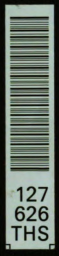

This is to certify that the

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THE EFFECT EXERCISE HAS ON THE PROGRESSION

OF DYSTROPHY IN THE SYRIAN HAMSTER

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Stanford A. Talcott

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of the requirements for

Master's degree in Zoology

Major professor

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THE EFFECT EXERCISE HAS ON THE PROGRESSION OF DYSTROPHY IN THE SYRIAN HAMSTER

By

Stanford A. Talcott

A THESIS

Submitted to
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ABSTRACT

THE EFFECT EXERCISE HAS ON THE PROGRESSION
OF DYSTROPHY IN THE SYRIAN HAMSTER

By

Stanford A. Talcott

Studies concerning the effect of exercise on the progression of muscular dystrophy in humans and experimental animals are characteristically nonquantitative and ill-defined in regard to the intensity of exercise, the period of time involved in an exercise program and the type of exercise employed. The purpose of this investigation was to quantitatively evaluate the effect of a moderate-intensity program of forced-swimming on the progression of the disease in the soleus muscle of dystrophic Syrian hamsters. Animals were forced to swim for one hour per day for 4, 8 and 12 weeks with 4% body weight attached. Muscle fibers were morphologically resolved into normal and pathological muscle fiber states which were used to quantify the effect of exercise on normal and dystrophic soleus muscle. In sedentary normal and normal forced-swum animals, fibers of the soleus muscle rarely exhibited myopathical lesions. In sedentary dystrophic animals, there was a continuous progression of the disease throughout the age of the animal. The data indicate that a moderate-intensity program of forced-swimming causes an initial acceleration of degenerative processes in the soleus muscle of dystrophic Syrian hamsters.

This research was supported by NSF Grant BNS 76-81406 and NIH Grant ROl NS10716.

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I acknowledge the use of the College of Osteopathic Medicine Electron Microscope Facility.

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TABLE OF CONTENTS

TABLE OF CONTENTS--continued Page

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES--continued

FIGURE Page

INTRODUCTION

Background

Mesocricetus auratus auratus, the Syrian hamster, was selectively bred initially by Dr. Rae Whitney. Presently, there are approximately 20 pedigreed strains which have been maintained from 5-25 generations through brother-sister matings (Homburger, 1962a). One of these lines, the BIO 1.50 line, shows a generalized polymyopathy and cardiac necrosis (Homburger, 1962b). Additional strains have been developed from the original BIO 1.50 line which also demonstrate myopathic lesions. These strains are BIO line 14.6, 53.58, and UM-X7.1.

An interesting development of these myopathic strains is the potential role the myopathy may play in elucidating the pathogenesis of human myopathies, specifically the dystrophies. In this regard, Homburger and Caulfield have investigated the basic histopathology and the mode of transmission of the myopathy. Other investigators have focused their attention on exercise therapy with current emphasis placed on the molecular biology of the membrane systems.

An initial study concerning the transmission of the disease involved crossing the original BIO 1.50 line with nondystrophic lines (Homburger, 1962a; 1962b). Autopsies were done at 55 to 62 days of age to evaluate animals which demonstrate myopathic lesions. In the F_1 generation all offspring failed to show pathological lesions. However, the F_2 generation produced a phenotypic ratio of three normal hamsters

to one dystrophic animal which is expected in the expression of a recessive autosomal gene. In another study performed by Homburger (1965) a new dystrophic line, the BIO 14.6 strain, was developed as the result of mating normal hamsters from the London School of Hygiene (LSH) and the BIO 1.50 dystrophic line. Biopsies were done on F_2 animals 60-100 days of age. The F_2 generation showed the expected phenotypic ratio of three normal to one dystrophic hamsters. A third study was performed (Homburger, 1966b) using the 14.6 and LSH lines with complete autopsies at various ages. The results of this study were in agreement with previous investigations. The genetic studies and the fact that both female and male hamsters are affected with equal frequency strongly suggest the disease is transmitted by a recessive autosomal gene and is not sex-linked. Human muscular dystrophies are usually recessive. However, they are often sex-linked (as in the psuedohypertrophic forms) and can be dominant as in the facioscapulohumeral form (Homburger, 1962b).

In addition, Homburger (1964) investigated seven strains of the Syrian hamster concerning body weight, organ weights, ear length, sex differences and histological observations. Comparisons between strains reveal no statistical differences among the parameters investigated except in the BIO 1.50 line. The BIO 1.50 strain when compared to normal strains reveal noticeable differences in organ weights. These are an enlarged heart and lung at 180 days of age and the testes and seminal vesicles were smaller than average at 56 and 180 days of age. Furthermore, the morphological characteristics of these strains showed

no abnormalities, except in the BIO 1.50 line that demonstrates a hereditary "dystrophic"-like myopathy and cardiopathy.

The normal life expectancy of the Syrian hamster is about 600-700 days of age, while in dystrophic animals death is reported as being between 200-280 days of age (Homburger, 1966a; 1970). Death is usually attributed to cardiac failure. Interestingly, this differs from most human dystrophies where death is usually the result of respiratory failure. The onset of the disease is manifested histologically before clinical symptoms are recognizable. The earliest onset is approximately at 20 days of age with nearly all animals showing myopathic lesions by 60 days of age (Homburger, 1972).

Clinical symptoms of onset are between 60-220 days of age (average is 180) and is expressed by unsteadiness of gait, predominant weakness of hindlimb muscle groups and difficulty in motion and coordination (Homburger 1962b).

Morphological and Ultrastructural Studies

Homburger (1966b) has investigated the severity of the disease as it relates to the age in normal LSH and the BIO 14.6 lines. The control LSH animals showed only occasional mild lesions in various ages of animals. A mild lesion is described as "greater than 4 centrally placed nuclei in transversally cut fibers or greater than 4 internally rowed nuclei in longitudinally cut fibers". The BIO 14.6 line animals from birth to 30 days of age showed mild or greater severity in 43.4% of the animals (cheek pouch retractor muscle). Whereas between 70 to

100 days of age, 94.0% of the animals showed mild or more marked lesions.

There is an assortment to the degree of lesion in any given striated muscle which implies muscle fibers continually show onset as others progress through the course of the disease. The descriptive pathology of these lesions has been investigated qualitatively and histologically by Homburger et al. (1962a; 1966a), and qualitatively and ultrastructurally by Caulfield (1966; 1972). Focal necrosis and central location of myonuclei are indicative of early lesions and onset. Later stages of disease show loss of striation and nuclear change, coagulation necrosis, and with eventual phagocytic infiltration.

The histological manifestations of onset have been reported by Homburger (1962a; 1962b). The focal degeneration of a muscle fiber was observed in the initial studies of Homburger (1962a; 1962b) as indicative of the earliest lesion in dystrophic hamster muscle fibers. Shortly thereafter, the subsarcolemmal nuclei become aligned in a central position in the muscle fiber. These nuclei were observed to increase in size and were juxtaposed to one another. These histological lesions were observed in dystrophic hamster muscle at 20 days or more of age. In addition, intact muscle fibers exhibited variation in size between 15 to 35 microns in diameter (Homburger, 1966a). Secondly, Homburger (1966a; 1966b) has established that the earliest lesion in hamster dystrophy was the appearance of a perinuclear halo around some subsarcolemmal nuclei. The nuclei were again observed to be centrally rowed with some perinuclear haloes fusing to each other. However, all centrally rowed nuclei were not surrounded by a halo. Homburger (1966a)

has noted that the appearance of a halo may be a fixation artifact. Muscle that is fixed in acetic-alcohol and then embedded in paraffin demonstrated the existence of a halo, whereas striated muscle that is frozen fixed or unfixed lack nuclei which are enveloped by a perinuclear halo.

The predominant morphological changes of myonuclei aligned in a central row of a muscle fiber are an increase in size and a large nucleolus (Homburger, 1962a). However, it should be noted that nuclei have different fates other than above. Myonuclei may also become enlarged and vesicular or shrunken and pyknotic both having a peripheral location. As the nuclei become aligned in fairly long chains there is a change in the staining affinity of the sarcoplasm showing a greater intensity of basophillia (Homburger, 1965). Initially, this increased basophillia is only around myonuclei, but as the disease progresses it spreads throughout the muscle fiber. Homburger has contributed this to the presence of RNA based on positive staining with pyronin-methyl green or gallocyanin, negative results after the addition of ribonuclease and positive results with acridine orange (Homburger, 1966a).

Parallelling the change in staining properties, surviving myoblasts were observed to be interspersed with degenerating muscle fibers, which may indicate that regeneration of myofibers is occurring as reported in other dystrophic animals (Telford, 1971).

In the description of advanced stages of the disease, the investigations have shown in greater detail structural alteration of the sarcoplasm, with occasional macrophage infiltration.

Pathological studies of dystrophic hamster muscle at the ultrastructural level have been investigated by Caulfield (1966; 1972). He found that in nonexercised, diseased animals the sarcotubular system is distended. An increased distension of this system was observed upon exercise in diseased and normal animals, but was more marked in the diseased animals. An early structural manifestation in exercised dystrophic hamster muscle was the deposition of lipid in between the rows of myofilaments. The lipid deposition is an isolated phenomenon and is not a characteristic of an entire fiber and adjacent muscle fibers may or may not exhibit this. The lipid deposition is similar to observations in ischemic muscle, Caulfield notes. Degeneration of the Z and I bands was observed in myopathic animals and degenerating fibers could be found adjacent to normal muscle fibers. It appears that actin filaments are lost before myosin filaments.

Following the degeneration of Z and I bands, the basement membrane remained intact, whereas the plasma membrane lost its continuity. Additional findings demonstrated that mitochondria may have mineral deposits and that leukocytes appear around or within muscle fibers. In addition, lipid deposition and Z and I band degeneration have been reported in other myopathies, denervation atrophy, ischemia and plasmocid intoxication.

Dystrophic Humans and Small Animals Under Forced-exercise

This portion of the review is a discussion of the few investigations on dystrophic humans and small animals when subjected to exercise.

Three phenomena, acting singly or in concert, are perhaps responsible for the changes associated with exercise in the skeletal muscle of dystrophic humans and animals and are as follows:

a) a change in the motor neuron's "trophic influence" upon the muscle fiber,

b) a redistribution of local circulation in the muscle fiber and/or

c) changes intrinsic to the muscle fiber that alters its own environment.

The results from studies on the effect exercise has on dystrophic humans are contradictory. Vignos (1963) and Vignos and Watkins (1966) report that exercise has a beneficial role in Duchenne, facioscapulohumeral and limb-girdle dystrophy on both muscle strength and an overall increase in functional ability. However, Johnson and Braddom (1971) report that exercise in facioscapulohumeral muscular dystrophy is deleterious. In patients with poliomyelitis, overwork of muscle fibers causes muscle deterioration, loss of strength and impaired innervation (Bennett, 1958).

Many investigators have used a combination of drug therapies and exercise programs in treating muscular dystrophy. The results of these investigations are contradictory. Dowben (1963) has reported that a combination of steroids and exercise causes an increase in muscle strength in facioscapulohumeral muscular dystrophy and a decrease or no change in patients afflicted with progressive muscular dystrophy. Fowler (1965) has reported that, when anabolic steroids are combined with exercise, this fails to prevent the progression of muscle weakness in Duchenne dystrophy, limb-girdle, or facioscapulohumeral dystrophy.

The above studies deal with dystrophic humans and lack quantitative data to support the general attitude of clinicians that exercise is beneficial to dystrophic humans. Therefore, investigators have turned their attention to dystrophic small animals where more controlled experimentation may be carried out.

Known dystrophic strains of animals have been used in approaching the problems pertaining to muscular dystrophy. However, there are few studies concerning the effects of exercise on dystrophic animals.

Acute exercise experiments have been performed and have shown an aggravation of the disease process. Homburger (1966a) showed that 1 to 4 hours of swimming could cause the death of dystrophic hamsters. This is probably the result of cardiac failure. Caulfield (1966) has observed in hamster dystrophy that a relationship exists between the strength and duration of exercise and the degree to which muscle fibers degenerate.

Wilson (1971) investigated the effect that forced-swimming has on dystrophic mouse muscle. Based on body weight, locomotor behavior and slight muscle hypertrophy, Wilson concluded that beneficial effects result from forced-swimming.

Solton (1962) observed an improved locomotor ability in dystrophic mice after a stress exercise program. This investigator observed that although dystrophic mice have impaired swimming ability the dystrophic animals improved their ability to swim after an initial declining period, suggesting that dystrophic mice have the potential to adapt to swimming stress.

Muscle contractile characteristics following exercise on a treadmill was observed by Taylor (1976) to significantly decrease the rate of tension and maximum tentanic tension developed in dystrophic mice muscle.

Physical activity was observed by Admundson (1966) to delay the onset and progression of dystrophy in chickens.

Although the Syrian hamster demonstrates muscular dystrophy, this experimental animal model shows a cardiomyopathy as well. Ho (1976) has demonstrated that dystrophic hamsters involved in an 8 week swimming program showed fewer and smaller myocardial lesions than sedentary dystrophic hamsters.

However, Howells (1974) has reported an increase in muscle fiber atrophy in the biceps brachii, extensor digitorum longus, and soleus muscle of dystrophic hamsters at 20 and 45 weeks of age when placed on a weight-lifting exercise regimen.

MATERIALS AND METHODS

This research project is a cooperative part of a much broader research investigation among three departments at Michigan State University; the Human Energy Research Laboratory of the Physical Education Department, the Neuromuscular Research Laboratory of the Department of Pathology, and the Department of Biochemistry.

I. Experimental Animals and Exercise Program

The experimental animals used in this study were male dystrophic Syrian hamsters of the BIO 14.6 and 53.58 lines. Control animals were normal random-bred Syrian hamsters. All animals were obtained from the Human Energy Research Laboratory at Michigan State University. This laboratory had originally obtained dystrophic and normal animals from the Jackson Memorial Laboratory, Bar Harbor, Maine.

The broader research project assigned animals to treatment groups defined by type and intensity of exercise regimen. The exercise regimen groups are as follows:

a) normal animals were confined to cages and were not assigned to any physical activity except for daily handling;

b) dystrophic animals confined to cages with only daily handling;

c) normal animals housed in cages and subjected to an exercise program; the exercise program was a daily 30 to 60 minutes of a progressive program of low, moderate, or high intensity exercise of swimming;

d) dystrophic animals housed in cages but followed an exercise program as in "c" above.

Each animal was randomly assigned to a control or swimming group. The animals in the swimming group were further subdivided into low, moderate, or high intensity exercise groups.

The present investigation utilized the dystrophic animals subjected to a moderate-intensity program of forced-swimming with high-intensity forced-swimming normal, sedentary normal and sedentary dystrophic animals serving as controls (Figure 1). In forced-swum dystrophic animals, a moderate-intensity program of forced-swimming was employed because dystrophic muscle may be less able to withstand stresses from a high-intensity program of forced-swimming. The maximum exercise was one hour of swimming with 4% body weight attached to the animals coat.

Each animal was confined to a cage. The control groups remained in their cages throughout the experiment, except for daily handling. The dimensions of all cages are 24 cm long X 18 cm wide X 18 cm tall.

The swimming was conducted in individual cylindrical tanks. The dimensions of each had a diameter of 28 cm and a height of 75 cm, the water depth reached 70 cm. The water temperature was held constant at 35 $\pm$ 1°C. The progressive exercise was conducted by increasing gradually each day the period of forced-swimming up to the 37th day. The 37th day was the maximum exercise and was maintained at this level for the duration of the experiment.

The animals began exercising at 35 days of age and sacrifices were made at 0, 4, 8, and 12 weeks. Thirty-five days of age was chosen

FIGURE 1. The experimental design is shown schematically. Animal genotype, age of the animal or the period of time involved in an exercise regimen and exercise regimen are the three independent variables considered in this investigation. (*-Four animals in each cell block were examined morphologically and data was collected at approximately 250X.)

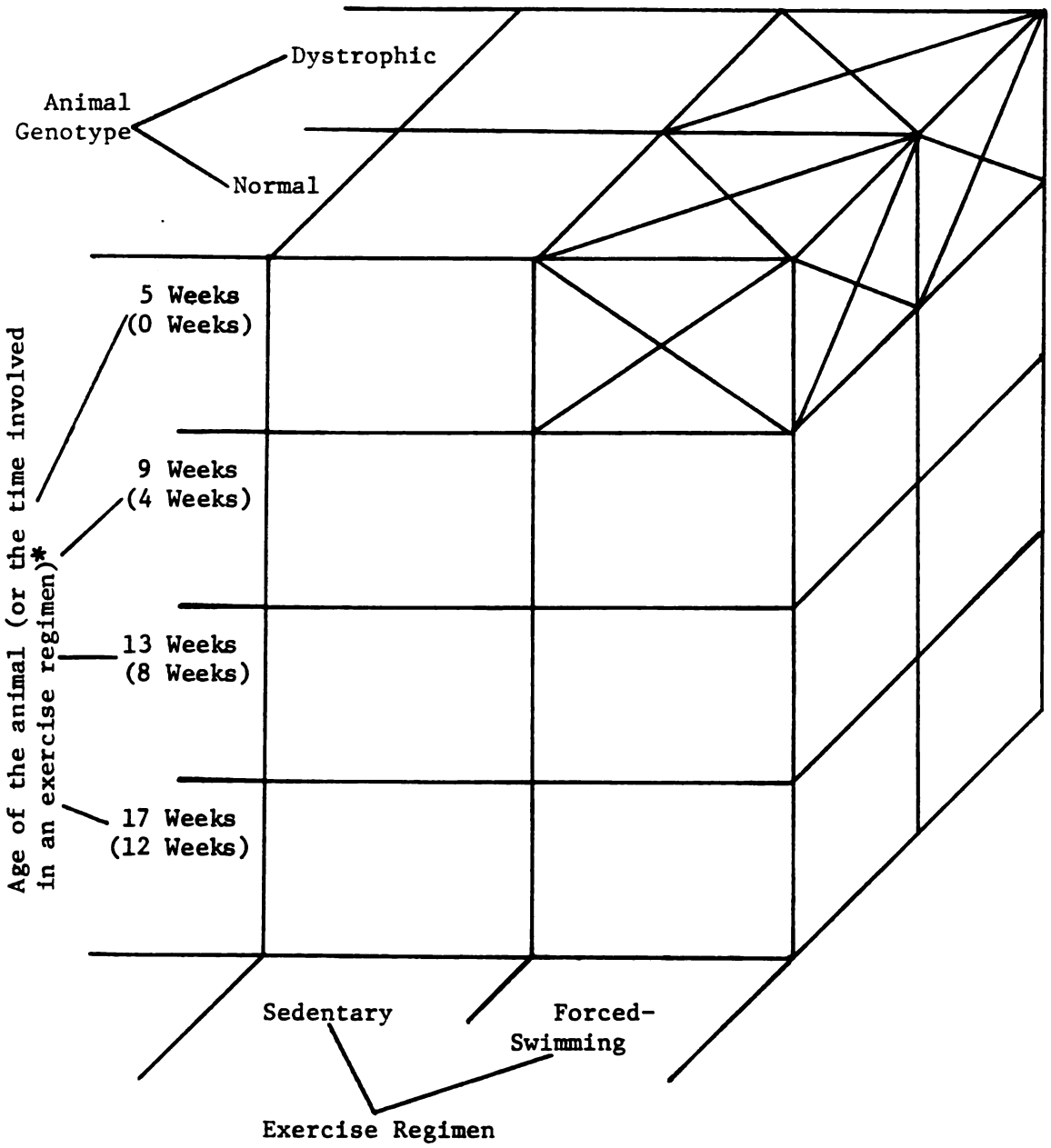

Animal Genotype
Dystrophic
Normal
5 Weeks (0 Weeks)
9 Weeks (4 Weeks)
13 Weeks (8 Weeks)
17 Weeks (12 Weeks)
Age of the animal (or the time involved in an exercise regimen)*
Sedentary
Forced-Swimming
Exercise Regimen

FIGURE 1

because the hamsters were 24 days old upon arrival from shipping and another 11 days allotted so the animals had time to adjust to laboratory conditions. Four, 8 and 12 weeks were chosen to see the changes that occur in the soleus muscle over a time course and the effects due to exercise (Figure 1).

II. Sacrifice and Dissection

At the time the animals were randomly assigned to treatment groups, they also were selected randomly for sacrifice at 0, 4, 8, and 12 weeks (Figure 1). The animals were sacrificed at about 70 hours after the last forced exercise treatment. In all experimental subgroups, 4 animals were sacrificed and quantitatively evaluated.

Hamsters were decapitated and the soleus muscle was isolated and dissected out by the following procedure:

a) the skin of the hindlimb was removed exposing the underlying muscle mass;

b) removal of the biceps femoris, semitendinosus and gracilis muscles;

c) with a pair of forceps the achilles tendon was clamped;

d) the nerve supply to the gastrocnemius and plantaris was removed;

e) the gastrocnemius and plantaris were removed by freeing their origins and insertions;

f) the nerve supply and connective tissue surrounding the soleus muscle was removed;

g) the insertion of the soleus muscle was cut and the muscle was lifted and the origin freed.

The muscle was placed on a piece of cardboard that was immersed in a solution of 4% glutaraldehyde in 0.1M sodium cacodylate buffer. Care was taken to obtain a muscle length that approximated the in situ resting length. From the dissected muscle the proximal and distal 1/3 of the soleus muscle was fixed, embedded and studied.

III. Preparation for Electron and Light Microscopic Investigation

Dissected muscle was prepared for light and electron microscopy by fixation for 3 hours in 4% glutaraldehyde (pH = 7.2-7.4) in 0.1M sodium cacodylate buffer (pH = 7.5). The samples were rinsed in 0.1M sodium cacodylate buffer (pH = 7.5). After a muscle was trimmed into segments, they were post-fixed for 1-2 hours in 2% OsO_4 in 0.1M sodium cacodylate buffer (pH = 7.5). The samples were rinsed in distilled water 3 times and dehydrated in an ascending series of ethanol. The percentage of ethanol used was 50, 70, 90, 95 and 3 times at 100%. The samples were then embedded in Spurrs plastic and placed in an oven at 60 to 70 degrees centigrade for 18 to 24 hours.

IV. Staining and Sectioning Procedure

Thick and thin sections were taken on a Sorvall MT2B ultramicrotome. Thick transverse and longitudinal sections were investigated. Cross sections were used in the collection of data, whereas longitudinal sections were used as an aid in evaluating the morphological changes in a muscle fiber observed in cross section. Thick sections

were stained with a 1-2% solution of methylene blue. Thin sections were stained with uranly acetate and .4% lead citrate and studied on a Phillips 201 electron microscope at 60 KV.

V. Morphological Quantitation

As stated above, the proximal and distal 1/3 of each soleus muscle was prepared for this investigation. In that the myopathy in the Syrian hamster is a progressive disease, pathological states were determined and used to evaluate quantitatively the effect exercise has on the progression of the disease. These states were resolved into progressive stages by morphological and ultrastructural description. Broadly defined, these states are normal muscle fibers, muscle fibers with centrally placed nuclei, atrophic fibers, degenerating fibers, macrophage invasion, coagulation necrosis and an unknown category. Criteria used in delineating these states is stated below.

State A Normal Muscle Fiber: Muscle fibers having one or more peripherally placed nuclei and demonstrate the uniform banding pattern characteristic of striated muscle.

State B Central Nuclei: Muscle fibers having one or more centrally placed myonuclei whose morphology is vesicular and swollen. The diameter and characteristic cross-striation appear similar to normal muscle fibers. (Figures 3a and 3b).

State C Atrophic: Muscle fibers with centrally located nuclei which are stellate in appearance. In addition, the muscle fiber is noticeably reduced in diameter and the morphology of the cross-striation appears ill-defined (Figure 4).

State D Degenerating Muscle Fibers: The predominant characteristic of this state is a significant decrease in the staining intensity of the sarcoplasm. In addition, these muscle fibers have an indented myonuclei and a further reduction in diameter. Ultrastructurally, these fibers appear to be going through processes that can be attributed to degenerative phenomena. Specifically, the degenerative characteristics are myofibrillar breakdown, retraction of the sarcolemma, undulating appearance of the basal lamina, an increase in ribosomes, and dark thickenings and discontinuity of the sarcolemma. Macrophage invasion is usually evident in between these muscle fibers (Figures 5 and 8).

State E Macrophage: These muscle fibers are characterized predominantly by mild to moderate macrophage invasion within a muscle fiber, centrally located myonuclei and no discernible myofibrillar organization. The outline of the muscle fiber appears intact. These muscle fibers appear smaller or normal in diameter as compared to State A which probably is the result of macrophage invasion.

State F Extensive Macrophage Invasion: Muscle fibers which appear smaller or normal in diameter and possess centrally located myonuclei. Extensive macrophage invasion is evident and an associated absence of myofibrillar organization (Figures 6a, 6b, and 9).

State G Hyaline (Coagulation Necrosis): These muscle fibers are larger than normal muscle fibers and are rounded in outline. These fibers are densely stained by methylene blue. In other fibers of this state, there are lightly-staining areas dividing darker staining areas or only a lightly-staining area throughout the fiber (Figures 7a and 7b). A centrally located myonuclei may be observed. Muscle fibers exhibiting these morphological characteristics are referred to as contracture clumps, coagulation necrosis, or hyaline fibers. Cullen and Fulthorpe (1974) have observed contracture clumps in muscle fibers from biopsies from cases of Duchenne muscular dystrophy. They have speculated that this change in muscle fiber morphology may be the central process in the breakdown of myofibrillar organization. Ultrastructurally, these fibers are characterized by a scarcity of cellular organelles with a sarcolemma and basal lamina surrounding the fiber.

State H Undeterminable: Muscle fibers which were not assigned to a specific state due to the following difficulties encountered in counting: 1) lack of myonuclei with normal banding pattern; 2) fixation artifact; 3) section which was overlapped; and 4) areas of the muscle fiber covered by stain precipitate.

The initial approach was to determine the percentage of muscle fibers resembling each state and then to apply statistics in comparing control and experimental animal groups. However, extreme variability in the percentage of muscle fiber states within a group existed and; therefore, the states were regrouped in the following way. The normal muscle fiber group, State A, remained as was originally planned. State B (Central Nuclei) and State C (Atrophic) were regrouped as a single state, State 2, representing early to moderate stages of the disease process. State D through F were regrouped into State 3 and represent later stages of disease. State G and H remained as was originally designed. State G became State 4 and State H became State 5. Figure 2 shows the new classifications of muscle fiber states and how they were constructed based on the original normal, pathological and undeterminable muscle fiber states.

VI. Data Collection

In the collection of data, an entire cross-sectional area of the proximal or distal 1/3 of the soleus muscle was taken to optimize the area used in this investigation. However, this procedure was not always feasible due to poor fixation, infiltration or as a result of trimming the block face in preparation for sectioning.

Thick sections were mounted on glass slides and stained with 1-2% methylene blue. From these slides, normal pathological and undeterminable muscle fiber states of forced-swum normal, forced-swum dystrophic, sedentary normal and sedentary dystrophic hamster soleus muscle were

counted on a Zeiss light microscope at 400X. In each sample of muscle, the average number of muscle fibers demonstrating a particular regrouped state and the total number of muscle fibers were determined based on 3 separate counts. The percentage (P) of muscle fibers of a particular normal or pathological state was determined by dividing the number of muscle fibers in a particular regrouped state (MF) by the total number of muscle fibers (TMF) minus the undeterminable (U) category $\frac{MF}{TMF-U} \times 100 = P$. The percentages were used in statistical evaluation.

VII. Statistics

A three-way analysis of variance and three two-way analyses of variance were employed to ascertain if the independent variables interacted in affecting the percentage of normal or pathological muscle fiber states. The SPSS-ANOVA statistical program was used and ran on a CDC 6500 computer at Michigan State University. The Kruskal-Wallis test was used to evaluate if the age of the animal or the period of time involved in an exercise regimen affected the percentage of normal or pathological muscle fiber states within an animal genotype group or exercise regimen group. Furthermore, the Mann-Whitney U test was used to determine if there was a significant difference in the population distribution in regard to the percentages of normal or pathological muscle fiber states within or between animal genotype or exercise regimen groupings over the age of the animals or the period of time invested in an exercise program.

Original Classification (used while collecting data)		Regrouped or New Classification (used for data analysis)	
Normal	State A	Normal	State 1
Central Nuclei	State B	Centrally Placed Myonuclei and/or	
Atrophic	State C	Atrophic	State 2
Degenerative	State D	Degenerative and Macrophage Invaded	State 3
Macrophage	State E		
Extensive Macrophage Invasion	State F		
Hyaline (Coagulation Necrosis)	State G	Hyaline (Coagulation Necrosis)	State 4
Undeterminable	State H	Undeterminable	State 5

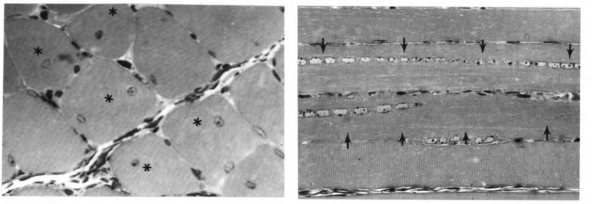
FIGURE 2. Original and regrouped normal and pathological muscle fiber states used while collecting data and for statistical analysis.

FIGURE 3. A. Centrally located myonucleus. A transverse section of muscle fibers having a centrally located myonucleus. These muscle fibers were grouped into State 2, i.e., muscle fibers having a centrally located myonucleus and/or a reduction in myofiber diameter. (*-Muscle fiber with centrally located myonucleus) 320X.

B. Longitudinal section of soleus muscle fibers demonstrating the central position of myonuclei. (arrows) 320X.

FIGURE 4. Atrophic muscle fiber. Note the reduction in myofiber diameter of the muscle fibers in the center as compared to fibers in the upper right. The nucleus of an atrophic muscle fiber usually has a stellate morphology. Muscle fibers resembling myofibers in the center of the photomicrograph were grouped into State 2, i.e., muscle fibers having centrally located myonuclei and/or a reduction in fiber diameter. (*-Atrophic muscle fiber) 320X.

FIGURE 5. Degenerating muscle fiber. The predominant characteristic of a degenerating muscle fiber is a decrease in the staining intensity of the sarcoplasm as compared to the darker staining muscle fibers. Degenerating muscle fibers were grouped into State 3, i.e., degenerating muscle fibers or muscle fibers invaded by macrophages. (*-Degenerative muscle fiber) 320X.



A. B.

Figure 3

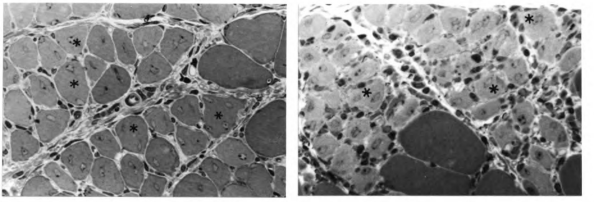
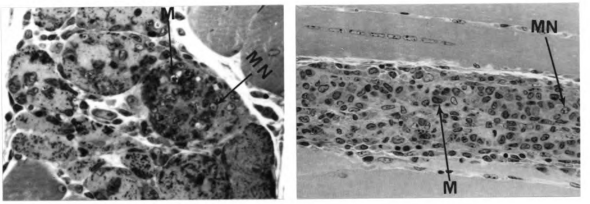


Figure 4 Figure 5

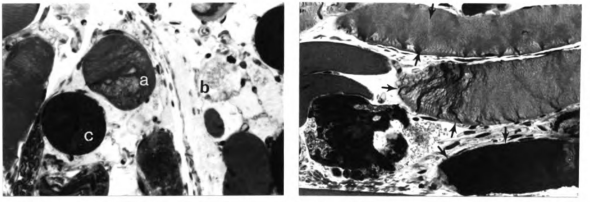
FIGURE 6. A. Extensive macrophage invasion. These muscle fibers have been invaded by macrophages and lack myofibrillar organization. Muscle fibers invaded by macrophages were grouped into State 3, i.e., degenerating muscle fibers or muscle fibers invaded by macrophages. (M-Macrophage; MN-Myonucleus) 320X.
B. Longitudinal section of a muscle fiber invaded by macrophages. (M-Macrophage; MN-Myonucleus) 320X.

FIGURE 7. A. Hyaline (coagulation) muscle fiber. Muscle fibers a, b, and c are considered to be hyaline muscle fibers. These muscle fibers were grouped into State 4, i.e., hyaline muscle fibers. 230X.
B. A longitudinal section of a hyaline muscle fiber. (arrows) 320X.



A. B.

Figure 6



A. B.

Figure 7

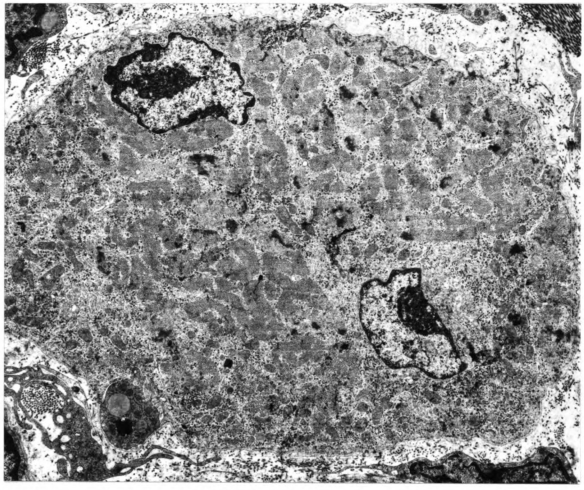


Figure 8. An electronmicrograph of a degenerating muscle fiber. 4,800X.

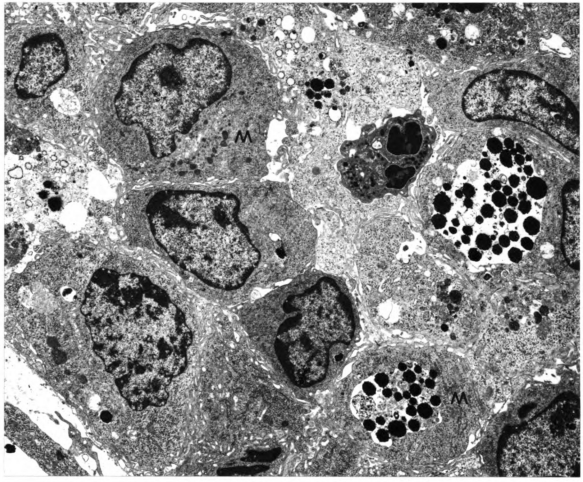


Figure 9. An electromicrograph demonstrating macrophage invasion around and within a muscle fiber. (M-Macrophage) 4,800X.

RESULTS

The analyses of variance, the Kruskal-Wallis test and the Mann-Whitney U test utilized data collected concerning the percentages of normal muscle fibers (State 1), muscle fibers which have centrally located myonuclei and/or reduction in fiber diameter (State 2), muscle fibers that were characterized as degenerative and/or ones invaded by macrophages (State 3) and hyaline (coagulation necrosis) muscle fibers (State 4). However, muscle fibers classified as undeterminable were not used in the application of the above statistical tests. This approach was followed to alleviate any problems encountered while working with data in the form of percentages. In particular transverse sections of soleus muscle, the percentage of undeterminable muscle fibers exceeded 20% (Table 1). This is largely due to the inability to distinguish between normal muscle fibers (State 1) and muscle fibers having a centrally located myonuclei. Primarily, these two muscle fiber states were resolved on the basis of the location of the myonucleus of the muscle fiber (i.e., peripheral or central). Therefore, when a muscle fiber lacks a myonuclei in a transverse section this usually necessitated classifying the muscle fiber as undeterminable (State 5). The data profiles of all normal and pathological muscle fiber states and the total number of muscle fibers in the soleus muscle of sedentary dystrophic, sedentary normal, forced-swum normal and forced-swum dystrophic over the entire age of the animal or the period of time

involved in an exercise regimen appears in Table 2 for further reference.

In applying the analyses of variance, the measurement used may not have been of sufficient strength to meet the assumptions underlying parametric statistical tests. Therefore, caution should be exercised when interpreting probability levels of significance. Certain theoretical groups believe that altered probability levels result from applying a parametric test while the model calls for a nonparametric test. However, other schools of thought believe that only slight deviations result in probability levels and does not lead to data misinterpretation based on empirical evidence.

Percentage of Normal Muscle Fibers by Animal Genotype, Age of the Animal and Exercise Regimen

The three-way analysis of variance demonstrated that animal genotype, age of the animal, and exercise regimen (i.e., whether hamsters led sedentary lives or hamsters that were forced to swim, see Materials and Methods), significantly interacted to influence the percentage of normal muscle fibers appearing in the soleus muscle in the Syrian hamster ($\alpha < .001$).

Since significant interactions occur in the three-way analysis of variance, three two-way analyses of variance were performed. The tests shown below point out animal genotype, age of the animal or the period of time involved in an exercise regimen, and whether a sedentary condition or forced-swimming program (exercise regimen) was implemented:

A. Animal Genotype-normal sedentary vs. dystrophic sedentary; Ages of the animals--5, 9, 13 and 17 weeks (Figures 10 and 11).

B. Animal Genotype-normal swim vs. dystrophic swim; Ages of the animals--5, 9, 13 and 17 weeks (Figures 12 and 13).

C. Exercise Regimen-dystrophic sedentary vs. dystrophic swim; Time participating in the exercise regimen--4, 8, and 12 weeks (Figures 14 and 15).

In each two-way analysis of variance, there was a significant effect exerted by the independent variables in altering the percentage of normal muscle fibers. Therefore, the Kruskal-Wallis test was used to determine the effect age of the animal has on the percentage of normal or pathological muscle fiber states within an animal genotype group or in an exercise regimen group. Furthermore, the Mann-Whitney test was employed to statistically evaluate the population distribution of a normal or pathological muscle fiber state at different ages or period of time invested in an exercise regimen within or between genotype or exercise regimen groupings. In addition, this approach was used, where applicable, in the analysis of the percentage of muscle fibers demonstrating central location of myonuclei and/or reduction of myofiber diameter (State 2), degenerative or macrophage invaded muscle fibers (State 3), and hyaline muscle fibers (State 4).

Percentage of Normal Muscle Fibers by Animal Genotype and Age of the Animal (A)

In sedentary normal animals (Figure 10), the normal muscle fiber population remained at a constant mean percentage of approximately 99%, as was expected. However, in the sedentary dystrophic group at 5 weeks, the mean percentage of normal muscle fibers was only 66%. In the sedentary dystrophic group (Figure 10), the Kruskal-Wallis test revealed

a significant difference ($\alpha < .001$) in the mean percentages of normal muscle fibers through all ages of the animals investigated. However, by the Mann-Whitney test no significant difference in the percentage of normal muscle fibers was noted between 5 and 9 weeks of age in the sedentary dystrophic animals. Initially, the percentage of normal muscle fibers remained approximately the same through 9 weeks of age in the soleus muscle of animals from the sedentary dystrophic group. However, the percentage of normal muscle fibers precipitously declined between 9 and 13 weeks ($\alpha < .014$) and increased between 13 and 17 weeks ($\alpha < .014$). In comparing normal sedentary and dystrophic sedentary animals at all ages investigated, the percentage of normal muscle fibers was significantly less in the sedentary dystrophic group ($\alpha < .014$). This leads to the conclusion that the animal genotype plays a predominant role in determining the percentage of normal muscle fibers (i.e., between sedentary normal and sedentary dystrophic animals), whereas the age of the animal exerts an effect in the sedentary dystrophic group.

Percentage of Normal Muscle Fibers by Animal Genotype and Age of the Animal (B)

In normal animals subjected to a swimming program (Figure 12), the normal muscle fiber population remained at approximately 99% through all ages investigated. In the forced-swimming dystrophic group (Figure 12), the Kruskal-Wallis test revealed a significant difference in the mean percentages of normal muscle fibers at all ages under investigation ($\alpha < .01$). In addition, the population of muscle fibers in the soleus muscle showed a continuous decrease in the percentage of

normal muscle fibers through 8 weeks of forced-swimming ($\alpha < .014$). Interestingly, a leveling off of the percentage of normal muscle fibers occurs between 8 and 12 weeks of forced-swimming. Again, the data indicate that the animal genotype determines to a large degree the percentage of normal muscle fibers, whereas the age of the animal is mainly responsible for changes observed in the forced-swimming dystrophic group.

Percentage of Normal Muscle Fibers by Exercise Regimen and Time in the Exercise Regimen (C)

In the comparison between sedentary and forced-swimming dystrophic hamster soleus muscle (Figure 14), the mean percentage of normal muscle fibers in the forced-swimming dystrophic group is significantly smaller ($\alpha < .014$) than in the sedentary dystrophic group after 4 weeks involvement in the exercise regimen. At later phases of the exercise regimen, the 2 groups failed to reveal significant differences in their percentage of normal muscle fibers. These data indicate that the initial effect of forced-swimming is to accelerate the degenerative process.

Percentage of Muscle Fibers with Centrally Placed Myonuclei and/or Atrophic Muscle Fibers by Animal Genotype, Age of the Animal and Exercise Regimen

In analyzing the percentage of muscle fibers assigned to State 2, the interaction between animal genotype, age of the animal and the type of exercise regimen was found to be insignificant ($\alpha < .175$). However, the two-way interactions, animal genotype-age of the animal and animal genotype-exercise regimen, were significant ($\alpha < .001$ and $\alpha < .046$, respectively). The interaction between the age of the animal and

whether the animal was sedentary or had been forced to swim was insignificant.

Percentage of State 2 Muscle Fibers by Animal Genotype and Age of the Animal (A)

In sedentary normal animals (Figure 10), the percentage of muscle fibers comprising State 2 rarely exceeded mean values above 1%. In the sedentary dystrophic group (Figure 10), the Kruskal-Wallis test revealed a significant difference ($\alpha < .01$) in the mean percentages of State 2 muscle fibers at all ages investigated. The percentage of muscle fibers assigned to State 2 demonstrated an increase between 5 and 17 weeks of age in the sedentary dystrophic animals ($\alpha < .014$). At all ages investigated, the sedentary dystrophic animals had significantly higher mean percentages of State 2 muscle fibers than control sedentary normal animals ($\alpha < .014$). In comparing these two groups, the data demonstrates that animal genotype and age of the animal are responsible in determining the percentage of muscle fibers belonging to State 2. However, the age of the animal has an effect only in the sedentary dystrophic group indicating a progressive increase of muscle fiber involvement.

Percentage of State 2 Muscle Fibers by Animal Genotype and Age of the Animal (B)

In normal swimming animals (Figure 12), the percentage of muscle fibers showing the morphological characteristics of pathological State 2 rarely exceeded mean values above 1%. In the forced-swimming dystrophic group (Figure 12), the Kruskal-Wallis test revealed a significant difference ($\alpha < .02$) in the mean percentages of State 2 muscle fibers at all animal ages investigated. In the dystrophic swim animals, muscle fibers

showing central location of myonuclei and/or reduction in fiber diameter demonstrated a significant increase in their percentage between 0 to 8 weeks of forced-swimming ($\alpha < .014$). By 12 weeks of forced-swimming the percentage of these fibers had leveled off. Once again, the genotype of the animal in part determines the percentage of muscle fibers belonging to State 2 and the age of the animal influences the percentage in the dystrophic forced-swimming group.

Percentage of State 2 Muscle Fibers by Exercise Regimen and Time in the Exercise Regimen (C)

The muscle fibers characterized by the central location of myonuclei and/or atrophy showed the following effect between dystrophic animal groups (Figure 14). Initially, the soleus muscle in the moderate-intensity forced-swimming dystrophic group had a higher percentage of these muscle fibers than in the sedentary dystrophic group ($\alpha < .014$). Thereafter, forced-swimming failed to alter the percentage of State 2 muscle fibers between exercise regimen groups at 8 and 12 weeks. In both groups, the percentage increases throughout the course of the investigation. This indicates that the initial effect of a moderate-intensity program of forced-swimming is deleterious to dystrophic soleus muscle in the Syrian hamster.

Percentage of Degenerative and Macrophage Invaded Muscle Fibers by Animal Genotype, Age of the Animal and Exercise Regimen

The three-way and all two-way analyses demonstrate that the percentage of degenerating muscle fibers or muscle fibers invaded by macrophages is not altered due to the interaction of the independent

variables. Interestingly, the analysis of variance shows the percentage of muscle fibers degenerating or infiltrated by macrophages is dependent only on animal genotype as the level of significance is $\alpha < .001$ (Figures 11, 13 and 15). This shows that the percentage of muscle fibers degenerating or those invaded with macrophages is dependent only on the animal genotype.

Percentage of Hyaline Muscle Fibers by Animal Genotype, Age of the Animal and Exercise Regimen

The three-way interaction can be considered to be significant ($\alpha < .056$) in view of the extreme variability that exists between animals within a group. This is probably due to the variable time of onset of muscle fiber involvement.

Percentage of Hyaline Muscle Fibers by Animal Genotype and Age of the Animal (A)

In this two-way analysis, the percentage of hyaline muscle fibers is dependent on the interaction between animal genotype and age of the animal, as the level of significance was $\alpha < .035$. Normal sedentary animals (Figure 11) rarely exceeded a mean value of 1% in percentage of hyaline muscle fibers. In the sedentary dystrophic animals (Figure 11), the Kruskal-Wallis test reveals no significant difference ($\alpha < .30$) in the mean percentages of normal muscle fibers through all ages of the animals investigated. The Mann-Whitney test showed that of all ages looked at, the sedentary dystrophic animals had significantly higher mean percentages of hyaline muscle fibers than the control sedentary normal animals ($\alpha < .014$). These data indicate that the animal genotype

in part determines the percentage of hyaline muscle fibers. In addition, the percentage of hyaline muscle fibers in sedentary dystrophic animals is not dependent on the age of the animal.

Percentage of Hyaline Muscle Fibers by Animal Genotype and Age of the Animal (B)

In the two-way analysis between normal and dystrophic swimming animals, no significant interaction was noted. The analysis shows a significant effect due to the animal genotype ($\alpha < .001$) in determining the percentage of hyaline muscle fibers (Figure 13). In addition, the Kruskal-Wallis test revealed no significant changes ($\alpha < .30$) in the mean percentage of hyaline muscle fibers throughout all ages in the forced-swum dystrophic animals (Figure 13). These data indicate that the percentage of hyaline muscle fibers is dependent on the genotype of the animal, i.e., dystrophic or normal.

Percentage of Hyaline Muscle Fibers by Exercise Regimen and Time in the Exercise Regimen (C)

In determining the percentage of hyaline muscle fibers, the two-way analysis of variance demonstrated that a significant interaction occurred between whether the animals were sedentary or swimming and their age. However, in both groups (Figure 15), the Kruskal-Wallis test failed to demonstrate significant differences in the mean percentage of hyaline muscle fibers at all ages ($\alpha < .30$). By the Mann-Whitney test, the percentage of hyaline muscle fibers between the swimming or sedentary dystrophic groups showed no significant difference at any age or period of time invested in an exercise regimen.

TABLE 1. Mean Number and Mean Percent of Undeterminable Muscle Fibers*

Muscle Fiber State	Age of the Animal (weeks)	Normal				Dystrophic			
		Sedentary		Forced-Swimming		Sedentary		Forced-Swimming	
		$\bar{X}$ Number	$\bar{X}$ Percent	$\bar{X}$ Number	$\bar{X}$ Percent	$\bar{X}$ Number	$\bar{X}$ Percent	$\bar{X}$ Number	$\bar{X}$ Percent
Undeterminable	5	256±29	33.5±3.4			212±29	25.0±3.1		
	9	285±65	35.2±2.5	316±69	44.4±1.3	202±50	21.5±2.9	79±22	12.3±1.0
	13	374±59	43.4±3.4	319±72	42.2±1.6	52±5	8.0±1.2	92±37	11.1±1.5
	17	258±29	40.9±4.6	295±82	42.0±0.9	50±6	13.2±2.1	62±9	9.9±1.3

*The sum of squared error (SSE) was used and appears to the right of the variable value.

TABLE 2. Mean Number, Mean Percent and the Total Mean Number of Soleus Muscle Fibers*,**

Muscle Fiber State	Age of the Animal (weeks)	Normal				Dystrophic			
		Sedentary		Forced-Swimming		Sedentary		Forced-Swimming	
		$\bar{X}$ Number	$\bar{X}$ Percent	$\bar{X}$ Number	$\bar{X}$ Percent	$\bar{X}$ Number	$\bar{X}$ Percent	$\bar{X}$ Number	$\bar{X}$ Percent
State 1 (normal)	5	516±93	98.4±1.3			451±69	69.7±6.0		
	9	551±68	99.6±0.2	384±73	98.8±0.7	502±84	66.0±5.5	245±9	43.7±3.2
	13	503±108	99.5±0.4	522±71	99.0±0.4	97±16	16.1±3.2	187±81	25.1±5.5
	17	367±64	97.2±0.2	400±109	99.0±0.2	86±11	25.6±3.2	135±25	23.9±2.8
State 2 (central/ atropic)	5	4±2	0.7±0.5			111±45	15.6±2.8		
	9	3±1	0.5±0.2	1±1	0.2±0.1	216±47	29.8±4.4	255±34	48.4±5.3
	13	2±2	0.4±0.4	5±2	1.0±0.4	323±80	53.0±10.0	445±100	63.4±5.1
	17	7±3	2.1±1.1	4±1	1.0±0.2	235±47	66.8±1.6	368±79	63.9±4.0
State 3 (Degener- ating/ Macrophage)	5	3±4	0.8±1.0			55±15	8.5±2.5		
	9	0±0	0.0±0.0	0±0	0.0±0.0	12±7	1.8±1.2	22±12	3.8±1.8
	13	1±1	0.1±0.1	1±1	0.1±0.1	47±28	6.6±2.9	56±36	7.7±4.7
	17	2±2	0.6±0.8	0±0	0.0±0.0	9±6	2.9±2.2	58±31	11.2±5.9
State 4 (Hyaline)	5	1±1	0.1±0.1			53±33	6.8±2.0		
	9	0±0	0.0±0.0	3±2	0.9±0.7	18±4	2.5±0.5	28±19	4.2±1.9
	13	1±1	0.1±0.1	1±1	0.1±0.1	124±56	23.4±12.2	28±18	4.0±2.4
	17	1±1	0.1±0.1	0±0	0.0±0.0	20±14	4.7±2.7	7±2	1.2±0.5
Total number muscle fibers (i.e., States 1-4)	5	597±122				671±147			
	9	553±68		388±73		748±115		549±126	
	13	506±109		530±72		590±108		714±179	
	17	374±60		404±111		350±66		566±85	

*The sum of squared error (SSE) was used and appears to the right of the variable value.
**The number of undeterminable muscle fibers was not used in compiling the above data.

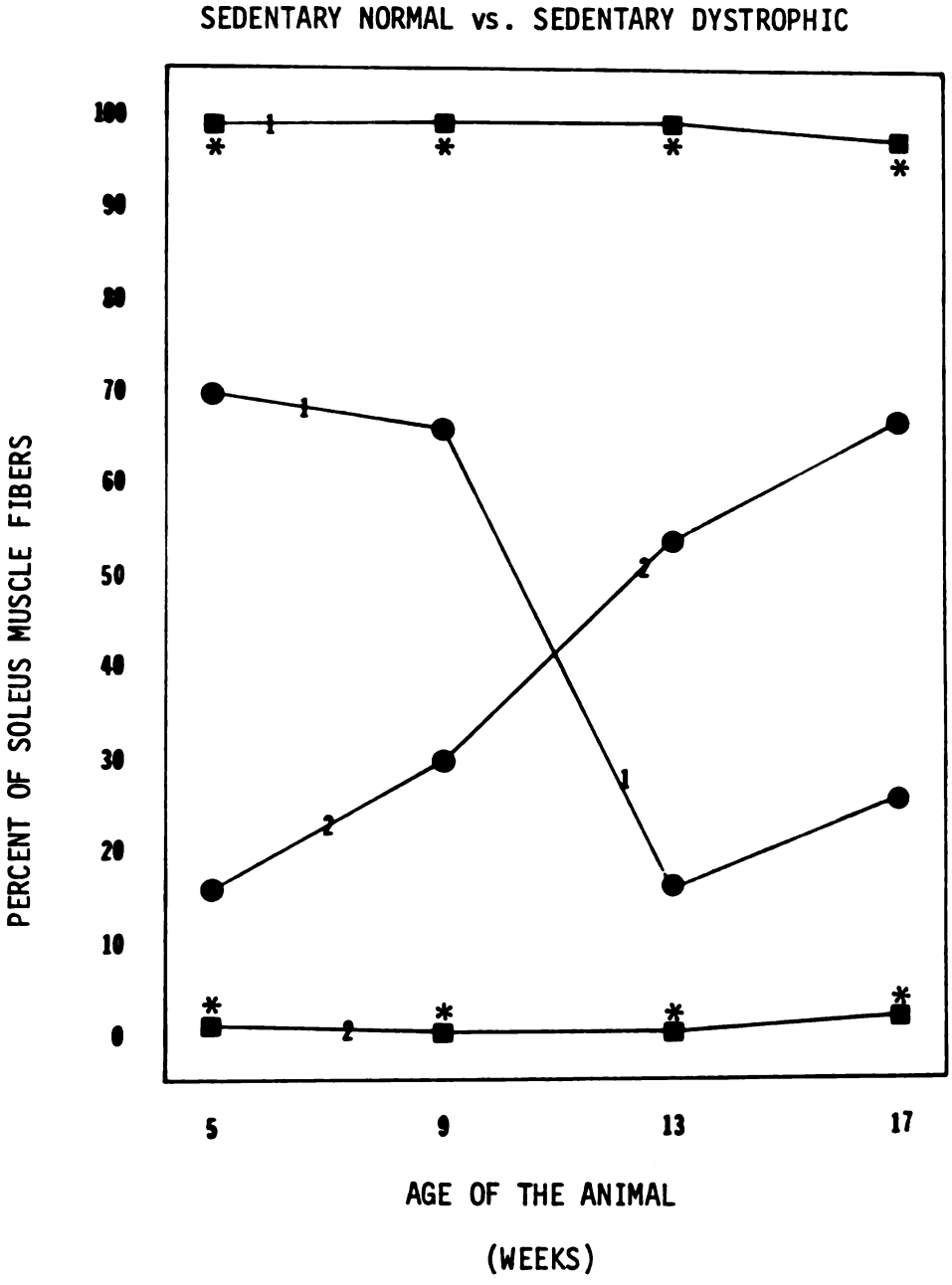


FIGURE 10. The mean percentage of normal muscle fibers (State 1) and muscle fibers having centrally located myonuclei and/or a reduction in myofiber diameter (State 2) in the soleus muscle of sedentary normal and sedentary dystrophic animals. *: indicates significant differences statistically as determined by the Mann-Whitney U test ($\alpha < .014$) between sedentary normal and sedentary dystrophic animals. (■-sedentary normal; ●-sedentary dystrophic; 1-State 1; 2-State 2)

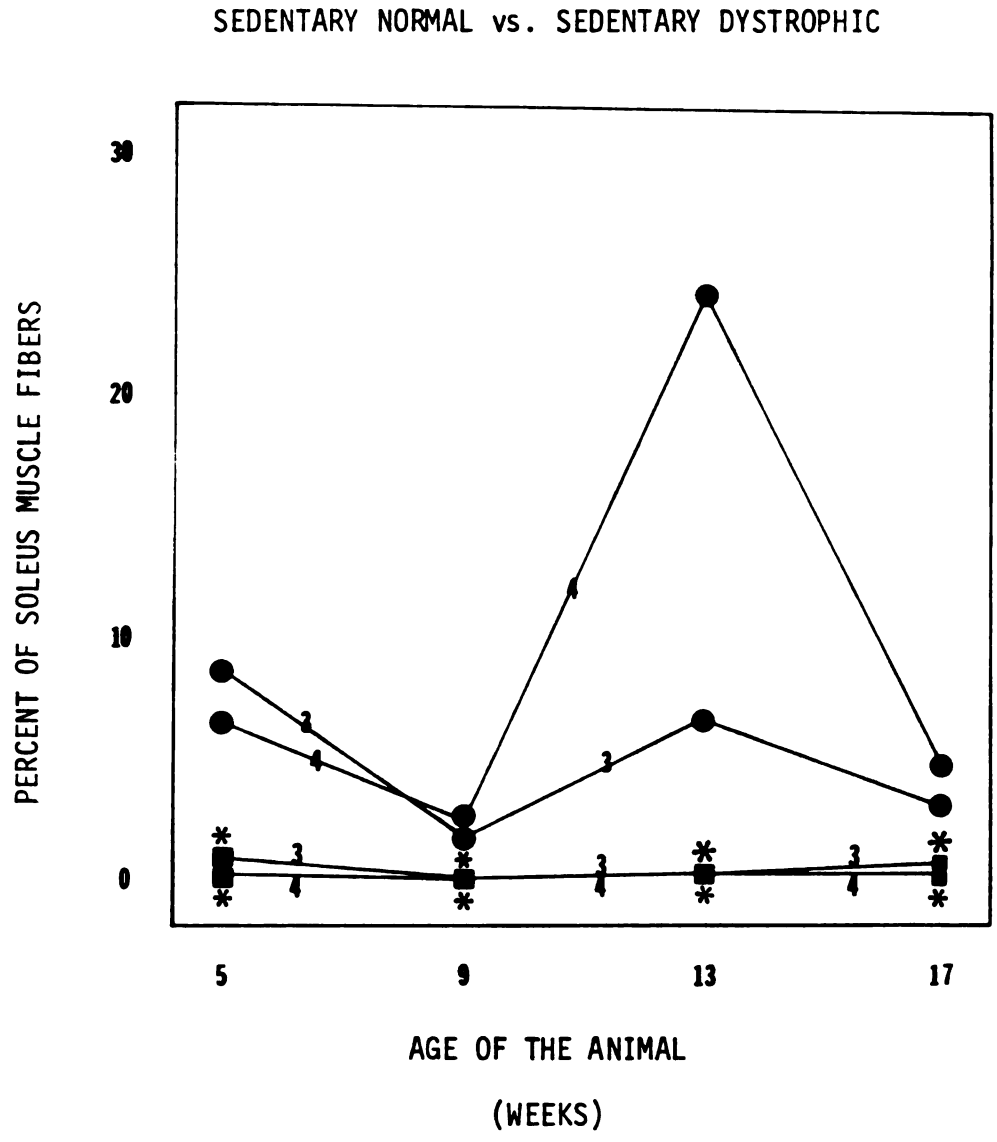


FIGURE 11. The mean percentage of degenerating muscle fibers and muscle fibers invaded by macrophages (State 3) and hyaline (coagulation) muscle fibers (State 4) in the soleus muscle of sedentary normal and sedentary dystrophic animals. *: indicates significant differences statistically as determined by the Mann-Whitney U test ($\alpha < .014$) between sedentary normal and sedentary dystrophic animals. (■-sedentary normal; ●-sedentary dystrophic; 3 -State 3; 4 -State 4)

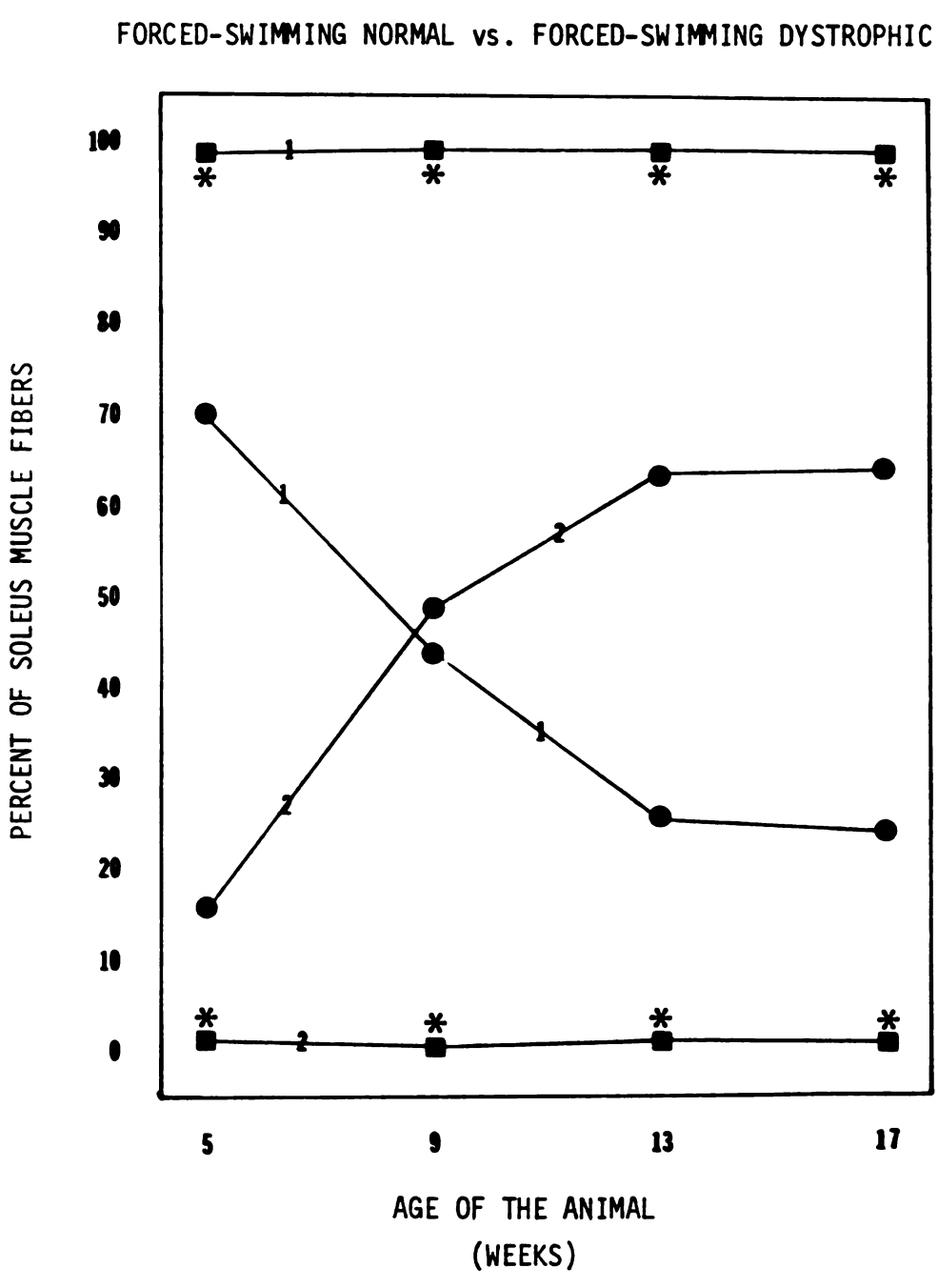


FIGURE 12. The mean percentage of normal muscle fibers (State 1) and muscle fibers having centrally located myonuclei and/or a reduction in myofiber diameter (State 2) in the soleus muscle of forced-swimming normal and forced-swimming dystrophic animals. *: indicates significant differences statistically as determined by the Mann-Whitney U test ($\alpha < .014$) between forced-swimming normal and forced-swimming dystrophic animals. (■-forced-swimming normal; ●-forced-swimming dystrophic; 1-State 1; 2-State 2)

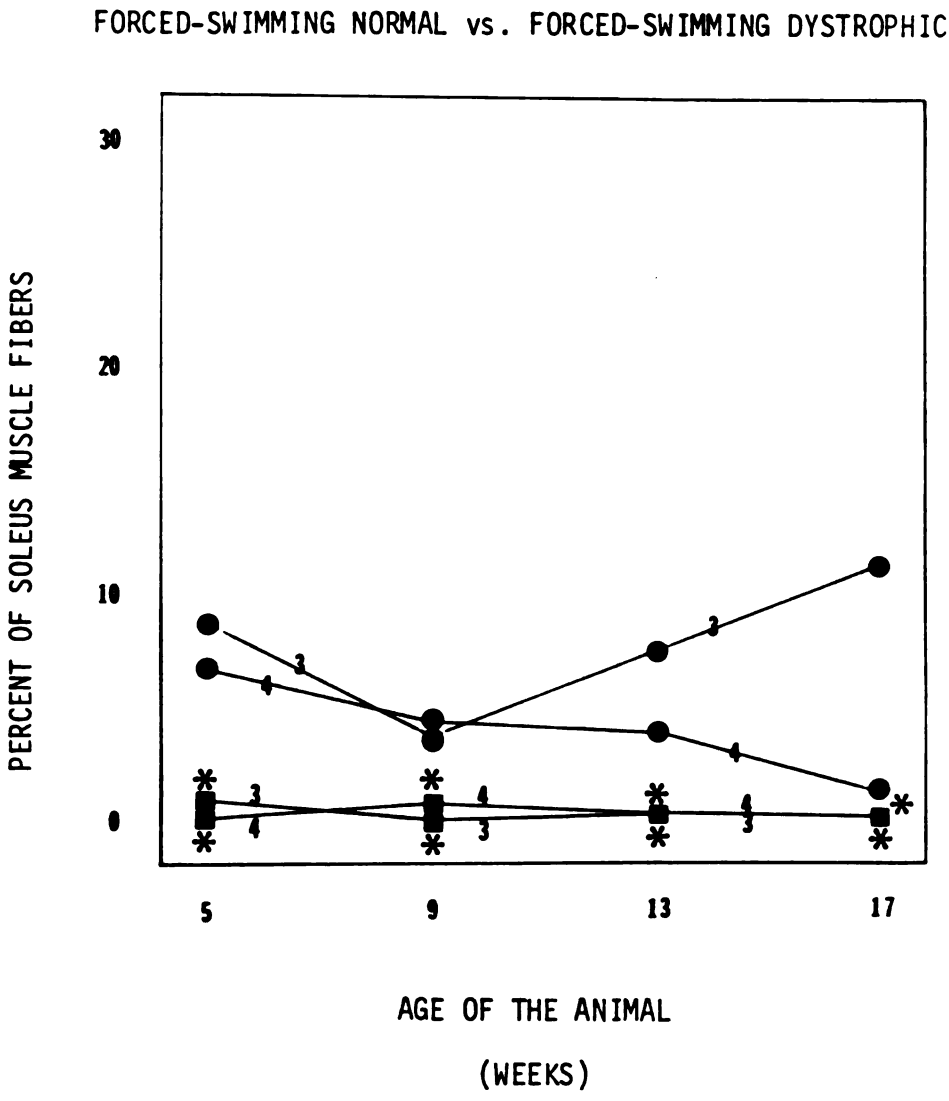


FIGURE 13. The mean percentage of degenerating muscle fibers and muscle fibers invaded by macrophages (State 3) and hyaline muscle fibers (State 4) in the soleus muscle of forced-swimming normal and forced-swimming dystrophic animals. *: indicates significant differences statistically as determined by the Mann-Whitney U test ($\alpha < .014$) between forced-swimming normal and forced-swimming dystrophic animals. (■-forced-swimming normal; ●-forced-swimming dystrophic; 3-State 3; 4-State 4)

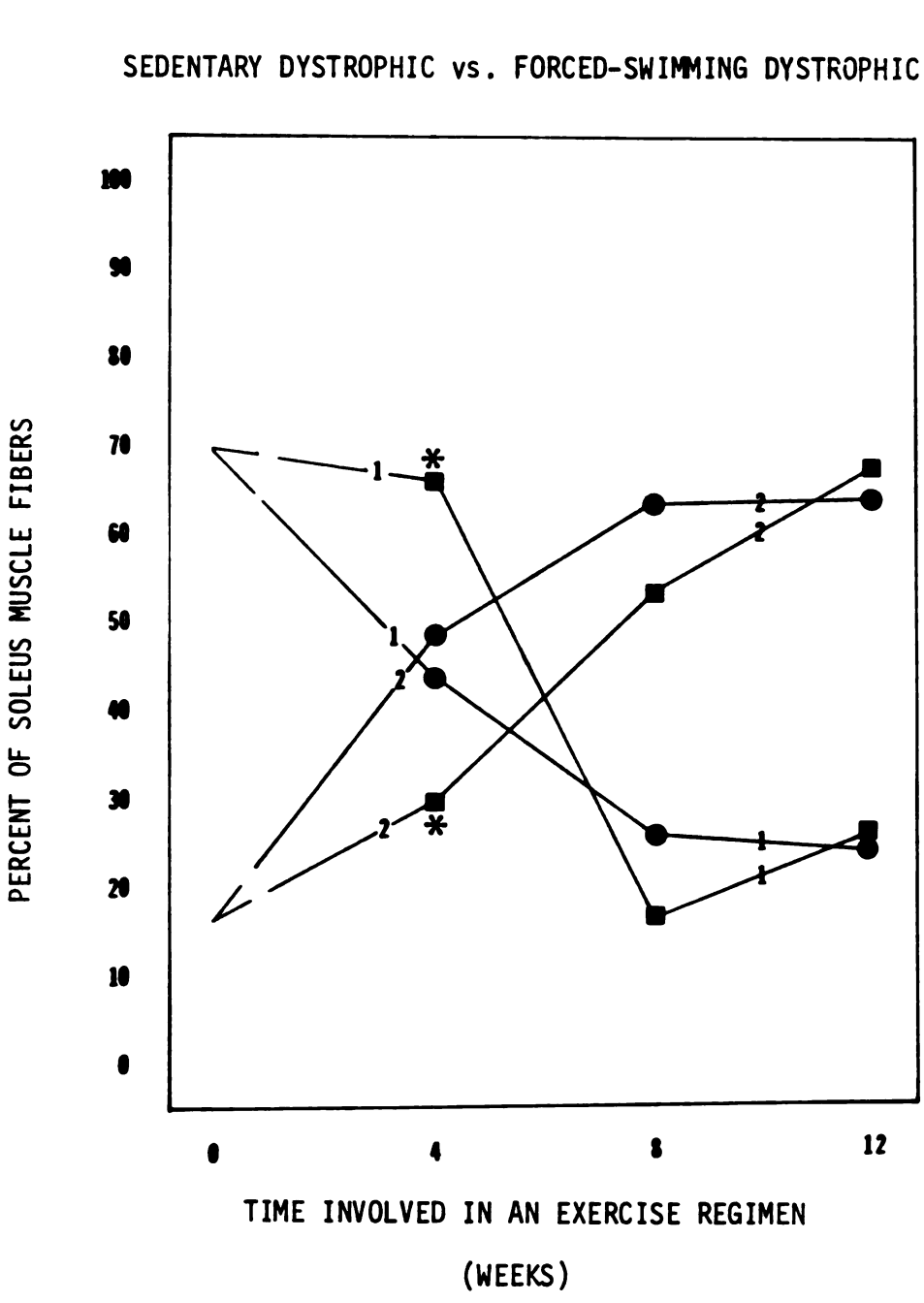


FIGURE 14. The mean percentage of normal muscle fibers (State 1) and muscle fibers having centrally located myonuclei and/or a reduction in fiber diameter (State 2) in the soleus muscle of sedentary dystrophic and forced-swimming dystrophic animals. *: indicates significant differences statistically as determined by the Mann-Whitney U test ($\alpha < .014$) between sedentary dystrophic and forced-swimming dystrophic animals. (■-sedentary dystrophic; ●- forced-swimming dystrophic; 1-State 1; 2-State 2)

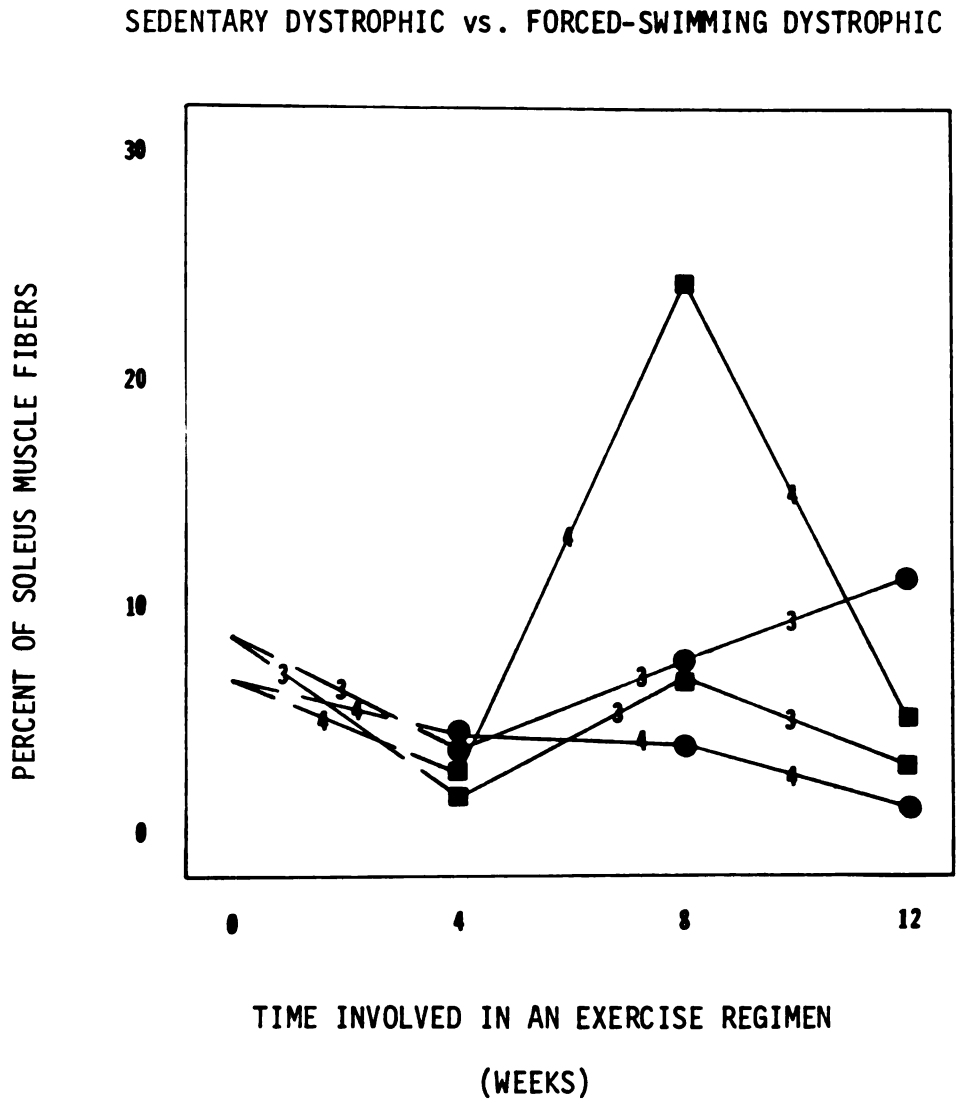


FIGURE 15. The mean percentage of degenerating muscle fibers and muscle fibers invaded by macrophages (State 3) and hyaline muscle fibers (State 4) in the soleus muscle of sedentary dystrophic and forced-swimming dystrophic animals. No significant differences were noted as determined by the Kruskal-Wallis and the Mann-Whitney U test between sedentary dystrophic and forced-swimming dystrophic animals. (■- sedentary dystrophic; ●- forced-swimming dystrophic; 3-State 3; 4-State 4)

DISCUSSION

Normal and pathological muscle fiber States were defined by morphological and ultrastructural criteria to quantitatively evaluate the effect that a program of forced-swimming has on the progression of dystrophy in the soleus muscle of dystrophic Syrian hamsters.

Before investigating this effect, it was important to evaluate to what extent soleus muscle fibers of normal Syrian hamsters showed pathological lesions due to sacrifice, dissection, fixation and/or embedding procedures or diet. These could possibly produce similar lesions as seen in dystrophic muscle. In that the percentage of normal muscle fibers remained at approximately 99% and pathological muscle fiber states rarely exceeded percentages above 1%, it was concluded that the soleus muscle of normal Syrian hamsters possessed few muscle fibers exhibiting a morphology similar to myopathical lesions in dystrophic hamsters.

Secondly, normal Syrian hamsters were placed on a high-intensity (see Materials and Methods) program of forced-swimming to evaluate if exercise was deleterious to soleus muscle fibers of normal Syrian hamsters. The data indicate that this intensity of forced-swimming was not detrimental to the muscle.

The soleus muscle of sedentary dystrophic hamsters was evaluated to determine the time course of the disease. This was based upon alterations in the percentages of muscle fibers belonging to normal and

pathological muscle fiber states. In addition, sedentary dystrophic animals were used as the control for comparisons with forced-swimming dystrophic hamsters.

In the sedentary dystrophic animals, the greatest rate of progression of the disease appears to occur between 9 to 17 weeks of age. This is based on a general decrease in the percentage of normal muscle fibers parallelled by an increase in the percentage of muscle fibers with centrally located myonuclei and/or a reduction in fiber diameter (State 2).

Three questions which should be asked at this point are:

1. What is the rate of progression of the disease from birth to 5 weeks of age in the soleus muscle of sedentary dystrophic hamsters?

2. What is the rate of progression of the disease from 5 to 9 weeks of age in the soleus muscle of sedentary dystrophic hamsters?

3. What accounts for the rise in the percentage of normal muscle fibers from 13 to 17 weeks of age in the soleus muscle of sedentary dystrophic hamsters?

Unfortunately, the data needed to assess the rate at which the disease progresses from birth to 5 weeks of age is unavailable through this investigation. However, if we assume that at birth few pathological lesions are evident, this suggests that a rapid progression exists from birth to 5 weeks. This is based on the fact that by the 5th week the percentage of normal muscle fibers was 70% and muscle fibers belonging to State 2 had risen to 16%. Homburger (1966a) has reported that few pathological lesions are seen at the time of birth and the earliest signs of onset usually appear between 20 and 60 days of age.

In assessing the progression of the disease from 5 to 9 weeks in sedentary dystrophic animals, the data are difficult to interpret because of an increase in the percentage of muscle fibers belonging to State 2, while the percentage of normal muscle fibers remains statistically unchanged. Although no significant differences were noted in the percentages of muscle fibers belonging to States 3 and 4 through the course of the investigation, the percentage of muscle fibers belonging to State 3 had declined from 8.5% to 1.9% and muscle fibers belonging to State 4 had declined from 6.6% to 2.5% from 5 to 9 weeks of age. This decline is probably the result of muscle fibers belonging to States 3 and 4 being removed from the muscle fiber population of the soleus muscle by macrophages. These decreases could mathematically affect the percentage of muscle fibers belonging to States 1 and 2, i.e., it would tend to increase their percentages. This is possibly what has occurred to significantly increase the percentage of State 2 muscle fibers at 9 weeks of age. If this is taken into account, one could interpret the data as a "slow-down" of the rate at which the disease progresses between 5 and 9 weeks of age. In addition, this phenomena may be the cause for the apparent increase in normal muscle fibers between 13 and 17 weeks of age, because of a corresponding decline in the percentage of muscle fibers belonging to States 3 and 4 over the same time span. It is also possible that the increase in the percentage of normal muscle fibers is the result of muscle fiber regeneration. Although quantitative studies have yet to be performed, Homburger (1966a) believes that muscle regeneration can occur extensively in dystrophic hamsters. Howells (1974) has noted that at

20 weeks of age few signs of regeneration are evident, whereas by 45 weeks of age extensive regeneration was visible.

The finding that no significant differences occurred in the percentages of muscle fibers belonging to States 3 and 4 is difficult to interpret in regard to the rate at which the disease progresses. In this investigation, the percentage of muscle fibers belonging to States 3 and 4 rarely exceeded 10%, an exception to this was found at 13 weeks of age where the mean percentage was 23.4% in State 4. This is consistent with Caulfield's (1966) observation that "the number of muscle fibers involved in the acute degenerative process at any one time is extremely low". Quite possibly, the resulting fluctuations may be explained by the interaction between muscle fibers beginning acute degenerative phenomena and/or the loss of muscle fibers from the entire soleus muscle population.

If we assume a rapid progression of the disease from birth to 5 weeks of age, a slower progression from 5 to 9 weeks of age and then a rapid progression from 9 to 17 weeks of age, it is interesting to speculate on the possibility of a phasic phenomenon of muscle fiber involvement. However, the author does acknowledge that the literature and prevailing opinion do not support such a hypothesis.

In the comparison of normal sedentary and dystrophic sedentary animals, and normal forced-swum and dystrophic forced-swum animals, the significant differences in the percentage of normal and pathological muscle fiber states between groups were found to be dependent on animal genotype, i.e., normal or dystrophic animals. However, in cases where a significant interaction was noted, animal genotype and the age of the

animal or the period of time involved in an exercise regimen influenced the percentage of normal or pathological muscle fiber states. In addition, the age of the animal or the period of time involved in an exercise regimen influenced the percentage of muscle fibers belonging to normal or pathological muscle fiber states in both groups of dystrophic animals. This effect is probably the reason why significant interactions were noted.

In comparing sedentary and forced-swum dystrophic hamsters, the data indicate that there was an initial acceleration of the degenerative processes in the forced-swimming dystrophic animals. This conclusion follows from the fact that at 4 weeks forced-swimming dystrophic animals had a significantly higher percentage of muscle fibers demonstrating centrally located myonuclei and a lower percentage of normal muscle fibers than sedentary dystrophic animals. However, it should be noted that no statistical differences were detected in the percentage of normal or State 2 muscle fibers at 8 and 12 weeks between sedentary and forced-swimming dystrophic animals. One may conclude from this that after an initial acceleration of degenerative phenomena in forced-swimming dystrophic soleus muscle a beneficial role of swimming may be assigned. In forced-swimming dystrophic animals, the leveling off of the percentage of normal muscle fibers may be due to the occurrence of muscle regeneration in dystrophic muscle. However, the reason for this leveling off remains obscure. Again, the finding of no significant differences in muscle fiber States 3 and 4 is probably the combined interaction of diseased muscle fibers evolving to later stages of disease

and/or the loss of muscle fibers from the entire soleus muscle fiber population.

It is interesting to speculate on the cause(s) which is (are) responsible for the apparent acceleration of degenerative processes in forced-swum dystrophic animals. In addition, alternate interpretations of the data are given below. Membrane defects in dystrophic animals, selective atrophy of specific muscle fiber types and muscle fiber splitting are discussed in reference to the above.

Currently, the breakdown of membrane systems in dystrophic muscle, e.g., specifically the sarcolemma, sarcotubular system and mitochondrial membranes, has gained considerable attention and momentum as the possible etiology of muscular dystrophy.

Dhalla and co-workers (1975) have approached the membrane defect theory by biochemical techniques in dystrophic hamsters. They have reported that mitochondrial calcium uptake and mitochondrial phosphorylation and respiratory rates were significantly lower in hind-leg muscle of 60-day-old dystrophic hamsters. In addition, sarcolemmal Ca^{++}-ATPase activity was found to be higher in the hind-leg muscle of 60-day-old dystrophic hamsters. Furthermore, dystrophic hamsters, that were 150 days old, had higher Ca^{++}-ATPase, Mg^{++}-ATPase, and Na^{+}-ATPase activities than normal control values.

Another study complementing the work by Dhalla was performed by Wrogemann and his co-workers (1979) which demonstrated an uncoupling of oxidative phosphorylation in skeletal muscle mitochondria of dystrophic hamsters. They have shown that mitochondria have an increased Ca^{++} concentration and believe this is responsible for the uncoupling of

oxidative phosphorylation. In addition, these mitochondria could easily be isolated from necrotic areas that have a higher order of magnitude of intracellular Ca^{++} concentration than normal values. Interestingly, these workers have set forth a "Calcium Overloading Hypothesis" which may possibly account for the myopathic lesions encountered in dystrophic hamsters. They have presented their hypothesis as follows:

> According to this model, the source of excess mitochondrial calcium is outside the cell, where calcium levels are from two to four orders of magnitude higher than in the cytoplasm. A defective plasma membrane allows for an increased net influx of this ion, which is largely taken up by mitochondria in fulfillment of their role in the maintenance of intracellular calcium homeostasis. The gradual overloading of these organelles with calcium leads to a vicious cycle of calcium overloading and energy depletion, which immediately precedes cellular death. When the compensatory mechanisms involved in maintaining low intracellular calcium levels are exhausted, the free calcium concentration rises. The consequences are hypercontraction of segments of muscle fibers and dissolution of Z-line material by proteases that require millimolar concentrations of calcium. Indeed, both hypercontraction and Z-line dissolution are considered early signs of nascent cellular necrosis.

Ward and co-workers (1979) have studied the sarcolemma in dystrophic hamster skeletal muscle by electrophysiological techniques. Ward has demonstrated that the mean resting membrane potential of a dystrophic muscle fiber is lower than that of normal control values. They have suggested that this may be the result of increased K^{+} permeability which would establish a lower intracellular K^{+} concentration in dystrophic hamster muscle. The authors suggest that this probably is the result of leaks (breakdown) in the sarcolemma. An alternate hypothesis suggested was that an increase in Na^{+} membrane permeability was responsible in lowering the resting membrane potential. In addition, this author reasons that it is equally plausible that both an

increase in Na^+ and K^+ membrane permeabilities may occur in dystrophic hamster muscle.

By morphological quantitation, Morki and Engel (1975) have approached the hypothesis that defects in membrane systems play a role in the pathogenesis of muscular dystrophy. In two cases of Duchenne dystrophy, they have reported that an ingress of horseradish peroxidase into the interior of a muscle fiber occurs at a significantly higher frequency than in control muscle, which may indicate a breakdown of the sarcolemma and increased membrane permeabilities. These authors suggest that this defect in the plasma membrane is an early one, but quickly add that this defect could be a secondary effect to a biochemical abnormality located elsewhere in a muscle fiber.

In interpreting the data, forced-swimming may have applied a stress too strenuous for dystrophic muscle to adapt to, leading to an acceleration of degenerative phenomena. What mechanism could possibly be responsible for stress accelerating the degeneration of "dystrophic" muscle fibers? Unfortunately, there are few studies concerned with degenerative processes in normal muscle fibers induced by swimming (exercise). However, the literature provides information on how muscle fibers adapt to an increased work load. In muscle fibers that have undergone compensatory hypertrophy, it is postulated that these muscle fibers take on the extra work load required for normal muscle function. In addition, muscle fiber splitting (hyperplasia) has been shown to occur in the soleus muscle of normal rats which swam twice daily with 4% body weight attached (Edgerton, 1969a). Ho (1979, personal communication) has reported that muscle fiber splitting occurs in the

adductor longus muscle of male albino rats involved in a weight-lifting exercise regimen. Once mature, these new muscle fibers would enable the muscle to perform adequately to the increased work load.

The investigations by Dhalla, Wrogemann, and Ward have provided evidence which demonstrates that membrane systems that are either directly or indirectly involved in muscle contraction and relaxation are defective in dystrophic muscle fibers. Therefore, as Wrogemann has proposed in his "Calcium Overloading Hypothesis", it seems plausible that energy pools in dystrophic muscle fibers would be diverted to maintaining homeostasis, particularly electrolyte balance, in a dystrophic muscle fiber. Parallelling this, one can logically assume that an increased energy demand would be placed on fibers of the soleus muscle in dystrophic hamsters in meeting the additional energy requirements for muscle contraction. In turn, this would draw energy away from energy pools available for reestablishing and maintaining homeostasis. Therefore, it may be that diseased muscle is less adaptable to stress resulting in degenerative processes similar to those of dystrophic muscle. What is the morphology of the muscle fibers involved in this model? These muscle fibers could resemble morphologically normal muscle fibers, but, nevertheless, fibers that have begun dystrophic changes, e.g., breakdown of the sarcolemma, and as yet show no visible myopathical lesions. In time, these muscle fibers would show the myonuclei in a central position. This could account for the increase in muscle fibers showing central location of myonuclei in forced-swum dystrophic hamsters after 4 weeks (9 weeks old) of forced swimming.

One may attempt to explain the initial acceleration of degenerative processes in forced-swum dystrophic hamsters by the alteration of muscle fibers to a particular fiber type that undergoes degenerative processes early in the course of the disease.

The atrophy of specific muscle fiber types has been investigated by Johnson and Pearse (1971) in the gastrocnemius muscle of dystrophic Syrian hamsters and their findings are summarized below. Of the three muscle fiber types found in the gastrocnemius muscle, Type II (high glycolytic) muscle fibers undergo the most pronounced atrophy in the early stages of the disease. By 60 days of age, Type II fibers had decreased in diameter from 60 to 33 microns. The Type III fibers showed an increased incidence of atrophy as the disease evolved, but are also capable of becoming hypertrophied. The Type I fibers had the least variation in diameter (atrophy) as compared to fiber Types II and III throughout the course of the disease.

The soleus muscle of normal rats consists primarily of Type I (slow) and Type III (intermediate) muscle fiber types (Edgerton, 1969a). In addition, swimming was reported by Edgerton (1969b) as having no effect in altering the proportion of muscle fibers showing specific fiber types after 52 days of forced-swimming.

In view of the information presented above, it is possibly contradictory to reason that swimming produced altered fiber types, which undergo atrophy earlier in the course of the disease in explaining the initial acceleration of degenerative processes in forced-swum dystrophic hamsters. In fact, it is difficult to say what effect altering muscle fiber type would have on the rate at which the disease progresses.

It may be that the "time" at which degeneration of a particular muscle fiber type occurs is determined during the initial maturation of the muscle fiber and altering muscle fiber type has no bearing on whether a fiber will undergo degenerative processes earlier than expected.

However, Howells (1974) has reported an increase in muscle fiber atrophy in the biceps brachii, extensor digitorum longus, and soleus muscle of dystrophic hamsters when placed on a weight-lifting exercise regimen. This resistive form of exercise is believed to increase the proportion of Type II fibers. Thus, this and the work by Johnson (fiber type) may account for Howells' conclusion that a weight-lifting exercise regimen accelerates the degenerative processes in dystrophic Syrian hamsters.

Finally, an explanation is given which may account for the initial increase in the percentage of muscle fibers showing atrophy and/or central location of myonuclei (State 2) at 4 weeks in the forced-swimming dystrophic hamsters, which significantly differed from muscle in sedentary dystrophic animals.

As mentioned earlier, muscle fiber splitting was reported to occur in the soleus muscle of normal rats involved in a forced-swimming program and in the adductor longus muscle of male albino rats (weight-lifting). As is most often the case, the morphology of a splitting muscle fiber has its myonuclei centrally located within the muscle fiber (Ho, 1979, personal communication). In that this would produce an increase in the number of muscle fibers resembling fibers of State 2, it is conceivable that the increase in the percentage of State 2 muscle fibers is the result of muscle fiber splitting. However, it is not

known unequivocally whether or not the intensity of exercise used in this investigation was strenuous enough to produce muscle fiber splitting. A question which should be raised at this point is: Why would muscle fiber splitting occur in the soleus muscle of forced-swum dystrophic hamsters, while the data regarding high-intensity forced-swum normal animals does not indicate that muscle fiber splitting occurred (i.e., there was no increase in the percentage of State 2 muscle fibers)? It is believed that an exercise stimulus must be of sufficient intensity to promote muscle fiber splitting. Therefore, even though muscle fiber splitting did not occur in normal animals under a "high-intensity" forced-swimming program, it may be that the "moderate-intensity" employed in the forced-swimming dystrophic animals was of sufficient intensity to induce the dystrophic soleus muscle to undergo increased muscle fiber splitting. It has been reported that muscle fiber splitting is a common feature in myopathic disorders (Schmalbruch, 1976 and Schwartz, 1976).

If muscle fiber splitting does occur in forced-swum dystrophic hamsters, it could modify our interpretation of the data. We could possibly conclude that swimming has no effect in accelerating or retarding the progression of the disease in the soleus muscle of dystrophic Syrian hamsters. Furthermore, it remains to be demonstrated quantitatively to what degree muscle fiber splitting occurs in the soleus muscle of dystrophic hamsters either through age or involvement in a forced-swimming program.

CONCLUSION

In sedentary normal hamsters, fibers of the soleus muscle rarely demonstrated myopathical lesions typically found in dystrophic hamster muscle. In addition, a high-intensity program of forced-swimming was found not to be detrimental to fibers of the soleus muscle of normal animals. It was concluded that a continuous progression of the disease existed in the soleus muscle of sedentary dystrophic hamsters. This was based on alterations in the percentage of normal and pathological muscle fiber states throughout the ages of the animals. Furthermore, alternate interpretations of the data were discussed in regard to the influence which fluctuations in the percentages of muscle fiber States 3 and 4 may have had on the percentages of normal muscle fibers and muscle fibers with a centrally located myonuclei and/or a reduction in fiber diameter. Speculation on a phasic phenomena of muscle fiber involvement was presented.

In the comparison of sedentary normal and sedentary dystrophic animals and forced-swum normal and forced-swum dystrophic hamsters, animal genotype and the age of the animal or the period of time invested in an exercise regimen were discussed in regard to the effect these variables had in determining the percentage of normal and pathological muscle fiber states. It was suggested that animal genotype was primarily responsible for differences noted between these animal groupings.

The data appear to indicate that a moderate-intensity program of forced-swimming causes an initial acceleration of degenerative processes in the soleus muscle of dystrophic hamsters. The possible cause(s) of this acceleration was discussed in reference to the membrane defect theory of muscular dystrophy and the adaptability of dystrophic muscle to stress. Furthermore, selective atrophy of specific muscle fiber types and muscle fiber splitting were topics surveyed as alternate explanations that may possibly explain for the initial acceleration of degenerative processes in the soleus muscle of dystrophic hamsters.

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