FIBER SIZE AND CAPILLARY TO FIBER RATIO IN THE GASTROCNEMIUS MUSCLE OF EXERCISED RATS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Rexford E. Carrow
1965

This is to certify that the

thesis entitled

Fiber Size and Capillary to Fiber Ratio in the

Gastrocnemius Muscle of Exercised Rats

presented by

Rexford E. Carrow

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Anatomy

Roger E. Brown
Major professor

Date September 15, 1965

ABSTRACT

FIBER SIZE AND CAPILLARY TO FIBER RATIO IN THE GASTROCNEMIUS MUSCLE OF EXERCISED RATS

by Rex E. Carrow

Thirty male rats (Sprague-Dawley), 25 days of age were placed in exercise cages for 7 days. The animals were assigned to one of three treatment groups: sedentary, voluntary exercise, and forced exercise. The table of random numbers was used to assign the animals to their various groups. For the next thirty-five days the sedentary group was permitted no exercise other than that allowed by their small individual cages. The voluntary group remained in activity cages while the forced group in addition to being in activity cages swam 30 minutes each day with lead weights equal to 2% of the body weight attached to their tails. At the end of the thirty-five days the animals were sacrificed. The hind limbs were injected with India ink, the gastrocnemius muscle was removed, embedded in gelatin and cut on the freezing microtome. The cross-sectional areas of the red and white muscle fibers from the gastrocnemius muscles were measured by using a polar planimeter. Ink filled capillaries were counted in conjunction with fiber measurements.

The results of measurements and counts for the sedentary, voluntary activity and forced exercise groups were compared statistically using correlations, analysis of variance and the Tukey procedures.

The average cross-sectional area of the red fibers per gram body weight in animals from the sedentary group was 3.82 square microns. In animals which had been forced to exercise the average red fiber area per gram body weight was 5.72 square microns.

The total per cent differences in the size of the white fibers (forced-sedentary) and red fibers (forced-sedentary) were 32.5 and 49.7 per cent respectively. Of the total differences found, 76.2 per cent was manifest in the animals permitted to exercise at will (voluntary group). Only a 56.8 per cent increase was produced in the red fibers of animals from the same group. By comparison, 23.6 per cent of the total white fiber difference and 43.1 per cent of the total red fiber difference (forced-sedentary) were found in the comparisons of the data from the forced exercise and voluntary exercise groups.

The mean capillary per fiber per gram of body weight (C/F/G) ratio for animals in the sedentary group was .80. In animals which had been forced to exercise the C/F/G

ratio was 1.0 while in the voluntary group it was .89.

The total per cent differences in C/F/G in red and white fibers (forced-sedentary) were 25 and 31 per cent. Of the total differences found 75 per cent was exhibited by the white fibers of the voluntary group and only 45 per cent was produced in the red fibers of animals of the same group. Fifty-five per cent of the total red C/F/G difference and 25 per cent of the total white C/F/G difference (forced-sedentary) were found when the data from the forced and voluntary exercise groups were compared.

These results showed that voluntary exercise produced a greater increase in size of the white than of the red fibers. The C/F/G ratio in conjunction with these fibers followed the same pattern. Under the conditions imposed by forced activity there was a relatively greater increase in the size of the red than of the white fibers. These differences were paralleled by commensurate changes in vascular supply.

The circulatory adjustments which accompany changes in red and white muscle fiber sizes with specific exercise regimens, guarantee a balance between effective blood flow and the immediate metabolic needs of the muscle tissue.

FIBER SIZE AND CAPILLARY TO FIBER RATIO IN THE GASTROCNEMIUS MUSCLE OF EXERCISED RATS

By

Rexford E. Carrow

A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Anatomy

1965

ACKNOWLEDGMENTS

The author is grateful to Dr. Roger E. Brown for the faith and encouragement he has expressed in me as a future teacher and research worker. His suggestions on the preparation of this thesis and the microtechniques involved were invaluable.

Grateful appreciation is extended to Dr. M. Lois Calhoun, Professor and Head of the Department of Anatomy. Her years of personal guidance and confidence in the author as an individual were primarily responsible for completion of this program.

Special thanks are also due Dr. Wayne VanHuss, Director of the Human Energy Research Laboratory for the acquisition of experimental animals and for his assistance with statistical evaluations and organization.

Many thanks to Dr. Esther M. Smith for her assistance with the photomicrographic equipment. Her patience in molding a graduate assistant into a teacher is greatly appreciated.

Special thanks to Drs. Charles W. Titkemeyer and Robert F. Langham for serving on the guidance committee and assuming the responsibility which this entails. The author

is also indebted to Drs. D. R. Swindler and A. W. Stinson for their constructive criticism of the manuscript.

Thanks are also extended to Dr. E. A. Roege for her technical advice, to Mr. Denver Baker, Miss Jean Pajot and Miss H. A. McCoy for their assistance in preparing the illustrations. To Mrs. L. J. Wahl and Mrs. L. L. Holmes for their assistance in preparing the manuscript.

The highest possible tribute is due my wife Pat who, with understanding, encouragement and sacrifice has contributed so much toward the completion of this work.

Many thanks to sons Rick and Tom who have often accepted the importance of this study in lieu of a much deserved camping trip.

VITA

REXFORD E. CARROW

Candidate for the degree of Doctor of Philosophy

Final examination: August 19, 1965. 10:00 a.m.

Dissertation: Fiber size and capillary to fiber ratio in the gastrocnemius muscle of exercised rats.

Outline of studies:

Major subject: Anatomy

Minor subject: Pathology

Biographical items:

Born: February 4, 1927. Mt. Pleasant, Michigan.

Undergraduate studies:

B.S., Michigan State University, 1953.

M.S., Michigan State University, 1960.

Professional experience:

Graduate Assistant, Department of Anatomy, College of Veterinary Medicine, Michigan State University, 1960-1961.

Instructor, Department of Anatomy, College of Veterinary Medicine, Michigan State University, 1961-1965.

Member of Society of Sigma Xi.

TABLE OF CONTENTS

1.

2.

3.

4.

5.

6.

LIST OF TABLES

LIST OF PLATES

LIST OF GRAPHS

INTRODUCTION

Movements of the body are a result of skeletal muscle contractions. The energy needed for contraction is obtained from external sources and brought to the individual muscle cells by the circulatory system. The great range of activity of the muscular system requires a blood flow which is capable of adjusting rapidly to provide adequate nutrients and energy rich materials when the occasion demands.

Although the muscular and circulatory systems have been the subjects of many investigations in themselves, their integrative potential has received comparatively little attention. Fewer yet are studies which have considered the relationships that exist between these two systems when they are subjected to various types of physical activity.

Furthermore, a review of the literature pertinent to skeletal muscle and its blood supply in exercised animals reveals that a great number of techniques as well as a variety of animals have been used in the investigations. In many cases the age, sex, or physical condition has been omitted from the report. In other instances the limited

number of animals used invalidated the statistics. As a consequence of the diversity in the materials and methods utilized there exists a great deal of confusion as to the exact meaning and interpretation of the results.

Because of these confusing data, the need for well controlled experiments on the relationships between skeletal muscle and its vascular supply is imperative. Of equal necessity is the critical examination of materials and methods in order to obtain accurate and meaningful data. The present study has been designed to control as many of the factors in question as possible.

ED

exis

tion

ject

mus

mus

ton

col

Bel

and

ext

PIE

ject

of

her

Ha

sig

por

Ha

REVIEW OF LITERATURE

RED AND WHITE MUSCLE FIBERS

Several types of skeletal muscle have been known to exist since ancient times, probably through the recognition of light and dark meat. Investigations on this subject have brought forth numerous terms, i.e., red and white muscles, light and dark muscles, granular and agranular muscles, large and small fibers, fast and slow fibers and tonic and tetnic fibers to describe the differences in coloration, size and speed of contraction. Reports by Bell (1911), Needham (1926), Denny-Brown (1929) and Smith and Giovacchini (1956) included excellent descriptions and extensive bibliographies on this subject.

FIBER SIZE

The sizes of individual muscle fibers has been the subject of a great many studies. In human embryos, the fibers of all skeletal muscles are of approximately the same dimensions and appear to grow at a uniform rate until birth (Halbran, 1894 and Greep, 1954). Soon after birth the fibers of certain muscles become larger than others. A report by Scott (1957) indicated that in the adult, each muscle fiber is two or three times as broad in cross-section

as in the child one year of age and that in the child there is a great variation between the sizes of the individual fibers. Similar results have been reported for the rat (Morpurgo, 1897).

Denny-Brown (1929), found that in the newborn kitten all muscles were opaque and the fibers were very small in diameter. All of the fibers in the gastrocnemius muscle measured 100 square microns in size. Approximately 50% of the fibers in the soleus muscle measured 100 square microns but the remaining fibers measured 220 square microns. Studies on several species of birds (George and Naik, 1957, 1958, 1959 and Denny-Brown, 1929), and the rat (Nachmias and Padykula, 1958) and (Stein and Padykula, 1962), also pointed out the variation in muscle fiber sizes in a single muscle. In these "mixed" muscles the smaller "red" fibers were found in the deeper portions, while the large "white" fibers were located at the periphery.

Denny-Brown's work on the pectoralis muscles of the pigeon, revealed that the smaller dark fibers averaged 900 square microns, and the cross-sectional areas of the large clear fibers varied between 3600 and 7200 square microns. In the same animal, the dark and light fibers of the superficial muscles of the leg were nearly the same size.

Martin et al. (1932) reported fiber sizes of 2300 and 2600 square microns in the semimembranosus and gracilis muscles of the dog. Valdivia (1958) worked with the guinea pig and found that red fibers were uniform in size and shape and averaged 1800 square microns in area. He mentioned that in mixed muscles the white fibers were more variable in size and shape and larger than the red fibers. In contrast, Paff's (1930) report on the guinea pig showed the largest fibers to be approximately half the size of those measured by Valdivia. In the rat and cat, he obtained measurements of 609 and 476 square microns respectively. Additional measurements on the dark and light muscle fibers in the gastrocnemius of the rat (Dellasanta, 1964) indicated cross-sectional determinations of 1353 and 2652 square microns. Smith and Giovacchini (1956) stated that Arloing and Lavocat, and Pakual found no differences in muscle fiber size, while Meyer and Graf observed that red fibers were larger than white ones. Stoel (1925) counted nearly 3 times as many white as red fibers per square millimeter of area. Watzka (1939) noted that white fibers shrink more than red ones during the process of fixation, however, in the fresh condition, they are nearly the same size.

INNERVATION AND FIBER SIZE

Another important characterization of individual muscle fibers is their speed of contraction. In 1929, Denny-Brown showed that all muscles in the two-week-old kitten are slow in contraction time, but a few weeks later the fibers have differentiated into the slow acting red type and the fast acting white type. Additional works of this nature (Buller et al. 1960a & b, Hess and Pilar 1963, and Vroba, 1963) indicated that innervation may play an important role in the differentiation of muscle fibers into fast and slow types.

Bach (1948) reported that in the rabbit the normally slow acting soleus can be made fast acting by exchanging its nerve supply with that of the originally fast acting, white, tibialis posterior muscle. In this reversal the white tibialis posterior muscle became slow acting and red. Bajusz (1964) quoted Graf and Kruger who reported that the type of innervation is one of the factors responsible for the differentiation of red and white fibers in speed and duration of contraction. In support of this Bajusz (1964) demonstrated that nerve impulses are not the same for the two types of fibers, and that there is relatively greater dependence of the white than of the red fibers on neuromuscular integrity.

In a symposium on "What we need to know about muscle" (Bennett et al. 1958), Denny-Brown reported that in the past there was evidence for a uniform speed of contraction in all fibers of any muscle, but now it seems possible that there is some variation from fiber to fiber in the larger muscles. He also mentioned that the sharp distinction of muscle fibers into red, pale, slow, fast, etc., makes it natural to seek two different types of muscular function corresponding to these differences.

In support of this, Bajusz (1963) pointed out that if red and white fibers differ with respect to speed and duration of contraction, it would be logical to assume a difference in innervation.

BLOOD SUPPLY TO MUSCLE

The differences in coloration between muscles and even muscle fibers brought forth investigations to determine the significance of the chromatic variation. Smith and Giovacchini (1956) quoted Ranvier who proposed that the deeper color of red muscles was due to some substance within the muscle fibers which could not be removed by exsanguination. More recently Millikan (1937) reported the substance to be myoglobin and that it acted as an oxygen reservoir, while

Lawrie

ably ha

Wh

muscle

the va

carrie

the ra

in det

red m

pointe

muscl

is re

impor

(C/F)

1925

squar

squa

cates

squa

on a

white

an e

eat

Lawrie (1952) mentioned that the red and white fibers probably had different mechanisms for the utilization of oxygen.

When attention shifted to the oxygen requirements of muscle, it was necessary to examine the relationship of the vascular bed with muscle fibers. Spalteholz (1888) carried out studies which described these relationships in the rabbit. Krogh (1919) first investigated the problem in detail. He found that there was 2.0 capillaries per red muscle fiber and 2.6 per white fiber. In addition, he pointed out that the number of functional capillaries in muscles is variable and dependent upon whether the muscle is resting or active at the time the counts are taken. The importance of methods in which the capillary to fiber ratios (C/F) are determined in exemplified by the work of Stoel in 1925 on the rabbit. His figure of 1525 capillaries per square millimeter of white muscle and 790 capillaries per square millimeter cross-sectional area in red muscle indicates that there are nearly twice as many capillaries per square millimeter in white muscle as in red muscle. However, on a C/F basis these figures revealed 0.7 capillaries per white fiber and 1.2 capillaries per red fiber. In contrast, an excellent study (Paff, 1930) on the rat, guinea pig and cat revealed that the number of capillaries per square

millimeter of cross-section of muscle ranged from 2341 to 3574. Duyff and Bouman (1927) also worked with the rabbit and reported a C/F ratio of 2.3 for red muscle and 1.3 to 1.5 for white muscle fibers. In Watzka's (1939) work on the rabbit and the hen, it was reported that red fibers had a higher concentration of capillaries than did white fibers. The only other study of this nature on birds was reported by Bosiger (1950) who found them 3.0 to 6.0 times as many capillaries per red fiber as for white fibers. A report on the influence of training on the number of capillaries in skeletal muscle by Petren et al. (1936) showed that the number of open capillaries in the gastrocnemius muscle of the guinea pig was considerably higher in trained animals than in controls. Valdivia (1958) worked with three groups of guinea pigs. Group A consisted of animals born at sea level in Peru. They were placed in pens and were permitted to exercise freely throughout their life span. Group B born in Wisconsin were maintained in small cages where movement was restricted. Group C guinea pigs were born in the Peruvian mountains and kept at an altitude of nearly 15,000 feet. In examining the C/F ratios in red muscles there was no significant difference found between groups A and B. Group C, the high altitude group, showed

an average increase of 1.4 capillaries per fiber over the other groups. C/F ratios calculated from the dog under resting conditions by Martin et al. (1932) produced a ratio of 0.64 while in animals exercised by electrical stimulation, it was 1.20. By using capillary dilators this ratio increased to nearly 2.5 capillaries per fiber. The authors made no mention of whether these counts were taken in relation to red or white fibers. A detailed investigation on the vascular patterns in red and white muscles of the rabbit was carried out by Smith and Giovacchini (1956). They found C/F ratios of 1.7 and 0.5 in red and white muscle respectively.

The variability in the numbers of capillaries per muscle fiber indicated in the foregoing discussion may be attributed to the different techniques used, and the conditions under which the relationships were obtained. It is also possible that internal mechanisms as yet unidentified may regulate the circulation in skeletal muscle (Uvnäs, 1960, Hyman, 1963 and Folkow, 1952). In fact it is possible that the results obtained by Smith and Giovacchini (1956) and Martin et al. (1932) may be attributed to such mechanisms.

After injection with India ink, these authors observed

that all muscles of the lower extremities turned black and appeared to be completely injected. However, upon microscopic examination of tissue sections, it was apparent that some areas of the muscle were filled while others were devoid of ink. Recent studies on the circulation to skeletal muscle (Barlow et al. 1961; Hyman and Lenthall, 1962; Hyman and Paldino, 1962; Hyman et al. 1963 and Renkin and Rosell, 1962) suggest that the circulation to skeletal muscle is influenced by several different mechanisms. The problems associated with incomplete injections will be discussed later in this thesis.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Fifty male albino rats (Sprague-Dawley) of the same age (25 days) were purchased as weanlings. They were numbered and placed in individual metal cages where they had the opportunity to become adjusted to the new surroundings. To avoid isolation stress (Hatch et al. 1963), the animals were handled daily, but allowed no exercise other than rest afforded by their confining quarters. All animals were fed ad libitum from a stock diet and had free access to water. The temperature in the animal room was maintained between 70 and 72 degrees Fahrenheit. Body weights were recorded for each animal daily throughout the experiment.

At 31 days of age, each animal was placed in a spontaneous exercise cage for seven days. On the basis of the degree of activity recorded on a counter during the last four days, the ten most active and ten least active animals were dropped from the study. The remaining thirty animals were assigned to one of three treatment groups: sedentary, voluntary exercise and forced exercise. The table of random numbers was used as the basis for assigning the animals to the various groups. Under this

procedure the animal possessing the first number chosen from the table was placed in the sedentary group, the second choice was placed in the voluntary group and the third selection in the forced group. This system was carried on until all thirty animals had been placed in one of the three treatment groups. They remained in their respective groups for the next 35 days (63 days from birth). During this time the animals in group I (sedentary group) were allowed no exercise other than that available in their individual standard cages (5"x5"x12"); animals in the voluntary group were housed in individual activity cages which had a freely rotating drum with counter. Group III (forced exercise group), in addition to living in activity type cages, were forced to swim for 30 minutes each day with lead weights equal to 2% of their body weight attached to their tails. The water was maintained at body temperature (38° C.) during the swimming period. At the end of the 35 day activity period all animals were anesthetized and subsequently injected with India ink. The gastrosnemius muscle was removed and processed for microscopic examination.

ANESTHESIA

Methoxyflurane* was chosen as the anesthetic agent because

*Pitman-Moore, Co., Indianapolis, Indiana

it has excellent muscle relaxant properties and does not alter heart rate or rhythm except at very deep anesthetic levels (North et al. 1961; Jones et al. 1962; and Bagwell and Woods, 1962). In addition, it has a maximum concentration of 3% in air, thus the depth of anesthesia can be controlled.

The animals were anesthetized in a special chamber which provided for CO_2 absorption and continuous circulation of the anesthetic agent (Heusner, 1965). The desired level of anesthesia was controlled by maintaining the gas in the chamber at a concentration of 3%. A Fisher Gas Partitioner* was used to analyze the gas mixture in the chamber and a model SR Sargent Recorder** was utilized to monitor the components resolved in the gas chromatograph accurately.

After the initial anesthesia, the animals were removed from the chamber for the necessary surgical and injection procedures. These usually averaged approximately 25 minutes per animal and the state of anesthesia was maintained by using minimum quantities of ether in a nose cone during the operative period.

* Fisher Scientific Co., Chicago 51, Illinois
**E. H. Sargent and Co., Detroit, Michigan

INJECT

Th

vascul

factor

The i

size

In ac

durin

tion

prev

tech

(19

the

lar

How

the

te

at

st

of

*p

INJECTION MATERIALS

The selection of a proper injection material for the vascular system is of particular importance since several factors must be controlled to produce accurate results. The injection mass must contain suspended particles of such size that they can enter the capillaries without difficulty. In addition, the material must remain in the vascular bed during fixation, embedding and sectioning. Rapid fixation is imperative in order to minimize shrinkage and to prevent autolysis.

With these points in mind, the Higgin's* ink injection technique described by Brown (1965) was used. Hartman et al. (1929) criticized the use of India ink because he observed the clumping of ink particles at the origina of open capillaries, thus, preventing the fluid from entering the vessels. However, Brown demonstrated that if care is taken to decant the ink as it is taken from the bottle, any flocculated material will be eliminated, and if the pH of the ink is held at 9.0, the carbon particles will remain in a colloidal state and will not aggregate and plug the smaller elements of the vascular tree.

*Higgins Ink Co., Brooklyn 15, New York

Immediately before the injection, commercial formalin (40%) was added to the India ink to a concentration of 2% formalin. This aided in a more rapid and even fixation of the muscle tissue and did not cause precipitation of the carbon particles. It should be noted that a concentration of more than 4% formalin does cause the particles to precipitate.

In order to insure a good injection with India ink, it is necessary to wash the vascular system free of blood to prevent flocculation of the ink and plugging of the vessels. Therefore, mammalian Ringer's solution, kept at 38° C., was perfused through the rear extremities prior to the injection of the ink.

INJECTION APPARATUS

The apparatus used for perfusion and injection is shown in Plate I. A sphygmomanometer was used to insure that the India ink was injected at a constant and even pressure. The pressure lead of the sphygmomanometer was connected to a glass T-tube whose branches were in turn connected to pieces of rubber tubing which led individually to flasks filled with India ink and Ringer's solution. With this arrangement, any pressure from the manometer would be equal in both flasks.

The filled flasks were immersed in a water bath held at 38° C. so the fluids would be at the normal body temperature of the animals at all times. A polyethylene tube, from both the perfusion and injection flasks was joined to make a common injection cannula. In this way, the same cannula could be used for both perfusion and injection. A two way valve (Point C, Plate I) placed at the union of the two plastic tubes provided a device by which the ink and perfusate could be turned on or off.

Although the apparatus described is easily constructed and gives excellent results, there are several problems to which the investigator must be aware. First, the stoppers in the fluid filled flasks must be firmly secured so they will not blow out when pressure is applied at the manometer. Second, the system should be inspected to see that it is completely free of bubbles. Finally, it is important that clamps be placed on the tubing between the manometer and flasks (Points A and B, Plate I). This allows for one side of the system to be closed while pressure is being applied to the opposite side. If the side containing the perfusate is left open while the ink is being injected, some of the ink will be drawn into the bottle containing Ringer's solution. This will cause the ink to precipitate and plug the vessels

during

if the

Perfusi

ink par

thereby

INJECTI

Se

and Cr

pressu

This w

Dellas

ber of

sures

approx

all pe

that

SURGI

F

ing a

inal

renal

clamp

during perfusion of the next animal. A similar problem exists if the injection side is left open during the perfusing period. Perfusing fluid drawn into the ink will not only cause the ink particles to flocculate, but it also dilutes the ink, thereby, making it difficult to identify in tissue sections.

INJECTION PRESSURE

Several authors (Durant, 1927; Byrom and Wilson, 1938; and Craig and Shintani, 1964) have noted the normal blood pressure of the rat to range from 100 to 120 mm of mercury. This was confirmed recently in a well controlled study by Dellasanta (1964) whose objective was to determine the number of capillaries open in skeletal muscle at injection pressures of 40, 120 and 200 mm Hg. The 120 mm level most nearly approximates the normal blood pressure of the rat, therefore, all perfusions and injections employed in this study were at that pressure.

SURGICAL AND INJECTION METHODS

Following anesthesia, a laparotomy was performed by making a three to four inch mid-saggital incision in the abdominal wall. The abdominal aorta was exposed caudal to the renal artery and closed off at this point with a bulldog clamp (Plate I). A small opening was made in the wall of

the vessel one inch caudal to the clamp, and a polyethylene tube approximately the same size as the distended diameter of the aorta was inserted caudad and secured with a ligature. As perfusion with mammalian Ringer's solution at 38° C. (body temperature) started, the inferior vena cava was cut to allow free flow. When the fluid issuing from the vena cava appeared was clear the valve (Point C, Plate I) was turned to shut off the perfusate and permit the introduction of the ink. Within seconds after the ink was injected the entire body below the cannulation point turned black. The animals remained alive during the injection procedure.

TISSUE PROCUREMENT AND PREPARATION

Immediately following injection, the left leg was removed by disarticulating the limb at the coxofemoral joint. The skin was carefully removed, exposing the gastrocnemius muscle.

The Achilles tendon was cut from its attachment on the fibular tarsal bone and a 7 gram paraffin coated lead weight was attached to this cut end. Then the muscle, with the weight attached, was suspended in 10% formalin for 24 hours. It was held in position at right angles to the upper and

lower leg with the femur and tibia supporting the weight by resting on two metal bars overlying the container of fixative, Plate II. This served to put a constant tension on the muscle fibers during fixation, and while it is not likely that normal tension was duplicated; nonetheless, considerable distortion was prevented. Also, this controlled tension gave uniformity to all the muscle sections and helped to validate the statistical analyses of the fiber measurements.

After fixation the muscle was removed from the bone infiltrated with 10% gelatin for 24 hours followed by 20% gelatin for another 24 hours.

Following infiltration the muscle was removed from the gelatin, placed on a millimeter ruler to determine the midpoint. The tissue was cut transversely at this point (Plate III). From previous work, Dellasanta (1964) it is known that an area which contains red and white fibers as well as a mixed fiber area, is located in the middle one-third of this muscle. The author also found the capillary concentration to be greatest at this location. To assure uniformity in both capillary counts and muscle fiber measurements the midpoint of each muscle was selected for making tissue sections.

The cut surface was placed face down in an embedding mold which was filled with 20% gelatin. This was refrigerated at

4° C. to harden. The solidified block was transferred to 10% formalin to denature the protein of the gelatin. This made the blocks firmer and easier to handle. Embedding in gelatin has proved to be most effective in obtaining sections because shrinkage caused by paraffin techniques are avoided (Valdivia, 1958).

The trimmed blocks were placed on the stage of a freezing microtome and sectioned at 16 microns. The sections were placed on a slide and coverslipped using 20% gelatin as mounting medium.

MEASUREMENTS

To determine accurate capillary to fiber ratios and to get repeatable muscle fiber measurements, a micro-projection method was utilized for recording the various microscope fields. The unstained sections were viewed on the 5x7 ground glass of a Bausch & Lomb, Model L, photomicrographic camera.* By using a 10X eyepiece and an 8 millimeter (20X) objective in the microscope and adjusting the bellows of the camera to a height of 55 centimeters a magnification of 296X at the viewing level was achieved. Frosted acetate sheets were superimposed over the ground glass and tracings of the various

*Bausch and Lomb Optical Co., Rochester, N.Y.

microscopic fields were made Areas which contained predominantly small red and large white muscle fibers (A and B, Plate III) were selected from each muscle for tracing.

A total of 50 muscle fibers from each zone were outlined carefully on the acetate and the ink-filled capillaries related to the muscle fibers were indicated on the tracings in their proper locations. Therefore, accurate and functionally realistic capillary to fiber ratios were determined by counting only those vessels associated with the muscle fibers used for cross-sectional measurements. Capillary counts were limited to vessels which measured nine microns or less. A tendon (Plates III and IV) was used as a landmark in locating fibers and capillaries in the red area. After the structures in this area were traced, the section was moved laterally by microscopic stage so that fibers and vessels in the white area came into view and could be reproduced in a like manner.

The tracings on the acetate sheet provided sharp boundaries of individual muscle fibers. The cross-sectional areas of the fibers were measured with a Keuffel and Esser compensating planimeter. Ten cross-sectional area measurements for each

of the

data w

STATIS

Th

sponta

pared

analy

(Guer

the a

sent

for

tion

Data

the

Tuk

the

th

in

of

me

la

of the fibers in the red and white areas were recorded. The data was subjected to various statistical analyses.

STATISTICS

The results of measurements and counts for the sedentary, spontaneous activity and forced exercise groups, were compared statistically using correlations (Edwards, 1964), analysis of variance (Scheffe, 1959) and the Tukey procedures (Guenther, 1964) (Appendix A). In the statistical analysis the average of the ten determinations was used as the representative value for that fiber. The fifty fibers measured for a single area were used as replications in the computation of correlations and analyses of variance. The Control Data 3600 Computer was utilized for the calculations. Where the analysis of variance indicated significance ($P = .05$), Tukey's method was used to evaluate the differences between the means of the three groups.

Analyses were completed comparing the three groups for the red and white fiber areas. Effects due to differences in fibers to differences in treatments and to combinations of muscle fibers and treatments were examined. The raw data measures, raw area measures per gram body weight and capillary to fiber ratios were analyzed (Appendix A).

GENERA

It

made u

are to

resist

only l

that c

additi

large

is the

1929;

1965;

Buller

mixed

format

electr

dition

In

exerci

and wh

Si

RESULTS AND DISCUSSION

GENERAL STATEMENT

It is well known that certain muscles of the body are made up of one type of fiber i.e., small, dark fibers that are tonic in nature and serve to maintain body tone by resisting the forces of gravity. Other muscles contain only large, pale fibers which come into play in situations that call for rapid responses of a short term nature. In addition, some muscles contain a combination of small and large fibers and are referred to as "mixed" muscles. It is the opinion of a number of investigators (Denny-Brown, 1929; Vroba, 1963; McPhedran et al. 1965; Wuerker et al. 1965; Henneman and Olson, 1965; Bajusz and Jasmin, 1963; Buller et al. 1960 a and b) that the two fiber types in mixed muscles are supplied by separate nerves. Most information of this type has been gathered as a result of electromyographic studies or in cases where diseased conditions have caused changes in the individual muscle fibers.

In the information which follows, the effects of selected exercise regimens on the size and capillarization of both red and white muscle fibers are considered.

Since muscle fibers are classified using a number of

criteria, it is pertinent to define the terminology used in this study. The classical small, slow acting are referred to as "red" fibers and the large, fast acting, as "white" fibers.

FIBER SIZES

The mean and relative muscle fiber sizes for both the control and experimental groups are shown in graphs I and II. The results of statistical analyses are presented in tables I-VI. Statistical information and basic data are presented in appendices A and B.

Correlation analyses (Appendix A) provided important information with regard to the design of the experiment. The low correlation values obtained by correlating the results for the animals' activity to the original selection indicated that the differences obtained in fiber sizes could be attributed to the exercise regimens to which the animals had been subjected and not to variations within the animals.

The average cross-sectional area of the red fibers per gram body weight (Table I) in animals of the sedentary group (SG) was 3.82 square microns. In animals which had been forced (FG) to exercise the average red fiber/gram body

weight was 5.72 square microns. The relative red fiber size of the naimals forced to exercise was 49.7 per cent larger than for the sedentary animals (Tables II, III). On the same basis the cross-sectional area of the white fibers/ gram body weight (Table I) of the forced group showed an increase of 32.5 per cent over those of the sedentary group (Tables II, III). In both instances the increases were significant ($F = 3.00$, $P = .05$; $F = 3.00$, $P = .05$).

When the relative red and white fiber sizes from the voluntary group were compared with those of the sedentary group, the relative fiber size differences of 24.8 and 28.2 per cent respectively were detected (Table II). These changes were statistically significant ($F = 3.00$, $P = .05$; $F = 3.00$, $P = .05$). When the voluntary and forced groups relative red and white fiber sizes were compared, no statistically significant differences were found.

The total per cent differences in the size of the white fibers (forced and sedentary) and red fibers (forced and sedentary) was 32.5 and 49.7 per cent, respectively (Table III). Of the total differences found 76.3 per cent was manifested in the animals permitted to exercise at will (voluntary group). Only a 56.8 per cent increase was produced in the red fibers of animals from the same group. By

Table I

Relative fiber sizes in sedentary and exercised animals

Animal group	Mean group wt. (gms.)	Mean fiber size/gms. (sq. microns)		S.D.	
		Red	White	Red	White
Sed.	314	3.82	6.24	0.89	0.93
Vol. ex.	295	4.90	7.79	1.33	2.16
For. ex.	262	5.72	8.27	1.75	1.81

Table II

Differences in fiber sizes (relative data)

Groups	Red fibers	Per cent difference	White fibers	Per cent difference
F-S	1.90	49.7	2.03	32.5
V-S	1.08	28.2	1.55	24.8
F-V	0.82	21.5	0.48	7.7

Table III

Relative differences in fiber size (relative data)

Groups	Red fibers	Per cent difference	White fibers	Per cent difference
F-S	1.90	49.7	2.03	32.5
V-S	1.08	56.8	1.55	76.3
F-V	0.82	43.1	0.48	23.6

comparison, 23.6 per cent of the total white fiber difference and 43.1 per cent of the total red fiber difference (forced-sedentary) were found in the comparisons of the data from the forced exercise and voluntary exercise groups.

It was apparent from these figures that voluntary exercise produced a greater increase in size of the white than in the red fibers. It was further evident that in the animals that were forced to exercise there was a relatively greater increase in the size of the red than of the white fibers.

A reasonable explanation for these changes might be found in the fact that during voluntary activity the white fibers were influenced the most and therefore, increased to a greater extent than did the red fibers. When animals were subjected to forced activity, the white fibers soon reached their maximum size, thus the increased load and constant stress was assumed by the red fibers. Under these conditions, the red fibers became relatively larger than did the white fibers. This may be what Morpurgo (1897) saw when he reported "the fibers that grow the most are those which were originally thinnest and therefore act as reserve material for growth."

The greater enlargement of white fibers in relation to voluntary activity and of red fibers with forced activity indicated that the two fiber types responded differently to the imposed exercise programs. While the present study did not take into consideration the types of innervation associated with the two types of muscle fibers in question, it is interesting to speculate on the greater increase in cross-sectional area of the red fibers when the animal is under the influence of one activity and of the white fibers when under the influence of another type of exercise.

Bajusz (1964) reported on the differences in red and white fiber sizes in rats and mice during the course of denervation atrophy and found that there was less change in red fibers than in white fiber sizes. He suggested therefore, that "the white fibers are more dependent on neural control than the red ones." Since the present study has shown that white muscle fibers demonstrated the greatest increase in size when subjected to voluntary activity, it is reasonable to expect that if Bajusz had used animals of this type, he would have seen a greater decrease in white than in red fiber sizes. On the other hand, if he had worked with highly trained animals, he might have seen a greater decrease in red than in white fiber sizes.

Denny-Brown (1929) stated that at birth all of the fibers of the gastrocnemius muscle of the cat were 100 square microns in cross-sectional area. He cited Banu who said that within 14 days many of these had increased to 220 square microns while at the same time the smaller fibers maintained their original sizes. These and other authors (Buller et al. 1960a and b; Hess and Pilar, 1963 and Vroba, 1963) indicated that the twitch and tension responses of these fibers increased with the age of the animal and thereby intimated that the tension applied as a result of the newly acquired activity may be responsible for the increase in differentiation in the size of the fibers. Although electromyographic recordings of the individual muscle fiber types were not obtained in this study, it seems evident that the increased muscular tension to which the animals were subjected in their exercise programs was responsible for the differences in muscle fiber sizes.

CAPILLARIES PER FIBER

GENERAL STATEMENT

Earlier in this paper it was pointed out that several investigators have delved into the problems associated with the blood supply to skeletal muscle. It was also emphasized that

a variety of animals and techniques had been employed in these studies. Furthermore, it was noted that in many cases caution was not taken to incorporate proper controls into the study. Such conditions have made it difficult to attach proper significance to the results obtained.

In the results which follow, the effects of selected exercise regimens on the numbers of capillaries per muscle fiber are presented.

RESULTS

The mean and relative capillary to fiber data for all groups of animals are shown in graphs III and IV. Results of statistical analyses are shown in tables IV-VI and the statistical information and basic data are given in appendices A and B.

Correlation values (Appendix A) for the capillaries per fiber (both red and white fibers) per gram body weight were obtained by correlating the results for the animals pre-experiment activity to the original matching. Since the animals were originally matched on the basis of the pre-experimental activity and then randomly placed into groups the low correlation values indicate that the differences in capillary to fiber ratios (C/F) could be attributed to

Table IV

Capillaries per fiber and capillaries per fiber per gram body weight

Animal group	Mean group wt. (gms.)	Total fibers		Total capillaries		Capillaries per fiber		Cap./fiber/gm. ($X10^{-2}$)	
		Red	White	Red	White	Red	White	Red	White
Sed.	314	500	500	1266	614	2.53	1.22	0.80	0.39
Vol. ex.	295	500	500	1326	701	2.65	1.42	0.89	0.48
For. ex.	262	500	500	1316	677	2.63	1.35	1.00	0.51

Table V

Differences in capillaries per fiber per gram body weight (relative data)

Groups	Red fibers	Per cent difference	White fibers	Per cent difference
F-S***	.20	25	.12	31
V-S**	.09	11	.09	23
F-V*	.11	12	.03	6

Table VI

Relative differences in capillaries per fiber per gram body weight (relative data)

Groups	Red fibers	Per cent difference	White fibers	Per cent difference
F-S	.20	25	.12	31
V-S	.09	45	.09	75
F-V	.11	55	.03	25

*** Forced to sedentary groups.
** Voluntary to sedentary groups.
* Forced to voluntary groups.

the exercise programs and not to differences within the individual animals.

The mean capillary per red and white fiber per gram of body weight (C/F/G) (Table IV and Graph IV) for animals in the sedentary group was .80 and .39 respectively. In animals which had been forced to exercise the mean C/F/G ratios were 1.0 and .51 while in the voluntary group it was .89 and .48. The relative C/F/G for the red fibers in animals of the forced group was 25 per cent greater than the same ratio in animals of the sedentary group. In comparison the C/F/G ratio for the white fibers in animals forced to exercise was 31 per cent larger than for similar fibers of the sedentary group (Tables V and VI). In both instances the differences were statistically significant ($F = 3.35$; $P = .05$; $F = 3.35$; $P = .05$) (Appendix A).

When the mean relative C/F/G ratios for the red and white fibers of the voluntary and forced groups were compared with those of the sedentary group, differences of 9 per cent were found in each case. Comparisons of the mean C/F/G ratios of the forced and voluntary groups revealed that the differences were not statistically significant.

The total per cent differences in C/F/G ratios in red and white fibers (forced-sedentary) were 25 and 31 per

cent. Of the total differences found 75 per cent was exhibited by the white fibers of animals in the same group. In comparison, 55 per cent of the total red C/F/G difference and 25 per cent of the total white C/F/G difference (forced-sedentary) is found when comparing the data from the forced and voluntary exercise groups (Table VI).

These figures indicate that there was an overall increase in the number of capillaries per fiber per gram body weight in association with both red and white muscle fibers. However, the greatest relative differences were produced in conjunction with the white fibers during voluntary activity and with the red fibers during forced activity.

Prior to presenting the results of the statistical analyses for the reader's perspective, a brief review of the literature on the vascular supply to skeletal muscle most pertinent to the current problem is presented.

Some authors (Krogh, 1919; Paff, 1930; Smith and Giovacchini, 1956; and Valdivia, 1958) have presented basic data on capillary to fiber ratios (C/F) in muscles which are made up of entirely red or white fibers. Results among these studies vary, but in general, it is recognized that red muscle which acts slowly but constantly has a greater number of capillaries per muscle fiber than does

white muscle which acts rapidly and for short periods of time. In addition, it has been found that active muscles are hyperemic when compared to inactive muscles (Petren et al. 1936; and Elsner and Carlson, 1962).

Since mixed muscles contain complements of fibers which are related to different activities it is of interest to see whether the C/F ratio maintains the same relationship that it does in muscles which contain only one type of fiber.

Reports by Krogh 1919, Millikan 1937, Lawrie 1952, 1953, Smith and Giovacchini 1956, Porter and Armstrong 1965 and others indicate the importance of the blood supply to muscle fibers in relation to the metabolic needs of the cells. Smith and Giovacchini (1956) found that red muscle was more vascular than white muscle. They suggested that since red muscle was also rich in myoglobin (which acts as an oxygen reservoir) (Millikan, 1937, Lawrie, 1952) that the combination of these entities provided an arrangement of double assurance. That is "those muscles which cannot function without a constant supply of oxygen apparently are equipped with a greater capillary bed as well as oxygen storing myoglobin."

These same authors and Porter and Armstrong (1965) who

reported on the sarcoplasmic reticulum in the various types of striated muscles strongly imply that all muscles do not have the same mechanisms for satisfying their metabolic needs.

Indeed, the figures presented in table VI point up the fact that under conditions of physical activity certain mechanisms, as yet unidentified, come into play which alter the blood supply within the muscle thereby providing an arrangement which is in line with the physiologic needs of the individual muscle fibers.

Under the influence of forced activity (forced-sedentary) total differences in C/F/G of 25 and 31 per cent were produced in the red and white fibers (Table VI). Of the total differences found, 75 per cent was exhibited by the white fibers of the voluntary group and only 45 per cent was produced in the red fibers of the same group. In light of reports (Denny-Brown, 1929, George and Naik, 1957, 1958, 1959) which indicate that the larger white fibers are more active during periods of exercise than are the smaller red fibers, the larger C/F/G in favor of the white fibers during voluntary activity is reasonable. This would assure that the increased metabolic needs of the white fibers were met.

Further insight into these differences is revealed by

the fact that white fibers contain little myoglobin, few mitochondria and an extensive sarcoplasmic reticulum (both of which are associated with the generation and exchange of energy rich materials). Therefore, they require a greater blood supply to satisfy their nutritional and energy requirements. In contrast, red fibers are rich in myoglobin, have many more mitochondria and the sarcoplasmic reticulum is less well developed. This arrangement provides conditions whereby the smaller C/F/G ratio maintains a constant environment and assures each tissue its necessary nutrients.

If the differences in C/F/G ratios for the red and white fibers of the voluntary group (Table VI) are compared with the differences in mean fiber sizes per gram body weight for the same group, (Table III), the following results are revealed.

Of the total difference in the size of the red fibers 56.8 per cent was expressed by the voluntary group. At the same time a 45 per cent difference of the total C/F/G ratio was produced in the same animals.

Similarly, the 76.3 per cent difference of the total mean fiber size per gram body weight in the white fibers was commensurate with a 75 per cent difference from the total in the C/F/G ratio for the group.

It is apparent that the red and white muscle fibers increased in size in response to voluntary exercise and furthermore, the increase in fiber size was accompanied by a comparable increase in blood supply.

Table VI also shows that 55 per cent of the total red C/F/G difference and 25 per cent of the total white C/F/G difference (forced-sedentary) was found when the data from the forced and voluntary groups were compared. These figures have an inverse relationship to those of the voluntary groups.

The figures in table III show that 43.1 per cent of the total difference in fiber size is related to the red fibers while table VI shows a total difference of 55 per cent in C/F/G for these fibers. The white fibers show a 23.6 per cent difference from the total relative difference in fiber size and a 25 per cent difference in C/F/G. Thus, under the conditions imposed by forced exercise, changes in muscle fiber sizes are also paralleled by changes in vascular supply.

The evidence presented here supports the work of Morpurgo, 1897; Petren et al. 1936 and others who have reported that the number of capillaries which can be opened is greater in muscles taken from trained than from untrained animals.

The differences seen in muscle fiber sizes and capillary to fiber ratios in response to forced exercise can be explained on the basis of usage. First of all, it is important to realize that the forced exercise program used in this experiment was of an endurance nature and not one which encompassed strength and speed. Therefore, it was an activity which was constant and of a low level of intensity as far as work was concerned.

Since small, red muscle fibers have been shown to be more susceptible to discharge and fire more often than large, white fibers, (Henneman and Olson, 1965; Buller et al. 1960a and b; Vroba, 1963) the greater total per cent difference in the red fiber sizes compared to white fiber sizes (Tables II and III) would be expected. Of further significance is the fact that the total per cent difference in C/F/G in relation to red fibers is greater than it is with white fibers (Tables V and VI). These results are in line with those of authors who have reported larger capillary to fiber ratios for red than for white muscle.

An additional point worthy of comment is that in our preparations, some areas of the muscle appeared to be well injected while others were devoid of ink (Plate VI).

Similar results have been reported by Martin et al.

(1932) for the dog, and Smith and Giovacchini (1956) for the cat. The earlier authors assumed that the fasciculus reacted as a circulatory unit or that there was alteration of vascular supply in terms of fasciculi; however, the latter found no evidence to support this idea.

Based on the results obtained by Dellasanta (1964) on the effects of various injection pressures on the numbers of open capillaries in skeletal muscle, it does not seem that this factor could be responsible for the condition in this study.

Studies on nervous control of blood vessels in skeletal muscle (Folkow, 1952; Uvnäs, 1960 and Barlow et al. 1961) indicate that there are two circulations through muscle and that they are differently controlled. Others (Hilton, 1959; Folkow, 1960 and Renkin and Rosell, 1962) point out the relationships of arterioles and pre-capillary sphincters to blood flow. These authors suggest that the pre-capillary sphincters monitor normal flow into the "effective circulation" at the samt time shunting more or less blood to the "by-pass" circulation as the situation demands. These circulatory adjustments in active skeletal muscle would tend to guarantee a balance between effective blood flow and the immediate needs of the tissues.

While the present report is not conclusive in this respect, it should be noted that the mechanisms involving the circulation to skeletal muscle are in harmony with the results of the present investigation which has demonstrated quantitative changes in circulation with specific exercise regimens. In light of this information, it is suggested that these mechanisms are responsible for the presence of ink filled capillaries in some areas of the muscle and total absence in others.

SUMMARY AND CONCLUSIONS

Thirty male rats (Sprague-Dawley), 25 days of age were placed in exercise cages for 7 days. From previous work, it is known that an area which contains red and white fibers as well as a mixed fiber area, is located in the middle one-third of this muscle. The author also found the capillary concentration to be greatest at this location. To assure uniformity in both capillary counts and muscle fiber measurements the mid-point of each muscle was selected for making tissue sections. For the next thirty-five days the sedentary group was permitted no exercise other than that allowed by their small individual cages. The voluntary group remained in activity cages while the forced group in addition to being in activity cages swam 30 minutes each day with lead weights equal to 2% of the body weight attached to their tails. At the end of the thirty-five day forced exercise period, the animals were sacrificed. The hind limbs were injected with India ink. The gastrocnemius muscle was fixed, embedded in gelatin and cut on the freezing microtome. The cross-sectional areas of the red and white muscle fibers from the gastrocnemius muscles were measured by using the polar planimeter. Ink filled capillaries were counted in conjunction with fiber measurements.

The results of measurements and counts for the sedentary, voluntary activity and forced activity groups were compared statistically using correlations, analysis of variance and the Tuckey procedures.

The average cross-sectional area of the red fibers per gram body weight in animals of the sedentary group was 3.82 square microns. In animals which had been forced to exercise, the average red fiber area per gram body weight was 5.72 square microns.

The total per cent differences in the size of the red fibers (forced-sedentary) and white fibers (forced-sedentary) was 49.7 and 32.5 per cent respectively. Of the total differences found, 56.8 per cent was manifest in the red fibers of animals permitted to exercise at will while a 76.2 per cent increase was produced in the white fibers of animals from the same group. By comparison, 23.6 per cent of the total white fiber difference and 43.1 per cent of the total red fiber difference (forced-sedentary) were found in the comparisons of the data from the forced exercise and voluntary exercise groups.

These findings support the work of Denny-Brown 1929, Buller et al. 1960a and b, Hess and Pilar 1963 and Vroba 1963 and have shown that the muscular tension imposed by the

various exercise programs was responsible for the differences in muscle fiber sizes.

In addition, the greater enlargement of white fibers in relation to voluntary activity and of red fibers with forced activity indicated that the two types of fibers responded differently to the imposed exercise programs.

The mean capillary per fiber per gram body weight (C/F/G) ratio for animals in the sedentary group was .80. In animals which had been forced to exercise, the C/F/G ratio was 1.0 while in the voluntary group it was .89.

The total per cent differences in C/F/G in red and white fibers (forced-sedentary) were 25 and 31 per cent. Of the total differences found only 75 per cent was exhibited by the white fibers of the voluntary group and only 45 per cent was produced in the red fibers of animals of the same group. Fifty-five per cent of the total red C/F/G difference and 25 per cent of the total white C/F/G difference (forced-sedentary) were found when the data from the forced and voluntary exercise groups were compared.

Comparisons of the differences in C/F/G ratios with the differences in mean fiber sizes per gram body weight indicated that of the total differences in the size of the red fibers, 56.8 per cent was expressed in the voluntary group.

At the same time, a 45 per cent difference of the total C/F/G ratio was produced in the same animals.

The 76.3 per cent difference of the total mean fiber size per gram body weight in the white fiber was commensurate with a 75 per cent difference from the total in the C/F/G ratio for that group.

Similarly, with forced exercise, the 43.6 per cent difference in white fiber size was accompanied by a 55 per cent difference in C/F/G ratio. The red fibers showed a 23.6 per cent difference from the total relative difference and a 25 per cent difference in C/F/G ratio. Thus, under conditions of voluntary and forced exercise, changes in muscle fiber sizes were paralleled by proportionate changes in vascular supply.

The comparable changes in muscle fiber sizes and C/F/G ratios are in line with the chemical myoglobin) and morphological (sarcoplasmic reticulum and mitochondria) relationships suggested by Milliken 1937, Lawrie 1952, 1953, and Porter and Armstrong 1965. Combined, these act to maintain a constant environment and satisfy the nutritional and energy requirements of the muscle tissue.

The fact that some areas of the muscle preparations appeared well injected while others were devoid of ink has

been noted in this study as well as by other authors. Adequate explanations of this phenomenon have escaped earlier investigators.

Important contributions to the solution of this problem may be found in the works of Folkow 1952, Uvnäs 1960 and Barlow et al. 1961, who reported that there are two circulatory systems in skeletal muscle and that they are differently controlled. In addition, Hilton 1959, Folkow 1960 and Renkin and Rossell 1962, suggested that pre-capillary sphincters monitored blood flow into the "effective circulation" and the same time shunted greater or lesser amounts of blood to the "by-pass circulation" as the situation demanded.

The present study which has demonstrated that quantitative changes take place in the circulation to skeletal muscle with specific exercise regimens are in agreement with these reports.

Furthermore, the quantitative changes shown in this report are in all probability the same as those reported by Martin et al. 1932 and Smith and Giovacchini 1956 and it is suggested that the presence of ink filled vessels in some areas of the muscle and total absence in others are expressions of mechanisms which affect the blood supply to

skeletal muscle as described by Folkow 1952, 1962, Hilton 1959, Uvnäs 1960, Barlow et al. 1961 and Renkin and Rosell 1962.

LITERATURE CITED

Bach, L. M. N. 1948. Conversion of red muscle to pale muscle. Proc. of Soc. Ex. Biol. and Med. 67:268-269.

Bagwell, E. E. and E. F. Woods. 1962. Cardiovascular effects of methoxyflurane. Anesthesiology 23:51-57.

Bajusz, E. 1964. "Red" skeletal muscle fibers: Relative independence of neural control. Science 145:938-939.

Bajusz, E. and G. Jasmin. 1963. Skeletal muscle diseases: Recent advances and some related basic problems. Canad. Med Ass J. 89:555-562.

Barlow, T. E., A. L. Haigh and D. N. Walder. 1961. Evidence for two vascular pathways in skeletal muscle. Clin. Sci. 20:367-385.

Bell, E. T. 1911. The interstitial granules of striated muscle and their relation to nutrition. Inter. Mschr. Anat. Physiol. 28:297-347.

Bennett, H. Stanley, A. Szent-Gyorgyi, D. Denny-Brown and R. D Adams. 1958. What we need to know about muscle. Symposium of inquiry. Neurology 8:64-79.

Bosiger, E. 1950. Vergleichende Untersuchungen uber die Brustmuskulatur von Hund, Wachtel and Star. Acta Anat. 10:385-429.

Brown, R. E. 1965. The pattern of the microculatory bed in the ventricular myocardium of domestic mammals. Am. J. Anat. 116:355-373.

Buller, A. J., J. C. Eccles and R. M. Eccles. 1960a. Differentiation of fast and slow muscles in the cat hind limb. J. Physiol. 150:399-416.

Buller, A. J., J. C. Eccles and R. M. Eccles. 1960b. Interaction between motoneurons and muscles in respect of characteristic speeds of their responses. J. Physiol. 150:417-439.

Byrom, F. B., and C. Wilson. 1938. A plethysmographic method for measuring systolic blood pressure in the intact rat. J. Physiol. 93:301-304.

Craig, John M., and Shoji Shintani. 1964. The failure of pregnancy to lower the blood pressure in rats with experimental hypertension. Lab. Invest. 13:378-380.

Dellasanta, L. 1964. Muscle fiber size and fiber to capillary ratio of the gastrocnemius muscle of sedentary adult rats. M. S. Thesis. Michigan State University, East Lansing, Michigan.

Denny-Brown, D. E. 1929. The histological features of striped muscle in relation to its functional activity. Proc. Roy. Sco. London 104:371-411.

Durant, R. R. 1927. Blood pressure in the rat. Am. J. Physiol. 81:679-685.

Duyff, J. W., and H. D. Bouman. 1927. Uber die Kapillarisation einiger Kaninchenmuskeln. Z. Zellforsch. 5:596-614.

Edwards, A. L. 1964. Statistical Methods for the Behavioral Sciences. Rinehart and Company, Inc., New York.

Elsner, R. W., and L. D. Carlson. 1962. Post-exercise hyperemia in trained and untrained subjects. J. Appl. Physiol. 17:436-452.

Folkow, B. 1952. A study of the factors influencing the tone of denervated blood vessels perfused at various pressures. Acta Physiol. Scand. 27:99-117.

Folkow, B. 1960. Range of control of cardiovascular system by the central nervous system. Physiol. Rev. 40, suppl. 4:93-99.

George, J. C., and R. M. Naik. 1957. The variations in the structure of the pectoralis major muscle of a representative type and their significance in the respective modes of flight. J. Anim. Morph. and Physiol. 4:23-32.

George, J. C., and R. M. Naik. 1958. The relative distribution and chemical nature of the fuel store of the two

types of fibers in the pectoralis major muscle of the pigeon. Nature 181:709-710.

George, J. C., and R. M. Naik. 1959. Studies on the structure and physiology of the flight muscles of birds. 4. Observations on the fiber architecture of the pectoralis major muscle of the pigeon. Biological Bulletin 116: 239-247.

Greep, R. O. 1954. Histology. The Blakiston Co., Inc., New York and Toronto. pp. 174.

Guenther, W. C. 1964. Analysis of Variance. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. pp. 112.

Halban, J. 1894. Die Dicke der querstreiften Muskelfasern und ihre Bedeutung. Anat. Hefte 3:267-286.

Hartman, Evans and Walker. 1929. Control of capillaries of skeletal muscle. Am. J. Physiol. 90:668-688.

Hatch, A., G. S. Wiberg, T. Balazs and H. C. Grice. 1963. Long term isolation stress in rats. Science 142:507.

Henneman, E., and C. B. Olson. 1965. Relations between structure and function in the design of skeletal muscles. J. Neurophysiology 28:581-598.

Hess, A., and G. Pilar. 1963. Slow fibers in the extraocular muscles of the cat. J. Physiol. 169:780-797.

Heusner, W. W. 1965. A specially built chamber for anesthetization of small animals. Unpublished.

Hilton, S. M. 1959. A peripheral conducting mechanism underlying dilatation of the femoral aretry and concerned in vasodilatation in skeletal muscle. J. Physiol. 149: 93-111.

Hyman, C., and R. L. Paldino. 1962. Local temperature regulation of microtissue clearance from rat skeletal muscle. Circ. Res. 10:89-93.

Hyman, C., and J. Lenthall. 1962. Analysis of clearance of intra-arterially administered labels from skeletal muscle. Am. J. Physiol. 203:1173-1178.

Hyman, C., R. L. Paldino and E. Zimmerman. 1963. Local regulation of effective blood flow in muscle. Circ. Res. 12:176-181.

Hyman, C. 1963. The circulation of blood through skeletal muscle. Pediatrics (Supplement) 32:671-679.

Jones, E. W., E. M. Jones, F. Stockton and C. Tigert. 1962. Observations on methoxyflurane anesthesia in the dog. J.A.V.M.A. 141:1043-1048.

Krogh, A. 1919. The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissues. J. Physiol. 52: 409-415.

Lawrie, R. A. 1952. Biochemical differences between red and white muscles. Nature 170:122-123.

Lawrie, R. A. 1953. Effect of enforced exercise on myoglobin concentration in muscle. Nature 171:1069-1070.

Martin, E. G., E. G. Woolley and M. Miller. 1932. Capillary counts in resting and active muscle. Am. J. Physiol. 100:407-416.

McPhedran, A. M., R. B. Wuerker and E. Henneman. 1965. Properties of motor units in a homogenous red muscle (soleus) of the cat. J. Neurophysiology 28:71-84.

Millikan, G. A. 1937. Experiments on muscle haemoglobin in vivo; the instantaneous measurement of muscle metabolism. Proc. Roy. Soc. B/123:218-241.

Morpurgo. B. 1897. Uber Activitals-Hypertrophie. Virchow's Arch. Path. Anat. 150:522-554.

Nachmias, Vivianne T., and H. A. Padykula. 1958. A histochemical study of normal and denervated red and white muscles of the rat. J. Biophysic. and Biochem. Cytol. 4:47-54.

Needham, D. 1926. Red and white muscle. Physiol. Rev. 6:1-27.

North, W. C., P. R. Knox, V. Vartanian and C. R. Stephen. 1961. Respiratory, circulatory, and hepatic effects of methoxyflurane in dogs. Anesthesiology 22:138-139.

Paff, G. H. 1930. A quantitative study of capillary supply in certain mammalian skeletal muscle. Anat. Rec. 46:401-405.

Petren, T., T. Sjostrand and B. Sylvan. 1936. Der Einfluss des Trainings auf die Haufigkeit der Capillaren in Herz- und Skeletmuskulatur. Arbeitsphysiol. 9:376-386.

Porter, K. R., and C. F. Armstrong. 1965. The sarcoplasmic reticulum. Scientific American. 212:72-81.

Renkin, E. M., and S. Rosell. 1962. Independent sympathetic vasoconstrictor innervation of arterioles and precapillary sphincters. Acta Physiol. Scand. 54:381-384.

Scheffe, H. 1959. The Analysis of Variance. John Wiley and Sons, Inc., New York. pp. 359-360.

Scott, J. H. 1957. Muscle growth and function in relation to skeletal morphology. Am. J. Phys. Anthro. 15:197-232.

Smith, D., and R. Giovacchini. 1956. The vascularity of some red and white muscle of the rabbit. Acta Anat. 28: 342-358.

Spalteholz, W. 1888. Die Vertheilung der Blutgefasse im Muskel. Abh. sachs. Ges. Wiss., math.-phys. Cl. 14: 509-528.

Stein, J. M., and H. A. Padukula. 1962. Histochemical classification of individual skeletal muscle fibers of the rat. Am. J. Anat. 110:103-124.

Stoel, G. 1925. Uber die Blutversorgung von Weissen und roten Kaninchemmuskeln. Z Zellforsch. 3:91-98.

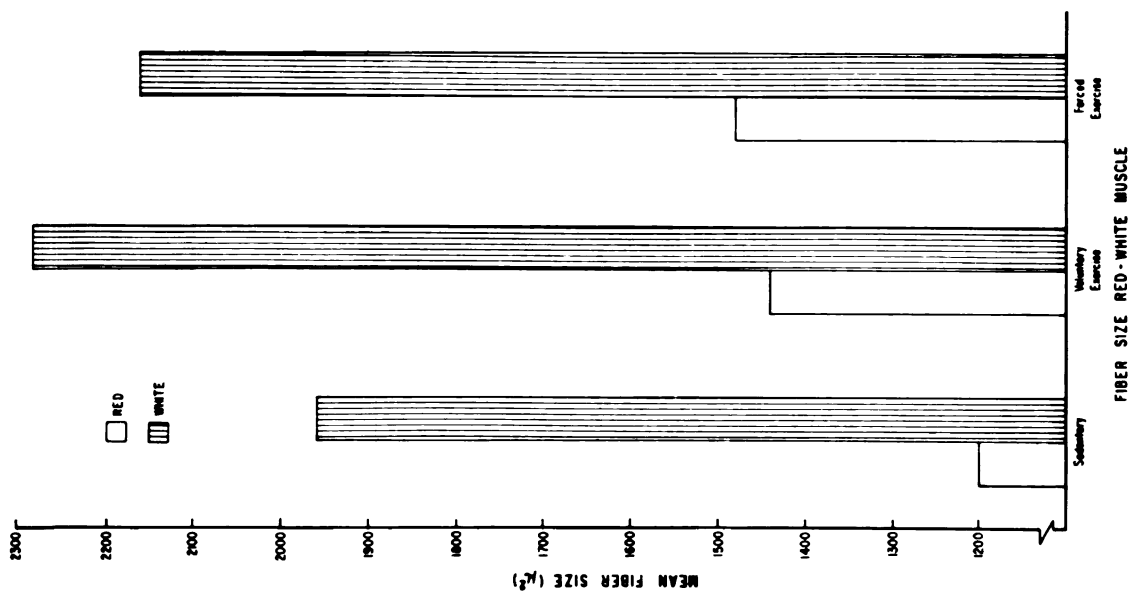
Uvnäs, B. 1960. Sympathetic vasodilator system and blood flow. Physiol. Rev. 40:(Suppl.4)69-76.

Valdivia, E. 1958. Total capillary bed in striated muscle of guinea pigs native to Peruvian mountains. Am. J. Physiol. 194:585-589.

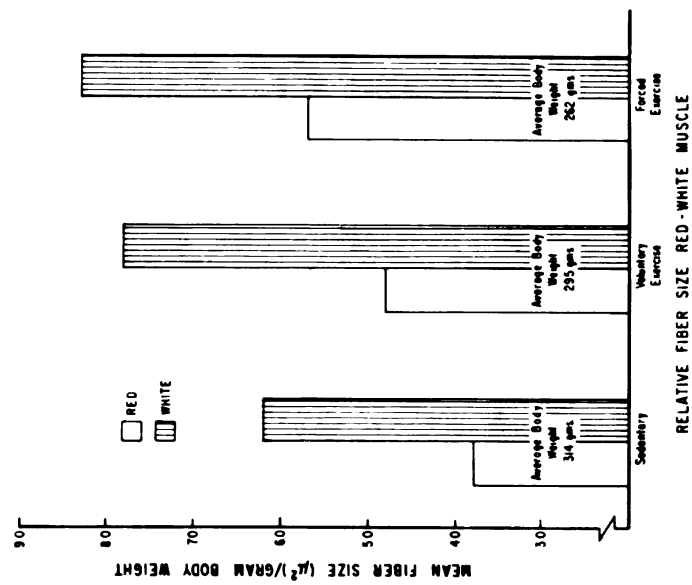
Vroba, G. 1963. The effect of motoneurone activity on the speed of contraction of striated muscle. J. Physiol. 169:513-526.

Watzka, M. 1939. "Weisse" und "rote" muskeln. Z. mikro.-anat. Forsch. 45:668-678.

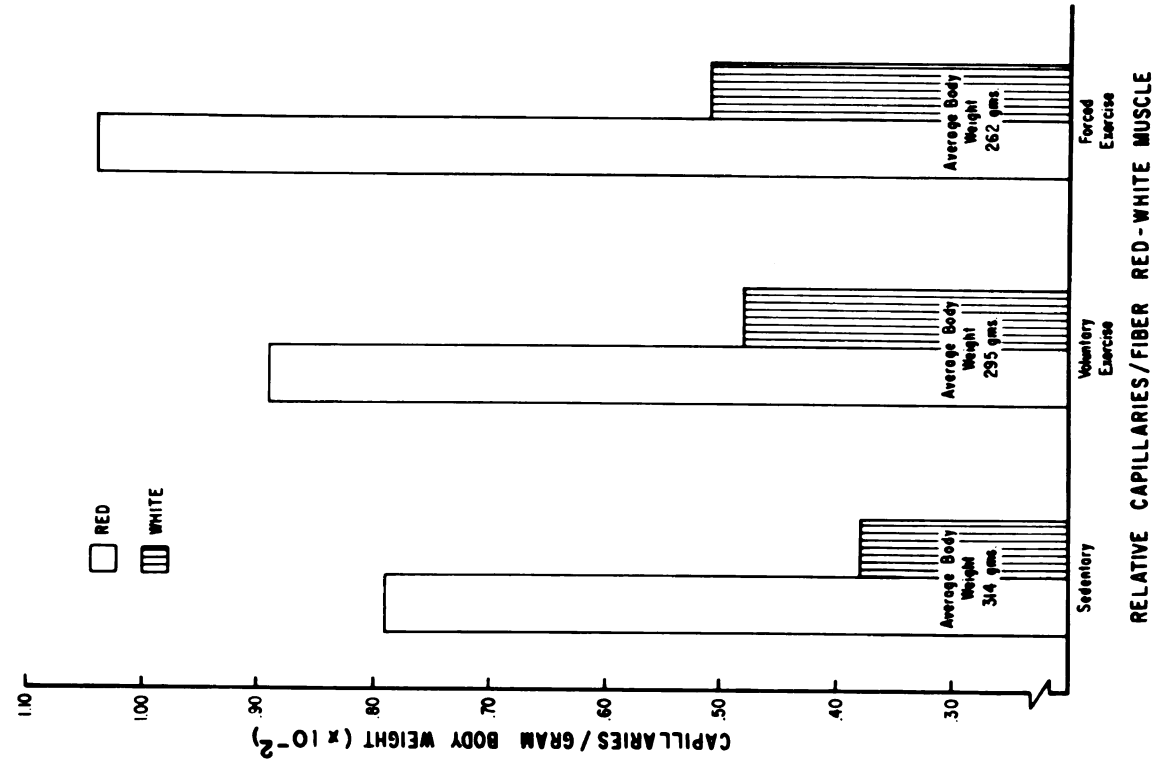
Wuerker, R. B., A. M. McPhedran and E. Henneman. 1965. Properties of motor units in a homogenous pale muscle (M. gastrocnemius) of the cat. J. Neurophysiology 28:85099.



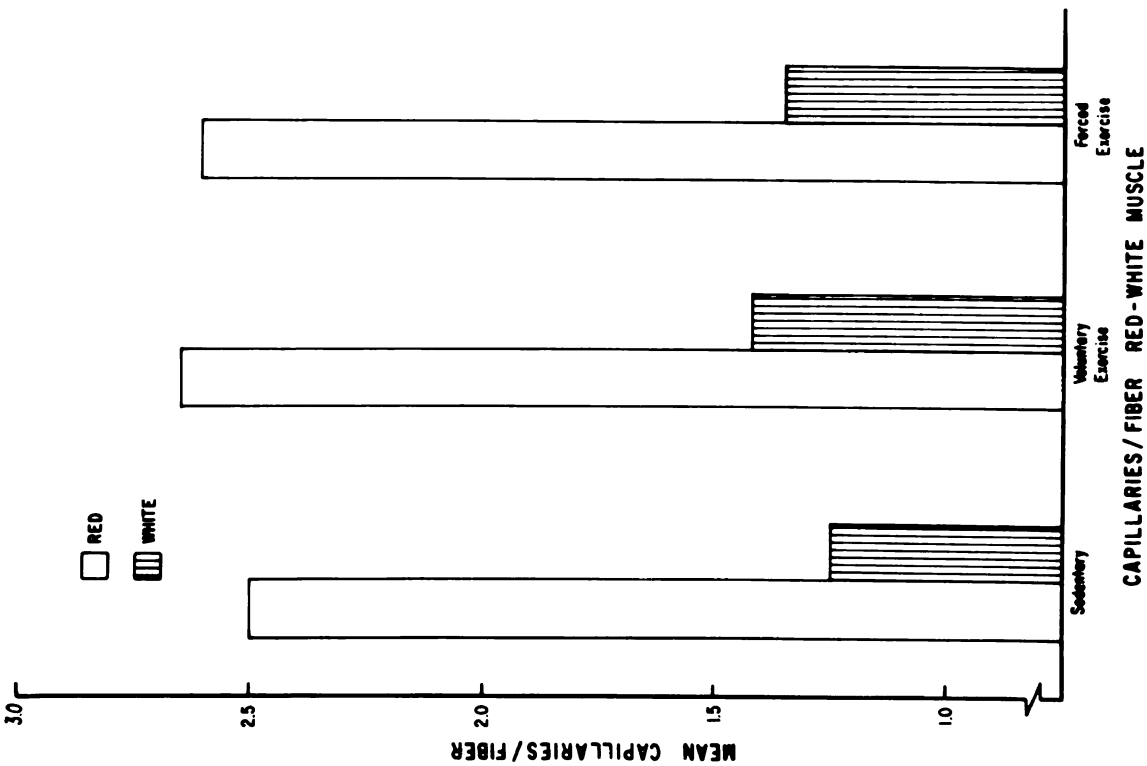
Graph 1



Graph 2



Graph 4



Graph 3

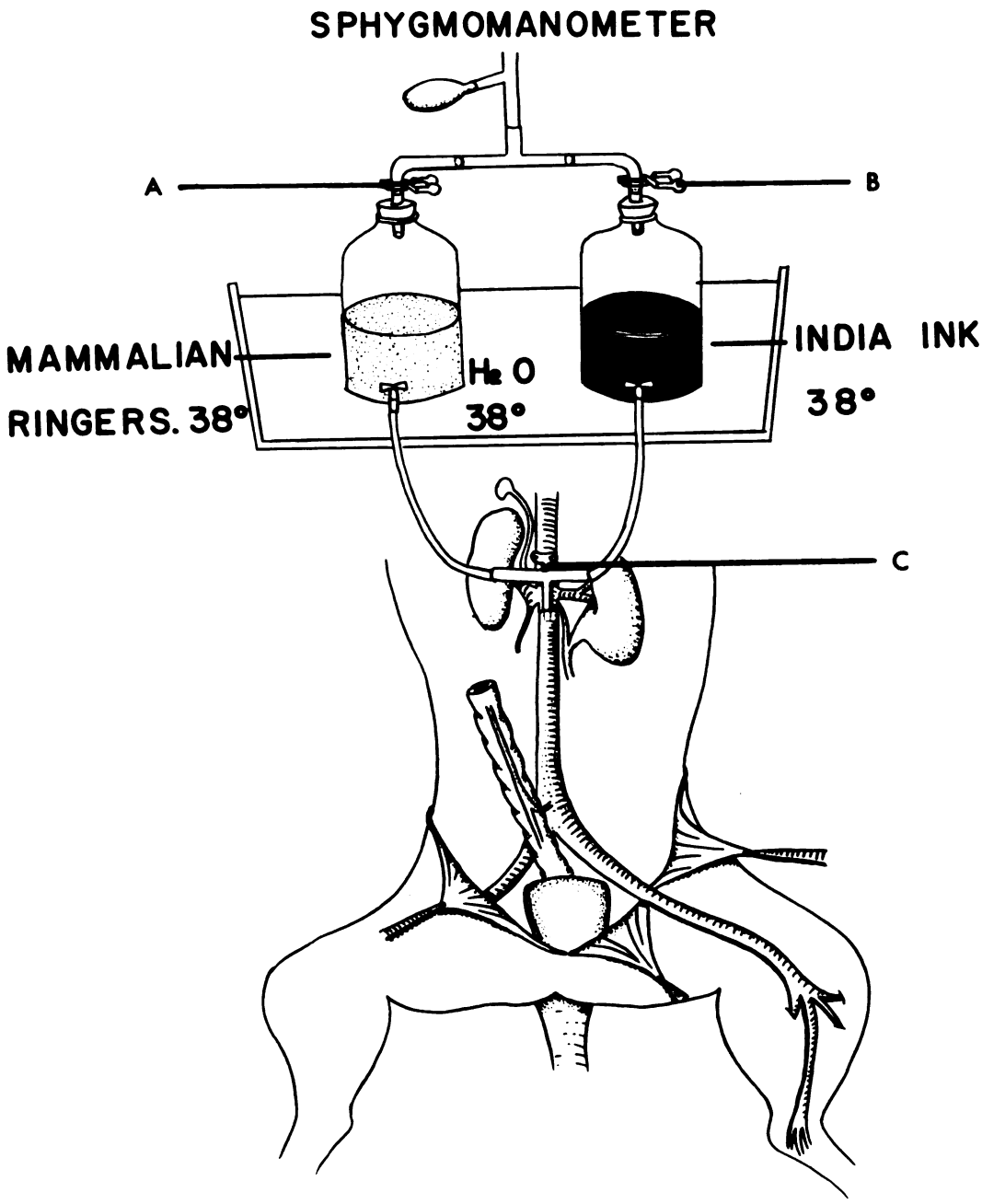

SPHYGMOMANOMETER
A
B
MAMMALIAN
RINGERS. 38°
H2 O
38°
INDIA INK
38°
C

Plate 1

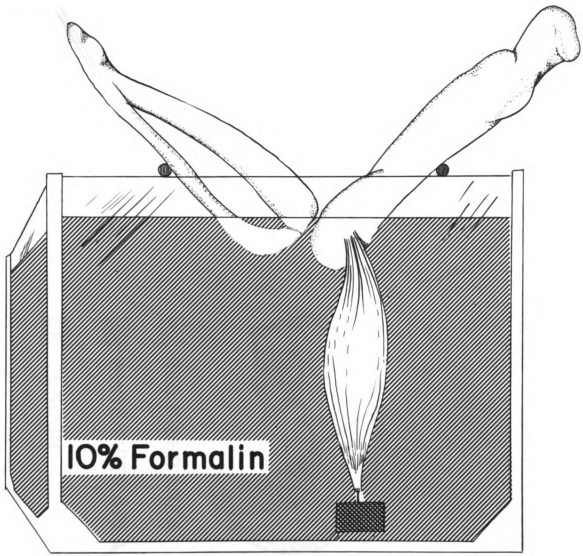


Plate 2

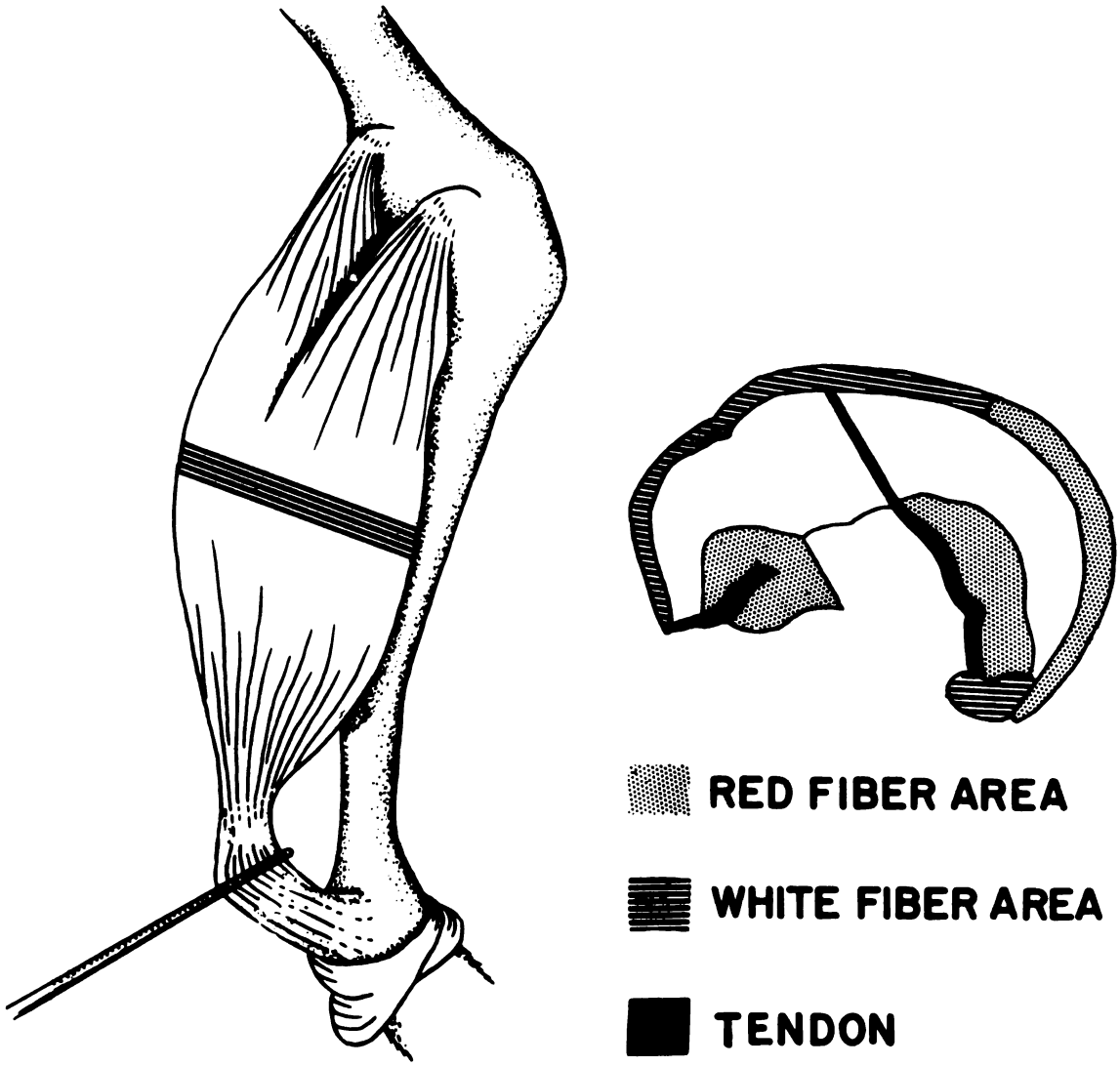


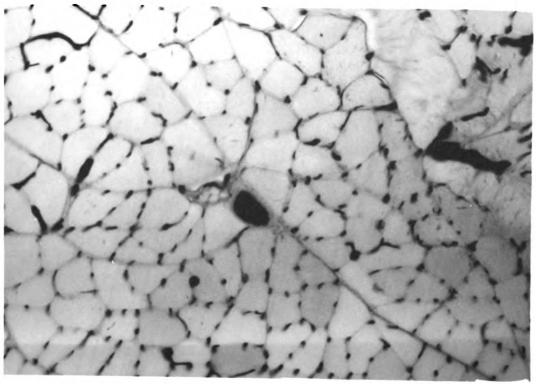
Plate 3

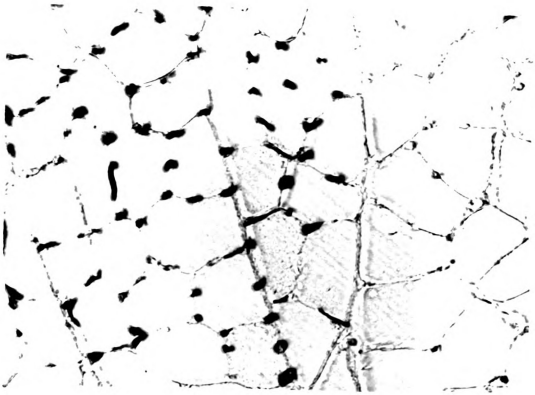
PLATE IV

Cross-sectional view of small, red muscle fibers and associated capillaries. (400 X).

PLATE V

Cross-sectional view of several adjacent fasciculi showing that some have capillaries filled with ink and others which are devoid of ink. (620 X).





APPENDIX A

Mean fiber sizes in sedentary and exercised animals

Animal group	Mean group wt. (gms.)	Mean fiber size/gms. (sq. microns)		S.D.	
		Red	White	Red	White
Sed.	314	1199	1959	283	283
Vol. ex.	295	1442	2284	386	580
For. ex.	262	1492	2163	447	470

Analysis of variance, red fibers, raw data

Source of variance	df	Mean square	F needed for significance	Experimental F
Effects due to differences in fibers	49	130595	F > 1.35	.89
Effects due to differences in treatment	2	12284824	F > 3.00	83.78*
Effects due to combinations of muscle fibers and treatments	98	99905	F > 1.25	.68
Error	1350	146632		
Total after mean	1499			

* Significant .05

Comparison of means--Tukey Test*

Group	$\bar{X}_{..j}$	$\bar{X}_{..j} - \bar{X}_F$	$\bar{X}_{..j} - \bar{X}_V$	$\bar{X}_{..j} - \bar{X}_S$
$\bar{X}F$	1492	0	51	293*
$\bar{X}V$	1441	-51	0	242*
$\bar{X}S$	1199	-293	-242	0

* Significant if > 231

Analysis of variance, red fibers, relative data

Source of variance	df	Mean square	F needed for significance	Experimental F
Effects due to differences in fibers	49	1.66	F > 1.35	.85
Effects due to differences in treatments	2	453.55	F > 3.00	233.71*
Effects due to combinations of muscle fibers and treatments	98	1.27	F > 1.25	.65
Error	1350	1.94		
Total after mean	1499			

* Significant .05

Comparison of means--Tukey Test*

Group	$\bar{X}..._j$	$\bar{X}..._j - \bar{X}_F$	$\bar{X}..._j - \bar{X}..._V$	$\bar{X}..._j - \bar{X}..._S$
$\bar{X}F$	5.73	0	.83	1.90 *
$\bar{X}V$	4.90	-.83	0	1.07 *
$\bar{X}S$	3.83	-1.90	-1.07	0

* Significant if > .84

Analysis of variance, white fibers, relative data

Source of variance	df	Mean square	F needed for significance	Experimental F
Effects due to differences in fibers	49	2.35	F > 1.35	.77
Effects due to differences in treatments	2	560.61	F > 3.00	184.14*
Effects to to combinations of muscle fibers and treatments	98	1.87	F > 1.25	.61
Error	1350	3.04		
Total after mean	1499			

* Significant .05

Comparison of means--Tukey Test*

Group	$\bar{X}_{..j}$	$\bar{X}_{..j} - \bar{X}_F$	$\bar{X}_{..j} - \bar{X}_V$	$\bar{X}_{..j} - \bar{X}_S$
$\bar{X}F$	8.27	0	.48	2.02*
$\bar{X}V$	7.79	-.48	0	1.54*
$\bar{X}S$	6.25	-2.02	-1.54	0

* Significant if > 1.06

Group correlations on fiber sizes

Red fibers
raw data

Groups	r
F-S***	.17
V-S**	-.10
F-V*	-.15

Red fibers
relative data

Groups	r
F-S	.05
V-S	.03
F-V	-.20

White fibers
raw data

Groups	r
F-S	.08
V-S	.01
F-V	-.04

White fibers
relative data

Groups	r
F-S	-.05
V-S	.07
F-V	-.14

*** F-S correlations of fiber sizes of forced and sedentary groups.
** V-S correlations of fiber sizes of voluntary and sedentary groups.
* F-V correlations of fiber sizes of forced and voluntary groups.

Analysis of variance, red fibers, capillary to fiber ratios, raw data

Source of variance	df	Mean square	F needed for significance	Experimental F
Effects due to treatments	2	.03908333	F > 3.35	1.61
Error	27	.02426407		
Total after mean	29			

* Significant .05

Comparison of means--Tukey Test*

Group	$\bar{X}_{..j}$	$\bar{X}_{..j} - \bar{X}_F$	$\bar{X}_{..j} - \bar{X}_V$	$\bar{X}_{..j} - \bar{X}_S$
$\bar{X}F$	2.63	0	-.01	.10
$\bar{X}V$	2.64	.01	0	.11
$\bar{X}S$	2.53	-.10	-.11	0

* Significant if > .55

Analysis of variance, red fibers, capillary to fiber ratios, relative data

Source of variance	df	Mean square	F needed for significance	Experimental F
Effects due to treatments	2	.00000975	F > 3.35	20.74*
Error	27	.00000047		
Total after mean	29			

* Significant .05

Comparison of means--Tukey Test*

Group	$\bar{X}_{..j}$	$\bar{X}_{..j} - \bar{X}_F$	$\bar{X}_{..j} - \bar{X}_V$	$\bar{X}_{..j} - \bar{X}_S$
F-S	.010064	0	.001055*	.002056*
V-S	.009009	-.001055	0	.000921*
F-V	.008088	-.002056	-.000921	0

* Significant if > .000803

Analysis of variance, white fibers, capillary to fiber ratios, raw data

Source of variance	df	Mean square	F needed for significance	Experimental F
Effects due to treatments	2	.09430333	$F > 3.35$	2.38
Error	27	.03961074		
Total after mean	29			

* Significant .05

Comparison of means--Tukey Test*

Group	$\bar{X}_{..j}$	$\bar{X}_{..j} - \bar{X}_F$	$\bar{X}_{..j} - \bar{X}_V$	$\bar{X}_{..j} - \bar{X}_S$
F-S	1.35	0	-.08	.12
V-S	1.42	.08	0	.20
F-V	1.22	-.12	-.20	0

* Significant if $> .70$

Analysis of variance, white fibers, capillary to fiber ratios, relative data

Source of variance	df	Mean square	F needed for significance	Experimental F
Effects due to treatments	2	.00000434	$F > 3.35$	7.03*
Error	27	.00000062		
Total after mean	29			

* Significant .05

Comparison of means--Tukey Test*

Group	$\bar{X}_{..j}$	$\bar{X}_{..j} - \bar{X}_F$	$\bar{X}_{..j} - \bar{X}_V$	$\bar{X}_{..j} - \bar{X}_S$
F-S	.005196	0	.000368	.001280*
V-S	.004828	-.000368	0	.000912*
F-V	.003916	-.001280	-.000912	0

* Significant if $>$ .000852

Group correlations on capillary to fiber ratios

Red fibers
raw data

Groups	r
F-S***	.16
V-S***	.30
F-V*	.55

Red fibers
relative data

Groups	r
F-S	.07
V-S	-.13
F-V	-.03

White fibers
raw data

Groups	r
F-S	-.27
V-S	.17
F-V	.20

White fibers
relative data

Groups	r
F-S	-.30
V-S	.34
F-V	.18

*** F-S correlations of capillary to fiber ratios of forced and sedentary groups.
** V-S correlations of capillary to fiber ratios of voluntary and sedentary groups.
* F-V correlations of capillary to fiber ratios of forced and voluntary groups.

APPENDIX B

BASIC DATA

Animal study number	Weight at sacrifice (gms.)	Mean daily revolutions	Red fibers cap./fiber	White fibers cap./fiber	Red mean fiber size (μ^2)
F 4	275	817	2.52	1.24	1205
F 6	244	634	2.38	1.38	1715
F21	247	1401	2.62	1.60	1685
F22	247	685	2.42	1.52	1534
F26	286	964	2.78	1.34	1349
F34	267	1086	2.64	1.12	1400
F37	274	729	2.86	1.68	1298
F39	261	862	2.66	1.04	1673
F42	271	870	2.82	1.28	1925
F47	246	741	2.62	1.34	1063
Mean	262	879	2.63	1.35	1492
V 4	275	5314	2.84	1.19	1785
V 6	293	3635	2.52	1.46	1170
V21	277	517	2.60	1.22	1228
V22	303	1221	2.48	1.18	1308
V26	329	497	2.64	1.36	1731
V34	288	1701	2.74	1.40	1708
V37	322	1562	2.92	1.80	1097
V39	320	2566	2.51	1.34	1693
V42	274	560	2.76	1.28	1275
V47	271	1032	2.46	1.96	1421
Mean	295	1860	2.65	1.42	1442
S 4	303	--	2.52	1.22	1110
S 6	298	--	2.56	1.10	1104
S21	308	--	2.48	1.14	1227
S22	332	--	2.52	1.16	1017
S26	295	--	2.46	1.28	1182
S34	329	--	2.44	1.18	1032
S37	327	--	2.90	1.34	1204
S39	334	--	2.56	1.42	1178
S42	337	--	2.34	1.24	1612
S47	279	--	2.54	1.20	1251
Mean	314	--	2.53	1.22	1199

Red fiber size (μ^2) per gm. body wt.	White mean fiber size (μ^2)	White fiber size (μ^2) per gm. body wt.	Red fibers cap./fiber per gm. body wt.	White fibers cap./fiber per gm. body wt.
4.3818	2084	7.5781	.0091	.0045
7.0286	2327	9.5368	.0097	.0056
6.8218	2141	8.6680	.0106	.0064
6.2105	2119	8.5789	.0097	.0061
4.7167	2170	7.5874	.0097	.0046
5.2434	2282	8.5468	.0098	.0041
4.7372	2522	9.2043	.0104	.0061
6.4099	2404	9.2107	.0101	.0039
7.1033	1994	7.3579	.0104	.0047
4.3211	1599	6.5000	.0106	.0054
5.6974	2163	8.2768	.0100	.0051
6.4909	2625	9.5454	.0103	.0043
3.9931	1861	6.3515	.0086	.0049
4.6498	1997	7.2093	.0093	.0044
4.3168	1960	6.4686	.0081	.0038
5.2613	1989	6.0455	.0080	.0041
5.9305	2792	9.6944	.0095	.0048
3.4068	2472	7.6770	.0090	.0055
5.2906	2363	7.3843	.0078	.0041
4.6532	1851	6.7554	.0100	.0046
5.2435	2882	10.6346	.0090	.0072
4.9236	2284	7.7766	.0089	.0048
3.6633	1850	6.1056	.0083	.0040
3.7046	1729	5.8020	.0085	.0036
3.9837	2087	6.7759	.0080	.0037
3.0632	1972	5.9397	.0075	.0034
4.0067	2033	6.8915	.0073	.0043
3.1367	1980	6.0182	.0074	.0035
3.6819	1945	5.9480	.0088	.0040
3.5269	2047	6.1287	.0076	.0042
4.7833	2010	5.9643	.0069	.0036
4.4838	1870	6.7025	.0091	.0043
3.8034	1959	6.2276	.0080	.0039