillaries in the young exercised animals increased with the increasing degree of cardiac hypertrophy. On the other hand, exercised adult animals produced no significant changes in the number of myocardial fibers or sarcoplasmic mass and showed a slight decrease of the myocardial capillary mass. Old exercised rats showed a decreased number of myocardial fibers and a decreased sarcoplasmic mass when compared to similar age controls.

The significant increase in HW in the exercised animals can also be explained with evidence of myocardial fiber hyperplasia. Sandritter and Scomazzoni (27) have shown, using DNA content determination, that myocardial fiber hyperplasia does occur for a short period after birth in the rat and the human.

A study conducted by Tomanek (31) showed that the hearts of trained animals had the same myocardial fiber diameters as did the controls but that the capillary to muscle fiber ratios were significantly greater in the trained animals. Hyperplasia is advantageous in that it increases the distance between capillaries and central myofibrils thereby facilitating diffusion.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The purpose of this study was to determine the effects of a strenuous running program on the morphologic and histopathologic parameters in the hearts of neonatal male and female albino rats. The training regimen employed a specifically designed treadmill.

Animals from each litter were randomly assigned to Control and Experimental groups. The treatments were initiated when the animnals were 10 days of age. Animals, both experimental and control, were sacrificed throughout the study approximately every week.

Body and heart weights were obtained as well as the heart weight to body weight ratios. Two histochemical procedures were used to evaluate the general degenerative foci and calcification. Histological cross-sections at two seperate levels in each heart were examined by light microscope for indications of degeneration.

The results showed that there were significant increases in the body weight of control animals throughtout

the period of the study. The increases were due to relatively low body weights in the trained animals and the age-dependent response of body weight.

The results also showed significant increases in the heart weight of control animals. Both treatment and age were significant factors. Here again the increases of heart weight were due to low heart weights in trained animals.

However, the heart weight to body weight ratio was inversely related with age. Only age had a significant effect on the difference between HW/BW ratio of control and experimental animals. The treatment used in this study did not produce significant hypertrophy in these young prepubertal animals.

These observations are supported by the fact that at the beginning of the treatment the animals were 10 days old (prepubertal), an age at which the growth rates of the heart and body are accelerated.

The histochemical data suggest that the training program had no significant effect on the histopathology of the hearts. Four animals showed some form of damage; two control animals and two experimental animals. Overall, the histopathological observations showed the hearts of the animals in both treatment groups to be normal. None of the lesions evidenced can be attributed to the training regimen.

Recommendations

When vigorous training programs are implemented:

1. Body weight must be regularly measured to insure a normal increase.

2. The extent of myocardial hypertrophy, if any, must be monitored to insure a beneficial effect of growth.

3. A relative heart weight to body weight ratio table must be devised for various ages, and a "norm" should be defined for each age.

LIST OF REFERENCES

REFERENCES

1. Anderson, W. A. D. Pathology (Volume 1). St. Louis: C. V. Mosby Company, 1971.

2. Bloor, C. M. and A. S. Leon. Interaction of age and exercise on the heart and its blood supply. Lab. Invest. 22:160, 1970.

3. Bonner, H. W., C. K. Buffington, J. J. Newman, R. P. Farrar, and D. Acosta. Contractile activity of neonatal heart cells in culture deived from offspring of exercised pregnant rats. Evr. J. Appl. Physiol. 39:1, 1978.

4. Edwards, J. E. Pathological spectrum of occlusive coronary artery disease. Lab. Invest. 5:475, 1956.

5. Flaim, S. F., W. J. Minteer, D. P. Clark, and R. Zelis. Cardiovascular response to acute aquatic and treadmill exercise in the untrained rat. J. Appl. Physiol. 46:302, 1979.

6. Gleeson, T. T., and K. M. Baldwin. Cardiovascular response to treadmill exercise in untrained rats. J. Appl. Physiol. 50:1206, 1981.

7. Gore, I., and W. Arons. Calcification of myocardium. Arch. Path. 48:1, 1949.

8. Hickson, R. C., G. T. Hammons, and J. O. Holloszy. Development and regression of exercise-induces cardiac hypertrophy in rats. Am. J. Physiol. 236:H268, 1979.

9. Ho, K. W. Effects of swimming on dystrophic syrian hamster heart. Exp. Path., Bd. 11:248, 1975.

10. Holloszy, J. O. Biochemical adaptation in muscle. J. Bio. Chem. 212:2278, 1967.

11. Kawamura, K., C. Kashii, and K. Imamura. Ultrastructural changes in hypertrophied myocardium of spontaneously hypertensive rats. Japanese Circulation Journal. 40:1141, 1976.

12. Kissling, G., and M. F. Wendt-Gallitelli. Dynamics of the hypertrophied left ventricle in the rat. Effects of physical training and chronic pressure load. Basic Res. Cardiol. 72:178, 1977.

13. Krayenbuehl, H. P. Effects of hypertrophy on contractile function in man. Basic Res. Cardiol. 72:184, 1977.

14. Linzbach, A. J. Heart failure and cardiac structure. Amer. J. Cardiol. 5:370, 1960.

15. MacIntoch, A. M., and K. M. Baldwin. Effects of repetitive exercise on neonatal rat skeletal muscle oxidative capacity. J. Appl. Physiol. 54:531, 1983.

16. Manual of Histological Staining Methods of the Armed Forces Institute of Pathology. New York: McGraw-Hill Book Co., 1968, p.176.

17. Paniagua, R., J. J. Vazquez, and N. Lopez-moratalla. Effects of physical training on rat myocarduim. An enzymatic and ultrastructural morpho-metric study. Rev. esp. Fisiol. 33:273, 1977.

18. Parizkova, J., M. Wachtlova, and M. Soukupova. The impact of different motor activity on body composition, density of capillaries and fibers in the heart and soleus muscles, and cell's migration in vitro in male rats. Int. Z. angew. Physiol. 30:207, 1972.

19. Penpargkul, S., A. Malhotra, T. Schaible, and J. Scheuer. Cardiac contractile proteins and sarcoplasmic reticulum in hearts of rats trained by running. J. Appl. Physiol. 48:409, 1980.

20. Penpargkul, S., A. Schwartz, and J. Scheuer. Effect of physical conditioning on cardiac mitochondrial function. J. Appl. Physiol. 45:978, 1978.

21. Porter, H., D. H. Doty, and C. M. Bloor. Interaction of age and exercise on tissue lactic dehydrogenase activity in rats. Lab. Invest. 25:572, 1971.

22. Resink, T. J., W. Gevers, T. D. Noakes, and L. H. Opie. Increased cardiac myosin ATPase activity as a biochemical adaptation to running training. Enhanced response to catecholamines and a role for myosin phosphorylation. J. Of Mol. and Cell. Cardiol. 13:679, 1981.

23. Retzlaff, E., J. Fontaine, and W. Furuta. Effect of daily exercise on life-span of albino rats. Geriatrics. 25:171, 1966.

24. Richter, G. W. and A. Kellner. Hypertrophy of heart. Cell Biol. 18:195, 1963.

25. Roberts, J. T., and J. T. Wearn. Capillary-muscle relationship in normal and hypertrophic hearts. Amer. Heart J. 21:617, 1941.

26. Rosahn, P. D. Weight of normal heart. Yale J. Biol. Med. 14:209, 1941.

27. Sandritter, W. and G. Scromazzoni. Deoxyribonucleic acid content and dry weight of normal and hypertrophic heart muscle fibers. Nature. 4927:100, 1964.

28. Schlesinger, M. J., and L. Reiner. Focal myocytolysis of heart. Amer. J. Path. 31:443, 1955.

29. Schmidt, F. A. Opinions on heart exercise and acute heart dilation. Phys. Educ. 11:10, 1957.

30. Tharp, G. D. and C. T. Wagner. Chronic exercise and cardiac vascularization. Eur. J. Appl. Physiol. 48:97, 1982.

31. Tomanek, R .J. Effects of age and exercise on the extent of the myocardial capillary bed. Anat. Res. 167:55, 1970.

32. Unge, G., S. Carlssom, A. Ljungquvist, G. Tornling, and J. Adolfsson. The proliferative activity of myocardial capillary wall cells in variously aged swimming-exercised rats. Acta Path. Microbiol. Scand. Sect. A. 87:125, 1979.

33. Van Huss, W. D., W. W. Heusner, J. Weber, D. Lamb, and R. Carrow. The effects of pre-pubertal forced exercise upon post-puberty physical activity, food consumption and selected physiological and anatomical parameters. International congress of psychology of sport. Reprint of Proceedings of the Congress, Rome, 1965.

APPENDICES

APPENDIX A

TRAINING PROGRAM

APPENDIX A

TRAINING PROGRAM

Age (in days)	Speed (m/min)	Incline (%)	Duration (min)	Animals killed Exp't	Con
10-12	2.7	0	15		4
13-14	2.7	5	15		
15-16	4.7	10	25	5	2
17-19	6.7	13	30		
20-24	9.9	20	45	5	2
25-29	16.1	20	50		
30-34	20.8	20	50	4	2
35-37	24.1	20	50		
38	no training			4	2
40-44	24.1	25	50		
45	no training			4	2
47-49	26.8	25	55		
50-51	26.8	25	60		
52	no training			4	2
54-58	26.8	25	60		
59	no training			4	2

APPENDIX B

HEART MUSCLE TISSUE PROCESSING PROCEDURE

APPENDIX B

MUSCLE TISSUE PROCESSING PROCEDURE

FIXING

Tissue after being removed from rats were numbered then fixed in 10% formalin fixative.

CUTTING

They were then cut transversely above the apex and both sections were placed in numbered cassettes.

PROCESSING

The cassettes with the tissues were processed in Vacuum Infiltration Processor (VIP):

> Tissues are first immersed in two sequential formalin solutions to fix the tissues to prevent structural changes. During these steps, the proteins (including enzymes) in the cell walls of the tissue are denatured, thus rendering them inactive. Following fixation, the tissues are dehydrated by subjecting them to an increasingly more concentrated series of alcohol immersions ending with absolute (100%) alcohol. These steps are necessary to embedding medium (paraffin) is not miscible with water. The tissues are then immersed in xylene to clear the alcohol and prepare the tissues for imprgnation with molten paraffin solutions: the first to remove the clearing agent and the second to empregnate the tissues with pure paraffin.

(Source: Operating Manual; V.I.P. Tissue-Tek III 1980 Miles Laboratories, Inc.)

The baskets of blocks are left in last cycle in vacuum for one hour before embedding. Embedding medium should be maintained at 55 degrees Celsius to 58 degrees celsius.

EMBEDDING

Tissues are embedded in molds of appropriate size and placed on a cold table in order. Molds are removed from blocks after solidification of paraffin, then cut.

CUTTING

The tissues were cut using a Leitz microtome set at a width of 5mm. Each block was cut to make 2 slides; one slide to be stained in H&E, and another in von Kassa's.

APPENDIX C

STAINING TECHNIQUES

APPENDIX C

STAINING TECHNIQUES

von Kassa's Silver Technique for Calcuim

1. Hydrate sections to distilled water.
2. Immerse slides in 5% silver nitrate solution.
3. Expose the slides to bright sunlight or ultraviolet light for 10-20 minutes, or to a 60 watt bulb at a range of 4 to 5 inches for 60 minutes. Stop exposure when calcuim salts are black-brown.
4. Wash slides in several changes of distilled water.
5. Remove unreacted silver with 5% sodium thiosulfate for 2 minutes. Rinse with distilled water.
6. Counterstain for 3 to 5 minutes with nuclear fast red.
7. Rinse slides well in several changes of distilled water.
8. Dehydrate, clear and coverslip.

Results: Calcuim salts--black to brown-black nuclei--red cytoplasm--pink

Note: Oxalate salts are usually believed to give a negative von Kassa reaction.

H&E Stainer

Slides are hooked on a moving chain while automatically regulated in and out of containers containing the following solutions. Each number represents one container. Each slide remains in each container for 10 seconds before proceeding to the next container.

1.	Inverted
2, 3, 4.	Xylene
5, 6.	100% Etoh
7.	95% Etoh
8.	70% Etoh
9.	Running water
10.	Distilled water
11, 12, 13.	Gill-3 Hematoxlin
14.	Running water
15.	Glacial Acetic Rinse 0.6%
16.	Running water
17.	Blueing agent
18.	Running water
19.	80% Etoh
20, 21, 22.	Eosin Y
23, 24, 25, 26, 27.	100% Etoh
28.	Xylene

APPENDIX D

PATHOLOGICAL RESULTS

APPENDIX D — PATHOLOGICAL RESULTS

SCALE: 1- slight damage
2- mild damage
3- extensive damage

AGE (days)	No.	EXTENT OF DAMAGE normal	1	2	3	inconclusive	COMMENTS
	1	X					
	2	X					
10							
	3	X					
	4	X					
	5	X					Fixation poor. Vacuolate nuclei.
	6	X					Sub-epicardial Dilatedcapillaries.
	7	X					" " "
15							
	8	X					" " "
	9	X					" " "
	10	X					
	11	X					Fixation poor Vacuolated nuclei loss of
	12	X					cross - striations.
	13	X					
22							
	14	X					
	15	X					
	16	X					
	17	X					
	18	X					
	19	X					
	20	X					
	21	X					
30							
	22	X					
	23	X					
	24		X				Focal loss of cytoplasm and cross striations.

APPENDIX D — PATHOLOGICAL RESULTS

SCALE: 1- slight damage
2- mild damage
3- extensive damage

AGE (days)	No.	EXTENT OF DAMAGE normal	1	2	3	inconclusive	COMMENTS
	25	X					
	26	X					
	27	X					
37	28	X					
	29	X					
	30	X					
	31	X					
	32	X					Poor fixation
45	33	X					
45	34	X					
	35	X					Good fixation
	36	X					
	37	X					
	38	X					Marked venous congestion.
	39	X					
52	40	X					Fragmental myocardian (fixation artifact).
	41				X		Large area of loss of muscle leukocytic infiltration. H & E? no slide.
	42	missing					
	43	X					
	44		X				Focal mast cell infiltrate
	45		X				" " " "
59	46	X					
	47	missing					
	48	missing					

APPENDIX D -- PATHOLOGICAL RESULTS

RATING SCALE

	AGE													
	10 days		15 days		22 days		30 days		37 days		45 days		52 days		59 days	
	Con	Ex	Con	Ex	Con	Ex	Con	Ex	Con	Ex	Con	Ex	Con	Ex	Con	Ex
Normal	3		2	4	2	5	2	4	1	4	2	4	2	4		2
1									1							2
2																
3															1	
Inconclusive																

APPENDIX E

MORPHOLOGICAL RAW DATA

APPENDIX E -- MORPHOLOGICAL RAW DATA

* DATA for Animals 1 - 3
not used for statistical analysis

Animal no.	Treatment	AGE (Days)	Body wt. gm.	Mean body wt. gm.	Heart weight mg	Mean heart wt. mg	HW body wt. mg/gm	Mean HW/Bw mg/g
1*	Con	10	27		138.3		5.12	
2*	Con	10	21	23.33	119.6	122.5	5.70	5.27
3*	Con	10	22		109.6		4.98	
4	Con	15	30		158.9		5.30	
				26.5		153.1		5.85
5	Con	15	23		147.3		6.40	
6	Ex	15	38		199.6		5.25	
7	Ex	15	37		201.7		5.45	
				34.75		194.15		5.63
8	Ex	15	29		184.7		6.37	
9	Ex	15	35		190.6		5.45	
10	Con	22	66		334.0		5.06	
				63.00		316.75		5.03
11	Con	22	60		299.5		4.99	
12	Ex	22	53		263.8		4.98	
13	Ex	22	57		269.6		4.73	
				56.60		269.16		
14	Ex	22	54		551.8		4.66	4.76
15	Ex	22	63		299.8		4.76	
16	Ex	22	56		260.8		4.66	
17	Con	30	102		449.5		4.41	
				108.50		498.6		4.59
18	Con	30	115		547.7		4.76	
19	Ex	30	103		462.1		4.49	
20	Ex	30	100		442.6		4.43	
				99.50		459.65		
21	Ex	30	99		454.9		4.59	4.63
22	Ex	30	96		479.0		4.99	
23	Con	37	159	157.00	633.8	622.6	3.99	3.97

APPENDIX E — MORPHOLOGICAL RAW DATA

24	Con	37	155	157.00	611.4	622.6	3.94	3.97
25	Ex	37	124	———	480.3	———	3.87	———
26	Ex	37	144		599.7		4.16	
				132.75		523.7		3.93
27	Ex	37	137		551.8		4.03	
28	Ex	37	126		463.0		3.67	
29	Con	45	223		803.5		3.60	
30	Con	45	152		598.3		3.94	
31	Ex	45	185	———	656.9	———	3.55	———
32	Ex	45	151	148.00	580.7	566.40	3.85	3.86
33	Ex	45	130		536.4		4.13	
34	Ex	45	126		491.6		3.90	
35	Con	52	267	———	969.4	———	3.63	———
				267.00		939.00		3.52
36	Con	52	267		908.6		3.40	
37	Ex	52	170	———	633.2	———	3.72	———
38	Ex	52	232		745.7		3.21	
				186.50		654.00		3.54
39	Ex	52	192		656.6		3.42	
40	Ex	52	152		580.5		3.82	
41	Con	59	243	———	903.7	———	3.72	———
				262.50		942.45		3.60
42	Con	59	282		981.2		3.48	
43	Ex	59	183	———	611.8	———	3.34	———
44	Ex	59	177		699.2		3.95	
				221.50		783.25		3.56
45	Ex	59	275		922.6		3.35	
46	Ex	59	251		899.4		3.58	

APPENDIX F

F-RATIOS AND P-VALUES

APPENDIX F

F-RATIOS AND P-VALUES

F-Ratios and P-values for Body Weight

Effect	F-Ratio	P
Treatment	12.133	.002
Age	60.780	.001
Treatment x Age	2.016	.096

F-Ratios and P-Values for Heart Weight

Effect	F-Ratio	P
Treatment	19.971	.001
Age	68.742	.001
Treatment x Age	2.918	.024

F-Ratios and P-Values for Heart Weight to Body Weight Ratio

Effect	F-Ratio	P
Treatment	.386	.539
Age	43.446	.001
Treatment x Age	.282	.941