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ALTERATIONS IN THE DEVELOPMENTAL PATTERN OF SKELETAL MUSCLE FIBRE TYPES IN RESPONSE TO ENDURANCE AND SPRINT TRAINING

by

BRENDA G. MACKIE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ALTERATIONS IN THE DEVELOPMENTAL PATTERN OF SKELETAL MUSCLE FIBRE TYPES IN RESPONSE TO ENDURANCE AND SPRINT TRAINING, submitted by BRENDA G. MACKIE in partial fulfilment of the requirements for the degree of Master of Science.

Howard A. Williams
Supervisor

Date August 13, 1976

ABSTRACT

A histochemical assessment of myosin ATPase, NADH diaphorase and α-GPD activity was used to identify the fibre composition of the soleus, plantaris II, and medial head of the gastrocnemius of 5 and 15 week old male Wistar rats. Fibres were classified as slow-oxidative (SO), fast-oxidative-glycolytic (FOG) or fast glycolytic (FG). The 10 weeks of aging resulted in a significant ($p < .05$) reduction in the complement of FOG fibres with concommitant increases in SO fibres in soleus and FG fibres in gastrocnemius. There was no significant developmental alteration in fibre types in the homogeneously fast twitch plantaris II.

The effects of sprint or endurance training on these patterns of differentiation as well as total body weight and wet muscle weights were investigated. Both training groups (N=10 for each) began training at 5 weeks of age. The endurance trained (ET) animals were gradually accustomed to a 30 minute run at [illegible] m/min., 8% grade. The sprint trained (ST) group were similarly adapted to an interval program of 10 bouts of 15 seconds duration followed by 20 seconds of rest at 80 m/min., 30% grade. Both groups were trained twice daily, 4 times per week for 10 weeks.

After the 10 week program, the ET and ST animals displayed a reduced body weight in relation to age matched controls but only in the ET group was this difference significant ($p < .05$). The wet weights of the plantaris and gastrocnemius from the ET rats were significantly ($p < .05$) lighter while the soleus and all three muscles from the ST group did not differ significantly from controls.

The fibre type composition in the ET group was not significantly different ($p > .05$) from the 15 week controls although tra[illegible]ed to

maintain the percentage of FOG fibres in the soleus and gastrocnemius. Sprint training elicited in a signfficant ($p < .05$) increment in the proportion of FOG fibres in soleus but resulted in a further augmentation of the FOG to FG developmental shift seen in the heterogeneous gastrocnemius. Neither training program altered the fibre type profile of plantaris II or the proportion of SO fibres in the medial gastrocnemius.

These findings indicate that fibre type adaptations to training are probably a reflection of the motor unit recruitment pattern which is, in turn, dependent upon the stimulus and the fibre composition of the exercising muscle. The evidence from this study tends to support the view that training during a time when development differentiation is still occurring may serve to partially offset the normal aging patterns.

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A special debt of thanks is owed to my advisor, Dr. Howard Wenger whose confidence and optimism proved as valuable as his guidance.

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INTRODUCTION

The elucidation of the metabolic profiles of skeletal muscle fibre types has involved an accumulation of knowledge that stretches back to the previous century. The importance of this knowledge in facilitating an understanding of the mechanisms of adaptation of skeletal muscle to a specific exercise stimulus has only recently been the subject of extensive investigation. The practical significance of all this research has, in turn, only just begun to be realized.

Most mammalian skeletal muscle is composed of three fibre types whose distinct biochemical and contractile properties have been described in detail (Barnard et al., 1971; Burke et al., 1971; Close, 1972; Peter et al., 1972). Following the system of nomenclature proposed by Peter et al. (1972) fibres are classified as either slow twitch-oxidative (SO), fast twitch-oxidative-glycolytic (FOG) or fast twitch-glycolytic (FG) in this study. Fast and slow twitch fibres may be distinguished by a qualitative histochemical staining procedure for alkaline stabile myosin adenosine triphosphatase (ATPase), an enzyme whose activity correlates highly with the speed of contraction of individual fibres (Barany et al., 1967). A similar assessment of reduced nicotinamide adenine dinucleotide (NADH) diaphorase and α-glycerophosphate dehydrogenase (α-GPD) can be used to further identify oxidative and glycolytic capacities respectively. The fibre composition of a skeletal muscle is variable depending on the particular muscle studied, the species of animal, its age, or state of training. Of all these

factors, the last one is subject to external manipulation and therefore has been of considerable interest to exercise physiologists.

Several authors have shown that endurance training elicits a variety of biochemical adaptations in both the oxidative and glycolytic metabolic pathways of skeletal muscle. These include an increase in the size and number of mitochondria and a two-fold increase in their ability to oxidize pyruvate (Baldwin et al., 1973; Barnard et al., 1970a; Gollnick and King, 1969; Holloszy, 1967). The activities of such oxidative enzymes as succinate dehydrogenase (SDH), NADH dehydrogenase, cytochrome oxidase (Baldwin et al., 1972; Edgerton et al., 1972; Kowalski et al., 1969; Holloszy, 1967), hexokinase (Holloszy et al., 1971, Morgan et al., 1971), and the ability of whole muscle to oxidize fatty acids (Baldwin et al., 1972; Paul and Issekutz, 1967) are significantly augmented as well.

Glycolytic enzyme adaptations are less consistently reported. No changes in phosphorylase, aldolase and pyruvate kinase have been documented while levels of phosphofructokinase (PFK), lactate dehydrogenase (LDH), and GPD appear to decrease in the muscles of endurance trained men (Morgan et al., 1971) and rats (Holloszy et al., 1971).

Histochemical analyses have provided the logic for some of the inconsistencies observed in both the direction and degree of adaptation by elucidating the specificity of the training response to different fibre types.

The work of several researchers has verified the increase in the proportion of fibres classified as highly oxidative in exercised rat muscle noted by Edgerton and Simpson (1969). In mixed muscle, an

increment in the percentage of FOG fibres at the expense of FG fibres has been shown to occur in endurance trained rats (Kowalski et al., 1969; Wilkinson et al., 1976), guinea pigs (Barnard et al., 1970; Faulkner et al., 1971, Maxwell et al., 1973), and the Lesser Bushbaby (Edgerton et al., 1972). The soleus, which contains only SO and FOG fibres, does not display any shift in its fibre population with a similar program (Edgerton et al., 1969; Maxwell et al., 1973).

The question of whether twitch times of fibres can be altered by training is a contentious issue. Most biochemical (Bagby et al., 1972; Edgerton et al., 1972) and histochemical (Barnard et al., 1970a; Edgerton et al., 1969; Edgerton et al., 1972; Syrovy et al., 1972) evidence indicates this is not the case in adult animals. Baldwin et al. (1975) disagree. They have found that myosin ATPase activity increases 20% in the FOG portion of vastus lateralis, declines by an identical amount in soleus and remains unaltered in the FG fibres of the vastus lateralis of endurance trained rats. The variation in the ATPase response may be dependent upon the age of the experimental animal (Gutmann and Hajek, 1971; Syrovy et al., 1972) or the intensity of the exercise program (Wilkerson and Evonuk, 1971).

The literature on the effects of sprint training on the fibre composition of skeletal muscle is scant. Staudte et al., (1973) have shown increases in the enzymes of glycogenolysis and glycolysis which were more pronounced in the slow soleus than in the fast rectus femoris of anaerobically trained rats. Histochemical evidence has suggested that the proportion of FOG fibres increases at the expense of FG fibres in a fast muscle (white gastrocnemius) or mixed muscle (plantaris III) and at the expense of SO fibres in soleus (Saubert et al., 1973;

Wilkinson et al., 1976). In contrast, Thorstensson et al. (1975) using human subjects could find no alteration in the fibre type population of the vastus lateralis after an 8-week sprint program.

Although there is lack of accord in many aspects of the literature, the fact that skeletal muscle response to training is specific not only to the nature of the stimulus but also to the anatomical location or action of the muscle in the exercise and to its fibre composition (Edgerton et al., 1972) is repeatedly borne out. This adaptation specificity is a function of the motor unit recruitment frequency elicited in the muscle by a particular exercise. Studies of glycogen depletion patterns during single bouts of activity in man (Gollnick et al., 1973; Gollnick et al., 1974, Thorstensson et al., 1975) and various animals (Armstrong et al., 1974; Armstrong et al., 1976; Edstrom and Kugelberg, 1968; Gillespie et al., 1974) have supported the view that the type and magnitude of training reflects the usage of each motor unit. Low intensity exercise calls upon FOG, then SO, and lastly FG fibres while short duration, sprint work recruits FG, FOG, and finally SO fibres (Burke and Edgerton, 1975). Adaptation could be expected according to this sequence, if the fibre composition of the muscle and fatigue factors are also considered.

Often, in many training studies, the age of the experimental animal has been overlooked or ignored as a possible confounding variable. The prenatal differentiation process in rat skeletal muscle continues postnatally. At birth, all fibres are relatively slow in comparison to the adult state (Gutmann et al., 1973) but contraction time increases during the first 3-5 weeks of life (Brown et al., 1973). The twitch time then stabilizes in SO fibres while further increasing in FOG and FG

fibres until the mature state is reached (Buller et al., 1960). Concommitant changes in myosin ATPase activity (Gutmann et al., 1973), glycolytic enzyme activity (Bass et al., 1970; Mann and Salafsky, 1970), and oxidative enzyme activity (Bass et al., 1970; Goldspink, 1969; Maxwell et al., 1973) occur in close association with twitch time differentiation. These enzymatic changes are reflected in the fibre type alterations such as those noted in the plantaris and soleus of young guinea pigs (Faulkner et al., 1971; Karpati and Engel, 1967; Maxwell et al., 1973) and rats (Wilkinson et al., 1976). In each case, a loss of FOG fibres and a corresponding increase in FG fibres in plantaris and SO fibres in soleus were seen with aging.

The few studies that have related fibre type adaptations to both training and aging have suggested that the exercise stimulus, rather than causing fibre type conversions, may actually preserve or maintain the fibre composition of non-mature muscle (Muller, 1974; Wilkinson et al., 1976). Stained sections of the plantaris of 14 week old trained guinea pigs have revealed a close resemblance to those of 6 week old controls (Maxwell et al., 1973). In rats, a similar relationship has been shown to exist between both the plantaris III region and soleus of a 15 week old exercise group and 10 week old controls (Wilkinson et al., 1976) and between 18 week old trained rectus femoris and 9 week controls (Muller, 1974). Generally, training seems to partially offset the normal loss of FOG fibres seen during development.

A further consideration is that some researchers have put forward the theory that young, developing tissue has a greater potential for adaptation than the mature counterparts (Schiaffino and Bormioli, 1973). This possibility has been investigated in human subjects as well

(Astrand et al., 1963; Ekblom, 1969).

It was the purpose of this study to confirm the aging patterns of skeletal muscle fibre types noted by previous authors. It was of further interest to investigate the differential effects of sprint and endurance training on the fibre composition of three distinctly different muscles and then relate any significant changes to the normal developmental pattern of differentiation of fibre types seen in the control groups.

METHODS AND PROCEDURES

EXPERIMENTAL ANIMALS

Thirty male Wistar rats were obtained at 4 weeks of age, numbered, and then randomly assigned to one of three groups: endurance trained, sprint trained, or control. A fourth group of 4 week old rats (N=10) was incorporated into the study a week prior to data collection. All animals were housed in individual, self-cleaning cages in an air conditioned room maintained at 22.5°C. As these animals are nocturnal, a reversal of the normal light/dark sequence was imposed (light 6 p.m. to 6 a.m.) to allow training during the work day. Throughout the experiment Purina Rat Chow and water were provided ad libitum. The daily ritual included rotation of cages, replenishment of food and water, changing of soiled papers and the handling of control animals.

TRAINING PROTOCOL

All experimental animals were exercised on a motor driven treadmill divided into 10 9.5 x 48 cm. compartments each with a shock grid at the back. After a preliminary week of orientation to the laboratory, training sessions were conducted 4 times weekly (Monday, Tuesday, Thursday, Friday), at approximately the same time morning and afternoon for 10 weeks.

The endurance program initially consisted of a 5 minute continuous run at 15 m/min., 8% grade. The speed was gradually increased to 30 m/min. in the first week after which the duration of the run was systematically lengthened by 2 minutes each training day to 30 minutes.

The sprint group began with 10 bouts of a 5 second run at 15 m/min. followed by a 20 second rest at a 30% grade. At the end of the first week, all rats were completing 10 15 second bouts at 30 m/min. after which the speed only was increased 5 m/min. daily to a maximum of 80 m/min. The animals were maintained at these respective training loads until sacrifice.

Body weights were recorded upon arrival, during the third and seventh weeks of training, and again just prior to sacrifice (Table I). A performance test was also conducted on both groups of animals. This involved a run to fatigue at 40 m/min. at the beginning of the ninth week of the program (Table III).

TISSUE COLLECTION, PREPARATION AND ANALYSIS

All animals were sacrificed by decapitation and the soleus, gastrocnemius and plantaris muscles immediately excised from the right hind limb. The proximal one third of each muscle was mounted in gummed cork and quickly frozen in isopentane cooled in liquid nitrogen and stored at -30°C for subsequent analysis. In the case of the gastrocnemius, the medial head was dissected free and that portion only was used. Wet weights of the whole muscle were obtained from the contralateral limb (Table I).

Serial sections 10 μ thick were cut and mounted on microscope slides in preparation for histochemical staining. The activity of NADH diaphorase was determined according to Dubowitz and Brooke (1973), the activity of ATPase according to Padykula and Herman (1955) as modified by Guth and Samaha (1969), and the activity of αGPD according to the method of Wattenberg and Leong (1960).

Fibre counts were made on the selected areas listed below:

1. Soleus: This muscle is approximately 80% SO and 20% FOG in the adult rat (Baldwin et al., 1973; Edgerton et al., 1969; Schiaffino and Bormioli, 1973; Syrovy et al., 1972).

2. Plantaris II: This region of the plantaris is exclusively fast twitch being composed of 50 to 75% FG and 25-50% FOG fibres (Edgerton et al., 1969; Wilkinson et al., 1975).

3. Gastrocnemius (medial head): This is a mixed muscle with a reported fibre population of 36% FG, 48% FOG, and 16% SO fibres (Barnard et al., 1970a; Muller, 1974).

An NADH diaphorase and ATPase slide for each muscle were simultaneously projected onto a white surface by two Tri-Simplex microprojectors (Bausch and Lomb) and identical areas of approximately 250-300 fibres outlined. Fibres were then counted and classified according to Peter et al. (1972) as previously described. Fibre population was expressed as a percentage of the total number of fibres counted. The glycolytic stain was used as an additional aid in identification.

These three particular muscle areas were chosen because, although they are all involved in plantar flexion of the foot, their fibre population is distinctly diverse. In addition, their gross structure and function in locomotion are different. The gastrocnemius-plantaris provides the propelling force in actions such as walking, running, or leaping, while the postural function of soleus is of more importance than its value as a prime mover (Davies and Davies, 1962). The structure of the muscle reflects these functions. Gastrocnemius is a bipennate muscle with shorter fibres, but more of them for its weight than soleus and can develop as much tension on a per weight

basis but has a limited contraction range. Soleus, on the other hand, is a longitudinal muscle which generates less tension but displays a greater degree of shortening (Henneman and Olson, 1965).

The two groups of control rats were 5 and 15 weeks of age at the time of sacrifice. The 15 week old trained animals were sacrificed 60 hours after their last exercise session to avoid any effects of acute exercise on the data.

STATISTICAL ANALYSIS

A one-way analysis of variance was used to test the hypothesis that there was no significant difference among the four groups in the mean percentage of each fibre type in each of the three muscles. One sample analysis may be outlined diagrammatically as follows:

		(DEPENDENT VARIABLE) Fibre Type Percentage
(INDEPENDENT VARIABLES)	5 week controls	
	15 week controls	
	Endurance	
	Sprint	

Where a significant F ratio ($p < .05$) was found, a Newman-Keuls test was employed to isolate the pairs of means which did differ significantly. Similar analyses were performed on the data summarizing the wet muscle weights and body weights of the four groups of animals.

RESULTS

Both the endurance and sprint trained groups of runners adapted easily to the training regimen in the initial stages of the experiment. The sprinters experienced some difficulties at the highest speeds and, consequently, injuries resulted in the loss of two animals from that group. The 30 minute continuous run at 30 m/min. was well within the capabilities of the endurance group (Appendix B, Table III) but one non-runner had to be eliminated from the study.

BODY AND MUSCLE WEIGHTS

The data in Table I are a summary of the mean body weights and mean wet weights of the left soleus, plantaris and gastrocnemius muscles for each of the four groups of rats at the time of sacrifice. An analysis of variance (Appendix B, Table VIII) and subsequent Newman-Keuls test on these means revealed that, while both experimental groups weighed substantially less than the 15 week controls, this difference was significant ($p < .05$) only in the endurance trained group. The body weights of the two training groups were not statistically different from each other although the sprint trained rats were, on the average, 30 grams heavier.

The wet weight of the soleus muscle was not altered by either exercise program but the weights of both the plantaris and gastrocnemius of the aerobic animals were significantly less ($p < .01$, $p < .05$) than those excised from controls (Table I). Sprint training did not affect

TABLE I

MEAN BODY WEIGHTS AND WET MUSCLE WEIGHTS (GRAMS) OF THE FOUR GROUPS AT TIME OF SACRIFICE

GROUP	N	BODY WEIGHT (g.)	MUSCLE WEIGHTS (g.) SOLEUS	PLANTARIS	GASTROCNEMIUS
5 WEEK CONTROLS	10	120.10 ± 1.07	0.044 ± .003	0.084 ± .044	0.434 ± .007
15 WEEK CONTROLS	10	407.38 ± 13.81	0.166 ± .035	0.370 ± .012	1.897 ± .031
ENDURANCE TRAINED	9	339.22 ± 24.92*	0.154 ± .010	0.289 ± .053*	1.581 ± .130*
SPRINT TRAINED	8	368.38 ± 15.89	0.160 ± .012	0.358 ± .020	1.824 ± .470

[a]Values are $\bar{X} \pm$ SEM

*Significantly different from 15 week controls ($p < .05$)

any of the three muscle weights as compared to age matched controls.

MUSCLE FIBRE POPULATION

Fibre typing. Fast and slow twitch fibres were easily distinguished by the ATPase stain in all muscle sections (Appendix C, Plates 1,2,5). Any discrepancies were resolved by referring to the matching GPD slide (Appendix C, Plate 4). Further identification of the oxidative capacity of fast fibres by NADH diaphorase staining intensity was somewhat more difficult. A decision was made to classify all fast twitch fibres with a darkly staining periphery fading to pale blue in the central area as FOG. Uniformly pale blue or white colored fibres were designated as FG (Appendix C, Plates 3,6). These criteria were used consistently throughout the fibre typing process.

Effects of aging. A significant decrease ($p < .001$) in the percentage of FOG fibres occurred in both the soleus and gastrocnemius muscles of the 15 week old control rats as a result of 10 weeks of aging. In soleus, this loss was coupled with a corresponding increase in the percentage of SO fibres (Fig. 1). The proportion of SO fibres in the medial head of the gastrocnemius, on the other hand, remained unchanged while the percentage of FG fibres was significantly ($p < .01$) elevated in the older animals (Fig. 2). The fast twitch portion of the plantaris did not display any significant change in fibre composition from 5 to 15 weeks (Fig. 3) but the trend towards an increase in the proportion of FOG fibres at the expense of FG fibres was directionally opposite to that seen in the two other muscles investigated.

Effects of training. The endurance program did not significantly alter the normal developmental pattern of muscle fibre types as displayed by

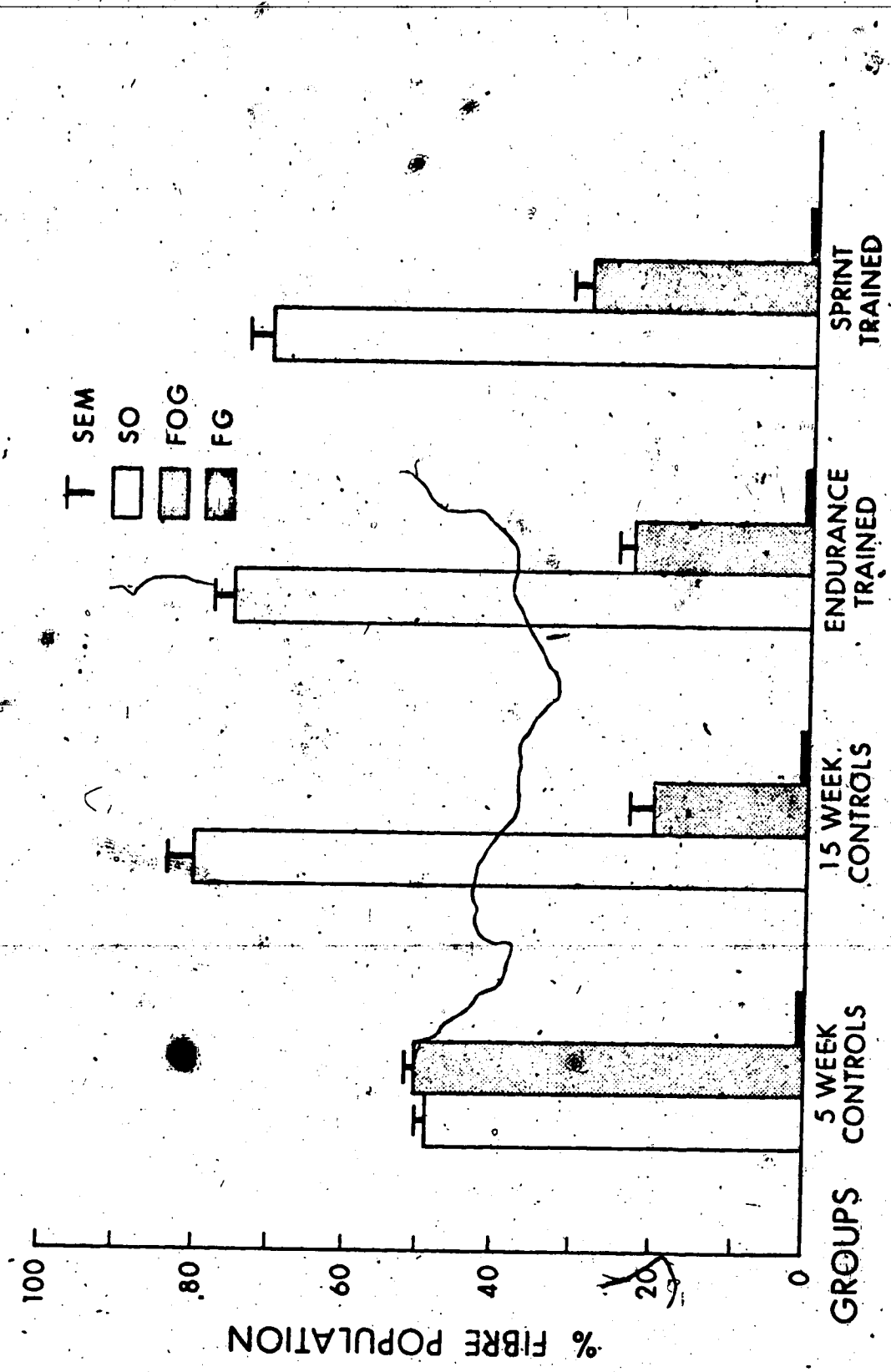

Figure 1: Mean (±SEM) percentage fibre population in the soleus of the four groups of rats.

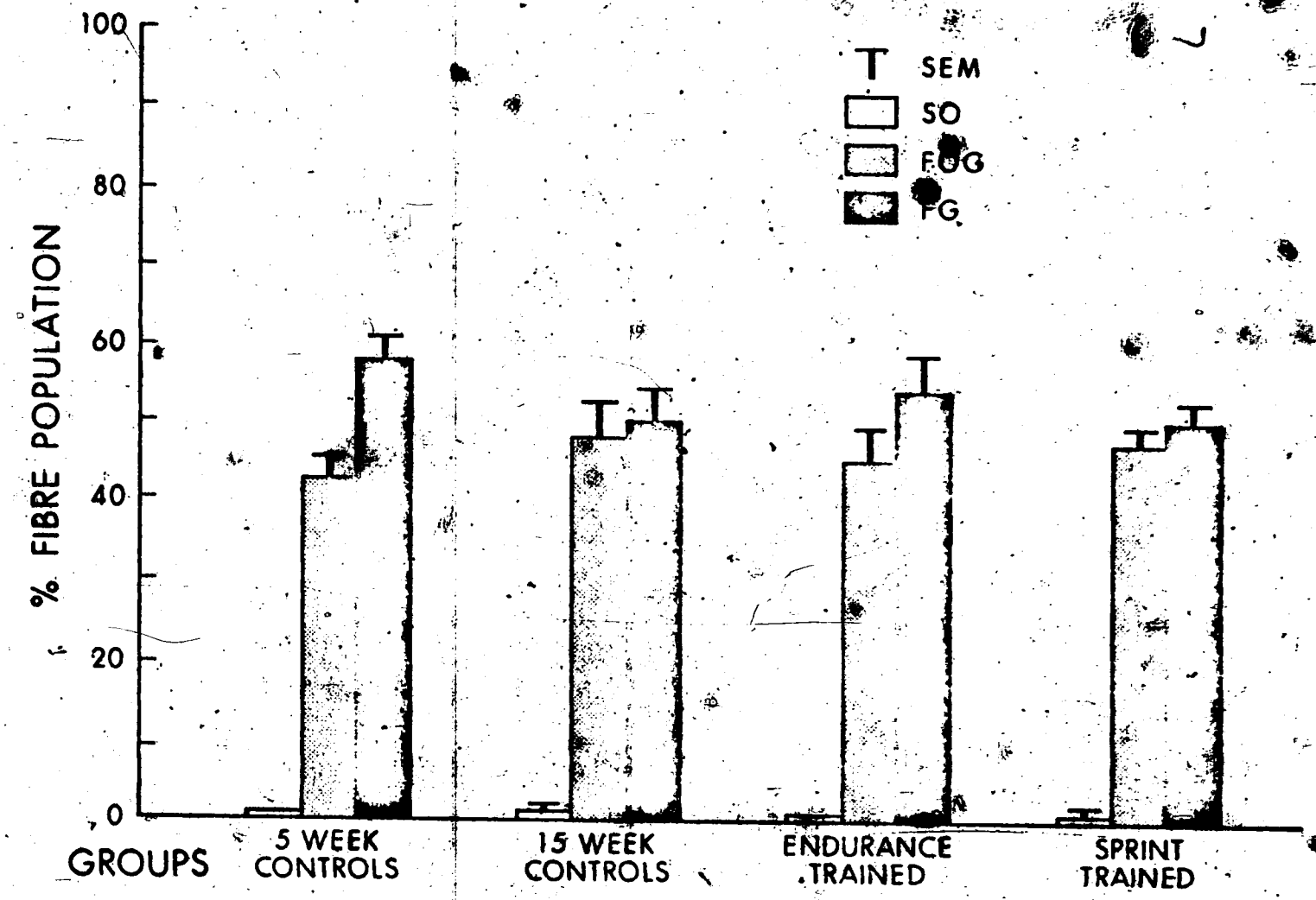

Figure 2: Mean (±SEM) percentage fibre population in the plantaris of the four groups of rats.

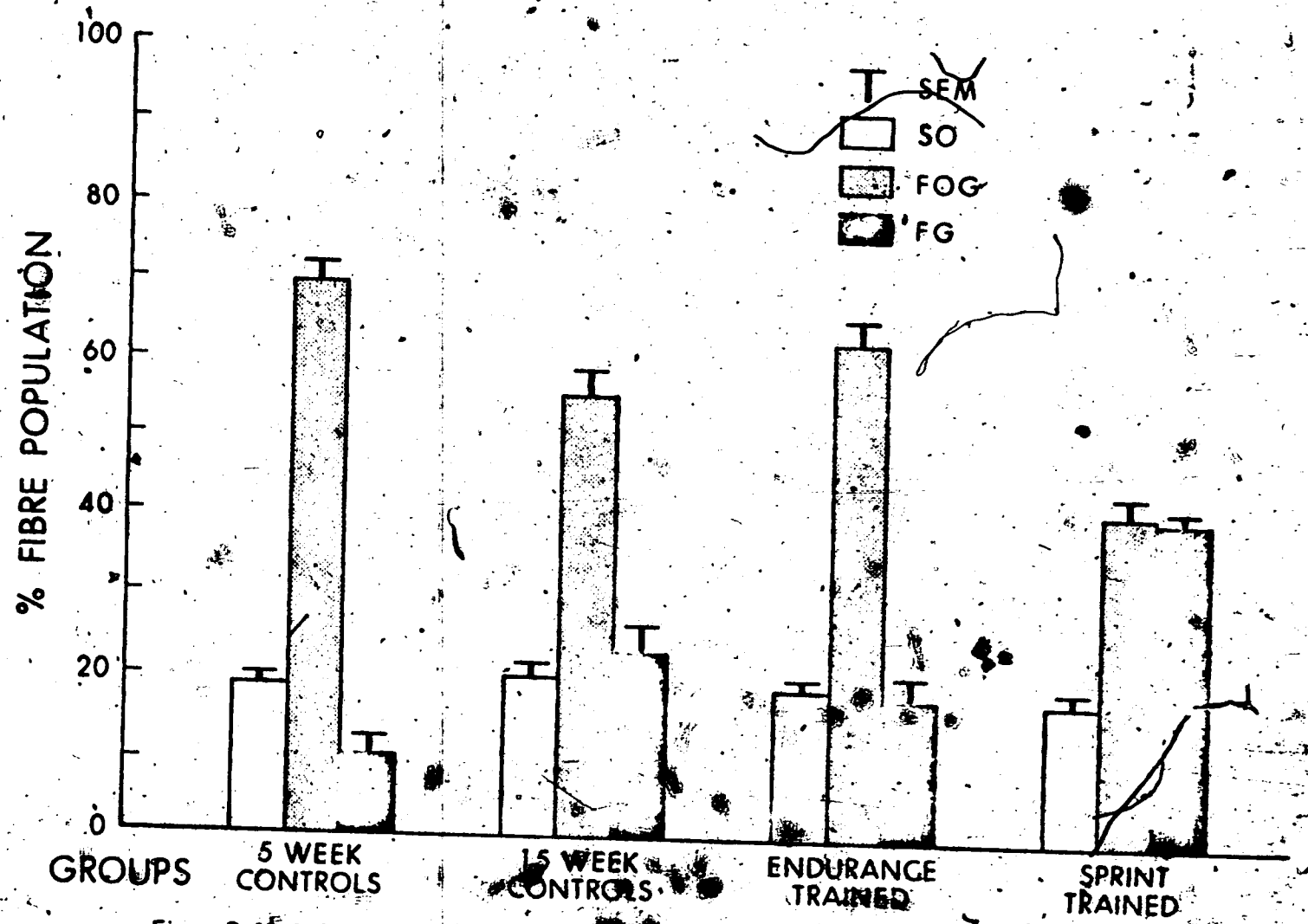

Figure 3: Mean (±SEM) percentage fibre population in the medial head of the gastrocnemius of the four groups of rats.

the control rats from 5 to 15 weeks. This was the case in all three muscles from the trained group. However, the tendency was for the endurance trained group to maintain a higher percentage of FOG fibres in the soleus (Fig. 1) and gastrocnemius (Fig. 3) and FG fibres in plantaris II (Fig. 2).

The fibre profiles of the soleus and gastrocnemius muscles were significantly different in the sprint trained rats as compared to their age matched counterparts. In soleus, the proportion of FOG fibres was significantly greater ($p < .01$) in the sprint exercised group (Fig. 1) reflecting a preservation of the non-mature pattern. In contrast, sprint training served to further accentuate the FOG to FG development shift seen in the gastrocnemius (Fig. 3). No changes occurred in the plantaris II region as a result of sprint training (Fig. 2).

The only differential effect of the two training programs on fibre population was seen in the gastrocnemius where the percentage of FOG fibres was significantly higher ($p < .01$) and the percentage of FG fibres lower ($p < .01$) in the endurance trained muscles.

A summary of significant differences in the mean percentages of each fibre type is contained in Table II. The raw data from which mean percentages were calculated appears in Appendix B, Tables IV-VII.

TABLE II

SUMMARY OF SIGNIFICANT DIFFERENCES IN MEAN FIBRE TYPE PERCENTAGES[a]

GROUP & MUSCLE	FIBRE TYPE %	GROUPS[b] C_5	C_{15}	ET	ST
SOLEUS					
C_5	SO 48.78(±0.88)	—	*	*	*
	FOG 51.22(±0.88)		*	*	*
C_{15}	SO 80.38(±2.63)		—	NS	*
	FOG 19.62(±2.63)			NS	*
ET	SO 76.73(±1.84)			—	NS
	FOG 23.27(±1.84)				NS
ST	SO 71.08(±2.38)				—
	FOG 28.92(±2.38)				
PLANTARIS					
C_5	FOG 42.53(±1.72)	—	NS	NS	NS
	FG 54.47(±1.72)		NS	NS	NS
C_{15}	FOG 48.07(±2.58)		—	NS	NS
	FG 50.50(±2.92)			NS	NS
ET	FOG 45.05(±2.78)			—	NS
	FG 54.30(±2.83)				NS
ST	FOG 48.16(±1.53)				—
	FG 51.34(±1.66)				
GASTROCNEMIUS					
C_5	SO 18.37(±1.06)	—	NS	NS	NS
	FOG 69.99(±1.96)		*	*	*
	FG 11.63(±1.45)		*	NS	*
C_{15}	SO 20.63(±1.37)			NS	NS
	FOG 56.19(±2.64)		—	NS	*
	FG 23.18(±3.03)			NS	*
ET	SO 18.82(±1.18)				NS
	FOG 62.93(±1.95)			—	*
	FG 18.24(±2.41)				*
ST	SO 18.02(±1.23)				
	FOG 41.04(±1.80)				—
	FG 40.94(±0.93)				

[a]Percentages are means ± SEM

[b]C_5 = 5 week controls, C_{15} = 15 week controls, ET = endurance trained, ST = sprint trained

*Significant at $p < .01$

DISCUSSION

BODY AND WET MUSCLE WEIGHTS

The significantly reduced mean body weight of the endurance group (339 g.) in comparison to age matched controls (407 g.) has been previously noted in rats (Hubbard et al., 1974; Syrovy et al., 1972; Wilkinson et al., 1976) and guinea pigs (Barnard et al., 1970a). Although the sprinters weighed, on the average, 40 grams less than controls, this difference was not significant ($p > .05$) (Table I). This finding is not in agreement with the data of Staudte et al. (1973) and Wilkinson et al. (1976) both of whom reported a decrease in the body weight of rats exposed to a sprint training program.

It is difficult to make conclusive statements on the effects of either type of training on body weight as the groups were not weight matched initially nor was food intake monitored or body composition assessed. However, it seems that the caloric expenditure required by regular exercise does contribute to a reduced weight gain especially in the endurance group where the total daily workload is probably greater. As Table I indicates, smaller muscle size as well as a lower percent body fat may account for the differences.

The non-significantly different wet weights of the soleus, plantaris and gastrocnemius of sprint trained rats as compared to controls (Table I) could imply a failure of the total muscle to undergo hypertrophy. Staudte et al. (1973) reported a similar occurrence in the rectus femoris and soleus of his sprint trained group. The relative

muscle weights (muscle weight ÷ body weight) of the soleus ($4.54X10^{-4}$) and gastrocnemius ($4.95X10^{-3}$) compare favorably to the values of $4.40X10^{-4}$ and $5.57X10^{-3}$ reported by Muller (1975) in 13 week old rats which had trained isometrically for 4 weeks. These relative muscle weights also did not differ significantly from controls. While the lack of gross muscle hypertrophy could be due to an insufficient training stimulus, it is possible that a localized hypertrophy of FG fibres only has occurred with FOG and SO fibres undergoing no change or even a slight decrease in size (Gordon et al., 1967). The net result would be no change in muscle size

The aerobic training program did not affect the soleus muscle which agrees with the findings of Syrovy et al. (1972), but caused a significant ($p < .05$) reduction in the wet weights of both the plantaris and gastrocnemius. This is in contradiction to the 11 to 14% increases reported by Muller (1974) in the soleus, rectus femoris and gastrocnemius of aerobically exercised rats.

The lack of weight loss in the soleus as contrasted to the loss in the other two muscles may be attributable to the higher percentage of SO fibres and hence to a more frequent contraction during the low intensity exercise. As a result, these active motor units would maintain larger fibre diameters than unused motor units. Maxwell et al. (1973) reached similar conclusions based on their observations of fibre areas in the soleus and plantaris of endurance trained guinea pigs compared to controls. Although hypertrophy with endurance training is well documented (Gordon et al., 1967) these reduced muscle weights are difficult to explain especially without supporting planimetry data.

HISTOCHEMICAL RESULTS

The mean percentage fibre populations (Fig. 1,2,3 and Appendix B,

Tables IV-VII) of the soleus (80.4% SO; 19.6% FOG), plantaris II (1.4% SO: 48.1% FOG: 50.5% FG) and medial gastrocnemius (20.6% SO: 56.2% FOG: 23.2% FG) of the 15 week control animals in this study fall within the ranges documented by other researchers in mature rats (Ariano et al.1973; Edgerton and Simpson,1969; Muller,1974; Saubert et al.1973; Wilkinson et al., 1976).

Unfortunately, there are very few sources of comparison for the fibre type data obtained from the 5 week controls. In the present study, the soleus was found to be almost equally comprised of SO and FOG fibres while the plantaris II contained only FOG and FG fibres, again in approximately equal proportions. Wilkinson et al. (1976) described similar distribution patterns in 6 week old rats. As yet, there is no corroborating data available to confirm the composition of the gastrocnemius.

A pronounced aging effect was seen in the soleus and medial gastrocnemius from 5 to 15 weeks while the fibre composition of the plantaris II region remained relatively unchanged (Figs. 1,2,3). The developmental increase in the proportion of SO fibres at the expense of FOG fibres in soleus was also noted by Wilkinson et al. (1976) in rats and by Maxwell et al. (1973) in guinea pigs. The 25% decline in biochemically determined ATPase activity in rat soleus from 30 to 365 days reported by Gutmann et al. (1974) would also seem to support the decrement in fast twitch fibres with age.

The FOG to FG fibre shift with no change in the SO component seen in the gastrocnemius during the 10 week aging period compares favorably with the alterations reported in other muscles of mixed fibre composition such as the deep portion of rat rectus femoris (Muller, 1974) plantaris II (Wilkinson et al., 1976) and guinea pig plantaris

(Maxwell et al., 1973). Although the precise controlling mechanisms for this differentiation process are still unclear, it is apparent that innervation and neural activity patterns play critical roles (Brown, 1973; Buller et al., 1960; Guth, 1968; Shafiq et al., 1972).

The failure of the fibre composition of any of the muscles from endurance trained rats to differ significantly from controls was somewhat surprising. A similar type of program has been shown to elicit FG to FOG fibre type conversions in guinea pig plantaris (Barnard et al., 1970a; Maxwell et al., 1973), rat plantaris (Edgerton et al., 1969; Wilkinson et al., 1976) gastrocnemius (Muller, 1974) and quadriceps (Baldwin et al., 1972). It may be erroneous to refer to this training adaptation as an actual fibre type conversion as the oxidative stain is strictly qualitative. An elevation of oxidative capacity above a critical level results in the classification of FG fibres as FOG. In addition, this process has been shown to be reversible with detraining (Faulkner et al., 1972). It should be noted that the aerobically trained gastrocnemius did display a higher percentage of FOG fibres than did their control counterparts but this difference was not of sufficient magnitude to be significant ($p > .05$). It could be that 10 weeks of training, 4 of which were devoted to gradually acclimatizing the rats to the final 30 m/min. run for 30 minutes was not of sufficient duration to elicit a significant training response. In addition, the 30 minute sessions twice per day amount to a considerably shorter training time than used by other researchers (Baldwin et al., 1972, 1973, 1975; Holloszy, 1967) and, as performance times (Appendix B, Table III) indicate, might not be a demanding enough stimulus to evoke adaptation.

The lack of change in the percentage of SO fibres in the gastrocnemius

is consistent with other findings in muscles of mixed fibre composition (Barnard et al., 1970a; Edgerton et al., 1969; Maxwell et al., 1973). Thus, no overt transformations of fast to slow twitch fibre types as discern ble by the myosin ATPase stain were shown.

Previous authors have found no change in the fibre composition of soleus and plantaris II in endurance trained rats (Baldwin et al., 1972), guinea pigs (Barnard et al., 1970a; Maxwell et al., 1973) and bushbabies (Edgerton et al., 1972). The results of the present study support these findings but are in contrast to the observations of Syrovy et al. (1972) and Wilkinson et al. (1976).

An adaptational response to endurance training of hypertrophy rather than fibre type changes in the soleus has been frequently noted (Edgerton et al., 1972, Maxwell et al., 1973). Since soleus contains only SO and FOG fibres, all motor units are probably equally recruited in low intensity exercise with no qualitative changes manifested as fibre type conversions occurring. Again, the exercise stimulus used in this experiment may not have been of sufficient intensity to elicit this response.

The FOG and FG fibres of plantaris II, on the other hand, are probably recruited minimally in endurance training and consequently no training adaptations would be expected.

It should be noted that higher percentage of FOG fibres in endurance trained soleus and gastrocnemius, although not significantly different from 15 week controls, may represent a tendency to maintain the fibre profiles of the younger animals as suggested by Muller (1974) and Wilkinson et al. (1976).

The sprint training program did not significantly alter the fibre

composition of plantaris II. This could be an analagous situation to the endurance trained soleus where all fibres are equally active in the particular exercise and consequently, no qualitative changes occur.

The mean percentages of FOG fibres in the soleus of the 15 week control and sprint groups were 19.6% and 28.9% respectively, values which compare closely to those reported by Saubert et al.,(1973) in age matched control and similarly trained experimental rats. The preferential usage of this fibre type in high intensity exercise is a reasonable explanation for this significant difference ($p < .01$). As the transformation of slow to fast twitch fibres is not consistent with the majority of the literature, it may be easiest to think of this occurrence as a preservation of the non-mature pattern.

The gastrocnemius of the sprinters displayed a significant increase (23.2% to 40.9%) in the proportion of FG fibres at the expense of FOG fibres but no change in the SO component. This shift was opposite in direction to the trend noted in the endurance trained muscle and a contradiction to the findings of Saubert et al. (1973) and Wilkinson et al. (1976). Rather than serving to maintain the fibre composition of the younger group, the training program seemed to further accentuate or accelerate the developmental pattern. It could be supposed that the preferential recruitment of FG followed by FOG fibres in the performance of the sprint work (which relies mainly on anaerobic metabolism as an energy source) resulted in a decrease in the oxidative capacity of the latter type below the criterion staining level. It should also be remembered that only the heterogeneous medial head of the gastrocnemius was sampled and these results may not reflect the adaptation of the muscle as a whole.

SUMMARY AND CONCLUSIONS

A summary of fibre population means and significant differences between groups appears in Table II. A significant developmental pattern was seen in both the soleus and medial gastrocnemius in rats as a result of aging from 5 to 15 weeks. A loss of FOG fibres with a concommitant increase in the proportion of SO and FG fibres in the soleus and gastrocnemius respectively was noted.

Of the two training groups, only the sprinters showed significant alterations ($p < .05$) from age matched controls in the fibre composition of the soleus and gastrocnemius muscles. The sprint training elicited a significant increase in the percentage of FOG fibres in soleus and FG fibres in gastrocnemius. There was a tendency, however, for the endurance trained rats to maintain the higher proportion of FOG fibres seen in the soleus and medial gastrocnemius of the younger animals. The proportion of SO fibres in the gastrocnemius remained unchanged in both training groups.

The homogeneously fast twitch plantaris II showed neither adaptations to training nor developmental alterations in its fibre type profile.

These results reinforce the principle of specificity of the training response. The magnitude, location and nature of the response seems to depend on the type of stimulus and the fibre composition of the working muscle. Both these factors determine the motor unit recruitment pattern which in turn is reflected in the specific adaptations (Burke and Edgerton, 1975). In the present study, an identical stimulus such as the sprint training program elicited two distinctly different fibre type alterations, a SO to FOG shift in soleus and a FOG to FG shift in

the medial head of the gastrocnemius. Two different types of training stimuli resulted in opposite trends in fibre type alterations in the same muscle such as was evidenced in the gastrocnemius.

The assessment of changes in mean fibre diameters and the total area occupied by each fibre type in the muscles of trained and control animals would help to more completely explain the nature of skeletal muscle fibre type adaptations to training.

Lastly, the need to relate training responses in skeletal muscle to developmental patterns when subjects are non-mature is reinforced. Rat muscle does not attain an adult state until approximately 17 weeks of age (Gordon et al., 1966; Close, 1972). Consequently, adaptations to intensive training conducted during this critical period may well differ from those seen where training is begun after the developmental pattern is completed. The work of Syrovy et al. (1972) and Schiaffino and Bormioli (1973) support this contention. If this is the case, the importance of commencing training at an early age in order to maximize the potential training response may have implications for young athletes and coaches.

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APPENDIX A

REVIEW OF LITERATURE

INTRODUCTION

Fibre type classification. It has long been known that mammalian skeletal muscle is composed of at least two distinctly different kinds of fibres, a dark orred type and a pale or white type. Needham (1926) has comprehensively reviewed the early literature concerning the functional differences between these two types noting the slow twitch time, resistance to fatigue and lower twitch tension in the red fibres and the reverse characteristics in white fibres.

Since that time, more sophisticated histochemical and biochemical techniques have revealed the existence in the skeletal muscle of most mammals of three fibre types each of which exhibits a distinct metabolic and contractile profile. Fibres may first be categorized as fast or slow twitch based on their contraction time or time to peak tension relative to other fibres in the same animal and then further identified by their oxidative and glycolytic capacities.

The contraction time dichotomy is histochemically demonstrable by staining for the presence of alkaline-stabile myosin ATPase as the activity of this enzyme has been shown to correlate highly with the contraction speed of a muscle fibre (Barany, 1967). Oxidative and glycolytic capacities can be qualitatively determined in a similar fashion via reactions involving enzymes critical to those two particular metabolic pathways (see Methods and Procedures).

Physiological criteria have also been used by some researchers

(Burke et al., 1971) to classify individual muscle fibres with identical results to the histochemical methods.

To date many systems of nomenclature (Burke and Tsairis, 1973; Edgerton and Simpson, 1969; Engel, 1962; Stein and Padykula, 1962) have been proposed and used creating considerable confusion in fibre classification. In this study, fibres will be typed according to Peter et al. (1972). Their method is explicit, designating individual fibres as slow twitch-oxidative (SO), fast twitch-glycolytic (FG), or fast twitch-oxidative-glycolytic (FOG). Numerical data on specific twitch times and enzyme activities are available from several sources (Keul, 1972; Saltin, 1973).

Fibre composition of skeletal muscles. The proportion of each fibre type present in any given muscle is dependent upon several factors. Comparative surveys (Ariano et al., 1973; Close, 1972; Dubowitz, 1968) have revealed that while fibre type composition of one muscle does vary among different species, considerable consistency does exist. A much greater variation can be seen in the different muscles of the same animal or even in different areas of the same muscle (Ariano et al., 1973; Bagby et al., 1972). These patterns are genetically determined and, for the most part, represent an evolutionary adaptation related to function (Henneman and Olson, 1965). Thus, postural muscles situated in predominantly axial positions tend to be more slow twitch and more highly oxidative while phasic types are found peripherally and are predominantly fast twitch and more glycolytic in nature (Keul, 1972).

RESPONSES OF SKELETAL MUSCLES TO ENDURANCE TRAINING

Biochemical assessment of oxidative adaptations. Many researchers have shown that numerous biochemical adaptations relating to energy supplying mechanisms can occur in the skeletal muscle of both man and animals as a result of training. In response to an endurance or aerobic program a general increment in the oxidative capacity of the muscles utilized is seen. Gordon et al. (1967) reported an increase in the sarcoplasmic proteins of the quadriceps of endurance trained rats while no change in myofibrillar protein was noted. Holloszy (1967) found that 12 weeks of treadmill running resulted in an increase in the size and number of mitochondria as well as a two-fold increase in their ability to oxidize pyruvate in rats. Holloszy's results are supported by those of Gollnick and King (1969), Barnard et al. (1970a); and Baldwin et al. (1972). Increases of similar magnitude have been found in such oxidative enzymes as SDH, NADH diaphorase, cytochrome oxidase, cytochromes a and c and citrate synthetase (Baldwin et al., 1972; Edgerton et al., 1972; Kowalski et al., 1969; Holloszy, 1967). Other adaptations to endurance training include significant elevations of hexokinase activity in rats (Holloszy et al., 1971) and in man (Morgan et al., 1971) as well as increase in the ability of whole muscle to oxidize fatty acids (Baldwin et al., 1972; Paul and Issekutz, 1967).

Histochemical assessment of oxidative adaptations. The previously mentioned studies employed biochemical assays of whole muscle homogenates to assess changes resulting from the training stimulus. Histochemical methods have further elucidated the specific location of these changes and the importance of noting the response of each fibre type to an

exercise program.

In rat muscle which is of a mixed fibre type such as plantaris III (Edgerton et al., 1969) or gastrocnemius, endurance training has been shown to elicit an increase in the proportion of fibres classified as highly oxidative (Edgerton and Simpson, 1969). Expressed in terms of fibre types, an augmentation in the number of FOG fibres at the expense of FG fibres is seen in exercised rats (Wilkinson et al., 1976), guinea pigs (Barnard et al., 1970a; Faulkner et al., 1971; Maxwell et al., 1973), and the non-human primate, Galago senegalensis, (Lesser Bushbaby) (Edgerton et al., 1972). These findings are supported by the work of Kowalski et al. (1969) who noted that the greatest increase in SDH activity in the quadriceps of endurance trained rats occurred in the anaerobic fibre types implying a FG to FOG fibre conversion had taken place.

Baldwin and coworkers (1972) also found a significant increment in the biochemically determined respiratory capacity of trained rat muscle however, it was of the nature of 200% in muscles which possessed distinctly different fibre type profiles. Thus, pre-training enzyme ratios were preserved in the soleus (SO), superficial quadriceps (FG) and deep quadriceps (FOG) muscle areas.

It is interesting to note that Edgerton et al. (1969) found that soleus which is 80-85% SO and 15-20% FOG at maturity in rats did not display any shift in its fibre population in response to endurance training. Rather, a uniform hypertrophy of all fibres has been reported (Maxwell et al., 1973).

It is questionable whether these adaptations are temporary transportations or permanent conversions. The work of Faulkner et al. (1972)

indicates that a period of detraining causes a regression of post-training values towards control values.

Contraction time and myosin ATPase adaptations. There is less agreement as to whether changes in contraction time of the whole muscle or in the ATPase staining pattern of individual skeletal muscle fibres occurs in response to endurance training. After 19 weeks of such training, Barnard et al. (1970b) could not find any changes in the contractile properties of the gastrocnemius-plantaris muscle group of adult guinea pigs. Histochemically this is supported by evidence negating any alteration in the myosin ATPase staining patterns of the individual fibres of mature rats (Bagby et al., 1972; Edgerton et al., 1969; Syrovy et al., 1972) or bushbabies (Edgerton et al., 1972). The majority of the evidence would substantiate the view that a conversion from fast to slow twitch fibres, or vice-versa, does not take place. However, these findings are contradicted by Baldwin et al. (1975) who reported that a strenuous program of treadmill running lasting 18 weeks or longer resulted in a 20% decrease in the myosin ATPase in the FOG fibre area of rat vastus lateralis, a quantitatively similar increase in the SO fibres of soleus and no change in the FG portion of the vastus.

Glycolytic adaptations. Although oxidative enzyme adaptations are the predominant response to endurance exercise, the activities of some glycolytic enzymes can also be affected and, again, alterations are fibre type specific. Baldwin et al. (1973) have reported that several glycolytic enzymes undergo changes which parallel those previously described for ATPase activity. The FOG portion of exercised rat quadriceps experienced a 20 percent decline in the activity levels of such enzymes as αGPD, PFK, pyruvate kinase (PK), LDH, and phosphorylase (Pase) while

the FG portion remained unaltered. In contrast, the SO fibres of the soleus underwent an 18-35 percent increase in these same enzymes.

RESPONSES OF SKELETAL MUSCLE TO SPRINT TRAINING

There has been comparatively little research on the specific effects of sprint training on skeletal muscle fibre types. Staudte et al.(1973) trained rats to run at 80 m/min. for 45 second intervals, 4 times daily. This ritual was conducted daily for 3 weeks. Subsequent assays of the soleus and rectus femoris muscles revealed that increases in enzymes of glycogenolysis and glycolysis were most pronounced in soleus while creatine kinase is elevated in that muscle alone. These authors also found a significant decrease in the isometric twitch contraction time of the soleus. These findings are in agreement with those of Saubert and coworkers (1973) who reported that only the soleus muscle of their sprint trained rats showed a consistent adaptational increase in Pase, PK, and PFK. Since neither the red and white portions of gastrocnemius nor the red area of vastus lateralis displayed any significant adaptation, they concluded that most skeletal muscles possessed sufficient anaerobic capacity to meet the demands of the heavy but short-term intermittant work load imposed. However, additional histochemical analyses revealed an increment in the proportion of FOG fibres at the expense of FG fibres in a fast muscle (white gastrocnemius) or at the expense of SO fibres in a slow muscle (soleus).

The same responses may not occur in humans as the sprint trained subjects tested by Thorstensson et al. (1975) showed no alterations in the fibre type composition of their vastus lateralis muscle after an eight week program. However, further investigations where training intensity, duration and frequency are manipulated are needed to make

conclusive statements.

TRAINING RESPONSE AND MOTOR UNIT RECRUITMENT PATTERNS

The phenomenon of training response specificity has been succintly summarized by Maxwell et al. (1973) who state "there is no generalized training response. The specific response in a given motor unit is dependent upon the recruitment pattern induced in that motor unit by the training program."

Since the motor unit is the true functionall element in muscle contraction and all its fibres are homogeneous in nature (Edstrom and Kugelberg, 1968), it may be more appropriate to speak of 'motor unit' types rather than 'fibre' types. Thus, any given type of exercise requires a specific combination of motor units that are best adapted to meet that demand. Burke and Edgerton (1975) further infer that the increase in usage of each unit will be reflected in the magnitude of the training effect.

Glycogen depletion studies. Studies of glycogen depletion patterns during single bouts of exercise have provided support for the theory of selective recruitment of motor units. In a mixed muscle of the rat, such as rectus femoris, low intensity running calls upon FOG, then SO, and lastly FG fibres in that order (Armstrong et al., 1974). As the speed is increased, FG fibres become selectively more depleted (Armstrong et al., 1974; Armstrong et al., 1976). Gillespie et al. (1974) have also reported that in a comparison between running and jumping in a non-human primate, the FOG fibres of a mixed muscle are depleted of their glycogen preferentially or, in other words, are used most in the former exercise and FG fibres used least. The reverse

pattern occurs in the latter exercise. Similar findings have been documented in human subjects by Gollnick et al. (1974). However, since FOG fibres are scarce in man, submaximal and supramaximal bicycle work preferentially deplete SO and FG fibres respectively.

Electromyography (EMG) evidence. Results from EMG investigations are in agreement with the glycogen depletion work. Smith et al. (1976) studied the electrical activity of cat soleus and gastrocnemius during various types of activity. They found that soleus was most active in quiet standing while slow running elicited activity in both muscles. As the speed of the run increased, the gastrocnemius became proportionally more active. In jumping it was this muscle, which is 80 percent fast twitch that accounted for almost all of the electrical activity.

Summary. The literature cited emphasizes that exercise is not a uniform stimulus to each and every motor unit in a working skeletal muscle. The degree to which any given unit will be recruited in contraction depends not only on the type of stimulus, that is its intensity or duration, but on the fibre composition of the whole muscle. Adaptations to repeated stimulation or training may be expected accordingly.

AGING EFFECTS ON SKELETAL MUSCLE FIBRE COMPOSITION

Contraction time and enzymatic differentiation. It is possible that some of the conflicting results regarding the response of skeletal muscle fibre types to training may be due to the potentially confounding variable of the age of the experimental animal. Most mammalian skeletal muscle undergoes a developmental differentiation after birth. The rate and degree of this process is largely dependent on the maturity of the species at birth (Gutmann et al., 1973; Gutmann et al., 1974) and

the specific muscle studied (Dubowitz, 1968; Gutmann and Melichna, 1972).

Immediately post natally, differentiation is evident to the extent that fibres may already be marked as fast or slow twitch in many species including the rabbit (Barany et al., 1967), kitten (Hammarberg and Kellerth, 1975), and rat (Brown, 1973; Close, 1964; Shafiq et al., 1972) but all fibres are slow relative to the adult state (Close, 1972; Guth, 1968). In neonatal rats, both fast and slow muscle fibres undergo a decrease in contraction time during the first 3 to 5 weeks (Brown, 1973; Gutmann et al., 1973). Contraction time further decreases in fast fibres while it levels off or increases in slow fibres until normal adult values are reached (Guth, 1968; Gutmann et al., 1973).

A concommitant increase in the biochemically determined ATPase activity has been shown to occur in developing fast fibres (Close, 1974; Dubowitz, 1968; Gutmann et al., 1973; Gutmann et al., 1974) and has been confirmed by histochemical data as well (Tomanek, 1975).

Increases in speed of contraction are parallelled by increments in the activities of such glycolytic enzymes as PK, aldolase (Mann and Salafsky, 1970), LDH and glyceraldehyde-3-phosphate dehydrogenase (Bass et al., 1970; Margreth et al., 1970). Bass et al. (1970) found decreases in the activities of some oxidative enzyme levels in developing fast muscle of chickens while Goldspink (1969) reported a similar pattern for SDH activity in laboratory mice. Mann and Salafsky (1970), however, found no alteration in the oxidative enzyme activities of developing kittens. These contradictory observations may be attributable to the fact that different experimental animals were employed in each case or related to the different rates of maturation of the nervous system of these animals (Close, 1972).

Aging and fibre type changes. Thus, it is not surprising that the fibre type composition of a given muscle is often not constant during the developmental period. In the guinea pig, age dependent changes in the fibre population of the plantaris (Faulkner et al., 1971; Maxwell et al., 1973) have been observed. In both muscles, a decrease in the percentage of FOG fibres was seen with a corresponding increase in the FG fibres of the predominantly fast plantaris and in the SO fibres of the slow soleus. A similar trend was described by Wilkinson et al. (1976) in the same muscles of two groups of control rats sacrificed at 5 and 15 weeks of age. Muller (1975) found no change in rat soleus muscle from 9 to 18 weeks of age but noted that both the high and low oxidative portions of rectus femoris displayed the FOG to FG fibre shift during those 9 weeks of growth. The significant FOG to SO fibre conversion seen by Wilkinson et al. (1976) in developing rat soleus was probably due to the initially younger age of the animals. It is interesting to note that no age related changes were seen in the psoas of guinea pigs (Maxwell et al. 1973) or in the plantaris II region of rats (Wilkinson et al., 1976), two muscles which contain only fast twitch fibres.

Aging and training. It would seem critical therefore, in light of the literature just cited, to relate data from experimental animals to that from age matched controls throughout any training study and yet this has been done in only a few instances. It is also of interest to view adaptations to training in relation to normal developmental patterns. It has been suggested that the exercise stimulus, rather than causing fibre type conversions, may actually preserve or maintain the fibre composition of non-mature muscle (Wilkinson et al., 1976). Muller (1975) agrees

stating:

> Adaptation takes place along the pathway of minimal 'expense'... The proportional adaptation of morphometrical parameters and the conservation of a juvenile state probably require less information than the removal of fibres or quantitative and especially qualitative transformation of fibres.

The limited experimental evidence available would tend to support this hypothesis. The staining pattern in sections of 14 week old endurance trained guinea pig plantaris muscle closely resembled that of 6 week old controls (Maxwell et al., 1973). A similar type of program preserved the pattern of a 9 week old rectus femoris in 18 week experimental rats (Muller, 1974). Wilkinson et al. (1976) found that the composition of the plantaris III region of both aerobically and anaerobically exercised rats at 15 weeks of age was not significantly different than the 10 week controls. The same was true for the soleus muscle but only in the aerobic animals.

In each case, training tended to maintain the proportion of FOG fibres or to partially offset their loss normally seen during development. A final consideration is that tissue may possess a greater potential for adaptation during the developmental period (Schiaffino and Bormioli, 1973). In support of this theory, Syrovy et al. (1972) have noted an increase of 17% in the ATPase activity of the soleus muscle of his young group of training rats only. The change took place in fibres already low in ATPase activity. As this is also a developmental occurrence, these authors suggested this relationship was more than just coincidental. Their observations are in agreement with those of Gutmann and Hajek (1971) who found a decrease in contraction time and concommitant elevation of ATPase activity in the extensor digitorum

longus of 20 day old rats forced to swim 4 to 6 hours daily for only 4 days. Again, more extensive research varying the age and species of the animal as well as the intensity of the training stimulus is needed to confirm this hypothesis.

APPENDIX B

TABLE III

RUNNING TIME (MIN.) TO EXHAUSTION AT 40 M/MIN. IN PERFORMANCE TEST

TRAINING GROUP	ANIMAL NUMBER	RUNNING TIME (min)
ENDURANCE	49	90
	50	85
	51	66
	52	37
	53	68
	55	150
	56	73
	57	112
	58	46
SPRINT	WOULD NOT RUN CONTINUOUSLY	
MEAN		81
SEM		34

TABLE IV

PERCENT FIBRE COMPOSITION IN 5 WEEK OLD CONTROL RATS

ANIMAL NUMBER	SOLEUS			PLANTARIS			GASTROCNEMIUS		
	SO	FOG	FG	SO	FOG	FG	SO	FOG	FG
112	46.80	53.20	0	0	37.66	62.34	18.52	77.78	3.70
113	52.65	47.35	0	0	50.42	49.58	17.60	72.28	10.11
114	48.12	51.88	0	0	35.77	64.23	24.59	65.84	9.56
115	51.65	48.35	0	0	44.67	55.33	13.53	76.69	9.77
116	47.02	52.98	0	0	39.66	60.34	18.84	70.20	10.96
117	49.44	50.56	0	0	35.67	64.33	21.67	56.65	21.67
118	49.04	50.96	0	0	46.15	53.85	15.60	69.15	15.25
119	52.78	47.22	0	0	38.28	61.72	13.85	73.76	12.41
120	43.75	56.25	0	0	47.80	52.20	17.73	74.25	8.03
121	46.50	53.50	0	0	49.19	50.81	21.82	63.33	14.85
MEAN	48.78	51.22	0	0	42.53	57.47	18.37	69.99	11.63
SEM	0.88	0.88	0	0	1.72	1.72	1.06	1.96	1.45

TABLE V

PERCENT FIBRE COMPOSITION IN 15 WEEK OLD CONTROL RATS

ANIMAL NUMBER	SOLEUS			PLANTARIS			GASTROCNEMIUS		
	SO	FOG	FG	SO	FOG	FG	SO	FOG	FG
59	—	—	—	—	—	—	13.39	68.62	17.99
60	72.27	27.73	0	5.45	53.64	40.91	23.87	68.39	7.74
61	79.15	20.85	0	.71	39.65	59.64	28.52	54.70	16.78
62	89.69	10.31	0	3.08	51.54	45.38	21.59	53.65	24.76
63	80.37	19.63	0	2.30	62.71	35.02	19.47	61.07	19.46
64	90.57	9.48	0	0	54.18	45.82	16.82	54.35	28.83
65	19.47	19.47	0	0	39.86	60.14	20.00	53.76	26.27
66	89.17	10.83	0	1.02	46.10	52.88	22.18	45.76	32.08
67	75.51	24.49	0	0	46.90	53.10	15.60	41.13	43.26
68	66.17	33.83	0	0.33	38.03	61.64	13.39	68.62	17.99
MEAN	80.38	19.62	0	1.43	48.07	50.50	20.63	56.19	23.18
SEM	2.63	2.63	0	0.58	2.58	2.92	1.37	2.64	3.03

TABLE VI

PERCENT FIBRE COMPOSITION IN 15 WEEK OLD ENDURANCE TRAINED RATS

ANIMAL NUMBER	SOLEUS			PLANTARIS			GASTROCNEMIUS		
	SO	FOG	FG	SO	FOG	FG	SO	FOG	FG
49	69.50	30.50	0	0	48.95	51.05	15.29	67.35	17.35
50	81.48	18.52	0	0	39.45	60.55	20.31	55.94	23.75
51	76.99	23.01	0	0	49.11	50.89	16.49	52.62	50.89
52	82.25	17.75	0	0	45.85	54.15	13.85	63.35	22.30
53	78.67	21.33	0	1.67	53.85	44.48	15.08	62.15	22.77
55	—	—	—	2.92	58.43	38.65	22.55	58.45	18.94
56	79.49	20.51	0	.66	34.29	65.71	24.38	65.76	9.85
57	66.95	33.05	0	.30	38.02	61.68	19.70	69.70	10.60
58	78.54	21.46	0	.32	37.54	62.14	21.72	70.59	7.69
MEAN	76.73	23.27	0	0.65	45.05	54.30	18.82	62.93	18.24
SEM	1.84	1.84	0	0.32	2.78	2.83	1.18	1.95	2.41

TABLE VII

PERCENT FIBRE COMPOSITION IN 15 WEEK OLD SPRINT TRAINED RATS

ANIMAL NUMBER	SOLEUS			PLANTARIS			GASTROCNEMIUS		
	SO	FOG	FG	SO	FOG	FG	SO	FOG	FG
39	63.04	36.96	0	0	42.12	57.87	22.27	34.01	43.72
40	65.46	34.54	0	.32	51.75	47.94	13.35	48.01	38.63
41	78.70	21.30	0	0	42.46	57.54	19.11	37.20	43.69
42	76.62	23.38	0	1.51	49.81	48.68	20.47	36.58	42.95
43	75.37	24.63	0	2.16	51.72	46.12	13.06	43.67	43.26
44	60.07	29.93	0	0	54.13	45.87	19.80	40.92	39.27
47	72.20	27.80	0	0	44.20	55.80	21.40	38.91	39.69
48	77.15	22.85	0	0	49.32	50.68	14.67	49.00	36.33
MEAN	71.08	28.92	0	0.50	48.16	51.34	18.02	41.04	40.94
SEM	2.38	2.38	0	0.28	1.53	1.66	1.23	1.80	0.93

TABLE VIII

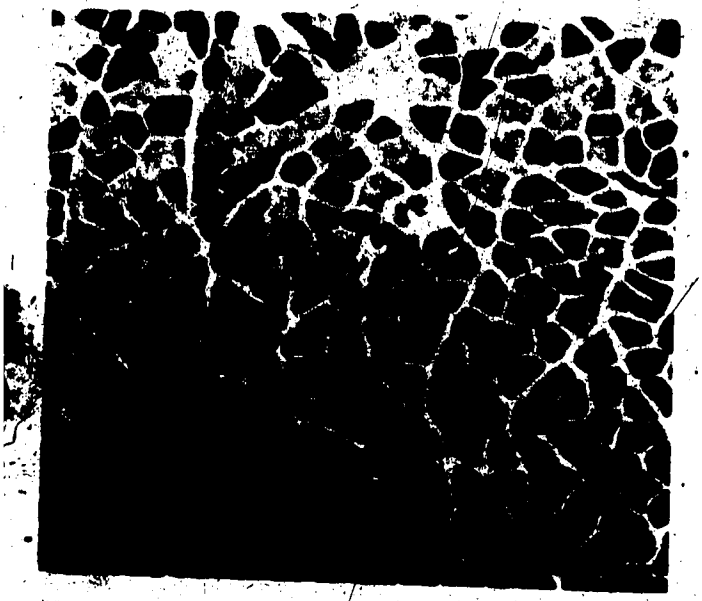
ANALYSIS OF VARIANCE: MEAN WET MUSCLE WEIGHTS AND BODY WEIGHT

VARIABLE	SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO	F RATIO
SOLEUS	BETWEEN	2	0.0006	.0003	0.439	0.649
	WITHIN	24	0.0171	.0007		
	TOTAL	26	0.0177			
PLANTARIS	BETWEEN	2	0.0351	0.0176	5.772	0.009
	WITHIN	24	0.0730	0.0030		
	TOTAL	26	0.1081			
GASTROCNEMIUS	BETWEEN	2	0.4695	0.2347	3.696	0.041
	WITHIN	23	1.4607	0.0635		
	TOTAL	25	1.9302			
BODY WEIGHT	BETWEEN	2	22204	11102	3.961	0.033
	WEIGHT	24	67271	2803		
	TOTAL	26	89475			

APPENDIX C

Plate 1 Myosin ATPase staining pattern in 5 week control soleus

Plate 2 Myosin ATPase staining pattern in 15 week control soleus



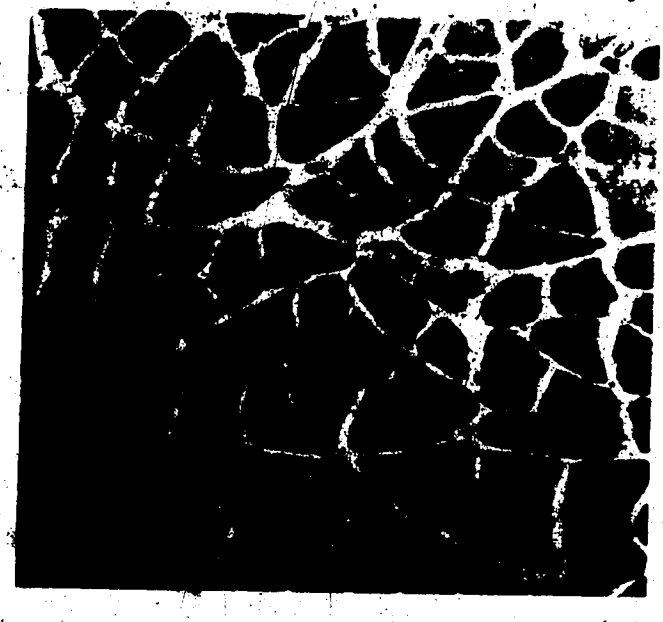


Plate 3 NADH - diaphorase staining pattern in 15 week old control soleus

Plate 4 α-GPD staining pattern in 15 week control medial gastrocnemius

Plate 5 - Myosin ATPase staining pattern in an endurance trained gastrocnemius

Plate 6 NADH diaphorase staning pattern in an endurance trained gastrocnemius (serial section to Plate 5)