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HISTOCHEMISTRY AND PHYSIOLOGY OF SKELETAL MUSCLE IN EXERCISE-TRAINED HAMSTERS

presented by

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has been accepted towards fulfillment
of the requirements for

M.A. degree in Physical Education

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Date Aug. 7, 1979

HISTOCHEMISTRY AND PHYSIOLOGY OF SKELETAL MUSCLE IN EXERCISE-TRAINED HAMSTERS

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A THESIS

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

MASTER OF ARTS

Department of Health, Physical Education and Recreation

1979

ABSTRACT

HISTOCHEMISTRY AND PHYSIOLOGY OF SKELETAL MUSCLE IN EXERCISE-TRAINED HAMSTERS

By

Ann Catherine Snyder

The purpose of this study was to determine the effects of aging and physical training on selected characteristics of normal hamster muscle. Twenty-seven animals were assigned to one of three groups: (a) zero-week control, (b) 12-week sedentary control, and (c) 12-week high-intensity endurance swimming.

The right leg soleus (SOL) and rectus femoris (RF) muscles were examined histochemically. Muscle fiber profiles and respective fiber sizes were determined. Corresponding muscles from the left leg were examined physiologically. Muscle strength, speed, rate of relaxation and fatiguability were determined.

The percentage of "fast" and "slow" fibers did not change in the central area of the SOL muscle or in the superficial area of the RF muscle. In the deep area of the RF muscle, however, the percentage of "fast" fibers was altered by both aging and training. Physiologically most characteristics were found to be affected by aging, but not altered by training.

This study is dedicated to my entire family, each and everyone of them, including the "fourth daughter", for their everlasting support and encouragement which have made it all possible.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to those people who gave of their time and expertise throughout the course of this study. Special thanks go to Dr. William Heusner, my advisor, whose interest in both me and my work are deeply appreciated. Thanks also go to Mr. Robert Wells who not only had the expertise to develop the physiological characteristics data collecting system used in this study, but also displayed neverending patience in explaining its operation to me. Thanks also go to Dr. Rexford Carrow who not only opened his laboratory to me but initiated my enthusiasm into the study of muscle. I would also like to thank Mrs. Barbara Wheaton for her time in teaching me the histochemical and histological techniques involved in the study of muscle.

A very special thank you goes to Kevin Scribner and Sandy Herman for their neverending assistance and stimulating conversations, both of which contributed greatly in the overall preparation of this thesis. The technical assistance of Dee Jakubiak and Joie Van Huss is also deeply appreciated, as is the assistance of Dr. Wayne Van Huss and Bonnie Smoak.

Thanks also go to Dr. Joseph Vorro for the use of his electronic digitizer which made possible the measuring of the muscle fiber areas.

A final thank you goes to all those people who were not only patient but also understanding throughout the preparation of this thesis.

TABLE OF CONTENTS

Page

APPENDICES

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

THE PROBLEM

Histochemically, biochemically and physiologically muscle fibers have been categorized into three basic types. Different schemes have been used to describe these types; however, the nomenclature of slow-twitch-oxidative (SO), fast-twitch-oxidative-glycolytic (FOG), and fast-twitch-glycolytic (FG) as devised by Peter et al. (276) has become widely used. The SO fiber type is slow contracting and has high oxidative capabilities. The FOG and FG fibers are both fast contracting. The FOG fibers have high oxidative and glycolytic capacities, while the FG fibers have only high glycolytic capacity.

The properties of muscle fibers have been found to change with development (52,57,82,83,90,91,130,177,178,184,204,205,229,234,242,316), immobilization (37,38,123,145,239,240), cross-innervation (56,59,61,174, 233,289,292,293), denervation (113,176,216,283), and electrical stimulation (2,21,33,53,63,94,149,175,181,227,270,277,278). Similar changes have been observed in the muscles of physically trained humans (3,27,76,92,93,95,160,161,216,280,321) and animals (13,41,114,115,119, 121,142,143,195,244,295,315,317). The degree of adaptation with physical training appears to vary with different species and exercise regimens (171,194,195,295,306,317). In particular, endurance-type training programs have been found to increase the aerobic capabilities of muscle fibers (3,27,92,93,95,128,160,216,321). A change in the

percentage of fiber types, toward more oxidative fibers, also has been observed with this type of training (76,92,93,160).

Purpose of the Study

The overall purpose of this investigation was to determine the effects of growth and development and physical training on selected physiological and histochemical characteristics of normal hamster soleus (SOL) and rectus femoris (RF) muscles. The animals were trained with a high-intensity endurance swimming program for 12 weeks. Muscle fibers from the right hindlimb were examined histochemically for muscle fiber type and size. Physiological characteristics, including data on both twitch and tetanic contractions, were determined for comparable muscles from the left hindlimb. Zero-week (7 weeks of age) and 12-week (19 weeks of age) sedentary animals served as pre- and post-training controls.

Need for the Study

This study was designed to fill the void which exists in the scientific literature concerning the histochemical and physiological characteristics of hamster muscle. Because descriptive work utilizing hamsters with muscular dystrophy (4,19,39,187,213,250,241,253,254,259, 260,327) is being carried out, comparative data on normal hamster muscle is necessary. Research also is being done on the effects of physical activity in dystrophic hamsters (39), and thus training data in unaffected animals is prerequisite to an understanding of the physiological adaptations that may occur under these conditions. In addition, the relationship between the physiological characteristics and the muscle fiber metabolic characteristics have rarely been studied

in hamster muscle (154). The effects of physical training on these parameters have never been determined.

Research Hypotheses

The main hypothesis of this study was that normal hamster muscle will adapt to a physical training program of swimming. Within this hypothesis are three subhypotheses:

1. Skeletal muscle fibers of hamsters will not change in size but will develop more aerobic metabolic capacity through an endurance swimming regimen.
2. Hamster muscle will become slower contracting and more resistant to fatigue through an endurance swimming regimen.
3. Physiological and metabolic characteristics of hamster muscle are related in both sedentary and trained animals.

Research Plan

Twenty-seven normal Syrian hamsters were used in this study. The animals were assigned randomly to one of the following groups: (a) zero-week control (n = 8), (b) 12-week sedentary control (n = 11), and (c) 12-week high-intensity endurance swimming (n = 8). The left and right SOL and RF muscles were evaluated.

Muscle fiber profiles and respective fiber sizes were determined using 30 adjacent fibers from the center of the SOL muscle and from the superficial and deep areas of the right RF muscle. The histochemical profiles were determined using the following stains: (a) adenosine triphosphatase at a pH of 9.4 (ATPase) as an indicator of contractile elements, (b) reduced nicotinamide adenine dinucleotide-diaphorase (NADH) for the demonstration of oxidative capacity, (c) α-glycero-phosphate dehydrogenase (αGPD), as an indicator of aerobic glycolysis,

(d) Sudan black B (SUD) for lipid storage, and (e) periodic acid-Schiff reaction (PAS) for glycogen storage.

Corresponding muscles from the left leg were used to evaluate selected physiological characteristics. Muscle strength, speed, rate of relaxation, and fatiguability were determined for both the SOL and RF muscles. The strength measures were corrected for body and muscle weight.

Limitations of the Research Plan

1. The results of this study can be applied only to normal hamsters.
2. Evaluation of the effects of the training program is limited by a lack of ability to determine the intensity of muscular activity when laboratory animals are subjected to a regimen of forced swimming.
3. The limited number of histochemical and physiological characteristics that could be determined do not provide a complete analysis of the metabolic adaptation of hamster muscle to physical training.
4. Due to the complexity of the sacrifice, some muscles were necessarily exposed to air for periods of up to four minutes between the time of removal of the tissue and the time of rapid freezing.
5. Due to the time required to obtain the physiological data on each muscle, the left SOL muscle was placed in Ringer's solution for approximately 15 minutes while the RF muscle was being tested.

CHAPTER II

RELATED LITERATURE

The purpose of this investigation was to determine the effects of physical training on the physiological and histochemical characteristics of normal hamster muscle. The literature related to this investigation will be reviewed in three main sections: (a) characteristics of muscle, (b) development of muscle, and (c) adaptation of muscle to physical training.

Characteristics of Muscle.

Muscle has been studied histochemically, biochemically, and physiologically. Thus, this section is subdivided to permit a separate examination of the literature resulting from each of these methods of investigation.

Characteristics of Muscle Determined Histochemically

Fiber types and sizes have been determined from the histochemical study of muscle. Therefore, this segment of the discussion will cover both entities.

Types of Muscle Fibers

The fact that there are different types of muscles has been known for many years. Ranvier (286) was cited by Kronecker and Stirling (226) in 1880 as one of the first to observe that there are "red" and "white"

muscles. Throughout the last 100 years, a great quantity of research has taken place involving muscles and muscle fibers. Histochemical techniques have revealed distinct metabolic characteristics for each of the fiber types (125). Furthermore, longitudinal sections of muscle fibers have shown that fibers are uniform throughout their entire length (106,107). This observation makes histochemistry appear to be an appropriate method for determining muscle fiber type.

Confusion has arisen due to inconsistencies within the literature concerning the nomenclature used to describe the various muscle fiber types. Table 1 contains a summary of the terms used to type muscle fibers.

Not only has the variation in nomenclature caused researchers difficulty, but the total number of fiber types has been widely disputed. Engel (134,135) and others (31,34,36,46,92,127,129,156,162,163,164,165, 174,188,210,211,215,225,228,234,271,288,289,300,304,312) contend that there are only two fiber types, while Brooke and coworkers (48,49) and many others (3,5,6,7,8,9,13,14,15,16,18,23,25,28,37,38,40,55,98,114,116, 118,121,122,137,138,140,150,151,160,166,167,178,180,183,208,212,213,214, 221,222,223,224,227,231,232,239,244,249,255,264,267,273,274,275,276,280, 282,283,291,295,308,311,313,322,330) have shown the existence of three fiber types. Observations by a few investigators (105,119,182,209,218, 224,257,258,290,296,307) have revealed as many as eight distinct types.

Since most researchers have agreed that three basic fiber types exist, that categorization will be adopted for the purpose of this review whenever practical. Table 2 contains a summary of the characteristics of the mammalian muscle fiber types using histochemical techniques. The nomenclature introduced by Peter et al. (276) has been used.

Table 1

Nomenclature of Muscle Fiber Types

SO	FOG	FG	5,6,121,124,137, 151,166,167,239, 276,280,295
I	IIA	IIB	3,48,49,98,138,231, 232,282
I	II		31,46,127,134,135, 156,188,210,211, 215,228,271,288, 289,300
III	II	I	291
RED	WHITE		36,225,234,304
INT	RED	WHITE	13,14,16,23,25,28, 37,114,116,118,140, 150,255,264,267,283
INT	I	II	249,313,322
SO	FO	F	38
C	B	A	55,227,330
I RED	II RED	II WHITE	222,223,224
SLOW TWITCH RED	FAST TWITCH RED	FAST TWITCH WH	18,40,122,160,172, 274,275
βR	αR	αW	7,8,9
β	α		174
F,G&H	D&E	A,B&C	290
I	III	II	213,214
I	INT	II	208
INT, MOD	RED	WHITE	119,258
SLOW TWITCH	FAST TWITCH		34,98,129,162,163, 164,165,312
RED	INT	WHITE	212
INT	RED TYPE I OXIDATIVE	WHITE TYPE II GLYCOLYTIC	221

Table 1 (continued)

Dm	Ld	Lm	Ll	257,258
ST	FTH		FT	180,308
HIGH OXIDATIVE SLOW (HS)	HIGH OXIDATIVE FAST (HF)		LOW OXIDATIVE FAST (LF)	244
S	FR		FF	183
SLOW TWITCH INT	FAST TWITCH RED		FAST TWITCH WHITE	25,273
SLOW TWITCH RED	FAST TWITCH INT		FAST TWITCH WHITE	25
B	C		A	311
SLOW TWITCH MOD. OXIDATIVE INT	FAST TWITCH HIGH OXIDATIVE RED		FAST TWITCH LOW OXIDATIVE WHITE	15
SLOW TWITCH OXIDATIVE	FAST TWITCH OXIDATIVE GLYCOLYTIC		FAST TWITCH GLYCOLYTIC	121

Table 2

Mammalian Muscle Fiber Characteristics Determined Histochemically

	SO	FOG	FG	Subjects	References
ATPase alkaline	L[a]	D[b]	D	Human, Rat, Cat, Hamster, Guinea Pig, Mouse, Bovine, Porcine, Rabbit, Pig, Horse	8,25,31,38,44, 49,69,70,72,74, 114,127,134,135, 140,167,180,210, 213,222,223,228, 235,239,244,248, 273,276,280,282, 301,311
ATPase acidic	D	L	L	Human, Rat, Cat, Pig	31,46,49,69,135, 228,280
Myosin ATPase	alkali-labile acid-stabile	alkali-stabile acid-labile	alkali-stabile acid-labile	Rat, Pig	77,223,330
ATPase	L	D	I[c]	Hamster	214
EDTA modified myosin ATPase	D	L	L	Human, Cat	70,288
SR-ATPase	L	D	D	Rabbit	222
AC-ATPase	D	L	I	Cat	70,74
UDPG-Glycogen Transferase	L		D	Human	134,135
PPL	L	D	D	Monkey, Human, Rat, Cat, Horse, Guinea Pig, Pig	31,36,116,127, 134,135,223,270, 279,304

Table 2 (continued)

	SO	FOG	FG	Subjects	References
PPL	L	L-I	D	Rat, Hamster, Mouse, Bovine, Porcine, Cat, Rabbit	8,208,213,214, 221,227,232,261
PPL	I	L	D	Pig	255
PAS	L	D	D	Cat, Guinea Pig, Horse	69,135,150,167, 276,308
PAS	L-I	L-I	I-D	Rat	232
αGPD	L	D	D	Cat, Human	70,134,135,161, 223,232,239,276
αGPD	L	I	D	Rat, Cat, Guinea Pig	114,116,273,301
αGPD	I	D	L	Mouse	264
Glycolytic	L	D	D	Cat, Human, Guinea Pig	74,127,267,322
Triosephosphate dehydrogenase	L	L	D	Rat	263
Glycerol-3-phosphate	L	I	I	Rabbit	235
LDH	L	I	D	Rat, Human, Cat, Guinea Pig, Rabbit	105,134,135,166, 263,276,301
LDH	I	I-D	L	Mouse	264
LDH	L	D	D	Cat	70

Table 2 (continued)

	SO	FOG	FG	Subjects	References
Esterase	D	I	L	Human, Rat	134,227
Esterase	L	D	D	Cat	70
Glutamic dehydrogenase	none	L	none	Mouse	264
Oxidative	I-D	D	L	Rat, Human	127,267,290
Oxidative	D	I	L	Human	48
SDH	D	I-D	L	Rat, Rabbit, Human, Monkey, Cat, Mouse, Bovine, Porcine, Horse, Guinea Pig, Pig	8,36,70,72,105, 134,135,138,180, 208,212,222,223, 227,244,280,291, 299,304,323,330
SDH	I	D	L	Rat, Rabbit, Guinea Pig	114,116,140,221, 235,263,264,273, 282,311
SDH	L	D	D	Rat	228
MDH	I	D	L	Rat	114,116,223,264, 276
NADH	L-I	D	L	Rat, Pig	38,49,114,116, 223,232,255,276
NADH	D	I-D	L	Human, Cat, Hamster, Horse, Pig	31,49,69,70,72, 105,134,135,167, 210,213,214,239

Table 2 (continued)

	SO	FOG	FG	Subjects	References
NADH	L	D	D	Rat	228
Cytochrome Oxidase	I-D	D	L	Rat, Rabbit, Human	134,135,223,235, 291
Glycogen Utilization	L	I	D	Rat	290
Lipid Metabolism	D	I	L	Human, Rat	127,290
OIL RED O	D	L	L	Human, Cat	70,134,135
SUD	D	I	L	Cat	212
SUD	D	D	L	Horse	308
GOM	I	D	L	Rat	114
Myoglobin	D	I-D	L	Mouse, Rat, Guinea Pig, Cat, Rabbit, Man	207,208,290

[a] L indicates a low staining intensity

[b] D indicates a high staining intensity

[c] I indicates a moderate staining intensity

The SO fiber is characterized by intermediate to high oxidative capability (as determined by succinic dehydrogenase--SDH, NADH, and malic dehydrogenase--MDH), low aerobic glycolytic activity (αGPD) and a slow contraction time (ATPase). Glycogenolytic (phosphorylase--PPL) activity and lactate formation (lactate dehydrogenase--LDH) are both low. Lipid storage as determined by SUD and Oil Red O is high in these fibers, while glycogen storage (PAS) has been found to be low.

The FOG muscle fiber has extensive metabolic capabilities as indicated by its intermediate to high oxidative, glycogenolytic and aerobic glycolytic activities. The FOG muscle fiber also has a fast time of contraction. Glycogen storage is high in this fiber, and lactate formation is intermediate to high. Lipid storage is intermediate.

The FG muscle fiber also is fast contracting and high in glycogenolytic activity, lactate formation and aerobic glycolytic activity. However, unlike the FOG fiber, the FG fiber is very low in oxidative enzyme activity as well as in lipid storage. Glycogen storage in the FG muscle fiber is high.

The mitochondrial content of the FOG fiber is high (273) which has been distinguished by the abundance of coarse diformazan granules seen with an NADH stain (7,25). The SO fiber has an intermediate content of mitochondria and thus uniformly dispersed diformazan granules. The FG fiber has low mitochondrial concentration and few diformazan granules.

Although the information given in Table 2 appears to be fairly consistent, many stains reappear in order to accommodate the varying staining patterns observed by different investigators. There appear to be several reasons why all muscle fibers can not unquestionably be categorized within one of the three main groups: (a) muscle fibers

from different species appear to be different, (b) muscle fibers from different locations within the same species have been found to be different, and (c) muscle fibers may actually be a continuum.

Guth and Yellin (173) and Yellin and Guth (330) concluded that muscle fiber types between species are different. Their work dealt with rats, cats, mice, guinea pigs, and rabbits. All muscles examined revealed somewhat similar fiber types; however, the intensity of the histochemical staining was different between the muscles. Engel and Irwin (133) and Sarnat et al. (298) found the following three types of frog muscle fibers: (a) a type of fiber that is high in oxidative capacity, glycogenolytic activity, and glycogen storage, (b) a second fiber type that is intermediate in the aforementioned categories, and (c) a third fiber type that is low in all three. These amphibious fiber types are all different from those reflected in Table 2.

Brooke and Kaiser (50) found that in the human biceps brachii the TYPE IIA fiber is an intermediate muscle fiber, while the TYPE IIA fiber of the rat gastrocnemius muscle is a red fiber when the same classifying systems are used to determine the muscle fiber types. Peter (273), aware of this discrepancy in the oxidative capacity between human and animal muscle fibers, suggested a terminology of fast-twitch white, fast-twitch intermediate and slow-twitch red for human muscle while using fast-twitch white, fast-twitch red and slow-twitch intermediate for animal muscle.

In studies involving rats and cats, the intensity of staining was different between two muscles of the same animal even though the fibers were classified as the same type (74,99,227,263). These studies used a variety of histochemical stains to compare the soleus with the

gastrocnemius (74,99), the rectus femoris (263), and the anterior tibial muscle (227).

A number of investigators (114,161,235,273,296) have proposed the existence of a continuous spectrum of muscle fibers. This point is supported by the studies within a single muscle of the same species in which some of the muscle fibers could not be classified as belonging to one of the three basic fiber types (105,119,182,209,218,224,228,257,258, 296,307). Some of these investigators (105,119,182,209,218,257,258,307) distinguished four fiber types, while Khan (224) found seven and Romanul (290) eight.

Muscle fiber types have been found to be distributed through a muscle (13,29,129,137,235,282,307). In many muscles the superficial area is composed of predominately FG fibers, while the deeper areas are primarily SO and FOG fibers (13,29,139,307). This observation applies to both human (129) and animal (13,29,137,235,282,307) subjects. Muscles from within and between species are composed of different percentages of fiber types. Table 3 summarizes the various percentages of muscle fiber types obtained by several investigators.

Even though variations have been found between different muscles, Karpati and Engel (217) found a nonsignificant difference when comparing the proportions of fiber types in right and left leg muscles of guinea pigs. The muscles examined were the gastrocnemius, plantaris and soleus. The largest difference was 4% which was found in the small head of the gastrocnemius.

Sizes of Muscle Fibers

The size of individual muscle fibers has been investigated by numerous researchers (47,48,69,72,116,210,211,212,214,227,229,241,269,

Table 3

Fiber Type Composition of Commonly Studied Muscles

Muscle	SO	FOG	FG	References
Soleus				
Human	64%	17%	19%	124
Human	89	7	4	6
Cat	100			5
Guinea Pig	100			5,25,273,276,289
Bushbaby	87	13		5
Slow Loris	72	7	21	5
Rabbit	96			276
Mouse	50	50		8
Biceps Brachii				
Human	25	32	43	55
Lateral Triceps				
Human	2	48	50	55
Gastrocnemius				
Human	52	16	31	127
Human	82	8	10	55
Medial Gastrocnemius				
Guinea Pig	12	50	38	25,273
Guinea Pig	22	54	24	5
Rat	4	58	38	5
Cat	25	61	14	5
Lateral Gastrocnemius				
Guinea Pig	12	56	32	5
Rat	5	58	37	5
Cat	18	66	16	5
Anterior Tibial				
Human	46	35	19	6
Rectus Femoris				
Guinea Pig	6	43	51	5
Rat	4	54	42	5
Cat	22	17	61	5
Bushbaby	8	48	44	5
Slow Loris	50	12	38	5
Adductor Muscles				
Rat	50	25	25	28
Vastus Lateralis				
Human	46	20	34	124
Vastus Intermedius				
Human	52	15	33	124

Table 3 (continued)

	SO	FOG	FG	References
Red Vastus				
Guinea Pig	4%	78%	18%	25,273,276
Flexor Hallicus Longus				
Guinea Pig	17	10	73	25,273
Flexor Digitorum Longus				
Guinea Pig	11	35	54	25,273
Guinea Pig	14	56	30	5
Rat	8	37	55	5
Cat	7	61	32	5
Bushbaby	7	38	54	5
Extensor Digitorum Longus				
Guinea Pig	6	57	37	5
Rat	3	38	59	5
Cat	14	55	31	5
Bushbaby	2	45	53	5
Plantaris				
Guinea Pig	6	73	23	5
Rat	6	41	53	5
Cat	26	46	28	5
Bushbaby	19	56	30	5

279,288,299,323). The studies on animals have shown that the three fiber types have different sizes (69,72,116,212,214,227,229,241,299,323). Johnson and Pearse (214) reported only that SO and FOG fibers are significantly smaller than FG fibers. Kugelburg (229) found the FG fibers to be largest, SO fibers intermediate, and FOG fibers the smallest. Most authors, however, have found the FG fibers to be largest, FOG fibers intermediate and the SO fibers the smallest (69,72,116,212,227, 241,299,323).

The results obtained from human data are not as clear as those from animals. Reniers et al. (288) and Polgar et al. (269) found FOG and FG (TYPE II) fibers to be larger than SO (TYPE I) fibers, while Jennekens et al. (211) found FOG and FG (TYPE II) fibers to be inconsistently different from SO (TYPE I) fibers. Polgar et al. (279) also found a nonsignificant difference between the sizes of SO, FOG and FG fibers in the soleus. Jennekens et al. (210) reported that in the biceps brachii and deltoid muscles FOG and FG (TYPE II) fibers were the largest, whereas in the rectus femoris and gastrocnemius muscles SO (TYPE I) fibers were the largest. Brooke and Engel (47) found FOG and FG (TYPE II) fibers to be larger than SO (TYPE I) fibers in males, but the converse was true for females. Brooke and Kaiser (48) in concurrence with this observation found the SO (TYPE I) fibers for males and females to be approximately equal in size, while the females' FOG and FG (TYPE II) fibers were much smaller than the males'.

Various factors such as the age (47), weight (47), and physical build (279) of the subject appear to have no significant effect on muscle fiber size once the growth period has ceased. However, Aherne et al. (1) found a trend of increasing fiber size with increasing body size.

Goldspink (152), looking at mice, and Vincelette and Jasmin (323), studying rats, found fibers of the same size to be approximately equal in enzymatic activity; whereas Hammarberg (182), in identifying four types of muscle fibers in cats, found a considerable overlap of fiber sizes between the types. In pig longissimus dorsi and trapezius muscles, Moody and Cassens (255) found little difference in muscle fiber sizes from one muscle to another.

The relationship in animal muscle fibers is linear between fiber diameter an myofibrillar number (152). A negative relationship exists between myofibrillar free sarcoplasm and muscle fiber size (152). Dulhunty et al. (111) found a significant correlation between the total capacity of the surface membrane and fiber size as well as between the total conductance of the surface membrane and fiber size.

Characteristics of Muscle Determined Biochemically

Until recently the biochemical evaluation of single muscle fibers was not possible. Therefore, most biochemists have used muscles which were homogeneous or fairly homogeneous as to fiber types. Tables 4 and 5 summarize the data on muscle which has been collected biochemically. Table 4 consists of the data obtained on muscles which were described as being "red" or "white". Table 5 consists of the data obtained on muscles which were characterized as containing predominantly SO, FOG or FG fiber types.

The biochemical analyses generally substatiate the histochemical data. Muscle containing predominantly SO fibers is highly oxidative (MDH, SDH, isocitrate dehydrogenase--IDH, cytochrome oxidase--CO), slow contracting (actomyosin ATPase, myosin ATPase, K^+, Na^+), low in glycolytic enzymes (phosphofructokinase--PFK, pyruvate kinase--PK) and

Table 4

Biochemical Evaluation of "Red" and "White" Muscle

	"RED"	"WHITE"	Subjects	References
Myosin ATPase	Low	High	Hamster, Guinea Pig	25, 154
Ca^{2+} ATPase	Low	High	Rabbit	20
ITPase	Low	High	Rabbit	20
EDTA-ATPase	Low	High	Rabbit	20
Actomyosin	Low	High	Rat, Guinea Pig	18, 25
AM-ATPase	Low	High	Rabbit	20
Total Creatine	Low	High	Hamster	154
Calcium Uptake	Low	High	Cat, Rat	43
Calcium Oxalate	Low	High	Cat, Rat	43
Sarcoplasmic Proteins	Low	High	Rabbit	20
Stroma Proteins	High	Low	Rabbit	20
Total PPL	Low	High	Human, Rat, Monkey, Rabbit	26, 36, 188
Active PPL	Low	High	Rat, Monkey	36
Glycogen	Low	High	Rat	28
Lactate Production	Low	High	Rat	28
Specific Activity of Lactate	High	Low	Rat	28
Total LDH	Low	High	Rat, Rabbit	26, 160
Oxidative Capacity	High	Low	Rat	13
Triosephosphate Dehydrogenase	Low	High	Rabbit	26
αGPD	Low	High	Rabbit	26
Hexosediphosphate	Low	High	Rabbit	26
Hexokinase	High	Low	Rat, Rabbit	26, 96
Citrate Synthase	High	Low	Rabbit	26

Table 4 (continued)

	"RED"	"WHITE"	Subjects	References
Alkaline Proteinase	High	Low	Hamster	253
Arylamidase	High	Low	Hamster	253
Cathepsin B1	Low	High	Hamster	253
Glycerol-3-Phosphate Dehydrogenase	Low	High	Rat	96
Carnitine Palmityltransferase	High	Low	Rat	220
Carnitine Acetyltransferase	High	Low	Rat	220
Total Carnitine	Low	High	Rat	220

Table 5

Muscle Fiber Characteristics Determined Biochemically

	SO	FOG	FG	Subjects	References
Myosin ATPase	Low	High	High	Guinea Pig	75, 120
Actomyosin ATPase	Low	High	High	Guinea Pig, Human	75, 120, 138
Arylsulfatase A	High	Int	Low	Guinea Pig	275
β-Acetyl Glucosamidase	High	Int	Low	Guinea Pig	275
β-Galactosidase	High	Int	Low	Guinea Pig	275
Acid Cathepsin	High	Int	Low	Guinea Pig	275
Acid Phosphatase	High	Int	Low	Guinea Pig	275
Total Lipids	High	Low	Low	Guinea Pig, Rabbit	144
Phospholipids	Int	High	Low	Guinea Pig	144
Cholesterol Content	High	Low	Low	Guinea Pig Rabbit	144
K^+	Low	High	High	Human	138
Na^+	Low	High	High	Human	138
Glycogen	Low	High	Int	Pig, Guinea Pig, Mice, Bovine	8, 75, 150
PPL	Low	Int	High	Guinea Pig, Rat	75, 99
Hexokinase	High	Int	Low	Guinea Pig	75
PFK	Low	High	High	Human	138
αGPD	Low	High	High	Guinea Pig	75
NAD-αGPD	Low	Int	High	Rat	99
Aldolase	Low	Int	High	Rat	99
Pyruvate Kinase	Low	Int	High	Rat	99
M-Type LDH	Low	Int	High	Guinea Pig, Rat	99, 274
H-Type LDH	High	Int	Low	Rat	99

Table 5 (continued)

	SO	FOG	FG	Subjects	References
Glucose-6-Phosphate Dehydrogenase	High	Int	Low	Rat	99
Glutamic-Oxalacetic Transaminase	High	Int	Low	Rat	99
Creatine Phosphokinase	Low	Int	High	Guinea Pig	75
SDH	High	Int	Low	Human	138
SDH	Int	High	Low	Guinea Pig	75
MDH	High	Int	Low	Rat	99
IDH	High	Int	Low	Rat	99
Cytochrome C	Int	High	Low	Guinea Pig	75
Cytochrome A	Int	High	Low	Guinea Pig	75
Cytochrome Oxidase	High	Int	Low	Rat	99

low in glycogenolytic enzymes (PPL). The SO muscle also is low in the formation of lactate (m-type LDH) and in αGPD which is needed for the regeneration of NAD^+ in glycolysis.

The FOG muscle is moderately to highly oxidative, moderately to highly glycolytic and fast contracting. The glycogenolytic and lactate formation enzymes for this muscle are moderately active, while the enzymes of the α-glycerophosphate shuttle are moderately to highly active.

The FG muscle is high in glycolytic and glycogenolytic activity, lactate formation, and α-glycerophosphate shuttle activity. Low oxidative enzyme levels and fast contraction time also are apparent in Table 5.

In agreement with the histochemical study by Karpati and Engel (217), Baldwin and Tipton (15) found high and very high correlations between biochemical analyses of the right and left tibialis anterior muscles in humans. The lowest correlation was found for lactic acid ($r = 0.86$), while total and free-carnitine each had a correlation coefficient of 0.99. Glycogen ($r = 0.91$), acyl-carnitine ($r = 0.91$) and phosphocreatine ($r = 0.92$) also were analyzed.

Thomson et al. (319) found no sex differences when studying the vastus lateralis muscle of humans. Their investigation included SDH, LDH, glycogen, and phosphagen concentration for either ATP or creatine phosphate.

Recently biochemical (138,139,193) and physiological (69,70,71,72, 73,74,75) data have been determined on individual muscle fibers and single motor units. The biochemical data on individual fibers correspond quite closely to that on whole muscle. The contractilability (ATPase) of the FOG and FG fibers was reported to be approximately 2.5

times higher than that of the SO fibers (138). Oxidative capacity (SDH) was found to be highest in SO fibers, intermediate in FOG fibers and lowest in FG fibers (138). The quantity of PFK was significantly higher in the FG and FOG fibers than in the SO fibers, while the reverse was true with the concentration of triglycerides (138). The amounts of glycogen in all three muscle fiber types were found to be similar (138).

Characteristics of Muscle Determined Physiologically

This section will deal with the physiological characteristics of muscles. The interrelationships of these characteristics and their dependence upon temperature also will be examined.

Physiological Characteristics of Muscle

Parallel to the finding of different muscle fiber types histochemically and biochemically was the observance of a difference in the physiological characteristics of muscle (226). Data are available on characteristics such as contraction time, tension produced, twitch/tetanus ratio and rate of fatigue. As was found with some muscles studied biochemically, many researchers categorized the muscles only as "fast" or "slow". The data collected in this fashion are presented in Table 6.

The distinguishing characteristics of a "slow" muscle are: slow contraction time, high mean tension, low twitch/tetanus ratio and slow rate of fatigue. A "fast" muscle exhibits the opposite characteristics: fast contraction time, low mean tension, high twitch/tetanus ratio and rapid fatigue.

With the advent of muscle histochemistry and the ability to differentiate the fibers into different types, muscle physiologists began to study muscles with a high percentage of one fiber type. In

Table 6

Physiological Characteristics of "Slow" and "Fast" Muscles

	Slow Muscles	Fast Muscles	Subjects	References
Mean Isometric Tension	High	Low	Rat	302
Rate of Tension Development	Low	High	Cat, Guinea Pig, Rabbit,	45,85,297, 302
Twitch/Tetanus	Low	High	Cat	62,256,297
Conduction Velocity	Low	High	Cat	58,59,62, 256
Total Action Potential	Low	High	Cat	58
Twitch Contraction Time	Low	High	Cat, Rabbit	58,62,226, 256
Rate of Fatigue	Low	High	Hamster	154
Maximum Velocity of Shortening	Low	High	Hamster	154
Electrical Threshold	Low	High	Human	112

conjunction with other technical advancements, the study of single motor units became possible.

A motor unit has been defined as being "an alpha-motoneuron and all of the skeletal muscle fibers that are functionally innervated by that neuron (126)." One muscle fiber usually is innervated by only one neuron (31,247); however, multiple innervations have been shown (247).

Burke and coworkers (69,70,71,72,73,74,75) have classified motor units into three types: (a) fast twitch fatigue sensitive (FF), (b) fast twitch fatigue resistant (FR), and (c) slow twitch fatigue resistant (S). As the appellations suggest the variables of contraction time and fatiguability were used in determining the motor unit types. More specifically, the variables used were a fatigue index and the presence or absence of "sag". The fatigue index is the ratio of maximum tension produced before and after two minutes of stimulation at a rate of 40/sec (70,73). This ratio is, therefore, a measure of the fatiguability of the motor unit. The phenomena of "sag" is characterized by an early tension maximum in the course of an unfused tetanus with a subsequent slight decline in tension to a lower plateau (70,73). This measure is an indication of contraction time since a fast fiber will reach an early tension maximum and then decline while a slow fiber will take a much longer time to reach its maximum tension. In using these two parameters to determine physiological motor unit types, Burke and coworkers (72,73) were able to classify all but 2-3% of the units they studied into S, FR, or FF types. Kugelberg (228), also studying single motor units, found that not all units could be classified into one of the three types. Although no percentage of unclassifiable units was given, only a few examples were noted and most units appeared to be one of the three types.

Table 7 summarizes the data collected with respect to contractile characteristics. From this table it can be seen that the S type motor unit is characterized as having a high fatigue index, no "sag" present, slow contraction time, slow axonal conduction time and slow one-half relaxation time. The FR motor unit has a high fatigue index, "sag" present, intermediate axonal conduction time, fast contraction time and fast one-half relaxation time. The FF motor unit has a low fatigue index, "sag" present, fast contraction time, fast axonal conduction time and fast one-half relaxation time.

Burke (64) originally found the twitch/tetanus ratio to be small in S motor units and large in FR and FF motor units. He later found this ratio to be small in S units, intermediate in FR units and large in FF motor units (70). Close (84), on the other hand, found the twitch/tetanus ratio to be virtually the same for all three types.

Aside from the twitch/tetanus ratio just discussed, no overlapping of the physiological characteristics between the three types of motor units was observed (72). Muscle fibers of the same motor unit type in the same muscle have identical histochemical profiles (42,69,70,72,134).

In comparing the physiological properties of motor units in the soleus and gastrocnemius muscles, Burke and coworkers (64,72,74) did find differences within the S type units. As compared to those in the gastrocnemius, the S units of the soleus muscle have a slower contraction time, slower conducting axons, no post tetanic potentiation of twitch tension, and a greater maximum tetanus tension output. Olson and Swett (266), however, concluded that one of the motor units found in the medial gastrocnemius muscle closely resembles the S motor units found in the soleus muscle.

Table 7

Physiological Characteristics
of Homogenius Muscles and Single Motor Units

	S	FR	FF	Subjects	References
Twitch	Slow	Fast	Fast	Guinea Pig, Cat, Human, Rat	25,55,64,66, 67,69,70,72, 75,84,228, 267,273
Peak Isometric Twitch Tension	Low	Int	High	Cat, Rat	64,228
Maximum Tetanic Tension	Low	Int	High	Cat	53,69,70,75
Maximum Isometric Tetanic Tension	Low	High	Low	Rat	84
Fatigue Index	> 0.75	> 0.75	< 0.25	Cat	53,69,70,73, 75
'Sag'	Absent	Present	Present	Cat	53,64,69,70, 73,75
Twitch/Tetanus	--- Virtually the same --			Rat	84
Twitch/Tetanus	Low	Int	High	Cat	70
Tetanus/Twitch	High	Low	Low	Cat	73
Motoneuron Input Resistance	High	Low	Low	Cat	64,65,66
Axonal Conduction Velocity	Slow	Faster	Fastest	Cat	64,66,70,75
EPSP	Long Dura-tion	Inter-mediate Duration	Short Duration	Cat	66
Post Spike Hyperpolariza-tions	Long	Short	Short	Cat	64
One-Half Relaxation Time	Slow	Fast	Fast	Rat	84
Tonic Firing	100%	70%	10%	Cat	66

Harris and Wilson (186) estimated there are 191±26 motor units in the tibialis anterior muscle of the mouse. This number is slightly higher than the 90 motor units Olson and Swett (265) found in the flexor digitorum longus muscle of a cat. Close (84) found an average of 40 motor units in the extensor digitorum longus muscle and 30 motor units in the soleus muscle of the rat. Of the 30 motor units in the soleus muscle, 3 were thought to be of the FR type with the remaining 27 being of the S type.

Burke and Tsairis (71) and Wuerker et al. (320) indicated that fast motor units are larger than slow motor units. In the cat medial gastrocnemius muscle, Burke and Tsairis (71) estimated each FF motor unit to contain 550-750 muscle fibers, each FR motor unit to contain 400-550 muscle fibers, and each S motor unit to contain at least 200 fibers. Burke and Tsairis (71) also estimated that this muscle contains approximately 170,000 muscle fibers. These data are considerably higher than the 118 average muscle fibers per motor unit that Brandstater and Lambert (42) found in the tibialis anterior muscle of the rat.

Muscle fibers of individual motor units have been found to be randomly distributed throughout the muscle (42,228). Brandstater and Lambert (42) found that 76% of the muscle fibers from one motor unit were not in contact with a muscle fiber from the same motor unit. Kugelberg (228) has stated that muscle fibers of a single motor unit may cover an area of 25-75% of the total muscle, but Brandstater and Lambert (42) found this area to be only about 12%.

Interrelationships of Physiological Characteristics of Muscle

Many investigations have been performed to determine the relationships of physiological characteristics to each other as well as to other parameters. Maximum tetanic tension is significantly related to the rate of tension development (35,318), and twitch contraction time is significantly related to the maximum rate of rise of twitch tension. However, contraction time is not correlated with the age of the subject (55,245), the twitch/tetanus ratio (84,256), or the size of the motor unit contracting (84). One-half relaxation time is significantly correlated with rate of tension development (318), rate of relaxation (318), muscle weight (318), and cross-sectional area (318) but is not correlated with the age of the subject (245). The sex of the subject does not appear to affect any of the physiological characteristics of muscle (55,245,318).

In accord with the histochemical studies (50,173,273,330) which determined that muscles of the same type may be different between species, Close (82) found that both "fast" and "slow" muscles of mice contract faster than do the same muscles in rats.

Although contraction time has been used historically to differentiate muscle types, recent questions have been posed as to the validity of this technique. Even though at least three different types of muscle fibers are known to exist, only two speeds of contraction have been identified (87). Therefore, when contraction time is used to differentiate muscle fibers at least one type of fiber is masked. Also, muscles and/or motor units of different types have been found to develop the same amount of tension while having different speeds of contraction (88,247,256). Close (83) concluded that the observed differences in contractile characteristics are related chiefly to the

intrinsic speed of shortening of the muscle fibers and, consequently, that the direct measure of contraction time is of little value when muscle fiber differentiation is sought.

Effects of Temperature on Physiological Characteristics

Researchers are in general agreement as to the proper muscle temperature to be maintained while measuring physiological characteristics. Most studies (35,45,57,60,64,65,68,84,88,104,184,186,246,266,293) have been performed with the muscles between 35-38^{o}C. Several studies were instrumental in establishing this protocol. Buller et al. (60), Close and Hoh (86) and Ranatunga and Lumper (284) all found that at temperatures below 35-38^{o}C muscles do not contract efficiently. These investigators showed that by decreasing the temperature to 10-15^{o}C time to peak tension and one-half relaxation time both were increased. With the decreased temperature maximum tetanus tension was decreased in both "slow" and "fast" muscles. Maximum twitch tension was decreased in "slow" muscle but was increased in "fast" muscle.

Ranatunga (285) found that different muscles respond in various ways to a 15^{o}C decrease in temperature. In his study the twitch/tetanus ratio was increased in the flexor digitorium longus muscle and was decreased in the soleus muscle. The maximum rate of tension was decreased in the flexor digitorum longus muscle and was increased in the soleus muscle.

Even though these results are generally accepted, a few discrepancies have been reported. In two studies maximum tension production was found to be independent of temperature through the ranges 10-36^{o}C (54) and 25-35^{o}C (83). Brown and VonEuler (51) found no relationship between a rise in muscle temperature and an increase in twitch tension.

Buchtal and Schmalbruch (55) found the contraction time of "fast" fibers to decrease 10% per degree C and "slow" fibers to decrease 7% per degree C when the muscle temperature was reduced from 32°C to 22°C.

Summary of Muscle Characteristics

In summary, whether differentiating muscle histochemically, biochemically or physiologically, at least three different kinds of muscle fibers have been found. Table 8 presents an abreviated composite analysis of these three fiber types.

Development of Muscle

Developing muscle also has been examined histochemically, biochemically, and physiologically. Thus, this section was subdivided to examine the literature resulting from each of these methods of analysis.

Histochemical Evaluation of Developing Muscle

The differentiation of muscle into specific fiber types appears at various stages in the development of different species of animals. After 50 days gestation lamb muscle can be differentiated into "slow" or "fast" fiber types via ATPase, and glycogen utilization (PAS) also is distinguishable in these animals (9). Only after 100 days of gestation do the oxidative capacity (SDH) and the glycogenolytic activity (PPL) of lamb muscle differentiate between fiber types (9). The normal gestation period of sheep is 147 days.

Beatty et al. (30) found "red" and "white" muscle fibers to be differentiated in foetal Rhesus monkeys at 120 days of gestation. At this age muscle fiber size also was different as "red" fibers were smaller than "white".

Table 8

Muscle Fiber Characteristics

	SO	FOG	FG
Oxidative Enzymes	High	Int-High	Low
Glycolytic Enzymes	Low	Int-High	High
Glycogenolic Enzymes	Low	Int-High	High
α-GPD Shuttle Enzymes	Low	Int-High	High
Lipid Metabolism	High	Low	Low
Contraction Time	Slow	Fast	Fast
Fatigue Index	High	High	Low
"Sag"	Absent	Present	Present
One-Half Relaxation Time	Slow	Fast	Fast
Tension Developed	Low	Int	High

Cosmos (90), in studying chicken embryos, found the breast muscle to be low in lactate formation (LDH) and the enzymes of the α-glycerophosphate shuttle (αGPD) but high in oxidative capacity (SDH). Cepina (80), however, found total LDH activity rises rapidly during the prenatal development of bovine foetuses.

In studying human foetuses, Dubowitz (109) and Strugalska and Fidzianska (314) reported muscle fibers to be differentiated and in a mosaic adult pattern by 26-30 weeks of gestation.

The muscle fibers of the newborn rat have been found to be undifferentiated (50,178,281,300). Two to three days after birth some differentiation is observable, and by seven days the fibers can be distinguished as "fast" or "slow" with the use of an ATPase stain (49, 50,108,300). Pullen (281) and Dubowitz (108), found that by 14 days of age rat muscle fibers assume a pattern similar to that of the adult animal. Other investigators (49,50,98,178,300) have found that approximately one month is needed before the final adult distribution is achieved.

Dubowitz (108) and Goldspink (153) found the development of mouse muscle to parallel that of the rat. Goldspink also found that by three weeks of age a relationship is established between the SDH concentration of an individual fiber and the size of the fiber. Smaller fibers contain more SDH than do larger fibers.

One-day-old pig muscle is differentiable with ATPase and PPL, but clear differentiation into the three fiber types does not occur until the fourth week (89). In the one-day-old pig an extreme range of fiber sizes exists (89). By the third week the muscle fibers are much more uniform in size, and from the fifth week on the FG fibers increase much more rapidly than do either the SO or FOG fibers (89).

Rabbit muscle fibers assume the adult pattern by 14-17 days after birth (235). In kitten muscle, on the other hand, the different fiber types can be distinguished at seven days of age (183).

A decrease in the oxidative capacity of some muscles has been found after an initial rise to a peak (90,91,229,234). This reversal of tricarboxylic acid cycle activity appears to be a part of the process of fiber type differentiation. Cosmos (90), in studying chicken breast muscle, found a decrease in SDH activity after the animals were one week old. Cosmos and Butler (91) noted a loss of approximately 60% in the SDH activity of the pectoralis muscle of the chicken at one month of age. By three months of age this muscle consists primarily of FG fibers. In contrast, the soleus muscle of the chicken shows little change in SDH activity after a peak is reached at one week of age (91). Lieberman et al. (234) found a 21% decrease in the percentage of red fibers in the diaphragm muscle of guinea pigs after six weeks of age.

Kugelberg (229) examined the soleus muscle of rats and found that an increase in SO fibers occurs over the first six months. At one month of age this ratio is 6:1. At five weeks of age the SO fibers comprise 66% of the soleus muscle; at thirty-four weeks of age 93% of the muscle is made up of SO fibers. This apparent gain in SO muscle fibers is not caused by the growth of new SO fibers since Kugelberg (229) and others (236,294) have found the total number of fibers in the soleus remains constant from 1 week through 14 weeks of age.

Kugelberg (229) found the rat soleus to have approximately 2,913 fibers and the rat extensor digitorium longus muscle to contain about 3,546 fibers (88). Hooper and McCarthy (201), in studying the mouse, found the biceps brachii muscle to have 1,172 fibers and the tibialis

anterior to have 1,694 fibers. Gonyea and Ericson (167) found 8,816 fibers in the flexor carpi radialis muscle of the cat.

In comparing the total number of fibers from the left and right muscles of guinea pigs, Karpatti and Engel (217) found differences of 15% between the medial gastrocnemius, 10% between the lateral gastrocnemius and 25% between the plantaris muscles. Gonyea (168) found no significant differences in the total number of muscle fibers between the right and left flexor carpi radialis muscles of cats.

Biochemical Evaluation of Developing Muscle

Even though no biochemical studies have been performed on foetal animal muscle, the studies performed on newborn animal muscle show trends similar to those observed with histochemical techniques. Svorvy and Gutmann (316) in studying rats, rabbits and guinea pigs found that myosin ATPase in the extensor digitorum longus muscle increases slightly from birth to adulthood, whereas that in the soleus muscle decreases until the adult level is one-half that of the new born. Hudlicka et al. (205) found that oxidative capacity (citrate synthase) and lipid content both decreased after birth, while lactate formation (LDH) had a distinct pattern in kittens by four days of age. In these kitten muscles glycogenolytic activity (PPL) was found to increase continuously after seven days of age, whereas the glycolytic enzyme triosephosphate dehydrogenase (TPDH) was found to increase only after 21 days of age (205). Enesco and Leblond (130) found DNA to increase with age in the medial gastrocnemius of rats. Hubbard et al. (204), also studying rats, found an increase in cytochrome c in the gastrocnemius and soleus muscles of animals between 39 and 87 days of age. The plantaris muscle of this age group showed no change in cytochrome c activity. However,

in animals between 87 and 124 days of age, all three muscles showed a decrease in cytochrome c (204).

Physiological Evaluation of Developing Muscle

At birth, the contraction and one-half relaxation times of "fast" and "slow" muscles are as nearly alike as they ever become, but even then they are not identical (52,81,242). After birth the contraction time of "fast" muscle has been found to decrease (52,57,82,83,177,178, 184,242) while that of "slow" muscle has been found to increase (57,177, 178,184,242), stay the same (82), or even decrease slightly (52,83). In cats, the one-half relaxation time decreases in "fast" muscles and increases in "slow" muscles (242) until the adult values are attained at around six to seven weeks of age (184).

Close (81,83) found that the twitch/tetanus ratio decreases during the development of rat soleus and extensor digitorum longus muscles. Buller and Lewis (57) found this ratio remains constant in the developing soleus of the cat while it increases in the flexor hallucis longus muscle. Mann and Salufsky (242) also found that this ratio increases in the tibialis anterior muscle of the cat.

A few studies have been performed on animal muscle comparing either young muscle to much older muscle or comparing two different groups of adult muscle. Maximum tetanic tension was found to increase in both "slow" and "fast" muscles of the rat and cat up to 14 to 18 weeks of age (81,242). In comparing the extensor digitorum longus muscles of adult hamsters at 180 and 360 days of age, Montgomery (254) found no differences in contraction time, one-half relaxation time, maximum tetanic tension, or twitch tension.

Adaptation of Muscle to Physical Training

Differences in muscle fiber populations have been observed in human athletes for many years (3,27,76,92,93,95,128,160,216,280,321). In general, athletes who participate in endurance events have a high percentage of SO fibers (3,27,92,93,95,128,160,216,321) and a high oxidative capacity in all fibers (76,92,93,160). Muscles from athletes engaging in sprint events have been found to have a high percentage of FG fibers (128,216,280,321).

Varying results have been obtained in regards to the muscle fiber sizes of these athletes. Anderson (3) studied endurance-trained athletes and found the FG and SO fibers to be the same size while the FOG fibers of these athletes were somewhat larger. Brodal et al. (44) reported that muscle fiber diameters were similar in trained and untrained muscles. Edstrom and Ekblom (128) found the mean cross-sectional areas of "red" fibers to be similar in weight-lifters, endurance runners and control subjects, but "white" fibers were significantly larger in weight-lifters than in the other two groups. Prince et al. (280) found both FG and FOG type fibers to be significantly larger in a group of weight-lifters than in a control group. Within the weight-lifters, the FG and FOG fibers were larger than the SO fibers. No fiber size differences were observed in any fiber type between distance runners and control subjects. Thorstensson et al. (321) found "fast" fibers to be larger than "slow" fibers in the vastus lateralis muscles of sprinters and jumpers, whereas Costill et al. (93) found "slow" fibers to be larger than "fast" fibers in the lateral head of the gastrocnemius muscle of distance runners.

Since most of the studies involving human muscle have dealt with the athlete after training, the question remains as to how much the

muscle can adapt to a training regimen. Consequently, much effort has been spent on the study of animal models in which data can be collected both before and after training.

Several approaches have been taken in studying trained animal muscle. Therefore, this section is broken down into the following subsections: (a) the effects of aerobic training, (b) the effects of anaerobic training and weight-lifting, (c) the effects of aerobic-anaerobic training, and (d) a comparison of anaerobic and aerobic training. A few studies on humans have been performed in which muscle has been obtained both pre- and post-training. These studies on humans are discussed with the work on animals where appropriate.

The Effects of Aerobic Training

Many different intensities and durations of aerobic exercise have been used in training animals. Training periods ranging from 15 minutes a day (17,22,324) to four hours a day (41,315) have been used, thus a total look at the aerobic continuum can be obtained. Differences also have been found in the types of activities used as some animals have been swum (41,79,114,115,157,169,172,189,230,262,315) while others have been run (13,79,119,121,142,143,157,169,192,200,244,329).

Most studies have shown that the body weights of exercised animals increase at a slower rate than do those of control animals during training (103,135,157,172,185,189,203,230,262,305), but several investigators have reported body weight to be unaffected by training (112,142,143,192, 200,244). Carrow *et al.* (97) and Gordon *et al.* (179) in studying rats that had either been run or swum, found the mean body weight of the runners to be significantly less than that of the controls while the mean body weight of the swimmers was not different from that of the

controls. This finding is in disagreement with the results obtained by other investigators who swam rats at the same intensity and for approximately the same duration but found a decreased gain in body weight (114, 157,172,189,230).

Several investigators have shown muscle weight to be unaffected by training (121,157,200,262). In these studies lesser bushbabies were run on a treadmill for six months (121), and rats were either swum (157, 262) or run (157,200) for seven (157,200) or ten weeks (262). Other investigators, however, have demonstrated a significant effect of training on muscle weight (114,192,203). The animals in these studies were either swum (114) or run (192). Maxwell et al. (244) and Yamaguchi et al. (329) found that training affects only specific muscles. Maxwell et al. (244) trained rats on a treadmill at 30 m/min for thirty to forty-five minutes over eight weeks and found the soleus muscles of the trained animals to be heavier than those of the control animals whereas the weights of the plantaris muscles were not different. Yamaguchi et al. (329) allowed rats to run voluntarily in running wheels for 30 days and found the weights of the flexor digitorum longus, tibialis posterior and triceps surea to be significantly affected by the training while the extensor digitorum longus, tibialis anterior, rectus femoris and vastus lateralis were not.

Through the use of histochemical techniques alterations in muscle fiber profiles have been observed after an aerobic training regimen is performed. These adaptations include primarily an increase in both glycolytic and oxidative activity in all three muscle fiber types, however, other changes have also been seen. Rats endurance trained either by swimming two-hour sessions twice daily for six weeks (41) or by running on a treadmill for 12 weeks (13), have been found to increase

both oxidative (13,41) and glycolytic (41) activity in all three muscle fiber types. Eriksson et al. (136), working with humans trained on a bicycle ergometer for six weeks, found no change in the contraction time of the vastus lateralis muscle as determined histochemically (ATPase). Glycogen (PAS), however, was stored equally well in all fibers while glycolytic (PFK) and oxidative (SDH and NADH) activities were increased at the end of the training period. Gollnick et al. (162) also trained humans on a bicycle ergometer at 75% of their aerobic power and found increased oxidative capacity in all muscle fiber types while glycolytic activity was increased only in the "fast" twitch fibers. Edgerton et al. (119) found higher levels of glycogen (PAS) in trained guinea pigs than in sedentary controls.

As alterations in the oxidative and glycolytic activities have been noticed so to have changes in the percentages of the different fiber types. Edgerton et al. (121) trained adult lesser bushbabies for six months on a treadmill and found no changes in glycolytic, glycogenolytic or lactate formation enzymes. However, an increase in the percentage of FOG fibers along with a proportionate decrease in the FG fibers of the tibialis anterior muscle was observed (121). No change was seen in the percentage of SO fibers in this muscle, and none of the fiber type percentages changed in the plantaris muscle. Other investigators (13,114,115,142,143,244) found that plantaris muscle adapts to both running (13,142,143,244) and swimming regimens (114,115). This adaptation was apparent from an increase in the FOG fibers and a decrease in the FG fibers (13,114,115). Syrovy et al. (315) trained rats by swimming them two hours every other day for nine weeks and found a decrease of 11.8% in the SO fiber population with a subsequent increase in the FOG fiber population of the soleus muscle. The Syrovy study is

distinguished by the fact that training was inauguarated at 14 days of age. This early starting age has been recognized by others as a distinct factor (13,114,115,244). In one study on adult humans Gollnick et al. (162) trained males for five months and observed that the percentages of muscle fiber types were not significantly altered.

Muscle fiber size has been shown to adapt to exercise (77,78,114, 121,142,143,162,200,324). Carrow et al. (78) found the mean cross-sectional area of the "red" fibers was increased 24% while the mean area of the "white" fibers was increased 10% in the gastrocnemius muscles of rats which were forced to swim but also could run voluntarily. Gollnick et al. (162), in a study of endurance-trained humans, determined that the cross-sectional area of "slow" twitch fibers was smaller than that of "fast" twitch fibers before training while the reverse was true after training. The ratio of "slow" twitch to "fast" twitch fiber areas increased from 0.82 before training to 1.11 after training. Walker (324) observed that 15 minutes of daily exercise produced hypertrophy, regardless of the intensity, while 5 minutes produced atrophy in mice that were trained for three and one-half weeks. Edgerton et al. (114) reported that fiber hypertrophy was dependent upon the muscle being examined. In both the soleus and plantaris muscles, FOG fibers were larger in trained lesser bushbabies than in control animals. The SO fibers were enlarged in only the plantaris muscle. The mean sizes of the FG fibers in the plantaris and soleus muscles and the size of the SO fibers in the soleus muscle were not different from those of untrained animals. On the other hand, Faulkner and coworkers (142,143) found the "red" and "white" fibers of trained guinea pigs to be smaller than those of sedentary animals. A possible explanation for this

observation is the hyperplasia which has been found in the muscles of endurance-exercised animals (79,117).

Even though fiber size seems to be affected by training, Holmes and Rasch (200) found no effect on the number of myofibrils per fiber in rat sartorius muscle. The distribution of the myofibrils within the muscle did suggest a training adaptation as a larger number were found at the origin and insertion of the muscle but not in the mid-portion.

In one training study the capillaries of FG and SO muscle fibers were not affected while those of the FOG fibers were significantly increased (238). Carrow et al. (78) studied rats forced to swim and also allowed voluntary running and found a 4% increase in the capillaries per "red" muscle fiber and a 10% increase in the capillaries per "white" muscle fiber. Later Carrow et al. (79) found no change in the capillary-to-fiber ratio of any muscle fiber type in rats which were allowed voluntary exercise, subjected to short, medium or long duration endurance running programs, or subjected to a long duration endurance swimming program.

Biochemically, several different enzymes and substrates of the metabolic pathways have been studied in relation to their adaptation to endurance exercise (22,39,103,121,136,141,144,158,162,172,174,182,185, 252,262,272,305,325). Muscle glycogen was found to increase with endurance training in both rats (262) and humans (121). Short et al. (305) found the glycogen concentration in trained rats to be higher in "red" fibers than in "white". Glycogen synthetase, an enzyme of glycogenesis, has been found to increase with endurance training. Glycogenolytic activity after endurance training is not as well defined. Baldwin et al. (14) found a slight decrease in glycogenolytic activity. Conversely, Gould and Rawlinson (174) observed no change in the PPL

activity of rats when pre- and post-trained muscles were studied.

Hexokinase has been found to increase in animals after endurance training (14,22,272). Barnard and Peter (22) and Peter et al. (272) found this increase to be approximately twofold. Glycolytic activity, as measured by PFK and glucose-6-phosphate (G-6-P), has been found to be unaltered by training in rats (185) and young boys (136). However, Gollnick et al. (162) found a significant training-related increase in PFK in the muscles of adult human males. Hearn and Wainio (190) trained rats and obtained an increase in unit and relative total aldolase activity but no change in actual total activity. Baldwin et al. (14), in looking at the glycolytic activity of specific muscle fiber types, found an increase in the SO fibers, a decrease in the FOG fibers, and no change in the FG fibers after endurance training.

Muscle lactate and the enzyme of lactate formation, LDH, have been shown either to decrease (14,158,305) or to remain the same (136,172, 185) with endurance training. These studies were performed on young boys (136) and rats (14,158,172,185,305); and the gastrocnemius (158, 185), soleus (14), adductor magnus (305), biceps femoris (172), vastus lateralis (136), and quadriceps (14) muscles were examined.

The oxidative capacity of endurance trained muscle, as measured by SDH (103,121,141,162,185,189,258), MDH (189,252) and citrate synthase (148,189), has been shown to increase in vastus lateralis (121,162), rectus femoris (258), gastrocnemius(103,141,148,185,189,252,258) and soleus (252,258) muscles. The subjects for the studies which found an increased oxidative capacity were humans (162), lesser bushbabies (121) and rats (103,141,148,185,189,252). However, the SDH activity in the semimembranosus muscles of endurance-trained lesser bushbabies was found to be no different than that in sedentary control animal (121).

Cytochrome oxidase (121) and cytochrome c (121,141,204) were found to be increased in the muscles of endurance-trained lesser bushbabies (121) and rats (141,204).

Eriksson et al. (136) exercised boys for 60 minutes three times per week, for four months, and found a significant increase in ATPase activity of the vastus lateralis muscle. Increases in ATPase also were seen in the predominantly SO and FOG muscles of endurance-trained rats while ATPase activity in muscles of predominantly FG fiber types did not increase (18,315). Wilkerson and Evonuk (325) also trained rats and found an ATPase increase in the gastrocnemius muscles of animals that had been swum to exhaustion every other day for either six or ten weeks but no increase in rats swum 30 minutes every other day for ten weeks. Edgerton et al. (121), Hearn and Gollnick (191) and Rawlinson and Gould (287) found no significant increase in the ATPase levels of trained animals. The muscles examined were the soleus (121), plantaris (121), tibialis anterior (121), and gastrocnemius (191).

Dohm et al. (101) found that total lipid and cholesterol levels were not changed in endurance-trained rats while free fatty acids and triglycerides were both reduced. Lipoprotein lipase was found to increase the greatest amount in FOG fibers, but increases also were seen in the SO and FG fibers of endurance-trained animals (40). Myoglobin, the transporter of oxygen in muscle, has been found to increase in endurance-trained hamster (39) and lesser bushbaby (121) muscles.

Mitochondrial concentration (159) and mitochondrial protein (252) have been reported to be significantly increased while total protein content (185,190) does not change with training. Helander (192) and Dohm et al. (102) both found sarcoplasmic proteins to be unaltered by training, whereas Gordon et al. (169) found a significant increase.

Dohm et al. (102) also found no change in the myofibrillar proteins, but Gordon et al. (169) detected a decrease. All of these studies were performed on rats.

Edgerton et al. (121), studying the physiological characteristics of the plantaris muscle of lesser bushbabies trained at running, found no change in either the twitch or tetanic tension. Furthermore, there was no training adaptation of the muscle in contraction time, one-half relaxation time, tetanus/twitch ratio, fusion frequency or rate of tension development.

Effects of Anaerobic Training and Weight-lifting

A search of the literature revealed very few studies dealing with only anaerobic training (258,304,310,320). Thorstensson et al. (320) trained students with maximal dynamic leg extensor exercises three times a week for eight weeks and found no changes in the percentages of the different fiber types utilizing the histochemical ATPase technique.

Muller (258) and Staudte et al. (310) did observe changes in sprint-trained animals. Body weight (248,310) and actual muscle weight (258) were both found to decrease in sprint-trained rats while relative muscle weight (258,310) was not significantly affected. The percentages of fiber types in the soleus muscle were not altered by sprint training. However, the "fast" fibers of the rectus femoris and gastrocnemius muscles decrease in oxidative capacity (SDH) (258).

Standte et al. (310) found increases in glycogenolytic (PPL), glycolytic (TPDH), and oxidative (citrate synthase, SDH) activities of both the rectus femoris and soleus muscles of sprint-trained rats. Hexokinase increases also were found in both muscles while creatine

kinase increased only in the soleus muscle (310). An indicator of fatty acid oxidation (3-hydroxyacyl-CoA dehydrogenase) was unchanged with training (310). In studying the physiological characteristics of these animals, Shield et al. (304) found the isometric twitch contraction time decreased while the maximum tetanic tension increased in both muscles.

Glycogenolytic activity (PPL) was found to increase in rats which were trained with weight-lifting; however, voluntary running produced a greater increase (225). Cytochrome oxidase was found to rise equally in both the weight-lifting and voluntarily run rats (225). Oxidative capacity (SDH) also was found to increase in both rats (225) and hamsters (202) while decreasing in the muscles of mice (309) trained in weight-lifting.

Roy et al. (296) found no change in the percentage of "fast" and "slow" muscles of rats that had been weight-lifting; however, the fiber profiles were more variable in the trained animals than in a group of sedentary animals. An unexpected finding of this study was that the trained animals showed a trend toward enhanced aerobic metabolism.

Muscle fiber hypertrophy has been observed in animals trained by weight-lifting (152,155,156,170). Gordon et al. (170) reported that only the "white" fibers hypertrophy, but Goldspink (152), Goldspink et al. (156) and Goldspink and Howell (155) found that all types of fibers increase in size. This increase was estimated to be approximately 30% of the average pretraining cross-sectional area (152) and was greater in the biceps brachii and extensor digitorum muscles than in the soleus muscle (155).

Another observation in the muscles of weight-lifting animals has been an increase in the total number of muscle fibers (168,196). Gonyea

(168) found an increase of 10-24% in the number of fibers in the flexor carpi radialis muscle of cats trained by weight-lifting. Ho et al. (196), in studying rats, found small split fibers with normal histochemical characteristics, adequate blood supplies and neural innervations. Goldspink and Howell (155) and Goldspink et al. (156), however, found no fiber splitting present in hamsters that had weight-lifted.

The Effects of Aerobic-Anaerobic Training

In some studies, animals have been trained both aerobically and anaerobically (10,11,16,23,24,32,97,100,179,180,182,197,198,199,206,257, 268). These studies usually have been designed to consist of endurance running with sprints interspersed throughout the training period. Body weight has been observed to be reduced with this type of training (10, 11,97,197). Absolute muscle weight has been found either to decrease (10,11,197) or to remain the same (24,268) while relative muscle weight has been reported to be unchanged (11).

Barnard et al. (23), using a histochemical NADH technique to differentiate the fibers in the medial gastrocnemius muscle, found an increase in the number of FOG fibers and decrease in the FG fibers. The number of SO fibers did not change. Guy and Snow (180) also found an increase in the percentage of FOG fibers, but they noted that both the FG and SO fiber populations were decreased. The subjects for this study were horses, and six different muscles were analyzed. Muller and Vogell (257) found an increase in the number of FOG fibers in the gastrocnemius muscle; however, both the rectus femoris and soleus muscles showed an increase in the number of SO fibers when an endurance-sprint type of training program was used with rats.

This type of training program has been found to increase both glycogenic (11) and glycogenolytic (206) activity as well as the activity of the enzyme hexokinase (16,206). Phosphofructokinase (182) and aldolase (180), both enzymes of glycolysis, were found to increase with training while PK (206), another enzyme of glycolysis, and aldolase (206) were unaltered. The activity of the α-glycero-phosphate shuttle (αGPD) was not changed (198); but LDH, the enzyme of lactate formation, was decreased with training (16,179,180,206). All oxidative enzymes studied (citrate synthase, IDH, ketoglutarate dehydrogenase, SDH, and MDH) increased in activity with training. The respiratory capacity, as measured by NADH (197), cytochrome c (197,198,199) and cytochrome oxidase (11,32,100,197,198), of animals trained with the endurance-sprint program was found to increase. Increases also were found in amino acid degradation (glutamate dehydrogenase) (199), fatty acid oxidation (11) and myoglobin concentration (268).

Baldwin et al. (16) studied homogeneous muscles and found hexokinase activity to be increased in all three fiber types. The activities of the enzymes αGPD, PPL, PFK, glyceraldehyde-3-phosphate dehydrogenase and PK increased in the SO fibers but decreased in the FOG fibers. Lactate dehydrogenase activity decreased in both the FOG and FG fibers.

Most physiological characteristics of guinea pigs trained with an endurance-sprint program were described as being "unremarkable" (24). These characteristics include time-to-peak tension, one-half relaxation time, tetanic-fusion frequency, twitch/tetanus ratio and rate of tension development. In contrast, an unusually high level of isometric tension was maintained by the trained muscles throughout a 60-minute stimulation (24).

A Comparison of Anaerobic and Aerobic Training

Recently, studies have been conducted which have utilized separate anaerobic and aerobic training programs, thus a direct comparison between the two types of training is possible. In studying human subjects that were endurance trained for 12 months or sprint trained for eight weeks, Sjodin et al. (306) found no change in the fiber composition of the vastus lateralis muscle. Total LDH activity was unchanged by sprint training while it was decreased with endurance training. Henderson and Reitman (194) also studied sprint- and endurance-trained humans and found no difference in the relative cross-sectional areas occupied by SO (TYPE I) and FOG and FG (TYPE II) muscle fibers. Oxidative activity (SDH) was found to increase 49% in the FOG and FG (TYPE II) fibers of the sprint-trained subjects and 32% in the SO (TYPE I) fibers of the endurance-trained subjects. Phosphofructokinase, an enzyme of glycolysis, was not changed with either training program.

Fitts and coworkers (146,147), studying sprint- and endurance-trained minature pigs, found no changes in the fiber type distribution of the biceps femoris and gracilis muscles following three and seven months of training. Muscle fiber size (146) and myoglobin concentration (147) also were similar in the two trained groups and a group of sedentary control animals.

Bagby et al. (12) found myosin ATPase, determined both histochemically and biochemically, to be unaltered after 11 weeks of either a sprint or an endurance training program in rats. Roy (295) trained rats and found an endurance program increases the aerobic ability of the plantar flexor muscles while a sprint program increases both the anaerobic and aerobic abilities. These results were based on the histochemical evaluation of contractile elements (ATPase), oxidative

capacity (SDH), lactate formation (LDH), and glycogen (PAS) and lipid (SUD) localization. In the same study Roy (295) found that eight weeks of training decreased both body and absolute soleus weights. Relative soleus weight was unchanged by training. Training also reduced the absolute plantaris weight, with the sprint-trained plantaris muscles weighing less than the endurance-trained. The relative plantaris weight was greatest in the endurance-trained rats and least in the sprint-trained animals.

Taylor (317) also found muscle adaptation when comparing sprint- and endurance-trained rats. In the medial gastrocnemius muscle, oxidative activity (SDH) was increased by a long endurance program after 12 weeks of training while it was increased by voluntary running after only four weeks. Glycogenolytic activity (PPL) increased in a moderate endurance group after eight weeks of training. Glycogen storage (PAS) increased in control, sprint and swim groups after four weeks, in a moderate endurance group after eight weeks, and in a voluntary running group after 12 weeks. Contractile elements (ATPase) increased in the voluntary running group after four weeks and in the endurance trained group after eight weeks. Similar changes were observed in both the plantaris and soleus muscles.

Hickson et al. (195) found a decrease in glycogenolytic capacity (phosphoglucomutase) and an increase in oxidative capacity (fumurase) in both endurance- and sprint-trained rats. These changes were observed in the plantaris, soleus, and white area of the vastus lateralis muscles after 16 weeks of training. A decrease also was observed in the LDH activity of the white area of the vastus lateralis muscle after 16 weeks of endurance training. In an eight-week training program, LDH decreased in both the soleus and the white area of the vastus lateralis muscles

of the sprint-trained animals. The vastus lateralis muscles of rats subjected to an endurance training program for eight weeks showed a decrease in LDH and phosphoglucomutase while phosphoglucoisomerase, an enzyme of glycolysis, was increased in the plantaris muscles. None of the enzyme differences between the sprint- and endurance-trained groups were significant after either 8 or 16 weeks of training.

Gordon et al. (171) found the body weights of rats trained by endurance running and weight-lifting to be significantly lower than those of sedentary control animals. Myofibrillar protein concentration also was decreased with endurance training while sarcoplasmic protein was increased. With sprint training the opposite results were observed. Myofibrillar protein concentration increased and sarcoplasmic protein concentration decreased.

CHAPTER III

METHODS AND MATERIALS

Experimental Animals

Twenty-seven random-bred male Syrian hamsters were used as experimental anaimals for this investigation. These animals were obtained in four shipments over four months. All animals were provided an environmental adjustment period before the treatment was commenced. Initiation of the treatments were begun when the animals were 35 days of age.

Treatment Groups

Upon arrival all animals were randomly assigned to one of three treatment groups: (a) zero-week control, (b) 12-week sedentary control, and (c) 12-week high-intensity endurance swimming. The zero-week control group was sacrificed at 35 days of age and thus designated as the pretreatment control group. The 12-week sedentary control group remained in their cages throughout the duration of the experiment and, therefore, served as a post-treatment control group. The 12-week high-intensity endurance swimming group was exercised five days a week for 12 weeks. A progressive training program was used with the amount of activity gradually increased up to the 37th day of training. By the 37th day of training, the animals were expected to complete a daily swim of one hour with 2-3% body weight attached (see appendix A for the complete training program). Each animal was swum in an individual

cylinderical tank having a diameter of 28 cm and a height of 75 cm (water depth 70 cm). The training program was performed between 1:00 p.m. and 5:00 p.m. All trained animals were weighed daily before being exercised. An animal was not swum if his body weight had decreased by more than two or three grams from the previous day.

Animal Care

Previous work in this laboratory has shown that nocturnal animals which are forced to exercise will perform best between four hours before and four hours after the lights are turned off in the animal living quarters. Thus, the lights in the animal quarters were turned off automatically each day at 1:00 p.m. and were turned on again at 1:00 a.m.

The animals were housed in sedentary cages (24 cm long by 18 cm wide by 18 cm tall). Throughout the experiment, all animals had access to water and a commercial hamster chow ad libitum.

Sacrifice Procedures

Six sacrifices were conducted over a six-month period. The two initial sacrifices involved only zero-week animals while the remaining four sacrifices involved animals in the 12-week groups. The sacrifices were conducted approximately 70 hours following the last exercise session. Only animals considered to be in good health were sacrificed. A performance criterion of swimming 75% of the sessions with weights attached 75% of the time was set for the trained hamsters.

Each animal was weighed and then sacrificed by decapitation. The heart and selected muscles of the left and right legs were removed immediately.

Left Leg Muscles

The left leg muscles were used for physiological characteristics. Since the instrumentation used in obtaining this data are new, explanations of both the physiological stimulating system and the exact sacrifice procedures will be dealt with in this section.

Physiological Characteristics Data Collecting System

The purpose of the physiological stimulating system were: (a) to maintain hamster muscles at a constant temperature (15°C) in Ringer's solution, (b) to stimulate the muscles electrically according to a predetermined program, and (c) to record the force curves resulting from the applied stimuli.

The stimulation chamber (Figure 1) consisted of a plastic dish (8.9 x 7.0 x 7.6 cm, inside) placed within a larger tank (22.9 x 12.7 x 16.5 cm, inside). The inner dish was filled with Ringer's solution, the outer tank with tap water. The temperature of the inner bath was maintained at 15 ± 1°C. In previous work performed in this laboratory it was observed that this temperature was optimal for the contractile program used.

Suspended within the inner bath were two stimulating electrodes, two tissue clamps and a gas dispersion tube for bubbling oxygen into the solution. Each of these was mounted on a plastic support (Figure 2) which slid into two slots on the outside of the larger tank. A second pair of slots held the assembly up and forward for ease in calibration and mounting tissue.

The tissue clamp arrangement is shown in Figure 3. The lower clamp was stationary and was intended to pinch a tendon. The upper clamp, designed to support a bone chip, was connected through a nylon insulator

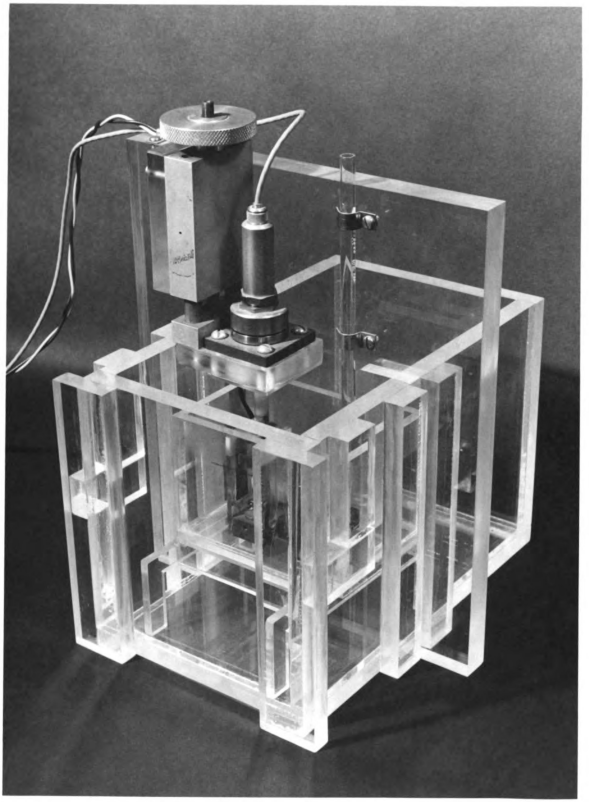


Figure 1

Physiological Characteristic Data Collecting System Stimulation Chamber

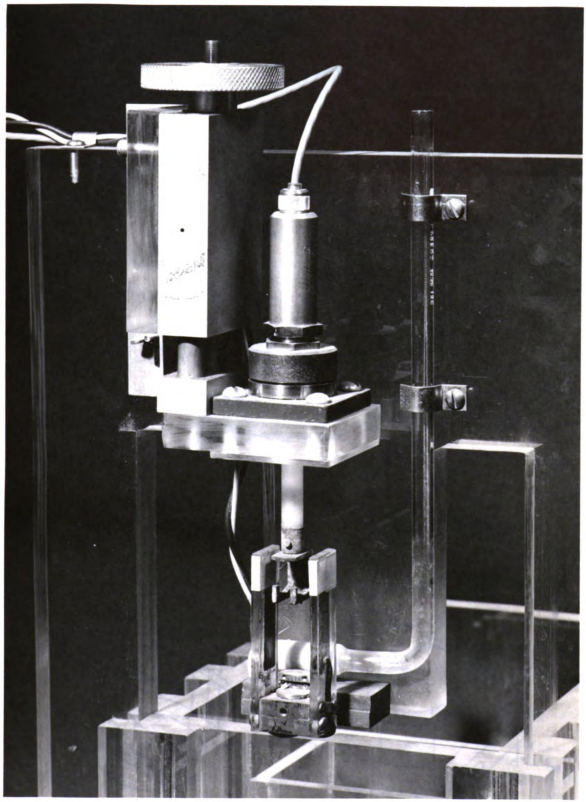
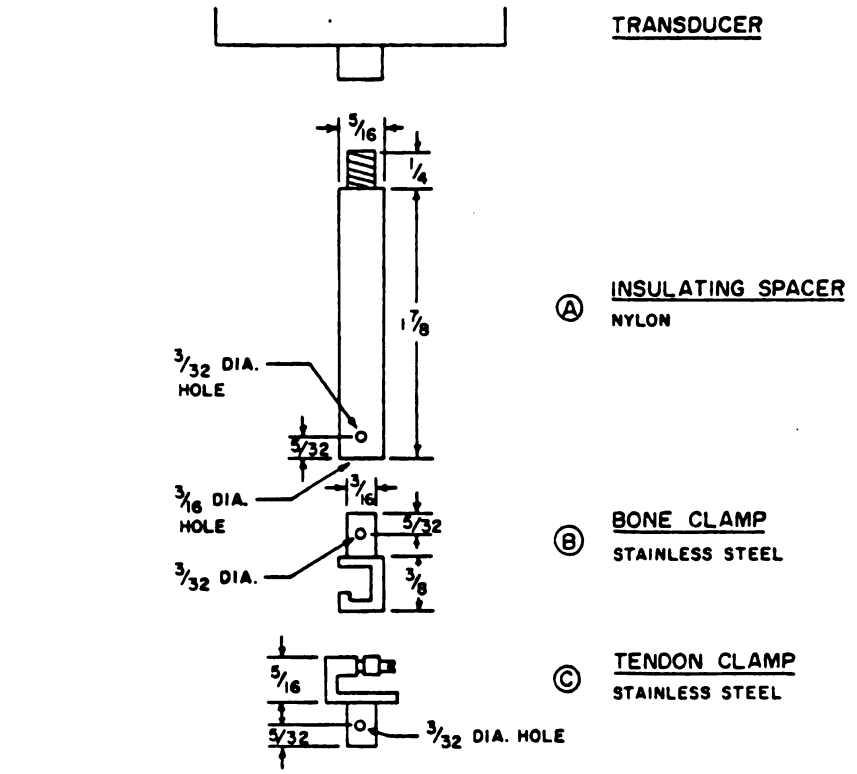


Figure 2

Electrode and Tissue Clamp Assembly of the Physiological Characteristic Data Collecting System



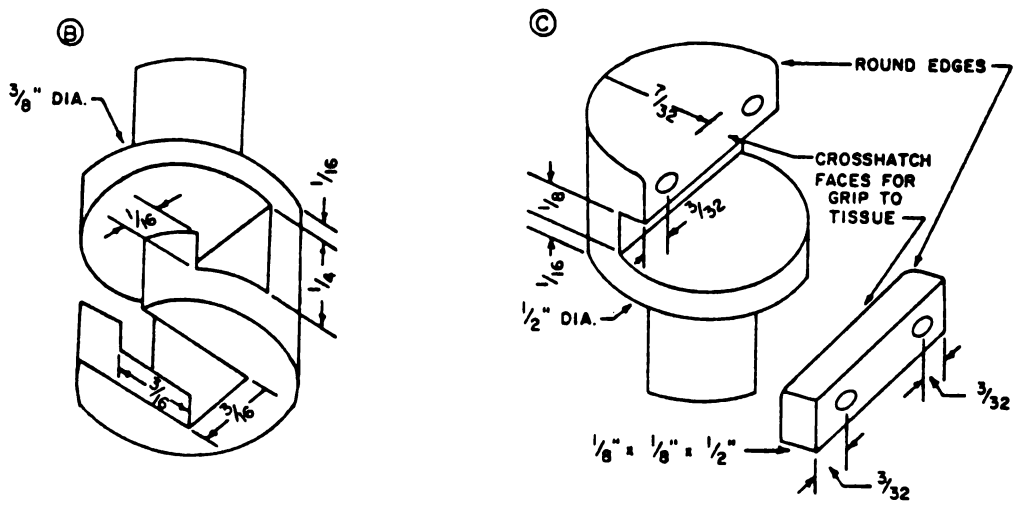


Figure 3

Tissue Clamp Assembly of Physiological Characteristic Data Collecting System

to a force transducer (Statham UC3 plus 5 pound load cell, UL4). The insulator was required to prevent unwanted current paths through the tissue. The transducer was raised or lowered by a micrometer adjustment (Figure 2) to account for differences in individual muscle lengths.

The electrodes (Figure 4) were made of 24 ga. sterling silver (1 cm x 4 cm) plates which were glued to plastic supports. Wire leads (18 AWG) were soldered to the back of each silver plate using ordinary techniques and solder. The plates were surrounded with a plastic liquid/powder mix: the same material was used to fill the lead holes. The result was electrodes which were mechanically rugged and had no exposed metal portion except the flat silver face.

Because unplated silver presents a very poor electrical interface to Ringer's solution, the electrodes were plated with AgCl to facilitate the flow of current between electrode and solution via an exchange of chloride ions. Each electrode was scrubbed with steel wool and placed in a .1 N solution of HCl with a dummy electrode (Figure 5). A current of 50 mA completed the process in 15-30 sec, as was evidenced by a color change to dull grey on the electrode surface. After 20 muscles had been stimulated, each electrode was cleaned and replated to avoid contamination of the surface.

The stimulus was applied to the muscle by a technique called massive stimulation. That is, current was passed between large electrodes through the muscle via the Ringer's solution. There was no direct contact between the electrodes and the tissue. This method had several advantages: (a) all parts of all available fibers contracted in unison which avoided propagation delays, (b) tissues remained in the physiological Ringer's solution at all times, and (c) no mechanical restrictions were imposed by electrodes attached directly to the muscles.

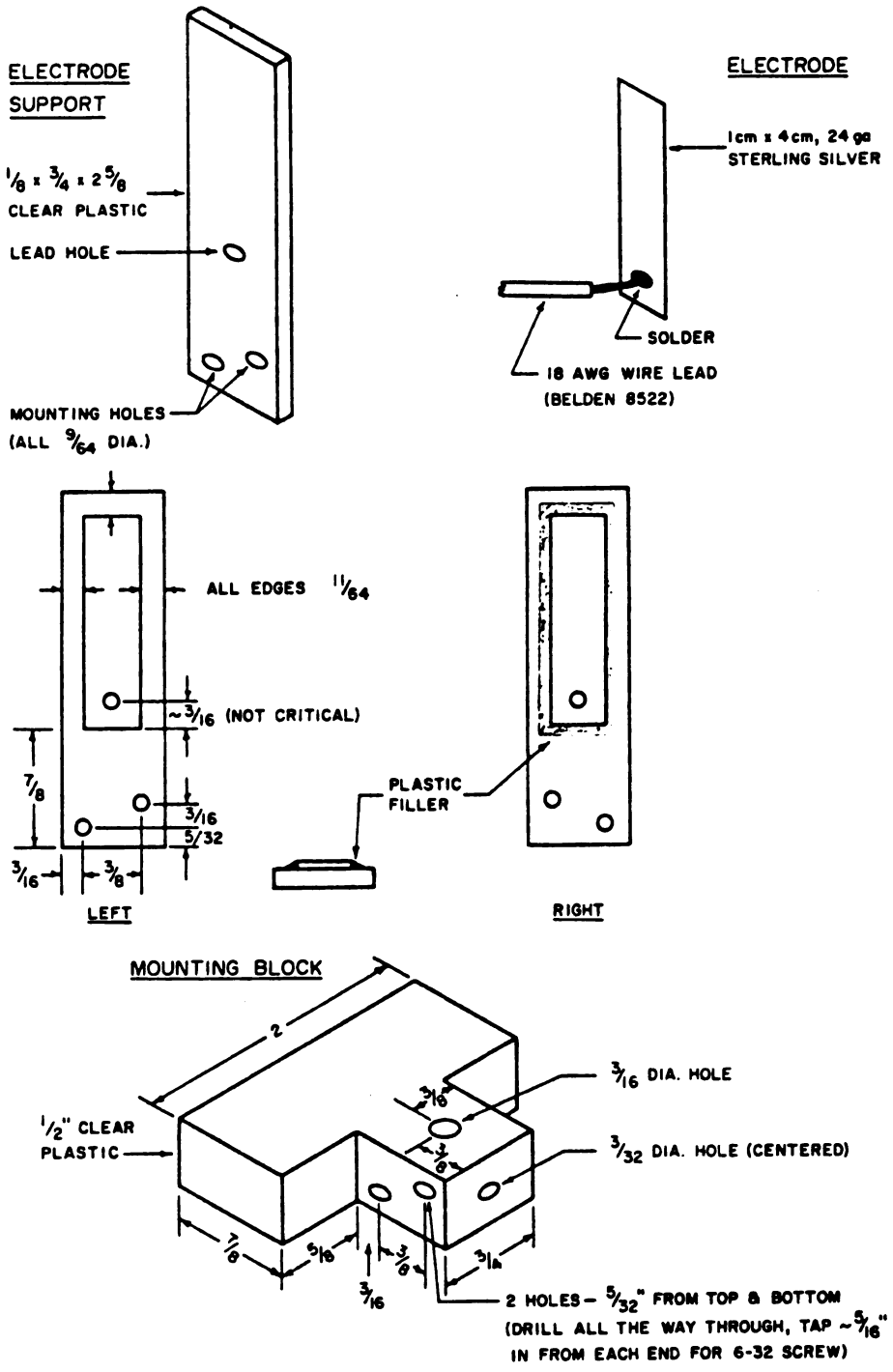


Figure 4

Electrode Assembly of Physiological
Characteristic Data Collecting System

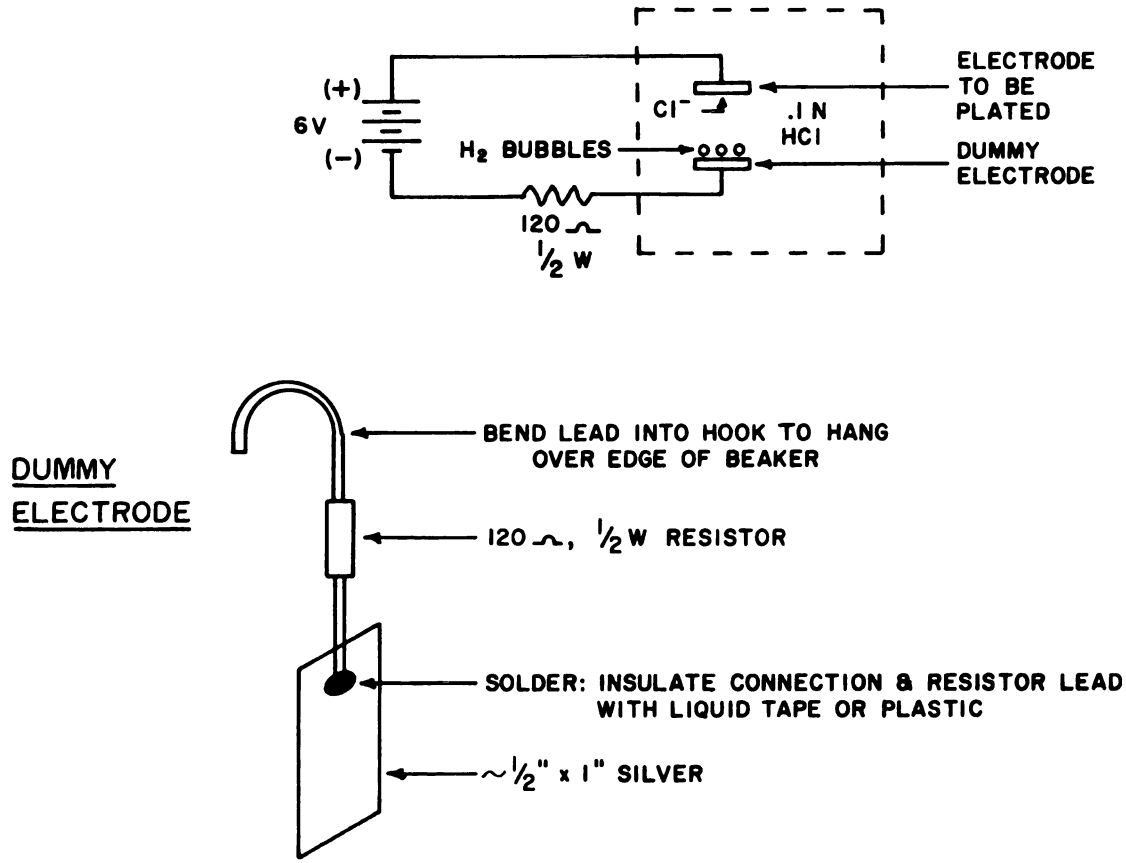


Figure 5

Electrode Plating Assembly for Physiological Characteristic Data Collecting System

Very high currents (1–4 amps) were required with massive stimulation because most of the current did not actually cross the muscle membranes. In our system, current pulses of 2 amps and 2 msec width were used: the polarity of each pulse (current direction) was opposite to that of the previous pulse so that net ionic flow was zero (Figure 6). This was necessary to avoid damage to tissues and electrodes.

The stimulator was custom made. The output stage was very similar to an audio amplifier. The pulse width and amplitude were controlled by analog circuits. Pulse frequency (for tetanus) was determined externally. The stimulator generated one 2-amp, 2-msec pulse whenever a trigger was received from a separate unit, the stimulus sequencer.

The function of the sequencer was to apply a fixed set of stimulations and rest periods to each muscle. The sequencer also controlled data rates to a digital recorder and generated start and stop signals so that data were not recorded during rest periods.

A mixed program of twitches and tetanus at various frequencies was desired to investigate such parameters as twitch/tetanus ratio, degree of fusion during tetanus and comparison of twitch characteristics before and after partial fusion of tetanus. The present stimulation program used 16 contractions. Contraction numbers 1-5 were twitches initiated with a single 2-msec pulse. Between each twitch there was a 10-sec rest with a 1-min rest following contraction number 5. Contraction numbers 6-11 were 2-sec tetanus contractions at frequencies of 10, 20, 50, 100, 200, and 500 pulses per second (PPS) respectively. Again the rest period between contractions was 10 sec with a 1-min rest period following contraction number 11. Contraction number 12 was a 30-sec tetanus at 50 PPS designed to show fatigue effects. After a 1-min rest three more twitch contractions, numbers 13-15, were performed. These

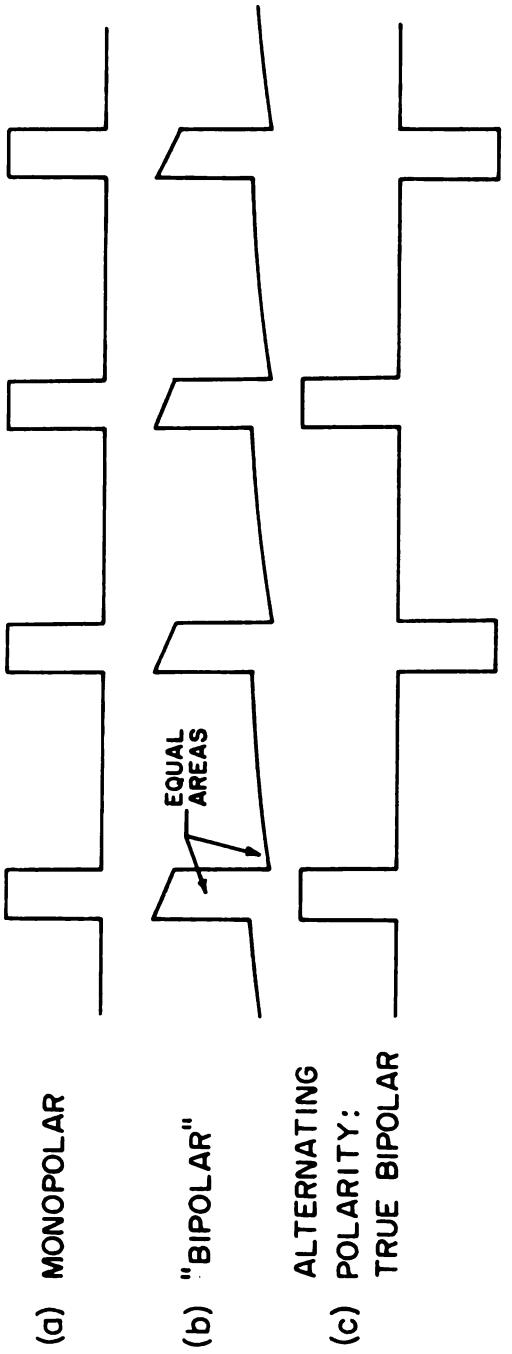


Figure 6

Stimulator Waveforms of Physiological Characteristic Data Collecting System

twitches were followed by another 1-min rest and a final 30-sec tetanus, contraction number 16.

The electrical output of the force transducer was connected to the signal processor which functioned to format the force data prior to recording. The signal was first amplified and filtered to remove high-frequency noise and vibrations. At this stage, calibration controls set two points on a scale of 0-100. In the next stage of signal processing, the force data were digitized to break up the analog range into 1000 steps, each step equal to 0.1 g. After digitizing, the force values were represented by numbers from 0-99.9. These numbers were re-converted to grams by the computer at a later time. The final stage of data collection consisted of recording these numbers on magnetic tape.

Data processing was accomplished in three stages: computing, plotting and typing. In the computing stage a Hewlett-Packard HP9810A calculator was used to first read and then smooth the data to reduce the effects of high-frequency noise and vibrations which occurred during the recording process. At the same time three additional data points were created for each twitch contraction to increase the time resolution from 2 msec to 0.5 msec. The smoothing routine also searched for several variables which were measured directly from the raw data. Seven contractions were plotted in three groups on a single page: twitches, 2-sec tetanus and 30-sec tetanus. In each group the largest peak tension was normalized to 1.0 units; the other contractions were plotted relative to it. A Hewlett-Packard HP-9862 plotter was used to obtain these plots. A Hewlett-Packard HP-9861 typewriter was also programmed so that the measured variables were printed along with several additional variables computed from them.

Left Leg Muscle Sacrifice Procedures

The left hindlimb was skinned. The RF muscle was removed and clamped immediately into the physiological stimulating device. The SOL muscle then was removed and placed in a dish containing Ringer's solution until it could be placed in the stimulating device. A small piece of bone was removed with each muscle to facilitate clamping the muscle into the stimulating system.

Initially the operator entered the animal number, checked the condition of the electrodes, checked the bath temperature, and calibrated the force transducer. The muscle then was mounted in the tissue clamps with its length adjusted to produce an initial tension of 1 g., and the muscle/electrode assembly was lowered into the Ringer's bath. At this time, the "start" button on the sequencer was pressed and the stimulation sequence proceeded until data was collected for all 16 contractions.

Right Leg Muscles

The right hindlimb was skinned. The right triceps surea (gastrocnemius and soleus) and plantaris muscles were removed as a complex. The RF muscle then was removed. All of the remaining procedures were performed on both the gastrocnemius-soleus-plantaris complex and the RF. Thus the general term "muscle" will refer here to either the complex or to the separate RF muscle.

After weighing, the muscle was rolled in talcum powder and cut into thirds. The center third was placed, distal end up, in 5% gum tragacanth on a small circular piece of cork. This unit, held with forceps, then was lowered for approximately 60 seconds into pre-cooled 2-methylbutane (isopentane). The isopentane was cooled to a viscous

fluid (-140 to -185°C) by liquid nitrogen. The frozen muscle unit then was wrapped in aluminum foil and stored in an ultra deep freeze (-70°C).

Histochemical Procedures

Before sectioning and histochemical proceedures were initiated, the muscle units were removed from the ultra deep freeze and allowed to warm in a cryostat (-10 to -20°C). The pieces of cork were frozen onto cryostat chucks using O.C.T. compound. Serial cross-sections were cut at 10 microns using an Ames rotary microtome-cryostat. Sections were mounted on cover glasses and air dried for at least one hour.

Glycolytic activity was studied through the use of αGPD (132). Oxidative capabilities were determined by NADH using nitro blue tetrazolium (NBT) as an electron acceptor (132). Myofibrillar adenosine triphosphatase (ATPase) at pH 9.4 was used to identify contractile elements (110). Lipid localization was determined by SUD (237) while a modified PAS reaction was used to determine glycogen storage (237).

Harris' alum hematoxylin and eosin (H&E) was applied to muscle sections for the identification of basic morphologic characteristics (237). Gomori's trichrome (GOM) (131) was used to identify connective elements while regenerating muscle fibers were identified by the Azure B bromide technique (326).

Tissue Analyses

In this study the total muscle was analyzed physiologically and the individual muscle fibers were analyzed histochemically.

Total Muscle

The muscles were analyzed physiologically to determine muscle strength, speed of contraction, relaxation characteristics, and fatigability. Twitch contractions, numbers 1 and 13, were analyzed along with tetanic contractions, numbers 12 and 16. Three 2-sec stimulations, contraction numbers 6, 7, and 9, also were studied.

Muscle strength was determined from the peak force (f = twitch, F = tetanus) developed during these seven contractions. This peak force was corrected for muscle weight and body weight to determine if either of these variables was related to muscle strength. The twitch/tetanus ratio (f/F) also was determined. The twitch value used was the largest force obtained from contraction numbers 1 and 13 while the tetanus value was the largest peak force obtained from contraction numbers 6, 7, 9, 12, or 16.

Twitch contraction time was used as a measure of muscle speed. Since Close (83) found contraction time to be influenced by the intrinsic speed of shortening of the muscle fibers, contraction time was evaluated after being corrected for tetanus peak force. Other indicators of muscle speed were found through the use of the twitch rising slope. A ratio of the twitch slope to the maximum tetanic force was used as one indicator. This value in effect normalized the average slope and eliminated the effects of the activation time of the muscle. The ratio of average slope to maximum slope for both twitches also was determined. Tetanic time to peak force was obtained for the 30-sec contractions (numbers 12 and 16).

The relative degree of fusion of the first two 2-sec contractions (numbers 6 and 7) served as an inverse indicator of contraction speed; a higher degree of fusion implied a slower muscle. This value was

corrected by the twitch/tetanus ratio to account for the activation time of the muscle. The first 2-sec contraction (number 6) also was used to obtain an estimate of "sag" as described by Burke and coworkers (70,73). This value was determined from:

$$\text{sag (grams)} = \frac{(f_2 - f_1) - 2.8 \text{ fusion (Contraction 6)}}{\text{Peak force of contraction 6}}$$

where: f_1 = largest value (grams) in first 0.4 sec

f_2 = smallest value (grams) between 0.2 and 2 sec

Measurements of f_1 and f_2 were manually made from the plot and thus included variations in force due to incomplete fusion. The term "2.8 fusion" was therefore subtracted so that fusion and sag were independent.

Relaxation time was obtained from the one-half relaxation times found after the two twitch contractions (numbers 1 and 13). No relaxation measures were determined after any of the other contractions since no others were included in the data collection program.

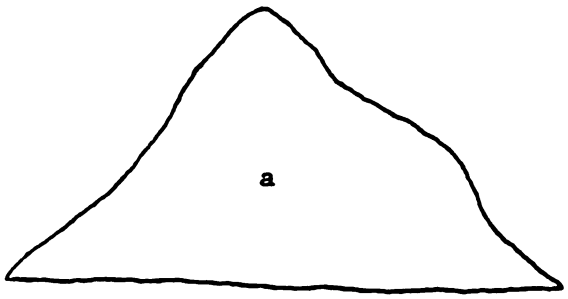
Three separate indexes of fatigue resistance were calculated. The first was a twitch fatigue index as determined by the ratio of peak force in contraction number 13 to peak force in contraction number 1. A tetanic fatigue index was obtained as the ratio of peak force in contraction number 16 to peak force in contraction number 12. The amount of fatigue within each of the two 30-sec tetanic contractions was evaluated by comparing the maximum peak force developed and the force obtained after 27.64 sec of contraction.

Individual Muscle Fibers

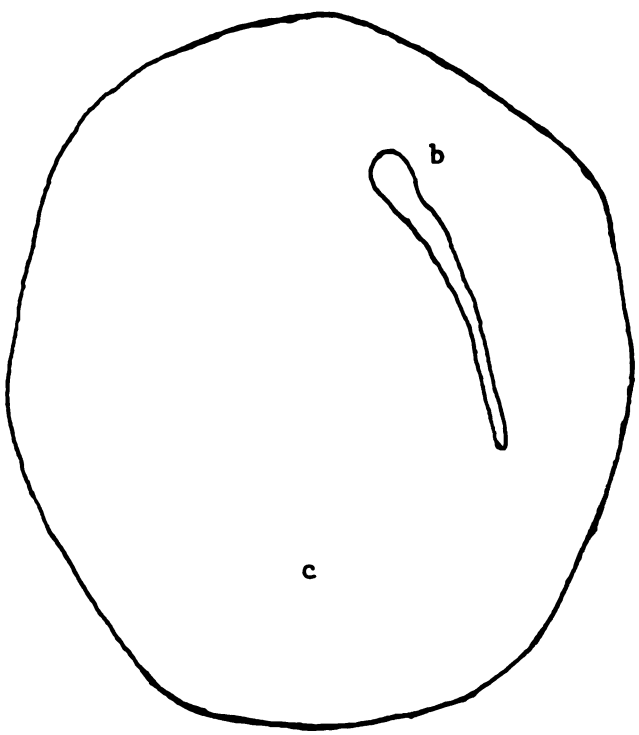
Since muscle fiber populations have been shown to be different within muscles, specific areas of the SOL and RF muscles were selected for this study (Figure 7). Pictures of each area were taken with a Zeiss photomicroscope. Muscle sections stained for ATPase were used for the SOL area. Two areas of the RF muscle were taken from the sections stained for NADH. These pictures were taken at a magnification of 40X and were further enlarged six to eight times. From each area 30 adjacent muscle fibers[1] were used to determine a muscle fiber profile. These fibers were located in the muscles stained for ATPase, αGPD, SUD, PAS, and NADH. The intensity of the stains was rated as follows: D = dark intensity, I = intermediate intensity, and L = light intensity.

Due to the appearance of a continuous spectrum of muscle fibers (114,161,235,273,296), our laboratory recently devised a new method of classifying muscle fibers (296). This technique uses the intensity of five histochemical stains to determine a fiber profile. Since ATPase has two intensities and the remaining stains (NADH, αGPD, PAS, SUD) all have three intensities, a total of 162 fiber profiles are possible with this technique. However, not all of these profiles are probable. From analyzing, condensing, and combining the fiber profiles, 12 metabolically feasible fiber types were determined (Table 9). The 12 fiber types in this classification included six different types of "fast" ("F", high

[1] In previous studies performed in this laboratory, a sample of 30 fibers was calculated to be more than necessary and sufficient for a four way (7 x 4 x 8 x 10) mixed-model nested analysis of variance when: (a) the probability of making a type I statistical error was limited to the .01 level, (b) the probability of making a type II statistical error was limited to the .05 level, (c) the minimum mean difference to be detected as significant was set at 0.5 standard deviations, and (d) moderate variability between subgroup means was assumed. Consequently, standard laboratory protocal now is to take readings on 30 fibers in each muscle area of interest.



Central (a) area of SOL muscle



Deep (b) and superficial (c) areas of RF muscle

Figure 7

Soleus and Rectus Femoris Areas Examined

Table 9

Metabolically Feasible Histochemical Fiber Types

FGL-FGgL
FG-FGg
F
FgL
FL
Fg
SGL-SGgL
SG-SGg
S
SgL
SL
Sg

ATPase) fibers and six different "slow" ("S", low ATPase) fibers. Any fiber with moderate to high glycogen storage (moderate to high PAS) had a "G" and/or a "g" as part of its designator. If the fiber could metabolize glycogen aerobically (moderate to high αGPD) the "G" symbol was used, whereas, the "g" symbol was used if the fiber could metabolize glycogen anaerobically. If the fiber could metabolize glycogen both aerobically and anaerobically, both "G" and "g" were used. Those fibers with moderate to high lipid storage (SUD) had an "L". A fiber with a high oxidative capacity (NADH) was not given a special designator letter but was accounted for when the fiber types were compiled.

All fibers were initially classified as either "fast" ("F") or "slow" ("S") with the remaining designators of the fiber type depending on the fibers metabolic characteristics. Due to the inability of a histochemical technique to distinguish between fibers with aerobic glycolytic activity, and those with both anaerobic and aerobic glycolytic activity, some fiber profiles have dual classifications. (Appendix B contains the characteristics of each muscle fiber profile.)

Three of the 12 fiber types resembled the fiber types which Peter et al. (276) found. The FGL-FGgL fibers which have high ATPase, moderate to high αGPD, PAS and SUD and low to high NADH is likened to the FOG muscle fibers. The FG-FGg fiber type with high ATPase, low PAS, moderate to high SUD and αGPD and low to high NADH is similar to the FG fiber type, while the Sgl muscle fibers having low ATPase and αGPD, moderate to high SUD and PAS, and low to high NADH are analogous to the SO fiber types.

Muscle fiber area was obtained for each fiber that was profiled. This area was determined through the use of a NUMONICS model 1224 electronic digitizer.

Analysis of Data

Fiber size was analyzed through the use of several one-, two-, and three-way analysis of variance routines on the Michigan State University Control Data 6500 Computer. A Student-Newman-Keuls test was used to evaluate differences between pairs of means whenever a significant ($P < .05$) F-ratio was obtained. All physiological data were analyzed using an apriori test calculated from a preliminary one-way analysis of variance routine.

CHAPTER IV

RESULTS AND DISCUSSION

Due to the complexity of the results this chapter is divided into the following subsections: (a) an analysis of the physical training performed by the 12-week high-intensity endurance swimming animals including the atmospheric conditions which were present during the training period, (b) an examination of the body and muscle weights of all animals, (c) a morphological and metabolic analysis of the muscles, (d) a physiological analysis of the muscles, and (e) a discussion aimed at relating the present findings with those of previous investigations reported in the literature.

Analysis of the Physical Training Performed

A performance criterion of swimming 75% of the sessions with the proper body weight attached 75% of the time was used to determine whether the animals were to be considered as trained. Seven of the eight trained animals performed better than this criterion and thus were considered to be physically trained with a high-intensity endurance swimming program. The mean and standard deviation for the number of swimming sessions completed by these seven animals was 56 ± 2.83 which represents 93% of the total number of sessions.

Since this study was part of a larger investigation, the training of the animals occurred in 12-week periods over a span of six months.

The mean air temperature throughout this time frame was 24.07 ± 1.86°C, while the average water temperature was 32.51 ± 1.19°C. The mean relative humidity was 31.44 ± 14.35% and the barometric pressure averaged 739.92 ± 7.11 mm Hg.

Analysis of Body and Muscle Weights

The body weights of the 12-week sedentary control animals were significantly greater than the body weights of either the 12-week high-intensity endurance swimming animals ($P < .05$), or the zero-week control animals ($P < .05$). The body weight (means and standard deviations) were 105.00 ± 5.36 g for the 12-week sedentary control animals, 90.86 ± 8.08 g for the 12-week high-intensity endurance swimming animals, and 50.50 ± 5.05 g for the zero-week control animals.

The absolute wet weights for both the SOL and RF muscles followed the same pattern as that observed in the body weights. That is, for the RF muscle the means and standard deviations were 0.2202 ± 0.0169 g for the 12-week sedentary control animals, 0.1881 ± 0.0164 g for the 12-week high-intensity endurance swimming animals, and 0.1243 ± 0.0328 g for the zero-week control animals. With the SOL muscle, the corresponding values for the 12-week sedentary control animals were 0.0216 ± 0.0028 g, those for the 12-week high-intensity endurance swimming animals were 0.0199 ± 0.0028 g, and those for the zero-week control animals were 0.0113 ± 0.0017 g. In general, the 12-week sedentary control animals had muscle weights that were significantly greater than those of either the 12-week high-intensity endurance swimming animals or the zero-week control animals. One exception was found when the SOL muscles of the 12-week sedentary control animals were compared with those of the trained animals. In all three animal groups the RF muscle

was significantly heavier than the SOL muscle (zero-week control, $P < .05$; 12-week sedentary control, $P < .05$; 12-week high-intensity endurance swimming, $P < .05$).

The relative muscle weights, that is the absolute muscle weights corrected for body weight, were not different between the animal groups for either of the two muscles. The means and standard deviations for the relative weights of the RF muscle were $2.097 \times 10^{-3} \pm 0.334 \times 10^{-3}$ in the 12-week sedentary control group, $2.073 \times 10^{-3} \pm 0.105 \times 10^{-3}$ in the 12-week high-intensity endurance swimming group, and $2.485 \times 10^{-3} \pm 0.712 \times 10^{-3}$ in the zero-week control group. With the SOL muscle the mean relative muscle weights were all approximately one-tenth the weight of the RF muscle (zero-week control = $2.27 \times 10^{-4} \pm 0.39 \times 10^{-4}$, 12-week sedentary control = $2.07 \times 10^{-4} \pm 0.35 \times 10^{-4}$, 12-week high-intensity endurance swimming = $2.17 \times 10^{-4} \pm 0.28 \times 10^{-4}$). The relative muscle weight of the RF muscle was significantly greater than that found with the SOL muscle at both age groups (zero-week, $P < .05$; 12-week, $P < .05$).

Histological and Histochemical Data

Each muscle was examined histologically to determine its morphological characteristics and histochemically to obtain the metabolic capabilities of its muscle fibers. Fiber size also was determined for each muscle fiber.

Results of Histological Examination

Through an evaluation of H&E, GOM and AZURE B stains, each muscle examined was found to be morphologically normal with no pathological conditions observed. In evaluating the H&E stain of the RF muscle, a large number of muscle fibers with central nuclei were noted near the centrally located connective tissue; however, since no regeneration was

apparent from the AZURE B stain these fibers were considered to be normal.

A few observable histological differences were noted between the two age groups. The zero-week animals had muscle fibers that were more rounded than those of the 12-week animals which tended to be either four or five sided. Also, the zero-week animals had small amounts of connective tissue around each muscle fasciculus, while the fasciculi of the 12-week animals were well defined.

Results of Histochemical Evaluation

In the histochemical evaluation of the muscles, the staining intensity of five stains was observed in 30 adjacent fibers. The five stains used were ATPase, NADH, αGPD, SUD, and PAS. The 30 fibers selected were considered to be typical for the muscle area observed. A superficial and a deep area were examined in the RF muscle while only the central SOL area was analyzed. Due to an inability to locate 30 adjacent fibers with all five stains in serial sections of the specified muscle areas, data were collected on only 24 RF muscles (seven zero-week control, eleven 12-week sedentary control, six 12-week high-intensity endurance swimming) and 22 SOL muscles (six zero-week control, ten 12-week sedentary control, six 12-week high-intensity endurance swimming).

Muscle Fiber Characteristics

A total of five muscle fiber types were found in the central SOL muscle area. In the deep area of the RF muscle nine muscle fiber types were observed while three were found in the superficial area of the RF muscle.

Central Soleus.--Three different types of "fast" fibers were observed in the central portion of the SOL muscle. The FGL-FGgL fibers were characterized as having high ATPase, moderate to high αGPD, PAS, and SUD, with NADH varying from low to high. This fiber type generally reflects the characteristics of the FOG muscle fiber. The FgL fiber type found in this muscle area had high ATPase with moderate to high PAS and SUD. The αGPD content was low with this fiber type; whereas, the staining intensity for NADH was similar to that of the FGL-FGgL fiber, and thus varied from low to high. The third type of "fast" fiber was the F fiber which was generally characterized as having high ATPase, moderate to high αGPD, varying SUD and NADH, and low PAS.

Two types of "slow" fibers, the SgL and SGL-SGgL fibers, were observed in the central SOL muscle. The SgL muscle fibers had low ATPase and αGPD, moderate to high SUD and PAS, and low to high NADH. The SGL-SGgL muscle fiber had similar characteristics except in aerobic glycolytic activity. The αGPD staining intensity was moderate to high with the SGL-SGgL muscle fibers. The characteristics of the SgL muscle fibers are similar to those of the SO muscle fibers.

Superficial Rectus Femoris.--Only "fast" fibers were observed in the superficial RF muscle. Two of these fiber types, the FGL-FGgL fibers and the F fibers, were the same as those previously described in the central area of the soleus muscle. The third type of "fast" fiber, the FG-FGg fiber type, was high in ATPase, moderate to high in αGPD and PAS, low to high in NADH, and low in SUD. These fibers are similar to the FG fibers.

Deep Rectus Femoris.--The deep area of the RF muscle exhibited a total on nine fiber types which included five types of "fast" fibers. The "fast" fiber types were the FGL-FGgL, FG-FGg, F, and FgL fibers previously analyzed, along with FL muscle fibers which were characterized by having high ATPase and moderate to high SUD staining intensity. In the FL muscle fibers the intensity of the NADH stain was found to vary from low to high while the intensities of the αGPD and PAS stains were low.

The "slow" fibers of the deep RF muscle area were the aforementioned SGL-SGgL and SgL fibers along with the S and SL fibers. The S fibers were generally characterized as having low ATPase and PAS, low to high NADH and SUD, and moderate to high αGPD. The SL fibers have the same staining intensities as the S fibers except the SL fibers have low levels of aerobic glycolysis.

Fiber Type Percentages

In accordance with the literature the FGL-FGgL (FOG), FG-FGg (FG), and SgL (SO) fiber types encompassed most of the muscle fibers profiled. The central area of the SOL muscle consisted of approximately 52% SgL fibers and 36% FGL-FGgL fibers. The superficial area of the RF muscle consisted of about 16% FGL-FGgL muscle fibers and 79% FG-FGg muscle fibers. The deep area of the RF muscle had 67% FGL-FGgL, 6% FG-FGg and 9% SgL muscle fibers.

Central Soleus.--The muscle fiber type distributions for the central SOL muscle area are presented in Table 10. This table shows that the overall percentages of the total number of "fast" and "slow" fibers did not change with either age or training. However, slight changes in

Table 10

Fiber Type Percentages of the Central Area of the Soleus Muscle

Fiber Type	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
FGL-FGgL	39.4%	36.0%	35.0%
F	0	1.7	0.6
FgL	1.1	3.3	6.1
SGL-SGgL	5.6	3.3	12.2
SgL	53.9	55.7	46.1
Fast	40.5	41.0	41.7
Slow	59.5	59.0	58.3
Non FGL-FGgL Non FgL	1.1	5.0	6.7
Non SgL	5.6	3.3	12.2

Table 11

Fiber Type Percentages of the Superficial Area of the Rectus Femoris Muscle

Fiber Type	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
FGL-FGgL	0 %	32.4%	16.7%
FG-FGg	97.6	67.0	71.7
F	2.4	0.6	11.7
Non FGL-FGgL Non FG-FGg	2.4	0.6	11.7

the percentages of individual fiber types were observed within the general catagories of "fast" and "slow" fibers. A slight trend of a decreasing percentage of FGL-FGgL muscle fibers was observed with age. A similar trend of an increasing percentage of FgL muscle fibers was seen. Small percentages of F fibers also were observed in the muscles of the 12-week sedentary control and 12-week high-intensity endurance swimming animals, but none was found in the central SOL muscle area of the zero-week control animals.

The SgL muscle fibers made up the largest percentage of any one fiber type in the central SOL muscle area for all three animal groups. The percentage of SgL muscle fibers was similar in the zero-week control and 12-week sedentary control animals but was much lower in the 12-week high-intensity endurance swimming group. This trend was coupled with a concommitant higher percentage of SGL-SGgL muscle fibers.

The number of fast muscle fibers not classified as FGL-FGgL or FG-FGg fibers steadily increased with both age and training. The percentage of "fast" fibers not classifiable into one of these two fiber types was 1.1% in the zero-week control animals muscle, 5.0% in the 12-week sedentary control animals muscle and 6.7% in the 12-week high-intensity endurance swimming animals muscle.

Superficial Rectus Femoris.--In the zero-week control animals the superficial RF muscle area consisted mainly of FG-FGg muscle fibers. The percentage of this type of fiber was decreased greatly in the 12-week sedentary control animals and was increased only slightly with training (Table 11). In the 12-week sedentary control animals the remaining muscle fibers were of the FGL-FGgL types, whereas in the 12-week high-intensity endurance swimming animals the rest of the fibers were either

of the FGL-FGgL type or of the F type. The F muscle fiber type only accounted for a small percentage of the fibers in this muscle area in the zero-week control and 12-week sedentary control animals' muscles.

Since the FGL-FGgL and FG-FGg fiber types account for two of the three fiber types found in this muscle area, the percentage of fibers not classifiable into one of these two types of muscle fibers was indicated by the percentage of F muscle fiber types. This percentage was found to be lowest in the 12-week sedentary control animals' muscles and highest in the trained animals' muscles.

Deep Rectus Femoris.--As observed in Table 12, the FGL-FGgL muscle fiber type made up the largest single fiber type population in the deep region of the RF muscle. In this muscle area the FGL-FGgL fiber type made up the majority of the fibers of the zero-week control animals' muscles, whereas a lesser percentage of the 12-week animals' muscles were made up of these fibers. To compensate for the decreased percentage of FGL-FGgL muscle fibers, the 12-week animals had more F and FgL fibers. The muscle fiber types FG-FGg and FL also were observed in this muscle area of the 12-week animals, while the muscles of the zero-week control animals were void of these types of fibers.

The SgL fiber type comprised most of the "slow" muscle fibers of the zero-week control and trained animals' muscles. The 12-week sedentary control animals' muscles also contained SgL muscle fibers but an almost equal percentage of SGL-SGgL and SL muscle fibers were observed in these muscles.

The percentage of "fast" fibers (and thus the percentage of "slow" fibers) varied among the three groups of animals. "Fast" fibers comprised 86.2% of the fibers observed in the zero-week control animals,

Table 12

Fiber Type Percentages of the Deep Area of the Rectus Femoris Muscle

Fiber Type	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
FGL-FGgL	83.8%	48.8%	67.8%
FG-FGg	0	7.3	13.3
F	0.5	15.2	2.2
FgL	1.9	4.8	4.4
FL	0	3.3	3.3
SGL-SGgL	1.9	6.1	0.6
S	0	0.6	0
SgL	11.4	8.2	8.3
SL	0.5	5.8	0
Fast	86.2	79.4	91.0
Slow	13.8	20.6	9.0
Non FGL-FGgL Non FG-FGg	2.4	20.0	6.6
Non SgL	2.4	12.5	0.6

79.4% of the total fibers observed in the 12-week sedentary control animals, and 91.0% of those reviewed in the trained animals.

The number of fibers not classifiable as either FGL-FGgL or FG-FGg also varied among the three groups of animals. A total of 20.0% of the "fast" fibers in the 12-week sedentary control animals were from fiber types other than FGL-FGgL or FG-FGg, while the 12-week high-intensity endurance swimming animals had 6.6% and the zero-week control animals had 2.4% of these "fast" fibers. A similar situation was found with the "slow" fibers as 12.5% of the 12-week sedentary control animals' muscle fibers, 2.4% of the zero-week control animals' muscle fibers, and 0.6% of the 12-week high-intensity endurance swimming animals' muscle fibers were not of the SgL fiber type.

Muscle Fiber Area

From an analysis of the muscle fiber area data, fiber size appears to be affected by the muscle area in which the fiber is located and the exercise treatment which the animal received. These data, which are presented in Table 13, show a significant effect of both variables.

In looking at the treatment of the animals, the zero-week control animals had muscle fibers that were significantly smaller (1261.34 ± 390.51 μ^2) than those found in the muscles of the 12-week high-intensity endurance swimming animals (2369.39 ± 769.53 μ^2), which were significantly smaller than the muscle fibers examined in the 12-week sedentary control animals (2790.06 ± 922.43 μ^2). The fibers obtained from the different muscle areas also varied in size, as the fibers of the two areas of the RF muscle were of similar size (deep RF = 2423.23 ± 1154.55 μ^2, superficial RF = 2351.51 ± 972.25 μ^2), whereas those from the SOL muscle were significantly smaller (1934.71 ± 747.26 μ^2).

Table 13

Analysis of Variance for Overall Effects for Muscle Fiber Area

Source of Variation	Sum of Squares	df	Mean Square	F Value	P Value
Main Effects	1,076,790,199	12	89,732,516	209.086	.001 *
Animal Treatment	941,626,782	2	470,813,391	1,097.044	.001 *
Muscle Area	83,941,116	2	41,970,558	97.796	.001 *
Fiber Type	99,911,189	8	12,488,899	29.100	.001 *
2-Way Interactions	127,851,166	22	5,811,417	13.541	.001 *
Animal Treatment - Muscle Area	33,327,784	4	8,331,946	19.414	.001 *
Animal Treatment - Fiber Type	7,378,961	12	614,913	1.433	.145
Muscle Area - Fiber Type	50,036,203	6	8,339,367	19.432	.001 *
3-Way Interactions	13,626,437	9	1,514,049	3.528	.001 *
Animal Treatment - Muscle Area - Fiber Type	13,626,437	9	1,514,049	3.528	.001 *
Explained	1,218,267,802	43	28,331,809	66.016	.001 *
Residual	882,364,054	2,056	429,165		
Total	2,100,631,856	2,099	1,000,777		

*Significant effect at the .05 level

Most of the interaction effects were significant in the analysis of fiber area. The only non-significant interaction was that of animal treatment with fiber type ($P = 0.145$).

Physiological Characteristics Data

Due to the many physiological characteristics which were obtained from each muscle, this section will deal primarily with those comparisons which were significantly different. Tables 14-21 contain the mean values and standard deviations of each physiological characteristic along with the F-values of the planned comparisons among the groups.

Table 14 demonstrates that with contraction number 1, the initial 2-msec pulse twitch, the only differences found were obtained in the peak force corrected for either body weight or muscle weight. The SOL muscle peak force/body weight and peak force/muscle weight were both significantly larger in the zero-week control animals than in the 12-week sedentary control animals ($P < .05$, $P < .05$; respectively). None of the other variables was significantly different among the groups for either of the muscles studied; and, therefore, no training adaptation was apparent from the data obtained with this contraction.

The data obtained from the 2-sec tetanus contractions (Table 15) show similar trends in the first two contractions, even though different stimuli were used. With contractions 6 and 7 (10 and 20 PPS; respectively) no differences were observed in the RF muscle; yet, the SOL muscles of the 12-week sedentary control animals produced a greater force than did those of the zero-week control animals ($P < .05$ for

Table 14

Physiological Characteristics Obtained from Contraction Number 1

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Peak Force (g)			
Rectus Femoris	8.1649 ± 2.6655	21.61 ± 21.56	19.414 ± 12.7672
		(F = 3.0311, df = 17)	(F = 0.0589, df = 16)
Soleus	3.3596 ± 0.9329	2.8544 ± 0.8369	2.8314 ± 1.5868
		(F = 1.4287, df = 16)	(F = 0.0010, df = 15)
Peak Force/Body Weight			
Rectus Femoris	0.1684 ± 0.0747	0.2035 ± 0.1961	0.2153 ± 0.1391
		(F = 0.2296, df = 17)	(F = 0.0191, df = 16)
Soleus	0.0669 ± 0.0189	0.0273 ± 0.0083	0.0301 ± 0.0163
		(F = 38.0566*, df = 16)	(F = 0.2389, df = 15)
Peak Force/Muscle Weight			
Rectus Femoris	71.4963 ± 33.2388	95.2118 ± 90.1627	103.7114 ± 68.9047
		(F = 0.5684, df = 17)	(F = 0.0450, df = 16)
Soleus	295.9286 ± 65.0371	134.6591 ± 44.7624	141.6417 ± 83.3094
		(F = 39.1951*, df = 16)	(F = 0.0518, df = 15)

Table 14 (continued)

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Contraction Time (msec)			
Rectus Femoris	84.5 ± 13.6277	91.45 ± 16.6049	103.64 ± 16.3980
		(F = 0.9386, df = 17)	(F = 2.3265, df = 16)
Soleus	148.0 ± 41.3511	132.5 ± 25.5245	144.3333 ± 13.3029
		(F = 0.9803, df = 16)	(F = 1.1017, df = 15)
One Half Relaxation Time (msec)			
Rectus Femoris	78.5 ± 20.0909	69 ± 13.1757	65.8571 ± 9.0725
		(F = 1.5578, df = 17)	(F = 0.3032, df = 16)
Soleus	253.2143 ± 67.4727	246.1818 ± 38.1460	230.5 ± 43.2296
		(F = 0.0808, df = 16)	(F = 0.5994, df = 15)

*Significant comparison at the .05 level

Table 15

Physiological Characteristics Obtained from 2-sec Contractions

	Contraction Number 6			Contraction Number 7			Contraction Number 9		
	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Peak Force (g)									
Rectus Femoris	25.0838 ± 13.6557	48.4345 ± 43.8182	41.5367 ± 32.5467	38.4863 ± 15.7142	86.1427 ± 71.9798	69.5343 ± 58.7189	41.4763 ± 14.3516	119.6500 ± 90.3387	88.8086 ± 71.6130
	(F = 2.0935, df = 17)	(F = 0.0316, df = 16)		(F = 3.3390, df = 17)	(F = 0.2022, df = 16)		(F = 5.7932*, df = 17)	(F = 0.5793, df = 16)	
Soleus	14.9616 ± 3.2544	19.0518 ± 4.0211	16.3500 ± 7.8498	17.7014 ± 3.0923	24.8709 ± 6.1952	20.5802 ± 9.1940	17.6129 ± 3.8555	28.2018 ± 6.8387	23.4373 ± 9.5291
	(F = 5.0837*, df = 16)	(F = 0.9050, df = 15)		(F = 7.9744*, df = 16)	(F = 1.3294, df = 15)		(F = 13.7812*, df = 16)	(F = 1.4328, df = 15)	
Peak Force/Body Weight									
Rectus Femoris	0.5336 ± 0.3695	0.4560 ± 0.3973	0.4609 ± 0.3590	0.8035 ± 0.4345	0.8122 ± 0.6587	0.7693 ± 0.6486	0.8467 ± 0.3371	1.1284 ± 0.8280	0.1814 ± 0.7865
	(F = 0.1816, df = 17)	(F = 0.0007, df = 16)		(F = 1.4061, df = 17)	(F = 0.0184, df = 16)		(F = 0.8176, df = 17)	(F = 0.1603, df = 16)	
Soleus	0.2956 ± 0.0526	0.1816 ± 0.0378	0.1748 ± 0.0804	0.3512 ± 0.0513	0.2367 ± 0.0580	0.2207 ± 0.0958	0.3487 ± 0.0636	0.2682 ± 0.0628	0.2517 ± 0.0988
	(F = 28.8240*, df = 16)	(F = 0.0573, df = 15)		(F = 18.1629*, df = 16)	(F = 0.1858, df = 15)		(F = 6.9828*, df = 16)	(F = 0.1734, df = 15)	
Peak Force/Muscle Weight									
Rectus Femoris	225.1400 ± 163.5802	211.9655 ± 178.3139	222.1314 ± 178.5798	336.2175 ± 179.4173	378.5000 ± 294.2962	371.8357 ± 324.3810	358.4350 ± 146.5182	528.0091 ± 370.0379	474.6629 ± 393.3642
	(F = 0.0207, df = 17)	(F = 0.0139, df = 16)		(F = 0.1290, df = 17)	(F = 0.0020, df = 16)		(F = 1.4899, df = 17)	(F = 0.0850, df = 16)	
Soleus	1323.9000 ± 274.1954	897.9182 ± 224.7633	817.8333 ± 409.6117	1569.9143 ± 261.6688	1176.6182 ± 329.7455	1030.1000 ± 480.4884	1572.0143 ± 370.9219	1336.4091 ± 374.7591	1176.9667 ± 502.3492
	(F = 12.9874*, df = 16)	(F = 0.2778, df = 15)		(F = 7.0664*, df = 16)	(F = 0.5577, df = 15)		(F = 1.7038, df = 16)	(F = 0.5553, df = 15)	

*Significant comparison at the .05 level

Table 16

Physiological Characteristics Obtained from Contraction Number 12

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Peak Force (g)			
Rectus Femoris	33.0612 ± 13.2560	63.1600 ± 60.9978	51.7219 ± 53.7949
		(F = 1.8559, df = 17)	(F = 0.1641, df = 16)
Soleus	17.6971 ± 3.8762	26.5082 ± 6.7631	21.3642 ± 8.7478
		(F = 9.7045*, df = 16)	(F = 1.8344, df = 15)
Peak Force/Body Weight			
Rectus Femoris	0.6875 ± 0.3497	0.5951 ± 0.5695	0.5725 ± 0.5972
		(F = 0.1642, df = 17)	(F = 0.0057, df = 16)
Soleus	0.3502 ± 0.0633	0.2522 ± 0.0634	0.2296 ± 0.0901
		(F = 10.2291*, df = 16)	(F = 0.3708, df = 15)
Peak Force/Muscle Weight			
Rectus Femoris	288.9888 ± 148.3484	278.9055 ± 262.0261	276.8400 ± 298.3020
		(F = 0.0164, df = 17)	(F = 0.0001, df = 16)
Soleus	1577.9714 ± 370.5737	1255.6091 ± 362.2333	1071.1667 ± 456.2417
		(F = 3.3299, df = 16)	(F = 0.8420, df = 15)

Table 16 (continued)

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Time to Peak (sec)			
Rectus Femoris	4.8512 ± 1.3458	9.8036 ± 2.3826	7.6343 ± 2.4044
		(F = 27.8066*, df = 17)	(F = 3.5220, df = 16)
Soleus	3.0043 ± 1.1310	7.0809 ± 3.7089	6.0417 ± 2.1821
		(F = 7.8322*, df = 16)	(F = 0.3898, df = 15)
Time from Peak to Max Negative Slope (sec)			
Rectus Femoris	16.3975 ± 3.0256	19.0436 ± 3.5552	18.6043 ± 3.6142
		(F = 2.8944, df = 17)	(F = 0.0645, df = 16)
Soleus	15.3250 ± 2.6304	22.7000 ± 5.1474	19.1400 ± 6.1326
		(F = 9.7307*, df = 10)	(F = 0.9708, df = 8)
Time from End Point to 1/2 End Point Force (sec)			
Rectus Femoris	15.9300 ± 4.1979	13.9400 ± 0	21.3800 ± 4.8199
		(F = 0.1926, df = 5)	(F = 1.7870, df = 2)
Soleus	107.5467 ± 39.1353	88.3550 ± 33.9482	80.7733 ± 19.8794
		(F = 0.3763, df = 6)	(F = 0.1067, df = 3)

*Significant comparison at the .05 level

Table 17

Physiological Characteristics Obtained from Contraction Number 13

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Peak Force (g)			
Rectus Femoris	1.6774 ± 1.0341	0.7046 ± 1.1420	1.1075 ± 1.3321
		(F = 3.9980, df = 17)	(F = 0.4691, df = 16)
Soleus	4.1527 ± 1.7136	2.0911 ± 0.8673	1.9224 ± 1.1862
		(F = 11.5709*, df = 16)	(F = 0.1139, df = 15)
Peak Force/Body Weight			
Rectus Femoris	0.0362 ± 0.0259	0.0068 ± 0.0115	0.0124 ± 0.0150
		(F = 11.2567*, df = 17)	(F = 0.7971, df = 16)
Soleus	0.0816 ± 0.0313	0.0198 ± 0.0079	0.0204 ± 0.0121
		(F = 40.2451*, df = 16)	(F = 0.0198, df = 15)
Peak Force/Muscle Weight			
Rectus Femoris	14.9686 ± 10.7259	3.1482 ± 5.2551	5.9917 ± 7.4523
		(F = 10.1723*, df = 17)	(F = 0.9029, df = 16)
Soleus	370.9 ± 161.5413	99.1945 ± 42.6643	94.2117 ± 54.8440
		(F = 28.9100*, df = 16)	(F = 0.0435, df = 15)

Table 17 (continued)

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Contraction Time (msec)			
Rectus Femoris	113.0000 ± 12.1155	90.6818 ± 24.9332	91.7857 ± 19.0675
		(F = 5.4140*, df = 17)	(F = 0.0099, df = 16)
Soleus	161.4286 ± 18.0033	125.8636 ± 11.7709	125.0000 ± 20.1221
		(F = 25.9957*, df = 16)	(F = 0.0127, df = 15)
One Half Relaxation Time (msec)			
Rectus Femoris	140.3125 ± 32.4686	495.0909 ± 300.0502	272.0714 ± 297.9427
		(F = 10.9184*, df = 17)	(F = 2.3756, df = 16)
Soleus	209.2143 ± 48.2259	164.0455 ± 22.0493	145.9167 ± 43.7726
		(F = 7.4213*, df = 16)	(F = 1.3253, df = 15)

*Significant comparison at the .05 level

Table 18

Physiological Characteristics Obtained from Contraction Number 16

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Peak Force (g)			
Rectus Femoris	9.9488 ± 3.9833	10.7888 ± 12.0866	13.8100 ± 15.5571
		(F = 0.0353, df = 17)	(F = 0.2153, df = 16)
Soleus	16.8100 ± 3.8161	22.9973 ± 6.6528	18.1113 ± 7.6254
		(F = 4.9440*, df = 16)	(F = 1.8959, df = 15)
Peak Force/Body Weight			
Rectus Femoris	0.2065 ± 0.1062	0.1024 ± 0.1150	0.1525 ± 0.1729
		(F = 4.0485, df = 17)	(F = 0.5529, df = 16)
Soleus	0.3324 ± 0.0628	0.2187 ± 0.0625	0.1944 ± 0.0787
		(F = 14.0963*, df = 16)	(F = 0.4901, df = 15)
Peak Force/Muscle Weight			
Rectus Femoris	91.5563 ± 58.7575	48.4009 ± 54.4576	73.3741 ± 86.4836
		(F = 2.7245, df = 17)	(F = 0.5958, df = 16)
Soleus	1537.0286 ± 436.2663	1091.5545 ± 352.7621	906.8167 ± 396.0272
		(F = 5.5654*, df = 16)	(F = 0.9797, df = 15)

Table 18 (continued)

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Time to Peak Force (sec)			
Rectus Femoris	3.6450 ± 1.4267	12.8136 ± 3.1480	12.6143 ± 2.8112
		($F = 58.3941^*$, df = 17)	($F = 0.0186$, df = 16)
Soleus	3.4986 ± 1.1608	10.9291 ± 4.4269	9.2083 ± 2.4435
		($F = 18.5193^*$, df = 16)	($F = 0.7635$, df = 15)
Time from Peak to Max Negative Slope (sec)			
Rectus Femoris	17.9050 ± 3.1784	21.2100 ± 6.6014	26.4275 ± 3.8203
		($F = 1.5573$, df = 12)	($F = 1.9974$, df = 8)
Soleus	15.3786 ± 2.8470	12.7685 ± 8.4723	14.5492 ± 6.5359
		($F = 0.2048$, df = 9)	($F = 0.2565$, df = 6)
Time from End Point to 1/2 End Point Force (sec)			
Rectus Femoris	19.6850 ± 6.0708	42.0800 ± 0	
		($F = 11.6643^*$, df = 5)	
Soleus	116.4986 ± 45.3892	223.4733 ± 33.3629	127.6367 ± 39.0435
		($F = 13.1798^*$, df = 8)	($F = 0.4471^*$, df = 4)

*Significant comparison at the .05 level

Table 19

Physiological Measures of Muscle Speed

	Zero-Week	12- Week Sedentary	12-Week High Intensity Swimming
(Peak Force x 100)/(Contraction Time x F) Contractions 1 & 13 (g/msec/g)			
Rectus Femoris	0.2425 ± 0.0249	0.2033 ± 0.0966	0.2293 ± 0.0336
		(F = 0.9097, df = 17)	(F = 0.4164, df = 16)
Soleus	0.1434 ± 0.0356	0.0858 ± 0.0278	0.0789 ± 0.0275
		(F = 14.8071*, df = 16)	(F = 0.2437, df = 15)
Average S1/Max S1 Contraction Number 1			
Rectus Femoris	0.5525 ± 0.0459	0.5389 ± 0.0844	0.5295 ± 0.0948
		(F = 0.1686, df = 17)	(F = 0.0487, df = 16)
Soleus	0.3645 ± 0.0621	0.3670 ± 0.0590	0.3737 ± 0.0843
		(F = 0.0074, df = 16)	(F = 0.0368, df = 15)
Average S1/Max S1 Contraction Number 13			
Rectus Femoris	0.3947 ± 0.0815	0.3422 ± 0.1056	0.3620 ± 0.0852
		(F = 0.9220, df = 10)	(F = 0.0981, df = 7)
Soleus	0.3645 ± 0.0473	0.3634 ± 0.0711	0.3373 ± 0.1065
		(F = 0.0011, df = 16)	(F = 0.3697, df = 15)

Table 19 (continued)

	Zero- Week	12- Week Sedentary	12-Week High Intensity Swimming
Fusion x F/f Contraction Number 6			
Rectus Femoris	0.7533 ± 0.3894	0.9348 ± 0.4300	1.3340 ± 0.2211
		(F = 0.8913, df = 17)	(F = 5.0900*, df = 16)
Soleus	0.0990 ± 0.0661	0.0776 ± 0.0988	0.0536 ± 0.0194
		(F = 0.2532, df = 16)	(F = 0.3345, df = 15)
Fusion x F/f Contraction Number 7			
Rectus Femoris	0.0800 ± 0.0624	0.0774 ± 0.0407	0.1195 ± 0.0419
		(F = 0.4540, df = 17)	(F = 0.5903, df = 16)
Soleus	0.0274 ± 0.0389	0.0250 ± 0.0438	0.0195 ± 0.0130
		(F = 0.0143, df = 16)	(F = 0.0880, df = 15)
Sag			
Rectus Femoris	-0.1239 ± 0.1539	-0.2084 ± 0.0842	-0.2500 ± 0.1079
		(F = 3.3262, df = 11)	(F = 0.7541, df = 14)

*Significant comparison at the .05 level

Table 20

Physiological Measures of Muscle Strength

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Force (F) (g)			
Rectus Femoris	43.5550 ± 16.5567	119.6500 ± 90.3387	88.8086 ± 71.6138
		(F = 5.4583*, df = 17)	(F = 0.5793, df = 16)
Soleus	18.4900 ± 3.1799	28.2436 ± 6.7280	23.4423 ± 9.5186
		(F = 12.6843*, df = 16)	(F = 1.4823, df = 15)
Force/Body Weight			
Rectus Femoris	0.8995 ± 0.4270	1.1284 ± 0.8280	0.9814 ± 0.7865
		(F = 0.5755, df = 17)	(F = 0.1875, df = 16)
Soleus	0.3664 ± 0.0482	0.2689 ± 0.0618	0.2520 ± 0.0985
		(F = 12.6117*, df = 16)	(F = 0.1853, df = 15)
Force/Muscle Weight			
Rectus Femoris	380.1725 ± 182.9737	528.0091 ± 370.0379	474.6629 ± 393.3642
		(F = 1.0731, df = 17)	(F = 0.0848, df = 16)
Soleus	1642.7143 ± 291.2875	1337.7455 ± 371.3176	1176.9667 ± 502.3492
		(F = 3.3753, df = 16)	(F = 0.5732, df = 15)

Table 20 (continued)

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Twitch/Tetanus (f/F)			
Rectus Femoris	0.2000 ± 0.0512	0.1771 ± 0.0678	0.2357 ± 0.0354
		(F = 0.6384, df = 17)	(F = 4.3827, df = 16)
Soleus	0.1811 ± 0.0339	0.1074 ± 0.0468	0.1119 ± 0.0353
		(F = 12.8888*, df = 16)	(F = 0.0420, df = 15)

*Significant comparison at the .05 level

Table 21

Physiological Measures of Muscular Fatigue Resistance

	Zero-Week	12-Week Sedentary	12-Week High Intensity Swimming
Peak Force Ratio - Twitch Contraction			
Rectus Femoris	0.1974 ± 0.0616	0.0246 ± 0.0321	0.0448 ± 0.0299
		(F = 63.2407*, df = 17)	(F = 1.7572, df = 16)
Soleus	1.2333 ± 0.4756	0.7788 ± 0.3574	0.6631 ± 0.1286
		(F = 5.3680*, df = 16)	(F = 0.5724, df = 15)
Peak Force Ratio - 30 sec Contraction			
Rectus Femoris	0.3099 ± 0.0728	0.1633 ± 0.0577	0.2531 ± 0.0444
		(F = 24.0061*, df = 17)	(F = 12.2388*, df = 16)
Soleus	0.9480 ± 0.0168	0.8575 ± 0.0547	0.8388 ± 0.0305
		(F = 17.7014*, df = 16)	(F = 0.5906, df = 15)
Ratio of End Force to Peak Force - Contraction Number 12			
Rectus Femoris	0.4953 ± 0.1109	0.6331 ± 0.1157	0.6378 ± 0.0874
		(F = 6.8048*, df = 17)	(F = 0.0082, df = 16)
Soleus	0.8194 ± 0.0412	0.8772 ± 0.0499	0.8413 ± 0.0350
		(F = 6.5147*, df = 16)	(F = 2.4152, df = 15)

Table 21 (continued)

	Zero-Week	12- Week Sedentary	12-Week High Intensity Swimming
Ratio of End Force to Peak Force - Contraction Number 16			
Rectus Femoris	0.5019 ± 0.0814	0.8089 ± 0.0662	0.8714 ± 0.0738
		(F = 68.6827*, df = 14)	(F = 2.9894, df = 13)
Soleus	0.8386 ± 0.0361	0.9249 ± 0.0280	0.9128 ± 0.0407
		(F = 30.9303*, df = 15)	(F = 0.5045, df = 14)
Percent Loss of Peak Force - Contraction Number 12			
Rectus Femoris	2.2125 ± 0.4715	2.0136 ± 0.4608	1.8043 ± 0.3350
		(F = 0.8453, df = 17)	(F = 1.0727, df = 16)
Soleus	0.7329 ± 0.1643	0.5764 ± 0.1929	0.7283 ± 0.1009
		(F = 3.1389, df = 16)	(F = 3.1798, df = 15)
Percent Loss of Peak Force - Contraction Number 16			
Rectus Femoris	2.0700 ± 0.2673	1.1588 ± 0.3201	0.8086 ± 0.3538
		(F = 38.1072*, df = 14)	(F = 4.0453, df = 13)
Soleus	0.6671 ± 0.1363	0.4140 ± 0.1049	0.4567 ± 0.1651
		(F = 18.7957*, df = 15)	(F = 0.4060, df = 14)

*Significant comparison at the .05 level

contraction number 6; $P < .05$ for contraction number 7). When analyzing the peak force corrected for body weight and muscle weight, the zero-week control animals produced more force (peak force/body weight: $P < .05$ for contraction number 6; $P < .05$ for contraction 7; peak force/muscle weight: $P < .05$ for contraction number 6; $P < .05$ for contraction number 7). With the third 2-sec tetanus stimulation, contraction number 9, slightly different results were obtained. Both the SOL and RF muscles from the 12-week sedentary control animals produced a greater peak force than did the muscles from the zero-week control animals. However, only the SOL peak force corrected for body weight was different between the zero-week control and the 12-week sedentary control animals, with the zero-week control muscles producing more force per body weight ($P < .05$). None of the other comparisons with these contractions was significantly different.

The SOL peak force obtained in contraction number 12, the first 30-sec tetanus, was greater in the 12-week sedentary control animals than in the zero-week control animals ($P < .05$) (Table 16). When considering body weight, the SOL peak forces were reversed as the zero-week control animals had a greater peak force/body weight than did the 12-week sedentary control animals ($P < .05$). The time to peak force was faster for both the SOL and RF muscles in the zero-week control animals than in the 12-week sedentary control animals ($P < .05$, $P < .05$; respectively). The time from peak force to the maximum negative slope was also faster for the SOL muscles in the zero-week control animals than in the 12-week sedentary control animals ($P < .05$). Except for the aforementioned differences for this contraction, none of the variables was found to be different in the RF muscle among the three groups. No

differences were observed among the three groups for either muscle in the peak force/muscle weight or in the time from the end point force to one-half the end point force. There was also no difference obtained between the data collected on the 12-week sedentary control and the 12-week high-intensity endurance swimming animals when analyzing either muscle.

As noted in Table 17 with contraction number 13, the second twitch contraction which was recorded, peak force was significantly greater for the SOL muscle in the zero-week control animals than in the 12-week sedentary control animals ($P < .05$). There was no difference with this variable when measuring the RF muscle. However, peak force corrected for both body and muscle weight was greater for the SOL and RF muscles in the zero-week control animals than in the 12-week sedentary control animals (peak force/body weight: $P < .05$, SOL; $P < .05$, RF: peak force/muscle weight: $P < .05$, SOL; $P < .05$, RF). The contraction time of this twitch was less for both the 12-week sedentary control animals' SOL and RF muscles when compared to their zero-week counterparts ($P < .05$, SOL; $P < .05$, RF). The one-half relaxation time of the SOL muscle was faster in the 12-week sedentary control animals than in the zero-week control animals ($P < .05$) but slower for the RF muscle in the 12-week sedentary control animals than in the zero-week control animals ($P < .05$). All of the comparisons between the 12-week sedentary control animals and 12-week high-intensity endurance swimming group were not significantly different.

For the SOL muscle, peak force for contraction number 16 was greater in the 12-week sedentary control animals than in the zero-week control animals ($P < .05$); however, peak force corrected for body and muscle weight was greater in the zero-week control animals than in the 12-week sedentary control animals (peak force/body weight, $P < .05$;

peak force/muscle weight, $P < .05$) (Table 18). None of these variables was different between the two groups when the RF muscle was tested. The time to peak force for both muscles was less in the zero-week control animals than in the 12-week sedentary control animals ($P < .05$, SOL; $P < .05$, RF). While the SOL and RF times from peak force to maximum negative slope were not different among any of the groups, the time from the end point force to one-half the end point force was shorter for both muscles in the zero-week control animals than in the 12-week sedentary control animals ($P < .05$, SOL; $P < .05$, RF). This time was also shorter in the SOL muscle from the 12-week high-intensity endurance swimming animals than in that from the muscles of the 12-week sedentary control animals ($P < .05$). This was the only training adaptation apparent from this contraction.

Other than the twitch contraction times already mentioned, only two measures of muscle speed were found to yield significant differences among the groups (Table 19). SOL muscle contraction time corrected for tetanus peak force was found to be faster in the 12-week sedentary control animals than in the zero-week control animals ($P < .05$). There was no difference with this variable for the RF muscle between these two groups. Another indicator of speed, the development of fusion during the first 2-sec contraction (contraction number 6), showed that the RF muscle developed fusion in the 12-week sedentary control animals faster than in the 12-week high-intensity endurance swimming animals ($P < .05$). None of the other contractile characteristics were significantly different between the 12-week sedentary control animals and the 12-week high-intensity endurance swimming animals.

The measures dealing with strength, defined as the largest peak force obtained from contraction numbers 6, 7, 9, 12, and 16, were found

to be significantly greater for both muscles in the 12-week sedentary control animals than in the zero-week control animals ($P < .05$, SOL; $P < .05$, RF) (Table 20). When this peak force value was corrected for body weight, the SOL muscle was still stronger in the 12-week sedentary control animals than in the zero-week control animals ($P < .05$); however, no difference was observed with the RF muscle. Peak tetanic force corrected for muscle weight was not different for either the SOL or the RF muscles when comparing these two groups of animals. The last measure of strength the twitch/tetanus ratio, was found to be greater for the SOL muscle in the zero-week control animals than in the 12-week sedentary control animals ($P < .05$). Again, this measure was not different between the two animal groups when the data obtained from the RF muscle was analyzed. Apparently, there was no significant adaptation to the exercise regimen as demonstrated by these strength measure with either muscle.

Table 21 contains the results of the fatiguability measures. Most of these measures were found to be significantly different between the muscles of the zero-week control animals and those of the 12-week sedentary control animals. Both the twitch and 30-sec contractions were found to be less fatigued in the muscles of the zero-week control animals as determined by the ratios of contraction numbers 13:1 and 16:12 (twitch: $P < .05$, SOL; $P < .05$, RF: 30-sec: $P < .05$, SOL; $P < .05$, RF). The only significant fatigue adaptation to occur with training was in the peak force ratio for the 30-sec contraction (16:12) of the RF muscles of the trained animals which was larger than that of the 12-week sedentary control animals ($P < .05$). In the 30-sec tetanic contractions, the ratio of end force to peak force showed greater fatigue to be characteristic of the zero-week control animals. This observation was

true for both the SOL and RF muscles and for both 30-sec contractions (contraction number 12: $P < .05$, SOL; $P < .05$, RF: contraction number 16: $P < .05$, SOL; $P < .05$, RF). The percentage loss of peak force was not different among the muscles of any of the animal groups for contraction number 12, while the percentage loss in contraction number 16 was greater for both muscles in the zero-week control animals than in the 12-week sedentary control animals ($P < .05$, SOL; $P < .05$, RF).

Discussion

The data concerning the body and muscle weights is in concert with that reported in the literature. That is, the weights increased with age, but these increases were retarded when a physical training program was imposed (108,114,141,157,185,189,203,230,262,305). Since no significant differences were found in the relative muscle weights, it appears that the SOL and RF muscles and the body developed at the same rate.

The percentages of "slow" and "fast" twitch fibers did not change in the SOL muscle with either growth or physical training. In the deep area of the RF muscles, however, changes in fiber type percentages were observed. In this area 13.3% of the fibers were "slow" twitch in the zero-week control animals, 20.6% of the fibers were "slow" twitch in the 12-week sedentary control animals, and only 9.0% of the fibers were "slow" twitch in the 12-week trained animals. Since the superficial RF muscle contains only fast fibers no changes in fiber percentages were observed in this muscle area.

The data obtained from the SOL muscle is in agreement with that in the literature which found no change in the percentage of "slow" and "fast" twitch fibers with growth (49,50,98,108,173,300). Thus, the data

obtained from the deep area of the RF muscle disagrees with this information. However, the data from the deep area of the RF muscle is in agreement with the literature which found an increase in the percentage of FOG (FGL-FGgL) muscle fibers after a period of physical training (12, 114,115,121,138,315). In these studies the increase in the percentage of FOG fibers was paralled by a decrease in the FG fiber population (13, 114,115,121,138) or SO fiber population (315). In the present experiment the increase in the FGL-FGgL fiber population was proportionate to the decrease in the F, SGL-SGgL and SL fiber populations.

In accordance with the literature, the FGL-FGgL (FOG), FG-FGg (FG), and SgL (SO) fiber types encompassed most of the muscle fibers profiled. However, as was previously noted other fiber types were observed which deviated from the three main fiber types.

In the zero-week control animals 6.7% of the SOL fibers were different from the three main fiber types (5.6% "slow", 1.1% "fast"). This percentage was 8.3 for the muscles from the 12-week sedentary control animals (3.3% "slow", 5.0% "fast") and 18.9 for those from the trained animals (12.2% "slow", 6.7% "fast"). It would appear that age slightly enhanced the ability of both the "fast" and "slow" fibers of the central area of the SOL muscle to metabolize glycogen anaerobically. Endurance training, on the other hand, caused an increase in both anaerobic and aerobic glycolytic metabolism of the slow fibers and a slight decrease in these activities of the fast fibers.

In the superficial area of the RF muscle, the fibers not classified as FGL-FGgL (FOG) or FgL (FG) accounted for 2.4% of the fibers in the zero-week control animals, 0.6% of the fibers in the 12-week sedentary control animals, and 11.7% of the fibers in the trained animals. With increasing age it would appear that this muscle area becomes more

oxidative while retaining its glycolytic activity. However, with training the amount of anaerobic and aerobic glycolytic activity was reduced while the oxidative capacity was not necessarily increased. The ability to store glycogen was decreased in these fibers.

The deep area of the RF muscle reflected the greatest alteration in the percentage of fibers varying from the three main fiber types. In the zero-week control animals, 4.8% of the fibers were found to deviate from the three main fiber types (2.4% "slow", 2.4% "fast). This percentage increased to 32.5% of the muscle fibers in the 12-week sedentary control animals (12.5% "slow", 20.0% "fast") but decreased to 7.2% of the observed fibers in the 12-week high-intensity endurance swimming animals (0.6% "slow", 6.6% "fast"). The age-related trend in this muscle area was for the "slow" fibers to have an increased ability to metabolize glycogen both anaerobically and aerobically, while the "fast" fibers had a reduced ability to metabolize glycogen aerobically. A reduction in the ability to store glycogen also was observed in both the "fast" and "slow" muscle fibers. With training the anaerobic and aerobic glycolytic ability of the "fast" muscle fibers was increased, but not enough to achieve the level observed in the zero-week control animals. The only training effect on the "slow" fibers was a decrease in the capacity to metabolize glycogen. Glycogen storage was increased in both fiber types with training.

The apparent shift in fiber profiles induced by age disagrees with the literature reviewed in which muscle fibers were found to have adult characteristics by at least three to four weeks of age (49,50,89,90,108 153,178,183,235,281,300). None of these earlier studies, however, was performed on hamsters. It should be noted that the zero-week control

animals in this study were five weeks old, well past the age when most animal muscle is developed.

There appear to be two separate possibilities to explain why the "fast" muscle fibers of the trained animals generally declined in their ability to metabolize and store glycogen while the "slow" fibers generally increased their capabilities of anaerobic and aerobic glycolytic metabolism. Initially, the tendancy could be an adaptation to the aerobic endurance training program to which the animals were subjected. This type of training program might stimulate the "slow" fibers to a greater extent than the "fast" fibers; thus the corresponding increase and decrease in glycolysis. However, similar training programs have been shown to increase the glycolytic activity in all three muscle fiber types (41,136,162,305). Thus this explanation does not seem probable. Second, the decline in the glycogen storage ability of the "fast" twitch fibers of these muscles could be attributed to the mode of sacrifice. In this study, immediately after decapitation the animals could be observed to go through a few spontaneous contractions which could have affected the stored glycogen levels. Marquez-Julio and French (243), in their study comparing muscles from rats which were either decapitated or anesthetized with pentobarbitol, found that the glycogen content was 40% less in the muscles of the decapitated animals. This would seem to be a possible explanation for the decrease in glycogen storage capacity in these animals, as similar exercise programs have been shown to increase the amount of glycogen stored in the trained muscles (119,136, 262). However, since all animal groups in this study were sacrificed by the same techniques this occurrance does not appear to be the primary cause for the lower glycogen levels in the fast twitch fibers of the trained animals.

Whether or not the oxidative capacity of the muscle fibers increased is difficult to discern from the present data. The deep area of the older animals RF muscle decreased in the percentage of FGL-FGgL muscle fibers and increased in the percentage of FG-FGg muscle fibers. This data is in agreement with that of earlier investigators who found the oxidative capacity of muscle fibers to decline with age (90,91,229,234). However, the animals in this study were older than the animals used in most of the previous studies. In the superficial area of the RF muscle the opposite trend was seen as the percentage of FGL-FGgL muscle fibers increased with age and the percentage of FG-FGg muscle fibers decreased with age. Since this observation is in conflict with the literature it is difficult to explain the exact nature of this reversal. With endurance training the percentage of FGL-FGgL fibers increased in the deep area of the RF muscle and decreased in the superficial area of this muscle. Since with similar training programs an increase in the oxidative capacity of the muscle has been found (13,41,103,115,121,136,141, 149,162,185,189,252,258), the explanation for the decrease in FGL-FGgL muscle fibers in the superficial area of this muscle is not readily apparent. Whether or not the other fiber types have increased or decreased in their oxidative capacity is difficult to analyze from the present data as the fiber profiles generally do not account for changes in the NADH staining intensity. As can be seen by examining the table of the metabolic characteristics for each type of fiber (Appendix B), fibers could have low, moderate, or high levels of oxidative activity and still have the same metabolic profile. Thus, an increase or a decrease in the oxidative capacity of a muscle fiber might go unnoticed with the present scheme of fiber profiles if all of the other metabolic characteristics of the muscle fiber remained the same.

The data obtained on the muscle fiber area appears to be in agreement with the literature concerning the effect of growth and development, but in disagreement concerning the effects of training. In previous studies (78,121,162,324) trained animals were found to have larger muscle fibers than sedentary animals. In the present experiment this observation was reversed, but is in agreement with the data presented by Faulkner and coworkers (142,143). The phenomenon could be due to the fact that the trained animals' body and muscle weights were less than those of the sedentary animals. The reason why the muscle fiber type had no effect on muscle fiber size is hard to explain as other investigations have shown the different muscle fiber types to have different muscle fiber sizes (69,72,116,212,214,227,229,241,288,299,323).

The main cause for differences in the physiological characteristics of the muscles appears to be the growth and development of these animals and not necessarily their performance in the high-intensity endurance swimming program. Generally, the 12-week sedentary control animals' muscles were stronger than those of the zero-week control animals. However, when the effects of either body weight or muscle weight were eliminated, the zero-week animals' muscles appeared to be stronger. This is in agreement with the results of Close (81) and Mann and Salafsky (242) both of whom found that peak tension rose with development in rats and cats up to 14-18 weeks of age.

In looking at contraction times, the twitch contraction times of the 12-week sedentary control animals' muscles were faster for both the SOL and RF muscles. This is in agreement with those investigators who found a decrease with age in contraction time of both "fast" twitch muscle fibers (52,57,82,83,177,178,184,242) and "slow" twitch muscle fibers (52,83). When examining the time to reach maximum tetanic tension in the

30-sec contraction, the zero-week control animals' muscles were faster than those of the 12-week sedentary control animals. These results were true with both 30-sec contractions (contraction numbers 12 and 16) obtained on either the SOL or the RF muscle.

The larger twitch/tetanus ratio found with the SOL muscle of the zero-week control animals, when compared to that of the 12-week sedentary control animals, is in agreement with the data presented by Close (81, 83). However, Close also found this relationship to hold true in the rat extensor digitorum longus muscle, a "fast" twitch muscle. No significant difference was observed in the twitch/tetanus ratio of the RF muscle in this study.

The muscles of the zero-week control animals tended to be more fatigue resistant than were those of the 12-week sedentary control animals. This was apparent from both the twitch and 30-sec contractions. In reviewing the literature no information was found dealing with the fatiguability of a muscle before and after a specified aging period.

Since the 12-week sedentary control and 12-week high-intensity endurance swimming animals were significantly different in only three of the 94 physiological characteristics obtained from the SOL and RF muscles, there appears to be no overall effect of the aerobic endurance training program on contractile characteristics. This lack of a training effect could be explained, at least in part, by the mode of exercise. When animals are forced to swim, many uncontrolable variables can affect the level at which the animals are trained. A few of these variables include: (a) the amount of air in the fur of the animals, (b) the body density of the animal, and (c) the efficiency or lack of efficiency in swimming skills of the animals. Thus the exact degree of physical training achieved may vary greatly among the animals. Of course, it is

possible too that these physiological characteristics simply are not altered by an endurance training program. Edgerton et al. (121) trained rats on a treadmill and found no significant differences between the muscles of the trained and untrained animals. The characteristics measured in that study included twitch and tetanic tension, contraction time, one-half relaxation time, twitch/tetanus ratio, fusion frequency and the rate of tension development.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The purpose of this study was to determine the effects of growth and development (aging) and physical training on selected histochemical and physiological characteristics of normal hamster muscle. Twenty-seven Syrian hamsters served as subjects in this study. These animals were assigned randomly to one of the following groups: (a) zero-week control (n = 8), (b) 12-week sedentary control (n = 11), and (c) 12-week high-intensity endurance swimming (n = 8).

Histochemical analyzes of the right leg muscles were performed on the central area of the SOL muscle along with the deep and superficial areas of the RF muscle. Muscle fiber profiles and respective fiber sizes were determined using 30 adjacent muscle fibers from each of the three muscle areas. Contractile elements of the muscle fibers were determined through the use of the ATPase stain. Oxidative capacity of these fibers was demonstrated with the NADH stain, while aerobic glycolytic activity was shown by the αGPD stain. Lipid and glycogen localizations were determined through the use of the SUD and PAS stains.

Physiological analyzes were conducted using the left leg SOL and RF muscles. In these analyzes many physiological characteristics were determined including muscle strength, speed, rate of relaxation, and

fatiguability. Some values, such as those from the strength measures, were corrected for body and muscle weight.

Histochemically, the percentage of "fast" and "slow" fibers did not change in the central area of the SOL muscle or in the superficial area of the RF muscle. In the deep area of the RF muscle, however, a decrease in the percentage of "fast" fibers was observed with aging while an increase in this percentage was seen with physical training.

Metabolically, the ability of the muscles to utilize glycogen anaerobically and aerobically was increased with age. With physical training the "slow" fibers of the central area of the SOL muscle and the "fast" fibers of the deep area of the RF muscle increased in their capacity to metabolize glycogen anaerobically and aerobically. The other fibers, including those of the superficial area of the RF muscle, decreased in their capacity to metabolize glycogen. The ability to store glycogen was decreased with growth and development in the "fast" and "slow" fibers of the deep area of the RF muscles. In the superficial area of the RF muscle of the trained animals, the "fast" fibers had a reduced amount of glycogen stored; whereas, both fiber types of this deep muscle area in the trained animals had an increased amount of stored glycogen.

Physiologically, most characteristics were found to be affected by aging but not altered by the animals performance in a high-intensity endurance swimming program. Generally, the muscles of the zero-week control animals were faster contracting and relaxing and also less fatiguing than the corresponding muscles of the 12-week sedentary control animals. The muscles of the older animals did develope more absolute force. However, when this force was corrected for either

muscle or body weight, the muscles of the zero-week control animals were stronger.

Conclusions

From the data obtained in this study the following general conclusions may be drawn:

1. The metabolic characteristics of the muscle fibers of the central area of the SOL muscle and the deep and superficial areas of the RF muscle were altered by growth and development and by the performance of a high-intensity endurance swimming program.
2. Muscle fiber size increased with age, but this increase was retarded when the animal was subjected to a high-intensity endurance swimming program.
3. The physiological characteristics of the muscles changed greatly with growth and development as these muscles became slower and stronger with age.
4. Animals who were subjected to a high-intensity endurance swimming program did not alter the physiological characteristics of their SOL and RF muscles.

Recommendations

1. In future studies dealing with the physical training of animals, a training method other than swimming should be used. Such methods could include running, jumping, or lifting regimens - all of which could be quantified.
2. In studies measuring muscle glycogen, the method of sacrifice should not be decapitation.

3. A revision of the muscle fiber profiles used in this study should be made before future studies are carried out in order to more fully incorporate the oxidative capacity of the muscle fiber into the muscle fiber profile.
4. Biochemical studies on comparable muscles should be performed to complete the evaluation of these muscles.
5. Studies involving the histochemical, biochemical, and physiological characteristics of single motor units of muscle from trained animals should be performed.

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APPENDICES

APPENDIX A

TRAINING PROGRAM

Table A-1

Training Program

Day	Percent Tail Weight	Time (min)
1	0	30
2	0	40
3	0	50
4	0	60
5	0	60
6	2	40
7	2	40
8	2	40
9	2	45
10	2	50
11	3	30
12	3	30
13	3	30
14	3	35
15	3	35
16	3	35
17	3	40
18	3	40
19	3	40
20	3	40
21	3	40
22	3	45
23	3	45
24	3	45
25	3	45
26	3	45
27	3	50
28	3	50
29	3	50
30	3	50
31	3	50
32	3	55
33	3	55
34	3	55
35	3	55
36	3	55
37	3	60
"	"	"
"	"	"
60	3	60

APPENDIX B

MUSCLE FIBER PROFILE CHARACTERISTICS

Table B-1

Muscle Fiber Profile Characteristics - Fast Fibers

ATPase High (D) Twitch Fast		Low SDH (L)			Mod SDH (M)			High SDH (D)		
		Low αGPD (L)	Mod αGPD (M)	High αGPD (D)	Low αGPD (L)	Mod αGPD (M)	High αGPD (D)	Low αGPD (L)	Mod αGPD (M)	High αGPD (D)
High PAS (D)	High SUD (D)	FgL	FGL FGgL	FGL FGgL	FgL	FGL FGgL	FGL FGgL	FgL	FGL FGgL	FGL FGgL
	Mod SUD (M)	FgL	FGL FGgL	FGL FGgL	FgL	FGL FGgL	FGL FGgL	FgL	FGL FGgL	FGL FGgL
	Low SUD (L)	Fg	FG FGg	FG FGg	F	FG FGg	FG FGg	F	FG FGg	FG FGg
Mod PAS (M)	High SUD (D)	FgL	FGL FGgL	FGL FGgL	FgL	FGL FGgL	FGL FGgL	FgL	FGL FGgL	FGL FGgL
	Mod SUD (M)	FgL	FGL FGgL	FGL FGgL	FgL	FGL FGgL	FGL FGgL	FgL	FGL FGgL	FGL FGgL
	Low SUD (L)	Fg	FG FGg	FG FGg	F	FG FGg	FG FGg	F	FG FGg	FG FGg
Low PAS (L)	High SUD (D)	FL	F	F	FL	F	F	FL	F	F
	Mod SUD (M)	FL	F	F	FL	F	F	FL	F	F
	Low SUD (L)	F	F	F	F	F	F	F	F	F

Table B-2

Muscle Fiber Profile Characteristics - Slow Fibers

ATPase Low (L) Twitch Slow		Low SDH (L)			Mod SDH (M)			High SDH (D)		
		Low αGPD (L)	Mod αGPD (M)	High αGPD (D)	Low αGPD (L)	Mod αGPD (M)	High αGPD (D)	Low αGPD (L)	Mod αGPD (M)	High αGPD (D)
High PAS (D)	High SUD (D)	SgL	SGL SGgL	SGL SGgL	SgL	SGL SGgL	SGL SGgL	SgL	SGL SGgL	SGL SGgL
	Mod SUD (M)	SgL	SGL SGgL	SGL SGgL	SgL	SGL SGgL	SGL SGgL	SgL	SGL SGgL	SGL SGgL
	Low SUD (L)	Sg	SG SGg	SG SGg	S	SG SGg	SG SGg	S	SG SGg	SG SGg
Mod PAS (M)	High SUD (D)	SgL	SGL SGgL	SGL SGgL	SgL	SGL SGgL	SGL SGgL	SgL	SGL SGgL	SGL SGgL
	Mod SUD (M)	SgL	SGL SGgL	SGL SGgL	SgL	SGL SGgL	SGL SGgL	SgL	SGL SGgL	SGL SGgL
	Low SUD (L)	Sg	SG SGg	SG SGg	S	SG SGg	SG SGg	S	SG SGg	SG SGg
Low PAS (L)	High SUD (D)	SL	S	S	SL	S	S	SL	S	S
	Mod SUD (M)	SL	S	S	SL	S	S	SL	S	S
	Low SUD (L)	S	S	S	S	S	S	S	S	S