HISTOCHEMICAL PROFILES OF RAT TRICEPS SURAE AND PLANTARIS AFTER SEVEN EXERCISE REGIMENS

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This is to certify that the

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presented by

James Francis Taylor

has been accepted towards fulfillment
of the requirements for

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Date November, 1971

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ABSTRACT

HISTOCHEMICAL PROFILES OF RAT TRICEPS SURAE AND PLANTARIS AFTER SEVEN EXERCISE REGIMENS

By

James Francis Taylor

This study was undertaken to determine the effects of seven different exercise regimens on the histochemical characteristics and distribution of selected muscle fibers of the triceps surae and plantaris muscles of albino rats.

One hundred and seventy-six 72-day-old, normal, male, albino rats (Sprague-Dawley Strain) were randomly assigned to one of the seven treatments. Treatments began after a 12 day adjustment period when all animals were 85 days of age. The treatment groups were sedentary control (CON); voluntary running (VOL); short-duration, high-speed endurance running (SHT); medium-duration, moderate-speed endurance running (MED); long-duration, low-speed endurance running (LON); electric stimulus control (ESC); and long-duration, low-intensity swimming (SWM). Treatments were administered once a day, Monday through Friday. All animals had access to food and water ad libitum. Seven animals, limited to the same duration, were weighed

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and sacrificed after zero, four, eight, and twelve weeks of training. The final sample consisted of 94 animals.

Animals were sacrificed under anesthesia by intraperitoneal injection of pentobarbital sodium. Pelikan ink was injected into the vascular system for capillary per muscle fiber calculations. The triceps surae and plantaris muscles were removed as a unit, and frozen in an isopentane-liquid nitrogen system. Fresh-frozen, distal-proximal serial cross sections, were cut at 10 microns using a rotary microtome-cryostat. Four histochemical procedures were utilized for identification of relative fiber type intensities of glycogen (periodic acid-Schiff), glycolytic enzyme (phosphorylase), oxidative enzyme (succinate dehydrogenase), and energy producing systems (adenosine triphosphatase). Fifty muscle fibers, from each specific intramuscular area of medial gastrocnemius, plantaris, and soleus muscles, were graded according to intensity and distribution patterns of the various histochemical procedures. The percentages of intensity ratings were recorded for individual animals.

The prominence of duration, as well as treatment, effects suggested that the seven different chronic physical activities had specific effects upon the alteration of fiber characteristics, but the effects were highly time dependent.

The results indicated there were diverse regional responses and patterns of change over time, to the same

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exercise stimulus. Four, eight, and twelve weeks of various activity programs produced metabolic alterations in proportions of fiber types in the medial gastrocnemius, plantaris, and soleus muscles.

In the soleus muscle area, similar periodic acid-Schiff (PAS) changes occurred for the voluntary group at four and eight weeks. The voluntary (VOL) group activity produced a significant increase in the percentage of intermediate ATP fibers at eight weeks, while the electric stimulus control (ESC) treatment produced a similar significant increase at four weeks, which was reversed at twelve weeks ($p < .20$).

In the plantaris muscle area increases in similar PAS intensities were found at four weeks for long (LON) and swimming (SWM) groups ($p < .20$). The electric stimulus control (ESC) and SWM programs produced significant increases in the percentage low intensity ATP fibers at twelve weeks, while the VOL treatment produced the same result at four weeks. The short (SHT) group showed a specific pattern of increasing intermediate ATP fibers from four to twelve weeks ($p < .20$).

The general adaptive patterns for the medial gastrocnemius muscle area showed that anaerobic fibers were able to acquire aerobic fiber characteristics and supported the hypothesis of specificity of alteration. The greatest relative rise in ATP occurred in the medium

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(MED) and long (LON) running groups. This change was observed between four and twelve weeks for the MED group and between zero and eight weeks for the LON group.

HISTOCHEMICAL PROFILES OF RAT TRICEPS SURAE AND PLANTARIS AFTER SEVEN EXERCISE REGIMENS

By

James Francis Taylor

A THESIS

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TABLE OF CONTENTS

Table

LIST OF TABLES

LIST OF PLATES AND FIGURES

VITA

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LIST OF ABBREVIATIONS

To prevent confusion, and for the sake of clarity, certain words and/or phrases are abbreviated throughout this thesis.

ATP	Intermyofibrillar adenosine triphosphatase
CDS	Cumulative duration of shock (seconds) received by both the experimental, and the control animals during all work periods of all bouts of a given training period.
CON	Sedentary control
CRW	Controlled Running Wheel
ESC	Electric stimulus control
EST	Expected swim time (minutes)
LON	Long-duration, low-intensity running exercise (long CRW program)
MED	Medium-duration, moderate-intensity running exercise (medium CRW program)
MG	Medial Gastrocnemius muscle
NBT	Nitro Blue Tetrazolium
PAS	Periodic Acid-Schiff
PER	Percent expected revolutions; PER = 100 TRR/TER

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VOL .

PET . . . . . .Percent expected swim time; PET = 100 STC/EST

PLT . . . . . .Plantaris muscle

PPL . . . . . .Phosphorylase

PSF . . . . . .Percent shock free time; PSF = 100 - (100 CDS/TWT)

SOL . . . . . .Soleus muscle

SDH . . . . . .Succinate dehydrogenase

SHT . . . . . .Short-duration, high-intensity running exercise (short CRW program)

STC . . . . . .Swim time completed

SWM . . . . . .Swimming exercise

TER . . . . . .Total expected revolutions that the animal would run, during all work periods of all bouts of a given training period, if he would run at the prescribed speed.

TRR . . . . . .Total number of revolutions run by the experimental animal, during all work periods of all bouts of a given training period.

TWT . . . . . .Total work time (sec) during all work periods of all bouts of a given training period.

VOL . . . . . .Voluntary running exercise

INTRODUCTION

General Comments

Classic investigations in the nineteenth century (Denny-Brown, 1929a) suggested possible correlations between certain cytological aspects of "red" and "white" muscle and functional activity. Since then numerous physiological and histological studies have documented differences between "red" and "white" muscle (Denny-Brown, 1929a; Jinnai, 1960; Beatty et al., 1963, 1966, 1967; Blanchaer, 1964; Gauthier and Padykula, 1966; Olson and Swett, 1966; Adams et al., 1967; Arangio and Hagstrom, 1969; Briskey et al., 1970; Sandow, 1970; Schiaffino et al., 1970). Recent histochemical investigations clearly show marked heterogeneity in glycolytic and oxidative properties between red and white muscle (Padykula, 1952; Glock and McLean, 1953; Takeuchi and Kuriaki, 1955; Wachstein and Meisel, 1955; Ogata, 1958; Nachmias and Padykula, 1958; Dubowitz and Pearse, 1961; Beckett, 1962, Ogata and Mori, 1964). Histochemical identification of enzymatically contrasting muscles, with even more unique fiber types, indicates the existence of differences in energy metabolism and energy supplying systems important to contractile function.

It is presently hypothesized that control of the metabolic and contractile properties of skeletal muscle is related to (a) the frequency of impulses in the motor nerve; (b) or the motoneurone release of "trophic" substances independent of electrical activity (Gutmann et al., 1956; Guth, 1968; Fex, 1969; Bass et al., 1970; Fex and Sonesson, 1970; Gutmann, 1970; Robert and Oester, 1970). Employing these basic tenents, investigations have shown that skeletal muscle properties are mutable in atrophy (Bajusz, 1964; Fischbach and Robbins, 1969, 1970; Brooks, 1970; Kauffman and Albuquerque, 1970; Klinkerfuss and Haugh, 1970), denervation (Needham, 1926; Nachmias and Padykula, 1958; Huls and Leonard, 1961; Gutmann, 1962; Pelligrini and Franzini, 1963; Hogan et al., 1965; Engel et al., 1966; Prewitt and Salafsky, 1967; Sreter, 1970), cross-innervation (Buller et al., 1960a, 1960b; Buller and Lewis, 1965a; Smith, 1965; Romanul and VanDerMeulen, 1966; Dubowitz, 1967a, 1967b, 1967c; Eccles, 1967; Hnik et al., 1967; Prewitt and Salafsky, 1967; Yellin, 1967; Gerebtzoff, 1968; Guth et al., 1968; Karpati and Engel, 1968b; Ogata et al., 1968; Buller and Mommaerts, 1969; Close, 1969; Robbins et al., 1969; Guth et al., 1970) and immobilization (Bach, 1948; Wells, 1969) as identified by anatomical, histochemical, biochemical, and physiological measures.

Recent evidence suggests that exercise has similar effects upon skeletal muscle properties (Gordon, 1967;

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Gordon et al., 1967a, 1967b; Holloszy, 1967; Barnard et al., 1970, 1971; Edgerton et al., 1969, 1970; Holloszy and Oscai, 1969; Kowalski et al., 1969; Lamb et al., 1969; Short et al., 1969; Ruhling, 1970; Spurway and Young, 1970; Campbell et al., 1971; Faulkner et al., 1971; Gollnick, 1971). This body of knowledge is difficult to interpret because exercise too often has been viewed as an entity, not as an aerobic-anaerobic continuum. Specificity of exercise as a reality was intimated in a related histochemical study on heart metabolism (Ruhling, 1970). To corroborate the emerging histochemical evidence of metabolic plasticity of skeletal muscle with exercise, a knowledge of the regional and temporal responses to identical and diverse physiological stimuli ultimately is necessary.

Statement of the Problem

This study was undertaken to determine the effects of seven different exercise regimens on the histochemical characteristics of selected muscle fiber areas of gastrocnemius and soleus (triceps surae), and plantaris muscles of normal, adult, male, albino rats.

Rationale

The extent to which the principle of specificity applies to a continuum of exercise is a fundamental physiological question which must be answered. Histochemical approaches to investigate this question are

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conspicuously absent. This study was designed to permit a rational statement, in histochemical terms, regarding distinctive patterns of metabolic alterations in rat skeletal muscle fibers that are associated with defined, reproducible exercise regimens.

It was postulated that cellular enzyme and substrate levels are determined by the frequency, intensity and duration of contraction, in accord with the metabolic requirements of the animal. It was believed also that these cellular responses are not only specific to the exercise requirements of the total muscle but are identifiable by specific muscle area, and even at the individual muscle fiber level, as identified in histochemical tissue sections.

Significance of the Problem

Histochemical investigations indicate that basic differences exist within, as well as between, classic muscle fiber types. Incorporation of methods of subjecting animals to different controlled regimens of reproducible exercise, which compare favorably with human exercise programs requiring aerobic, anaerobic, and aerobic-anaerobic adaptations, should yield: (a) differential histochemical fiber type alterations produced by specific regimens of physical activity, (b) new evidence on general patterns of adaptation to specific exercise regimens and the effects of different durations of the various exercise

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programs, (c) a body of knowledge for intelligent prescription of exercise programs at the muscle fiber level, and (d) a stimulus for investigations to elucidate the cellular mechanisms involved in exercise induced adaptations.

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REVIEW OF PERTINENT LITERATURE

This review focuses on the fundamental anatomical, physiological, biochemical and histochemical differences of vertebrate skeletal muscle fibers; the effect of surgically induced conditions on these muscle fiber parameters; and a consideration of related biochemical and histochemical studies on skeletal muscle response to various exercise programs.

Red, Intermediate, and White Skeletal Muscle Fibers

Application of diverse biological techniques to "white" and "red" muscle established new dimensions for the identification of at least three fiber types.

Anatomical Differences

The main differences in fiber types are found in the content, form, and distribution of the constituent cellular organelles and associated tissues. In the red fiber large spherical mitochondria, with numerous fenestrated sheet cristae and dense matrices, form an aggregate oxidative machinery layer between the sarcolemma and the contractile substance (Van Breemen, 1960; Padykula and

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Gauthier, 1963; Gauthier and Padykula, 1966; Murata and Ogata, 1969; Ogata and Murata, 1969a, 1969b). In the interior of the fibers on either side of the Z line smaller filamentous mitochondria are aligned in the region of the I band. Prominent small mitochondria form longitudinal chains in the intermyofibrillar space.

In the white fiber, few mitochondria are dispersed in the subsarcolemmal space or the intermyofibrillar space, while small to medium mitochondria, with few cristae and less dense matrices, are occasionally located in the region of the I band. No striking differences exist between size and structure of the peripheral and central mitochondria (Padykula and Gauthier, 1963).

The intermediate fiber occasionally has aggregates of mitochondria in the subsarcolemmal space. Accumulations of smaller mitochondria, with fewer cristae, as intermyofibrillar chains, are not as conspicuous as found in red fibers (Gauthier and Padykula, 1966; Shafiq et al., 1966). Lipid droplet concentration is directly related to mitochondrial density (Gauthier and Padykula, 1966).

The sarcoplasmic reticulum of individual red fibers has received conflicting description. Schiaffino et al. (1970) described a well developed sarcoplasmic reticulum in the red fibers of the rat soleus muscle. Padykula and Gauthier (1970) and Nishiyama (1965a, 1965b), however, stated that red fibers in the diaphragm and intercostal

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muscles have poorly developed sarcoplasmic reticulum. The assumption that mitochondrial rich fibers have poorly defined sarcoplasmic reticulum is not true in all muscles. The view that both mitochondrial rich and mitochondrial poor fibers are fast, and have comparably rich development of sarcoplasmic reticulum is consistent with the proportion of motor units with relatively fast speeds of contraction for rat soleus (Close, 1967a, 1967b).

In the red and white fibers triads occur at the A-1 band junctions, longitudinal tubules extend from the terminal cisternae toward the H band level to form a transverse network (Schiaffino et al., 1970).

The intermediate fiber of extensor digitorum longus and soleus muscles and the red fiber of the diaphragm occasionally have T tubules with only one junctional cristerna (dyad), instead of the familiar triad structure. Sparse longitudinal tubules extend from the junctional cisternae into the A band, with limited branching at the H band level (Ogata, 1964; Padykula and Gauthier, 1970; Schiaffino et al., 1970).

The red fiber usually has more myonuclei (Needham, 1926), wider Z line (Gauthier, 1969; Schiaffino et al., 1970), longer sarcomere length (Schiaffino et al., 1970), more sarcoplasm (Denny-Brown, 1929a; Gauthier and Padykula, 1966), and smaller diameter (Denny-Brown, 1929a), and less connective tissue (Beatty et al., 1966) than

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white fibers. Triglyceride droplets are directly related to mitochondrial density, and are more abundant in red than white fibers (Gauthier and Padykula, 1966).

While each en plaque endplate is situated in the middle of the fiber, differences exist in the neruomuscular junction. The red fiber endplate has the least sarcoplasmic and axoplasmic surface, at each contact relatively discrete and separate axonal terminals are small and elliptical, junctional folds are shallow, flat and sparse. The white fiber has long flat axonal terminals and the profile of junctional folds increases in complexity. The intermediate fiber in the diaphragm has large axonal terminals with the most widely spaced and deepest junctional folds (Ogata, 1964; Ogata and Murata, 1969a, 1969b; Padykula and Gauthier, 1970). A clear cut difference in the form and volume of the junctional invagination is evident.

The capillary to fiber ratio is directly proportionate to oxidative metabolism in red, intermediate and white fibers (Nishiyama, 1965b; Romanul, 1965; Carrow et al., 1967; Romanul and Pollock, 1969; Mai et al., 1970).

Physiological Differences

Physiological studies performed on whole muscle preparations conform well with morphological investigations in establishing slow and fast twitch muscles.

At birth all muscles in the kitten are uniformly slow twitch, and speed of shortening of sarcomeres

increases two to three fold within three to four weeks (Denny-Brown, 1929a, 1929b; Buller et al., 1960a; Close, 1967a, 1967b; Close and Hoh, 1967). Slow muscles in contrast to fast muscles, show little change in speed of shortening with maturation. Close (1967a, 1967b) examined the dynamic properties of muscles in different species and found that neither the fast nor the slow muscles had the same intrinsic speeds. In this connection at least three types of motor units have been identified in skeletal muscles (Henneman and Olson, 1965; Wuerker et al., 1965; Close, 1967b; Edström and Kugelberg, 1968, 1969). In a histochemical study on the effect of contraction induced by low frequency stimulation, Edström and Kugelberg (1969) and Kugelberg and Edström (1968) showed that repetitive stimulation of ventral root, single nerve fiber or entire nerve trunk, produced preferential changes in phosphorylase and glycogen in muscle fibers. Mapping of the motor unit showed highly intermingled phosphorylase and glycogen negative fibers, with a more pronounced influence in white fibers. Wuerker et al. (1965), found differences in contraction speeds of individual motor units. This information tends to support the concept of a homogeneous character of the motor unit, as a natural result of the uniform neural control exerted by its motoneurone.

In gastrocnemius and plantaris muscles, the type A motor unit has large, low resistance motoneurones

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innervating fast twitch muscle units with relatively large tension output (Somjen et al., 1964; Burke, 1968). In the soleus muscle the type B motor unit is characterized by smaller, higher resistance motoneurones innervating slow twitch muscle units with small tension output (Burke, 1968). The type B unit characteristics are known from work on the cat soleus muscle, which has only intermediate fibers (Henneman and Olson, 1965). A group of intermediate units remains unidentified, of which some may belong to type C. Close (1967b) showed in rat soleus muscle, that of 30 units identified on basis of contraction time, 3 were intermediate (17.5 milliseconds) and 27 slow (38 milliseconds).

Generally fast muscles develop high initial tension and contract rapidly (18-129 milliseconds) with conduction velocity of 95 meters per second and an axon diameter 13.6-8.3 microns. Slow muscles contract slowly (58-193 milliseconds) with conduction velocities of (51-81 meters per second) and axon diameter 6.3-2.7 microns (Henneman and Olson, 1965; Wuerker et al., 1965; Eberstein and Goodgold, 1968).

McComas and Thomas (1968) observed for human gastrocnemius muscle a contraction speed of 117.6 milliseconds, which appears to be considerably slower than that of slow muscles in other species, such as cat and rabbit.

Rapidly contracting phasic fibers are usually located near the subcutaneous surface and slow contracting tonic

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fibers near the deep axial surface (Gordon and Phillips, 1953). Physiological data tend to indicate that "redness" of a muscle reflects a slow rate of contraction, while "whiteness" reflects a fast contraction rate. Buchthal and Schmalbruch (1970) found that histograms of contraction times agreed with histochemical findings in triceps surae muscles. In the tibialis anterior muscle, red fibers constituted half the fiber population, and half of the contraction times were longer than 60 msec. However, exceptions exist. For example, Hall-Craggs (1968) observed that the thyroarytenoid muscle was an extremely fast twitch muscle, with histochemical characteristics, that predicted slow twitch, whereas, the cricothyroid muscle was a slow twitch muscle, with histochemical characteristics found in fast limb muscles. Illustrating functional activity can only be equated to the activity of individual fibers after the fiber population has been ascertained.

Blood flow to individual red and white muscle has received little attention. Investigators have found that flow is three times greater in red than white limb muscles. A direct relationship exists between functional blood flow, both for substrate supply and removal, capillary per fiber, myoglobin concentration and the duration of the contraction (Reis and Wooten, 1970).

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Biochemical Differences

The classic postulate regarding the metabolic differences between red and white muscle states that white (tetanic) muscle, capable of rapid but brief contractions, primarily utilizes the glycolytic system of the sarcoplasm and associated membranes for energy production, whereas red (tonic) muscle, which can contract for prolonged periods, relies on glycolytic and other oxidative mechanisms (Beatty et al., 1963). In regard to energy-supplying metabolism, possibly the metabolic type of a muscle does not cause a distinct type of function, but rather the function of a muscle implies the development of a distinct type of energy (Moody and Cassens, 1968).

White muscle has high levels of activity of the enzymes of glycolysis and glycogenolysis; aldolase, phosphorylase, pyruvate kinase and lactate dehydrogenase. Red muscle has high levels of glycogen synthetase, myoglobin, hexokinase, acetoacetate, Beta-hydroxy acyl Co-A dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase, glucose-6-phosphate dehydrogenase, cytochrome oxidase, and glutamic oxaloacetic transaminase (Blanchaer, 1964; Dawson and Romanul, 1964; Stubbs and Blanchaer, 1965; Beatty et al., 1966; Bocek et al., 1966; Prewitt and Salafsky, 1967; Sigel and Pette, 1969; Jeffress and Peter, 1970). Lawrie (1952, 1953) found in various species that high myoglobin content was associated with high capacity for

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aerobic energy-rich phosphate formation, particularly cytochrome oxidase. White muscle is relatively impoverished in myoglobin and cytochrome oxidase. Myoglobin speeds diffusivity of oxygen into red muscle, and serves to meet sustained demand for oxygen. During sustained contraction red and intermediate fibers achieve a steady state in which work output, blood flow and utilization of blood oxygen are constant (Wittenberg, 1970).

While most investigators (Stubbs and Blanchaer, 1965; Pande and Blanchaer, 1971) claim that only white muscle has high endogenous muscle glycogen, high glycogen levels have been reported for red muscle (Gillespie et al., 1970). The concentration of glycogen varies only within a narrow range under normal conditions (DiMauro et al., 1970). The polysaccharide influences the proportion of phosphorylase a and b and glycogen synthetase and thus, its own catabolic and anabolic enzymes. High tissue levels of glycogen increase the proportion of phosphorylase a and exert a double feed back mechanism controlling glycogen synthesis and degradation. Prenatally, the pathway of glucose to glycogen, favors glycogen formation in red and white muscle and neonatally is more active in red muscle (Bocek et al., 1966, 1969). Gutmann et al. (1969) stated that glycogen breakdown was determined by the number of frequency of arriving impulses, and that metabolic recovery processes were dependent upon the functional

state of nerve centers. Glycogen concentration only rises above the initial level after high frequency stimulation. Bass et al. (1955) and Stubbs and Blanchaer (1965) found that stimulation produced a significant conversion of phosphorylase b to a in only white muscle. White muscle consumes more glycogen and forms lactate from pyruvate to a greater extent than red muscle (Domonkos, 1961; Domonkos and Latzkovits, 1961a, 1961b). The oxygen consumption of red muscle is three times that of white muscle (Domonkos and Latzkovits, 1961a). Decreasing hydrogen ion concentration increases glycolysis, but does not affect respiratory capacity in red and white muscle (Domonkos and Latzkovits, 1961a).

Failure of red muscle to utilize glycolysis does not impair its efficiency. Reserve fat depots and circulating free fatty acids can be readily used by red muscle (Romanul, 1964; Reis and Wooten, 1970). Pande and Blanchaer (1971) found high mitochondrial respiratory rates with pyruvate in both red and white muscle. Compared to pyruvate, respiratory rate with acetylcarnitine was only slightly slower in red, but nearly half as great in white muscle mitochondria. White muscle oxidizes carbohydrate rather than fat, and in vigorous exercise glycogenolysis and carbohydrate metabolism predominates over β-oxidation and fat metabolism.

The contractile myosin from fast muscles has higher enzymatic activity than slow muscle (Margreth et al., 1970; Perry, 1970). Guth and Samaha (1969) reported that actomysin, isolated from cat fast muscle had threefold greater adenosine triphosphatase (ATPase) activity, and suggested that neural regulation was based upon specific type, rather than the activity, of an enzyme. The capacity of the sarcoplasmic reticulum to remove calcium from the cytoplasm, and form ATP from creatine phosphate is greater in fast (white) muscle. The converse applies to slow muscle, as ATP is formed by the oxidative activity of mitochondria.

Perry (1970) noted that myosin from skeletal muscle of new born rabbits was similar to that of adult red muscle, and showed that the capacity of the cell to develop a greater tension resulted from a rise in the number of myofibrils, and hence myosin molecules. Forced activity in new born rabbits caused an early peak of ATPase activity of the sarcoplasmic reticulum. Thus, specialization appears in part to be an adaptation to activity pattern characteristic of the particular muscle.

Histochemical Differences

Maturation of the species is a decisive factor in differentiation of fiber types. Man and guinea pig have full differentiation of fiber types at birth. In the mouse, rat, chicken, rabbit and pig differentiation occurs postnatally (Dubowitz and Pearse, 1960, 1961; Dubowitz,

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1965, 1967a, 1967b, 1967c; Cooper et al., 1970; Ashmore and Doerr, 1971a). In the mammalian embryo all muscle fibers are equal in size and uniformly non-differentiated histochemically (Denny-Brown, 1929a; Bowden and Goyer, 1960). Investigators propose that the various histochemical types of muscle fibers develop as: separate populations (Wirsen and Larsson, 1964; Nystrom, 1968), two populations with a possible common origin for red and intermediate fibers (Germino et al., 1965; Fenichel, 1966), intermediate fibers as precursors of white fibers (Vincelette and Jasmin, 1969), or fibers from a common pool (Dubowitz, 1965; Cooper et al., 1970).

Basic biochemical differences exist between red and white muscles. The advent of histochemical techniques produced a valuable tool for identification of muscle fiber types at the tissue level. In adult muscle, histochemical localization of enzyme systems and other chemical constituents revealed the existence of two, three or more fiber type groups. Dubowitz and Pearse (1960) suggested the subdivision of muscle into two fiber types on the reciprocal relationship between phosphorylase and oxidative content. Stein and Padykula (1962) defined A, B, and C muscle fiber types from individual fiber profiles, using PAS, SDH, non-specific esterase and two adenosine triphosphatase techniques. The previous investigators' muscle fiber profile was based mainly on the amount and

distribution pattern of diformazan particles of succinate dehydrogenase. Romanul (1964) described a spectrum of eight muscle fiber types, which could be broadly divided into three groups by correlating the relative intensities of individual histochemical procedures. Guth and Yellin (1971) also reported that, in general, three categories are evident. However, careful analysis revealed diverse combinations of enzyme activities and more than three histochemical fiber types in mammalian skeletal muscle. Other investigators favor the basic classification of only two fiber types (Engel, 1962, 1965; Karpati and Engel, 1968a, 1968b; Brooke and Engel, 1969; Brooke and Kaiser, 1970). From the preceding, various nomenclatural schemes have emerged. These are summarized in Table 1.

White muscle fibers are generally characterized by high glycogen, low lipid, low oxidative enzymes, low myoglobin, high adenosine triphosphate, high M-type lactate dehydrogenase, and high phosphorylase. Red muscle fibers are generally characterized by low glycogen, high lipid, high oxidative enzymes, low phosphorylase, H-type lactate dehydrogenase, and high myoglobin. Intermediate fibers have moderate glycogen, moderate lipid, high oxidative enzymes, and high myoglobin (Briskey et al., 1970).

The nomenclature should be based on the muscle fiber properties being examined and result in a clear cut differentiation of fiber types (Brooke and Kaiser, 1970).

TABLE 1.--Nomenclatural schemes for classifying fiber types.

Types of Muscle Fibers			Reference
White	Intermediate	Red	Jinnai, 1960; Padykula and Gauthier, 1963; Beatty et al., 1963; Dawson and Romanul, 1964; Bocek et al., 1966; Moody and Cassens, 1968; Baker and Laskin, 1969; Edgerton et al., 1969; and Short et al., 1969.
A	B	C	Stein and Padykula, 1962; Henneman and Olson, 1965; Eversole and Standish, 1970; and Yellin and Guth, 1970.
II	I	II	Karpati and Engel, 1968a.
II	I	I	Brooke and Kaiser, 1970.
I[a]	II[a]	III[a]	Romanul, 1964; and Guth and Yellin, 1971.

[a]Spectrum of fiber types involved.

The relative, or semi-quantitative, histochemical reactivity can only be indicative of the amount of accumulated end-product. However, direct relative comparison can be made between fibers in the same muscle (Engel, 1965; Nystrom, 1966, 1968).

Skeletal Muscle Fiber Response to Experimentally Induced Conditions

The requirements placed upon striated muscle in specific anatomical locales, and the characteristics of the fiber types involved have been shown to change under surgically induced conditions.

Denervation

Engel et al. (1966) found that white fibers are preferentially affected by denervation, and fiber metabolic differences are no longer maintained. Nachmias and Padykula (1958) found that succinate dehydrogenase decreased in red fibers in both soleus and biceps femoris muscles, and white fibers were essentially unaffected. Guth and Watson (1967) electrophoretically determined that after denervation the protein pattern of the plantaris muscle resembled that of the soleus muscle, while the denervated soleus muscle did not change; but when reinnervated by the nerve supplying the plantaris muscle the soleus muscle pattern resembled the plantaris muscle. Sreter (1970) stated that denervation affects both white and red muscles,

although the changes in the latter are smaller and confined to adenosine triphosphatase activity; and indicated that calcium uptake did not change significantly in red, as contrasted to profound change in white fibers. Bajusz (1964) found that red muscle fiber sarcoplasmic granularity was preserved longer. Smith (1965) showed that glycolytic activity was reduced, and β-oxidation enzymes were not reduced as much as mitochondrial enzymes. The basic metabolism resembled cardiac muscle in utilizing ketones and fatty acids after denervation.

Cross-innervation

The cross-innervation studies involve both changes in specific innervation and physiological activity of the neuromuscular apparatus. Close (1964, 1965, 1969) reported that changes in intrinsic speeds of shortening are brought about by a direct effect on the contractile material in fast muscle. Following cross-innervation of fast and slow muscle, investigators (Buller et al., 1960a, 1960b; Engel, 1965; Dubowitz, 1967a, 1967b, 1967c; Prewitt and Salafsky, 1967; Guth et al., 1968, Karpati and Engel, 1968b; Ogata et al., 1968; Fex, 1969; Robbins et al., 1969; Mann and Salafsky, 1970) reported an interconvertibility of energy metabolism, contraction times, and capillary to fiber ratios. After cross-union there was no appreciable change in conduction velocity, as control and experimentally cross-united slow and fast nerve fibers

had the same conduction velocity on both sides. Muscle fibers tend to exert little appreciable influence on the time characteristics of motoneruones under these conditions. Several postulates are possible: (1) special differentiating action of innervation by slow motoneruones, with long after hyperpolarization, is due to the slow frequency of discharge (10-20/second); (2) muscles become fast when activated at higher frequencies; (3) or specific chemical transmitter produced from large and small motoneurones influence differentiation. High frequency discharge to slow motor units would merely serve to fatigue the muscle with no effective return in a higher contraction tension.

Buller et al. (1960a, 1960b) found that differentiation of fast muscles was unaffected by spinal cord transection and dorsal rhizotomy, while differentiation of slow muscle was greatly depressed and contraction times resembled normal fast muscle. Guth et al. (1970) reported that after cross-innervation the qualitative type of actomyosin ATPase synthesized formerly by fast muscle was that type formerly characteristic of slow muscle, and visa versa. Actomyosin adenosine triphosphatase seems to be specific pH dependent and labile, and suggests genomic control regulated by neural input.

Directional transformation of muscle fiber types seems to be specific, as fast (white) muscle transforms

to slow (red and intermediate), slow muscle (intermediate) changes reversibly with slow (red), but slow muscle (intermediate and red) rarely converts to fast (white) (Buller and Lewis, 1965a, 1965b, Dubowitz, 1967a; Hnik et al., 1967; Guth, 1968; Guth et al., 1968). Differentiation rather than dedifferentiation indicates interaction between neural factors and other physiological influences. Mommaerts et al. (1969) agreed that after cross-union transformation of red to white muscle appeared negligible.

Cross-innervation histochemical muscle fiber type studies showed a reversal of enzyme profiles, and density of capillary network present in the fibers of their normal counterparts (Romanul and Van Der Meulen, 1966, 1967; Robbins et al., 1969; Romanul and Pollock, 1969). A general tendency exists for cross-innervated muscle to assume the variety of muscle fiber size and histochemical fiber type relationship observed under normal fast and slow neuromuscular conditions. One striking feature in reinnervated and cross-innervation muscle was the absence of the typical mosaic pattern and presence of "type grouping" pattern, possibly brought about by abundant collateral branching of nerve fibers (Yellin, 1967; Karpati and Engel, 1968b).

Prewitt and Salafsky (1967), after cross-innervation of nerves to soleus and flexor digitorum longus muscles, found an increase in pyruvate kinase and aldolase, and

decreases in malate dehydrogenase and isocitrate dehydrogenase, in the soleus muscle. Conversely, in the flexor digitorum longus muscle, Edström and Kugelberg (1968) induced muscle contractions by shock stimulation (10/second) and found decreased phosphorylase and glycogen content in white and intermediate muscle fibers. Red muscle fibers showed increased phosphorylase and glycogen content.

Tenotomy

Bach (1948) reversed positions of red and white muscle tendons and found conversion of red to pale muscle. Guth and Yellin (1971) using histochemical tissue techniques found profound changes in actomyosin ATPase, and succinate dehydrogenase in soleus and plantaris muscles, following excision of several muscles of the posterior compartment. Vrbova (1962, 1963, 1966) and others (McMinn and Vrbova, 1962; Salmons and Vrbova, 1969, Shafiq et al., 1969) concluded that, with disuse, the soleus muscle became a fast muscle, and no appreciable change occurred in the plantaris muscle. Fischback and Robbins (1969, 1970) found that rapid atrophy alone in slow muscle was not sufficient to cause a change in contractile properties. Nelson (1969) could not confirm the findings of Vrbova and co-workers.

Skeletal Muscle Fiber Response to Exercise Biochemical and Histochemical Studies

The literature in this area is difficult to interpret due to vastly different intensities and durations of exercise (for verification see Table 2). Most investigations have controlled duration (Holloszy, 1967; Edgerton et al., 1969), but few have controlled intensity (Ruhling, 1970).

Biochemical Studies

Studies to selectively correlate the effects of exercise on the myofibrillar and sarcoplasmic protein content and aerobic and anaerobic capacities are inconclusive (Jeffress and Peter, 1970). Prolonged running increases only the sarcoplasmic proteins (Kendrick-Jones and Perry, 1965; Gordon et al., 1966, 1967a, 1967b), whereas forceful exercise increases the number of myofibrillar proteins (Helander, 1961; Kendrick-Jones and Perry, 1967).

Short et al. (1969) trained rats for eight weeks by submaximal running (13.7 m/min) on a motor-driven drum, four hours daily with 5 minutes rest between each 30-minute period, six days per week. Examination of adductor magnus in vitro from these animals showed increased creatine and decreased lactate levels in the red portion, but proportionately less than that observed in the white area. Red muscle from trained animals had greater glycogen concentration. The decreased lactate

TABLE 2.--Comparison of training programs used by various investigators.

TABLE 2.--Comparison of training programs used by various investigators.

Reference	Species	Mode of Exercise	Velocity ft/sec[a]	Dairy Ex. Period	Duration	Specifications
Holloszy, 1967; Holloszy and Oscai, 1969	Rat: Wistar, SPF	Treadmill, 8° incline	1.20 1.69 2.29	20 min 120 min 6 or 12 min	12 wks 5 days/ wk	Initial load, 2-10 min. periods. Load progressively increased up to 120 min. at 1.69 ft/sec with 30 sec or 1 min. sprints at 2.29 ft/sec every 10 min.
Edgerton et al., 1969	Rat	Swimming		30 min 60 min	52 days	Duration controlled; weighted animals.
Edgerton et al., 1969	Guinea Pig	Treadmill, zero grade	1.46	5, 10 min all out	Acute	Continuous
Barnard et al., 1969	Guinea Pig	Treadmill, zero and 2% grade	1.13 2.69	25 min	20 wks 5 days/ wk	Complex program: velocities ranged from 1.13 to 2.69 ft/sec.
Taylor, 1971 Ruhling, 1970	Rat: Sprague Dawley	Controlled Running Wheel (48" circumference)	2.0	50 min: 4 bouts x 12.5 min	12 wks 5 days/ wk	Individual CRW programs described in Appendix A.
			4.0	40 min: 5 bouts x 8 min	12 wks 5 days/ wk	
			5.5	40 min: 8 bouts x 5 min	12 wks 5 days/ wk	

[a]Values are all expressed as standard unit (ft/sec).

accumulation might reflect a reduced rate of glycolysis, increased oxidation of alpha-glycerophosphate, increased pyruvate oxidation, utilization of lactate in glycogen resynthesis, or removal of lactate by increased blood flow to the muscles of conditioned animals. Rawlinson and Gould (1959) swam three different age group rats daily for eight weeks for one and two 30-minute periods, and concluded from biceps femoris homogenates that the total activity of creatine phosphokinase, adenosine triphosphatase, lactate dehydrogenase, malate dehydrogenase, and phosphorylase enzymes was not affected by either swimming program in any of the groups.

Lawrie (1952) exercised rats at 2.0 ft/sec and found an increase in myoglobin with increased duration of treatment. Over a short period, severe exercise elicits no such response, although glycogen reserves are diminished. Hearn and Wainio (1956) found no changes in succinate dehydrogenase levels in the gastrocnemius muscle after swimming rats 30 minutes daily for up to eight weeks. The latter authors suggested that existing aerobic systems are capable of meeting stress induced by moderate exercise. Yakovlev et al. (1963) reported that the enzymes of aerobic metabolism were the first to increase during training and the last to decrease on cessation of training.

Kendrick-Jones and Perry (1965, 1967) and Lamb et al. (1969) suggested that chronic exercise increased the capacity of skeletal muscle to work anaerobically. The previous authors noted that treadmill exercise caused an increase in hexokinase and glycogen content, and increased the capacity to phosphorylate glucose both during and after exercise periods. Using intense activity, Kendrick-Jones and Perry (1965, 1967) found significant increases in creatine phosphokinase and other sarcoplasmic proteins. These authors suggested that the ability of the myofibril to split adenosine triphosphate was not as rate limiting as was the supply of adenosine triphosphate to the enzymatic center.

Gollnick and King (1969) swam rats and found increased mitochondria in the gastrocnemius muscle. Running to exhaustion prior to sacrifice produced marked swelling (uncoupled oxidative phosphorylation) in mitochondria, whereas, swimming to exhaustion produced no such change. It seems that chronic exposure to exercise increases the oxidative capacity in some forms of activity because of the addition of more metabolic apparatus. Holloszy (1967) and Holloszy and Oscai (1969) found an increased capacity of the mitochondrial fraction from the gastrocnemius muscle to oxidize pyruvate after a strenuous program of treadmill running. Mild exercise did not effect the level of succinate dehydrogenase. Increase in both mitochondrial

enzymatic activity and electron transport capacity was associated with a concomitant rise in capacity to produce adenosine triphosphate. Holloszy (1967) observed a change in the mitochondrial content, and suggested that the composition of the cristae, rather than a simple uniform increase in size or number of mitochondria accounted for the increased aerobic capacity. Wilkerson and Evonuk (1971) examined calcium-activated adenosine triphosphatase of the rat gastrocnemius muscle after acute and chronic programs of swimming. The specific activity of gastrocnemius muscle myosin ATPase was only significant when contrasting control and exhaustive programs at six and ten weeks. No difference was found for mild exercise at ten weeks. Previous failure to find significance, as Holloszy (1967) contended, might reflect the relative midlness of the program, since rats can easily perform 30 minutes of swimming. One might also speculate that adequate programs for anaerobic adaptation have not been utilized.

Histochemical Studies

Studies regarding histochemical changes in muscle as a result of training have steadily emerged within the last several years. In general, these studies support metabolic alteration of fiber type with various exercise programs.

Investigating the effect of two different duration running programs on hypertrophy and transformation of fiber type in the tibialis anterior muscle, Man-i et al. (1967) reported that white and red fibers hypertrophied (21% and 11%, respectively) in only the 50-day program. No evidence of change in distribution of various Sudan Black B fiber types with either training program was noted.

Investigators (Kowalski et al., 1969; Spurway and Young, 1970) using low-intensity, repetitive running and weight lifting programs supported an aerobic metabolic adaptation at the interregional fiber level. Kowalski et al. (1968) found, using subjective histochemical measures, an increase in phosphorylase in weight lifting and an even greater increase in running rats. Both exercises resulted in equal increments for succinate dehydrogenase and cytochrome oxidase. The greatest relative rise of succinate dehydrogenase occurred in muscle fibers regarded as predominantly anaerobic (white) only with running.

In a similar study using young mice, Spurway and Young (1970) indicated that running increased the proportion of fibers with high succinate dehydrogenase, while weight-lifting decreased this proportion and raised the percentage of moderate fibers.

The pioneering investigations (Barnard et al., 1970, 1971; Edgerton et al., 1969, 1970; Edgerton and Simpson,

1969, 1971) have produced the most extensive attempts to elucidate the influence of exercise on fiber type alteration. In an early investigation, Edgerton et al. (1969) swam 100-day-old rats for 52 days (one and two 30-minute exercise bouts each day) and found the proportion of intermediate and red fibers in the soleus muscle unaltered in oxidative enzymes, and myosin adenosine triphosphatase patterns. Moderately and heavily exercised rats showed an increase in the proportion of fibers having high intensity malate dehydrogenase, succinate dehydrogenase, and nicotinamide adenine dinucleotide diaphorase in the plantaris muscle (white and mixed fiber areas). Faulkner et al. (1971) determined the number of red and white fibers with succinate dehydrogenase from the plantaris muscle of 6-week and 45-week-old guinea pigs, subjected to low-speed, high-repetition treadmill running. Total fiber counts from the plantaris muscle revealed 14-week and 45-week animals had 20% fewer fibers than 6-week. Training for 8 weeks enhanced the oxidative capacity by increasing the number of red fibers, and prevented attrition of muscle fibers.

In a later comprehensive histochemical, biochemical and physiological study, Edgerton et al. (1969, 1970) examined the effects of prolonged progressive, low-resistance, intermittent, repetitive exercise on gastrocnemius, plantaris and soleus muscles. The

5-day per week complex training program for adult male guinea pigs was progressively increased each week through 9 weeks of training (velocities ranged from 20.8 to 49.3 m/min). Guinea pigs trained to 21 weeks followed the ninth week program. After 9 weeks of training the mitochondria isolated from gastrocnemius and plantaris muscles showed no significant adaptation, while the 18-week group showed significantly increased mitochondrial protein concentration. The percentage of histochemically demonstrated red muscle fibers was significantly increased. No significant differences in myosin ATPase, or contractile properties were observed.

After acute exercise, electrical stimulation of the plantaris muscle, caused selective depletion of glycogen and phosphorylase content in aerobic (red) fibers (Edgerton et al., 1970). The proportions of phosphorylase negative fibers increased with the duration of the exercise. Kugelberg and Edström (1968) induced muscular contraction with low frequency stimulation and found that changes in phosphorylase and glycogen content were most pronounced in white muscle fibers, less in intermediate, and least in red muscle fibers. After one and two hours of stimulation, glycogen negative fibers were identified, but phosphorylase negative fibers were absent. Edgerton et al. (1970) investigated the question further using guinea pigs trained for 9 and 18 weeks. Muscular

contraction induced by electrical stimulation caused total phosphorylase activity to be selectively depleted in white fibers; less in trained than non-trained animals. These data suggest different effects for normally exercised and electrically stimulated muscles. The mitochondrial and glycolytic adaptations might have had a sparing effect on the metabolic characteristics.

Campbell et al. (1971) obtained biopsies from the longissimus muscle of Chester white pigs trained on a treadmill at 2.2 ft/sec for two weeks, and reported an increase in the number of alkali-stabile ATPase fibers. Edgerton et al. (1969) and Rawlinson and Gould (1959) using histochemical and biochemical techniques, have reported no changes in myosin adenosine triphosphatase fiber type or activity.

Recently Dorn et al. (1970) using histochemical techniques reported an increase in glycolytic enzymes and a decrease in oxidative enzymes for fibers of the rat soleus and rectus femoris muscles after forced exercise.

MATERIALS AND METHODS

Experimental Animals

One hundred and seventy-six normal, 72-day-old, male, albino rats (Sprague-Dawley Strain)[1] were brought into the laboratory in four shipments. Each animal was randomly assigned to one of seven treatment groups and then allowed 12 days to become acclimated to laboratory conditions before treatments began.

Treatment Groups

The seven treatment groups used in this study were as follows:

Control (CON)

The animals assigned to the control group received no special treatment and were housed in standard, individual, sedentary cages (24 cm. x 18 cm. x 18 cm.) during both the adjustment and treatment periods.

Voluntary (VOL)

The animals in the voluntary exercise group received no special treatment during either the adjustment period

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[1]Obtained from Hormone Assay Laboratory, Chicago, Illinois.

or the treatment period but were housed in standard, individual voluntary-activity cages. These dages were identical to individual sedentary cages except that the animals were allowed access to freely revolving activity wheels (13 cm. wide and 35 cm. in diameter). Individual daily records of total revolutions run (TRR) were recorded on the attached revolution counter.

Short (SHT)

The animals assigned to the short group were housed in individual, voluntary-activity cages during the adjustment period and in individual sedentary cages during the treatment period. These animals were subjected to a short-duration, high-speed endurance program of interval running. The program was progressive in nature. That is, the intensity of the program was gradually increased until on the thirty-seventh day of training, and thereafter, the animals were expected to complete eight bouts of exercise with 2.5 minutes of inactivity between bouts. Each bout consisted of six repetitions of 10 seconds of work alternated with 40 seconds of rest. During the work intervals, these animals ran at the relatively fast speed of 5.5 ft/sec. (for compete program, see Appendix A-1).

Medium (MED)

The animals in the medium group were housed under the same conditions as the SHT animals. However, the medium (MED) group was subjected to a medium-duration,

moderate-speed endurance program of progressive interval running. By the thirty-seventh day of training, these animals were expected to complete five 8-minute bouts of exercise with 5 minutes of inactivity between bouts. Each bout consisted of eight repetitions of 30 seconds of work alternated with 30 seconds of rest. During the work intervals, these animals ran at the moderate speed of 4.0 ft/sec. (for complete program, see Appendix A-2).

Long (LON)

The animals in the long group were housed under the same conditions as the SHT and MED groups. However, the long (LON) group was subjected to a long-duration, low-speed endurance program of progressive interval running. By the thirty-seventh day of training, these animals were expected to complete four 12.5-minute bouts of exercise with 2.5 minutes of inactivity between bouts. Each bout consisted of one repetition of 12.5 minutes of work with no rest periods. During the work intervals, these animals ran at the slow speed of 2.0 ft/sec. (for complete program, see Appendix A-3).

Electric Stimulus Control (ESC)

These animals were housed in individual voluntary-activity cages during the adjustment period and in individual sedentary cages during the treatment period. Each ESC animal was permanently paired with a short (SHT) animal. Only SHT animals were paired with an

electric-stimulus-control group (ESC). The SHT program was selected because these animals received slightly more shock than the animals on either of the other two forced-running programs. During the SHT animal's treatment period, the ESC animals were placed into an attached adjacent stimulus control cage (21.5 cm. long, 14 cm. wide, and 10.5 cm. high) with a grid floor comparable to that of the controlled running wheel (CRW). Each ESC animal was exposed to the same total light stimulus and electrical shock as its SHT counterpart.

Swimming (SWM)

These animals were housed in individual voluntary-activity cages during the adjustment period and in individual sedentary cages during the treatment period. Animals were swum (28 to 32°C) in individual cylindrical tanks which measured 28 cm. in diameter and 76 cm. in depth. On the last four days of the eighth week of this program, each animal was expected to swim one 60-minute bout with an attached tail weight equal to 3% of the animal's weight (for complete program, see Appendix A-4).

Duration Groups

To provide chronological perspective of treatment effects, animals were sacrificed at zero, four, eight, and twelve weeks after the initiation of treatments. The respective training requirements (see Appendix A-1 through

A-4), for the four-week (20 training days), and the eight-week (40 training days) duration groups (SHT and ESC, MED, LON, and SWM) were progressively increased. The SHT and ESC, MED, LON, and SWM twelve-week groups followed their respective thirty-seventh day schedules during each of the last 23 training days (see Appendix A-1 through A-4).

Treatment Procedures

Treatments began after a 12-day adjustment period when all animals were 85 days of age. Animals designated as zero week control (CON) were sacrificed at the end of the adjustment period. The SHT and ESC, MED, LON, and SWM experimental treatments were conducted once a day, between 12:30 p.m. and 5:30 p.m., Monday through Friday, in the Human Energy Research Laboratory, Michigan State University, East Lansing, Michigan. Animal body weights for SHT, MED, LON, and ESC groups were recorded before and after each treatment period. Only pretreatment dry weights were taken for SWM animals.

For each animal in the VOL group, total revolutions run during the previous 24 hours were recorded, Tuesday through Friday between 10:00 a.m. and 11:00 a.m.

The SHT, MED, and LON groups and one of the control treatments (ESC) received treatment in the CRW described as " . . . a unique animal-powered wheel which is capable of inducing small laboratory animals to participate in highly specific programs of controlled, reproducible

exercise" (Wells and Heusner, 1971). During the first 40-minute learning period in the CRW, the animals ran in response to shock. The low-intensity, controlled shock current was applied to the animal through the grid running surface of the wheel. By the end of the third learning period, most animals were conditioned to run in response to a light stimulus which preceded a shock stimulus.

Initially, animals were placed in individually braked running wheels. For each running period a light above the wheel signaled the start of a work interval and remained on for a predetermined time, the acceleration period. Loss of the light stimulus and application of the shock stimulus occurred for animals not obtaining prescribed wheel speeds during the acceleration period. Animals running slower than the specified speed had the light and shock sequence repeated. The light was turned off and no shock current stimulus was applied for animals obtaining or exceeding prescribed wheel speed. During the work periods, the wheel was free to turn, while during the rest periods, the wheel was automatically braked to prevent spontaneous activity. A typical running program consisted of alternate work and rest periods.

Total revolutions run (TRR) and cumulative duration of shock (CDS) were recorded from a controlled running wheel (CRW) attached result unit after each treatment period for SHT, MED, and LON groups while ESC used SHT values. These values, with total expected revolutions

(TER) and total work time (TWT) (see Appendix A), were used to calculate percent expected revolutions (PER) and percent shock free time (PSF). For the SWM group, swim time completed (STC) was recorded after each treatment period and used with expected swim time (EST) (see Appendix A-4) to calculate percent expected swim time (PET).

Animal Care

Since rats are normally more active at night than during daylight hours, the light sequence in the animal quarters was automatically timed to reverse the rat's active period by having the lights off between 1:00 p.m. and 1:00 a.m. Thus animals were trained during their active phase and at convenient times.

Standard procedures designed to maintain a relatively constant environment for the animals, such as daily handling, temperature and humidity control, and regular cage cleaning, were observed. Throughout the experiment, all animals had access to water and a commercial animal diet[1] ad libitum.

Sacrifice Procedures

Fourteen biweekly sacrifices of seven animals of the same treatment duration were conducted from November 11,

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[1]Wayne Laboratory-Blox, Allied Mills, Inc., Chicago Illinois.

1970 to May 24, 1971. The two initial sacrifices involved only zero week animals. For all other sacrifices, animals were selected after their last treatment on Friday for sacrifice on the following Monday. Only animals subjectively determined in good general health were selected. Performance criteria of 75 percent expected revolutions (PER) and 75 percent shock-free time (PSF) were set for the controlled running programs. Only those SHT, MED, and LON animals whose performance values were above these criteria were selected for sacrifice. Proximity to a mean percent expected swim time (PET) value of 100 was used as a sacrifice selection criteria to the original animals resulted in a final sample of 96 animals. The final cell frequencies are indicated by treatment and duration of treatment in Table 3. These sacrifices involved one CON animal plus pairs of animals from one of the following experimental trios; VOL-MED-LON or SWM-SHT-ESC. This sacrifice schedule was judged most compatible with treatment schedules.

Animals were weighed and then sacrificed under anesthesia by intraperitoneal injection, 4 mg/100 g body weight of Halatal[1] (pentobarbital sodium 64.8 mg).

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[1]From Jensen-Salsbery Laboratories, Division of Richardson-Merrell, Inc., Kansas City, Missouri.

TABLE 3.--Final cell frequencies by treatment and duration of treatment.

Treatment	Duration of Treatment			
	0-week	4-week	8-week	12-week
Control	2	4	4	4
Voluntary	2	4	4	4
Electric Stimulus Control	2	4	4	4
Short	2	4	4	4
Medium	2	4	4	3
Long	2	4	3	4
Swimming	2	4	4	4

Laparotomy, gentle rotation of the abdominal viscera, and partial removal of parietal peritoneum were performed to permit withdrawal of 1-2 ml. of blood from the caudal vena cava. After syringe exchange, 4 ml. of Pelikan ink[1] were injected into the vascular system for subsequent capillary per muscle fiber calculations. After 3 minutes of *in vivo* ink circulation, the heart was removed and preserved for future study.

The right hindlimb was skinned and the superficial posterior crural muscles were exposed by reflecting the overlying tissues. The right triceps surae (gastrocnemius and soleus) and plantaris muscles were removed as a unit. Similar procedures were followed for the left hindlimb except the gastrocnemius, soleus, and plantaris muscles were separated. The left tibialis anterior muscle, the nerve to the left soleus muscle and the lumbar segments of the spinal cord were also removed. Only the right triceps surae and plantaris muscles were used in the present study, the remaining tissues mentioned above were preserved for analysis by other members of the research team.

Upon removal, the right triceps surae and plantaris unit was rolled in talcum powder. The unit was held with forceps and lowered, for approximately 60 seconds into

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[1]Obtained from John Henschel and Co., Inc., Farmington, Long Island, New York.

pre-cooled 2-methylbutane (isopentane). The isopentane had been previously cooled to a viscous fluid (-140 to -185°C) by liquid nitrogen. The frozen muscles were stored in aluminum 35 mm. film containers in a cryostat at -20°C until sectioning and histochemical procedures were initiated. Within 24 hours sandwich blocks (10 mm. thick) were ablated from the mid-portions of the units with a pre-cooled stainless steel knife. The sandwich blocks were frozen onto cryostat chucks using 5% gum tragacanth. Fresh-frozen, distal-proximal serial cross sections, were cut at 10 microns using a rotary microtome-cryostat.[1] Sections were mounted on cover glasses and air-dried for at least one hour.

Histochemical Procedures

Glycogen localization was studied as described by McManus (1946) using the periodic acid-Schiff reaction (PAS). Phosphorylase (PPL) was examined by Takeuchi's (1958) method of inclusion product of polysaccharide and iodine (clathrate). Succinate dehydrogenase (SDH) localization was determined using NBT[2] (2,2'-di-p-nitrophenyl-5,5'-diphenyl-(3,3'-dimethoxy-4,4'-biphenylene)-ditetrazolium chloride) as the electron acceptor (Barka and Anderson, 1963). Intermyofibrillar adenosine

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[1] International-Harris Microtome--Cryostat, Model CTI International (IEC) Equipment Co., Needham Heights, Mass.

[2] Sigma Chemical Company, St. Louis, Missouri.

triphosphatase (ATP) localization was investigated employing the technique described by Wachstein and Meisel (1957) in calcium-formol fixed fresh frozen sections. Control sections were included periodically to verify specific localization patterns.

Incubation times for SDH, ATP and PPL were 45 min, 60 min, and 180 min respectively. Mounting media for the previous sections was glycerin-jelly while PAS and hematoxylin and eosin (H & E) sections were mounted in Histoclad.

Histological Procedure

Harris' alum Hematoxylin and Eosin (Gridley, 1960) was applied to fresh frozen sections for morphological characteristics.

Tissue Analysis

Since muscle fiber populations were known to be different intermuscularly and intramuscularly, specific areas of the medial gastrocnemius, plantaris, and soleus muscles were selected for study (see Figure 1). The types of muscle fibers observed in control animals in the areas selected were as follows: the medial gastrocnemius muscle (area I) had high incidence of white fibers; the plantaris muscle (area II) consisted principally of red and intermediate fibers with occasional white fibers; and the soleus muscle (area III) had predominantly intermediate

fibers with red fibers interspersed. Histochemical characteristics are depicted for these areas in Figures 2 through 12.

Histochemical

Microscopic evaluation of succinate dehydrogenase (SDH), intermyofibrillar adenosine triphosphatase (ATP) phosphorylase (PPL), and glycogen (PAS) phenotypes was determined from a group of 50 adjacent muscle fibers in the three distinctive areas for each animal. To insure data collection from identical fibers, each group of 50 fibers was traced from the SDH section using a microprojector[1] at (x208) magnification. Fibers in serial sections for each histochemical procedure were identified on the original tracing and rated according to the following schema for SDH, ATP and PAS: 1 = dark intensity, 2 = intermediate intensity and 3 = light intensity. For PPL the schema was 1 = blue-violet color, 2 = violet-red to brown-purple color, 3 = yellow to reddish brown.

Control animals were used to establish individual sacrifice standards on the basis of reaction intensity and color for the four histochemical procedures. Except for the control animals, analyses were performed without knowledge of the treatment groups. In every case, data collection was completed before the ensuing biweekly sacrifice.

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[1]Prado Universal, Ernst Leitz GMBH Wetzlar, Germany.

PLATE I

Figures 2 through 6 are sections taken from control animals.

FIGURE 1

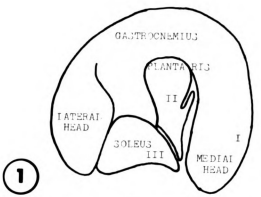
A schematic view of the posterior superficial crural musculature. Histochemical profiles were classified from intramuscular areas identified as I, II, and III. (6X).

FIGURES 2, 4, 6

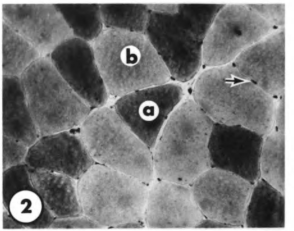
Soleus. PPL, SDH, and ATP respectively. Serial sections illustrate phenotypic properties of red (a) and intermediate (b) fibers. Capillaries (Figures 2 and 6, arrows) and characteristic subsarcolemmal accumulation of diformazan particles (Figure 4, arrow) are prominent. (400X).

FIGURES 3, 5

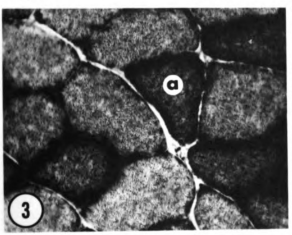
Soleus. SDH and ATP, respectively. End-product localization identifying typical intermyofibrillar pattern for red (a) fiber. (500X).


GASTROCNEMIUS
PLANTARIS
II
LATERAL HEAD
I
SOLEUS
III
MEDIAL HEAD

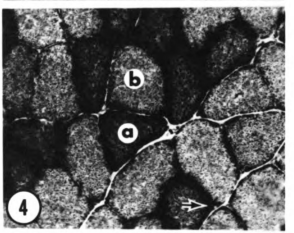
1


b
a

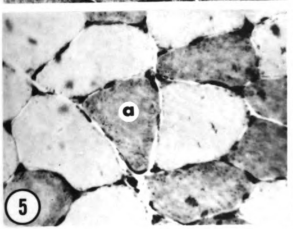
2


a

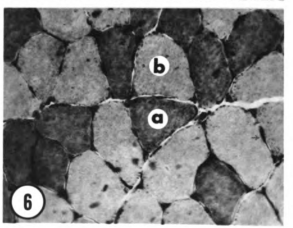
3


b
a

4


a

5


b
a

6

PLATE II

Figures 7 through 12 are sections taken from control animals.

FIGURE 7

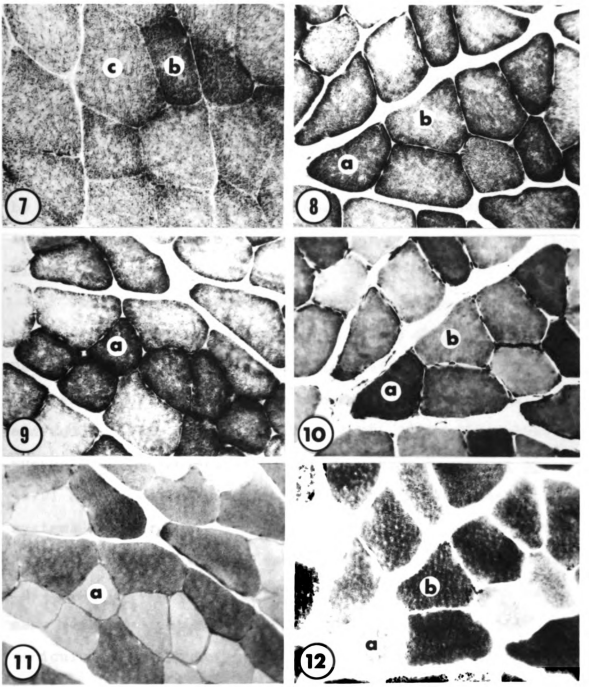
Medial gastrocnemius. SDH. The large fiber (c) with a paucity of subsarcolemmal and intermyofibrillar deposition of diformazan particles, is characteristic of white fibers. The intermediate fiber (b) has slight subsarcolemmal and moderate intermyofibrillar end-product deposition. (500X).

FIGURES 8, 10, 12

Plantaris. SDH, ATP, and PPL, respectively. Serial sections illustrating phenotypic properties of red (a) and intermediate (b) fibers. Note the marked heterogeneity of fibers in this muscle compared to soleus (Figures 2, 4, 6). (400X).

FIGURES 9, 11

Plantaris. SDH and PAS, respectively. Serial sections illustrating phenotypic properties of a red (a) fiber. (400X).


c
b
7
b
a
8
a
9
b
a
10
a
11
b
a
12

Histochemical Interpretation

General synopses of the localization and reaction intensity of end-product in comparable red, intermediate and white skeletal muscle fibers are indicated in Table 4. The significance of these histochemical results can only be interpreted as relative metabolic profile characteristics brought forth by the particular procedural conditions.

Various techniques (Padykula and Herman, 1955, 1965; Engel, 1962; Padykula and Gauthier, 1963; Gauthier and Padykula, 1965; Gauthier, 1967; Karpati and Engel, 1968a; Severson et al., 1968; Brooke and Engel, 1969; Farrell and Fedde, 1969; Guth and Samaha, 1969, 1970; Tice, 1969; Eversole and Standish, 1970; Meijer, 1970; Samaha et al., 1970) have revealed different adenosine triphosphatase systems and enzymes. In this investigation the Wachstein-Meisel lead-precipitation technique localized predominantly intermyofibrillar ATP. The intermyofibrillar region has many membrane systems (mitochondria, sarcoplasmic reticulum, and tubular systems) which hydrolyse adenosine triphosphate or similar engery-rich compounds (creatine phosphate) to link endergonic processes to others that are exergonic.

The measure for succinate dehydrogenase (SDH) was used as an indicator of an oxidation-reduction step in the tricarboxylic acid cycle. This enzyme catalyzes the

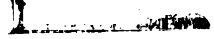


TABLE 4.--Localization and reaction intensity of end-product in red, intermediate, and white skeletal muscle fibers (cross section).

Enzyme or Substrate	Cross Section		Probable Cellular Constituent	Reaction end-product Intensity		
	Myofibrills	Inter-myofibrillar		Red	Intermediate	White
Succinate dehydrogenase (SDH)	0	-	mitochondria	dark(1)	medium(2)	light(3)
Intermyofibrillar adenosine triphosphatase (ATP)	0	-	mitochondira	dark(1)	medium(2)	light(3)
Phosphorylase (PPL)	0	-	aqueous sarcoplasm	medium(2) (violet red)	light(3) yellow, reddish-brown)	dark(1) (blue-violet)
Periodic acid-Schiff (PAS)	0	-	aqueous sarcoplasm	light(3)	medium(2)	dark(1)

removal of two hydrogens from succinate to fumarate. These electrons are carried to the electron transport system by flavin adenine dinucleotide. Histochemically, NBT served as the electron acceptor and localization occurred primarily on mitochondria membranes (Padykula, 1952; Wachstein and Meisel, 1955; Scarpelli and Pearse, 1958; Beckett and Bourne, 1960; Novikoff et al., 1961; Germino et al., 1965; Brooke and Engel, 1966; Nystrom, 1966). This aerobic measure was used as an indicator of resistance to fatigue.

Glycogen (PAS) localization and reciprocal phosphorylase (PPL) represented relative anabolic and catabolic processes of a control system. Phosphorylases in vivo catalyze reactions leading to formation of glucose-1-phosphate from glycogen in the presence of phosphates. The in vitro reversibility of the reactions has been used for the histochemical demonstration of phosphorylase activity (Takeuchi and Kuriaki, 1955; Takeuchi, 1958; Takeuchi and Sasaki, 1970a, 1970b). This concentration was measured by the amount of newly formed polyglucose.

These procedures taken together yield a metabolic inventory, but did not necessarily include the rate limiting steps in the various metabolic pathways. It was unlikely that the histochemical procedures examined were altered in similar fashion.

Statistical Procedures

Data were analyzed by treatment groups and durations by (CDC 3600) computer. Calculations were performed for means, standard deviations, and simple correlation coefficients for training performance, environmental conditions, and pre- and post-treatment body weights. Since diameters of wheels attached to the voluntary-activity cages were less than CRW diameters, daily TRR values of VOL animals were multiplied by a calibration factor, 0.9163, to equate TRR values for VOL, SHT, MED, and LON groups.

Mean percent values were calculated for each histochemical rating and the arc sine transformation (angular transformation) was applied to insure that histochemical data met the variance homogeneity assumption of analysis of variance (ANOVA) (Sokal and Rohlf, 1969). This procedure was repeated for each combination of area and histochemical technique.

The mean percent values were then analyzed by a two-way, fixed effects ANOVA model. Complex Scheffé contrasts were employed to determine the specific significant categories within each of the independent variables, treatment and duration of treatment. For those categories which were significant, standard Scheffé contrasts were used to identify the particular cell means responsible for the observed significance. The

probability of committing a Type I error (α) was set at .05 for the two-way ANOVA. The probability of committing a Type II error (β) was set at .25. Significance levels for the Scheffé tests was held at $p = .20$ (Scheffé, 1959; Guenther, 1964).

RESULTS

Training Results

Treatments

On the basis of the programmed increase of total expected revolutions (TER) with duration of treatment (see Appendix A-1 through A-3), animals of the long (LON) group had the largest mean daily total revolutions run (TRR) increase, medium (MED) next, and short (SHT) the least. Figure B-4 (see Appendix B), shows that, on the basis of mean daily TRR, the SHT, MED, and LON animals met their respective program requirements. Mean daily TRR of voluntary (VOL) animals did not display a consistent position relative to the programmed running groups for the first five weeks of training although a definite trend toward a position between the SHT and MED groups was evident from six to twelve weeks. This observation was limited in that the speed and duration of wheel revolutions run by VOL animals cannot be equated to those of SHT, MED, and LON animals.

Figures B-1, B-2, and B-3 (see Appendix B) show that SHT, MED, and LON animals generally maintained

percent expected revolution (PER) values above 80, thus exceeding the criterion PER level, 75, of acceptable execution of program requirements. This level of performance compared favorably with other groups of animals subjected to similar training programs (Ruhling, 1970). Figures B-1, B-2, and B-3 also show that the animals generally responded to light, rather than electrical shock, stimuli. Comparisons across treatment duration of percent shock free time (PSF) values for SHT, MED and LON groups showed that SHT animals and their electric stimulus control (ESC) received the most electrical shock.

Almost without exception, percent expected swim time (PET) values for swimming (SWM) animals were 100 (Table B-2, see Appendix B). Therefore, PET was not plotted across duration of SWM treatment.

Treatment Environment and Body Weight Values

Table B-1 (see Appendix B) shows that SHT, MED, and LON animals were exercised under conditions of relatively constant air temperature and barometric pressure and low humidity. These values did not affect PER and PSF as reflected in the low correlations among these parameters. However, animals with relatively high pre-treatment body weights tended to display low PER and PSF. Animals showing relatively large weight losses tended to display high PER and PSF. The high correlation coefficient between PER

and PSF (Table B-1) confirmed the nearly parallel plot of these values in Figures B-1, B-2, and B-3 (see Appendix B).

The SWM animals (Table B-2, see Appendix B) were exercised under environmental conditions, including water temperature, comparable to those of SHT, MED, and LON animals. None of these conditions or pre-treatment body weight values were highly correlated with PET.

Histochemical Results

Individual mean percent values of histochemical ratings are tabulated in Appendix C by animal number, treatment and duration of treatment. The cell mean percent values of histochemical ratings are depicted by treatment and duration of treatment in Figures E-1 through E-3 (see Appendix E) for the muscles examined.

Overall Analysis of Variance for Sin^{-1} Percent Histochemical Fiber Ratings.--The overall two-way analysis of variance by area, histochemical procedure and rating are located in Tables D-1 through D-3 (see Appendix D).

Complex and Standard Scheffé Analysis for Sin^{-1} Percent Histochemical Ratings.--Complex and standard Scheffé contrasts for specific dependent category variable

differences within the two independent variables, treatment and duration of treatment[1] are located in Tables 5, 6, 7, 8, and 9, 10, 11, 12, respectively. The prominence of duration, as well as treatment effects, suggested that the seven different chronic physical activities had specific effects upon the metabolic characteristics, but the effects were highly time dependent.

Medial Gastrocnemius.--The presence of significant treatment-duration effects of exercise were evident at four, eight, and twelve weeks. Similar SDH and ATP changes occurred for VOL at four weeks, and for LON at eight weeks with ATP and at twelve weeks with SDH. Increases in PAS were found in CON, SHT, MED, and VOL. This effect occurred for CON and SHT at four weeks, for MED at eight weeks, and VOL at twelve weeks. The SHT and SWM groups showed a training effect with PAS measure at four weeks. Increases in the percentage of intermediate phosphorylase fibers was seen for ESC at four weeks, and for MED at eight weeks; while the ESC treatment reverted to control percentages of phosphorylase fibers at eight and twelve weeks. Similar ATP changes appeared at twelve weeks for MED and SWM.

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[1]The animals assigned to zero week had received no experimental treatment. Differences between dependent categories were not significant. Dependent category values were pooled, disregarding treatment assignment, to increase the power of the Scheffé contrasts within duration of treatment. These animals were designated as zero week CON (N = 14).

TABLE 5.--Summary of Scheffé contrasts for area/histochemical Sin^{-1} percent ratings within duration.

Area	Rating	Histochemical Procedure	Duration (wk)		
			4	8	12
Medial Gastrocnemius	1	SDH	S	S	N
	2	SDH	N	N	N
	3	SDH	N	N	N
	1	ATP	N	S	N
	2	ATP	N	N	N
	3	ATP	N	N	N
	1	PPL	S	N	N
	2	PPL	S	N	N
	3	PPL	N	N	N
	1	PAS	S	N	S
	2	PAS	S	N	N
	3	PAS	N	N	S
Plantaris	1	SDH	N	N	N
	2	SDH	N	N	N
	3	SDH	N	N	N
	1	ATP	N	N	N
	2	ATP	N	N	N
	3	ATP	N	N	N
	1	PPL	N	N	N
	2	PPL	N	N	N
	3	PPL	N	N	N
	1	PAS	N	N	N
	2	PAS	N	N	N
	3	PAS	N	N	N
Soleus	1	SDH	N	N	N
	2	SDH	N	N	N
	3	SDH	N	N	N
	1	ATP	N	N	N
	2	ATP	N	N	N
	3	ATP	N	N	N
	1	PPL	N	N	N
	2	PPL	N	N	N
	3	PPL	N	N	N
	1	PAS	N	N	N
	2	PAS	N	N	N
	3	PAS	N	N	N

N = Not significant; S = Significant at .20 level.

TABLE 6.--Summary of Scheffé contrasts for histochemical Sin^{-1} percent ratings for statistically significant treatments at four weeks in medial gastrocnemius muscle.

Scheffé Contrast	Histochemical Procedure	SDH			ATP			PPL			PAS		
	Rating	1	2	3	1	2	3	1	2	3	1	2	3
CON-VOL		S+						N	N		N	N	
CON-SHT		N						N	N		S-	N	
CON-MED		N						N	N		N	N	
CON-LON		N						N	N		N	N	
CON-ESC		N						S-	S+		N	N	
CON-SWM		N						N	N		N	S+	
VOL-SHT		S-						N	N		N	N	
VOL-MED		N						N	N		N	N	
VOL-LON		N						N	N		N	N	
VOL-ESC		N						S-	S+		N	N	
VOL-SWM		N						N	N		N	N	
SHT-MED		N						N	N		N	N	
SHT-LON		N						N	N		N	N	
SHT-ESC		N						S-	S+		N	N	
SHT-SWM		N						N	N		N	N	
MED-LON		N						N	N		N	N	
MED-ESC		N						S-	S+		N	N	
MED-SWM		N						N	N		N	N	
LON-ESC		N						N	N		N	N	
LON-SWM		N						N	N		N	N	
ESC-SWM		N						S+	S-		N	N	

N = Not significant.

Contrasting significant Scheffé contrasts right to left: S- = decrease in percentage of fibers for a given histochemical procedure/rating at .20; S+ = increase in percentage of fibers for a given histochemical procedure/rating at .20.

TABLE 7.--Summary of Scheffé contrasts for histochemical Sin^{-1} percent ratings for statistically significant treatments at eight weeks in medial gastrocnemius muscle.

Scheffé Contrast	Histochemical Procedure	SDH			ATP			PPL			PAS		
	Rating	1	2	3	1	2	3	1	2	3	1	2	3
CON-VOL		N			N								
CON-SHT		N			N								
CON-MED		N			N								
CON-LON		N			N								
CON-ESC		N			N								
CON-SWM		N			N								
VOL-SHT		N			N								
VOL-MED		N			N								
VOL-LON		N			N								
VOL-ESC		S+			N								
VOL-SWM		N			N								
SHT-MED		N			N								
SHT-LON		N			N								
SHT-EST		N			N								
SHT-SWM		N			N								
MED-LON		N			N								
MED-ESC		N			N								
MED-SWM		N			N								
LON-ESC		N			N								
LON-SWM		N			S-								
ESC-SWM		N			N								

N = Not significant.

Contrasting significant Scheffé contrasts right to left: S- = decrease in percentage of fibers for a given histochemical procedure/rating at .20; S+ = increase in percentage of fibers for a given histochemical procedure/rating at .20.

TABLE 8.--Summary of Scheffé contrasts for histochemical Sin^{-1} percent ratings for statistically significant treatments at twelve weeks in medial gastrocnemius muscle.

Scheffé Contrast	Histochemical Procedure	SDH			ATP			PPL			PAS		
	Rating	1	2	3	1	2	3	1	2	3	1	2	3
CON-VOL											N		N
CON-SHT											N		N
CON-MED											N		N
CON-LON											N		N
CON-ESC											N		N
CON-SWM											N		N
VOL-SHT											N		N
VOL-MED											S+		S-
VOL-LON											N		N
VOL-ESC											S+		S-
VOL-SWM											N		N
SHT-MED											N		N
SHT-LON											N		N
SHT-ESC											N		N
SHT-SWM											N		N
MED-LON											N		N
MED-ESC											N		N
MED-SWM											N		N
LON-ESC											N		N
LON-SWM											N		N
ESC-SWM											N		N

N = Not significant.

Contrasting significant Scheffé contrasts right to left: S- = decreast in percentage of fibers for a given histochemical procedure/rating at .20; S+ = increase in percentage of fibers for a given histochemical procedure/rating at .20.

TABLE 9.--Summary of Scheffé contrasts for area/histochemical Sin^{-1} percent ratings within duration of treatment.

Area	Rating	Histochemical Procedure	Treatment Group						
			CON	VOL	SHT	MED	LON	ESC	SWM
Medial Gastrocnemius	1	SDH	N	S	N	N	N	N	N
	2	SDH	N	N	N	N	S	N	N
	3	SDH	N	S	N	N	S	N	N
	1	ATP	N	S	N	N	S	N	N
	2	ATP	N	N	N	N	N	N	S
	3	ATP	N	N	N	S	S	S	N
	1	PPL	N	N	N	N	N	S	N
	2	PPL	N	N	N	S	N	S	N
	3	PPL	N	N	N	N	N	S	N
	1	PAS	S	S	N	S	N	N	N
	2	PAS	S	N	N	N	N	N	N
	3	PAS	N	S	N	N	N	N	N
Plantaris	1	SDH	N	N	N	N	N	N	N
	2	SDH	N	N	N	N	N	N	N
	3	SDH	N	N	N	N	N	N	N
	1	ATP	N	N	S	N	N	N	N
	2	ATP	N	N	S	N	N	N	N
	3	ATP	N	S	N	N	N	S	S
	1	PPL	N	N	N	N	N	N	N
	2	PPL	N	N	N	N	N	N	N
	3	PPL	N	N	N	N	N	N	N
	1	PAS	N	N	N	N	N	N	S
	2	PAS	S	N	N	N	S	N	N
	3	PAS	N	N	N	N	N	N	S
Soleus	1	SDH	N	N	N	N	S	N	N
	2	SDH	N	N	N	N	S	N	N
	3	SDH	N	N	N	N	N	N	N
	1	ATP	N	N	N	N	N	S	N
	2	ATP	N	S	N	N	N	S	N
	3	ATP	N	N	N	N	N	N	N
	1	PPL	N	N	N	N	N	N	N
	2	PPL	N	N	N	N	N	N	N
	3	PPL	N	N	N	N	N	N	N
	1	PAS	N	N	N	N	S	S	N
	2	PAS	N	N	N	N	N	N	N
	3	PAS	N	S	N	N	N	S	S

N = Not significant; S = Significant at .20 level.

TABLE 10.--Summary of Scheffé contrasts for histochemical Sin^{-1} percent ratings for statistically significant duration of treatment in medial gastrocnemius muscle.

Scheffé Contrast		Histochemical Procedure	SDH			ATP			PPL			PAS		
		Rating	1	2	3	1	2	3	1	2	3	1	2	3
CON-OWK	CON-4WK											S+	S-	
CON-OWN	CON-8WK											N	N	
CON-OWK	CON-12WK											N	N	
CON-4WK	CON-8WK											N	N	
CON-4WK	CON-12WK											N	S-	
CON-8WK	CON-12WK											N	N	
VOL-OWK	VOL-4WK		S+		S-	S+						N	N	
VOL-OWK	VOL-8WK		N		N	N						N	N	
VOL-OWK	VOL-12WK		N		N	N						S+	S-	
VOL-4WK	VOL-8WK		S-		N	N						N	S-	
VOL-4WK	VOL-12WK		S-		S+	S-						N	S-	
VOL-8WK	VOL-12WK		N		N	N						N	N	
MED-OWK	MED-4WK							N			N	N		
MED-OWK	MED-8WK							N			S+	S+		
MED-OWK	MED-12WK							S-			N	N		
MED-4WK	MED-8WK							N			N	N		
MED-4WK	MED-12WK							N			N	N		
MED-8WK	MED-12WK							N			N	N		
LON-OWK	LON-4WK			N	N	N		N						
LON-OWK	LON-8WK			N	N	S+		S-						
LON-OWK	LON-12WK			S+	S-	N		N						
LON-4WK	LON-8WK			N	N	S		N						
LON-4WK	LON-12WK			S+	S-	N		N						
LON-8WK	LON-12WK			N	N	N		N						
ESC-OWK	ESC-4WK								S-	S+	S+			
ESC-OWK	ESC-8WK								N	N	N			
ESC-OWN	ESC-12WK								N	N	N			
ESC-4WK	ESC-8WK								S+	S-	N			
ESC-4WK	ESC-12WK								S+	S-	N			
ESC-8WK	ESC-12WK								N	N	N			
SWM-OWK	SWM-4WK						N							
SWM-OWK	SWM-8WK						N							
SWM-OWK	SWM-12WK						S+							
SWM-4WK	SWM-8WK						N							
SWM-4WK	SWM-12WK						N							
SWM-8WK	SWM-12WK						N							

N = Not significant

Contrasting significant Scheffé contrasts right to left: S- = decrease in percentage of fibers for a given histochemical procedure/rating at .20; S+ = increase in percentage of fibers for a given histochemical procedure/rating at .20.

TABLE 11.--Summary of Scheffé contrasts for histochemical Sin^{-1} percent ratings for statistically significant duration of treatment in plantaris muscle.

Scheffé Contrast		Histochemical Procedure	SDH			ATP			PPL			PAS		
		Rating	1	2	3	1	2	3	1	2	3	1	2	3
CON-OWK	CON-4WK												N	
CON-OWK	CON-8WK												N	
CON-OWK	CON-12WK												S-	
CON-4WK	CON-8WK												N	
CON-4WK	CON-12WK												S-	
CON-8WK	CON-12WK												S-	
VOL-OWK	VOL-4WK							S+						
VOL-OWK	VOL-8WK							N						
VOL-OWK	VOL-12WK							N						
VOL-4WK	VOL-8WK							N						
VOL-4WK	VOL-12WK							S-						
VOL-8WK	VOL-12WK							N						
SHT-OWK	SHT-4WK					N	N							
SHT-OWK	SHT-8WK					N	N							
SHT-OWK	SHT-12WK					N	N							
SHT-4WK	SHT-8WK					N	N							
SHT-4WK	SHT-12WK					S-	S+							
SHT-8WK	SHT-12WK					N	N							
LON-OWK	LON-4WK											S+		
LON-OWK	LON-8WK											N		
LON-OWK	LON-12WK											N		
LON-4WK	LON-8WK											N		
LON-4WK	LON-12WK											N		
LON-8WK	LON-12WK											N		
ESC-OWK	ESC-4WK							N						
ESC-OWK	ESC-8WK							N						
ESC-OWK	ESC-12WK							S+						
ESC-4WK	ESC-8WK							N						
ESC-4WK	ESC-12WK							N						
ESC-8WK	ESC-12WK							N						
SWM-OWK	SMW-4WK							N			S+			S-
SWM-OWK	SWM-8WK							N			N			N
SWM-OWK	SWM-12WK							S+			N			N
SWM-4WK	SWM-8WK							N			N			N
SWM-4WK	SWM-12WK							N			N			N
SWM-8WK	SWM-12WK							N			N			N

N = Not significant.

Contrasting significant Scheffé contrasts right to left: S- = decrease in percentage of fibers for a given histochemical procedure/rating at .20; S- = increase in percentage of fibers for a given histochemical procedure/rating at .20.

TABLE 12.--Summary of Scheffé contrasts for histochemical Sin^{-1} percent ratings for statistically significant duration of treatment in soleus muscle.

Scheffé Contrast		Histochemical Procedure	SDH			ATP			PPL			PAS		
		Rating	1	2	3	1	2	3	1	2	3	1	2	3
VOL-OWK	VOL-4WK						N							S-
VOL-OWK	VOL-8WK						S+							N
VOL-OWK	VOL-12WK						N							N
VOL-4WK	VOL-8WK						N							S+
VOL-4WK	VOL-12WK						N							N
VOL-8WK	VOL-12WK						N							N
LON-OWK	LON-4WK		N	N								N		
LON-OWK	LON-8WK		N	N								N		
LON-OWK	LON-12WK		S+	S-								S+		
LON-4WK	LON-8WK		N	N								N		
LON-4WK	LON-12WK		S+	S-								N		
LON-8WK	LON-12WK		N	N								N		
ESC-OWK	ESC-4WK					S-	S+					N		N
ESC-OWK	ESC-8WK					N	N					S+		S-
ESC-OWK	ESC-12WK					N	N					N		N
ESC-4WK	ESC-8WK					N	N					N		N
ESC-4WK	ESC-12WK					S+	N					N		N
ESC-8WK	ESC-12WK					N	N					N		N
SWM-OWK	SWM-4WK													N
SWM-OWK	SWM-8WK													S-
SWM-OWK	SWM-12WK													N
SWM-4WK	SWM-8WK													N
SWM-4WK	SWM-12WK													N
SWM-8WK	SWM-12WK													N

N = Not significant.

Contrasting significant Scheffé contrasts right to left: S- = decrease in percentage of fibers for a given histochemical procedure/rating .20; S+ = increase in percentage of fibers for a given histochemical procedure/rating .20.

Plantaris.--None of the Scheffé contrasts within the duration groups for category variables were significant. Between durations similar PAS changes were found at four weeks for LON and SWM. ESC and SWM groups produced significant increase in the percentage of low ATP fibers at twelve weeks, while VOL produced the same result at four weeks. The CON showed a change in the percentage of intermediate PAS fibers at twelve weeks. The SHT group activity produced a significant increase in the percentage of intermediate ATP fibers from four to twelve weeks.

Soleus.--None of the Scheffé contrasts within the duration groups for category variables were significant. Between durations the LON endurance group required twelve weeks to produce a directly related change in SDH and PAS. Similar PAS changes occurred for VOL at four and eight weeks. VOL activity produced a significant increase in the percentage of intermediate ATP fibers at eight weeks. Similar changes were produced in the ESC group at four weeks but such changes were reversed at twelve weeks.

General Patterns of Metabolic Response.--The prominence of duration, as well as treatment, effects suggested further insight into category variables for relative analysis of the regional, temporal and specific response to identical and diverse physiologic stimuli. Frequency weighted scores were calculated (frequency of fibers times the intensity rating of the enzyme or

substrate). These cell weighted values are presented by area, treatment and duration in Figure 13.

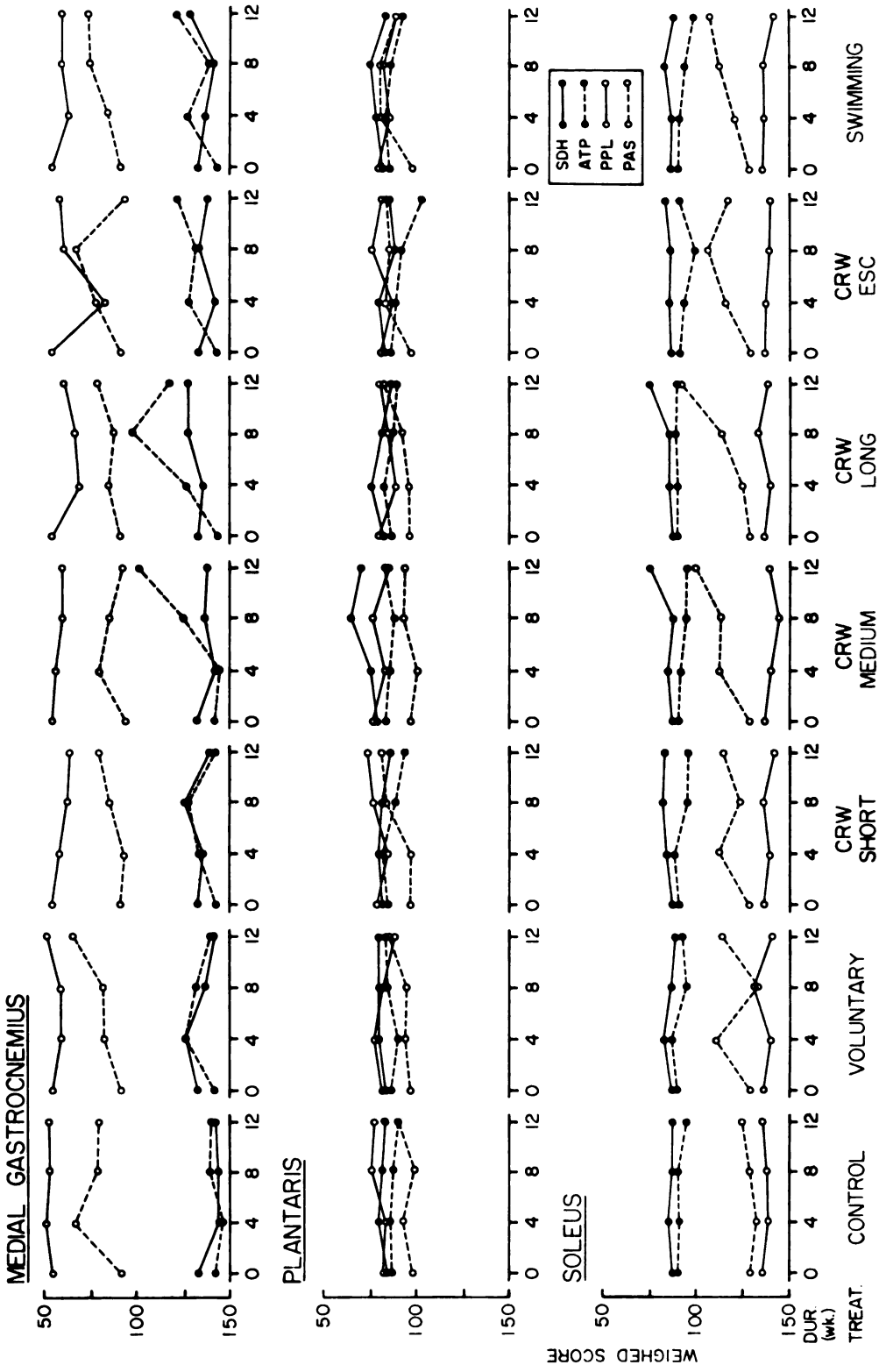

Figure 13.-- Frequency of Fiber Ratings (Weighted Score) Presented by Area, Treatment and Duration of Treatment for SDH, ATP, PPL and PAS

DISCUSSION

The attainment of combinations of seven distinct levels and four durations of chronic physical activity permitted meaningful interpretation of the histochemical and exercise related data. The control (CON) animals were confined to individual sedentary cages, and therefore represented a low level activity group. The controlled running and swimming programs were designed to produce divergent activity levels (see Appendix A-1 through A-4). The performance values (Figures B-1 through B-3, and Tables B-1 and B-2, see Appendix B) established that the short (SHT), medium (MED), long (LON), and swimming (SWM) groups represented four different physiological types and intensities of chronic physical activity. Figure B-4 (see Appendix B) indicated a separation of activity levels, on the basis of mean daily total revolutions run (TRR), for voluntary (VOL), short (SHT), medium (MED), and long (LON) groups.

The decision to pair electric stimulus control (ESC) and SHT animals was correct in that the SHT animals received the largest amount of electrical shock (see

Figures B-1, B-2, and B-3, Appendix B). The ESC animals served as a shock control group, and the CON animals served as an environmental living control. However, the amount of muscular activity induced in the ESC animals was not known due to the static physical response of the ESC animals to the noxious stimuli.

The histochemical evaluations were made on fiber types[1] selected from distinct intramuscular areas of medial gastrocnemius, plantaris, and soleus muscles. It was known that muscle fibers vary intermuscularly and intramuscularly. The histochemical procedures utilized elicited markedly distinct patterns and intensities for the individual muscle cells in these areas.

This study represented an initial step in establishing histochemical profiles for individual fibers. Further, this study provided information on the alteration of fiber characteristics and distribution in response to various types and durations of physical activity.

It was hypothesized that the histochemical profiles of enzymes and substrates would indicate adaptive responses by area to the different levels of chronic activity.

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[1]The term histochemical fiber type implies a static situation. This writer, however, acknowledges a classification on a more dynamic basis for individual cullular metabolic activities. In general, distinct intensity relationships occur between the various histochemical measures.

The results obtained were somewhat unexpected in relation to those of previous investigations (Edgerton et al., 1969; Kowalski et al., 1969; Dorn et al., 1970; Spurway and Young, 1970; Campbell et al., 1971). In the present study, regional and temporal patterns of change frequently were different for the various exercise regimens. The prominence of duration, as well as treatment, effects suggested that the seven different chronic physical activities had specific effects upon the alteration of fiber characteristics and fiber type distribution, but the effects were highly time dependent. Since the program requirements were progressively increased, it was observed that weekly changes in programs brought about concomitant changes in fiber type profiles. Recent evidence suggested that muscle cells continually undergo alteration in adaptation to functional demands (Baker and Laskin, 1969; Guth and Yellin, 1971). It seems reasonable that cardiovascular and respiratory factors interact with autoregulatory, hormonal, and neurotrophic processes to influence the regulation of muscle cell metabolism. The present results supported the concept of alteration of fiber types as an effect of physical activity.

Patterns of Adaptation

Various patterns of adaptation are suggested from the weighted frequency values for individual areas by treatment and the duration of treatment as shown in

Figure 13. It was apparent that the three areas examined reflected ranges of both local aerobic and anaerobic related responses. A number of adaptive processes evidently occurred in exercising muscle. However, these processes did not take place in a continuous linear fashion, but seemed to proceed concomitantly with metabolic need.

Soleus.--The patterns of adaptation in Figure 13 showed significant temporal changes for periodic acid-Schiff's reaction (PAS) in soleus fibers for all the experimental groups. In addition, VOL and SHT groups showed cyclic increases and decreases in glycogen levels. Seemingly, an increase in the frequency of stimulation caused an increase in glycogen content. It was interesting that the long-duration, low-intensity LON and SWM group and medium duration, moderate intensity MED groups showed progressive increases in glycogen content. The increased glycogen content in soleus might be the result of increased glycogen synthetase activity (synthesis) or decreased utilization (degradation) of existing glycogen stores. Both the present results and other findings (Bass et al., 1955; Kugelberg and Edström, 1968; Gutmann, 1970) suggested that highly repetitive low frequency discharge to slow motor units served to increase glycogen content. Extension of these programs over a longer treatment period should partition the adaptive responses.

Plantaris.--The muscle fiber population examined in the plantaris showed increased glycogen content for VOL, SHT, MED, LON, and SWM groups at different durations of treatment. The SWM and ESC groups showed marked increases in glycogen content between zero and four weeks, whereas, the SHT and MED groups showed marked increases in glycogen content between zero and eight weeks. The periodicity of glycogen values illustrated that specificity of training was a reality. The plantaris thus reflected that adaptive mechanisms proceed in phases according to the particular overload stress. For most measures, the heterogeneous population sampled seemed resistant to change.

Medial Gastrocnemius.--It was evident that white (anaerobic) fibers acquired red (aerobic) fiber characteristics, as a response to physical activity. The running groups, MED and LON, had the greatest proportional increases in intermyofibrillar adenosine triphosphatase (ATP) and succinate dehydrogenase (SDH) fibers (see Figure 13). Previously, Carrow et al. (1967), found an increase in capillary per fiber ratio and Ashmore and Doerr (1971b) supported an increase in blood flow in white muscle with repetitive exercise. Ashmore and Doerr (1971b), as well as other authors (Edgerton et al., 1969; Kowalski et al., 1969; Barnard et al., 1970) hypothesized white to red fiber transformation after

repetitive exercise. Mai et al. (1970) failed to show increased capillary per fiber ratio for white fibers and indicated that an increase in capillarity in the white zone was due to an increase in capillarity of red fibers. Reference to these facts, indicated that the transformation of white to red fiber characteristics was probably facilitated by circulatory mechanisms.

The histochemical changes resulting from this study, where several intensities and durations of exercise were considered separately and in combination, supported the concept of "specificity of exercise." Other authors (Edgerton et al., 1969; Kowalski et al., 1969; Dorn et al., 1970; Spurway and Young, 1970; Barnard et al., 1970) worked with durations from nine to eighteen weeks, and intensities of 2.7 ft/sec. or less, and reported increases in oxidative fiber populations but no changes in fiber proportions of adenosine triphosphatase or phosphorylase. In contrast, this study showed changes occurred for phosphorylase, glycogen, oxidative enzymes, and intermyofibrillar adenosine triphosphatase at durations of four, eight, and twelve weeks when the velocities were essentially doubled (4.0 ft/sec. and 5.5 ft/sec.).

It should be noted that biochemical homogenate studies (Holloszy, 1967; Barnard et al., 1970) have failed to show changes in adenosine triphosphatase after exercise periods as long as eighteen weeks. The present

histochemical fiber analysis did reveal changes in adenosine triphosphatase fiber populations. This finding could be attributable to the intensity of the programs used in this study; however, diverse regional histochemical responses to the same stimuli point out that whole muscles should not be studied as biochemical entities.

From the results of this study, it can be speculated that the intracellular components are capable of selective adaptation. If this is the case, the basic unit underlying specificity of adaptation must be either the muscle fiber or the highly intermingled motor units.

Interregional Comparisons

Several common statements can be made regarding the areas examined. The fibers selected for study in the soleus, plantaris and medial gastrocnemius muscles represented distinct populations. These populations revealed diverse responses to the same exercise stimuli (see Figure 13). The metabolic profiles of the muscle areas under consideration confirmed previous findings (Buller and Lewis, 1965a; Hnik et al., 1967; Guth, 1968; Ashmore and Doerr, 1971b) that muscle metabolic characteristics can change. The previous authors established that after a variety of surgically induced conditions, which modified the neural input to the muscle, alterations occurred in energy metabolism. In this study, high work loads

produced similar results in intact animals. Continual use of leg muscles in endurance type activity, resulted in white fibers acquiring red-fiber characteristics.

The presence of significant ($P < .05$) training, duration, and interaction effects of exercise on metabolic profiles strongly supports the fundamental principle of muscle fiber mutability and the hypothesis of specificity of alteration. It should be noted that the observed differences were specific not only to the types, intensities and durations of exercise employed, but also to the fiber populations studied. For example, in Figure 13, the greatest relative rise in ATP occurred in the medium (MED), and long (LON) running groups. The observed ATP changes in these two groups took place only in the medial gastrocnemius muscle and between four and twelve weeks for the MED group and between zero and eight weeks for the LON group.

The changes observed reflect the fact that muscle-fiber metabolic characteristics can be altered under normal physiological conditions. These alterations in normal intact animals are in line with the suggestion by Guth and Yellin (1971) that plasticity is the rule rather than the exception.

Medial Gastrocnemius.--Endogeneous glycogen changes paralleled previous histochemical and biochemical findings (Beatty et al., 1966; Edgerton et al., 1970). Voluntary

(VOL), SWM, and LON groups tended to concentrate glycogen, whereas the SHT and MED groups had less concentration, indicating active glycogenolysis and glycogenesis. The phosphorylase values were fairly steady over the twelve-week treatment period for the VOL, SWM, and LON groups. It was reasonable that more glycogen was broken down with higher frequency activity than lower frequency activity. Sources of carbon other than glycogen are probably utilized as white fibers assume characteristics of red fibers. Fats and free fatty acids are easily obtained and broken down by oxidative mechanisms. The increased indices reflecting energy-supplying systems (SDH and ATP) for VOL at four weeks indicated such a transformation. Further, the dramatic increase in ATP for the LON group at eight weeks probably was necessary to supply energy for sustained contraction. The lack of significant changes in the SHT group would seem to suggest that the recovery period was of sufficient length for resynthesis of enzymes and substrates.

Plantaris.--The fact that the fibers tended to become "redder" with the SDH index in response to endurance activity was not totally unexpected. Since Edgerton et al. (1969) and Kowalski et al. (1969) reported increases in the proportions of red fibers as identified by malate dehydrogenase and succinate dehydrogenase.

The only significant histochemical differences found were in endogeneous glycogen and intermyofibrillar ATP. This normally red and intermediate fiber population assumed the characteristics of a more uniform population of intermediate fibers, again suggesting that red and white fiber characteristics can approach each other. For the most part, this heterogeneous area had sufficient enzymatic capacities to maintain equilibrium under varied exercise stresses.

Soleus.--In the soleus muscle, phosphorylase and succinate dehydrogenase fiber profiles were relatively unchanged by the exercise programs. The red fibers of the soleus muscle had both anaerobic and aerobic capacities. This finding suggested that these fibers, although in the minority, were endowed with enzymes to support both aerobic and anaerobic metabolism and were able to provide the necessary requirements for both short and sustained activity. The results confirmed the earlier histochemical findings of Edgerton et al. (1969).

The duration effects were limited to PAS, ATP, and SDH. All experimental treatments produced an increase in PAS values at four and twelve weeks in contrast to those found at zero weeks. Increased glycogen content was more evident in red muscle. This increase was probably due to higher levels of glycogen synthetase (Beatty et al., 1963; Gillespie et al., 1970).

The controlled running groups showed increased SDH intensity at twelve weeks, but only the LON result was statistically significant. The LON group probably required higher resistance to fatigue, and an appropriate alteration occurred. Overall, it appeared that the soleus contained glycolytic and oxidative enzymes in a sufficient number of fibers to cope with the exercise stresses afforded by these programs. Reed (1971) in an accompanying study on succinate dehydrogenase and motor endplate cholinesterase, found significant duration effects for the controlled running groups in the soleus SDH measure. This effect was seen at twelve weeks for the LON group, and agreed with the present findings. Extension of the treatment period might reveal even more prominent treatment-duration differences.

It was obvious that in examining metabolic adaptation to physical stress at the tissue level multiple factors must be considered. These factors include: age of animals, confinement of animals, types of treatment, duration of the treatment, muscle or region of muscle examined, and histochemical methods. Furthermore, studies incorporating only one type or duration of treatment to test the hypothesis that muscle adapts selectively to physical activity may be either positive or negative in their findings. A variety of treatments and durations of

treatment, as incorporated in this study, provided chronological evidence to distinguish the various patterns of response.

Limitations of this Study and Suggestions for Future Studies

Limitations of the Study

1. The relative histochemical reactivity can only be indicative of the amount of accumulated end-product, elucidating phenotypic patterns, rather than necessarily reflecting the amount or location of the reaction product.
2. The interpretation of results is restricted to normal, adult, male, albino rats of the Sprague-Dawley strain.
3. The subjective classification for the specific muscle areas examined are restricted to analysis of that particular area.
4. Data were collected and analyzed from only three subjectively defined muscle areas.
5. The application of histochemical procedures necessarily limited the collection of physiological and biochemical data.

Suggestions for Future Studies

1. A similar study should be completed which encompasses a greater variety of low and high intensity physical activity. The training period should be extended to determine the duration of treatment effect. A six-month program might yield interesting findings.

2. Any further histochemical study should be correlated with appropriate physiological analysis of the motor unit and quantitative enzyme assays.

3. Study of additional enzymes is necessary for a more complete inventory to extend the present findings. The rate limiting step enzymes (lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase) should be investigated.

4. The cellular modifications should be examined by transmission electron microscopy.

5. Analysis of histochemical profile of the motor unit should be delineated in an entire muscle; rather than area analysis.

6. The possibility of change in numbers of fibers per unit area should be investigated.

SUMMARY AND CONCLUSIONS

Summary

This study was undertaken to determine the effects of seven different exercise regimens on the histochemical characteristics and distribution of selected muscle fibers of the triceps surae and plantaris muscles of albino rats.

One hundred and seventy-six 72-day-old, normal, male, albino rats (Sprague-Dawley Strain) were randomly assigned to one of seven treatments. Treatments began after a 12-day adjustment period when all animals were 85 days of age. The treatment groups were sedentary control (CON); voluntary running (VOL); short-duration, high-speed endurance running (SHT); medium-duration, moderate-speed endurance running (MED); long-duration, low-speed endurance running (LON); electric stimulus control (ESC); and long-duration, low-intensity swimming (SWM). Treatments were administered once a day, Monday through Friday. All animals had access to food and water ad libitum.

Only animals subjectively determined to be in good general health were selected for sacrifice. Performance criteria of 75 percent expected revolutions (PER) and

75 percent shock-free time (PSF) were set for controlled running programs. Only those short (SHT), medium (MED), and long (LON) animals whose performance values were above these criteria were selected for sacrifice. Proximity to a mean percent expected swim time (PET) value of 100 was used as a sacrifice selection criterion for the swimming (SWM) program. Seven animals, limited to the same duration, were weighted and sacrificed on Mondays after zero, four, eight, and twelve weeks of training. The final sample consisted of 94 animals.

Animals were sacrificed under anesthesia by intraperitoneal injection of pentobarbital sodium. Pelikan ink was injected into the vascular system for capillary per muscle fiber calculations. The triceps surae and plantaris muscles were removed as a unit, rolled in talcum powder and frozen in an isopentane-liquid nitrogen system. Fresh-frozen, distal-proximal serial cross sections, were cut at 10 microns using a rotary microtome-cryostat. Four histochemical procedures were utilized for identification of relative fiber type intensities of glycogen (periodic acid-Schiff), glycolytic enzyme (phosphorylase), oxidative enzyme (succinate dehydrogenase), and energy producing systems (adenosine triphosphatase). Fifty muscle fibers, from each specific intramuscular area of medial gastrocnemius, plantaris, and soleus muscles, were graded according to intensity and distribution patterns of the various

histochemical procedures. The percentages of intensity ratings were recorded for individual animals.

The prominence of duration, as well as treatment, effects suggested that the seven different chronic physical activities had specific effects upon the alteration of fiber characteristics, but the effects were highly time dependent.

The results indicated there were diverse regional responses and patterns of change over time to the same exercise stimulus. Four, eight, and twelve weeks of various activity programs produced metabolic alterations in proportions of fiber types in the medial gastrocnemius, plantaris, and soleus muscles.

In the soleus muscle, similar periodic acid-Schiff (PAS) changes occurred for the voluntary group at four and eight weeks. Voluntary (VOL) group activity produced a significant increase in the percentage of intermediate ATP fibers at eight weeks, while the electric stimulus control (ESC) treatment produced a similar significant increase at four weeks, which was reversed at twelve weeks ($p < .20$).

In the plantaris muscle similar increases in PAS were found at four weeks for long (LON) and swimming (SWM) groups ($p < .20$). The electric simulus control (ESC) and SWM programs produced significant increases in the percentage of low intensity ATP fibers at twelve weeks,

while the VOL treatment produced the same result at four weeks. The short (SHT) group showed a specific pattern of increasing intermediate ATP fibers from four to twelve weeks ($p < .20$).

The general adaptive patterns for the medial gastrocnemius muscle showed that anaerobic fibers were able to acquire aerobic fiber characteristics and supported the hypothesis of specificity of alteration. The greatest relative rise in ATP occurred in the medium (MED) and long (LON) running groups. This change was observed between four and twelve weeks for the MED group and between zero and eight weeks for the LON group.

Conclusions

1. Histochemical profiles, for medial gastrocnemius, plantaris, and soleus muscle areas can be influenced differentially by specific types and durations of physical activity.
2. Alterations in histochemical profiles can be enzyme or substrate specific.
3. Selective adaptation occurs at the individual muscle fiber level, or by motor units, but not homogeneously in an entire muscle complex.

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APPENDICES

APPENDIX A

TRAINING PROGRAMS

TABLE A-1.-- Standard eight-week, short-duration, high-speed endurance training program for postpubertal and adult male rats in controlled running wheels.

Wk.	Day of Wk.	Day of Tr.	Acc-eler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repe-ti-tions per Bout	No. of Bouts	Time Bet-ween Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revo-lu-tions *TER*	Total Work Time (sec) *TWT*
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
	5=F	5	2.0	00:10	10	40	3	5.0	1.2	2.0	49:30	600	1200
2	1=M	6	1.5	00:10	10	28	4	5.0	1.2	2.5	51:40	700	1120
	2=T	7	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	3=W	8	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	4=T	9	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	5=F	10	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
3	1=M	11	1.5	00:10	15	27	4	5.0	1.2	3.0	59:00	810	1080
	2=T	12	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	3=W	13	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	4=T	14	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	5=F	15	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
4	1=M	16	1.5	00:10	20	23	4	5.0	1.2	3.5	59:40	805	920
	2=T	17	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	3=W	18	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	4=T	19	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	5=F	20	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
5	1=M	21	1.5	00:10	25	20	4	5.0	1.0	4.0	60:00	800	800
	2=T	22	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	3=W	23	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	4=T	24	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	5=F	25	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
6	1=M	26	1.5	00:10	30	16	4	5.0	1.0	4.5	55:40	720	640
	2=T	27	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	3=W	28	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	4=T	29	2.0	00:10	35	10	5	5.0	1.0	5.0	54:34	625	500
	5=F	30	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
7	1=M	31	2.0	00:10	35	10	5	5.0	1.0	5.0	54:35	625	500
	2=T	32	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	3=W	33	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	4=T	34	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	5=F	35	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
8	1=M	36	2.0	00:10	35	7	8	2.5	1.0	5.0	54:50	700	560
	2=T	37	2.0	00:10	40	6	8	2.5	1.0	5.5	52.10	660	480
	3=W	38	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	4=T	39	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480
	5=F	40	2.0	00:10	40	6	8	2.5	1.0	5.5	52:10	660	480

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSF* and *PER* scores of 75 or higher during the final two weeks. Alterations in the work time, rest time, repetitions per bout, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.

Standard short-duration, high-speed endurance maintenance program for postpubertal and adult male rats in controlled running wheels.

Acc-eler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repe-ti-tions per Bout	No. of Bouts	Time Bet-ween Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revo-lu-tions *TER*	Total Work Time (sec) *TWT*
1.5	00:30	30	6	3	5.0	1.0	4.0	26:30	540	540

TABLE A-2.-- Standard eight-week, medium-duration, moderate-speed endurance training program for postpubertal and adult male rats in controlled running wheels.

Wk.	Day of Wk.	Day of Tr.	Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:15	15	19	4	5.0	1.2	2.0	52:00	570	1140
	5=F	5	2.5	00:15	15	19	4	5.0	1.2	2.0	52:00	570	1140
2	1=M	6	2.0	00:15	15	19	4	5.0	1.2	2.0	52:00	570	1140
	2=T	7	2.0	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
	3=W	8	1.5	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
	4=T	9	1.5	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
	5=F	10	1.5	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
3	1=M	11	1.5	00:15	15	19	4	5.0	1.2	2.5	52:00	712	1140
	2=T	12	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
	3=W	13	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
	4=T	14	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
	5=F	15	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
4	1=M	16	1.5	00:15	15	18	4	5.0	1.2	3.0	50:00	810	1080
	2=T	17	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
	3=W	18	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
	4=T	19	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
	5=F	20	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
5	1=M	21	1.5	00:15	15	18	4	5.0	1.0	3.5	50:00	945	1080
	2=T	22	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	3=W	23	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	4=T	24	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	5=F	25	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
6	1=M	26	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	2=T	27	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
	3=W	28	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
	4=T	29	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
	5=F	30	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
7	1=M	31	1.5	00:20	20	11	5	5.0	1.0	4.0	55:00	1100	1100
	2=T	32	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
	3=W	33	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
	4=T	34	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
	5=F	35	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
8	1=M	36	1.5	00:25	25	9	5	5.0	1.0	4.0	55:25	1125	1125
	2=T	37	1.5	00:30	30	8	5	5.0	1.0	4.0	57:30	1200	1200
	3=W	38	1.5	00:30	30	8	5	5.0	1.0	4.0	57:30	1200	1200
	4=T	39	1.5	00:30	30	8	5	5.0	1.0	4.0	57:30	1200	1200
	5=F	40	1.5	00:30	30	8	5	5.0	1.0	4.0	57:30	1200	1200

This standard program was designed using male rats of the Sprague-Dawley Strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSF* and *PER* scores of 75 or higher during the final two weeks. Alterations in the rest time, repetitions per bout, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the training program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.

Standard medium-duration, moderate-speed endurance maintenance program for postpubertal and adult male rats in controlled running wheels.

Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/ sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
2.0	00:10	40	4	6	2.5	1.0	5.5	28:30	330	240

TABLE A-3.-- Standard eight-week, long-duration, low-speed endurance training program for postpubertal and adult male rats in controlled running wheels.

Wk.	Day of Wk.	Day of Tr.	Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	1.5	40:00	---	---
1	1=M	1	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	2=T	2	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	3=W	3	3.0	00:10	10	40	3	5.0	1.2	1.5	49:30	450	1200
	4=T	4	2.5	00:20	10	30	2	5.0	1.2	1.5	34:40	450	1200
	5=F	5	2.5	00:30	15	20	2	5.0	1.2	1.5	34:30	450	1200
2	1=M	6	2.0	00:40	20	15	2	5.0	1.2	2.0	34:20	600	1200
	2=T	7	2.0	00:50	25	12	2	5.0	1.2	2.0	34:10	600	1200
	3=W	8	1.5	01:00	30	10	2	5.0	1.2	2.0	34:00	600	1200
	4=T	9	1.5	02:30	60	4	2	5.0	1.2	2.0	31:00	600	1200
	5=F	10	1.0	02:30	60	4	2	5.0	1.2	2.0	31:00	600	1200
3	1=M	11	1.0	02:30	60	4	2	5.0	1.2	2.0	31:00	600	1200
	2=T	12	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	3=W	13	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	4=T	14	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	5=F	15	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
4	1=M	16	1.0	05:00	0	1	5	2.5	1.2	2.0	35:00	750	1500
	2=T	17	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	3=W	18	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	4=T	19	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	5=F	20	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
5	1=M	21	1.0	07:30	0	1	4	2.5	1.0	2.0	37:30	900	1800
	2=T	22	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	3=W	23	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	4=T	24	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	5=F	25	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
6	1=M	26	1.0	07:30	0	1	5	2.5	1.0	2.0	47:30	1125	2250
	2=T	27	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	3=W	28	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	4=T	29	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	5=F	30	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
7	1=M	31	1.0	10:00	0	1	4	2.5	1.0	2.0	47:30	1200	2400
	2=T	32	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	3=W	33	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	4=T	34	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	5=F	35	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
8	1=M	36	1.0	10:00	0	1	5	2.5	1.0	2.0	60:00	1500	3000
	2=T	37	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	3=W	38	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	4=T	39	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000
	5=F	40	1.0	12:30	0	1	4	2.5	1.0	2.0	57:30	1500	3000

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PSF* and *PER* scores of 75 or higher during the final two weeks. Alterations in the work time, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training programs.

Standard long-duration, low-speed endurance maintenance program for postpubertal and adult male rats in controlled running wheels.

Acceleration Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repetitions per Bout	No. of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min: sec)	Total Exp. Revolutions *TER*	Total Work Time (sec) *TWT*
1.0	12:30	0	1	2	2.5	1.0	2.0	27:30	750	1500

TABLE A-4.-- Standard eight-week, endurance, swimming training program for postpubertal and adult male rats.

Wk.	Day of Wk.	Day of Tr.	Per Cent Tail Weight	Expected Swim Time (min) *EST*
1	1=M	1	0	30
	2=T	2	0	40
	3=W	3	C*	50
	4=T	4	C	60
	5=F	5	C	60
2	1=M	6	2	40
	2=T	7	2	40
	3=W	8	2	40
	4=T	9	2	45
	5=F	10	2	50
3	1=M	11	3	30
	2=T	12	3	30
	3=W	13	3	30
	4=T	14	3	35
	5=F	15	3	35
4	1=M	16	3	35
	2=T	17	3	40
	3=W	18	3	40
	4=T	19	3	40
	5=F	20	3	40
5	1=M	21	3	40
	2=T	22	3	45
	3=W	23	3	45
	4=T	24	3	45
	5=F	25	3	45
6	1=M	26	3	45
	2=T	27	3	50
	3=W	28	3	50
	4=T	29	3	50
	5=F	30	3	50
7	1=M	31	3	50
	2=T	32	3	55
	3=W	33	3	55
	4=T	34	3	55
	5=F	35	3	55
8	1=M	36	3	55
	2=T	37	3	60
	3=W	38	3	60
	4=T	39	3	60
	5=F	40	3	60

*C = clothes pin only.

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 90 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have *PET* scores of 75 or higher during the final two weeks. Alterations in the per cent tail weight or expected swim time can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a severe, sudden exercise stress upon the animals and will seriously impair the effectiveness of the training program.

Standard endurance swimming maintenance program for postpubertal and adult male rats.

Per Cent Tail Weight	Expected Swim Time (min) *EST*
2	40

APPENDIX B

TRAINING PERFORMANCE AND ENVIRONMENTAL CONDITIONS

TABLE B-1.--Treatment environment and body weight values for short, medium and long groups.

Variable	N[a]	Mean	Stan. Dev.	Simple Correlations					
				Air Temp.	% Humidity	Bar. Press.	Pre-Treat. Body Wgt.	% Body Wgt. Loss	PER
Air Temp. (°C)	2048	23.4	1.99						
% Humidity	2048	21.0	7.34	0.193					
Bar. Press. (mmHg)	2048	739.3	5.67	-0.137	-0.137	-0.292			
Pre-Treat. Body Wgt. (g)	2035	350.0	44.16	0.027	0.281	-0.039			
% Body Wgt. Loss	2035	2.2	0.74	0.078	-0.007	0.025	-0.256		
PER	2048	107.5	28.54	0.010	0.003	0.008	-0.437	0.348	
PSF	2048	89.2	8.09	0.005	0.005	0.027	-0.265	0.285	0.605

[a]Total training days for all animals.

TABLE B-2.--Treatment environment and body weight values for swimming group.

Variable	N[a]	Mean	Stan. Dev.	Simple Correlations				
				Water Temp.	Air Temp.	% Humidity	Bar. Press.	Pre-Treat. Body Wgt.
Water Temp. (°C)	477	31.9	0.43					
Air Temp. (°C)	477	22.9	1.43	-0.113				
% Humidity	477	21.3	7.47	0.293	0.180			
Bar. Press. (mmHg)	477	739.4	5.19	0.003	-0.205	-0.398		
Pre-Treat. Body Wgt. (g)	477	370.0	49.82	0.156	0.256	0.046	0.024	
PET	477	99.3	2.79	-0.049	0.003	-0.095	0.013	0.124

[a]Total training days for all animals.

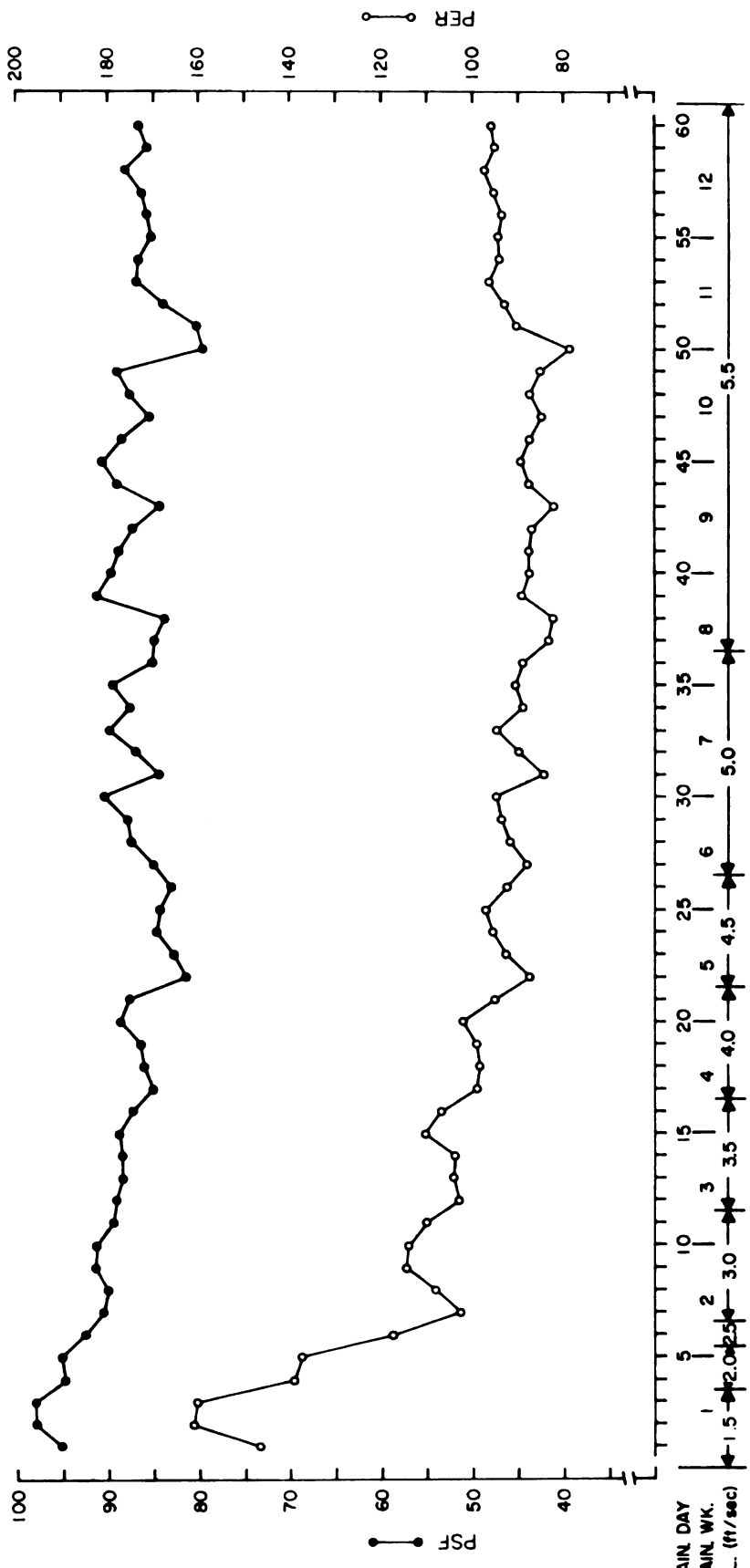

Figure B-1.--Mean Daily Per Cent Shock Free Time (PSF) and Per Cent Expected Revolutions (PER) for CRW SHORT

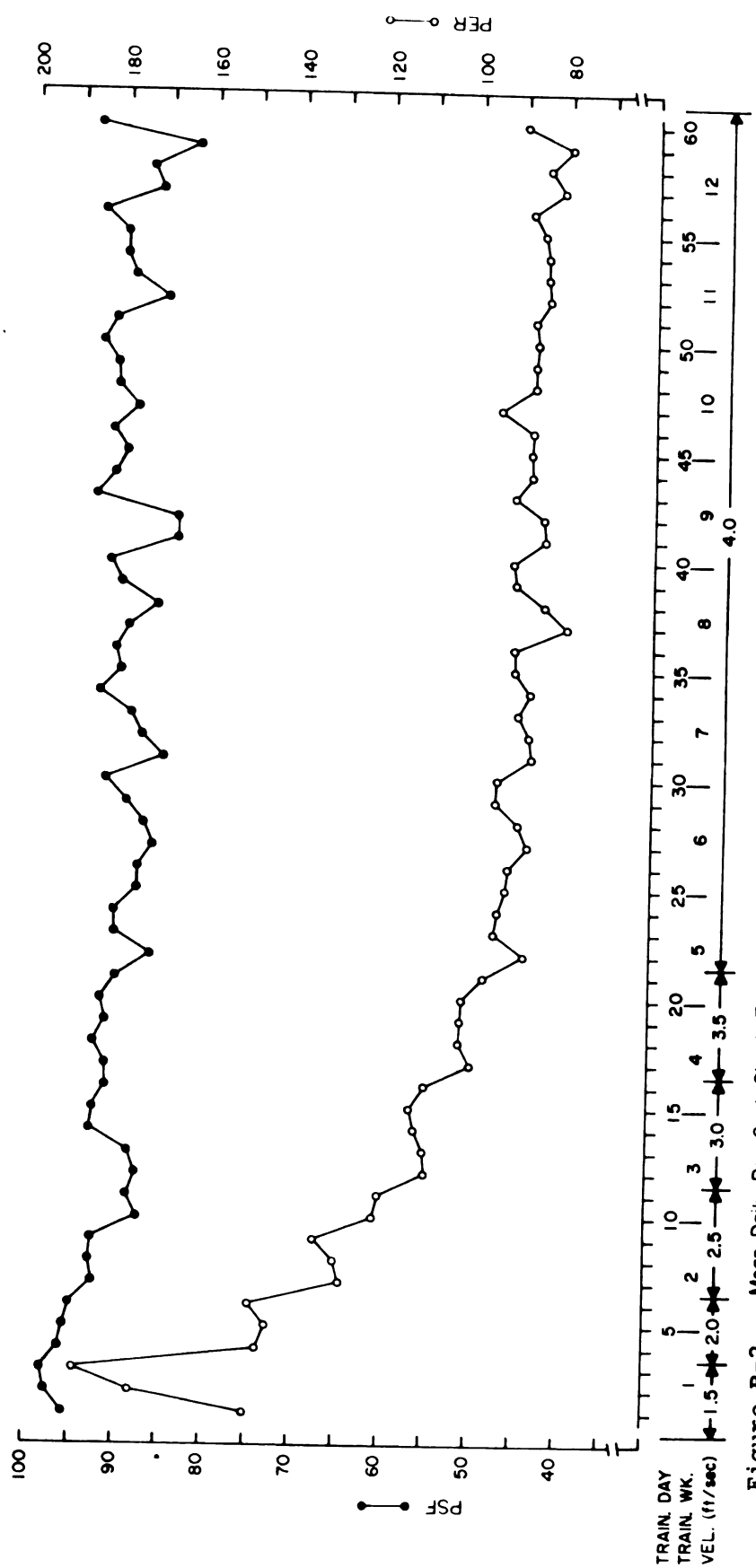

Figure B-2.--Mean Daily Per Cent Shock Free Time (PSF) and Per Cent Expected Revolutions (PER) for CRW MEDIUM

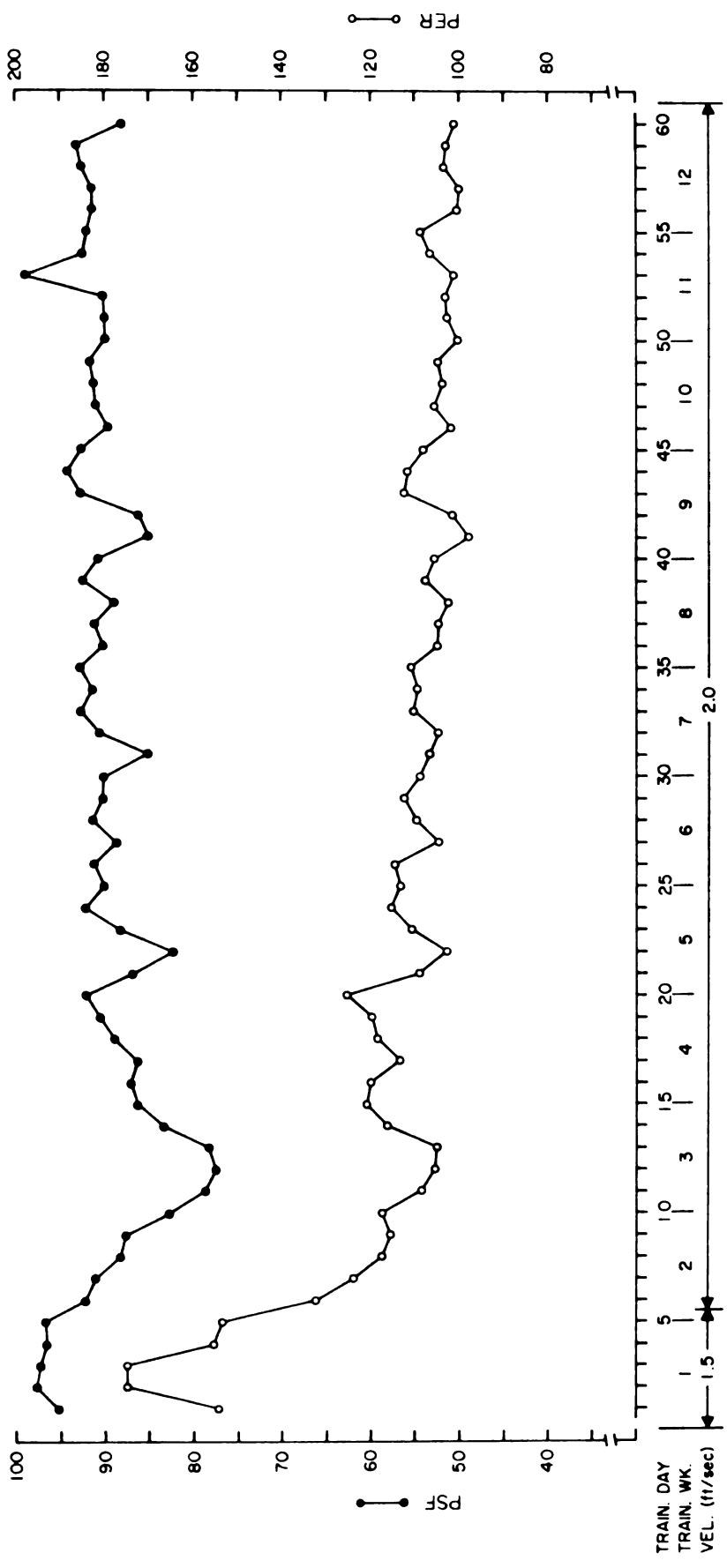

Figure B-3.-- Mean Daily Per Cent Shock Free Time (PSF) and Per Cent Expected Revolutions (PER) for CRW LONG

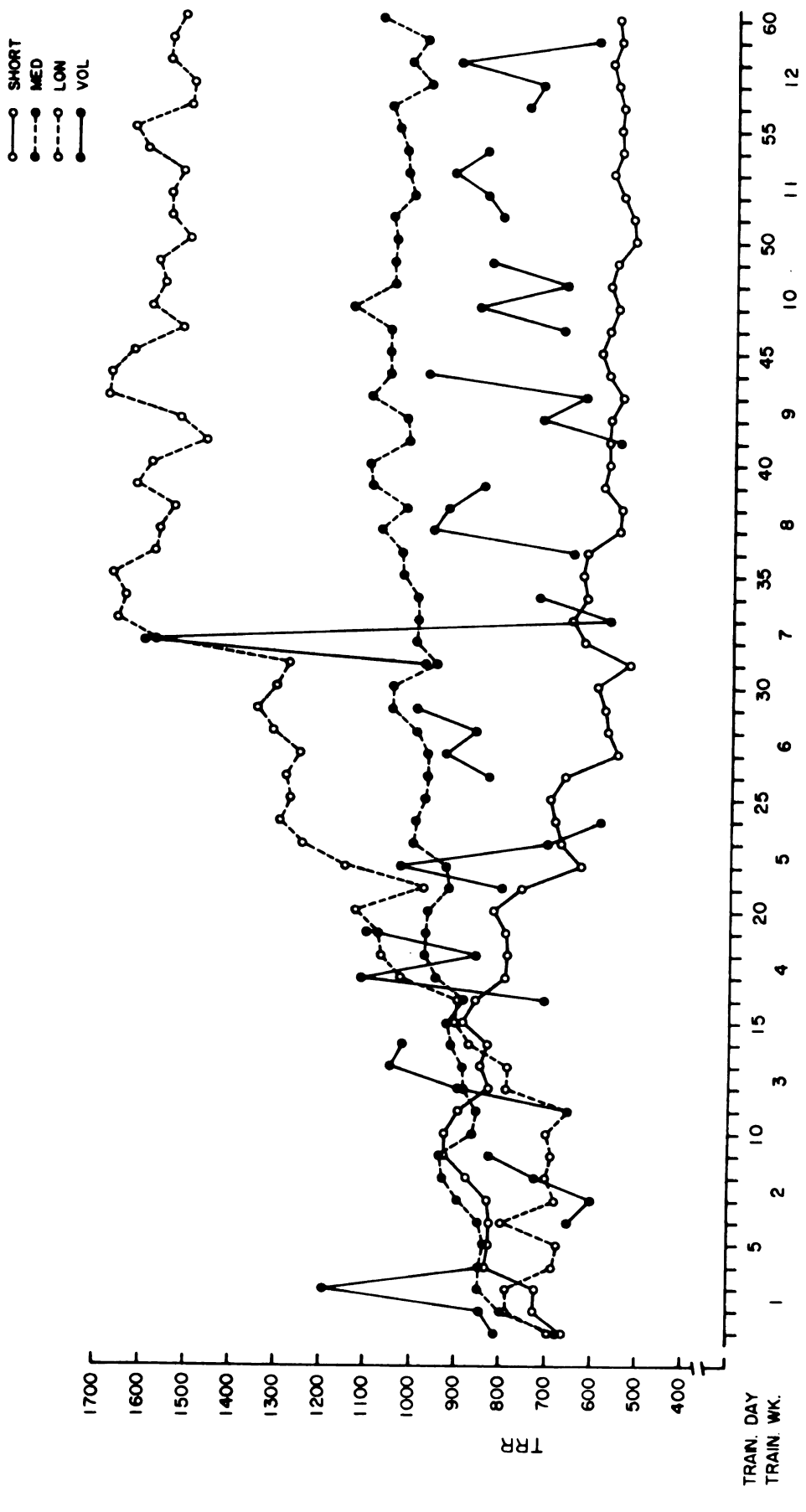

Figure B-4.--Mean Daily Total Revolutions Run (TRR) for VOLUNTARY and CRW SHORT, MEDIUM, and LONG

APPENDIX C

HISTOCHEMICAL FIBER TYPE RAW DATA

TABLE C-1.--Histochemical ratings for medial gastrocnemius muscle presented by animal number, treatment, and duration.

Animal Number	Treatment	Dur. (Wk.)	Phenotypic Rating/Histochemical Procedure											
			1				2				3			
			SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS
006	1	0	02	00	47	05	10	06	03	45	38	44	00	00
009	1	0	02	00	47	05	10	06	03	45	38	44	00	00
002	1	4	00	00	45	07	10	07	05	40	40	43	00	03
053	1	4	00	00	49	49	02	02	00	00	48	48	00	00
100	1	4	00	00	49	49	02	02	00	00	48	48	00	00
140	1	4	00	00	49	32	04	02	00	18	46	48	00	00
005	1	8	04	01	49	24	00	05	01	22	46	44	00	04
052	1	8	00	00	49	03	01	04	01	47	49	46	00	00
099	1	8	01	01	45	37	03	03	05	13	46	46	00	00
139	1	8	05	05	45	34	00	12	05	12	45	33	00	04
003	1	12	03	01	45	12	05	16	05	35	42	33	00	02
051	1	12	03	01	45	13	05	16	05	35	42	33	00	02
098	1	12	02	00	48	39	04	02	02	11	44	48	00	00
138	1	12	00	00	49	24	11	06	00	26	39	44	00	00
007	2	0	01	00	41	07	05	05	09	31	45	45	00	12
057	2	0	01	00	41	07	05	05	09	21	45	45	00	12
069	2	4	10	07	40	29	25	18	09	14	15	25	01	07
070	2	4	10	07	40	29	25	18	09	14	15	25	01	07
147	2	4	00	00	49	12	03	03	00	38	47	47	00	00
149	2	4	10	07	40	29	25	18	09	14	15	25	01	07
064	2	8	00	09	41	15	13	07	09	35	38	34	00	00
068	2	8	00	06	42	07	09	05	08	43	41	39	00	00
148	2	8	00	00	41	24	07	16	09	26	43	34	00	00
150	2	8	01	01	39	30	18	18	10	19	31	31	01	01
062	2	12	00	00	45	43	04	04	05	07	46	46	00	00
072	2	12	00	00	45	43	04	04	05	07	46	46	00	00
145	2	12	00	00	49	28	11	09	00	22	39	41	00	00
152	2	12	00	00	49	28	22	24	00	22	38	26	00	00
055	3	0	08	00	38	24	09	13	12	26	24	22	29	00
058	3	0	02	03	49	15	13	17	01	32	35	30	00	03
014	3	4	00	00	43	11	04	08	07	39	46	42	00	00
015	3	4	00	00	49	13	05	08	00	37	45	42	00	00
107	3	4	00	00	41	05	49	31	09	45	00	19	00	00
108	3	4	00	00	34	05	20	18	16	45	30	30	00	00
020	3	8	00	00	44	06	06	08	06	44	44	42	00	00
022	3	8	00	00	47	15	27	17	03	35	23	34	00	00
106	3	8	15	05	25	27	08	23	22	19	27	22	03	04
110	3	8	07	07	25	21	16	22	22	27	27	21	03	02
018	3	12	15	05	25	27	08	23	22	19	27	22	03	03
021	3	12	00	00	29	12	15	01	21	38	35	49	00	00
101	3	12	00	00	49	28	06	01	01	24	44	49	00	00
104	3	12	00	00	49	26	06	01	00	24	44	49	00	00
059	4	0	01	00	49	03	06	06	00	47	43	44	00	00
011	4	0	00	00	49	27	13	06	00	15	37	44	00	08
074	4	4	00	00	40	14	09	09	10	36	41	41	00	00
077	4	4	00	00	40	12	14	14	10	38	36	36	00	00
154	4	4	00	00	49	19	03	01	01	31	47	49	00	00
159	4	4	00	00	49	19	03	01	00	31	47	49	00	00

TABLE C-1.--Continued.

Animal Number	Treatment	Dur. (Wk.)	Phenotypic Rating/Histochemical Procedure											
			1				2				3			
			SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS
075	4	8	00	00	44	23	15	22	06	25	35	28	00	02
080	4	8	01	01	44	23	14	21	05	25	35	28	01	02
162	4	8	00	00	45	30	06	22	05	20	44	28	00	00
164	4	8	01	01	29	27	19	23	21	23	30	26	00	00
073	4	12	00	27	33	30	15	22	17	20	35	01	00	00
076	4	12	03	07	38	12	06	40	12	38	41	03	00	00
153	4	12	00	00	49	00	15	16	00	25	35	34	00	25
010	5	0	02	00	47	05	10	06	03	45	38	44	00	00
056	5	0	00	00	41	07	05	05	09	31	45	45	00	12
088	5	4	00	00	31	18	21	19	19	32	29	31	00	00
089	5	4	00	16	20	16	19	29	25	12	31	05	05	11
170	5	4	03	00	29	24	08	12	21	22	39	38	00	04
172	5	4	00	00	47	25	06	07	03	25	44	43	00	00
091	5	8	06	08	43	20	12	08	07	24	32	34	00	06
092	5	8	00	26	41	03	20	22	09	42	30	02	00	05
166	5	8	00	11	31	31	25	33	10	15	25	06	09	04
085	5	12	01	10	35	26	49	00	15	24	00	40	00	00
095	5	12	04	30	37	23	46	20	13	27	00	00	00	00
171	5	12	00	00	38	26	12	14	12	21	38	36	00	03
176	5	12	00	00	45	22	12	17	05	23	38	33	00	05
012	6	0	00	00	49	16	18	01	00	34	32	45	49	00
060	6	0	00	00	49	25	05	01	00	25	45	49	00	00
026	6	4	00	09	09	14	11	00	40	23	39	41	01	03
027	6	4	00	09	09	14	11	00	40	23	39	41	01	03
119	6	4	00	00	09	29	05	49	40	21	45	00	01	00
120	6	4	00	00	46	29	04	08	04	21	46	42	00	00
032	6	8	05	01	41	30	08	17	08	19	37	32	01	01
034	6	8	05	01	41	30	08	17	08	19	37	32	01	01
118	6	8	05	01	41	30	08	17	08	19	37	32	01	01
122	6	8	05	01	41	30	08	17	08	19	37	32	01	01
030	6	12	04	03	39	19	11	32	11	31	35	15	00	00
033	6	12	04	03	39	19	11	32	11	31	35	16	00	00
113	6	12	00	00	49	00	00	00	00	30	49	49	01	20
116	6	12	00	03	47	13	14	32	00	34	36	15	03	03
008	7	0	00	00	49	20	11	00	00	30	39	49	00	00
054	7	0	05	07	39	25	10	12	05	21	30	31	06	04
041	7	4	00	00	35	12	17	17	14	37	33	33	01	01
046	7	4	00	00	35	12	17	17	14	37	33	33	01	01
135	7	4	00	08	41	26	11	24	09	24	39	18	00	00
136	7	4	00	00	47	17	02	02	03	33	48	48	00	00
045	7	8	00	00	46	29	13	12	14	21	37	38	00	00
047	7	8	00	00	46	29	13	12	04	21	37	38	00	00
131	7	8	00	00	30	27	09	14	20	21	41	36	00	02
132	7	8	00	00	42	23	01	03	07	27	49	47	01	00
037	7	12	00	00	43	49	06	07	07	01	44	43	00	00
038	7	12	06	08	36	45	09	40	14	05	35	02	00	00
125	7	12	00	00	45	00	22	22	02	44	28	28	03	06
126	7	12	13	04	40	20	12	25	10	30	25	21	00	00

LEGEND: 1 = Dark staining intensity; 2 = Medium staining intensity; 3 = Light staining intensity; SDH = Succinate dehydrogenase; ATP = Intermyofibrillar adenosine triphosphatase; PPL = Phosphorylase; PAS = Periodic Acid-Schiff.

TABLE C-2.--Histochemical ratings for plantaris muscle presented by animal number, treatment, and duration.

Animal Number	Treatment	Dur. (Wk.)	Phenotypic Rating/Histochemical Procedure											
			1				2				3			
			SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS
006	1	0	24	17	19	10	21	21	21	31	05	12	10	09
009	1	0	07	16	28	11	35	34	16	27	08	00	06	12
002	1	4	25	13	15	17	23	35	24	31	02	02	11	12
053	1	4	19	19	32	11	28	31	12	34	03	00	06	05
100	1	4	26	28	22	19	16	17	21	30	08	05	07	01
140	1	4	25	12	32	16	24	35	11	30	01	03	07	04
005	1	8	24	18	24	03	22	24	21	42	04	08	05	05
042	1	8	23	20	24	07	21	25	23	31	06	05	03	12
099	1	8	24	16	35	22	23	32	19	23	03	02	06	05
139	1	8	24	16	35	22	23	32	09	23	03	02	06	05
003	1	12	19	12	28	24	31	37	15	15	00	01	07	11
051	1	12	19	12	28	24	31	37	15	15	00	01	07	11
098	1	12	24	15	28	25	26	35	09	07	00	00	13	18
138	1	12	14	17	34	24	28	25	13	16	08	08	03	10
007	2	0	12	07	27	13	36	41	13	33	08	02	10	14
057	2	0	24	23	23	16	26	27	25	24	00	00	02	10
069	2	4	21	16	27	12	27	24	14	36	02	10	09	02
070	2	4	30	18	34	00	18	19	11	30	02	13	05	20
147	2	4	27	15	33	28	23	31	12	20	00	04	05	02
149	2	4	28	23	22	17	22	22	24	28	00	05	04	05
064	2	8	26	17	29	00	23	29	15	22	01	04	06	28
068	2	8	23	21	24	22	21	22	22	22	06	07	04	06
148	2	8	29	26	21	16	19	22	22	33	02	02	07	01
150	2	8	18	20	28	26	32	28	16	18	00	02	06	06
062	2	12	22	22	22	24	28	28	22	21	00	00	06	05
072	2	12	28	26	20	16	22	24	23	29	00	00	07	05
145	2	12	24	24	21	19	22	21	18	29	04	05	11	02
152	2	12	20	11	21	19	26	34	18	29	04	05	11	02
058	3	0	22	15	28	23	23	35	15	20	05	00	07	07
055	3	0	24	22	29	00	26	28	21	30	00	00	00	20
014	3	4	24	21	16	00	26	29	27	43	00	00	07	07
015	3	4	19	18	28	10	30	32	18	25	01	00	04	15
107	3	4	23	21	24	20	27	29	18	26	00	00	08	04
108	3	4	25	22	26	19	22	25	18	18	03	03	06	13
020	3	8	09	16	19	11	41	34	27	37	00	00	04	02
022	3	8	29	19	29	24	21	31	17	26	00	00	04	00
106	3	8	25	14	28	26	24	35	15	20	01	01	07	04
110	3	8	18	06	35	19	29	41	11	23	03	03	04	08
018	3	12	13	10	31	21	37	40	14	19	00	00	05	10
021	3	12	16	07	25	16	33	42	22	34	01	01	03	00
101	3	12	17	07	37	27	31	42	07	16	02	01	06	07
104	3	12	17	07	37	27	31	42	07	16	02	01	16	07
011	4	0	19	17	26	21	17	13	19	23	14	20	05	06
059	4	0	24	21	32	20	26	29	18	27	00	00	00	03
074	4	4	24	22	15	12	26	28	21	38	00	00	14	00
077	4	4	20	16	17	12	29	27	30	22	01	07	03	16
154	4	4	31	17	29	17	19	33	15	11	00	00	06	22
159	4	4	27	12	29	17	22	36	15	11	01	02	06	22
075	4	8	41	20	16	16	09	24	28	19	00	06	06	15
080	4	8	27	18	26	28	23	31	19	11	00	01	05	11
162	4	8	22	13	28	23	28	32	17	19	00	15	05	08

TABLE C-2.--Continued.

Animal Number	Treatment	Dur. (Wk.)	Phenotypic Rating/Histochemical Procedure											
			1				2				3			
			SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS
164	4	8	37	15	99	18	16	32	99	12	07	03	99	20
073	4	12	34	22	24	99	16	28	22	99	00	00	04	99
076	4	12	37	23	14	17	10	26	25	33	03	01	11	00
153	4	12	14	13	31	28	34	35	11	14	02	02	08	08
010	5	0	27	11	24	07	23	39	18	34	00	00	08	09
056	5	0	16	13	28	24	34	37	11	19	00	00	11	07
088	5	4	23	24	12	13	27	26	27	00	00	00	11	37
089	5	4	25	24	17	17	25	26	21	28	00	00	12	15
170	5	4	29	13	26	14	17	36	19	29	04	01	05	07
172	5	4	27	43	26	35	20	11	18	06	03	36	06	09
091	5	8	17	16	14	22	18	23	30	16	15	11	06	12
092	5	8	35	23	23	06	14	26	18	41	01	01	09	03
166	5	8	22	14	32	26	28	36	15	14	00	00	03	10
085	5	12	04	30	37	23	46	20	13	27	00	00	00	00
095	5	12	37	20	18	20	12	30	26	30	00	00	06	00
171	5	12	09	14	30	21	41	32	10	22	00	04	10	07
176	5	12	15	14	21	26	31	32	21	13	04	04	08	11
012	6	0	19	19	22	09	25	31	18	32	16	00	10	09
060	6	0	20	16	27	07	30	34	15	34	00	00	08	09
026	6	4	16	14	16	20	34	36	22	21	00	00	12	09
027	6	4	16	14	16	20	34	36	22	21	00	00	12	09
119	6	4	25	12	30	23	25	35	10	24	00	03	10	03
120	6	4	29	16	30	23	21	32	14	24	00	02	06	03
032	6	8	25	14	29	13	23	34	11	25	02	02	10	12
034	6	8	15	13	33	35	10	30	15	24	25	07	02	01
118	6	8	25	14	29	13	23	34	11	25	02	02	10	12
122	6	8	17	14	31	34	31	31	13	14	02	05	06	02
030	6	12	20	06	25	27	30	29	14	21	00	15	11	02
033	6	12	20	06	25	27	30	29	14	21	00	15	11	02
113	6	12	21	06	22	23	25	29	24	21	04	15	04	06
116	6	12	10	18	29	24	38	32	11	04	02	00	10	22
008	7	0	33	28	19	00	17	22	21	20	00	00	10	30
054	7	0	22	18	29	19	28	32	11	23	00	00	10	08
041	7	4	14	15	22	22	36	35	17	27	00	00	11	01
046	7	4	29	27	23	28	21	23	17	22	00	00	10	00
135	7	4	20	19	23	19	30	29	20	25	00	02	07	06
136	7	4	25	18	30	19	25	29	14	30	00	03	06	01
045	7	8	18	13	24	22	32	24	22	27	00	13	04	01
047	7	8	39	23	28	18	11	25	18	30	00	02	04	02
131	7	8	22	28	24	31	26	19	17	19	02	03	09	00
132	7	8	27	14	23	19	19	33	16	24	04	03	11	07
037	7	12	17	16	20	19	23	28	22	24	10	06	08	07
038	7	12	20	14	24	14	24	31	16	32	06	05	10	04
125	7	12	23	14	24	19	25	33	18	22	02	03	08	09
126	7	12	28	15	20	18	22	26	14	26	00	09	16	06

LEGEND: 1 = Dark staining intensity; 2 = Medium staining intensity; 3 = Light staining intensity; SDH = Succinate dehydrogenase; ATP = Intermyofibrillar adenosine triphosphatase; PPL = Phosphorylase; PAS = Periodic acid-Schiff.

TABLE C-3.--Histochemical ratings for soleus muscle presented by animal number, treatment, and duration.

Animal Number	Treatment	Dur. (Wk.)	Phenotypic Rating/Histochemical Procedure											
			1				2				3			
			SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS
006	1	0	09	07	00	00	41	43	12	20	00	00	38	30
009	1	0	16	12	00	00	34	38	17	19	00	00	33	31
002	1	4	13	37	00	00	37	42	14	26	00	00	36	24
053	1	4	13	09	00	00	37	41	10	07	00	00	40	43
100	1	4	12	07	00	00	38	43	07	07	00	00	43	43
140	1	4	12	11	00	00	38	39	11	28	00	00	39	22
005	1	8	13	09	00	00	37	41	08	26	00	00	42	24
052	1	8	16	14	00	00	34	36	15	26	00	00	35	24
099	1	8	07	06	02	00	43	44	06	17	00	00	42	33
139	1	8	17	14	00	00	33	36	16	17	00	00	37	33
003	1	12	14	00	00	03	36	40	08	28	00	00	42	19
051	1	12	14	10	00	03	36	40	08	28	00	00	42	19
098	1	12	14	09	00	00	36	41	41	10	00	00	09	40
138	1	12	10	02	00	00	40	48	04	29	00	00	46	21
007	2	0	07	08	00	00	43	42	08	10	00	00	42	40
057	2	0	21	20	00	00	29	30	22	37	00	00	28	13
069	2	4	24	16	00	01	26	34	15	44	00	00	35	05
070	2	4	09	09	00	09	41	41	17	36	00	00	33	05
147	2	4	16	12	00	00	34	38	12	26	00	00	38	24
149	2	4	22	17	00	00	28	33	16	32	00	00	34	18
064	2	8	17	03	00	00	33	47	06	04	00	00	44	46
068	2	8	15	13	00	00	35	37	14	13	00	00	36	17
148	2	8	10	03	00	00	40	47	48	28	00	00	02	22
150	2	8	10	02	00	00	40	48	05	24	00	00	45	26
062	2	12	12	13	00	00	38	37	12	36	00	00	38	14
072	2	12	15	12	00	00	35	38	09	46	00	00	41	04
145	2	12	06	02	00	00	44	48	06	22	00	00	44	28
152	2	12	11	07	00	00	39	43	09	47	00	00	41	03
058	3	0	17	06	00	00	33	44	17	24	00	00	33	26
055	3	0	15	10	00	00	35	40	20	09	00	00	30	41
014	3	4	08	10	00	00	42	40	10	09	00	00	40	41
015	3	4	19	16	00	00	31	34	16	38	00	00	34	12
107	3	4	15	09	00	07	35	41	06	23	00	00	44	20
108	3	4	24	12	00	07	26	38	15	23	00	00	35	20
020	3	8	08	03	00	00	42	47	00	03	00	00	49	47
022	3	8	15	07	00	01	35	43	10	29	00	00	40	20
106	3	8	16	08	00	02	34	42	37	43	00	00	13	05
110	3	8	26	05	00	00	24	45	11	32	00	00	39	18
018	3	12	12	07	00	00	38	43	08	30	00	00	42	20
021	3	12	10	04	00	04	40	46	07	46	00	00	43	00
101	3	12	14	03	01	02	36	47	07	24	00	01	43	24
104	3	12	14	03	99	02	36	47	99	24	00	00	99	24
011	4	0	11	10	00	00	39	40	11	33	00	00	39	17
059	4	0	09	11	00	00	41	39	10	32	00	00	40	18
074	4	4	07	05	00	05	43	45	13	42	00	00	37	03
077	4	4	10	09	00	00	40	41	08	21	00	00	42	29
154	4	4	27	11	01	02	23	39	11	27	00	00	39	21
159	4	4	17	08	00	02	33	42	11	27	00	00	39	21
075	4	8	14	06	00	00	36	44	11	32	00	00	39	18
080	4	8	34	05	00	00	16	45	08	33	00	00	42	17
162	4	8	02	02	00	00	48	48	02	38	00	00	48	12

TABLE C-3.--Continued.

Animal Number	Treatment	Dur. (Wk.)	Phenotypic Rating/Histochemical Procedure											
			1				2				3			
			SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS	SDH	ATP	PPL	PAS
164	4	8	01	08	01	00	49	42	02	28	00	00	48	22
073	4	12	34	03	00	15	16	47	30	33	00	00	20	02
076	4	12	18	03	00	05	32	47	02	43	00	00	48	02
153	4	12	22	08	00	00	28	42	00	37	00	00	49	13
010	5	0	09	07	00	00	41	43	12	20	00	00	38	30
056	5	0	14	14	00	00	36	36	14	14	00	00	36	36
088	5	4	16	14	00	00	34	36	15	20	00	00	35	30
089	5	4	13	10	00	00	37	40	10	06	00	00	40	44
170	5	4	11	09	00	01	39	41	11	39	00	00	39	10
172	5	4	11	03	00	02	39	47	07	37	00	00	43	11
091	5	8	14	12	00	02	36	38	11	34	00	00	39	14
092	5	8	10	08	00	00	40	42	20	42	00	00	30	08
166	5	8	21	16	00	00	29	34	20	31	00	00	30	19
085	5	12	14	06	00	24	36	14	10	26	00	00	40	00
095	5	12	43	18	00	17	07	31	23	33	00	01	27	00
171	5	12	19	00	00	00	31	49	10	45	00	00	40	05
176	5	12	29	12	00	00	21	38	16	49	00	00	34	01
012	6	0	13	13	00	00	37	37	17	26	00	00	33	24
060	6	0	16	13	00	00	34	37	17	39	00	00	33	11
026	6	4	11	08	06	00	39	42	09	46	00	00	35	04
027	6	4	12	05	00	00	38	45	12	28	00	00	38	22
119	6	4	27	02	00	01	23	48	06	28	00	00	44	22
120	6	4	09	09	00	01	41	41	14	28	00	00	36	22
032	6	8	30	03	02	15	30	47	15	35	00	00	33	00
034	6	8	20	14	00	00	30	36	14	17	00	00	36	33
118	6	8	03	05	00	00	47	45	05	44	00	00	45	06
122	6	8	13	04	00	05	37	46	08	43	00	00	42	02
030	6	12	14	07	01	01	36	43	12	40	00	01	38	10
033	6	12	14	07	00	00	36	43	12	40	00	00	38	10
113	6	12	20	16	00	00	30	34	06	20	00	00	44	30
116	6	12	20	12	00	00	30	38	15	34	00	00	35	16
008	7	0	16	06	00	00	34	44	13	04	00	00	37	46
054	7	0	11	11	00	00	39	39	14	10	00	00	36	40
041	7	4	16	15	00	00	34	15	16	41	00	00	34	09
046	7	4	11	13	05	00	39	37	11	04	00	00	34	46
135	7	4	16	08	00	01	34	42	12	27	00	00	38	22
136	7	4	12	07	00	05	38	43	08	32	00	00	42	13
045	7	8	26	00	01	05	24	49	17	44	00	00	33	01
047	7	8	13	06	00	00	37	44	17	09	00	00	33	41
131	7	8	17	11	00	06	33	39	14	44	00	00	36	00
132	7	8	13	08	00	02	37	42	09	28	00	00	41	20
037	7	12	10	03	00	00	40	47	05	49	00	00	45	00
038	7	12	13	02	00	17	37	39	06	33	00	09	44	00
125	7	12	10	04	00	00	40	45	09	30	00	00	41	20
126	7	12	16	07	00	00	34	43	12	26	00	00	38	24

LEGEND: 1 = Dark staining intensity; 2 = Medium staining intensity; 3 = Light staining intensity; SDH = Succinate dehydrogenase; ATP = Intermyofibrillar adenosine triphosphatase; PPL = Phosphorylase; PAS = Periodic acid-Schiff.

APPENDIX D

STATISTICAL TABLES FOR MEDIAL GASTROCNEMIUS, PLANTARIS, AND SOLEUS MUSCLES

TABLE D-1.--Two-way analysis of variance tables for histochemical Sin^{-1} percent ratings in medial gastrocnemius muscle.

Source	SS	df	MS	F	P
		SDH--Rating 1			
A (Training)	278.50	6	46.42	0.663	N
B (Duration)	375.60	3	125.20	1.789	N
AB (Training-Duration)	2666.78	18	148.15	2.117	S
Error	4618.13	66			
Total	7939.01	93			
		SDH--Rating 2			
A (Training)	2532.17	6	422.03	2.713	S
B (Duration)	188.68	3	62.89	0.404	N
AB (Training-Duration)	3361.93	18	186.77	1.200	N
Error	10267.14	66	155.56		
Total	16349.92	93			
		SDH--Rating 3			
A (Training)	2667.07	6	444.51	2.288	N
B (Duration)	308.80	3	102.93	0.530	N
AB (Training-Duration)	4513.04	18	250.72	1.290	N
Error	12824.75	66	194.31		
Total	20313.66	93			
		ATP--Rating 1			
A (Training)	1081.71	6	180.28	1.736	N
B (Duration)	825.91	3	275.30	2.650	N
AB (Training-Duration)	2653.00	18	147.39	1.419	N
Error	6856.08	66	103.88		
Total	11416.70	93			

TABLE D-1.--Continued.

Source	SS	df	MS	F	P
		ATP--Rating 2			
A (Training)	1269.91	6	211.65	0.973	N
B (Duration)	2035.83	3	678.61	3.119	N
AB (Training-Duration)	4336.55	18	240.92	1.107	N
Error	14361.30	66	217.60		
Total	22003.59	92			
		ATP--Rating 3			
A (Training)	3012.10	6	502.02	1.760	N
B (Duration)	3056.55	3	1018.85	3.572	S
AB (Training-Duration)	5935.16	18	329.73	1.156	N
Error	18827.70	66	285.27		
Total	25831.51	93			
		PPL--Rating 1			
A (Training)	2129.71	6	354.95	3.039	S
B (Duration)	1216.22	3	405.41	3.471	S
AB (Training-Duration)	3849.16	18	213.84	1.831	S
Error	7707.98	66	116.79		
Total	14903.07	93			
		PPL--Rating 2			
A (Training)	2303.00	6	383.83	2.797	S
B (Duration)	1647.30	3	549.10	4.001	S
AB (Training-Duration)	5550.69	18	308.37	2.247	S
Error	9057.11	66	137.23		
Total	18558.10	93			

TABLE D-1.--Continued.

Source	SS	df	MS	F	P
PPL--Rating 3					
A (Training)	2073.86	6	345.64	4.401	S
B (Duration)	204.52	3	68.17	0.868	N
AB (Training-Duration)	2242.67	18	124.59	1.587	N
Error	5183.02	66	78.53		
Total	9704.07	93			
PAS--Rating 1					
A (Training)	1248.36	6	208.06	0.983	N
B (Duration)	1355.36	3	451.79	2.134	N
AB (Training-Duration)	7207.63	18	400.42	1.891	N
Error	13973.05	66	211.71		
Total	23784.40	93			
PAS--Rating 2					
A (Training)	1061.75	6	176.96	0.968	N
B (Duration)	1082.29	3	360.76	1.974	N
AB (Training-Duration)	5686.19	18	315.90	1.728	N
Error	12064.01	66	182.79		
Total	19894.24	93			
PAS--Rating 3					
A (Training)	892.24	6	148.71	1.714	N
B (Duration)	150.87	3	50.30	0.580	N
AB (Training-Duration)	2846.52	18	158.14	1.823	N
Error	5726.43	66	86.76		
Total	9616.06	93			

LEGEND: 1 = Dark staining intensity; 2 = Medium staining intensity; 3 = Light staining intensity; SDH = Succinate dehydrogenase; ATP = Intermyofibrillar adenosine triphosphatase; PPL = Phosphorylase; PAS = Periodic acid-Schiff; N = Not significant; S = Significant at .05 level.

TABLE D-2.--Two-way analysis of variance tables for histochemical Sin^{-1} percent ratings in plantaris muscle.

SDH--Rating 1					
A (Training)	494.77	6	82.46	1.258	N
B (Duration)	487.40	3	162.47	2.478	N
AB (Training-Duration)	651.54	18	36.20	0.552	N
Error	4327.76	66	65.57		
Total	5961.47	93			
SDH--Rating 2					
A (Training)	462.27	6	77.04	1.120	N
B (Duration)	568.34	3	189.45	2.755	N
AB (Training-Duration)	919.42	18	51.08	0.743	N
Error	4538.62	66	68.77		
Total	6488.65	93			
SDH--Rating 3					
A (Training)	797.91	6	132.99	1.291	N
B (Duration)	458.49	3	152.83	1.484	N
AB (Training-Duration)	1789.46	18	99.41	0.965	N
Error	6799.02	66	103.02		
Total	9844.88	93			
ATP--Rating 1					
A (Training)	467.27	6	77.88	2.189	N
B (Duration)	236.19	3	78.73	2.213	N
AB (Training-Duration)	931.73	18	51.76	1.455	N
Error	2348.05	66	35.58		
Total	3983.24	93			

TABLE D-2.--Continued.

Source	SS	df	MS	F	P
ATP--Rating 2					
A (Training)	719.53	6	119.92	3.278	S
B (Duration)	103.66	3	34.55	0.945	N
AB (Training-Duration)	1276.12	18	70.90	1.938	N
Error	2414.42	66	36.58		
Total	4513.73	93			
ATP--Rating 3					
A (Training)	1107.25	6	184.54	2.257	N
B (Duration)	761.83	3	253.94	3.106	N
AB (Training-Duration)	2113.78	18	117.43	1.436	N
Error	5395.76	66	81.75		
Total	9378.62	93			
PPL--Rating 1					
A (Training)	220.49	6	36.75	0.816	N
B (Duration)	123.90	3	41.30	0.917	N
AB (Training-Duration)	713.80	18	39.66	0.881	N
Error	2971.24	66	45.02		
Total	4029.43	93			
PPL--Rating 2					
A (Training)	205.60	6	34.27	0.789	N
B (Duration)	58.59	3	29.53	0.450	N
AB (Training-Duration)	437.32	18	24.30	0.559	N
Error	2867.33	66	43.44		
Total	3568.84	93			

TABLE D-2.--Continued.

Source	SS	df	MS	F	P
PPL--Rating 3					
A (Training)	597.69	6	99.62	2.965	S
B (Duration)	210.61	3	70.20	2.090	N
AB (Training-Duration)	628.23	18	34.90	1.039	N
Error	2217.44	66	33.60		
Total	3653.97	93			
PAS--Rating 1					
A (Training)	565.25	6	94.21	0.747	N
B (Duration)	1665.81	3	555.27	4.403	S
AB (Training-Duration)	1728.90	18	96.05	0.762	N
Error	8323.35	66	126.11		
Total	12283.31	93			
PAS--Rating 2					
A (Training)	521.79	6	86.97	0.858	N
B (Duration)	350.76	3	116.92	1.154	N
AB (Training-Duration)	2333.08	18	129.62	1.279	N
Error	6686.36	66	101.31		
Total	9891.99	93			
PAS--Rating 3					
A (Training)	157.11	6	26.19	0.208	N
B (Duration)	737.77	3	245.92	1.950	N
AB (Training-Duration)	3350.16	18	186.12	1.476	N
Error	8322,63	66	126.10		
Total	12567.67	93			

LEGEND: 1 = Dark staining intensity; 2 = Medium staining intensity; 3 = Light staining intensity; SDH = Succinate dehydrogenase; ATP = Intermyofibrillar adenosine triphosphatase; PPL = Phosphorylase; PAS = Periodic acid-Schiff; N = Not significant; S = Significant at .05 level.

TABLE D-3.--Two-way analysis of variance tables for histochemical Sin^{-1} percent ratings in soleus muscle.

Source	SS	df	MS	F	P
		SDH--Rating 1			
A (Training)	153.92	6	25.65	0.340	N
B (Duration)	128.76	3	42.92	0.568	N
AB (Training-Duration)	1244.60	18	69.14	0.916	N
Error	4983.19	66	75.50		
Total	6510.47	93			
		SDH--Rating 2			
A (Training)	153.92	6	25.65	0.340	N
B (Duration)	128.76	3	42.92	0.568	N
AB (Training-Duration)	1244.60	18	69.14	0.916	N
Error	4983.19	66	75.50		
Total	6510.47	93			
		SDH--Rating 3			
A (Training)	4.74	6	0.79	1.183	N
B (Duration)	2.63	3	0.88	1.311	N
AB (Training-Duration)	14.80	18	0.82	1.231	N
Error	44.07	66	0.67		
Total	66.24	93			
		ATP--Rating 1			
A (Training)	268.70	6	44.78	0.647	N
B (Duration)	910.17	3	303.39	4.385	S
AB (Training-Duration)	1237.38	18	68.74	0.994	N
Error	4566.37	66	69.19		
Total	6982.62	93			

TABLE D-3.--Continued.

Source	SS	df	MS	F	P
		ATP--Rating 2			
A (Training)	196.25	6	32.71	0.743	N
B (Duration)	441.42	3	147.14	3.343	S
AB (Training-Duration)	910.28	18	50.57	1.149	N
Error	2905.11	66	44.02		
Total	4453.06	93			
		ATP--Rating 3			
A (Training)	24.02	6	4.00	0.429	N
B (Duration)	60.91	3	20.30	2.176	N
AB (Training-Duration)	82.91	18	4.61	0.494	N
Error	615.88	66	9.33		
Total	783.72	93			
		PPL--Rating 1			
A (Training)	61.04	6	10.17	0.668	N
B (Duration)	32.79	3	10.93	0.718	N
AB (Training-Duration)	107.57	18	5.98	0.393	N
Error	1004.99	66	15.23		
Total	1206.39	93			
		PPL--Rating 2			
A (Training)	1102.99	6	183.83	1.652	N
B (Duration)	685.77	3	228.59	2.054	N
AB (Training-Duration)	1289.19	18	71.62	0.644	N
Error	7345.38	66	111.29		
Total	10423.33	93			

TABLE D-3.--Continued.

Source	SS	df	MS	F	P
PPL--Rating 3					
A (Training)	929.89	6	154.98	1.488	N
B (Duration)	605.99	3	201.99	1.939	N
AB (Training-Duration)	1106.64	18	61.48	0.590	N
Error	6876.72	66	104.19		
Total	9519.24	93			
PAS--Rating 1					
A (Training)	370.06	6	61.68	0.699	N
B (Duration)	614.27	3	204.76	2.318	N
AB (Training-Duration)	1567.42	18	87.08	0.986	N
Error	5827.86	66	88.30		
Total	8379.61	93			
PAS--Rating 2					
A (Training)	2610.10	6	435.02	2.058	N
B (Duration)	2883.87	3	961.29	4.548	S
AB (Training-Duration)	3222.52	18	179.03	0.847	N
Error	13949.12	66	211.35		
Total	22665.61	93			
PAS--Rating 3					
A (Training)	3182.20	6	530.37	1.894	N
B (Duration)	5443.59	3	1814.53	6.480	S
AB (Training-Duration)	6189.84	18	343.88	1.228	N
Error	18482.22	66	280.03		
Total	33297.85	93			

LEGEND: 1 = Dark staining intensity; 2 = Medium staining intensity; 3 = Light staining intensity; SDH = Succinate dehydrogenase; ATP = Intermyofibrillar adenosine triphosphatase; PPL = Phosphorylase; PAS = Periodic acid-Schiff; N = Not significant; S = Significant at .05 level.

APPENDIX E

HISTOCHEMICAL FIBER TYPE MEAN DATA

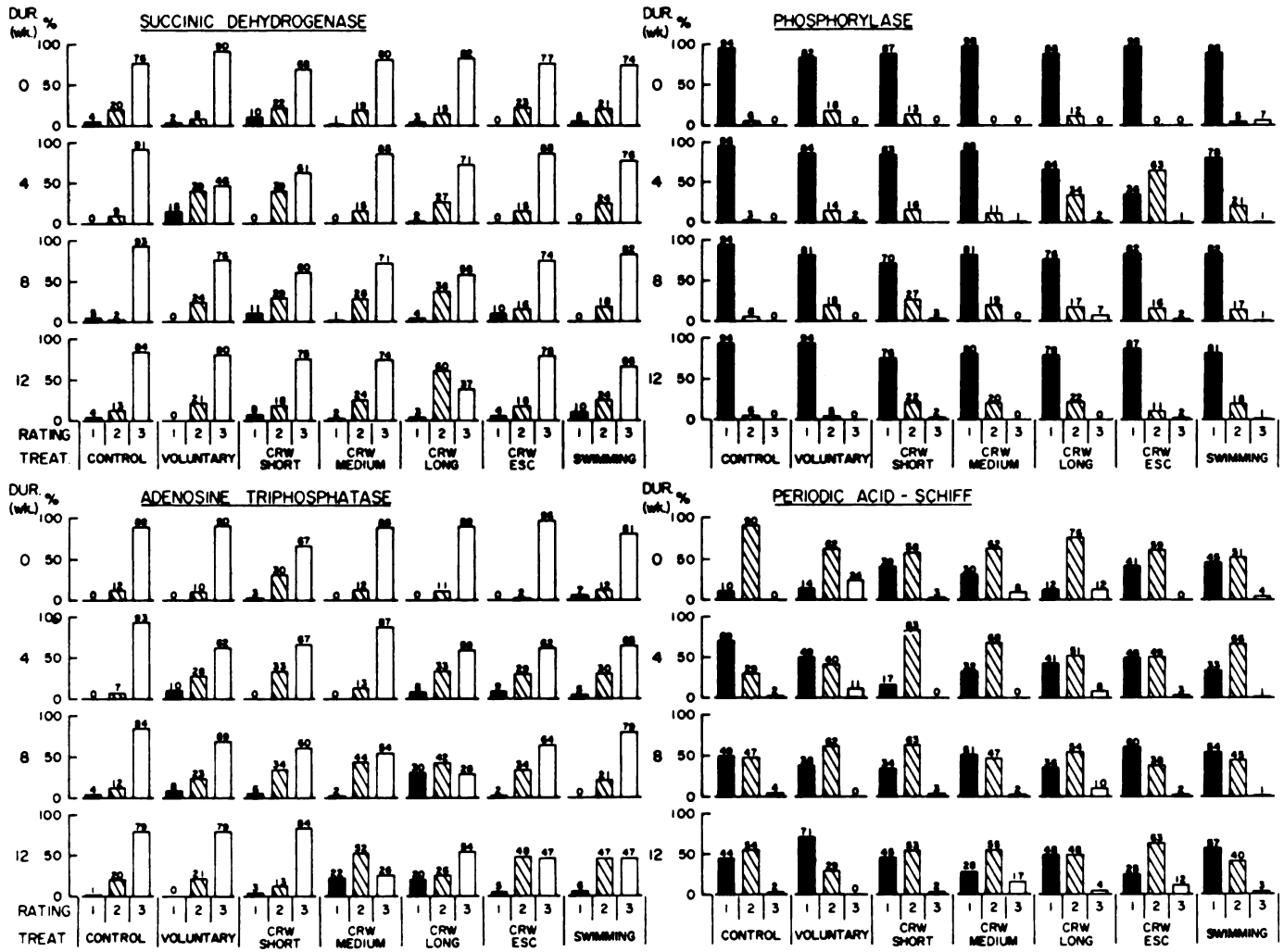

Figure E-1.-- Mean Per Cent Histochemical Fiber Ratings for MEDIAL GASTROCNEMIUS by Treatment and Duration of Treatment

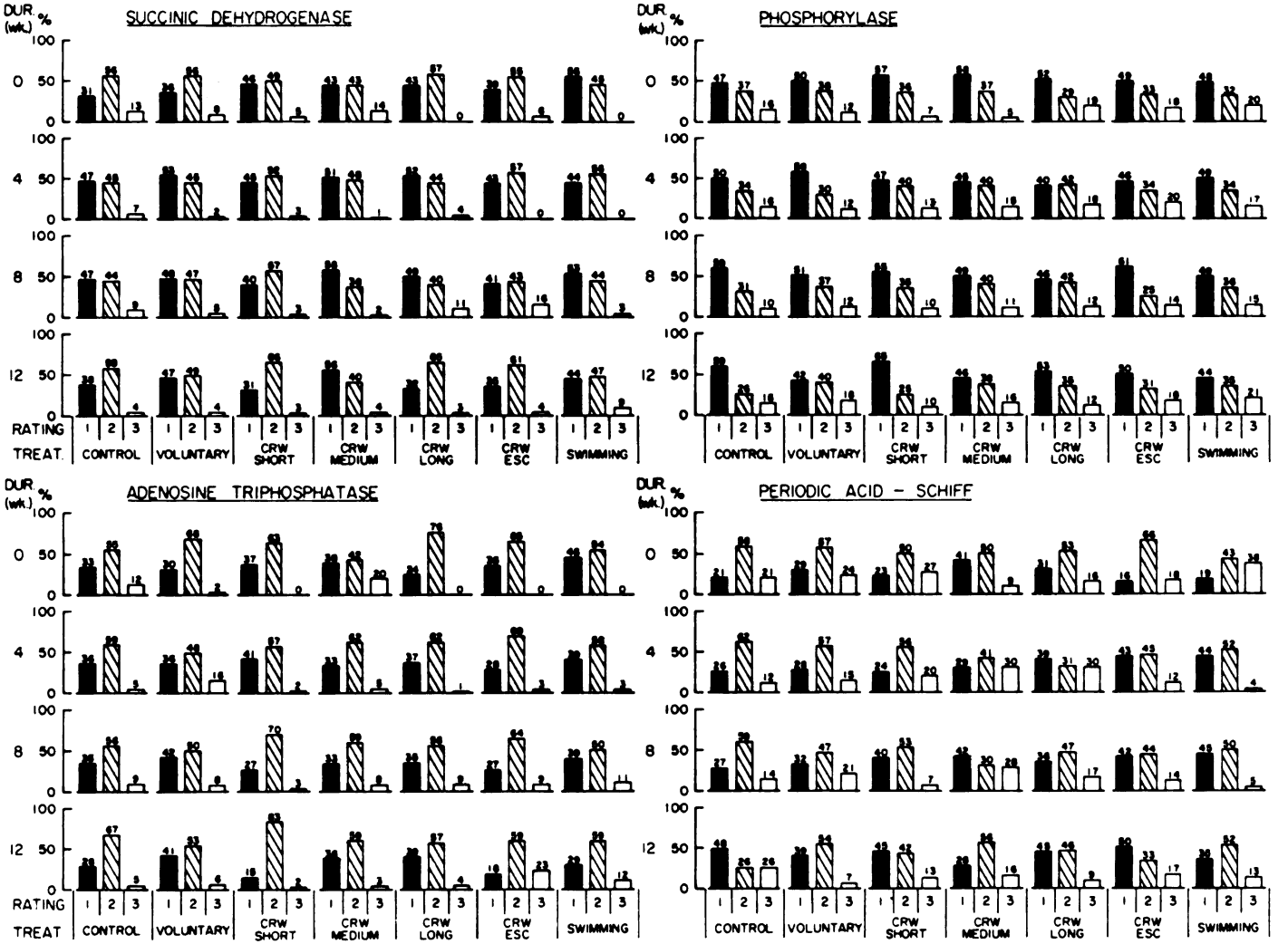

Figure E-2.-- Mean Per Cent Histochemical Fiber Ratings for PLANTARIS by Treatment and Duration of Treatment

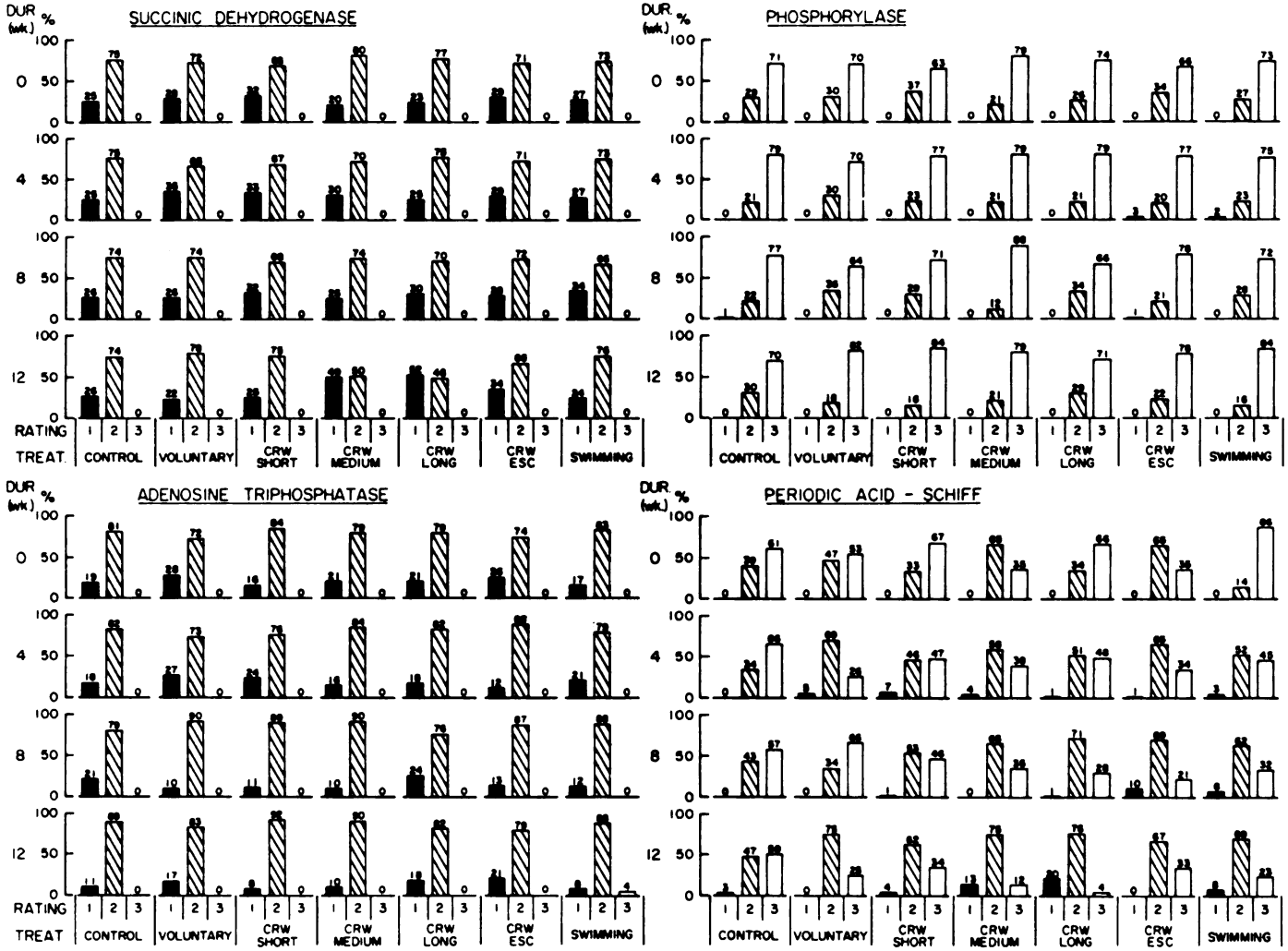

Figure E-3.-- Mean Per Cent Histochemical Fiber Ratings for SOLEUS by Treatment and Duration of Treatment