SPECIFIC CHANGES IN A HISTOCHEMICAL PROFILE OF RAT HINDLIMB MUSCLE INDUCED BY TWO EXERCISE REGIMENS

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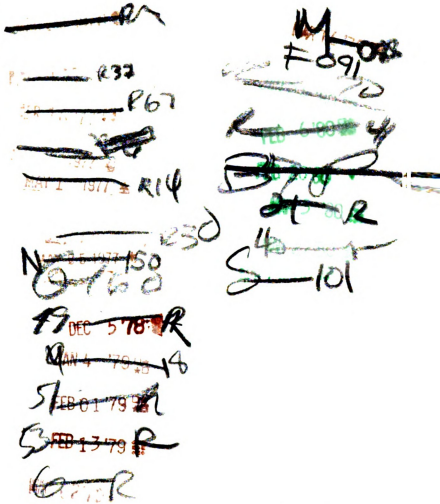

ABSTRACT

SPECIFIC CHANGES IN A HISTOCHEMICAL PROFILE
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By

Roland Richard Roy

This investigation was undertaken to determine the effects of eight weeks of sprint (SPT) or endurance (END) training on a histochemical profile of the various fiber types found in the hindlimbs of adult male albino rats (Sprague-Dawley strain). Two muscle areas were selected for study on the basis of homogeneity of fiber type: the central portion of the soleus which is composed primarily of slow-twitch oxidative (SO) fibers and the posterior part of the plantaris which consists mainly of fast-twitch glycolytic (FG) fibers with some fast-twitch oxidative glycolytic (FOG) fibers interspersed. Histochemical profiles were determined using the reactions of adenosine triphosphatase (ATPase 9.4) as an indicator of contractile speed, lactic dehydrogenase (LDH) to reflect lactate fermentation activity, succinic dehydrogenase (SDH) to indicate Krebs cycle activity, and Sudan Black B (SUD) and periodic acid-Schiff (PAS) to localize intracellular fat and glycogen respectively.

Roland Richard Roy

A histochemical photometer was used to obtain objective photometric evaluations in serial cross-sections for a group of 30 adjacent muscle fibers from each of the two muscle areas investigated. Chi-square analyses, within muscle areas for each stain, revealed significant ($P < .01$) differences between distributions in all treatment comparisons except that for SDH in the plantaris. In general, the exercise-induced metabolic adaptations were similar in the SO soleus and FG-FOG plantaris areas.

The SPT and END exercise regimens each produced a number of alterations in the histochemical profiles of the muscle cells. Both training regimens resulted in decreased staining intensities for ATPase 9.4 and increased reactivities to SDH staining. The SPT program specifically enhanced LDH and PAS staining reactions, whereas END training produced a large group of fibers staining darkly with SUD. In effect, the END training program resulted in an increased aerobic capacity of the muscle cells while the SPT program enhanced both their aerobic and anaerobic metabolic capacities.

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By

Roland Richard Roy

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DEDICATION

To my wife, Sharon, and our
two boys, Stephen and Michael.

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CHAPTER I

THE PROBLEM

Histochemical techniques are used to categorize skeletal muscle fibers according to various metabolic characteristics. In conjunction with biochemical, physiological and anatomical observations, histochemical profiles have helped to identify at least three major fiber type categories. Many systems of fiber-type classification have evolved, but the nomenclature of Peter et al. (235) of fast-twitch glycolytic (FG), fast-twitch oxidative glycolytic (FOG) and slow-twitch oxidative (SO) seems to be the most comprehensible and is supported in the current literature (39).

Single-cell characterization can only be accomplished with histochemical and morphological techniques. No biochemical or physiological method has been perfected for determining individual fiber profiles. Biochemists and muscle physiologists usually depend upon histochemical analyses for selection of skeletal muscles, or parts of muscles, which are fairly homogeneous in composition. Recently, fiber populations of several mammalian muscles have been categorized according to their percentage of FOG, FG and SO fibers (4,24,65,88,117,173,235,261,262).

Fiber types, even in adult animals, are not immutable. There is evidence to indicate that muscle cells undergo continual alteration

throughout life in adaptation to changing functional demands. For example, the metabolic profile of rat skeletal muscle can be modified in response to the functional overload induced by incapacitation of synergistic muscles (135,259), by inactivity (196,249), and by immobilization (31,89,247,248).

The nervous system plays a primary role in determining adaptive changes in the metabolic and physiological characteristics of skeletal muscle (59,131,134,281,296). Mutability of muscle fibers was first indicated in studies involving surgical alterations of the innervating nerves. Following denervation of mature fast and slow muscles, the enzymatic differences between muscle fibers gradually disappear. That is, the fibers lose their metabolic differentiation (18,38,74,151,177, 271). Twitch times of muscles composed mainly of FG and FOG fibers are considerably lengthened, while muscles formed mainly by SO fibers may show either a slight decrease in the speed of contraction or a slight increase (138,140,193). When these muscles are reinnervated with their own nerves, there is no metabolic dedifferentiation (64,74, 171,172,253). However, cross-innervation of fast and slow muscles results in a shift of the energy metabolism of the muscle fibers (38, 41,42,43,58,64,77,241,252,253).

Alterations of normal discharge patterns of the innervating nerve also produce significant changes. Fast muscles, electrically stimulated at normal rates of discharge for tonic fibers, become markedly slower in their contraction times (231,239,255), are more resistant to fatigue (231) and exhibit significant shifts in their enzyme activities (90,231,249).

Neural control of skeletal muscle differentiation seems to be regulated by the transport of specific neurotrophins from the neurons to the muscle via axoplasmic transport. Evidence for this transsynaptic transfer of specific neuroproteins has been accumulating (2,183,184).

Various regimens of physical training have produced specific changes in the fiber profiles of skeletal muscles. Prolonged programs of endurance exercise have resulted in significant increases in the aerobic metabolic capacity of all fiber types (155,158). Furthermore, increases in the percentages of SO and FOG fibers, especially in predominately FG muscles, have been reported (21,78,83,194,198,208). Exercise programs for laboratory animals that are solely dependent upon anaerobic metabolic processes are yet to be developed. However, studies utilizing training programs with relatively high anaerobic components have produced increases in anaerobic metabolic capacity and shifts in SO muscles toward higher FOG fiber populations (122,257,272). Isometric training has produced specific changes in enzyme activities and fiber type populations that are dependent upon the specifications of the training program used (96,97,159,185,209,270,297).

Statement of the Problem

In light of the evidence for mutability of muscle fibers, this study was undertaken to determine the effects of two very strenuous training programs on the histochemical profiles and distributions of various fiber types. Supplementary data were obtained on performance criteria, body weights, and muscle weights.

Research Plan

Normal adult male rats (Sprague-Dawley strain) were used as subjects. For each animal, a common area containing thirty adjacent fibers in each of the left soleus and plantaris muscles were studied. Several anatomical landmarks were used to help locate homologous areas in all tissue sections. The fibers selected were chosen as being typical of those in the central portion of the soleus and the medial posterior portion of the plantaris.

The two training regimens were modifications of Controlled-Running Wheel routines previously reported from this laboratory (286, see Appendix A). The modified programs, an endurance running routine (END) and a sprint running routine (SPT), represented attempts to stimulate selectively either aerobic or anaerobic metabolic processes in the experimental animals. At the termination of the study, the END animals were running continuously for one hour at the relatively slow speed of 36 m/min. The END program was expected to produce increases in aerobic metabolic capacity. The SPT program consisted of alternated work and rest periods. The animals ran at speeds of up to 108 m/min, but the work periods were limited to 15 sec. Anaerobic metabolic pathways were expected to be taxed by the SPT program. The exercise treatments were administered five days per week for eight weeks.

Histochemical profiles were determined using an adenosine triphosphatase (ATPase 9.4) reaction as an indicator of contractile speed. The lactate dehydrogenase (LDH) reaction was used to show lactate fermentation activity. The succinic dehydrogenase (SDH) reaction was

selected to indicate tricarboxylic acid cycle activity. Localization of fat and glycogen as substrates was demonstrated by the use of Sudan Black B (SUD) and periodic acid-Schiff (PAS) stains respectively.

Rationale

Current literature has indicated that exercise consists of a continuum of specific activities each of which elicits a specific response within the organism (29,99,125,126,127,128,202,242). The two training regimens used in this study were designed to provide functional overloads of the aerobic and anaerobic ends of this continuum.

The act of running in the rat involves plantar flexion of the foot. The soleus and plantaris muscles are both involved in plantar flexion and therefore were assumed to be highly active during the training programs. The muscle areas were selected for homogeneity of fiber-type populations. The soleus in the rat has been reported to contain 84% SO, 16% FOG, and 0% FG fibers (4). The central portion of the soleus has been shown to be predominately SO (78). The posterior part of the rat plantaris has been observed to contain mainly FG fibers with some FOG fibers interspersed (78).

It was postulated that the response of the different fiber types would be specific to the functional demands of the training programs. The selection of histochemical procedures was made to insure a reasonably inclusive fiber profile. Enzymes involved in aerobiosis (SDH) and anaerobiosis (LDH) reflect different metabolic pathways. ATPase

reaction indicates the contractile properties of the various fiber types. Substrate levels are indicated by PAS (glycogen) and SUD (fat).

Significance of the Problem

Metabolic fiber profiles have become valuable tools for assessing the functional state of individual muscle fibers. Specific adaptations in fiber metabolism have been shown to be induced by exercise and various surgical techniques and have been observed in numerous neuromuscular disorders. The study of exercise-related alterations, by fiber types, may provide insight into the mechanisms of these metabolic adaptations.

Limitations of the Study

1. The results of this study are restricted to the soleus and plantaris muscles of normal male albino rats.
2. The training programs used may not have stimulated purely aerobic or anaerobic metabolic processes.
3. Histochemical methods to evaluate precise quantitative enzyme concentrations in individual muscle fibers are not available at the present time.
4. The limited number of histochemical techniques that were used cannot be expected to provide a complete picture of all exercise-related metabolic adaptations.

5. A control for the shock stimulus used to motivate the animals to run was not included in the investigation. However, previous experience in this laboratory suggests that the stimulus has no effect on histochemical or morphological parameters in the plantar flexor muscles.
6. Three sessions for the sectioning and staining of tissues were conducted. A single session included all animals from one treatment group. Intersession variability in staining reactions may have accounted for some of the histochemical differences observed. This confounding factor may be important especially for the highly pH sensitive ATPase reaction.

CHAPTER II

REVIEW OF RELATED LITERATURE

Skeletal muscle fibers have been classified into three broad categories or fiber types according to their histochemical, morphological, physiological and biochemical characteristics (48,207,235,238, 280). These characteristics are not fixed; individual fibers are known to be dynamic with regard to fiber type (60,135). Various regimens of physical activity have produced marked changes in both metabolic and contractile profiles (122,156,158,208,233). The direction and extent of enzymatic adaptations have been dependent on the specifications of the training programs used.

To facilitate a discussion of fiber-type mutability, the following review of literature is divided into two main sections with several subdivisions. A general description of the three fiber types will be presented under the first main heading. Histochemical and morphologic characteristics of single muscle fibers, biochemical correlates in muscles of nearly homogeneous fiber type, and physiological data utilizing whole muscle and single motor unit preparations will be discussed. The second major part will deal with the adaptations of these fiber types to different exercise regimens. Histochemical and biochemical changes will be emphasized.

Fiber Types

Differences between individual muscle fibers can be seen best by histochemical and morphologic techniques. Biochemical methods are not available at present to establish single-fiber enzyme profiles, and physiological measures of contractile speed will differentiate only between fast-twitch and slow-twitch contractile elements.

Histochemical Characteristics and Differences

Enzyme histochemistry is a specialty that forms a connecting link between two methods of approaching the investigation of tissues: histology and biochemistry (171). When stained histochemically, individual muscle fibers show different degrees of coloration. These differences generally are thought to persist throughout the length of an individual fiber (66,72,92,98,273), but this hypothesis recently has been challenged (287). Serial cross-sections of muscle can be stained by different histochemical reactions to obtain metabolic profiles of individual fibers (93). It is important to note that relative degrees of fiber staining do not necessarily represent relative levels of enzyme activity (92). A histochemical reaction can only be indicative of the amount of accumulated end-product. Therefore, direct relative comparisons of histochemical staining intensities should be restricted to fibers in the same muscle of the same species (5,28,217,295).

In the adult mammal, skeletal muscle fibers may be differentiated by a variety of histochemical techniques. Combinations of these stains plus biochemical reactions, physiological properties, and morphologic

characteristics have prompted investigators to categorize fibers according to several schema (see Table 1). The descriptive taxonomy introduced by Peter et al. (235) of fast-twitch glycolytic (FG), fast-twitch oxidative glycolytic (FOG), and slow-twitch oxidative (SO) seems to have emerged as the most useful and has been adapted by a number of investigators. This classification system will be used throughout the current report.

Table 2 summarizes the information that is now available concerning relative histochemical staining intensities of the three fiber types. Clearly, substrate levels in FG fibers are characterized by a high glycogen content as reflected by the PAS stain and by a low lipid content as reflected by the SUD stain. Aerobic capacity is assumed to be low since stains for localizing the activity of oxidative enzymes such as SDH, malate dehydrogenase, and NADH-diaphorase have minimal intensities and the myoglobin content is low. Anaerobic capacity is thought to be high because of the maximal staining reactions of anaerobic enzymes such as M-lactate dehydrogenase, triosephosphate dehydrogenase, mitochondrial α-glycerophosphate dehydrogenase, and phosphorylase. The high myosin ATPase reaction at pH 9.4 confirms the fast-twitch characteristic of FG fibers.

The histochemical profile for FOG fibers is quite different. These fibers are high in glycogen and lipids, have high to moderate reactions for most aerobic and anaerobic enzyme stains, and are fast contracting.

SO fibers react strongly to most indicators of aerobic metabolism. These fibers exhibit the lowest glycogen content and only a moderate

Table 1. Classifications of Fiber Types Used by Histochemists

Classification System			References
Fast-twitch glycolytic (FG)	Fast-twitch oxidative glycolytic (FOG)	Slow-twitch oxidative (SO)	4,85,88,102,117,207,208, 209,235,237
II	II	I	35,36,37,38,72,73,94,98, 165,167,177,178,179,180, 181,223
I	II	III	135,251
α	αβ	β	133,287,294
White	Red	Intermediate	21,22,26,66,78,80,82,83, 100,101,110,112,114,116, 170,206,212,215,224,254, 258,266
A	C	B	95,149,256,273
α-White	α-Red	β-Red	5,6
Fast-twitch White	Fast-twitch Red	Slow-twitch Intermediate	24,104,233,280
Fast-twitch	Fast-twitch	Slow-twitch	10,120,123,124,125,126, 162,175
Low Oxidative Fast	High Oxidative Fast	High Oxidative Slow	198
White	Red	Medium	218

Table 2. Histochemical Metabolic Profile for the Three Fiber Types in Mammals

Metabolic Characteristics	Fiber Type: Fast Glycolytic (FG)	Fast Oxidative Glycolytic (FOG)	Slow Oxidative (SO)	References
Myoglobin Content	L[a]	H	H[b]	165,166,251,258
NADH-Diaphorase	L	H	I[c]	80,235
Glycogen Localization	I-H	H	L	116,214
Periodic acid-Schiff (PAS)	H	H	L	116,214,235
Phosphorylase	H	H	L	80,82,214,235,293
Hexokinase	L	I	H	233
Triosephosphate Dehydrogenase	H	L	L	215
Lactate Dehydrogenase (M) (M-LDH)	H	L-I	L	214,215,218,233, 234
Lactate Dehydrogenase (H) (H-LDH)	L	H	I	234,262,263,273
Mitochondrial α-Glycerophosphate Dehydrogenase	H	I-H	L	80,235,297
Succinic Dehydrogenase (SDH)	L	H	I	3,80,95,143,144, 179,180,212,215, 218,258,273,284, 293,297
Malate Dehydrogenase	L	H	I	80,82,218,235
Lipid Localization	L	H	I-H	109,143
Sudan Black B (SUD)	L	H	I	109,143,262
Myosin Adenosine Triphosphatase	H	H	L	78,84,132,133, 179,293
Mitochondrial Adenosine Triphosphatase	L	H	I	116,180
Myofibrillar Adenosine Triphosphatase (ATPase) at pH 9.4	H	L	H	80,91,95,253,273
pH sensitivity of myofibrillar ATPase	acid labile alkali stable	acid labile alkali stable	acid stable alkali labile	37,132,256,296
Formaldehyde sensitivity of myofibrillar ATPase	sensitive	stable		132,273
Creatine Phosphokinase	H	H	H	179,180

[a] L indicates a low staining reaction.

[b] H indicates a high staining reaction.

[c] I indicates a moderate staining reaction.

amount of lipid material. High myoglobin content and moderate to high reactions for oxidative enzymes are indicated. Stains for anaerobic enzyme activity are light. The reaction with myosin ATPase at pH 9.4 is low and reflects the slow-twitch characteristic of these fibers.

Morphological Characteristics and Differences

Morphological differences in skeletal muscle fiber types are found at both the gross and ultrastructural levels. Qualitative and quantitative disparities in cellular content and in the distribution and form of constituent organelles and inclusions are clearly evident. In addition, surrounding and associated tissues are quite variable among fiber types.

Mitochondria.--One of the primary differences among fiber types is found in the number, form and distribution of mitochondria. All fiber types have mitochondria arranged in pairs opposite the I bands (112,219). However, the FOG fibers contain many large, interfibrillar mitochondria that are arranged in rows. These spherically-shaped organelles contain dense matrices with closely packed cristae (110,111, 112,224,226,280). Subsarcolemmal and perinuclear aggregations of mitochondria are typical of the FOG fiber (112,114,280).

Sparsity and smallness of mitochondria distinguish the FG fiber type from other types. Interfibrillar mitochondria are scarce and interfibrillar rows are absent. A few mitochondria may occupy perinuclear regions. Subsarcolemmal organelles usually occur individually. Paired mitochondria at the I bands are present, but they are smaller and have fewer cristae and less dense matrices than do those of the FOG

fibers (112,224).

Compared to those in FOG fibers, the mitochondria within the SO fiber are fewer, more pleomorphic and have less opaque matrices (280). Subsarcolemmal and perinuclear chains are present, but they are shorter and less conspicuous than in FOG fibers (219,265).

Lipid and Glycogen Inclusions.--Lipids are numerous in both FOG and SO fibers but extremely rare in FG muscle cells (280). A direct relationship seems to exist between mitochondrial density and triglyceride droplets (109).

Abundant glycogen permeates the sarcoplasm of all fiber types but is most prominent in the I band region of FG fibers (280). This observation may be related to the phasic nature of FG fibers.

Myofibrils.--FOG fibers generally have the smallest cross-sectional dimensions, and FG fibers have the largest. SO fibers are intermediate in size (68).

The M line is more prominent in FOG and FG fibers than it is in SO fibers (280).

The width of the Z line, measured at comparable sarcomere lengths, usually is reported to be greatest in SO fibers and smallest in FG fibers (112,209,254,280). Indications are that wide Z lines may be associated with tonic muscle contractions (280). However, recent work has determined that Z-line width is highly variable and may differ in the same fiber types of separate muscles of the same species (114,115) and in the same muscle between species (113). The significance of this finding is not yet clear.

Sarcoplasmic Reticulum and Transverse System.--An extensive reticular network pervades the FG fiber. This network consists primarily of longitudinal components at the A band, but it has numerous broad expansions and transversely or obliquely oriented components at the I band (280). A compact arrangement of broad parallel tubules is present at the H band (112).

The reticulum in the FOG fiber consists of a plexus or fenestrated collar in the A band region between successive T tubules and a less extensive component at the I band (280). An elaborate network of narrow tubules is present at the H band.

The sarcoplasmic reticulum of the SO fiber is less extensive than that of either the FOG or FG fiber (30,280). The observation that fast-twitch fibers (FOG and FG) have a more extensive sarcoplasmic reticulum correlates well with their physiological characteristics (103).

Neuromuscular Junction.--Obvious morphological differences in neuromuscular junctions exist between the three fiber types. The FG nerve terminal is the largest and is characterized by many long thin branches which are relatively straight and have numerous small pearl-shaped swellings along their course (182,285). The profile of junctional folds reveals increasing complexity as the folds extend towards the sarcoplasm (210,227). The site of contact has a large surface area (111) with deep wide folds (210).

The small FOG nerve terminal possesses only a few short thick branches with more elongated swellings (285). The junctional folds have a relatively small number of branches which are shallow and flat (210).

The SO terminal possesses intermediate characteristics in terms of the number and size of terminal branches and swellings (285) and the size and form of junctional folds (210,227).

Capillarity.--Tomanek et al. (280) recently found higher capillary to fiber ratios for SO and FOG fibers than for FG fibers in guinea pig soleus and vastus lateralis muscles. Other investigators have reported no differences in capillarity between fiber types (130,195). Due to the differences in staining and counting techniques used, the current results on capillary to fiber ratios are inconclusive. There is general agreement, however, that capillarization is directly related to the oxidative metabolism of the muscle fiber (54,130,160,161,195,213, 244,252,253,280).

Biochemical Characteristics and Differences

Biochemical assays have been used to substantiate some of the inferences drawn from histochemical staining reactions. Since no biochemical technique has been devised to determine enzyme profiles in single muscle fibers, biochemists have used whole muscles or portions of muscles which have been identified histochemically as being relatively homogeneous (see Table 3). Table 4 summarizes some of the literature dealing with biochemical determinations in homogenates of predominately one fiber type.

Fast-twitch glycolytic fibers are dependent chiefly upon anaerobic carbohydrate metabolism. These fibers have a high glycogen content and exhibit high levels of glycogenolytic (phosphorylase), glycolytic (phosphofructokinase, pyruvate kinase, glyceraldehyde 3-phosphate

Table 3. Representative Values for Fiber Type Composition of Several Muscles Commonly Used in Histochemical, Biochemical and Physiological Investigations

Muscle	Species	Fiber Types FOG	FG	SO	Reference
Gastrocnemius	rat	37	58	5	4
Superficial Vastus	rat	0	100	0	11
Deep Vastus	rat	70	0	30	11
Soleus	rat	16	0	84	4
Extensor Digitorum Longus	rat	59	38	3	4
Plantaris	rat	53	41	6	4
Tibialis Anterior	rat	66	32	2	4
Biceps Brachii	rat				
central		61	23	16	297
peripheral		29	51	20	297
Rectus Femoris	rat	54	42	4	4
Lateral Gastrocnemius	guinea pig	32	56	12	4
Medial Gastrocnemius	guinea pig	50	38	12	24
Red Vastus	guinea pig	78	18	4	4
White Vastus	guinea pig	23	77	0	4
Soleus	guinea pig	0	0	100	4
Semimembranosus	lesser bushbaby	33	66	1	4
Vastus Lateralis	lesser bushbaby	13	87	0	4
Plantaris	lesser bushbaby	30	51	19	4
Soleus	lesser bushbaby	13	0	87	4
Tibialis Anterior	lesser bushbaby	45	43	12	4

Table 4. Enzyme Activity Levels and Substrate Concentrations Determined Biochemically in Muscle Homogenates of Predominately One Fiber Type

Metabolic Characteristics	Fiber Type: Fast Glycolytic (FG)	Fast Oxidative Glycolytic (FOG)	Slow Oxidative (SO)	References
Myoglobin Content	L[a]	H[b]	H	235
Cytochrome a	L	H	I[c]	11,235
Cytochrome c	L	H	L-I	11,235
Glycogen Content	I-H	H	L	14,24,235,245
Glycogen Synthetase	L	H		168
Phosphorylase	I-H	H	L	13,25,62,235,257
Hexokinase	L	I-H	H	25,62,232,235
Phosphofructokinase	H	I	L	62,235,257
Triosephosphate dehydrogenase	H	L		25
Glyceraldehyde 3-phosphate dehydrogenase	H	I	L	235
Pyruvate kinase	H	I	L	235,257
Lactate dehydrogenase	H	I	L	25,62,235
α-Glycerophosphate dehydrogenase	H	I-H	L	25,62,235
Citrate Synthase	L	H	I	11,25
Succinic dehydrogenase	L	H	I	235,257
Total Lipid Content	L	I	H	103
Triglyceride Content	L	H	L-I	14,245
Carnitine Palmityltransferase	L	H	I-H	11,12
3-Hydroxyacyl CoA	L	H		25
3-Hydroxybutyrate dehydrogenase	L	I	H	290
3-Ketoacid CoA-transferase	L	I	H	290
Acetoacetyl-CoA thiolase	L	I	H	290
Lipoprotein lipase	L	I	H	32
Palmitate Oxidation	L	H	I	11
Pyruvate Oxidation	L	H	I	11
Myosin adenosine triphosphatase	H	H	L	235,277

[a] L indicates a low enzyme activity.

[b] H indicates a high enzyme activity.

[c] I indicates a moderate enzyme activity.

dehydrogenase and triosephosphate dehydrogenase), and lactate fermentation (lactate dehydrogenase) enzyme activities. High values of α-glycerophosphate dehydrogenase activity suggest an important role for the α-glycerophosphate shuttle system in the regeneration of NAD for glycolysis. Aerobic capacity is limited as is shown by low succinate dehydrogenase, citrate synthase, and cytochrome activities as well as low myoglobin content. Fat metabolism is relatively unimportant in these fibers. Low total lipid and triglyceride contents and low levels of activity of β-oxidation enzymes (β-3-hydroxyacyl CoA and carnitine palmityl transferase) are found. Low lipoprotein lipase levels also suggest little dependence on exogeneous fat stores. Fast-twitch contractile characteristics are indicated by high levels of myosin adenosine triphosphatase (myosin ATPase).

An opposite pattern of enzyme activities is found in the slow-twitch oxidative fibers. The fact that SO fibers are slow contracting is shown by the low levels of myosin ATPase activity. Metabolically, these fibers appear to rely predominately on aerobic mechanisms. Total lipid content is high in these fibers, but it should be noted that triglyceride levels are relatively low. Intermediate to high activities of the enzymes of fatty acid oxidation and high levels of lipoprotein lipase activity indicate a heavy reliance on fat metabolism. High β oxidation levels substantiate this observation. Cytochrome and myoglobin levels are high as are the activities of the citric acid cycle enzymes. Glycogenolytic, lactate fermentation, and glycolytic enzyme activities are minimal and glycogen content is low. As expected,

hexokinase activity is an exception since it seems to vary directly with respiratory capacity (25,62).

Fast-twitch oxidative glycolytic fibers appear to have the highest capacity for aerobic metabolism. Succinic dehydrogenase and citrate synthase activities as well as cytochrome levels and myoglobin concentrations are greatest in these fibers. Moderate total lipid and high triglyceride concentrations indicate a capacity for fat storage, and high activities of lipoprotein lipase and the fatty acid oxidation enzymes reflect high rates of fat metabolism. FOG fibers also are characterized by a moderate to high anaerobic capacity. They have the highest glycogen concentration with moderate activity levels of the glycolytic enzymes. Phosphorylase activity is high and α-glycerophosphate dehydrogenase and lactate dehydrogenase activities are moderate. In summary, these fibers have adequate capacity for glycogenolysis, glycolysis and oxidative phosphorylation with a fast speed of contraction.

Physiological Characteristics and Differences

Histochemical and biochemical studies suggest the existence of marked differences in contractile characteristics between fiber types. These differences have considerable physiological importance.

Fast- and Slow-twitch Characteristics.--It is now well-established that the histogenesis of striated muscle in mammals leads to the formation of limb buds which at first are uniformly slow contracting (60,68, 207). Further differentiation into fast and slow muscles occurs later, but the developmental changes differ between muscles within the same

animal and in corresponding muscles between different species (40,56, 139,176). Differentiation in the rat appears to be brought about by a relative shortening of contraction time in potential fast muscles (e.g., extensor digitorum longus), there being little or no change in eventual slow muscles (e.g., soleus) (60). However, histochemical findings in the soleus muscles of the guinea pig, rabbit and cat reveal a mixed fiber pattern with a predominance of fibers having high ATPase activity (FOG and FG fibers) at birth, and many fibers having low ATPase activity (SO fibers) in adult animals (139,176,217). This slowing of contraction time in the soleus follows a different time course in each species and appears to be dependent upon the level of maturation at birth (139).

Biochemical studies have shown that there are proportional changes in the intrinsic speed of contraction and the myosin ATPase level during ontogenetic differentiation of vertebrate fast and slow muscles (60). Barany and Close (17) and Barany (16) reported that specific activity of myosin ATPase is correlated with contraction time in adult muscle, and Guth and Samaha (132) demonstrated that actomyosin ATPase measured biochemically is correlated with the histochemical myofibrillar ATPase at pH 9.4. These observations have been substantiated by other investigators (24,88). Using the myosin ATPase reaction at pH 9.4, it has been shown that the SO fibers are slow contracting while both the FOG and FG fibers have fast contraction times (24,80,84,132, 293).

Motor Unit Characteristics.--The contractile elements of skeletal muscle are organized into functional entities called "motor units". A motor unit consists of a group of muscle fibers and the single motoneurone innervating them (76,264). Each motor unit appears to be homogeneous with regard to muscle fiber type (33,48,90,149,199,292), and the fibers are scattered and intermingled with fibers of other motor units (33,45,90). The dynamic properties of motor units found in "slow" and "fast" muscles are quite different (57,60). There is evidence from animal studies that the size of the motor unit and its contractile properties are related in some way to the diameter of the innervating motor axon (1,148,149,199,292). However, this is not always the case (276,292). Alpha motor neurons have been divided into slow (S) and fast (F) types on the basis of distinctive twitch properties of the muscle fibers they innervate (199,292), but these neurons are indistinguishable in terms of their histochemical profiles since all are high in phosphorylase and low in SDH (50,51).

Direct investigation of the histochemical, morphologic and physiologic characteristics of mammalian muscle fibers has become possible using a variety of techniques that are based on the classical work of Kugelberg and Edstrom (90,187). These investigators developed a technique for the histochemical mapping of the muscle fibers belonging to a single motor unit using depletion of fiber glycogen following repetitive electrical stimulation. This technique permits the identification of stimulated fibers in PAS-stained sections as being unstained fibers outlined against the stained fibers of surrounding unstimulated

motor units. The process does not affect the staining properties of the stimulated fibers with other histochemical reactions and thus allows for fiber typing with serial sections.

Burke et al. (44,47), using a modification of this technique, have presented evidence which suggests that motor units of the medial and lateral heads of the gastrocnemius of the cat may be classified into three nonoverlapping groups. These motor-unit groups are based on fatigue characteristics and contractile speed. The three groups are as follows: type FR, fast contracting and fatigue resistant; type FF, fast contracting and fast fatiguing; and type S, slow contracting and fatigue resistant. It seems to be a reasonable extension of the existing histochemical information to assume that the FF, FR, and S motor units contain FG, FOG and SO muscle fibers respectively.

The histochemical data presented by Kugelberg (188,189) on rat hindlimb substantiates the ability to categorize motor units into three groups corresponding to muscle fiber types. In the anterior tibial muscle, Kugelberg (189, p. 9) identified a Type I motor unit that corresponds to the S group of Burke et al. (44), a Type IIA motor unit that corresponds to the FF group, and Types IIB and IIC that together correspond to the FR group. A similar histochemical profile in the soleus of the rat revealed motor units of only Type I or the S group and Types IIB and IIC or the FR group (188).

It is of interest to note that indirect estimates of the percentage of motor units belonging to each group in the medial gastrocnemius of the cat have been reported by Burke and Tsairis (45) to be:

55% FF, 20% FR, and 25% S. The values compare favorably with those for the muscle fiber population that were determined histochemically by Ariano et al. (4): 61% FG, 14% FOG, and 25% SO. Other data support these findings (49,201,275).

It has been known for a long time that slow muscles are employed in slow contractions and in the maintenance of posture, whereas fast muscles are used primarily in quick phasic movements (68). This principle should hold true for slow and fast motor units within any given muscle (47,220). In a recent study, Stephens and Stuart (275) observed the recruitment of motor units in the cat medial gastrocnemius in response to different intensities of electrical stimulation. At low contraction strengths, motor units which were largely fatigue resistant were stimulated; at high contraction strengths, motor units which were fast contracting and less fatigue resistant were recruited. The functional interpretation and importance of this dual role was emphasized. The medial gastrocnemius is a muscle which participates in a broad range of activities. Fatigue-resistant units (of the S and maybe FR groups) could be well-adapted to maintain long sustained contractions as needed in standing. Rapidly contracting units (of the FF and FR groups) may be required for phasic activities such as jumping and running. Animal exercise studies of motor unit recruitment have supported the concept of task-specific recruitment patterns (86,87,117, 198).

Fast- and slow-twitch units have been demonstrated by stimulating single motoneurones in man (267). Milner-Brown and co-workers (201,274)

provided direct evidence that human motor units of the first dorsal interosseous muscle of the hand are recruited during increasing voluntary contraction in an orderly fashion. They also observed that the number of additional motor units recruited for a given increment in force declines sharply at high levels of voluntary force. This suggests that even though the high threshold units generate more tension, the contribution of recruitment to increases in voluntary force declines at higher force levels.

Metabolic Adaptations to Physical Training

The fact that muscle fiber types are mutable was first established in studies involving surgical alterations of motor nerves. Denervation (18,38,74,151,177,186,271), reinnervation (64,74,171,172,253), and cross-innervation (38,41,42,43,58,64,77,252,253) all have been shown to produce marked metabolic changes in muscle fibers. The obvious conclusion is that muscle fiber type is under neural control (131,281). This concept has been supported by direct stimulation of intact motor nerves (90,231,239,249,255). Evidence is accumulating that axoplasmic flow may be a regulating factor (2,183,184).

Regardless of the nature of the control mechanism(s), alterations of nerve discharge patterns clearly produce significant changes in the metabolic characteristics of muscle fibers. It might be expected, therefore, that noninvasive physiological conditions which affect nerve function would produce similar changes in muscle fibers. Inactivity (196,249) and immobilization (31,89,248) are two such conditions that

have been shown to modify the metabolic profile of rat skeletal muscle. The effects of different regimens of exercise on fiber type have been studied in some detail (see Table 5).

Endurance Training--Biochemical Alterations

Prolonged programs of endurance training, performed regularly, have resulted in significant increases in the aerobic capacity of all fiber types (156,158).

Myoglobin and Cytochrome Levels.--Myoglobin, which stores oxygen and enhances its rate of diffusion through the cell wall, has been shown to be increased by endurance exercise in mixed muscles of the rat (191,228) and in the FG portion of the vastus lateralis muscle of the lesser bushbaby (85). This rise may account for a portion of the increase in maximal oxygen uptake that occurs in response to prolonged endurance training (158).

Cytochrome a (cytochrome oxidase) and cytochrome c (ferrocytochrome c-oxygen oxidoreductase) activities are elevated in endurance-trained rats (8,11,29,70,152,154,204,221,290), guinea pigs (23), and lesser bushbabies (85). All three fiber types are equally involved. The magnitude of the changes found in the rats was greatest, but this may be due to interspecies variations and/or differences in training programs.

The question of the significance of the elevated cytochrome levels has been debated (23,106,236). After a 12-week treadmill program of running, untrained and trained guinea pigs were run to exhaustion in a single bout of exercise (23,236). Performances were not

Table 5. Comparison of Animal Training Programs Used by Various Investigators

Principal Investigators	Reference	Species	Initial Wt and/or Age	Mode of Exercise	Velocity m/min	Length of Daily Exercise Program	Duration of Program	Specifications
Holloszy	152	rat	6 wks	Motorized	22	120	12 wks	Initially ran two 10-min
Baldwin et al.	13		90-110 gms	treadmill	31		5 days/wk	bouts at 22 m/min; pro-
Molé et al.	205			8° incline	42			gressively increased up
Winder et al.	289							to 120 min at 31 m/min
Baldwin et al.	11							with 12 sprints at 42
Molé et al.	204							m/min each lasting 30 to
Oscai & Holloszy	221							60 sec interspersed at
Holloszy & Oscai	153							10-min intervals
Molé & Holloszy	203							
Holloszy et al.	154							
Pattengale & Holloszy	228							
Borensztajn et al.	32							
Holloszy et al.	297							
Dohm et al.	69							
Winder et al.	291							
Baldwin et al.	15			15° incline			18-24 wks	
Winder et al.	290							
Dohm et al.	70							Only one bout for the first 6 wks
Dohm et al.	71						9 wks	Omitted last 3 wks
Muller	208	rat	6 wks	Motor driven treadmill 15° incline	36	120	12 wks 6 days/wk	Progressive in nature; similar to reference 152
Askew et al.	9	rat	110 gms	Motor driven treadmill 8° incline	29.5 56.5	120	12 wks 5 days/wk	Initially ran one 10-min bout at 29.5 m/min; progressively increased to 120 min at 29.5 m/min with 60 sec sprints at 56.5 m/min every 10 min
Askew et al.	8							
Askew et al.	7	rat	5 wks 110 gms	Motor driven treadmill 8% grade	29.5	120	7 wks 5 days/wk	Progressive in nature

continued

Table 5--continued

Principal Investigators	Reference	Species	Initial Wt and/or Age	Mode of Exercise	Velocity m/min	Length of Daily Exercise Program	Duration of Program	Specifications
Froberg et al.	108	rat	320 gms	Motor driven treadmill	37	80	15 wks	Training intensity gradually increased
Lawrie	191	rat	120 days	Mechanical driven rotary drum	36	120	8 wks	
Gollnick & Innuzzo	121	rat	160-250 gms	Motor driven works wheel	37.5	50	7-10 wks 5 days/wk	Intensity progressively increased to reach maximum capacity of rats
Short et al.	266	rat	150-200 gms	Motor driven rotary drum	13.7	240	7-8 wks 6 days/wk	5 min rest during each 30-min period, progressive in nature
Edgerton et al.	85	lesser bushbaby	250 kgs	Motor driven treadmill 4° grade	43	60 120	6 mo	Learning variability; 3 animals ran 120 min for two 60-min sessions/day
Jeffress et al. Peter et al.	168 232	guinea pig	600 gms 424 gms	Motor driven treadmill	32	30	alternate days for 21 days	
Campbell et al.	52	pig	--	Motor driven treadmill	40	120	2 wks	Two 1-hour periods/day
Barnard et al.	21	guinea pig	500 gms	Motor driven treadmill 0-2° grade	27.5 40 49.3	25	21 wks	Progressive in nature up to 9 weeks; warm-up at 27.5 m/min; continuous runs at 40 m/min; alternate days of continuous running and sprint running plus continuous running; complex program

continued

Table 5--continued

Principal Investigators	Reference	Species	Initial Wt and/or Age	Mode of Exercise	Velocity m/min	Length of Daily Exercise Program	Duration of Program	Specifications
Barnard & Peter Peter & Barnard	23 236	guinea pig	500 gms	Motor driven treadmill 0° grade	27.5 36.8 60	50	12 wks 5 days/wk	Similar to Barnard et al. (21); slightly higher intensity and longer duration; complex program
Faulkner et al. Lieberman et al. Faulkner et al. Maxwell et al.	100 194 101 198	guinea pig	300 gms, 6 wks 6,14,40 wks 6 wks 6 wks	Motor driven treadmill	30	60 30	8 wks	Progressive in nature
Bagby et al.	10	rat	175-200 gms	Motor driven wheels	28.4 80.4	60 18	11 wks 5 days/wk	Progressive in nature; sprint groups alternated 30-sec run; 30-sec rest for 18 bouts
Fitts et al. Fitts et al.	104 105	miniature pig	8 wks 5 kgs	Motor driven treadmill 5-10% grade	95 198	60 10	7 mos 5 days/wk	Progressive in nature; sprint group ran 10 to 60-sec sprints at 198 m/min and 10% slope; endurance group ran at 95 m/min for 60 min at 5% slope
Huston et al.	163	rat	100 gms	Motor driven treadmill 8° grade	29.5 53.6	120	12 wks 5 days/wk	Initially ran one 10-min bout at 29.5 m/min; progressively increased up to 120 min at 29.5 m/min for 30 sec every 10 min
Saubert et al.	257	rat	175-200 gms	Motor driven treadmill	16.1 80.5	18	11 wks 4 days/wk	3-min warm up; 30-sec sprints; 30-sec rest; initially 5 sprints at 16.1 m/min; progressively up to 18 sprints at 80.5 m/min
Fitts et al.	106	rat	6 kgs	Motor driven treadmill 15% grade	32.2	10 30 60 120	13 wks 5 days/wk	Progressive in nature; final program length is variable

continued

Table 5--continued

Principal Investigators	Reference	Species	Initial Wt and/or Age	Mode of Exercise	Velocity m/min	Length of Daily Exercise Program	Duration of Program	Specifications
Staudte et al.	272	rat	46 days 135 gms	Motor driven treadmill 30° incline	80	3	21 consecutive days	Four 45-sec bouts with at least 60 min rest between each bout; incline and speed progressively increased
Kowalski et al.	185	rat	17 wks	Voluntary wheel; "weight lifting"	--	240-360	6 wks	Weight lifters climbed a vertical ascent of 40.6 cm 50 times daily with up to 75% body wt attached
Spurway and Young	270	mice	"young"	Voluntary wheel; weight lifting	--	--	40-60 days	Weight lifters must reach up to obtain food and water; no additional load
Exner et al. Exner et al.	96 97	rat	53 days	isometric chamber 60° incline	--	< 5-min exhaustion	25-35 consecutive days	Training consisted of 3 sessions twice daily 12 hr apart; load individualized to maintain~5 min isometric work
Howells and Goldspink	159	hamster	4,9,14,60 wks	weight-lifting	--	--	5 wks	Weightlifting consisted of pulling down a counterweighted basket to obtain food
Muller	209	rat	6 wks	isometric chamber 60° incline	--	--	4 wks 6 days/wk	Training consisted of 3 sessions twice daily 12 hr apart; load individualized to maintain~5 min isometric work
Zika et al.	297	rat	15 days	tonic stress on ladders	--	240	4-6 mos 5 days/wk	

continued

Table 5--continued

Principal Investigators	Reference	Species	Initial Wt and/or Age	Mode of Exercise	Velocity m/min	Length of Daily Exercise Program	Duration of Program	Specifications
Gollnick *et al.*	119	rat	244 gms	swimming	--	60	35 consecutive days	Initially 30 min; add 5 min each day to reach 60 min
Gollnick and Hearn Hearn and Gollnick	118 147	rat	340 gms	swimming	--	30	35 consecutive days	
Syrovy *et al.*	277	rat	14 days 105 days	swimming	--	120	alternate days for 8-9 wks	5% body weight attached
Gould and Rawlinson Rawlinson and Gould	129 243	rat	8,11 and 15 wks 8 wks	swimming	--	30	6 wks 5 days/wk	
Hearn and Wainio Hearn and Wainio	145 146	rat	250 gms	swimming	--	30	5-8 wks	
Wilkerson and Evonuk	288	rat	367 gms	swimming	--	30 min exhaustion	alternate days 10 wks alternate days 6-10 wks	Exhaustion groups had 5% body weight attached
Edgerton *et al.* Edgerton *et al.*	78 81	rat	100 days	swimming	--	30 60	52 days 6 days/wk	Sedentary forced had one 30-min swim with 3% body wt attached; voluntary forced had two 30-min swims with 4% body wt attached plus access to revolving drum at will

different for the two groups. Yet, the trained animals had much higher levels of the cytochromes. The correlation coefficient between cytochrome c activity and running time to exhaustion was a low 0.37. These data indicate that cytochrome levels are not good indicators of aerobic capacity. However, Fitts et al. (106) challenged this position. Rats run on a standard endurance treadmill program demonstrated significant correlations between cytochrome c, citrate synthase and respiratory capacity in the gastrocnemius muscle and the duration of a run to exhaustion. Differences in training procedures seem to be the cause of this discrepancy.

Glycogenolytic and Related Enzymes.--In an early study small increases in phosphorylase activity were demonstrated in the biceps region of the hindlegs of rats swum for 15 weeks (129). Subsequent studies have verified that changes in phosphorylase levels do take place. Huston et al. (163) reported an increased activity in phosphorylase in gastrocnemius homogenates of trained rats. Baldwin et al. (13) found an increase in activity in the predominately SO soleus muscles of rats trained on a treadmill program. A decrease in the FOG deep quadriceps and no change in the FG superficial quadriceps also were reported. However, Edgerton et al. (85) trained lesser bushbabies on a treadmill for six months and found no change in phosphorylase activity in the mixed semimembranosus or the FG vastus lateralis. It appears that phosphorylase activity may be affected mainly in the SO fibers which have the lowest initial values.

Increases in LDH activity in the biceps region of the hindlimbs of rats (243) and increases in aldolase activity in the gastrocnemius muscles of rats (146), swum from 5 to 15 weeks, were reported in early studies. These findings were surprising in view of the very light stress that a swimming program imposes on animals. Gollnick et al. (118,119) refuted these findings when they found no change in the LDH activity of the gastrocnemius in rats swum for seven weeks.

With prolonged running of rats on a treadmill, Baldwin et al. (13) and Holloszy et al. (155) found changes in the glycolytic enzymes that are fiber-type specific. Hexokinase activity increased greatly in FOG muscle (deep red quadriceps), less in SO muscle (soleus), and slightly in FG muscle (superficial white quadriceps). This enzyme was unique in that it was the only glycolytic enzyme to increase in all three fiber types. However, the finding was expected since hexokinase activity tends to vary directly with respiratory capacity (25,62). Increases in hexokinase activity were found in whole gastrocnemius homogenates of rats trained on a treadmill (163). Similar increases were reported in the red (FOG) and white (FG) portions of the vastus lateralis muscles of guinea pigs run on a treadmill (20,190,232). The changes were of the same magnitude in both parts of the vastus muscle.

Phosphofructokinase, pyruvate kinase, and LDH all increased from 18 to 35% in the soleus and decreased approximately 20% in the red quadriceps of rats trained by endurance running on a treadmill (13). The only change in the white quadriceps was a 15% decrease in LDH. Molé et al. (205), using the same training program, reported no

physiologically significant shifts of LDH isozyme patterns in the various fiber types. Edgerton et al. (85) also found no change in LDH activity in either the FG vastus lateralis or the mixed semimembranosus after six months of training. However, their results were from the lesser bushbaby and species specificity needs to be investigated.

The glycerol phosphate shuttle is involved in unidirectional transport of reducing equivalents into the mitochondria of muscle cells. NAD-linked (cytoplasmic) and FP-linked (mitochondrial) α-glycerophosphate dehydrogenases catalyze the first step of the reaction on either side of the mitochondrial membrane. This shuttle system is extremely important in the regeneration of NAD for glycolysis during anaerobic metabolism. The findings from exercise studies are inconclusive regarding the alterations that may occur in NAD-linked α-GPD activity. Baldwin et al. (13) reported a significant increase in the SO soleus, a significant decrease in the FOG red quadriceps and no change in the FG white quadriceps of the treadmill-trained rat. These results are consistent with the changes reported in glycolytic enzymes and phosphorylase (13,155). However, other studies utilizing chronic activity of low (85,153) and high (272) intensity have shown no α-GPD effect in a variety of muscles and species (rat gastrocnemius and rectus femoris, and lesser bushbaby vastus lateralis and semimembranosus).

Tricarboxylic Acid (TCA) Cycle Enzymes.--Early work by Hearn and Wainio (145,146) indicated that changes in TCA intermediates might accompany endurance training. Moderate swimming programs resulted in increases in aldolase (146) and SDH (145) activities in the rat

gastrocnemius. Similarly, a moderate program of treadmill running increased SDH activity 30% in the rat gastrocnemius (121).

More recent work has supported these results. The activity of citrate synthase, which catalyzes the primary rate-limiting step of the TCA cycle (192, p. 453), has been shown to increase two-fold in all types of muscle with prolonged endurance training (11,70,154,289,290). Other TCA cycle enzymes including NAD-linked mitochondrial isocitrate dehydrogenase (70,154,203), aconitase (157), and succinic dehydrogenase (70,152,154) have shown parallel two-fold increases in activity. Smaller significant rises have been reported for α-ketoglutarate dehydrogenase (154) and malate dehydrogenase (70,154,205). The only exception to increased TCA cycle enzyme activity with endurance training was reported by Edgerton et al. (85) who found no SDH change in the SO soleus of the lesser bushbaby. However, there was a 20% increase in the FG part of the vastus lateralis muscle.

A recent study by Benzi et al. (29) has given support to the concept of specificity of training effects. SDH, cytochrome c and cytochrome oxidase activity levels changed in relation to the daily workload and the total training time. More work in this area is needed.

ATPase and Enzymes of Oxidative Phosphorylation.--The immediate source of energy required for muscular contraction is derived from the hydrolysis of ATP to ADP, a process that is catalyzed by adenosine triphosphatase. ATP stores are limited and must be replenished constantly by oxidative phosphorylation in the presence of oxygen. Under anaerobic conditions, phosphocreatine becomes a primary source of the high-energy

phosphate needed for ATP resynthesis. ATP-creatine transphosphorylase, now commonly known as creatine phosphokinase or creatine kinase, catalyzes the transfer of high-energy phosphate to ADP to form ATP. A secondary source of ATP regeneration is the myokinase reaction in which adenylate kinase catalyzes the transfer of high-energy phosphate from one molecule of ADP to another to form ATP plus AMP. Combined with glycolysis, these two reactions supply the needed ATP during anaerobic muscular contraction.

Oscai and Holloszy (221) have shown that endurance training specifically increases oxidative phosphorylation without affecting the anaerobic ATP regenerating systems. Mitochondrial ATPase activity, used as a measure of mitochondrial coupling factor 1 (F_1), increased two-fold in gastrocnemius muscle homogenates of endurance-trained rats. At the same time, the levels of mitochondrial and cytoplasmic adenylate kinase and creatine phosphokinase were unchanged.

Myosin ATPase activity levels have been shown to be correlated with speed of muscle contraction (16,17), and the specific activity of myosin ATPase in "white" muscle was found to be two to three times greater than in "red" muscle (16). Several groups of investigators have studied the effects of prolonged endurance exercise on myosin ATPase activity with conflicting results. Early studies utilizing moderate swimming programs showed little or no change in homogenates of rat gastrocnemius muscle (147,243). Similar results were reported by Bagby et al. (10) for rats trained 11 weeks on a treadmill. Syrovy et al. (277) observed an increase in myosin ATPase activity in the

soleus of young rats swum for several weeks, but no changes in adult soleus or extensor digitorum longus muscles. Wilkerson and Evonuk (288) used mild and exhaustive programs of swimming for either 6 or 10 weeks. The rats trained for both durations of the exhaustive program demonstrated increased specific activities of myosin ATPase in gastrocnemius homogenates.

Recently, Baldwin et al. (15) investigated the adaptation of actomyosin ATPase in specific muscle fiber types to endurance running. Initial concentration levels were maintained after 18 weeks of training. Specific activity levels of actomyosin ATPase were increased in the SO soleus, decreased in the FOG red vastus lateralis, and unchanged in the FG white vastus lateralis. The reported changes paralleled earlier findings on glycogenolytic enzymes (13).

Enzymes Involved in Fatty Acid and Ketone Metabolism.--Plasma free fatty acids (229) and plasma triglyceride fatty acids (107,164) have been shown to be important substrates for oxidation by skeletal muscle during exercise. Major increases in the levels of enzymes involved in the activation, transport and β oxidation of long-chain fatty acids (11,69,71,108,157,203,204) and in the levels of enzymes involved in ketone oxidation (8,289,290,291) have supported these observations.

Molé et al. (204) reported a doubling of palmityl CoA synthetase, carnitine palmityl transferase, and palmityl CoA dehydrogenase activities in mixed muscle homogenates (quadriceps plus gastrocnemius) of rats trained on an endurance running program. The rates of palmitate oxidation by whole muscle homogenates and by mitochondrial fractions

from the leg muscles also were found to increase two-fold. Identical observations had been reported earlier for gastrocnemius homogenates (203).

To determine which fiber types participate in the exercise-related increase in fat metabolism, Baldwin et al. (11) repeated the study of Molé using homogenates of the SO soleus, the FOG deep red quadriceps, and the superficial white quadriceps. The rate of palmitate oxidation and the activity level of carnitine palmityl transferase increased approximately two-fold in all three fiber types. Consequently, the relative capacities of the different fiber types for fat metabolism remained unchanged.

Ketone oxidation also is affected by an endurance program (8,289, 290,291). Winder et al. (289,290) found a two-fold to three-fold increase in the rates of D-β-hydroxybutyrate and acetoacetate oxidation in gastrocnemius muscle homogenates under conditions of uncontrolled respiration. D-β-hydroxybutyrate dehydrogenase, 3-ketoacyl-CoA transferase and acetoacetyl-CoA thiolase, key enzymes in ketone metabolism, all increased significantly with training. Recently, Winder et al. (291) have shown that endurance training affects ketone metabolic pathways in the three fiber types differently. The levels of 3-hydroxybutyrate dehydrogenase activity increased slightly in FG, 2.6-fold in SO and 6-fold in FOG fibers. Acetoacetyl-CoA thiolase activity increased approximately 40-45% in all fiber types, and 3-keto acid CoA-transferase activity increased 2-fold in FOG and FG muscle, but only 26% in SO muscle. This exercise-induced increase in the capacity of

skeletal muscle to oxidize ketones could play a major role in preventing ketosis in the exercising animal (8,291).

In a related study, Borensztajn et al. (32) investigated the effects of prolonged endurance training on the activity of lipoprotein lipase in the same three muscles. This enzyme is responsible for the uptake of chylomicrons by skeletal muscle. Initial control measurements revealed the highest activities to be in SO muscle. The soleus had activities which were 14 to 20 times greater than that in the FG white quadriceps and 2 times greater than that in the FOG red quadriceps. Twelve weeks of training resulted in a four-fold increase in lipoprotein lipase activity in the FOG muscle and two-fold increases in the SO and FG muscles. The greater rise found in the FOG muscle may reflect selective recruitment of these fibers during treadmill running. In contrast to these findings, Askew et al. (7) found no significant change in lipoprotein lipase activity in the quadriceps muscles of rats trained for seven weeks on a treadmill.

Endurance Training--Histochemical Alterations

Histochemical techniques have been used to determine muscle fiber metabolic profiles before and after specific training programs. Recruitment patterns induced by various work tasks and loads may be studied in this manner.

Faulkner and co-workers (100,101,194,198) studied the effects of chronic exercise on the distribution of fiber types in hindlimb muscles of the guinea pig. The exercise regimen consisted of daily running on a motor-driven treadmill at 0% grade with a maximum speed of 30 m/min.

The animals were exercised 30 to 45 min per day for eight weeks. SDH and myofibrillar ATPase were used to classify fiber types. In adult sedentary animals, the composition of the plantaris muscle was determined to be 53% FG, 36% FOG, and 11% SO. The soleus was found to be 100% SO and the psoas was 2% FG, 33% FOG, and 66% SO (198). These values agree closely with the findings of other investigators (4) and reflect three vastly different muscle fiber distributions. Training specifically affected the composition of the plantaris muscle but had no effect on the others. An increase in the proportion of FOG fibers, a decrease in the proportion of FG fibers, and no change in the proportion of SO fibers was reported for the plantaris. The results are consistent with those found in other studies of the effects of endurance exercise (21,83).

Reversability of exercise-induced fiber changes in the guinea pig was observed with 16 weeks of detraining (101). Selective atrophy and degeneration of FG fibers may have occurred, but the regression effect was attributed to a loss of mitochondrial density in FOG fibers which then were reclassified as FG fibers.

It should be noted that the percentage of red fibers (presumably FOG) has been found to be significantly increased in the diaphragm of endurance-trained animals (194).

The productive group of Edgerton, Barnard, Peter and their coworkers (20,21,22,23,24,78,79,80,81,82,83,84,85,86,87,88,89,102,103, 116,117,168,169,190,232,233,234,235,236,237) pioneered the early work of physiological, histochemical and biochemical correlational studies

and made many contributions to the concepts of mutability of fiber types and motor unit recruitment during exercise.

Edgerton et al. (78) subjected male albino rats to a prolonged swimming program. No significant alterations in percentages of fiber types were found in the soleus. However, the plantaris muscles of the exercised animals had a greater proportion of fibers with high malate, SDH, and NAD-diaphorase staining reactions than did those of the sedentary controls. These changes were observed in two areas of the plantaris. One area had a mixed-fiber population and the other was composed predominately of FG fibers. No changes were found in the proportion of SO fibers with weak myosin ATPase reactions.

Morphological changes also were investigated in these animals (81). Necrotic, angular and split fibers were observed in the soleus muscle but not in the gastrocnemius or plantaris muscles of all groups including the control group. The number of split fibers was the same for the three groups, but the total number of subfibers increased with the intensity of exercise. Split fibers have been reported in several other training (55,208) and surgically overloaded muscle studies (141, 142,283).

In a series of classical papers Barnard et al. (21,22,23) reported the histochemical, biochemical and physiological changes induced by an endurance training program in guinea pig hindlimb muscles. After 18 weeks of training, the mitochondrial yield had significantly increased in the gastrocnemius and plantaris (21). Histochemical analysis (NADH-diaphorase) revealed a significant conversion of FG to FOG fibers in

the central "red" and peripheral "white" areas of the medial gastrocnemius. The percentage of SO fibers did not change (21). Contractile properties as measured in the *in situ* gastrocnemius-plantaris muscle preparation revealed no exercise effect (22).

Edgerton *et al.* (82) ran guinea pigs on a treadmill at 1.6 km/hr for 5 min, 10 min, or until exhaustion. With increasing durations of acute exercise, the percentage of fibers lacking phosphorylase activity increased. Selective depletion of phosphorylase content was found in the red fibers (presumably FOG) of the plantaris muscle. No consistent changes were found in the soleus. This finding reflects the homogeneous SO fiber population in the soleus (4) which shows negligible phosphorylase activity even in controls.

Edgerton *et al.* (79,83) studied this depletion phenomenon further. Guinea pigs were trained on a progressive program of intermittent running for 20 weeks. Indirect electrical stimulation caused total phosphorylase to be selectively depleted in FG fibers. The effect was less in trained (86%) than in untrained (97%) animals. The histochemical depletion of phosphorylase was paralleled by glycogen depletion which was measured by spectrophotometric readings of PAS staining intensities. These PAS results support the findings of Kugelberg and Edstrom (187).

Recently, Edgerton *et al.* (85,117) have attempted to extend their findings to a nonhuman primate, the lesser bushbaby. These animals were trained to run or jump on a motor-driven treadmill. After six months of endurance running, fewer glycogen-depleted fibers were found in the plantaris muscles of trained than untrained animals following 15

min of electrical stimulation (85). This finding reaffirms the training-related resistance to fatigue reported for the guinea pig (83). The other biochemical, histochemical and physiological data also were in agreement with the results of previous work on guinea pigs (21,22,23). Endurance running produced increases in SDH and cytochrome a and c activities. Myoglobin content was enhanced. There was an increased proportion of FOG fibers, at the expense of FG fibers, in the tibialis anterior but not in the soleus. Glycogenolytic enzyme concentrations and contractile properties were not altered. No significant changes in myosin nor actomyosin ATPase activities were found. In general, the results supported those of other histochemical (10) and biochemical (147,243) studies.

A single 5-min to 15-min bout of running at 1.75 m/min or jumping at 2.4 to 2.9 m/min was used to determine the pattern of motor unit recruitment during specific types of exercise (117). Glycogen depletion was assessed by the PAS stain. FOG fibers were preferentially depleted in the vastus lateralis and gastrocnemius muscles after running. Jumping affected mainly the FG fibers in these two muscles. Both exercise regimens depleted the FOG fibers in the soleus. The findings suggest that the recruitment pattern of specific types of motor units is related to the nature of the specific movement being performed. Recent work has indicated that this also is the case in humans (124,125,126, 162).

In a comprehensive investigation, Muller (208) attempted to determine the temporal progress of mutability in muscle fibers. Young female

rats were exercised on a motor-driven treadmill six days a week for periods of 3, 6 and 12 weeks. At the end of the study, the mean fiber areas in the soleus, gastrocnemius and rectus femoris muscles of the exercised and control animals were not different. However, progressive splitting of SO fibers was seen in the soleus muscles of the trained rats at 3 and 6 weeks. Fiber splitting was not evident in the control animals. This observation conflicts with that of Edgerton et al. (81) who noted that a minimal amount of splitting is to be expected even in untrained animals. Muller (208) also reported a significantly decreased percentage of fast-twitch fibers in the soleus muscle. This decrease presumably was caused by the transformation of FOG fibers to SO fibers. Small but similar endurance-training effects were observed in the predominately FOG and FG areas of the gastrocnemius and rectus femoris muscles. The general trend of adaptation was from FG to FOG to SO fibers. The conversion of fast-twitch to slow-twitch fibers was not found in several earlier biochemical (147,243) and histochemical (10, 52,85) studies. However, recent evidence indicates that myosin ATPase activity may be altered in response to specific exercise regimens (15, 137).

Sprint Training

A program of sprint running could be expected to provide considerable stimulation for the anaerobic metabolic mechanisms. Unfortunately, due to the inherent difficulties associated with training animals at high running velocities, relatively little work has been done to date with this type of exercise.

Saubert et al. (257) trained adult male rats on a treadmill at speeds of up to 80.5 m/min for 11 weeks. Glycogenolytic and glycolytic mechanisms were affected but only minimally. Phosphorylase activities were unchanged in the FG white portion of the gastrocnemius, the FOG red portion of the gastrocnemius, the red vastus, and the mixed rectus femoris muscles. The only change in phosphorylase activity was a 70% increase in the SO soleus.

Hexokinase activity increased 50% in both the mixed rectus femoris and the soleus muscles. No changes in phosphofructokinase, pyruvate kinase, triosephosphate dehydrogenase, or lactate dehydrogenase activities were reported except in the soleus where there was a 35% increase in pyruvate kinase. In a parallel study by Staudte et al. (272), running at 80 m/min produced a 17% increase in triosephosphate dehydrogenase activity of the rat soleus.

The slight anaerobic adaptation of the soleus muscle was evident in the ATP regenerating system. Creatine phosphokinase activity increased 12% in the soleus but remained unchanged in the rectus femoris (272). Bagby et al. (10) also ran rats for 11 weeks at speeds up to 80.4 m/min and found myosin ATPase activity was unchanged in homogenates of the mixed gastrocnemius muscle. No alterations in the percentages of FG, FOG, or SO fiber types were observed.

Possible explanations for these relatively small changes include: (a) the FG and FOG fibers of the rat already may be equipped metabolically to handle an anaerobic stress; or (b) the running speed of 80 m/min may not be fast enough to act as a pure anaerobic stimulus. The latter

hypothesis is supported by the fact that histochemical fiber typing techniques revealed a significant increase in the percentage of FOG fibers, with an accompanying decrease in FG fibers, for the white portion of the gastrocnemius (257). There was an accompanying shift towards an FOG fiber population in the soleus muscle.

Fitts et al. (104) studied several histochemical parameters in the miniature pig following a sprint-running program known to have physiologically measurable training effects. No changes in fiber types were observed, and the investigators concluded that the histochemical techniques were not sensitive enough to distinguish metabolic adaptations.

The effects of sprint training on aerobic metabolism are not clear at this time. No change in myoglobin content in any fiber type of sprint-trained miniature pigs was reported by Fitts et al. (105). Staudte et al. (272) found increases of 20% in citrate synthase activity in homogenates of both the mixed rectus femoris and the SO soleus muscles. However, no changes have been observed in a variety of muscles assayed for SDH activity (257,272). Although not enough information is available to draw firm conclusions, it appears that current sprint programs for animals may have a substantial aerobic component.

Isometric, Weight Lifting, and Miscellaneous Training

Three parallel studies have been conducted to determine the effects of an isometric training program on histochemical and biochemical profiles of exercised skeletal muscle (96,97,209). Male and female

rats were forced to climb a 60° incline and support a predetermined amount of weight until exhaustion (approximately 5 min).

Activities of several enzymes of glycolysis, glycogen metabolism, fatty acid oxidation, lactate fermentation, and the ATP regeneration system were determined in homogenates of the rectus femoris and soleus muscles (96,97). Changes in anaerobic enzymes were evident. Creatine phosphokinase, glycogen phosphorylase, and triose phosphate activities increased in the rectus femoris and decreased in the soleus. Lactate dehydrogenase also decreased in the soleus. Contraction times became faster in the rectus femoris and slower in the soleus muscles of the females (96).

Histochemical changes in the female rats were studied using SDH and myofibrillar ATPase to classify muscle fibers (209). The percentage of FOG fibers decreased at the cost of the FG fibers in rectus femoris but not in the soleus or the lateral head of the gastrocnemius. The percentage of SO fibers did not change significantly in any of the muscles studied.

Together, these parallel studies would indicate that a rise in anaerobic capacity occurs in predominately FG-FOG muscle which is subjected to isometric training (96,97,209). This increased anaerobiosis may be modulated by a shift of FOG to FG fibers (209). A concomitant decrease in aerobic capacity might accompany such a shift (270). However, conflicting results have been reported by other investigators (159,185,297).

Howells and Goldspink (159) devised a counter-weighted basket which the animal had to pull down to obtain food. Hamsters subjected to this regimen for five weeks had increased SDH levels in the mixed biceps brachii, the slow soleus, and the fast extensor digitorum longus muscles. Similar increases in SDH values were reported by Zika et al. (297) in the biceps brachii of young rats subjected to tonic stress on a ladder for four to six months. Significantly elevated levels of α-glucanphosphorylase and nonspecific esterases also were found. There were no changes in LDH or mitochondrial α-glycerolphosphate dehydrogenase values.

Kowalski et al. (185) trained adult female rats on a weight lifting program of vertical climbing with an attached load for six weeks. Six preselected regions of the quadriceps muscles were investigated histochemically. Weight lifting resulted in overall increases in SDH, phosphorylase and cytochrome oxidase in all six regions regardless of the fiber-type population.

The increases in oxidative enzymes observed by Howells and Goldspink (159), Zika et al. (297), and Kowalski et al. (185) suggest that the various training programs used in these studies may have had a common aerobic component.

CHAPTER III

METHODS AND MATERIALS

Gross measurements of total-body oxygen debt and oxygen uptake have been used to reflect human metabolic responses to physical activity. Exhaustive sprint running leads to an increased tolerance of oxygen debt which presumably reflects a greater capacity for the generation of muscular energy via anaerobic metabolism. Training regimens based on this type of running are characterized by maximal workloads and relatively short bouts of repeated exercise. In contrast, distance running is thought to be dependent chiefly upon oxidative muscle metabolism and tends to increase total-body oxygen uptake capacity. Moderate or light workloads and relatively long bouts of continuous exercise are typical of endurance training programs. This study was designed to investigate cellular-level alterations in two preselected areas of the plantar flexor muscles of the male albino rat following eight weeks of sprint and endurance training.

Experimental Animals

Forty-two normal male albino rats (Sprague-Dawley strain) were obtained from Hormone Assay, Inc., Chicago, Illinois. They were received at weekly intervals in three shipments of 15, 12, and 15 animals

respectively. Each shipment was designated as a separate treatment group. A standard period of 12 days was allowed for adjustment to laboratory conditions. The treatments were initiated when the animals were 84 days old. The application of selection criteria (to be discussed later) reduced the final sample to a total of 27 animals.

Research Design and Treatment Groups

This study was conducted as a one-way design with three treatment groups of nine animals each. The duration of the treatment period was eight weeks. The three treatment groups were as follows.

Control Group

The 12 animals in the second shipment constituted the control (CON) group. These animals received no special treatment and were housed in individual sedentary cages (24 cm x 18 cm x 18 cm) during both the adjustment period and the treatment period.

Sprint Group

The sprint running (SPT) group was comprised of the 15 animals in the first shipment. Each of these animals was housed in an individual voluntary-activity cage (sedentary cage with access to a freely revolving activity wheel) during the adjustment period and in an individual sedentary cage during the treatment period. The SPT animals were subjected to an interval training program of high-intensity sprint running (Appendix A). The workload of the SPT program was gradually increased until on the 27th day of training, and thereafter, the animals were

expected to complete six bouts of exercise with 2.5 min of inactivity between bouts. Each bout included five 15-sec work periods alternated with four 30-sec rest periods. During the work periods, the animals were required to run at the relatively fast speed of 108 m/min.

Endurance Group

The endurance running (END) group was composed of the 15 animals in the third shipment. These animals were housed under the same conditions as the SPT animals. The END animals were subjected to a demanding program of distance running (Appendix A). The workload was progressively increased so that on the 30th day of training, and thereafter, the animals were expected to complete 60 minutes of continuous running at 36 m/min.

Training Procedures

The SPT and END groups were trained in a battery of individual controlled-running wheels (CRW). This apparatus has been 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. (286)

Animals learn to run in the CRW by avoidance-response operant conditioning. A low-intensity controlled shock current, applied through alternating grids comprising the running surface, provides motivation for the animals to run. A light above the wheel signals the start of each work period. The animal is given a predetermined amount of time (acceleration time) to attain a prescribed running speed. If the

animal does not reach the prescribed speed by the end of the acceleration time, the light remains on and shock is applied. As soon as the animal reaches the prescribed speed, the light is extinguished and the shock is discontinued. If the animal responds to the light and attains the prescribed running speed during the acceleration time, the light is extinguished immediately and shock is avoided. If the animal fails to maintain the prescribed speed throughout the work period, the light-shock sequence is repeated. Most animals learn to react to the light stimulus after only a few days of training.

A typical training session consists of alternated work and rest periods. The wheel is braked automatically during all rest periods to prevent spontaneous activity. The brake is released and the wheel is free to turn during work periods.

Performance data are displayed for each animal in terms of the total meters run (TMR) and the cumulative duration of shock (CDS). The TMR and the total expected meters (TEM) are used to calculate the percentage of expected meters (PEM):

$$PEM = 100\ (TMR/TEM)$$

PEM values are the chief criteria used to evaluate and compare training performances. A secondary criterion is provided by the percentage of shock-free time (PSF) which is calculated from the CDS and the total work time (TWT):

$$PSF = 100 - 100\ (CDS/TWT)$$

In this study, all exercise treatments were administered once a day, Monday through Friday, between 12:30 p.m. and 5:30 p.m.

Animal Care

All housing cages were steam-cleaned every two weeks. Standard procedures for daily CRW cleaning and maintenance were observed.

The animals received food (Wayne Laboratory Blox) and water ad libitum.[1] A relatively constant environment was maintained for the animals by daily handling as well as by temperature and humidity control.

The animals were exposed to an automatically regulated daily sequence of twelve hours of light followed by twelve hours without light. Since the rat normally is a nocturnal animal, the light sequence was established so that the lights were off between 1:00 p.m. and 1:00 a.m. and on between 1:00 a.m. and 1:00 p.m. This lighting pattern altered the normal day-night schedule for the animals so that they were trained during the active phase of their diurnal cycle.

Body weights of the SPT and END animals were recorded before and after each training session. The CON animals were weighed weekly.

Sacrifice Procedures

Anticipated limitations of time and personnel restricted the number of animals that could be handled at sacrifice to 12 in each

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[1]The three groups of animals used in this study were the placebo groups for a larger diet-training investigation. Seven days a week, between 7 p.m. and 9 p.m., each animal was given approximately .1 cc of 5% sugar solution/100 gms body weight, by oral syringe. Administration of the placebo was begun the day prior to the initiation of treatments and was terminated the day prior to sacrifice. Since all of these animals received the same dietary treatment, the effect of the placebo can not be evaluated. However, the internal validity of this study could not have been affected.

treatment group. Since one of the inherent purposes of the study was to compare various parameters in two groups of highly trained animals and a group of untrained animals, three extra rats originally were included in the SPT and END groups. Twelve animals were selected for sacrifice from each of these two groups on the basis of their health and their training performance throughout the treatment period. Only animals subjectively determined to be in good health were chosen. Because the training requirements were extremely vigorous, no absolute minimal performance criteria were established. However, individual daily records of PEM and PSF values were examined, and those animals making the best adaptations to the training regimens were selected for sacrifice. All 12 CON animals were judged to be healthy and were sacrificed.

Three sacrifice periods of two-days duration (Monday and Tuesday) were established. All animals within a treatment group were killed during a single sacrifice period (i.e., six animals each day). The trained animals were killed either 72 or 96 hr after their last exercise bouts were completed. This procedure was followed to eliminate any transient effects of acute exercise. The animals were either 140 or 141 days old at sacrifice.

Final body weights were recorded immediately prior to sacrifice. Each animal was anesthetized by an interperitoneal injection (4 mg/100 gm body weight) of a 6.48% sodium pentobarbital (Halatal) solution. The right hindlimb was skinned and the superficial posterior crural muscles were exposed by reflecting the overlying tissue. The right triceps

surae (gastrocnemius and soleus) and plantaris muscles were removed as a block. Similar procedures were used on the left hindlimb except that the plantaris and soleus muscles were separated, individually weighed, and discarded.

Upon removal, the right muscle block was rolled in talcum powder. The block was held with forceps, gently stretched to approximate its physiological length, and quick frozen in 2 methylbutane (isopentane). The isopentane had been precooled to a viscous fluid (-140 to -160° C.) by liquid nitrogen. The frozen muscles were stored in aluminum 35-mm film containers at -20° C until sectioning and histochemical procedures could be initiated. Using precooled stainless steel knives, sandwich blocks approximately 10 to 15 mm thick were cut from the mid-portions of the frozen muscles. The sandwich blocks were oriented distal end up and frozen onto cork strips using 5% gum tragacanth. The cork strips were used to attach the muscle blocks onto cryostat chucks for sectioning. Fresh-frozen serial cross-sections, 10 micra thick, were cut using a rotary microtome-cryostat (International-Harris Microtome). Sections were picked up on cover glasses and fan-dried for at least one hour.

Histochemical Procedures

Succinic dehydrogenase (SDH) reactivity was used as an indicator of aerobic capacity and resistance to fatigue. In the Krebs cycle, succinate is oxidized to fumarate by SDH. The covalently bound flavin adenine nucleotide picks up the two hydrogens removed and transports them to the electron transport system. SDH is bound firmly to the

mitochondrial membrane, and thus it also is a good indicator of mitochondrial distribution. In this study, SDH localization was demonstrated using nitro blue tetrazolium (NBT) as the electron acceptor. The method has been described by Barka and Anderson (19, p. 313). NBT yields a colored precipitate of diformazan when it is reduced and the formazan deposition observed with the light microscope indicates the localization of oxidative enzymes (216). A direct correlation between the qualitative histochemical classification by staining intensity for SDH and the quantitative measurements of SDH activity has been reported (27).

Lactate dehydrogenase (LDH) reversibly oxidizes lactate to pyruvate in the last step of glycolysis. It is found in all cells which are capable of glycolysis. Five isozymes have been isolated biochemically each consisting of one or a combination of two polypeptide chains designated as M (muscle) or H (heart) (67). All LDH isozymes catalyze the same reaction but have different activity levels. LDH localization was determined in this study using NBT as the electron trap and nicotinamide adenine dinucleotide (NAD^+) as the cofactor (230, p. 911). Staining intensity was assumed to be an indicator of lactate fermentation capacity. Because of the rather uniform intermyofibrillar network that is associated with this enzyme, it does not differentiate fiber types as well as do some other glycolytic enzymes. This lack of discriminative ability perhaps is due to the fact that LDH is a water soluble enzyme which may be present in the aqueous sarcoplasm (35).

Myosin adenosine triphosphatase (ATPase) localization was investigated by the method of Padykula and Herman (223) as modified by Guth

and Samaha (132) and presented by Dubowitz and Brooke (75, p. 32). The reaction is one in which both the preincubation of the tissue section and the incubation in the ATP mixture are carried out at a pH of 9.4. Under these conditions, the reaction develops in the myofibrils, and the intermyofibrillar network seems to dissolve out of the tissue section at some stage during the reaction (75, p. 32). The localization of this ATPase in the myofibrils has been substantiated by selective extraction procedures (260). Myosin ATPase is an enzyme involved in the hydrolysis of ATP to ADP with the release of a high energy bond available for muscle contraction (192). A direct correlation between myosin ATPase activity and speed of muscle contraction has been demonstrated biochemically (16,17,60) and substantiated histochemically (24, 53,88,132). Fast-twitch fibers (FOG and FG) stain darkly and slow-twitch fibers (SO) stain lightly at a pH of 9.4.

Glycogen localization was determined using the periodic acid-Schiff (PAS) reaction (197, p. 132). Previous studies have shown that spectrophotometric measures of glycogen content are correlated highly with PAS staining intensity in frozen tissue when the PAS response is evaluated either by microphotometric methods or by subjective ratings (117,188).

Lipid localization was demonstrated using the Sudan Black B (SUD) method (197, p. 126). SUD, a colorant, is soluble in absolute alcohol and has a high affinity for fatty material.

Harris' alum hematoxylin and eosin (H & E) was applied to the fresh-frozen sections to facilitate observations of morphological characteristics (197, p. 29).

Incubation times were varied according to the staining procedure. The mounting medium for the ATPase, SDH and LDH sections was glycerin-jelly. PAS, SUD and H & E sections were mounted in permount (Histoclad).

Muscle Areas

Histochemical evaluations were performed on two muscle areas in this study. These areas were selected to represent two different fiber populations. The central portion of the soleus (area 1) normally is composed primarily of SO fibers. Only a few FOG fibers are present. The posterior part of the plantaris (area 2) consists mainly of FG fibers with some FOG fibers interspersed (78).

Histochemical Evaluations

Each histochemical stain was evaluated objectively with the use of a Histochemical Photometer (HCP) at a magnification of 80X. The operator of the HCP is able to isolate a photometric beam on the center of a single muscle fiber in the projected image of a muscle cross-section. The photometer registers the percentage of light that is transmitted through the fiber on a scale from 0 to 100. The percentage of light transmitted is converted to percentage of light absorbed so that higher HCP readings reflect higher values of substrates and enzymes. Repeated measures of the same fibers on different days have shown the average percent error for the HCP to be $\pm$ 0.3%.

Photometric evaluations of each stain were determined for a group of 30 adjacent muscle fibers[1] from both muscle areas in each animal. The planned analysis of data imposed the requirement that histochemical values had to be taken on the same fibers in all serial cross-sections from a given animal. Fiber tracings of the SDH sections were matched with the projected images of the other sections to insure that this requirement was satisfied. Although 12 animals in each treatment group were sacrificed, identical fibers could be found throughout the serial cross-sections of only 9 animals per group. In each of the other cases, various artifacts prevented conclusive fiber identification in one or more of the sections. Consequently, the final sample was limited to a total of 27 animals.

The HCP values obtained on cross sections from the control animals served as reference standards for the histochemical stains. All photometric determinations of each stain were performed at the same time without knowledge of the treatment groups.

Analysis of Data

The body weights and the absolute and relative muscle weights were analyzed using a one-way fixed-effects analysis of variance routine on

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[1] In a previous study, a sample of 30 fibers was calculated to be more than necessary and sufficient for a four-way (7x4x8x10) mixed-model nested analysis of variance that was run on HCP data when: (a) the probability of making a type I statistical error was limited to the .01 level, (b) the probability of making a type II statistical error was limited to the .05 level, (c) the minimal mean difference to be detected as significant was set at 0.5 standard deviations, and (d) a moderate variability between subgroup means was assumed. Consequently, standard laboratory protocol now is to take readings on 30 fibers in each muscle area of interest.

the Michigan State University Control Data 6500 Computer (CDC 6500). Newman-Keuls tests were used to evaluate differences between pairs of means whenever a significant ($P \leq .05$) F-ratio was obtained.

The histochemical data for each stain were plotted by treatment group and muscle area. A Chi-square contingency analysis (ACT routine) was used to determine if there were any significant differences ($P \leq .01$) between frequency distributions for treatment groups within muscle areas.

CHAPTER IV

RESULTS AND DISCUSSION

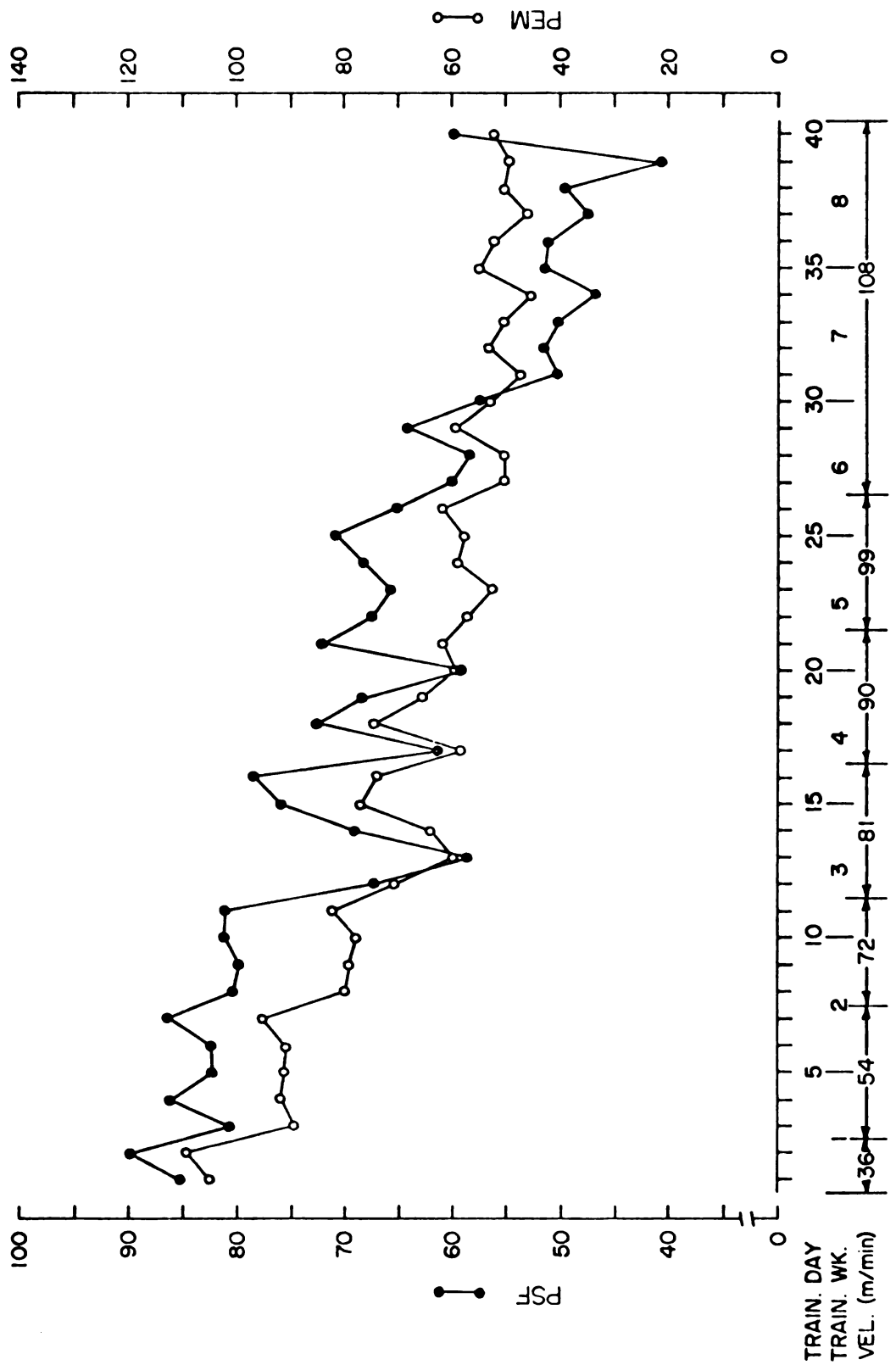
The material in this chapter is organized into four main sections. The first part deals with the training results from the Controlled-Running Wheel (CRW) programs and includes a summary of basic statistics for the percentage of body weight lost during the daily exercise periods, the environmental factors that operated during training, and the data obtained on the two performance criteria. Body and muscle weight results at sacrifice are given next. A major section is devoted to the histochemical data which are presented by muscle area. Finally, a discussion is offered that attempts to relate the present findings to those of other investigations reported in the literature.

Training Results

The sprint (SPT) and endurance (END) Controlled-Running Wheel (CRW) training programs are presented in Appendix A. These programs are modified versions of standard regimens routinely used in the Human Energy Research Laboratory, Michigan State University, East Lansing, Michigan. The modifications were incorporated in an attempt to design strenuous exercise programs which would specifically stimulate anaerobic or aerobic metabolic processes in individual muscle cells. The performances

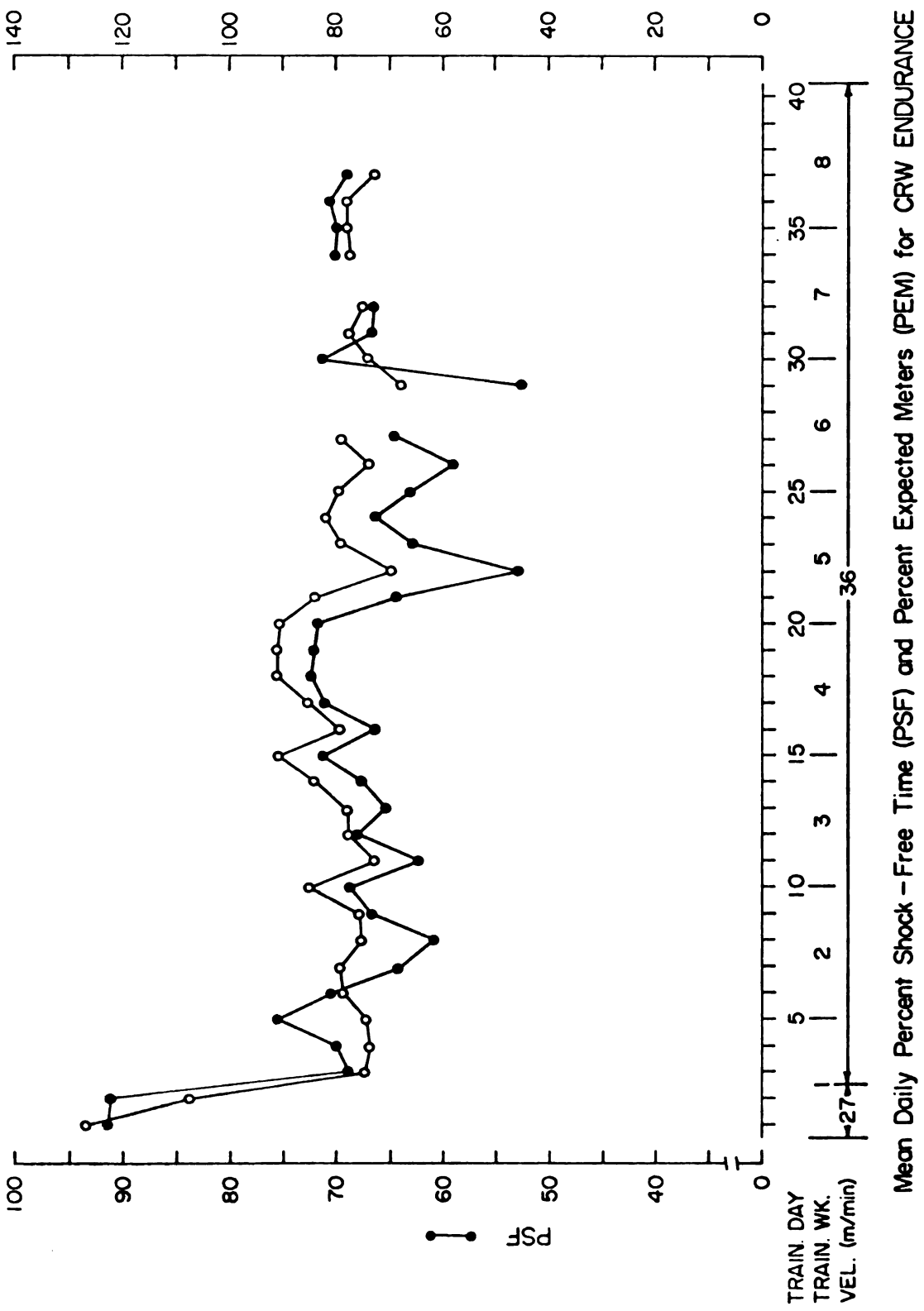
of the animals were evaluated using the percentage of expected meters (PEM) and the percentage of shock-free time (PSF) as criterion measures.

The performance data for the SPT group are presented in Figure 1. Progressive increases in the required running velocity were made rapidly. From the beginning of the fourth week of training to the end of the program, the animals were expected to run at velocities ranging from 90 to 108 m/min (see Figure 1 and Appendix A, Table A-1). No comparable exercise programs for small animals has been found in the literature. The results indicate the animals could not maintain the program requirements. PEM and PSF values fell to approximately 50 and 40 respectively during the last three weeks of training. Several possible explanations could account for these relatively poor performance data. The required running velocities may have been too fast, but observations during the training sessions revealed that the animals were capable of sprinting at the desired speeds. Low PEM and PSF values might suggest that the animals responded to the unconditioned shock stimulus rather than to the conditioned light stimulus. Improper initial training and defects in the CRW equipment could lead to such a learning problem, but the END animals learned to run under the same conditions and had no such difficulties (see Figure 2). A lack of control of environmental factors affecting training performance might have accounted for these results. This is particularly true for air temperature and percent humidity, but again the END data make this explanation improbable (see Appendix B). The most likely cause of the low PEM and PSF values is that the SPT regimen may have produced a state of



Mean Daily Percent Shock-Free Time (PSF) and Percent Expected Meters (PEM) for CRW SPRINT

Figure 1



Mean Daily Percent Shock-Free Time (PSF) and Percent Expected Meters (PEM) for CRW ENDURANCE

Figure 2

overtraining. The data in Figure 1 support this hypothesis. Increases in the required velocity were expected repeatedly from the SPT animals before they were fully adapted to the previous velocity. The constant additional stress could have resulted in overtraining.[1]

The training data for the END group are shown in Figure 2. PEM values were 70 or higher on all but one day and averaged 81.3. PSF values were above 60 on all but three days. The mean PSF value was 68.4. These results indicate that the animals were able to maintain the daily requirements of the END program relatively well.

The END animals ran at the relatively slow speed of 36 m/min. Periods of continuous running were progressively increased to 60 min at the end of five weeks of training and were maintained at this level for the remainder of the eight week program (see Appendix A, Table A-2). The single bout of exercise was determined subjectively to result in daily physical exhaustion of the animals. Repeated exposure to this level of stress could have resulted in a mild state of overtraining. On the average, the rats lost 2.7% body weight during each training session (see Appendix B, Table B-2). Body weight data were used to award an unplanned recovery day on Wednesday of each of the last three weeks of training. The animals were run on the 39th and 40th days of the program, but the results were not recorded due to a technician error.

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[1] Supplementary data on hindlimb bone weights of the animals (to be reported elsewhere) also suggest an overtraining phenomenon. The bones of the SPT group were approximately 40% lighter than those of the CON group. This observation was totally unexpected and does not agree with the results of previous work in which less strenuous training regimens were used (282).

The requirements of the END program appear to be similar to those of the training protocol used in the experiments conducted by Holloszy and a number of other investigators (see Table 4). In those studies animals ran continuously for periods of up to two hours at 31 m/min. The discrepancy in the duration of time the animals could run probably is due to the different modes of training. Holloszy and co-workers used a motor-driven treadmill, whereas the CRW used in the present study is animal-powered. The animals must displace the mass of the running wheel during the acceleration period and then maintain the rotation of the wheel at some required speed for the entire program. At any given running velocity, the CRW is a more demanding exercise module than the motor-driven treadmill. The metabolic changes produced by these two pieces of apparatus need not coincide.

Body and Muscle Weight Results at Sacrifice

At the end of eight weeks of exercise, the trained animals were significantly smaller than the sedentary control animals (see Table 6). The difference in body weight between the SPT and END groups of animals was not statistically significant. Both trained groups were approximately 20% lighter than the CON group. These results are in agreement with those of previous studies using the CRW (150,278) and support the general observation that strenuous exercise slows the usual gain in body weight seen in the male rat over time (21,63,100,152). The slower rate of weight gain is usually attributed to an increase in caloric expenditure associated with exercise and, in some instances, to a

Table 6. Analysis of variance for overall treatment effects and Newman Keul's tests of paired comparisons for body weight at sacrifice and absolute and relative muscle weights.

Dependent Variable	Treatment Means CON	SPT	END	F Value	P Value	Newman Keul's Test**
Body Weight at Sacrifice (g)	517.2	409.9	420.6	52.722	<0.0005*	SPT = END < CON
Absolute Soleus Weight (g)	0.200	0.160	0.164	6.801	0.005*	SPT = END < CON
Absolute Plantaris Weight (g)	0.501	0.365	0.456	20.744	<0.0005*	SPT < END < CON
Relative Soleus Weight (g x 10^{-3})	0.387	0.390	0.391	0.009	0.991	
Relative Plantaris Weight (g x 10^{-3})	0.968	0.892	1.082	11.232	<0.005*	SPT < CON < END

*Significant overall treatment effect at the 0.05 level.

**Newman Keul's Tests were run at the 0.05 level of significance.

significant reduction in food intake (63,222). In the present study, however, these parameters were not monitored.

The absolute soleus weights followed the same pattern as was found for the body weight data. The CON group had a significantly larger mean soleus weight than did either of the trained groups. The absolute soleus weights of the SPT and END groups were not significantly different. Consequently, relative soleus weights were not different among the three treatment groups.

The absolute plantaris weights of the three treatment groups were significantly different. The plantaris muscles of the CON animals were the largest, those of the END animals were intermediate in weight, and those of the SPT animals were the smallest. When body weight was taken into consideration, this relationship resulted in relative plantaris weight being largest in the END group and smallest in the SPT group.

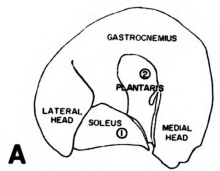
Histochemical Results

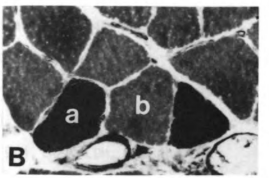
Histochemical photometer (HCP) readings were obtained for ATPase 9.4, SDH, LDH, PAS and SUD taken on 30 adjacent fibers in each of two muscle areas. All muscle sections selected were determined histologically normal as reflected by the routine H and E stain. Serial cross-sections were used, and the HCP readings were taken on the same 60 fibers in all five sections from a given animal. The fibers were selected as being typical of those in the central portion of the soleus muscle and the medial posterior portion of the plantaris muscle (see Plate I, A).

PLATE I

A: Schematic view of a cross-section of the superficial posterior crural musculature. Histochemical profiles were determined for the intramuscular areas identified as 1 and 2.

B-F: Serial-section histochemical profile for area 1 in the soleus muscle of a control animal. B through F show ATPase 9.4, SDH, LDH, PAS and SUD stains respectively. Fiber a is FOG; fiber b is SO. (X 125)


GASTROCNEMIUS
PLANTARIS
LATERAL HEAD
SOLEUS
MEDIAL HEAD
A


a
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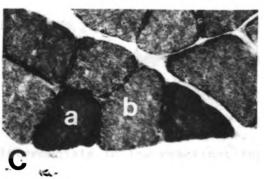
B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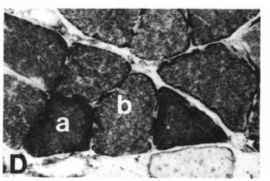
b
C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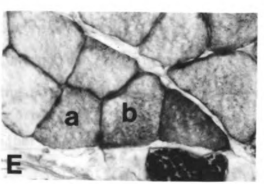
D


a
b
E

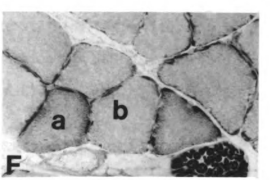

a
b
F

PLATE I

Fiber-type Profiles

Histochemical profiles for individual fibers in the two muscle areas were established from the serial cross-sections.

Soleus Muscle (Area 1).--Two fiber types were identified histochemically in the soleus muscle (Area 1). The FOG fibers were characterized by high reactions to the ATPase 9.4, SDH, LDH, PAS and SUD staining procedures (see fiber a in Plate I, B-F). This profile confirms the metabolic heterogeneity previously reported for the FOG fiber type (see Tables 2 and 4). The SO fibers stained less intensely with all of the histochemical procedures (see fiber b in Plate I, B-F).

Plantaris Muscle (Area 2).--The plantaris muscle (Area 2) exhibited three fiber types. The FG fibers had high reactions to ATPase 9.4, intermediate to low reactions for LDH, SDH and PAS, and low reactions to SUD (see fiber a in Plate II, A-E). The FOG fibers stained darkly with all five histochemical indicators (see fiber b in Plate II, A-E). Low reactions to ATPase 9.4, intermediate staining intensities for SDH, LDH and PAS, and intermediate to high reactions to SUD characterized the SO fibers (see fiber c in Plate II, A-E).

Fiber-type Distributions

Percent frequency distributions for HCP readings of the five stains were constructed by treatment groups within muscle areas. This procedure was carried out in an attempt to identify any treatment effects on metabolic characteristics and fiber-type distribution. A total of 30 frequency distributions were established (i.e., five

PLATE II

Serial-section histochemical profile for area 2 in the plantaris muscle of a control animal. A through E show ATPase 9.4, SDH, LDH, PAS and SUD respectively. Fiber a is FG; fiber b is FOG; fiber c is SO. (X 125)

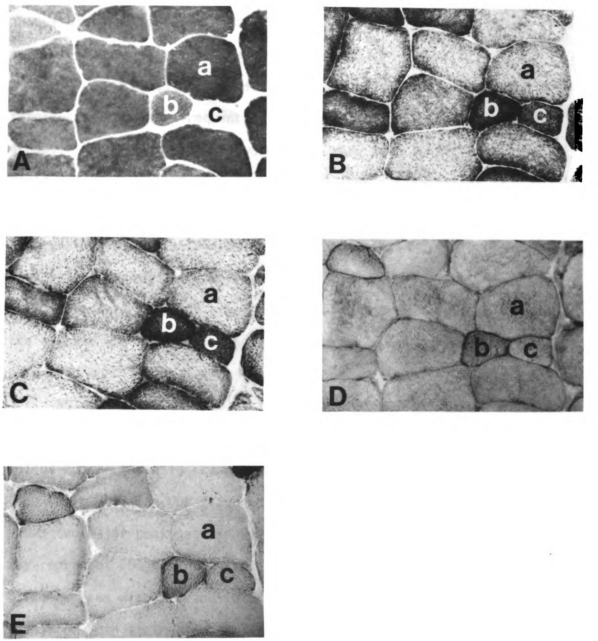


PLATE II

stains times two muscle areas times three treatment groups). Each frequency distribution represents 270 muscle fibers (i.e., 30 fibers from each of nine animals in a treatment group).

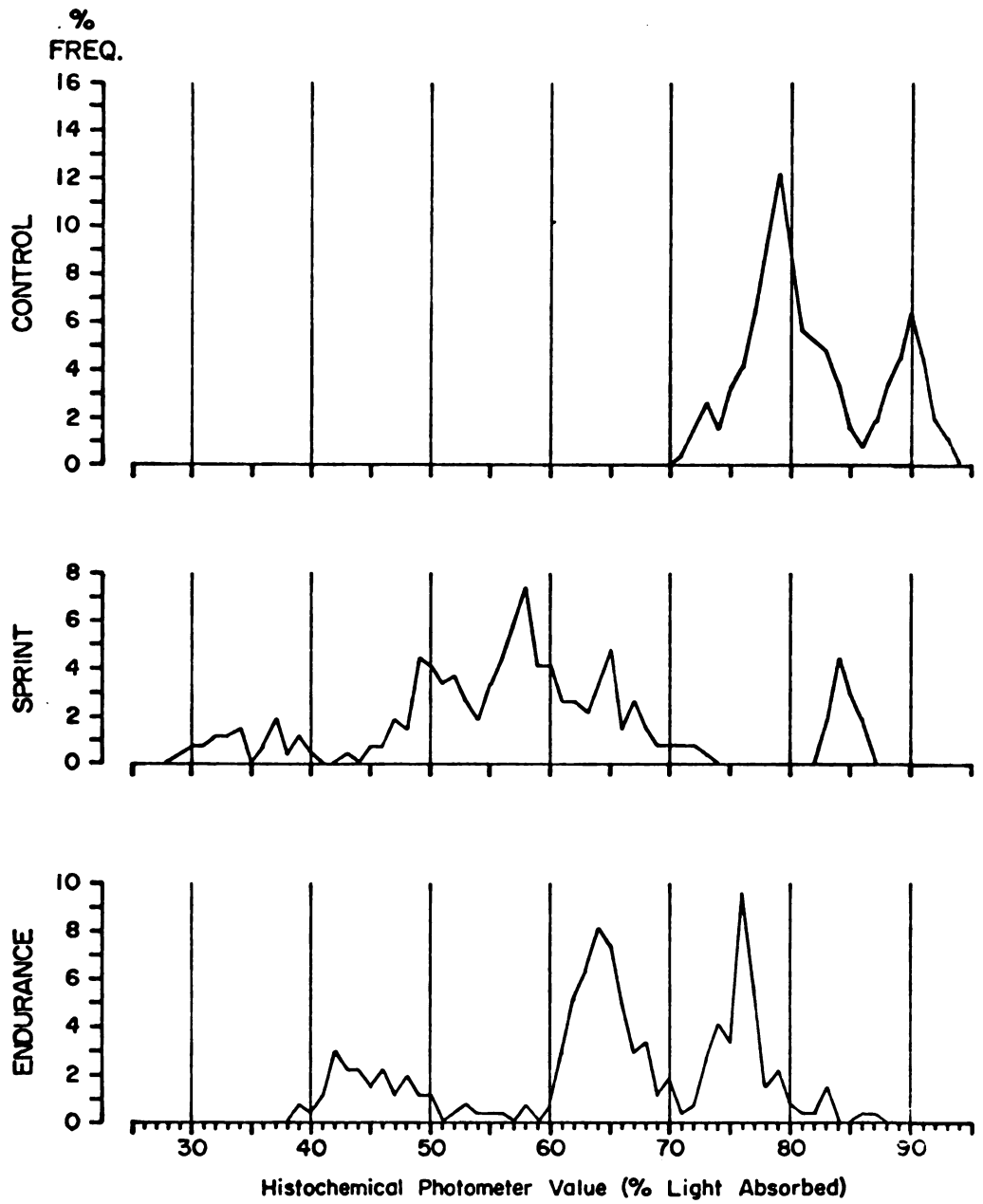
Chi-square tests were used to determine overall significant differences between the frequency distributions of the three treatment groups. Supplementary Chi-square tests were used to evaluate differences between pairs of distributions whenever a significant ($P < .01$) overall value was obtained for a given procedure. In all analyses, observations had to be pooled near the ends of the distributions to achieve the minimum expected frequencies required for the Chi-square test. Standard grouping procedures were followed. Although some of the extreme HCP values were pooled for calculation purposes, raw data were used when the frequency distributions were graphed.

Soleus Muscle (Area 1).--The results of the Chi-square analyses for differences between frequency distributions of HCP readings in the soleus muscle (Area 1) show that all comparisons were highly significant (see Table 7). The CON frequency distribution for ATPase 9.4 (Figure 3) shows two major peaks at the upper end of the graph. The smaller peak may represent FOG fibers and the larger peak SO fibers. This interpretation agrees with previous histochemical findings (4). The distributions for both trained groups were shifted to the left and were quite dispersed. The SPT distribution was shifted more than the END distribution. Physiologically, these findings suggest that a slowing of contraction speed and an increase in resistance to fatigue may have occurred with training. Another possibility, the existence of a simple

Table 7. Chi-square Analyses for Overall and Paired Treatment Effects on Frequency Distributions of Five Histochemical Stains in the Soleus Muscle (Area 1)

Histochemical Stain	Overall Treatment Comparisons		Paired Treatment Comparisons					
			CON vs SPT		CON vs END		SPT vs END	
	df	Chi-square	df	Chi-square	df	Chi-square	df	Chi-square
ATPase 9.4	46	839.1*	23	462.6*	23	353.1*	23	252.1*
SDH	50	162.6*	25	70.2*	25	87.4*	25	88.0*
LDH	58	259.9*	29	112.7*	29	87.7*	29	186.0*
PAS	34	505.6*	17	307.6*	17	35.9*	17	306.2*
SUD	44	150.9*	22	48.6*	22	91.2*	22	71.3*

*Significant comparison at the .01 level.



Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for ATP 9.4 in Soleus Muscle Fibers (Area I)

Figure 3

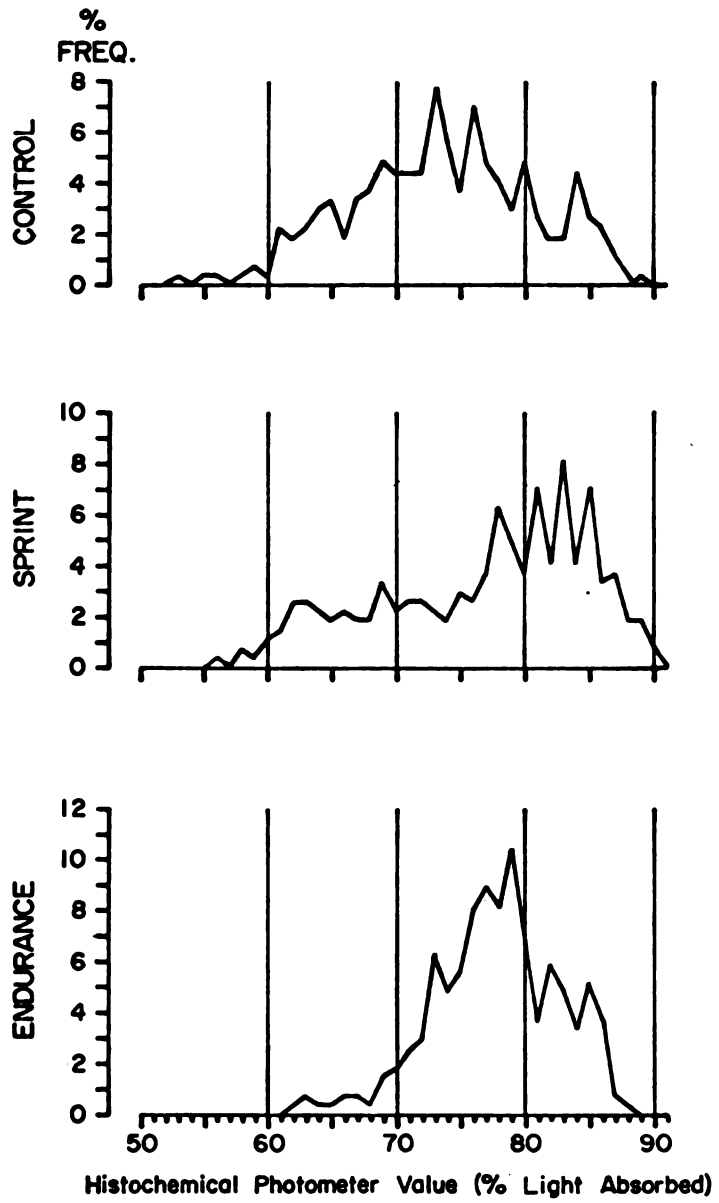
exercise-induced enzyme depletion, cannot be ruled out from the present data. Considering the time between the last exercise bout and sacrifice, however, such an effect seems to be unlikely.

An apparent third group of fibers with relatively low values of ATPase 9.4 can be seen in all three treatment distributions. This observation is interesting although it cannot be explained at the present time.

The SDH distributions (Figure 4) were shifted to the right with both types of training. This adaptation would indicate that increases in aerobic metabolism took place. The shift is more apparent in the END group than in the SPT group.

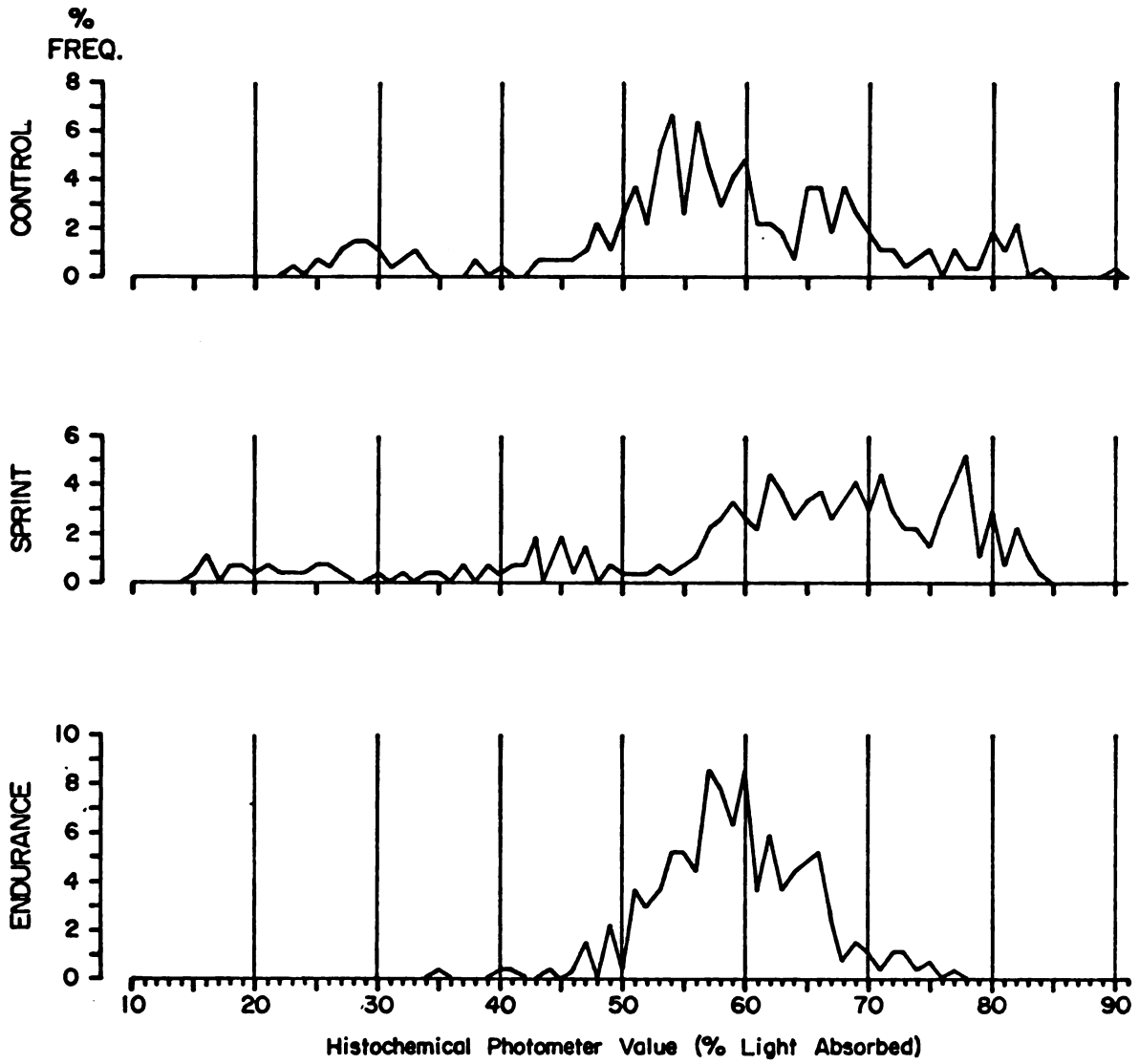
LDH is an index of lactate fermentation and is used as a marker for anaerobic metabolism. With training, LDH reactions in individual muscle cells generally were increased (Figure 5). The SPT training program produced the largest overall shift in HCP readings, whereas an additional effect of the END program was to decrease the range of values. Note, however, that the distribution for the END trained animals showed a small shift towards high LDH values. Evidence indicates that endurance exercise may alter the metabolic profile of red (SO-FOG) muscle to resemble cardiac muscle. That is, the red muscle may assume the ability to utilize lactate as an energy source (174). This phenomenon may be reflected in the increased LDH activity in the SO soleus.

SPT training resulted in marked increases in glycogen stores as reflected by the PAS stain (Figure 6). This finding is consistent with the observed increase in LDH reactions for the SPT group since anaerobic



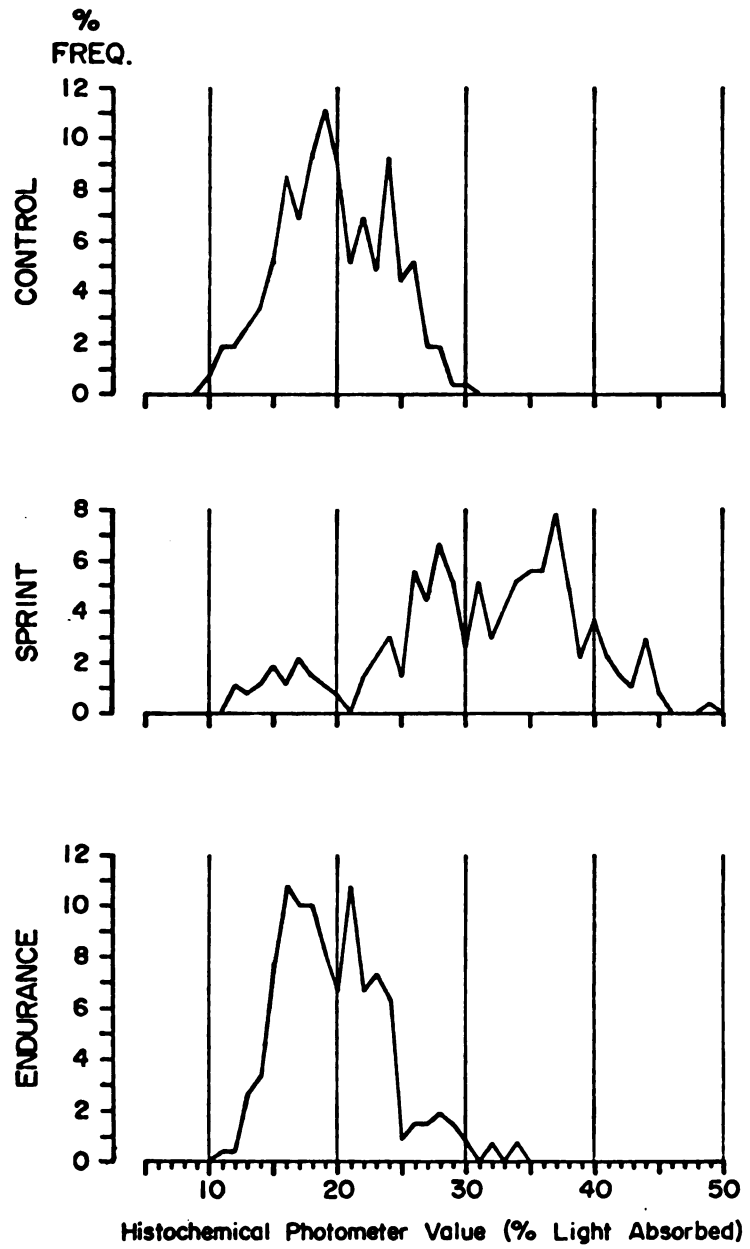
Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for SDH in Soleus Muscle Fibers (Area I)

Figure 4



Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for LDH in Soleus Muscle Fibers (Area 1)

Figure 5



Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for PAS in Soleus Muscle Fibers (Area I)

Figure 6

metabolism is known to depend primarily on glycogen as a substrate (122). The PAS distribution was shifted only slightly to the right with END training. The small change implies a relatively trivial role of glycogen as a substrate in prolonged activity.

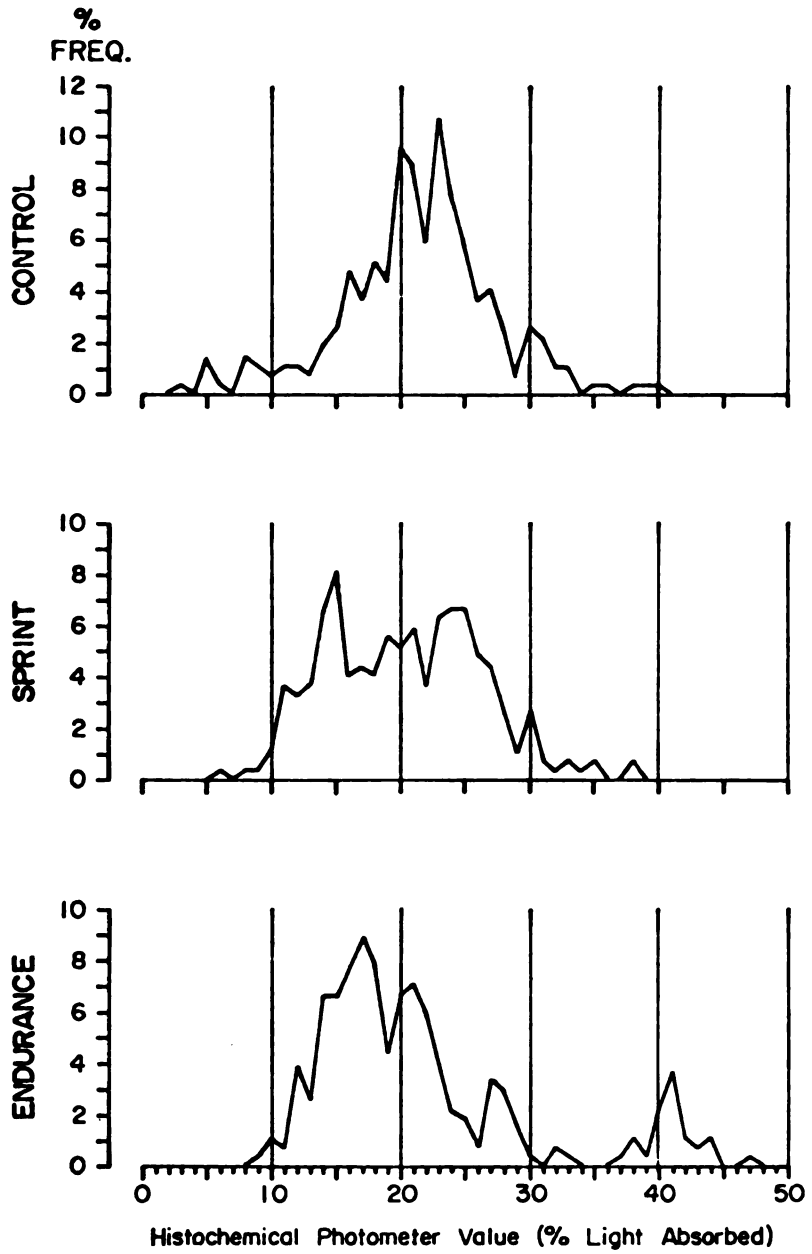
Fat metabolism becomes increasingly important during physical activity of long duration (107,108,164). With END training, approximately ten percent of the soleus muscle fibers demonstrated a high reaction to SUD staining (Figure 7). However, a large increase in the number of fibers exhibiting low HCP readings also was evident. The distribution of values for the SPT group was shifted slightly to the left.

Pictorial representations of the phenotype changes observed in the soleus muscle are shown in Plate III.

Plantaris Muscle (Area 2).--The Chi-square analyses for differences between frequency distributions of HCP readings in the plantaris muscle (Area 2) are summarized in Table 8. All but one of the comparisons were significant at the .01 level.

Nearly all of the plantaris muscle fibers in the CON animals had HCP values between 80 and 90 for ATPase 9.4 (Figure 8). Homogeneity of staining reaction was expected since this muscle area is composed predominately of fast contracting fibers. The frequency distributions of the two trained groups were shifted to the left and were widely dispersed. The general pattern of training effects was the same as that seen in the soleus muscle.

Significant increases in SDH staining intensity (Figure 9) were observed in both the SPT and the END groups. A similar pattern was



Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for SUD in Soleus Muscle Fibers (Area I)

Figure 7

PLATE III

Representative phenotype changes in the histochemical profile of the soleus muscle (Area 1) following eight weeks of strenuous training. The first column (A, D, G, J and M) contains cross-sections from CON animals. The center column (B, E, H, K and N) contains sections from SPT animals. The last column (C, F, I, L and O) contains sections from END animals. The sections are not serial. (X 33)

A-C: ATPase 9.4 sections. Note the general decreases in staining intensity and the reduced number of dark staining fibers in the trained animals.

D-F: SDH sections. There is an exercise-related increase in both the SO and the FOG fibers. This is particularly apparent in the END animal.

G-I: LDH sections. The same general pattern is seen here as with SDH. However, in this case the SPT animal has the highest staining reaction.

J-L: PAS sections. The SPT animal has a large number of fibers with high staining intensity. Reaction levels in the END animal are similar to those in the CON animal.

M-O: SUD sections. There are many dark staining fibers in the END animal.

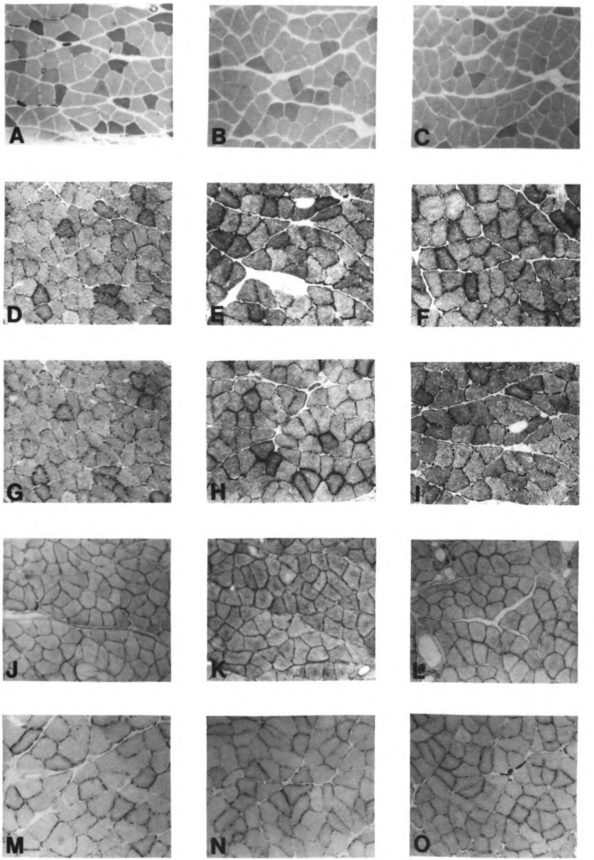
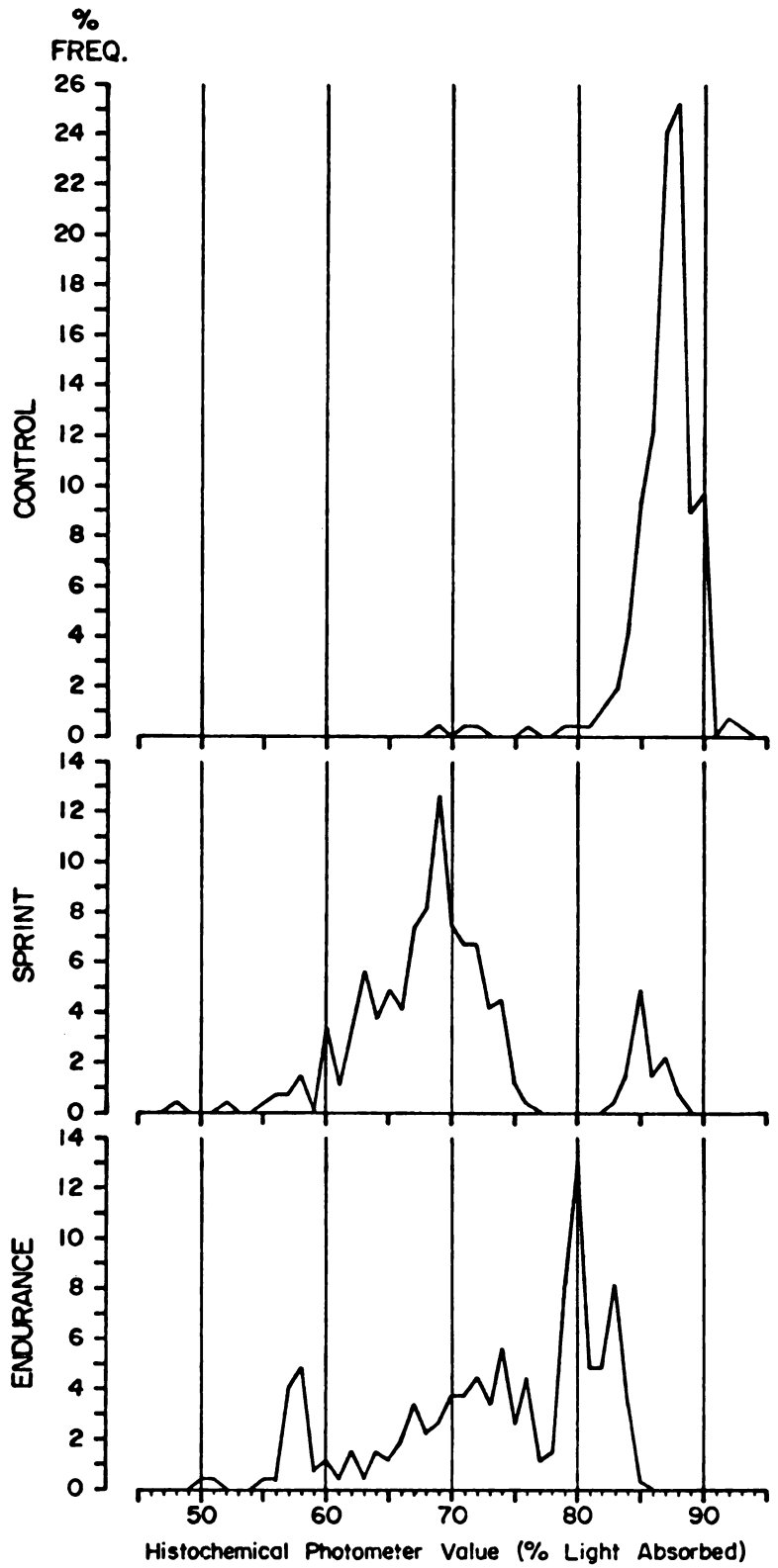


PLATE III

Table 8. Chi-square Analyses for Overall and Paired Treatment Effects on Frequency Distributions of Five Histochemical Stains in the Plantaris Muscle (Area 2)

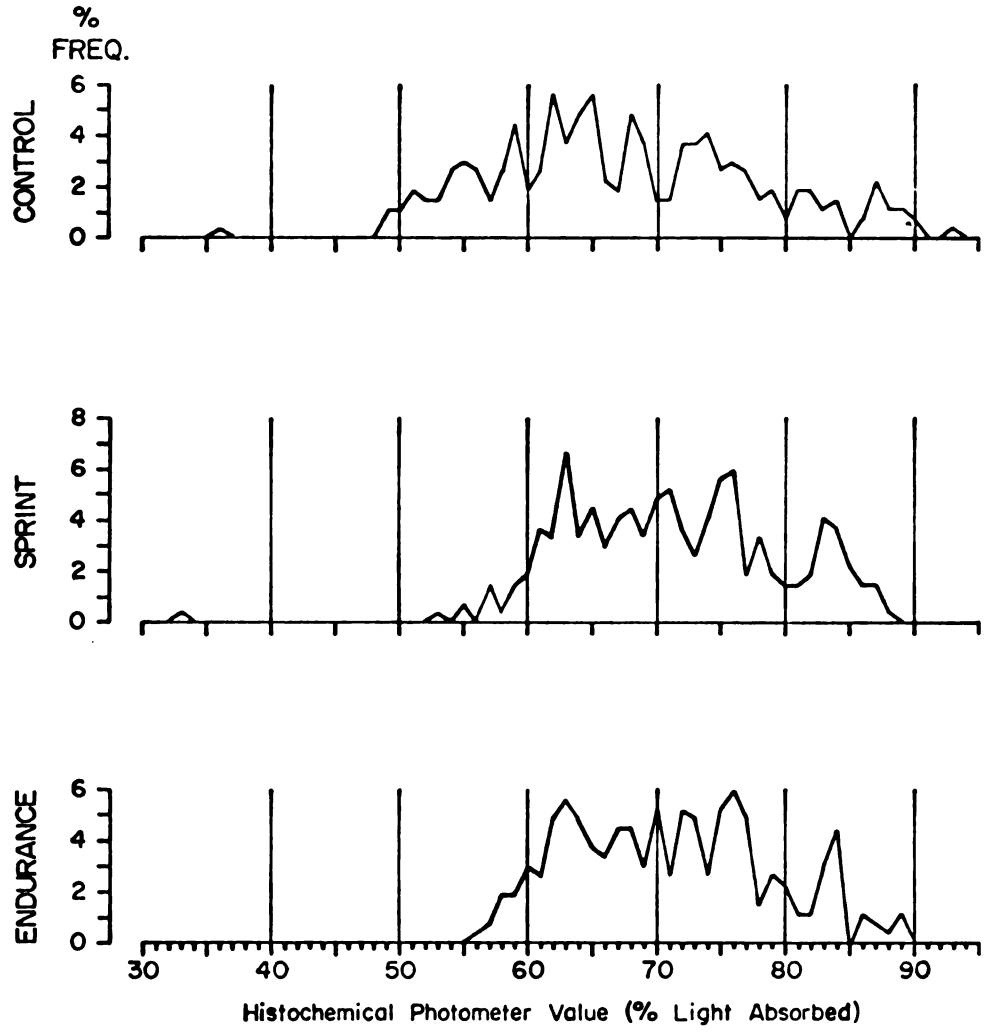
Histochemical Stain	Overall Treatment Comparisons		Paired Treatment Comparisons					
			CON vs SPT		CON vs END		SPT vs END	
	df	Chi-square	df	Chi-square	df	Chi-square	df	Chi-square
ATPase 9.4	34	909.3*	17	432.8*	17	463.5*	17	213.0*
SDH	58	136.2*	29	80.2*	29	74.8*	29	24.7
LDH	72	157.7*	36	63.5*	36	84.1*	36	103.2*
PAS	32	623.1*	16	413.1*	16	112.8*	16	285.5*
SUD	42	227.8*	21	100.5*	21	108.1*	21	78.5*

*Significant comparison at the .01 level.



Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for ATP 9.4 in Plantaris Muscle Fibers (Area 2)

Figure 8



Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for SDH in Plantaris Muscle Fibers (Area 2)

Figure 9

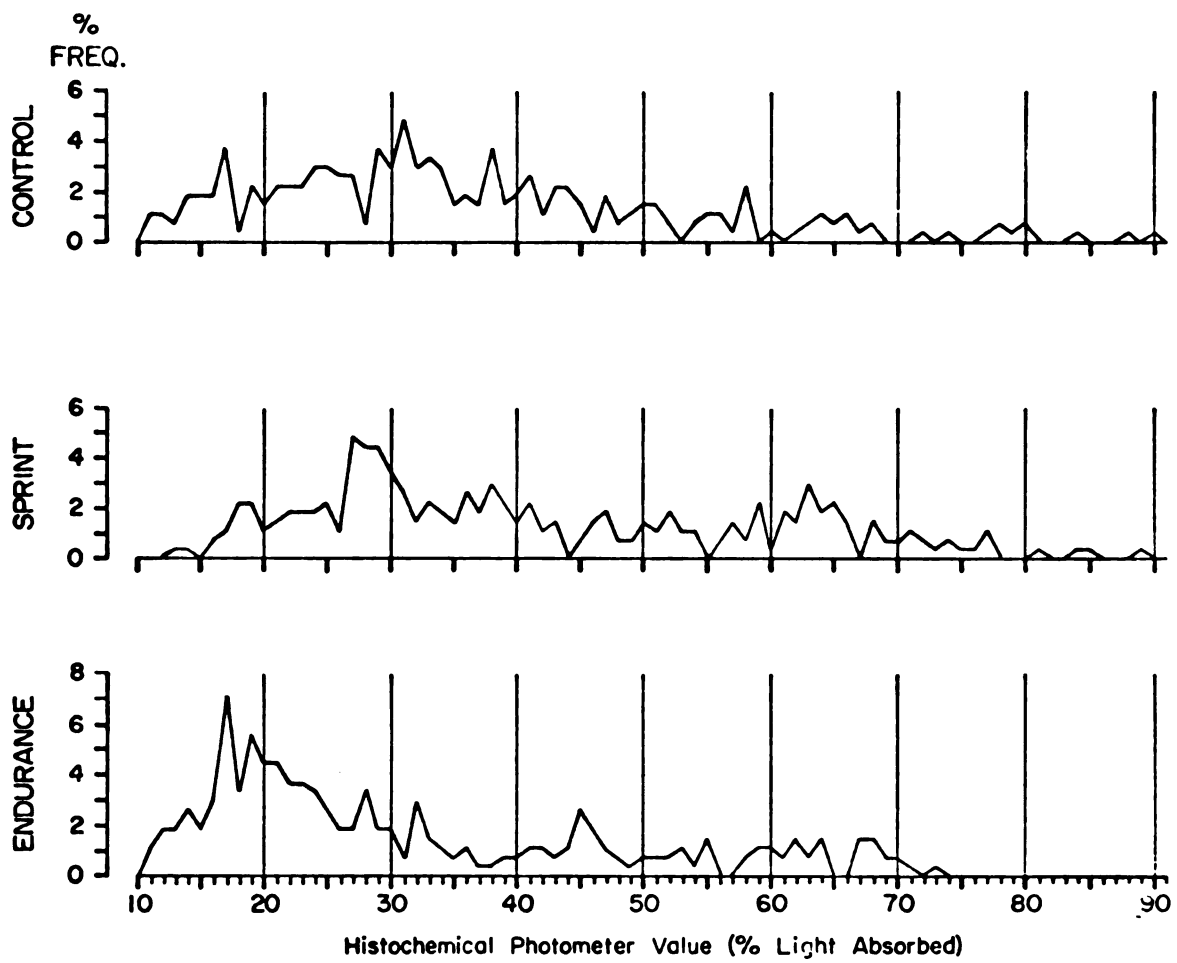
found in the soleus muscle. Although the SPT program originally was designed specifically to stimulate anaerobic metabolic processes, it is clear that both training programs had large aerobic components.

The range of HCP readings for LDH (Figure 10) was greater than for any other stain. This pattern was found in the soleus muscle also and suggests that the LDH reaction is quite variable within fiber types. Since the LDH stain used in this study is not isoenzyme specific, these diverse values were anticipated. M-type and H-type LDH could be expected to respond differently to the specific exercise programs. With SPT training, a general shift to the right was observed. The END running program resulted in a large number of fibers having low LDH reactions.

SPT training caused a large increase in PAS staining intensity (Figure 11). This adaptation would indicate an enhanced ability of the fast contracting fibers to store glycogen after a program of strenuous running. END training also produced a shift to the right, but the change was much less pronounced than it was in the SPT animals.

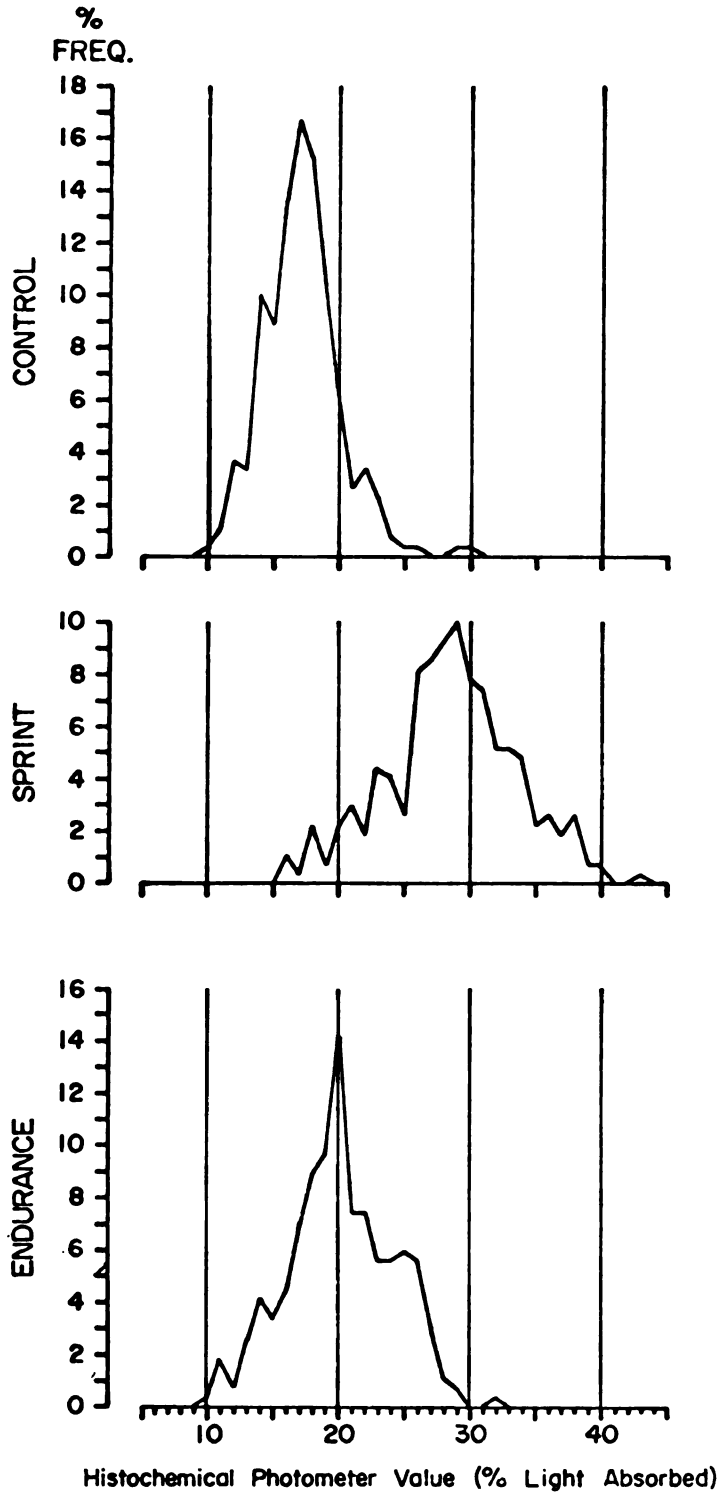
Both training programs caused the distribution of HCP readings for SUD to be shifted towards the middle and to be concentrated at the lower end of the continuum (Figure 12). A larger percentage of low SUD values were found in the END group than in the SPT group.

Plate IV gives a pictorial representation of the phenotype changes observed in the plantaris muscle.



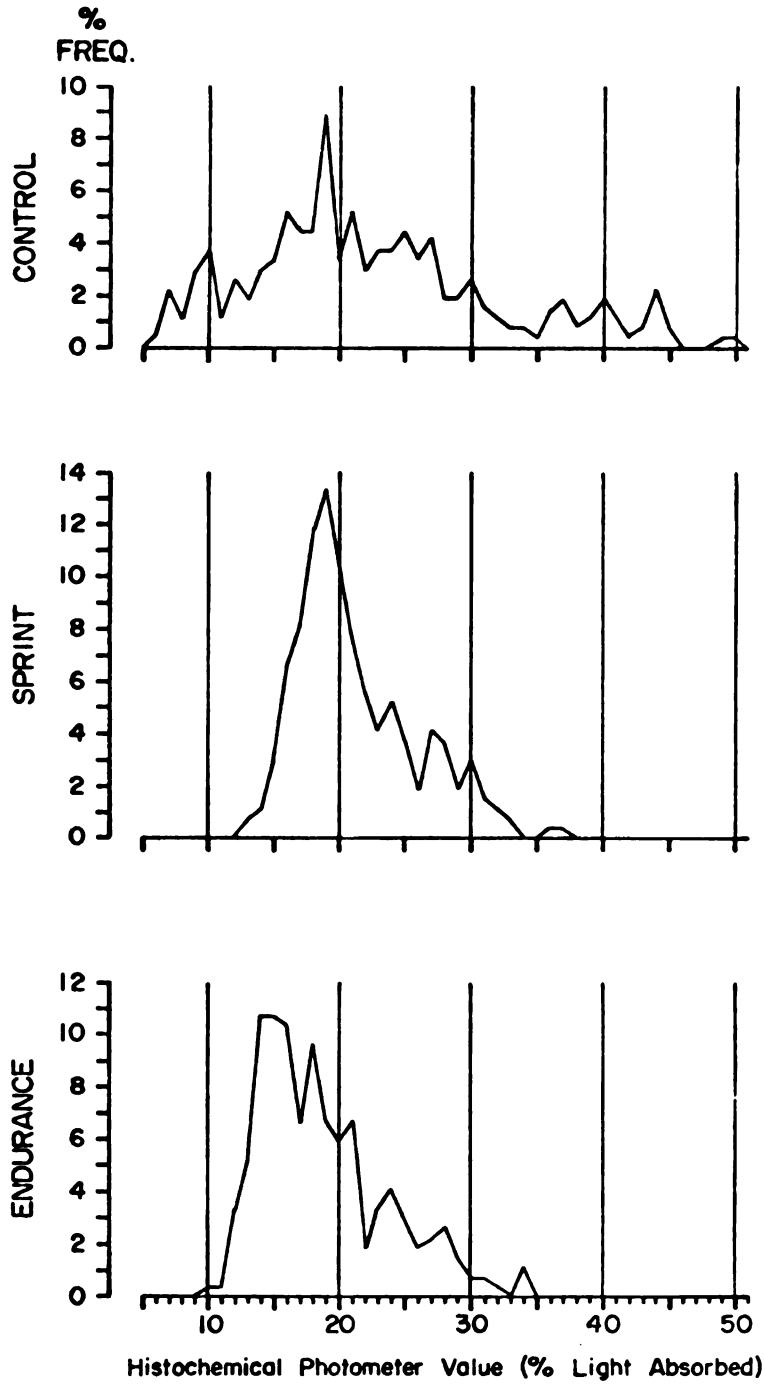
Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for LDH in Plantaris Muscle Fibers (Area 2)

Figure 10



Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for PAS in Plantaris Muscle Fibers (Area 2)

Figure 11



Percent Frequency Distributions, by Treatment Groups, of Histochemical Photometer Values for SUD in Plantaris Muscle Fibers (Area 2)

Figure 12

PLATE IV

Representative phenotype changes in the histochemical profile of the plantaris muscle (Area 2) following eight weeks of strenuous training. The first column (A, D, G, J and M) contains cross-sections from CON animals. The center column (B, E, H, K and N) contains sections from SPT animals. The last column (C, F, I, L and O) contains sections from END animals. The sections are not serial. (X 33)

A-C: ATPase 9.4 sections. A similar pattern to that found in the soleus is seen. Note the general decrease in staining intensity in both the SPT and END animals.

D-F: SDH sections. An increased reaction is seen in both SO and FOG fibers with training. The percentage of FOG fibers may be increased.

G-I: LDH sections. Dark staining fibers are increased in the SPT animal and decreased in the END animal.

J-L: PAS sections. Many fibers exhibit intermediate to dark reactions in the SPT animal.

M-O: SUD sections. A moderate number of dark fibers can be seen in both of the trained animals.

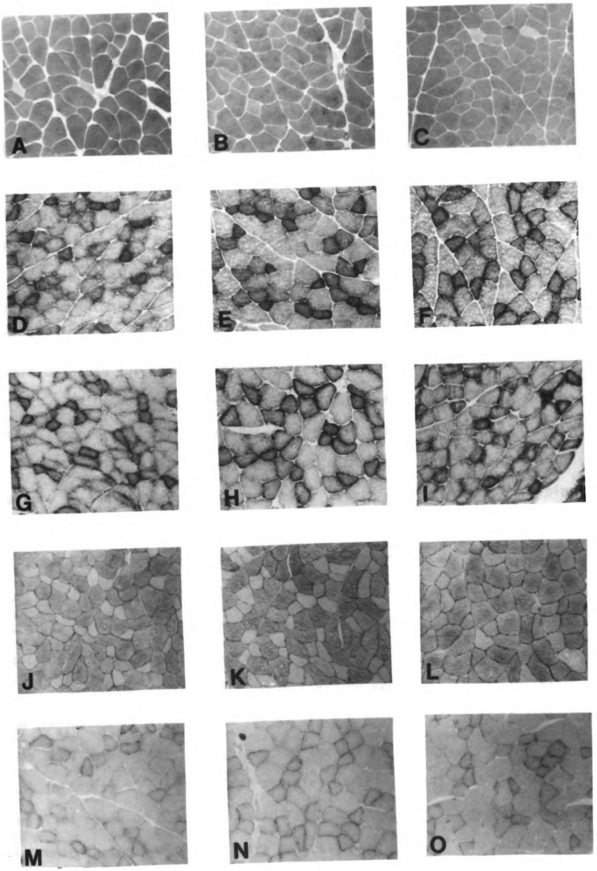


PLATE IV

Discussion

The two muscle areas investigated have markedly different fiber-type populations. The central portion of the soleus muscle (Area 1) is composed primarily of SO fibers with some FOG fibers interspersed. The medial posterior portion of the plantaris muscle (Area 2) consists almost entirely of fast-twitch fibers, and a majority of these are FG.

Serial cross-sections revealed typical patterns of relative staining intensities for the fiber types within each muscle area. However, different intensities of reaction to given staining procedures were obtained for the same fiber types in the two areas. Consequently, fiber-type comparisons between areas were not warranted. This observation supports the results of previous investigations (185,278).

The SPT and END exercise regimens each produced a number of alterations in the histochemical profiles of muscle cells that were training-program specific. In most cases, however, the differences between the SPT and END effects were in the magnitude rather than in the direction of the shifts. Surprisingly, most of the distribution changes were similar in both magnitude and direction in the two muscle areas. Some notable exceptions were observed.

The tissue sections from both training groups had large increases in the number of fibers with low ATPase 9.4 values. This adaptation was seen in both the slow soleus and the fast plantaris areas. Since the intensity of histochemical staining at this pH has been shown to parallel speed of contraction and fatiguability, the results suggest an increase in the number of fibers possessing relatively slow contractile

properties and high resistance to fatigue. The decreases in staining intensity could have resulted from an actual transformation of fiber types, a decreased reaction across the muscle cells of one or more fiber types, or some combination of these possibilities.

The frequency distributions of the quantitative HCP readings for ATPase 9.4 show that the staining intensities within categorically determined groups of "dark" and "light" fibers are not homogeneous. There are gradations of both "dark" and "light". These gradations become very apparent in the graphs of the trained animals where the ranges of values are large. It is possible that an increased range of values reflects a progressive adaptive process in the contractile properties of the muscle.

Recent biochemical (15) and histochemical (208) studies have demonstrated exercise-induced changes in the ATPase activity of specific muscle fiber types. Baldwin et al. (15) reported significant changes in the specific activity of actomyosin ATPase in rat skeletal muscle homogenates after 18 weeks of endurance running on a treadmill. Similarly, Muller (208) found a decrease in the percentage of fast-twitch fibers in the soleus muscles of rats run for 12 weeks on a treadmill. The change in fiber-type composition was attributed to a transformation of fast-twitch fibers to slow-twitch fibers.

Physiological studies involving motor unit composition and recruitment also support the present findings. Motor units composed of muscle fibers with light ATPase 9.4 staining characteristics have been shown to be fatigue resistant (44,46). These units are involved in

maintaining prolonged low levels of physical activity (117). The observed decreases in ATPase 9.4 staining intensities found in this study suggest that both the END and the SPT training programs produced enhanced capacity for aerobic work.

A word of caution is appropriate regarding the evaluation and interpretation of ATPase findings. The ATPase reaction is highly pH sensitive. That sensitivity may have affected the results of the current study to some unknown degree. The three groups of animals used in the study were all involved in a larger diet-training experiment. The research design of the parent study required the tissues of the CON, SPT and END groups to be processed separately. The potential bias inherent in following such a protocol is obvious. However, the ATPase changes observed in this study were so striking that it appears they must have been due, at least in part, to the training programs.

Histochemical and biochemical procedures for demonstrating SDH reactivity are used routinely to indicate the aerobic capacity of individual muscle cells (see Table 2). Several studies using low-intensity exercise as a stimulus have reported increases in the activities of most tricarboxylic acid cycle enzymes (80,100,154,194,198,297). In this study, SDH staining intensity was enhanced in both muscle areas by both training regimens. This finding is in agreement with the ATPase 9.4 results and suggests that the SPT program has an aerobic component which is at least as great as that of the END program.

Metabolic adaptations specific to sprint-type training have recently been reported. Biochemical assays have shown a 70% increase

in phosphorylase activity (257), a 35% increase in pyruvate kinase activity (257), and a 17% increase in triosephosphate dehydrogenase activity (272) in soleus muscle homogenates of sprint-trained rats. Corresponding changes were not found in predominately FG, FOG or mixed muscles. In the present study, an increase in the glycogenolytic capacity of the muscle cells of the SPT animals was indicated by an increased number of high PAS and LDH readings. The shifts in distributions were greater in the soleus than in the plantaris. Since FG and FOG fibers have been shown to have higher initial levels of glycogenolytic and glycolytic enzymes than do SO fibers (see Tables 2 and 4), these findings are consistent with current knowledge.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study was undertaken to determine the effects of two strenuous training regimens on a histochemical profile of various fiber types. Two muscle areas were selected for study on the basis of homogeneity of fiber type: the central portion of the soleus (a predominately SO area) and the posterior part of the plantaris (an FG-FOG area). Normal male adult rats (Sprague-Dawley strain) were used as subjects. The two training regimens were modifications of Controlled-Running Wheel routines previously reported from this laboratory (286). The modified programs, an endurance running routine (END) and a sprint running routine (SPT), represented attempts to stimulate selectively either aerobic or anaerobic metabolic processes in the experimental animals. Histochemical profiles were determined using the reactions of ATPase 9.4 as an indicator of contractile speed, LDH to reflect lactate fermentation activity, SDH to indicate Krebs cycle activity and, SUD and PAS to localize intracellular fat and glycogen respectively.

Forty-two animals were brought into the laboratory and randomly assigned to CON, SPT and END treatment groups. An eight-week treatment period began when the animals were 84 days of age. Selected animals

were sacrificed 72 to 96 hours after their last training session.

Each histochemical stain was evaluated objectively with the use of a Histochemical Photometer (HCP). Photometric evaluations were determined in serial cross-sections for a group of 30 adjacent muscle fibers from each of the two muscle areas investigated. Selection criteria developed for training performance and staining characteristics resulted in a final frequency of nine animals per treatment group.

The histochemical data for each stain were plotted by treatment group and muscle area and statistically analyzed for distribution differences using a Chi-square contingency analysis (ACT routine).

All comparisons of frequency distributions by treatments were significant ($P < .01$) except that for SDH in the plantaris (Area 2). In most cases, the changes caused by training were similar in both magnitude and direction in the two muscle areas investigated. That is, the exercise-induced metabolic adaptations were similar in the SO soleus and the FG-FOG plantaris areas.

The SPT and END exercise regimens each produced a number of alterations in the histochemical profiles of the muscle cells. Both training regimens resulted in decreased staining intensities for ATPase 9.4 and increased reactivities to SDH staining. The SPT program specifically enhanced LDH and PAS staining reactions, whereas END training produced a large group of fibers staining darkly with SUD. In effect, the END training program resulted in an increased aerobic capacity of the muscle cells while the SPT program enhanced both their aerobic and anaerobic metabolic capacities.

Conclusions

The results of this study have led to the following conclusions:

1. A wide range of staining intensities for histochemically demonstrated metabolic markers can be found within each muscle fiber type.
2. The SPT and END training programs produced similar increases in the aerobic capacity of muscle cells in the two areas investigated. This adaptation was indicated by the increased staining reactions for SDH.
3. The contractile properties of the muscle cells involved were altered by both training regimens. This change was reflected by decreased reactivity to the ATPase 9.4 staining procedure.
4. The SPT training program resulted in specific anaerobic metabolic adaptations as indicated by the enhanced staining reactions to the LDH and PAS techniques.

Recommendations

1. The present study should be repeated with the intersession staining factor eliminated.
2. In any follow-up study using the SPT program, additional anaerobic metabolic markers should be included. The response of enzymes such as phosphorylase and phosphofructokinase would be helpful in evaluating the metabolic adaptations to sprint training.
3. The specifications of the SPT program should be refined to produce as specific an anaerobic effect as possible. In addition, other high-intensity exercise regimens for animals should be developed.

4. Correlative morphological, biochemical, histochemical and physiological studies are needed for complete muscle evaluations.
5. Power-type events for animals must be designed to add to the present knowledge of the metabolic adaptations resulting from activities across the endurance continuum. High-jumping and weight-lifting programs should be developed for this purpose.
6. Circulatory adjustments in skeletal muscle produced by anaerobic training should be investigated.
7. The metabolic adaptations in animals resulting from exercise should be substantiated in human subjects via muscle biopsy and energy metabolism studies. These results then should be extended to the applied clinical and training areas.
8. The effects of specific exercise regimens on the rate of flow and the composition of axoplasmic transport materials should be studied. In addition, the entire area of trophic relationships within the neuromuscular unit must be explored in relation to exercise.
9. Studies involving the recruitment patterns of motor units during specific types of exercise should be continued.

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APPENDICES

APPENDIX A

TRAINING PROGRAMS

Table A-1. Modified Eight Week Sprint 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 (m/min)	Total Time of Prog. (min: sec)	Total Exp. Meters TEM	Total Work Time (sec) TWT
0	4=T	-2	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1	5.0	0.0	27	40:00	---	---
1	1=M	1	2.0	00:10	10	10	8	2.5	1.2	36	42:50	480	800
	2=T	2	2.0	00:10	10	10	8	2.5	1.2	36	42:50	480	800
	3=W	3	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
	4=T	4	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
	5=F	5	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
2	1=M	6	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
	2=T	7	1.5	00:10	15	10	8	2.5	1.2	54	49:50	720	800
	3=W	8	1.5	00:15	30	6	7	2.5	1.2	72	43:00	756	630
	4=T	9	1.5	00:15	30	6	7	2.5	1.2	72	43:00	756	630
	5=F	10	1.5	00:15	30	6	7	2.5	1.2	72	43:00	756	630
3	1=M	11	1.5	00:15	30	6	7	2.5	1.2	72	43:00	756	630
	2=T	12	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
	3=W	13	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
	4=T	14	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
	5=F	15	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
4	1=M	16	1.5	00:15	30	6	6	2.5	1.2	81	36:30	729	540
	2=T	17	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
	3=W	18	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
	4=T	19	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
	5=F	20	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
5	1=M	21	2.0	00:15	30	5	6	2.5	1.2	90	32:00	675	450
	2=T	22	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
	3=W	23	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
	4=T	24	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
	5=F	25	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
6	1=M	26	2.0	00:15	30	5	6	2.5	1.2	99	32:00	743	450
	2=T	27	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	3=W	28	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	4=T	29	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	5=F	30	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
7	1=M	31	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	2=T	32	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	3=W	33	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	4=T	34	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	5=F	35	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
8	1=M	36	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	2=T	37	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	3=W	38	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	4=T	39	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450
	5=F	40	2.0	00:15	30	5	6	2.5	1.2	108	32:00	810	450

This training program is a modified version of a standard program designed using male rats of the Sprague-Dawley strain (150,278).

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.

APPENDIX A--continued

Table A-2. Modified Eight Week Endurance Training Program for Postpubertal and Adult Male Rats in Controlled-Running Wheels

Wk.	Day of Wk.	Day of Tr.	Acceler-ation Time (sec)	Work Time (min: sec)	Rest Time (sec)	Repeti-tions per Bout	No. of Com-plete Bouts	Par-tial Bouts (min: sec)	Time Be-tween Bouts (min)	Shock (ma)	Run Speed (m/min)	Total Time of Prog. (min: sec)	Total Exp. Meters TEM	Total Work Time (sec) TWT
0	4=T	-2	3.0	40:00	10	1	1		5.0	0.0	27	40:00	---	---
	5=F	-1	3.0	40:00	10	1	1		5.0	0.0	27	40:00	---	---
1	1=M	1	2.0	02:30	0	1	6		2.5	1.2	27	27:30	405	900
	2=T	2	2.0	02:30	0	1	6		2.5	1.2	27	27:30	405	900
	3=W	3	1.5	05:00	0	1	3		5.0	1.2	36	25:00	540	900
	4=T	4	1.5	05:00	0	1	3		5.0	1.2	36	25:00	540	900
	5=F	5	1.5	05:00	0	1	3		5.0	1.2	36	25:00	540	900
2	1=M	6	1.5	05:00	0	1	3		5.0	1.2	36	25:00	540	900
	2=T	7	1.0	07:30	0	1	2		5.0	1.2	36	20:00	540	900
	3=W	8	1.0	07:30	0	1	2		2.5	1.2	36	17:30	540	900
	4=T	9	1.0	07:30	0	1	2		1.0	1.2	36	16:00	540	900
	5=F	10	1.0	15:00	0	1	1		0.0	1.2	36	15:00	540	900
3	1=M	11	1.0	15:00	0	1	1	05:00	1.0	1.2	36	21:00	720	1200
	2=T	12	1.0	15:00	0	1	1	07:30	1.0	1.0	36	23:30	810	1350
	3=W	13	1.0	15:00	0	1	1	10:00	1.0	1.0	36	26:00	900	1500
	4=T	14	1.0	15:00	0	1	1	12:30	1.0	1.0	36	28:30	990	1650
	5=F	15	1.0	15:00	0	1	2		1.0	1.0	36	31:00	1080	1800
4	1=M	16	1.0	15:00	0	1	2	05:00	1.0	1.0	36	37:00	1260	2100
	2=T	17	1.0	15:00	0	1	2	07:30	1.0	1.0	36	39:30	1350	2250
	3=W	18	1.0	15:00	0	1	2	10:00	1.0	1.0	36	42:00	1440	2400
	4=T	19	1.0	15:00	0	1	2	12:30	1.0	1.0	36	44:30	1530	2550
	5=F	20	1.0	15:00	0	1	3		1.0	1.0	36	47:00	1620	2700
5	1=M	21	1.0	15:00	0	1	3	05:00	1.0	1.0	36	52:00	1800	3000
	2=T	22	1.0	15:00	0	1	3	07:30	1.0	1.0	36	54:30	1890	3150
	3=W	23	1.0	15:00	0	1	3	10:00	1.0	1.0	36	57:00	1980	3300
	4=T	24	1.0	15:00	0	1	3	12:30	1.0	1.0	36	59:30	2070	3450
	5=F	25	1.0	15:00	0	1	4		1.0	1.0	36	63:00	2160	3600
6	1=M	26	1.0	15:00	0	1	4		1.0	1.0	36	64:00	2160	3600
	2=T	27	1.0	30:00	0	1	2		5.0	1.0	36	65:00	2160	3600
	3=W	28	1.0	30:00	0	1	2		2.5	1.0	36	62:30	2160	3600
	4=T	29	1.0	30:00	0	1	2		1.0	1.0	36	61:00	2160	3600
	5=F	30	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
7	1=M	31	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	2=T	32	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	3=W	33	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	4=T	34	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	5=F	35	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
8	1=M	36	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	2=T	37	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	3=W	38	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	4=T	39	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600
	5=F	40	1.0	60:00	0	1	1		0.0	1.0	36	60:00	2160	3600

This training program is a modified version of a standard program designed using male rats of the Sprague-Dawley strain (150,278).

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 in the animals and will seriously impair the effectiveness of the training program.

APPENDIX B

BASIC STATISTICS FOR TRAINING DATA

Table B-1. Basic Statistics for Percentage of Body Weight Loss, Environmental Factors and Performance Criteria for the Sprint Running Group

				Simple Correlations				
Variable	N[a]	Mean	Standard Deviation	Air Temp.	Percent Humidity	Bar. Press.	Percent Body Weight Loss	PEM
Air Temp. (°F.)	340	73.1	4.6					
Percent Humidity	340	38.6	12.3	.122				
Bar. Press. (mm Hg)	340	740.7	4.2	-.255	-.717			
Percent Body Wgt Loss	340	1.7	.6	-.041	-.182	.029		
PEM	340	66.2	25.1	-.197	-.477	.266	.100	
PSF	340	66.5	22.3	-.287	-.339	.155	.038	.840

[a]Total training days for all animals.

Table B-2. Basic Statistics for Percentage of Body Weight Loss, Environmental Factors and Performance Criteria for the Endurance Running Group

				Simple Correlation				
Variable	N[a]	Mean	Standard Deviation	Air Temp.	Percent Humidity	Bar. Press.	Percent Body Weight Loss	PEM
Air Temp. (°F.)	314	73.9	4.0					
Percent Humidity	314	47.1	10.6	.147				
Bar. Press (mm Hg)	314	739.5	3.8	-.290	-.679			
Percent Body Wgt Loss	314	2.7	1.0	.455	.131	-.173		
PEM	314	81.3	19.3	-.258	-.263	.323	-.060	
PSF	314	68.4	19.4	-.282	-.120	.174	.759	.759

[a]Total training days for all animals.