GLYCOGEN DEPLETION PATTERNS
IN RAT SKELETAL MUSCLE
INDUCED BY TREADMILL EXERCISE
AT SELECTED SPEEDS AND GRADES

BY

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THESIS

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ABSTRACT

Forty eight male Wistar rats with mean initial body weights of 163 grams were randomly assigned to one of eight different treatment groups. Two series of experiments were conducted on a Quinton (model 42-15) motor driven rodent treadmill.

Series I consisted of exercise bouts performed at speeds of 25, 35, 45 and 55 m/min. at 0% incline. Series II consisted of exercise bouts performed at a constant speed of 35 m/min. but at selected grades of 0, 20, and 40% incline. In each series, the distance covered was equal for all groups in that series.

Glycogen depletion patterns induced as a result of the series I exercise bouts were also reproduced generally in those exercise bouts of the series II group. From the results of the present study, it was concluded that glycogen depletion patterns are affected by changes in both speed and incline of a motor driven treadmill. Since this relationship has been shown to exist in running done on the level and with grade, it appears that changes in intensity are responsible for the different glycogen depletion patterns observed in these situations. The trend towards predominantly SO and FOG fiber depletion at low to moderate intensity work, and predominantly FG depletion at the higher intensities of work, was established but, this effect appeared to be more distinct in those groups of animals that ran at selected inclines.

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CHAPTER I

THE PROBLEM

Introduction

Mammalian skeletal muscle is composed of fibers which are characterized by differences in contractile, metabolic, and functional parameters (Close, 1972). It is generally accepted, at the present time, that mammalian skeletal muscle contains two major fiber type categories which can be histochemically distinguished on the basis of differences in myofibrillar ATPase activity (Dubowitz and Pearse, 1960; Edstrom and Nystrom, 1969; Gollnick _et_ _al_., 1972a, 1972b). As myosin ATPase activity is closely related to contractile speed (Barany _et_ _al_., 1965), these two categories can be designated as consisting of either slow-twitch (low levels of myofibrillar ATPase activity) or fast-twitch (high levels of myofibrillar ATPase activity) fibers (Gollnick _et_ _al_., 1972a, 1972b; Barnard _et_ _al_., 1971). Because of the metabolic demands and mechanisms implicated in these two pools of muscle fibers, often the terms slow-twitch and slow-oxidative (SO) are used synonymously, as are the terms fast-twitch and fast-glycolytic (FG). Although this specific two-fiber profile has been shown to exist in human skeletal muscle, much experimental evidence supports the fact that other mammalian skeletal muscle (ie. prosimian, rat, mouse etc.) is composed of, in addition to these two basic types, yet a third muscle fiber type (Edgerton and Simpson, 1969; Armstrong et al., 1974, 1975: Khan et al., 1974: Gillespie et al., 1974). This third muscle fiber type is an intermediate type of fiber and has been termed fast-oxidative-glycolytic (FOG), as it shows both high levels of myofibrillar ATPase activity (Burke and Edgerton, 1975) as well as heavy staining for oxidative enzyme potential (Peter et al., 1972).

This distinction between fast-twitch and slow-twitch fibers with differing enzymatic and structural characteristics is basic to the idea or concept of motor unit (ie. specific fiber) recruitment. Furthermore, the findings of Henneman and Olson (1965), Grimby and Hannerz (1968), Hannerz (1974) and Tanji and Kato (1973) with electromyographic recordings of single motor units also seem to indicate the presence of two metabolically and characteristically different muscle fiber pools which, via the cerebral centers, are capable of being selected in advance with respect to recruitment order that appears most appropriate for the work intended (Hannerz and Grimby, 1973).

Because of metabolic demand, all fibers do, to some extent, undergo exercise-induced glycolytic degradation of fuels. Consequently, each fiber type has been shown to possess some store of carbohydrate (glycogen) within the fibers themselves (Beatty et al., 1963: Gillespie et al., 1970; Dubowitz and Pearse, 1960). The loss of this fiber glycogen, a substrate which serves as an important energy source for muscular contraction, has been used as an indicator of fiber recruitment in exercise and single motor unit recruitment (Edgerton et al., 1970; Burke et al., 1971; Edgerton and Hewitt, 1972a). This loss in fiber glycogen has been demonstrated histochemically by the technique known as the Periodic Acid-Schiff (PAS) reaction. The PAS reaction gives an indication of the relative glycogen content of specific fibers and has been important in the recognition and understanding of glycogen depletion patterns induced as a result of exercise. This histochemical evidence suggests that the mechanism postulated by Henneman and Olson (1965) exists where, at low to moderate stimulation intensity, those fibers with the smallest motorneurons (SO<FOG) are probably predominantly recruited and where those fibers with the largest motorneuron size (FG) are called into play only at the higher intensities of stimulation. Support for this hypothesis comes from other authors who have shown that the effect of exercise on the glycogen store is dependent on the work load employed (Hultman _et_ _al_., 1971; Saltin and Karlsson, 1971), and from the analysis of glycogen depletion patterns in exercising rats (Armstrong _et_ _al_., 1974, 1975; Edgerton and Simpson, 1969), prosimians (Gillespie _et_ _al_., 1974), guinea pigs (Edgerton and Simpson, 1969) and humans (Piehl, 1974; Edgerton _et_ _al_., 1975; Gollnick _et_ _al_., 1973a, 1973b, 1974a, 1974b; Costill _et_ _al_., 1973).

Rationale

Because of the many advantages that work with an animal model offers over the use of human subjects, many of the exercise related studies are relying on these experimental animals for their supply of 'subjects'. However, in studies where a motor driven rodent treadmill is used little is known concerning the effects of specific exercise regimens on the skeletal muscle fiber recruitment patterns of these animals at different speeds or grades. Such a profile could be established in an experimental situation by histochemical demonstration of the glycogen depletion patterns in rat hind limb skeletal muscle fibers throughout graded work bouts of increasing intensity achieved by means of a change in speed or incline of a rodent treadmill. In this way, selected changes in workload can show their effects upon recruitment shifts in specific fiber selection.

The need for such a profile in order to be able to establish specific high-intensity anaerobic or low-intensity aerobic exercise programs, as well as to pinpoint the intensity of work which seems to bring about the recruitment shift from the aerobic to anaerobic fiber populations, is immediate and will no doubt assist researchers in identifying an exercise situation which will more precisely induce in their experimental animals that effect which they are seeking.

Purpose

The present study was designed to determine the effects of changing the intensity of a workload by variations in speed of a motor driven rodent treadmill from 25 - 55 m/min., and elevation from 0 - 40% grade, on glycogen depletion patterns demonstrated histochemically in the plantaris muscle of male Wistar rats.

Definitions

Motor Unit - A motorneuron and all those muscle fibers which it innervates.

Slow-Oxidative Fiber - Those fibers which are histochemically demonstrated by low levels of myofibrillar ATPase activity, and high levels of oxidative enzyme activity (NADH-Diaphorase). (SO)

Fast-Oxidative-Glycolytic Fiber - Those fibers which are histochemically demonstrated by high levels of myofibrillar ATPase activity, as well as high levels of oxidative enzyme activity (NADH-Diaphorase). (FOG)

Fast-Glycolytic Fiber - Those fibers which are histochemically demonstrated by high levels of myofibrillar ATPase activity, but low levels of oxidative enzyme activity (NADH-Diaphorase). (FG)

PAS Dark Fiber - Those fibers which stain darkest with the PAS reaction (see reference photomicrographs which follow). (D)

PAS Moderate Fiber - Those fibers which show an intermediate level of PAS staining intensity between those of Dark and Light classification (see reference photomicrographs which follow). (M)

PAS Light Fiber - Those fibers which show very little staining with the PAS reaction (see reference photomicrographs which follow). (L)

PAS Negative Fiber - Those fibers which show no staining with the PAS reaction (see reference photomicrographs which follow). (Neg)

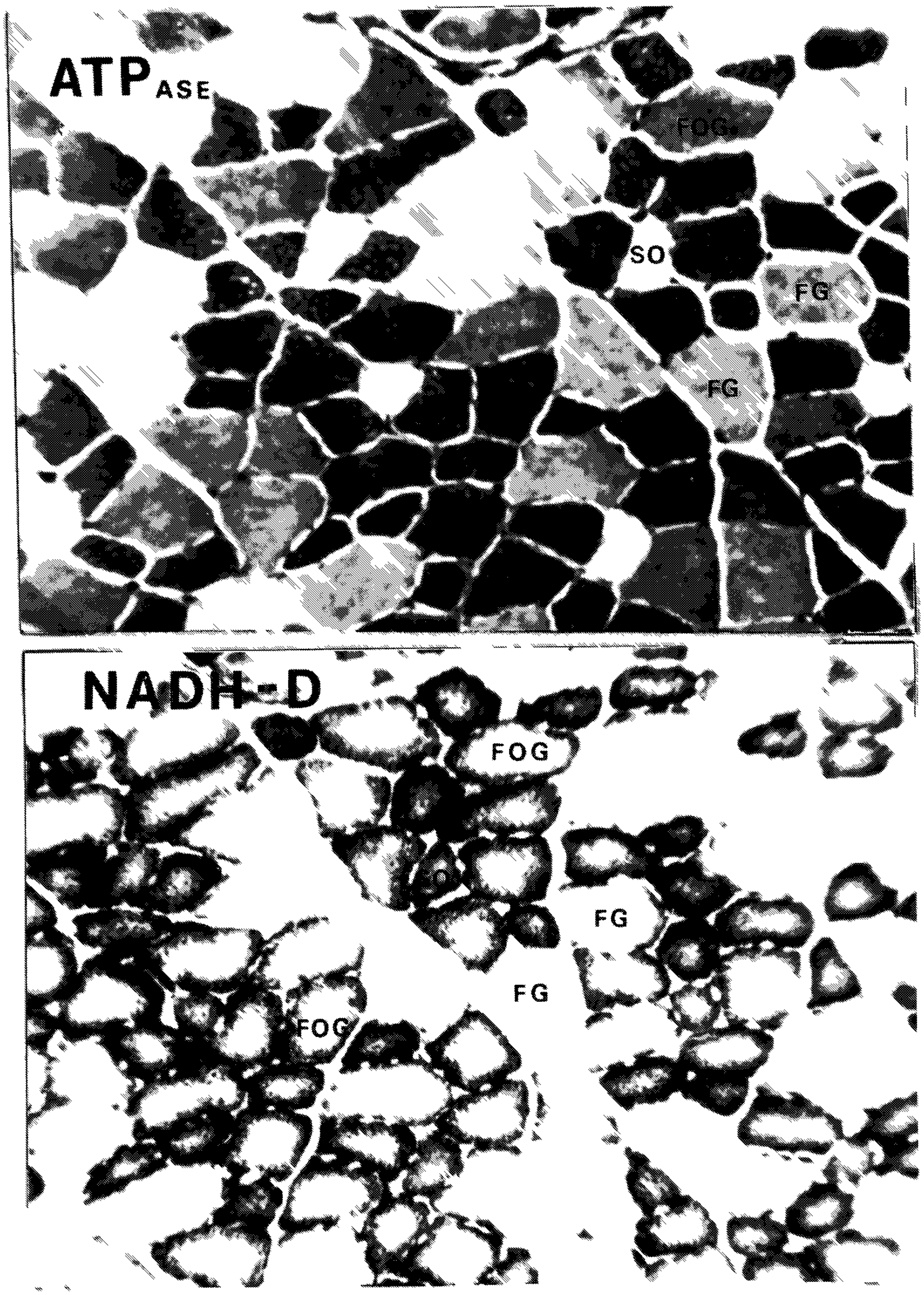

Figure 1: Serial Sections of Rat Plantaris Muscle of Unexercised Control Animal. Note that the SO fibers are light in ATPase staining and dark in NADH-D staining, FOG fibers are dark in ATPase staining and dark in NADH-D staining and, FG fibers are dark in ATPase staining and light in NADH-D staining. Reference photomicrographs demonstrate the system used to classify the individual fibers into the three types used in this study. (x115)

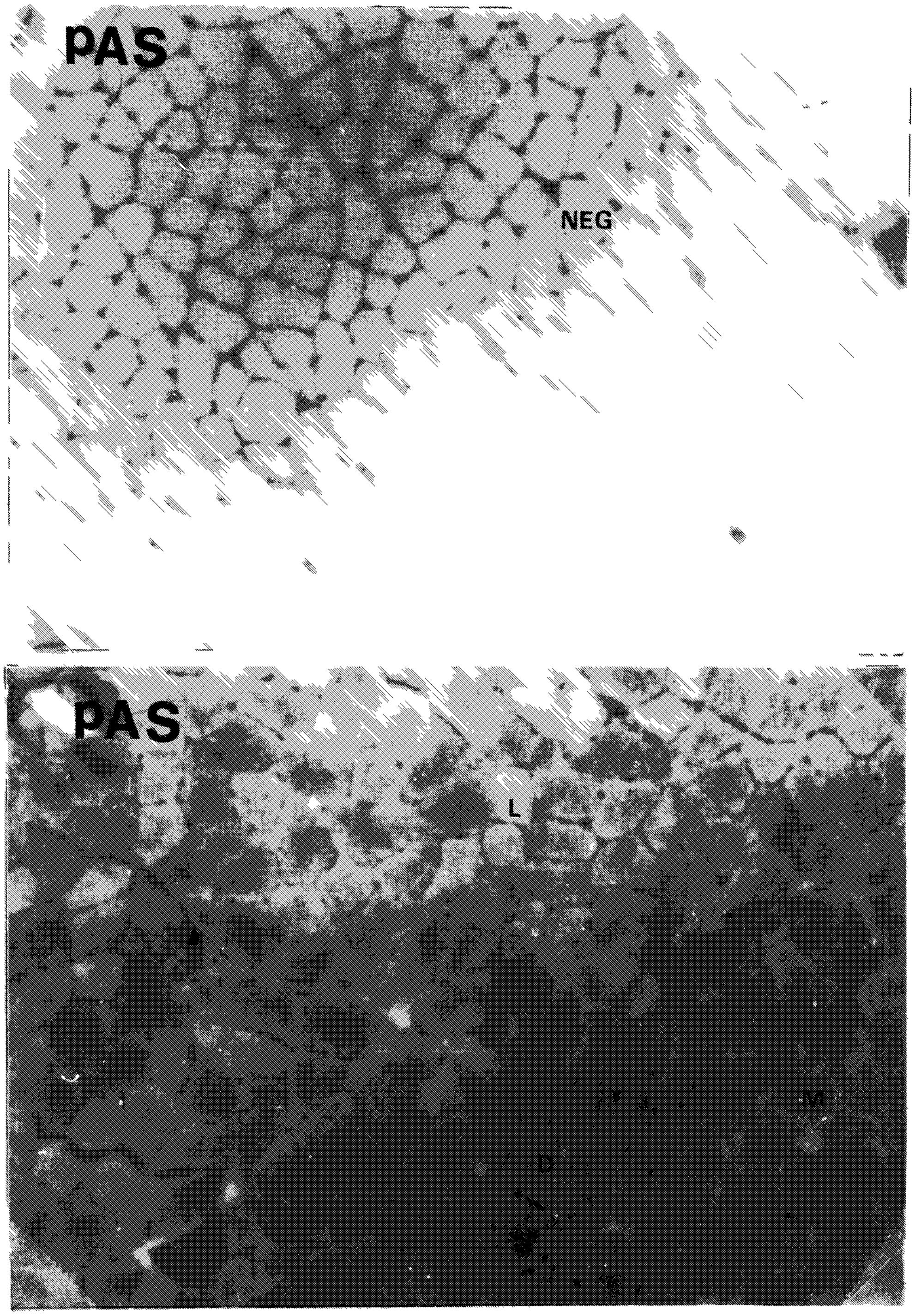

Figure 2: Serial Sections of Rat Plantaris Muscle. Photomicrograph showing PAS Neg fiber was taken from an exercise depleted animal, while the other photomicrograph was taken from a normally fed, unexercised control animal. Reference photomicrographs (stained by the PAS technique) demonstrate the system used to classify the individual fibers as Dark (D), Moderate (M), Light (L), and Negative (Neg) in relative glycogen content. (x115)

CHAPTER II

REVIEW OF RELATED LITERATURE

Introduction

Within this chapter, literature concerning the enzymatic and structural profiles of the different fiber types present in mammalian skeletal muscle will be presented. Material dealing specifically with the slow-oxidative fiber will then be discussed, and followed by sections dealing with fast-glycolytic and fast-oxidative-glycolytic fiber types. In the final section, evidence from studies considering glycogen depletion patterns in relation to recruitment shift will be examined and followed by a summary.

Enzymatic and Structural Profiles of the Different Fibers

It has been shown that mammalian skeletal muscle contains at least two major fiber types which can be distinguished as fast-twitch and slow-twitch on the basis of myofibrillar ATPase activity (Dubowitz and Pearse, 1960; Edstrom and Nystrom, 1969: Gollnick et al., 1972a, 1972b, 1973a, 1973b; Engel et al., 1974). These findings are in agreement with results from other authors (Henneman and Olson, 1965) who have shown that the different sizes of the motorneurons in the spine can have a functional significance, and that the motorneurons are connected to different types of fibers. These cells have different thresholds and their recruitment patterns are related to the strength of the electrical stimuli (Henneman and Olson, 1965) and the organization of the excitatory and inhibitory synaptic systems impinging on the interneuronal cells in question (Burke and Edgerton, 1975). Similarly, work by Kugelburg and Edstrom (1968) supports these results and also indicates that those fibers in the same motor units have identical enzymatic profiles.

Because the distinction between fast-twitch and slow-twitch fibers with differing enzymatic and structural characteristics is basic to the idea or concept of motor unit recruitment, some of the properties which characterize each specific type of fiber and best adapt it for the function which it must perform, and the type of metabolic condition under which it must work, will be presented in the following sections.

The Slow-Oxidative Fiber

When considering the environment in which a primarily aerobically metabolizing fiber must function, it is important for these fibers to demonstrate relatively high oxidative potentials. From histochemical data, slow-twitch (ST) fibers of mammalian skeletal muscle appear to have two to three times the oxidative capacity of the fast-twitch (FT) fibers (Dubowitz and Pearse, 1960; Gollnick et al., 1972a, 1972b, 1973a, 1973b). A five-fold difference has been reported between 'red' and 'white' rat skeletal muscle by Baldwin and his co-workers (1972a). The specific nomenclature used by the authors of these studies was born from the fact that fiber type differences were only assessed on the one hand (FT-ST), on the basis of myosin ATPase and, on the other hand (Red-White), on the basis of physical colour of the whole muscle.

The difference in oxidative potential seems to be due to a number of contributing factors. Patriarca and Carafoli (1969) have shown that in red muscle (SO,FOG) the amount of calcium (a prerequisite for contraction) in mitochondria is high and the sarcoplasmic calcium low. This calcium relationship seems to be a property of the sarcoplasmic reticulum which is characteristic to this type of muscle. The authors suggest that mitochondria may have a role in the intracellular movements of calcium associated with contraction and relaxation in red muscle. The myoglobin content of red muscle also is two to five times higher than that of white muscle (Malekina _et_ _al_., 1966; Harthshorne and Perry, 1962; McPherson and Tokunaga, 1967; Dawson and Romanul, 1964), and various investigators have reported that red muscle fibers have a higher mitochondrial content and mitochondrial protein than white fibers (Porter and Armstrong, 1965; Patriarca and Carafoli, 1969).

The red muscle fibers are considered to be fat-utilizing, as opposed to the white glycogen-utilizing fibers (Vallyathan _et_ _al_., 1970) and contain not only lipid inclusions which white fibers do not, but also show five times as much hydroxyacyl-CoA dehydrogenase activity and are able to oxidize ketone bodies at a rate which is two to three times higher than in white muscle tissue (Beatty _et_ _al_., 1959). Since the activity of hexokinase in red muscle is higher than in white (Peter _et_ _al_., 1968; Bass _et_ _al_., 1969) it appears that tonic muscle fibers better utilize free glucose whereas phasic fibers, because of their high phosphorylase activity (Dubowitz and Pearse, 1960), rely more upon glycogen metabolism.

Golarz de Bourne and Bourne (1966) have demonstrated five different lactate dehydrogenase (LDH) isoenzymes in skeletal muscle cells. Further observations of human muscle by other authors showed that white fibers contain predominantly the M form of LDH which is responsible for the conversion of pyruvate to lactate in anaerobic glycolysis, whereas red fibers are particularly rich in the H form of LDH, responsible for the conversion of lactate to pyruvate in aerobic glycolysis in the heart and liver (Dawson and Kaplan, 1965; Karlsson _et al_., 1974).

Finally, the work of Romanul (1965), Carrow _et_ _al_. (1967) and Mai and co-workers (1970) establishes the existance of a greater capillary to fiber ratio, with a subsequent increased blood flow potential, in slow-oxidative fibers specificaly.

Thus, these findings would seem to favor the uptake and oxidation of blood glucose and free fatty acids (FFA) by the oxidative fibers and accentuate the 'oxidative potential' difference between both oxidative (SO,FOG) and non-oxidative (FG) fiber types.

The Fast-Glycolytic Fiber

It has been shown that FT (FG) fibers of mammalian skeletal muscle generally have a higher glycolytic and lower oxidative capacity than ST (SO) fibers as judged histochemically and by enzyme activities (Dubowitz and Pearse, 1960; Bass _et_ _al_., 1969; Domonkas and Lotzkovits, 1961; Gollnick _et_ _al_., 1972a, 1972b, 1973a, 1973b). In support of these findings, Dubowitz and Pearse (1960) have reported also that the FT fibers of human skeletal muscle contain more stored glycogen than the ST fibers. This same relationship between glycogen content of FT and ST fibers is also supported by other authors (Gillespie et al., 1970; Beatty et al., 1963; Ogata, 1960).

With respect to contractile prerequisites, Patriarca and Carafoli (1969) have shown that the calcium pool associated with the sarcoplasmic reticulum is higher in white than in red muscle, and reports indicate that phasic (white) muscles contain about twelve percent more total protein (Taskar and Tulpule, 1964; Barany et al., 1965) and twenty-five percent less noncollagenous protein than tonic (red) muscle (Prewitt and Salafski, 1967). Thus, this higher component of structural protein found in the FT fibers could in part be responsible for the capacity of these FT fibers for the development of the greater tension which is characteristic of this particular fiber type.

Finally, Barany (1967) has demonstrated that the intrinsic speed of muscle contraction, with and without load, is a characteristic property of the ATPase activity of myosin in the skeletal muscle of several mammalian species. Subsequently, Taylor and co-workers (1974) have shown that the ATPase hydrolysis in the fast contracting fibers has higher maximal velocity as compared to the ATPase which predominates in the more slowly contracting fibers.

This high glycolytic - low oxidative fiber is in complete contrast to the SO fiber seen previously, and although it is capable of fast contraction and rapid tension development, it posesses little of the oxidative qualities which could render it fatigue resistant.

The Fast-Oxidative-Glycolytic Fiber

The third muscle fiber type which has been shown to exist in mammalian skeletal muscle (Edgerton and Simpson, 1969; Armstrong et al., 1974, 1975; Khan et al., 1974; Gillespie et al., 1974) is a type of fiber which has been termed fast-oxidative-glycolytic as it shows both high levels of myofibrillar ATPase activity (Burke and Edgerton, 1975) as well as heavy staining for oxidative potential (NADH-Diaphorase)(Peter et al., 1972).

The high glycolytic aspect of the FOG fiber profile is supported by work from other authors which has shown that, in guinea pig vastus lateralis, the FOG fiber has a higher glycogen content than even the FG fiber (Gillespie et al., 1970) as well as moderate LDH activity and high phosphorylase and alpha glycerophosphate dehydrogenase activity (Peter et al., 1972). This, as the authors suggest, implies an important role for the alpha glycerophosphate shuttle system in regenerating nicotinamide adenine dinucleotide (NAD) for glycolysis in both the FOG and FG fibers.

On the other hand, much evidence also exists to support the highly oxidative potential of the FOG fiber. In fact, the oxidative enzymatic capacity of the FOG fiber type seems to indicate that it is better equipped for oxidative metabolism than even the SO fiber. Cytochrome C content, an index of mitochondrial content, is approximately nine times greater in guinea pig FOG fibers than in FG, and even three times greater than in SO fibers (Burke and Edgerton, 1975). Likewise, the oxidative enzyme activity of succinate dehydrogenase is higher in FOG than in SO fibers in rat skeletal muscle (Nolte and Pette, 1972) and a similar relationship has been shown to exist with respect to myoglobin content in guinea pig muscle (Peter et al., 1972). Finally, Reis et al. (1969) in cat, and Mai and his co-workers (1970) in guinea pig have shown that FOG fibers have higher capillary to fiber ratios than FG fibers, and thus, the richer blood supply which they receive can enhance their aerobic oxidative potential and subsequent resistance to fatigue.

Glycogen Depletion Patterns and Muscle Fiber Recruitment

Although all of the physiological and physical characteristics of the individual skeletal muscle fibers presented in the previous sections have been reported by various authors, one might ask whether or not any direct evidence of recruitment at the fiber or motor unit level has been shown. Such evidence of the selectivity of fiber recruitment by type of work has been reported. The techniques involved in establishing the theories of specific fiber recruitment include electromyographic studies (Henneman and Olson, 1965; Hannerz and Grimby, 1973; Grimby and Hannerz, 1968; Hannerz, 1974), biochemical studies on homogeneous muscles (Bass *et al.*, 1969), and histochemical glycogen depletion studies on human and animal skeletal muscle fibers (Armstrong *et al.*, 1974, 1975; Costill *et al.*, 1973, 1974; Gillespie *et al.*, 1974; Gollnick *et al.*, 1973a, 1973b, 1974a, 1974b). For the purposes of this review, only the latter evidence will be considered in detail.

The loss of fiber glycogen, a substrate which serves as an important energy source for muscular contraction, has been used as an indicator of fiber recruitment in exercise and single motor unit recruitment (Edgerton *et al.*, 1972b; Burke *et al.*, 1971; Edgerton and Hewitt, 1972a; Edgerton and Lehto, 1972c).

It has been postulated by some authors that during normal activity, fibers are probably recruited in the manner outlined by

Henneman and Olson (1965). It was suggested that, at low to moderate stimulation intensity, those fibers with the smallest motorneurons are probably predominantly recruited, and those fibers with the largest motorneurons called into play only at the higher intensities of stimulation. This concept supports the hypothesis that at low to moderate work intensity, oxidative fiber groups are probably used to a greater extent and the glycolytic fibers added only when the work load exceeds the capacity of the oxidative fibers.

Gollnick and his co-workers (1973a) observed that at the end of a work bout to exhaustion on a bicycle ergometer, at a workload requiring sixty to seventy percent of Max $\dot{V}O_2$, total muscle glycogen was very low. It was determined by PAS stain that the ST fibers were depleted first, and, as the exercise progressed, the FT fibers also became depleted. Thus it seems that all muscle fibers are used at some time during an exercise bout to exhaustion and suggests that there exists a preferential early utilization of ST fibers during prolonged intense exercise, with a secondary recruitment of FT fibers occurring as the ST fibers become depleted of their glycogen stores. The authors suggest a mechanism whereby such depletion patterns could be explained. One interpretation of these results put forth by Gollnick and his co-workers is that those fibers losing PAS staining first were recruited initially and, as they became depleted of glycogen, their metabolic rate, and therefore ability to develop tension, was impaired to a point where additional motor units had to be activated to sustain the work. Another interpretation involves a synchronous activation of motor units with a very intermittent use of those containing FT fibers. Thus, the FT fibers might be activated in work of relatively moderate intensity for only short intervals of time at the point(s) of peak tension. It is possible that both of these mechanisms are active in determining recruitment patterns in skeletal muscle fibers during activity.

In support of these findings, Karlsson, Diamant and Saltin (1971) observed a depletion of the ATP and CP stores relative to work load while the muscle lactate concentration increased faster at higher workloads concomitant with a tendency to an increased glycolysis. Likewise, Hultman et al. (1971) and Saltin and Karlsson (1971) also have shown that the effect of exercise on the glycogen store is dependent on the workload employed. The anaerobic energy output however, seemed to start when the phosphagens were lowered to approximately seventy percent of the basic values which occurred during steady state work at workloads in excess of fifty percent of Max $\dot{V}O_2$. These results tend to indicate that the body can use stored ATP and CP within the cells for some proportion of the initial work and that, after these reserves of high energy phosphates are depleted, the glycolytic machinery becomes involved at work loads in excess of fifty percent Max $\dot{V}O_2$.

With work of an even higher intensity (pedalling a bicycle ergometer at 90 or 104 rpm at a resistance calculated to require an energy expenditure of 150 percent of the subject's maximal aerobic power), Gollnick and his associates (1973b) showed that the first fibers to become depleted of their glycogen stores were the low-oxidative, high-glycolytic FT fibers. This would seem to suggest an early recruitment of these fibers during heavy exercise. This pattern is in direct contrast to that seen during prolonged, moderately intense exercise when the high-oxidative slow-twitch fibers are the first to become depleted of their glycogen reserves. In this way, even though FT fibers were recruited at the onset of exercise, ST fibers were probably also utilized in these exercise bouts, but because of their greater aerobic capacity, they would not deplete their glycogen reserves as rapidly as the FT fibers. Likewise, perhaps the lower glycolytic potential of the ST fibers may also have prevented such a rapid rate of glycogen utilization.

With work of varying intensity and at varying pedalling rates on the bicycle ergometer, the results of Gollnick, Piehl and Saltin (1974b) point to a primary reliance upon ST fibers during submaximal, endurance exercise, with the FT fibers being recruited after the ST fibers are depleted of glycogen. During exertion requiring energy expenditure greater than the maximal aerobic power, both fiber types appear to be continuously involved in carrying out the exercise. This relationship holds true in work by Costill _et_ _al_. (1973, 1974) during distance running and during prolonged running on the level or uphill, respectively, and Gollnick _et_ _al_. (1974a) with isometric contractions in man.

Similar findings appear to be the case as well in studies where animal models are used. Gillespie _et_ _al_. (1970), in a study involving a running and jumping program in a prosimian (Galago Senagelensis), determined that glycogen loss was related to the specific type of exercise used. In the running program (a low intensity workbout) depletion in the SO fibers was more pronounced than in the FOG fibers, and even more so than in the FG fibers (SO>FOG>FG). On the other hand, with the jumping program (a high intensity workbout), the order of the depletion pattern was directly opposite to that seen as a result of the running program (FG>FOG>SO). Thus, these findings would strongly suggest that the recruitment pattern of specific types of motor units is related to the nature of the specific movement performed.

In support of these findings, Armstrong and his associates (1974, 1975) in two studies using highly sprint-trained rats, showed that total muscle glycogen decreased as exercise intensity increased (in these two particular studies by increases in speed). At low running speeds (less than fifty m/min) the greatest decrease in PAS staining was demonstrated in the FOG and SO fibers whereas, at higher running speeds (in excess of fifty m/min), the greatest decrease in PAS staining was in fact shown to be in the FG fibers. The authors suggest that at low work intensities, primary reliance is upon oxidative fibers for contractile activity and that the anaerobic fibers are only recruited at higher intensity work levels or when aerobic fibers become depleted of glycogen in prolonged, low intensity work.

This situation also has been shown to exist in rats which are exposed to swimming as a mode of exercise stimulus (Armstrong et al., 1975). Armstrong and his co-workers (1975) do however speculate that some high oxidative fibers may be active at all work intensities but that FG fibers are only recruited at higher intensities where the number of these active FG fibers is directly proportional to the amount of tension necessary to do the work. This is consistent then with the Henneman and Olson theory presented in earlier sections. This reliance of FG fibers on anaerobic energy production and the relative unavailability of substrates from the blood would be expected to result then in a markedly higher rate of glycogen catabolism in the FG fibers than in either the FOG or SO fibers during contraction. For this reason, prolonged use of FG fibers would not be possible as glycogen stores are limited and complications arise as a result of metabolic acidosis.

Finally, in a study conducted by Terjung (1976) using a motor driven rodent treadmill and a group of 12 wk. endurance-trained rats, training programs varying in intensity by changes in the percent grade of the treadmill and duration of the workbouts resulted in similar recruitment patterns as demonstrated biochemically by the assessment of the increase in oxidative marker enzymes (citrate synthetase) brought about as a result of these different training programs. In this study, speed was kept constant. At the lower percent grade (10%), the training regimen was of low to moderate intensity and increases in the oxidative markers occurred primarily in the FOG and SO fibers. However, with the speed unchanged, at a higher elevation of the treadmill (30%), predominantly FG fibers were shown to have increased their complement of these oxidative markers. This increase in elevation would seem to reflect a workbout of a higher intensity.

Although glycogen depletion patterns have been used widely as indicators of specific fiber recruitment, it must be recognized that certain limitations do exist with this technique. In the first place, the PAS stain used in determining glycogen utilization indicates qualitative differences in fiber glycogen content but not quantitative differences. At dark staining intensity (high glycogen content) differences cannot be determined as all dark fibers, whether some have more glycogen than others, appear to be as dark one as another.

Secondly, glycogen depletion in different muscle fiber types depends not only on the usage of particular motor units, but also on the possible utilization of other energy sources in addition to glycogen. In regard to this fact, glycogen depletion patterns induced as a result of extended periods of submaximal work are probably even less precise as an index of fiber usage because of such factors as glycogen resynthesis, mobilization of fatty acids, and vasoregulatory adjustments (Burke and Edgerton, 1975). And finally, although examination of glycogen depletion patterns gives us a final picture of the relative glycogen content of the different fibers, nothing is known about the order in which these fibers are recruited. Yet, despite these limitations, this technique remains one of the most widely used by researchers working in this field.

Summary

Thus, in summary, the literature seems to indicate that histochemical glycogen depletion patterns implicate the oxidative fibers (FOG and SO) of mammalian skeletal muscle as being preferentially used in prolonged, moderately-intense exercise, and that FG fibers are progressively recruited as the glycogen of the oxidative fibers becomes depleted, or when there is an increase in intensity great enough to require the use of these FG fibers.

The differences in the type of fibers that are depleted of glycogen are a reflection of the flexibility of the motorneuronal recruitment seen earlier. The consistent patterns of differential glycogen loss related to specific types of muscle fibers support the hypothesis that the motorneuronal recruitment pattern is not determined solely by motorneuron size and that man, as most primates, has the ability to utilize those motor units that are best suited to the demands of the movement to be performed.

During an aerobic or long-duration low-intensity event, endurance is a more critical factor than tension development, whereas, in anaerobic or short-duration high-intensity events, high tension output is essential, and necessarily endurance must be sacrificed.

Although this relationship seems to be firmly established in the literature at the present time, nothing is known concerning the specificity involved in the method of increasing the intensity or stress under which a muscle must operate. Whether or not there is a difference in fiber type recruitment specific to exercise intensity still needs to be determined.

CHAPTER III

RESEARCH METHODOLOGY

Introduction

The first section in this chapter deals with the selection and grouping of subjects that were used in this study. Then, the acclimation and training regimen imposed on the subjects are outlined and the testing protocol presented. Finally, the sacrifice procedures, histochemical analysis, and statistical methods are presented.

Selection of Subjects

Forty-eight seven week old male Wistar rats (Biobreeding Inc.) with mean initial body weights of 163 grams were used in this experiment. Six animals were randomly assigned to one of eight different treatment groups (see Table 1) and acclimated as described below.

Acclimation

The animals used in this study were obtained at seven weeks of age and maintained on standard laboratory chow and water *ad libitum* in individual cages with a 12h/12h light/dark cycle. Animals were kept sedentary or assigned to one of the treatment groups described in Table 1. All of the experimental animals ran on a calibrated rodent treadmill (Quinton model 42-15) six days per week for two weeks. The animals ran only during their 12 hour dark cycle, as this is their most active period. The running program was designed to bring the animals to a level where they were capable of exercise of thirty

Table 1

Experimental Group Classifications

Treatment Group	(n)	Experimental Conditions		
		Speed m/min	Grade % incline	protocol
EX-C	(6)	0	0	
S-25	(6)	25	0	continuous
S-35	(6)	35	0	continuous
S-45	(6)	45	0	continuous
S-55	(6)	55	0	continuous
G-0	(6)	35	0	intermittent
G-20	(6)	35	20	intermittent
G-40	(6)	35	40	intermittent

Table 2

Acclimation and Training Regimen

Day	Exercise Duration Repetitions	Work/Rest Interval(sec.)	Speed m/min.	Grade % inc.
1	1	300 / 0	0	0
2	1	300 / 0	10	0
3	1	300 / 0	18	0
4	15	60 / 60	25	0
5	15	60 / 60	30	0
6	15	60 / 60	35	0
7		REST		
8	15	30 / 30	40	0
9	15	30 / 30	45	0
10	15	30 / 30	45	0
11	15	30 / 30	50	0
12	15	30 / 30	55	0
13	15	30 / 30	55	0
14		REST		
15		REST		
16	EXPERIMENTAL DAY			

seconds duration followed by thirty seconds of rest, at a speed of 55 m/min, over a period of fifteen consecutive trials (see Table 2). Following this two week acclimation period, the animals were given a two day rest to allow for complete glycogen refilling of muscle (Terjung, 1974) and the third day, the experiment was concluded as described in the next section.

Testing Protocol

Two series of experiments were completed. In series I, running on a rodent treadmill at a zero percent grade with increases in treadmill speed was the exercise stimulus. In series II, running at a constant speed, with increases in the percent elevation of the treadmill, comprised the exercise stimulus.

For series I, the animals performed the bout of exercise which conformed to that outlined in Table 1 for groups S-25 to S-55 inclusive. These groups represented the series of work bouts done at zero percent elevation but at increasing speeds (25 to 55 m/min). All rats, prior to their actual work bout, received a one minute preliminary walk on the treadmill at a constant speed of 10 m/min and elevation of zero percent.

The six animals of the highest speed group (S-55) were then made to run continuously, in groups of two, to exhaustion at a speed of 55 m/min and elevation of zero percent. The criterion for exhaustion used was that the animal fell back onto the shock grid (constantly set at a dial reading of 55 V.), and remained there for a period of ten seconds or greater, three consecutive times after being removed from the grid and being replaced on the treadmill. Once this occurred, if the animal, when placed on its back on a flat surface, failed to immediately right itself, it was considered to be exhausted. The animals ran in groups of two because each animal was timed. This was done to ensure that the time and actual distance covered for each animal was exact (ie. the clock was stopped during the time the animal was on the shocking grid as well as being checked for exhaustion). The distances covered by each animal were then recorded and the mean distance run by the animals in this group was then calculated. The remaining 3 treatment groups ran this same distance at speeds of 25, 35, and 45 m/min at zero percent elevation. Upon completion of the exercise bout, the animals were sacrificed and the tissues removed and processed as outlined in the following section.

For series II, the animals performed the bout of exercise which conformed to that outlined in Table 1 for groups G-0 to G-40 inclusive. These groups represented the series of workbouts done at a constant speed of 35 m/min but at increasing percent elevations of the treadmill (0 to 40 %). Here also, all rats, prior to their actual work bouts, received a one minute preliminary walk on the treadmill at a constant speed of 10 m/min and elevation of zero percent.

The six animals of each percent elevation group (G-0, G-20, G-40) ran intermittently with thirty seconds of work followed by thirty seconds of rest until they had achieved three minutes and thirty seconds of actual work time at a constant speed of 35 m/min and at elevations of 0, 20, and 40% incline, respectively. Upon completion of the exercise bouts the animals were sacrificed and the tissues removed and processed as outlined in the following section.

The remaining group of six animals (EX-C) performed the same acclimation and training regimen (Table 2) as the animals in the other treatment groups, with the exception of the run on the experimental day. These animals served as exercised controls. All animals were sacrificed and the tissues removed and processed as outlined in the following section.

Sacrifice and Histochemical Analysis

Upon completion of the exercise bouts, the animals were sacrificed by decapitation and exsanguinated. The plantaris muscle from both of the hind limbs of each animal was dissected out, rolled in talcum powder, mounted on specimen holders in OCT embedding medium (Ames Tissue-Tek), and frozen in isopentane cooled in liquid nitrogen. The tissues were then placed into separate containers and stored in dry ice (-40°C) until histochemical analysis was performed.

For analysis, serial sections 10μ thick were cut in a cryostat (IEC Minotome) at -20°C, mounted on clean, dry cover slips without adhesive, and left to air dry for a period of at least fifteen minutes. Myofibrillar adenosine triphosphatase (ATPase) and reduced nicotinamide adenine dinucleotide diaphorase (NADH-Diaphorase) activities were demonstrated by the technique of Padykula and Herman (1955) as modified by Guth and Samaha (1969) and Khan et al. (1974)(Appendix A), and Novikoff and co-workers (1961), respectively.

Muscle fibers were classified as fast-twitch-glycolytic (FG), fast-twitch-oxidative-glycolytic (FOG), or slow-twitch-oxidative (SO) with the system described by Peter and co-workers (1972). The relative glycogen content in the muscle fibers was subjectively estimated from photomicrographs taken under the light microscope of the PAS reaction (Pearse, 1961). The fibers were rated as dark (D), moderate (M), light (L), or negative (Neg) using a system similar to that proposed by Kugelberg and Edstrom (1968) as described by Gollnick and his co-workers (1972c), and reference photomicrographs as described in the section dealing with 'Definitions' (p. 5).

Statistical Methods

A field of muscle fibers was chosen and photographed under the light microscope from histochemical sections of the right plantaris muscle stained for ATPase, NADH-Diaphorase, and PAS for every experimental animal according to the following four criteria:

1. that the field chosen contain at least 200 fibers,
2. that the field be chosen to provide the greatest number of SO fibers as possible,
3. that the histochemical serial sections be as clean as possible,
4. and that the field chosen be as representative as possible in PAS staining intensity of the entire muscle.

The muscle fibers of each photomicrograph were then rated as SO, FOG, and FG from the histochemical stains for ATPase and NADH-D, and as D, M, L, or Neg in PAS staining intensity, using the reference photomicrographs (Figures 1,2).

As the data classified in this manner was expressed as frequency data, the results were analysed using the following statistics. In order to determine whether or not the mean percentage of SO, FOG, and FG fibers with respect to the mean total number of fibers per group was homogeneous when compared across the eight different groups, the test of Chi Square was applied. The Chi Square test was also used to determine whether or not the mean total number of fibers per group was significantly different across the eight treatment groups, and to test for reliability and objectivity in the rating of muscle fibers for each of the fiber type classifications and PAS staining intensities. Finally, a One Way Analysis of Variance was performed on the body weight data of the experimental animals in order to determine whether or not there were any significant differences in this parameter across each of the eight treatment groups.

CHAPTER IV

RESULTS AND DISCUSSION

1. Results

The purpose of this study was to investigate the effects of changing the intensity of a workload by variations in speed of a motor driven rodent treadmill from 25 - 55 m/min., and elevation from 0 - 40% grade, on glycogen depletion patterns demonstrated histochemically by the PAS reaction in the plantaris muscle of male Wistar rats. The results were analyzed as follows: a) the training regimen, which includes training performance and body weight data, b) reliability, c) objectivity, d) homogeneity of fiber types, and, e) PAS staining intensity of the various fiber types expressed as means for each group.

a) Training Regimen

The training program was of 12 days duration and was designed to bring the animals to a level where they were capable of exercise of thirty seconds duration followed by thirty seconds of rest, at a speed of 55 m/min, over a period of fifteen consecutive trials (Table 2).

Animals 1 and 35 were eliminated during the training regimen as they performed poorly on the treadmill and received many shocks. Animal 39 was eliminated on the experimental day as it could not perform the bout of work assigned to it (S-45). As a result of this, the number of experimental animals in treatment group S-45 was reduced to five.

Table 3 represents the statistical treatment of the body weight data for each of the treatment groups on the experimental day.

TABLE 3

ANOVA TREATMENT OF BODY WEIGHTS FOR EACH GROUP (grams)

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F Ratio	P*
Among Groups	7	2152.8	307.6	1.12	ns`
Within Groups	36	9896.1	274.9		
Total	43	12048.9			

* P - Probability ` ns - not significant

There is no statistically significant difference between the body weights for each group of animals on the experimental day.

The individual data for the increases in body weight for each of the experimental animals over the course of the acclimation and training regimen is tabled in Appendix B together with the graph representing the growth of the rats as related by the mean body weight (± SD) of all the animals for each day of the training program.

b) Reliability

Reliability was established by a test-retest method on the same group of three photomicrographs chosen at random. A two day period separated the test day from that of the retest. On both occasions, muscle fibers were classified as either SO, FOG, or FG using the ATPase and the NADH-D stains, and within each of these classifications, as D, M, L, or Neg with the PAS stain. Tables 4 and 5 represent the test-retest data (frequencies), and the statistical treatment of these data, respectively.

TABLE 4

TEST-RETEST VALUES FOR RELIABILITY IN CLASSIFICATION OF STAINING INTENSITY OF SKELETAL MUSCLE FIBERS

Treatment	Fiber Type	PAS Staining Intensity D	M	L	N
TEST	SO	0	0	7	14
	FOG	0	14	40	58
	FG	0	37	5	0
RETEST	SO	0	0	8	13
	FOG	0	12	36	60
	FG	0	38	8	0

TABLE 5

CHI SQUARE TEST OF RELIABILITY DATA

Fiber Type	PAS Staining Intensity D TEST-RETEST	M TEST-RETEST	L TEST-RETEST	N TEST-RETEST
SO			$X^2 = 0.97$ $_{.95}X^2_2 = 5.99$	$X^2 = 0.06$ $_{.95}X^2_1 = 3.84$
FOG		$X^2 = 0.16$ $_{.95}X^2_1 = 3.84$		
FG				

There is no statistically significant difference among the classification of fibers as PAS D, M, L, or Neg in each of the fiber type categories between the test and retest treatments.

c) Objectivity

Objectivity was established by a comparison of the rating results (using the reference photomicrographs) of the author and another experienced rater on three serial photomicrographs chosen at random. Muscle fibers were classified as either SO, FOG, or FG using the ATPase and the NADH-D stains, and within each of these classifications, as D, M, L, or Neg with the PAS stain. Tables 6 and 7 represent the Rater 1 - Rater 2 data (frequencies), and the statistical treatment of these data, respectively.

TABLE 6

RATER 1 - RATER 2 VALUES FOR OBJECTIVITY IN CLASSIFICATION OF STAINING INTENSITY OF SKELETAL MUSCLE FIBERS

Treatment	Fiber Type	PAS Staining Intensity			
		D	M	L	N
RATER 1	SO	0	0	7	14
	FOG	0	14	40	58
	FG	0	37	5	0
RATER 2	SO	0	0	10	11
	FOG	0	13	28	69
	FG	0	40	4	0

TABLE 7

CHI SQUARE TEST OF OBJECTIVITY DATA

Fiber Type	PAS Staining Intensity			
	D	M	L	N
	RATER1-RATER2	RATER1-RATER2	RATER1-RATER2	RATER1-RATER2
SO			$X^2 = 1.71$ $_{.95}X_2^2 = 5.99$	$X^2 = 0.89$ $_{.95}X_1^2 = 3.84$
FOG		$X^2 = 0.12$ $_{.95}X_1^2 = 3.84$		
FG				

There is no statistically significant difference among the classification of fibers as PAS D, M, L, or Neg in each of the fiber type categories between Rater 1 and Rater 2.

d) <u>Homogeneity of Fiber Types</u>

The data in Appendix C represent the frequencies of rat plantaris muscle fibers which stained PAS D, M, L, or Neg within each fiber type category, for each animal as well as the mean frequencies per group.

Homogeneity of the mean percentages of each of the three different fiber types with respect to the mean total number of fibers for each group, was tested across the eight treatment groups by the application of the Chi Square test. Chi Square was also used to test for homogeneity in the mean total number of fibers per group across all treatment groups.

Table 8 represents the mean frequency data for each fiber type as a percentage of the mean total number of fibers within that group, as well as the mean total number of fibers for each group, and the statistical treatment of these data.

TABLE 8

MEAN FREQUENCY DATA FOR EACH FIBER TYPE EXPRESSED AS PERCENTAGES OF THE MEAN TOTAL NUMBER OF FIBERS PER GROUP, AND CHI SQUARE ANALYSIS OF THE DATA.

Group	Mean % Number of Fibers / Group			Mean Total Number of Fibers / Group	(±SD)
	SO	FOG	FG		
EX-C	10.9	77.3	11.8	238	±56.2
G-0	12.7	71.6	15.6	275	±47.9
G-20	11.4	68.5	20.1	273	±42.5
G-40	12.0	78.7	9.3	301	±33.0
S-25	12.9	67.4	19.7	264	±69.7
S-35	11.5	58.5	30.0	217	±54.2
S-45	14.5	66.7	18.8	297	±53.9
S-55	10.3	75.0	14.7	300	±77.7
All Groups $_{.95}X^2_7 = 14.07$	$X^2 = 1.02$	$X^2 = 4.36$	$X^2 = 16.04$ $p \leq 0.05$	$X^2 = 23.97$ $p \leq 0.05$	
All Groups except S-35 $_{.95}X^2_6 = 12.59$			$X^2 = 6.5$	$X^2 = 11.51$	
All Groups except G-40 $_{.95}X^2_6 = 12.59$			$X^2 = 10.9$		

There exists a statistically significant difference among the mean % number of FG fibers, as well as among the mean total number of fibers, across all of the eight treatment groups ($p \leq 0.05$). In order to find out which groups in the FG and Total categories were responsible for these significant differences, each group, in turn, was dropped and the Chi Square test applied to the seven remaining groups. From the statistical analysis presented in Table 8, the significant differences in the % FG category were shown to exist in groups S-35 and G-40, and the significant difference in the mean total number of fibers was shown to be in group S-35.

e) PAS Staining Intensity of Fiber Types

Data in Appendix D represent the mean frequencies of rat plantaris muscle fibers which stained PAS D, M, L, or Neg as percentages of the mean total number of fibers within each fiber type category. Figure 3 depicts these relationships graphically. Figures 4 and 5 represent the histochemical glycogen depletion patterns of one of the animals in treatment group G-20 and G-40, respectively.

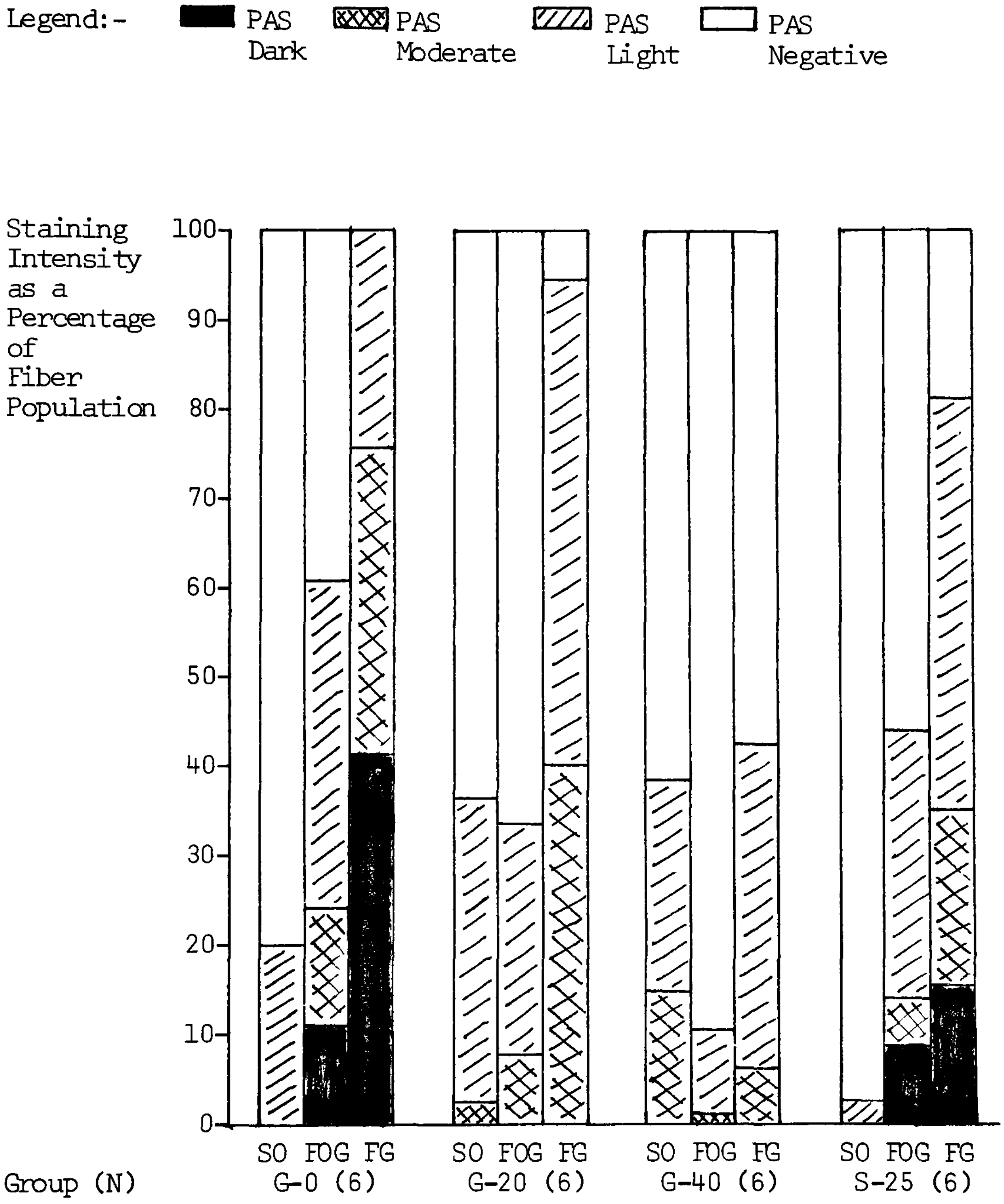

Figure 3: - Mean Ratings of Staining Intensity of Rat Plantaris Muscle Fibers Expressed as a Percentage of the Mean Fiber Population for Each Treatment Group

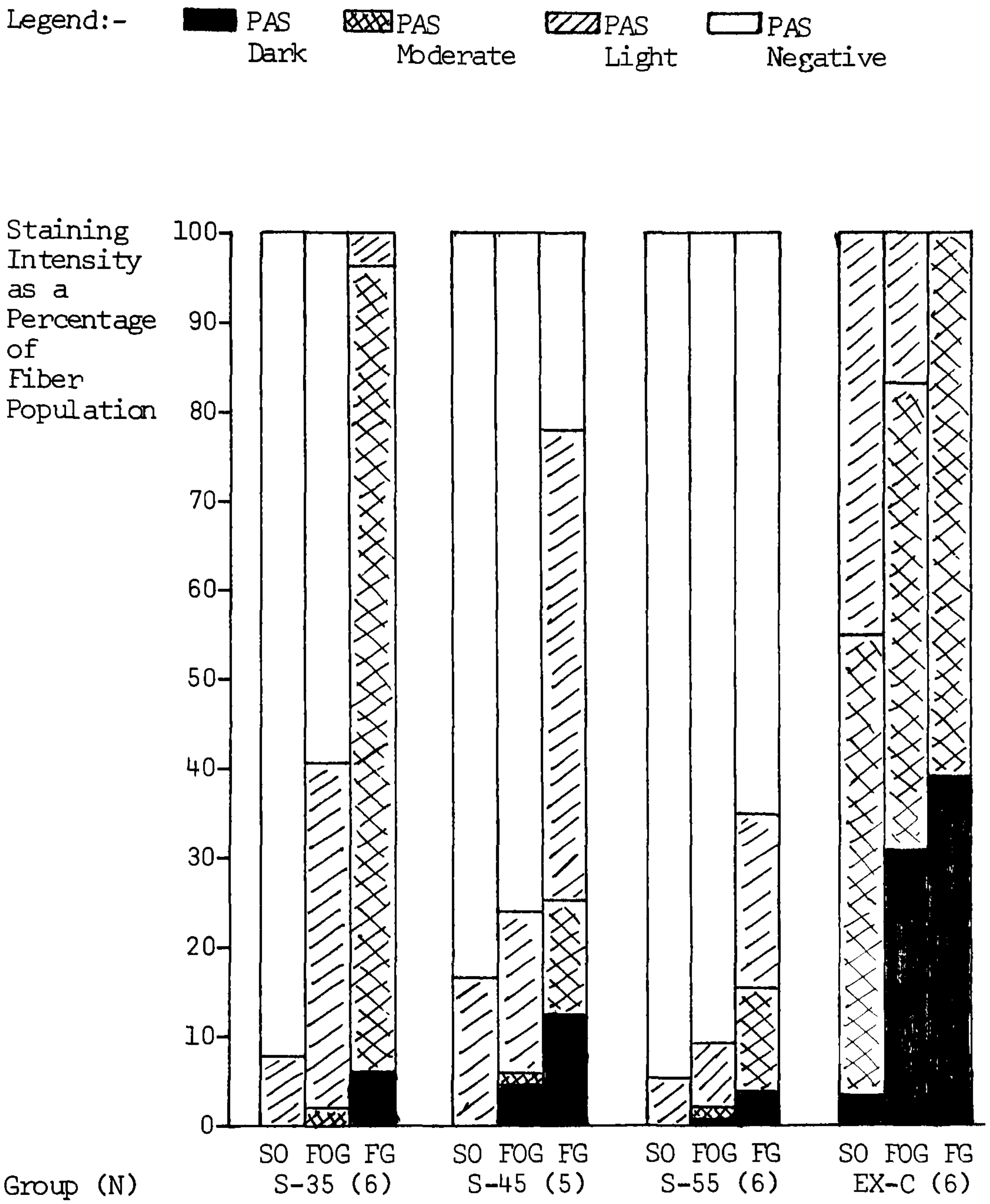

Figure 3: - Mean Ratings of Staining Intensity of Rat Plantaris Muscle Fibers Expressed as a Percentage of the Mean Fiber Population for Each Treatment Group

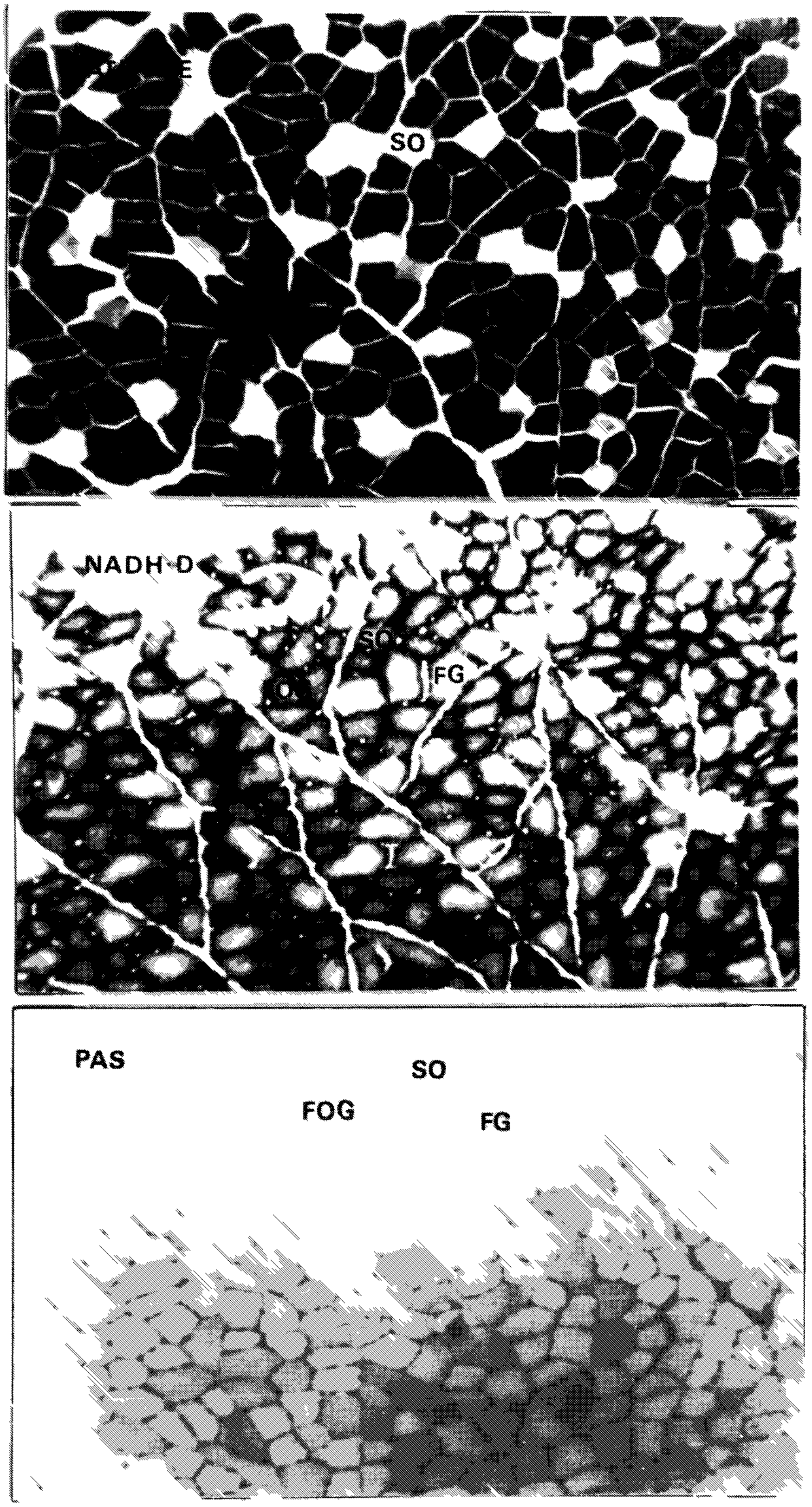

Figure 4: - Serial Sections of Rat Plantaris Muscle of an Animal (23) which ran for 3:30 sec. intermittently with 30 sec. of work followed by 30 sec. of rest up an incline of 20% elevation. Note the selective depletion of glycogen in the SO and FOG fibers. (x115)

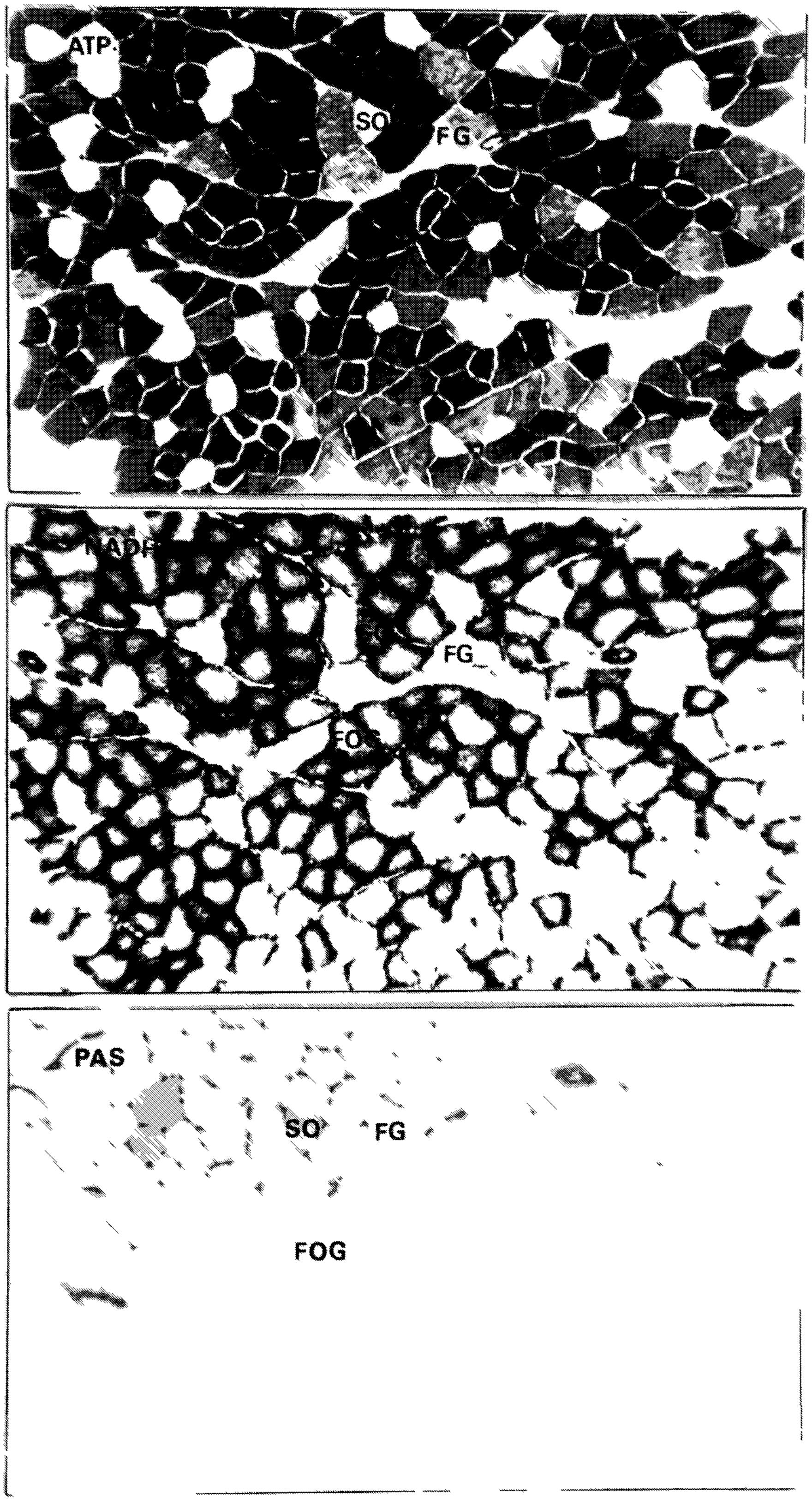

Figure 5:- Serial Sections of Rat Plantaris Muscle of an Animal (50) which ran for 3:30 sec. intermittently with 30 sec. of work followed by 30 sec. of rest up an incline of 40% elevation. Note the selective depletion of glycogen in the FG and FOG fibers. (x115)

2. Discussion

a) Training Regimen

The acclimation and training regimen designed was successful in bringing about the desired performance in the experimental animals. The program did bring the animals to a level where they were capable of exercise of thirty seconds duration followed by thirty seconds of rest, at a speed of 55 m/min. over a period of fifteen consecutive trials. The length of the training program in this study (2 weeks) was of a much shorter duration than those programs used by Terjung (1976) (12 weeks) with a group of endurance-trained rats, and Armstrong et al. (1974, 1975) (10 weeks) with a group of sprint-trained rats. The reason behind the selection of this particular training regimen lies in the fact that in most training studies where rats are made to exercise to exhaustion, or at high intensities on a treadmill or in training wheels, relatively well-trained animals are used. Very little information exists concerning acute studies of exercise-induced changes in skeletal muscle in relatively untrained animals. This type of information could help to clarify the ongoing, chronic changes that occur in a training program which result in the physiological profile observed in those animals which are said to have achieved a trained state.

b) Homogeneity of Fiber Types

In order to determine homogeneity within each fiber type across the eight treatment groups, the test of Chi Square was applied to the mean frequency data for each fiber type expressed as percentages of the mean total number of fibers per group (Table 8). As it can be seen from the Chi Square values for the SO (X^2 = 1.02) and FOG (X^2 = 4.36) populations, there existed no significant differences among the individual group mean percentages of each fiber type. In this way, the PAS staining patterns characteristic of each group, for the SO and FOG populations, can be attributed to a treatment effect upon each group and not to any sampling effect which may have been introduced by the selection of the histochemical fields chosen to be photomicrographed.

This however was not the case when considering the percent number of FG fibers as well as the mean total number of fibers for each group. As it can be seen from the Chi Square analysis in Table 8 for the FG (X^2 = 16.04) and mean total fiber populations (X^2 = 23.97), there does exist some significant difference(s) among the individual group mean percentages for the FG population and among the mean total number of fibers per group.

Upon further analysis it was determined that the FG fiber population differences were attributed to the mean percentage of FG fibers for the treatment groups S-35 and G-40. In the case of the mean total number of cell types per group, the source of the significance was group S-35.

It must be kept in mind then, when discussing the staining patterns characteristic of these particular groups for the FG fiber population (S-35, G-40), as well as that for the mean total number of fibers (S-35), that a sampling effect was introduced over and above the treatment effect imposed by the experimental day protocol for each of these two groups.

The reason for these sampling effects is, however, unknown. One possible explanation for this situation which might be put forth, is that one of the main criteria for field selection was that the histochemical field should contain as many SO fibers as possible. There was however, no requirement as to the FG fiber proportion in those same fields. This could account, in part, for the variability in FG population seen in some of these groups. Together with this, the fact that FG fibers are significantly larger than the FOG and SO fibers could also account for the significant difference seen in the mean total number of fibers (217) in the S-35 treatment group (ie. the higher the proportion of larger fibers in a field - the fewer the total number of fibers in that field). This sampling effect could help to explain the apparent inconsistency seen in the PAS staining intensities observed in the mean FG population of treatment group S-35. From the glycogen depletion patterns seen in group S-35, there seems to be less of a depletion in FG fibers at 35 m/min. than at a speed of 25 m/min. This increase in staining intensity seen in the FG fiber population of group S-35 however, could be attributed to the fact that, because the mean percentage of FG fibers in this group is significantly greater (and the FG fiber population stains darkest of all the three fiber types in the control sections), the probability of obtaining FG fibers which are more darkly stained, is greater in this situation.

Likewise, as the mean percentage of FG fibers in group G-40 (9.3%) was significantly lower when compared to the percent FG fibers for the other groups, this size effect could help to explain the high (301) but non-significant mean total number of fibers seen in treatment group G-40.

c) PAS Staining Intensity of Fiber Types

In this study, exercise induced histochemical glycogen depletion patterns were used to determine qualitatively the apparent involvement, in exercise, of each of the individual fiber types present in mammalian skeletal muscle. The qualitative assessment of relative glycogen depletion patterns was performed on histochemical sections stained by the PAS technique.

The first part of this section will deal with the glycogen depletion patterns brought about as a result of changes in speed, at 0% incline, and the latter part, with those glycogen depletion patterns elicited as a result of exercise at selected grades but the same speed.

From the work of Armstrong et al. (1974, 1975) with a group of 12 week sprint-trained rats, it was suggested that, at low to moderate speeds with no incline, the oxidative fibers (SO, FOG) support most of the work, but that when the intensity of work was increased (to approximately 39 m/min.) or when the oxidative fibers became glycogen depleted during prolonged low intensity exercise, FG fibers appeared to become involved and progressively, as the intensity increased, to make a major contribution to the exercise. This hypothesis receives support from conclusions reached by Henneman and Olson (1965) about the mechanisms of motor unit recruitment in skeletal muscle. They found that the efferent neurons with the smallest diameter

(SO<FOG<FG) are the most excitable, and thus, that the participation of a motor unit in graded muscular activity was a function, in addition to other factors, of the size of its motorneuron.

With the criterion speed then of approximately 40 m/min. (which seemed to produce a workbout of sufficient intensity to cause this recruitment shift into the FG fiber pool), the range of speeds was set for this study to include speeds of only 15 m/min. to either side of this point (25-55 m/min.). This was done because it was felt that there was no need to include speeds of up to 80.5 m/min. (as Armstrong and his co-workers had done) to show some FG fiber involvement.

Generally, the results of this study are in agreement with the findings of Armstrong and his co-workers (1974, 1975). Some differences do however exist. In this study, in the plantaris muscle of the rats sacrificed at rest, 39% of the FG fibers stained dark for glycogen and the rest were rated as moderate. Likewise, 32% of the FOG fibers were rated as PAS dark with 52% being moderate and 17% light. For the SO fibers only 4% were rated as PAS dark, 54% were moderate and 42% light (Appendix D). These resting values vary markedly from those reported by Armstrong and his group. The resting levels of glycogen in the FG population in this study are lower, and in the SO and FOG categories higher, on the average, than those reported by Armstrong and his co-workers (1974, 1975).

One possible explanation for these findings could be related to the type of training undergone by each group of animals. The animals used by Armstrong and his team were relatively highly sprint-trained (10 weeks) as compared to the animals trained for only two weeks, used in this study. As the type of training undergone by Armstrong's animals was of a very high intensity (interval work at 80.5 m/min.), in the latter stages of the program, it can be assumed that the major part of the work done was supported by FG fiber involvement. As a result of this type of situation the amount of carbohydrate stored as glycogen in these fibers due to the training effect could be expected to be much higher than the stored in the other two types of fibers. On the other hand, in the present study, the animals were relatively untrained with the little training they had performed to this time tending to support the rationale for higher glycogen reserves being stored in the more oxidative SO and FOG fibers.

Points of similarity which emerge however, when comparing the results of this study with those of Armstrong et al. (1974, 1975) concern the trends seen in each of the different fiber types with regard to the glycogen depletion patterns induced at increasing speed in exercise. At the slower speeds used in this study in the FG fiber population, there is a depletion pattern indicative of a relatively minor involvement of FG fibers. As the exercise intensity increased (ie. as speed increased) the depletion pattern observed in the FG fibers indicated a trend to a seemingly greater glycogen depletion of this fiber type in supporting the work done. The exception seen in this pattern was group S-35. As outlined previously (p. 42) however, a sampling effect which resulted in a significantly greater number of FG fibers may have, in this situation, suppressed FG fiber depletion since FG fibers are normally dark or moderately rated. This trend was also substantiated by Armstrong and his co-workers, except for the one point discussed below.

The animals of the two slowest speed groups in Armstrong's studies (22.5, 28.4 m/min) showed virtually no difference in the FG relative glycogen estimation when compared to the rested controls. The reason for this discrepancy could be attributed to the fact that firstly, the animals in Armstrong's study were much better trained than those used in this study and secondly, that the distance covered by those animals used in this study was approximately three times the distance covered by the animals in the former studies (162.2 vs 471.0 m). Thus, the total amount of work done by the animals in this study could explain the lower relative glycogen levels seen in the FG fiber population at the slowest speeds.

In the FOG fiber population, basically the same relationship exists as that demonstrated in the FG category. At the slowest speed, (25 m/min) FOG fiber glycogen depletion, although markedly less than the control situation, is not complete. But, as the intensity of work increased, so too did the FOG fiber glycogen depletion - to a point where 90% of this fiber population was rated as PAS negative at a speed of 55 m/min.

The trend shown by the FOG fiber population in the studies conducted by Armstrong _et_ _al_. (1974,1975) however, was not the same. The animals used in their studies showed FOG fiber glycogen depletion patterns where 90% of the fibers were rated as PAS negative at the slowest speed (22.5 m/min.), and where this same pattern did not change across all of the running speeds tested (22.5 - 80.5 m/min.). The reason for the higher relative glycogen levels present in the FOG fiber population of the animals used in the present study at the slowest speed (25 m/min.), could be explained by the fact that, as mentioned earlier, the resting control levels of glycogen stored in the FOG fiber are indeed higher in these animals, and secondly, that at this slower speed, the greater depletion of the FG fibers seen as a result of doing the work, could be, in part, responsible for the sparing effect of glycogen stored in the FOG fibers by reducing the load under which they must perform.

Finally, the trend observed in this study, when considering the SO fiber population, is essentially the same as that seen in those studies by Armstrong <u>et al</u>. (1974, 1975). At the slowest speeds, the SO fibers showed the most depletion (indicative of the greatest involvement) with a tendency to a slightly lesser degree of depletion as the intensity of the work load increases. This slight sparing effect can presumably be attributed to the increased FOG and FG involvement at these higher intensities.

In summary then, the data suggest that the more oxidative fibers (SO, FOG) support most of the work at the slower running speeds but that, when the intensity of work increases, or when the oxidative fibers become depleted of their glycogen stores in the more prolonged, low intensity exercise bouts, the FG fibers appear to progressively make more of a major contribution to the exercise in question. In short, some high oxidative fibers are used at all speeds, but FG fiber involvement is primarily seen only at the higher speeds.

The literature available on studies using animals exercised by inclined running on the rodent treadmill is scanty. Studies by Terjung (1976) and Baldwin et al. (1973) are the only two pieces of work encountered in the course of this review which have used the rat model and such an experimental protocol. Unfortunately however, the inclines used in the present study were based upon pilot work and not upon the inclines used in those studies mentioned above. (20, 40% incline). A comparison with the results from these studies will, therefore, not be made directly to those obtained in the present study. Trends which have been shown to exist however, in those previous studies, will be mentioned and compared to those trends which have been observed in these experiments.

The work done by Terjung (1976) with a group of endurance-trained rats (12 weeks) was not an acute study, but more of a training study where animals ran 6 days/week for a period of 12 weeks at a speed of approximately 27 m/min. according to one of the following four protocols:

1. 10% elevation for 2 hr/day
2. 10% elevation for 4 hr/day
3. 30% elevation for 40 min/day
4. 30% elevation for 2 hr/day

They were sacrificed at the end of this period to allow for biochemical assays of specific muscles.

The activity of Cytochrome C and Citrate Synthase (CS) (a mitochondrial marker enzyme) was assessed in muscles of markedly different fiber populations. CS activity in the fast-twitch-red (FOG) fibers increased in all groups over the sedentary control and it was concluded that these fibers were used to a great extent in all of the training programs. In the fast-twitch-white (FG) fiber however, there was only a slight increase in CS activity over the control when the 10% programs were used, but a statistically significant increase in CS activity in both of the groups trained at 30% elevation. This increase was however, not as high as that shown in the FOG fibers. In the slow-twitch-red (SO) fibers, CS activity increased in all groups with the greatest increase occurring in the one trained at 30% for 2 h/day. Terjung (1976) thus concluded, in part, that more intense exercise (30%) builds upon the performance of previously used FOG fibers and additionally involves the low oxidative FG fibers.

Baldwin _et_ _al_. (1973) in a study where groups of rats were made to run up an 8° incline (approximately 13% elevation) at a selected number of speeds including 1 mph (27 m/min.), arrived at much the same conclusions that Terjung (1976) did a number of years later.

In short, the FOG fibers are the main muscle fibers involved during mild to moderate physical activity that can be maintained for prolonged periods of time, whereas, FG fibers are used to a much greater extent during the more strenuous running required by the greater treadmill incline. This is similar to the inference made by Baldwin _et_ _al_. (1973) and Armstrong _et_ _al_. (1974, 1975) for running at increased speeds but with unchanged inclines.

This same relationship (ie. increased FG fiber utilization at higher inclines) was also shown to exist in the present study using relatively untrained animals in an acute series of experiments.

When considering the mean PAS staining patterns graphically represented in figure 3, or the data in Appendix D, it can be seen that in the treatment group G-0, the degree of SO fiber depletion is very extensive, whereas, the FG fiber profile differed very little from those of the rested controls. The FOG fiber population on the other hand, showed a moderate amount of glycogen depletion when compared to the rested controls. At the same speed but at 20% elevation the greatest depletion appeared to occur in the FOG fiber population. An extensive amount of SO fiber depletion was still seen (although less than at the 0% elevation) as well as an even greater amount of FG fiber depletion when compared to the 0% elevation group. At 40% elevation however, the mean PAS staining intensities for the different fiber types has shown a slight change. The SO fibers seem to show less of a glycogen depletion than at either of the two other percent elevation treatment groups. But, the FOG and FG fiber categories seem to be even more depleted at this elevation, than at the 0 and 20% elevations.

It must be remembered though that the sampling effect shown to exist in the FG population in this group (smaller percentage of FG fibers), could render the staining patterns observed in this fiber type artifactual. It is possible that the reduced number of FG fibers could have resulted in an exaggeration of the glycogen depletion seen in this fiber type. It is unlikely however, because of the fact that no FG fibers were rated as darkly stained in the 20% elevation group (G-20), that darkly stained fibers would be seen in those muscles which had covered the same distance at an incline of 40% (G-40). It can be logically assumed then that the trend which can be seen in this fiber type as a result of increasing intensity by increases in grade (ie. FG fiber depletion) would be substantiated.

Thus, it can be seen that the general recruitment pattern demonstrated in the series of experiments done on the level at speeds of 25 to 55 m/min. (ie. SO and FOG fiber depletion at the lower intensities of work with FG fibers being called into play mainly at the higher intensities of work), has also been reproduced in the series of experiments at selected grades of 0, 20, and 40% elevation. In the present series of experiments however, FG fiber depletion came about primarily as a result of a greater load (ie. treadmill incline) and not because of an increased speed. The reason for this recruitment shift from the more oxidative fibers to the fast-glycolytic ones could in part be due to the fact that the 40% incline experimental protocol required a power output in excess of the capabilities of the FOG fibers, and in this way resulted in the greater FG fiber recruitment.

Though the actual mechanism of the recruitment shift is unknown at the present time the question still exists as to whether, after this recruitment shift has occurred, the more oxidative fibers (in particular the SO fibers) are still recruited, or simply that their metabolic rates are greatly reduced. If this preferential selection of fiber types in exercise does indeed exist, and at the higher workloads the SO fibers become less utilized or tend to 'drop off', a glycogen sparing effect should be seen in these fibers and a histochemical picture not much different from the control sections should exist.

It has been stated however, when comparing the mean staining patterns for each group, that this recruitment shift was not clearly demonstrated, even though the progressively greater depletion of FG fibers at the higher inclines was substantiated. But, if a closer examination of the histochemical glycogen depletion pattern of several of the individual animals used in the grade work is made, several striking relationships do emerge.

Figures 4 and 5 represent serial photomicrographs for muscle sections of animal 23 belonging to treatment group G-20, and animal 50 belonging to the treatment group G-40. It must be pointed out here, that the distance covered by each of these animals as well as the speed at which this distance was covered, was identical. The only difference between the running protocols assigned to each of these animals remains the grade at which they performed the work. It can be seen when examining the individual frequency data for PAS staining intensity for each of these animals in Appendix C, as well as the photomicrographs in figures 4 and 5, that a striking reversal in the PAS staining pattern seems to have occurred. In animal 23 (of the G-20 group) the histochemical glycogen depletion pattern shows a SO fiber population which is quite depleted, as is the FOG fiber population, but a relatively undepleted FG fiber population.

In contrast however, with animal 50 (of the G-40 group) the SO fiber population is relatively unchanged as compared to the controls, but both the FOG and FG fiber populations show quite extensive depletion. These results would seem to suggest that in this animal (50) a shift in recruitment pattern of fiber types used to do the work has indeed occurred, and caused a sparing effect of glycogen in the SO fibers that is indicative of either a possible use of other substrates, a shut down in utilization of this fiber type, or at least a very marked reduction in their metabolic rate. Moreover, there was a tendency, although not as distinct, for animals 2 and 17 of the G-40 group to show a similar pattern to that of animal 50, in glycogen depletion. The reason for the lack of such a distinct 'reversal' in the other animals in treatment group G-40 could be associated to the phenomenon of individual threshold levels of fiber recruitment. It is possible that, in those animals which did not show this type of selectivity in fiber recruitment, the stress imposed on them by the running protocol used for this group was not quite intense enough (elevated enough) to elicit this type of skeletal muscle response.

In conclusion then, a similar trend in glycogen depletion patterns to that seen with running at increasing speeds on the level, seems to have been reproduced with work at selected grades.

With the grade running however, specific results establish the possible existence of complete recruitment shift from SO to FG (and FOG) fibers with an apparent disuse of the SO fiber type, or at least a very marked reduction in metabolic rate. More work however is needed to clarify this point.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

Conclusions

The purpose of this study was to investigate the effects of changing the intensity of a workload by variations in speed of a motor driven rodent treadmill from 25-55 m/min., and elevation from 0-40% grade, on glycogen depletion patterns demonstrated histochemically by the PAS reaction in the plantaris muscle of male Wistar rats.

Glycogen depletion patterns induced as a result of the series I exercise bouts were also reproduced generally in those exercise bouts of the series II group. From the results of the present study, it was concluded that glycogen depletion patterns are affected by changes in both speed and incline of a motor driven treadmill. Since this relationship has been shown to exist in running done on the level and with grade, it appears that changes in intensity are responsible for the different glycogen depletion patterns observed in these situations. The trend towards predominantly SO and FOG fiber depletion at low to moderate intensity work, and predominantly FG depletion at the higher intensities of work, was established, but this effect appeared to be more distinct in those groups of animals that ran at selected inclines.

Recommendations

Because training programs of different work intensities and durations represent a potentially useful technique for assessing the extent of fiber type involvement during exercise, further studies of the effect of varying these two parameters in exercising animals are recommended where, in particular, the range and selection of grades at which the work is to be done, should be made more extensive.

Based on the results of this study, it is recommended that variations in the incline parameter be used to induce the more specific recruitment of individual fiber types in exercise.

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APPENDIX A

HISTOCHEMICAL TECHNIQUE FOR ATPase

MYOSIN ADENOSINE TRIPHOSPHATASE STAIN (ATPase)

- Procedure of Padykula and Herman, as modified by Guth and Samaha. Exp. Neurol. V 25, p. 138-152, 1969.

SOLUTIONS: * volumes are indicated, concentrations are final

I Fixative - * made up to 20 ml.

a) 5 % Formaldehyde (analar chemicals)
1.0 ml. into 20 ml.

b) Sucrose (m.w. 342.3)
2.3276 gr. into 20 ml. (340.mM)

c) Calcium Chloride (m.w. 110.99)
0.1509 gr. into 20 ml. (68.0 mM)

d) Sodium Cacodylate (m.w. 214.02)
0.856 gr. into 20 ml. (200.0 mM)

** make up to 20 ml. with distilled water.
** pH taken to 7.6 with HCl. (in cold - 4°C)
** To be made up fresh.

II Fixative Rinse: Cold (4°C) Distilled Water.

III Pre-incubation Medium: * made up to 25 ml.

a) Calcium Chloride
0.1 gr. into 25 ml. (36.0 mM)

b) 2, amino-2, methyl-1, propanol (m.w. 89.14)
12.5 ml. of 0.2 M stock buffer into 25 ml. (0.1 M)

** make up to 25 ml. with distilled water.
** pH adjusted to 10.4 with HCl. (in cold)
** To be made up fresh.

IV Incubation Medium: * made up to 25 ml.

a) Propanol Buffer
12.5 ml. of 0.2 M stock buffer into 25 ml. (0.1 M)

b) Calcium Chloride
0.0499 gr. into 25 ml. (18.0 mM)

c) Disodium ATP (m.w. 551.2)
0.062 gr. into 25 ml. (4.5 mM)

** Make up to 25 ml. with distilled water.
** pH adjusted to 9.4 with HCl.
** To be made up fresh.

V <u>0.2 M Buffer Stock:</u> * make up to 75 ml.

a) 1.3371 gr. of 2, amino-2, methyl-1, propanol buffer into 75 ml. (0.2 M)

VI <u>0.1 M. Buffer Rinse</u>: * make up to 100. ml.

a) 50. ml. of 0.2 M stock diluted with 50. ml. of distilled water (0.1 M)

** pH adjusted to 9.4 with HCl
** To be made up fresh.

VII <u>1% Calcium Chloride</u>:
1.0 gr. $CaCl_2$ in 100. ml. of distilled water

VIII <u>2% Cobaltous Chloride</u>: (m.w. 237.95)
2.0 gr. $CoCl_2$ in 100. ml of distilled water

IX <u>1% Ammonium Sulphide</u>: (assay 23.4%)
1.0 ml. $(NH_4)_2S$ in 99. ml. of distilled water.

<u>NB</u>: Above solutions VII, VIII, and IX must be stored in brown glass bottles at 4°C if made up as stock.

MYOSIN ATPase PROCEDURES

1) Fixative - 10 min in cold (4°C) fixative medium
2) Wash - 4 x 3 min cold distilled water rinse
3) Pre-incubation - 15 min. in cold pre-inc. medium
4) Incubation - 45 min in 37°C incubation medium
5) 1% $CaCl_2$ - 3 x 30 sec rinse
6) Drain and blot
7) 2% $CoCl_2$ - 1 x 3 min wash
8) 0.1 M buffer rinse - 4 x 30 sec rinse (re-establish pH)
9) 1% $(NH_4)_2S$ (Ammonium sulphide) - 1 x 3 min
10) Tap water rinse (cold) - 1 x 5 min
11) Ascending alcohols - ethanol
    95% alcohol - 1 x 1 min
    99% alcohol - 2 x 1 min
12) Xylene - 1 x 2 min
13) Mount - Permount

APPENDIX B

BODY WEIGHT DATA

Table 9

Body Weights of Experimental Animals during Acclimation and Training Regimen*

Day	Animal Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
In	175	160	165	174	151	165	159	165	149	162	170	177	154	167	171	173	165	169	163	165	168	156	162	161	149
1	212	198	198	211	184	201	195	205	181	195	205	208	186	203	212	210	197	205	199	202	205	189	197	191	176
2	214	198	205	213	189	205	198	208	183	206	209	212	192	203	215	215	197	206	203	204	206	193	201	191	177
3	221	205	212	215	194	207	203	213	185	211	213	217	196	207	219	220	201	210	205	205	212	193	203	196	182
4	225	204	214	215	196	211	206	218	187	217	216	217	197	208	222	221	203	210	211	207	216	195	206	199	183
5	229	210	221	220	202	216	211	223	191	223	221	223	200	213	226	228	209	212	216	210	220	199	212	206	189
6	240	218	233	228	209	224	221	234	199	228	228	232	208	220	233	236	214	221	221	214	226	204	218	212	192
7												REST													
8	257	237	248	239	223	239	230	252	216	245	239	244	219	235	248	250	224	233	239	222	237	219	232	221	205
9	271	245	260	245	233	247	240	263	227	253	248	253	230	244	257	262	239	244	254	239	248	231	241	237	214
10	273	244	263	247	233	248	239	260	227	258	246	256	228	243	258	263	236	245	254	235	248	229	245	237	219
11	279	251	270	254	243	256	246	268	236	264	255	264	236	253	262	270	240	250	261	244	253	233	251	244	226
12	287	257	279	260	247	261	252	272	243	269	257	268	242	257	265	275	246	254	268	247	256	236	258	247	230
13	293	266	283	265	254	266	261	279	253	275	261	275	248	264	268	281	248	258	278	250	265	242	261	257	238
14												REST													
15												REST													
16								EXPERIMENTAL DAY																	

Key: In - Day on which animals were received
* - Weights of all animals are expressed in grams

Body Weights of Experimental Animals during Acclimation and Training Regimen*

Day	Animal Number																							
	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
In	160	172	149	174	167	151	170	169	175	155	166	152	149	157	172	171	171	165	152	158	170	154	152	164	162
1	187	206	178	204	195	176	200	211	208	193	206	189	176	191	213	211	205	195	178	187	206	185	180	194	180
2	191	205	184	208	200	178	201	215	213	198	210	194	178	195	218	216	207	203	180	190	211	187	185	199	194
3	193	211	185	212	211	183	203	218	221	202	215	199	183	201	221	218	213	207	184	195	213	191	191	206	195
4	194	213	188	216	208	188	206	223	225	206	221	208	189	202	226	225	219	212	185	202	219	194	191	211	200
5	201	218	194	218	210	194	209	226	229	214	227	211	195	205	230	232	224	216	188	208	223	200	197	214	211
6	205	227	198	222	216	198	216	233	237	221	233	218	200	210	239	236	232	225	193	216	230	206	204	222	212
7	REST																								
8	216	241	207	234	226	213	225	247	255	235	244	233	215	221	255	252	242	242	202	232	237	211	215	238	231
9	226	250	217	246	236	226	239	261	270	243	256	243	227	231	265	269	252	251	210	236	243	220	225	244	234
10	228	254	218	240	238	226	238	259	271	244	257	244	227	234	268	270	252	253	212	238	246	220	229	251	245
11	231	258	224	247	245	232	243	267	279	251	263	254	235	239	279	276	257	262	217	247	250	228	235	257	250
12	235	266	230	251	249	239	251	272	283	259	271	259	241	243	286	284	263	269	221	252	255	231	241	262	254
13	243	264	234	258	256	242	255	277	291	261	275	264	246	249	293	288	271	271	225	257	256	239	244	267	257
14	REST																								
15	REST																								
16	EXPERIMENTAL DAY																								

Key: In - Day on which animals were received
* - Weights of all animals are expressed in grams

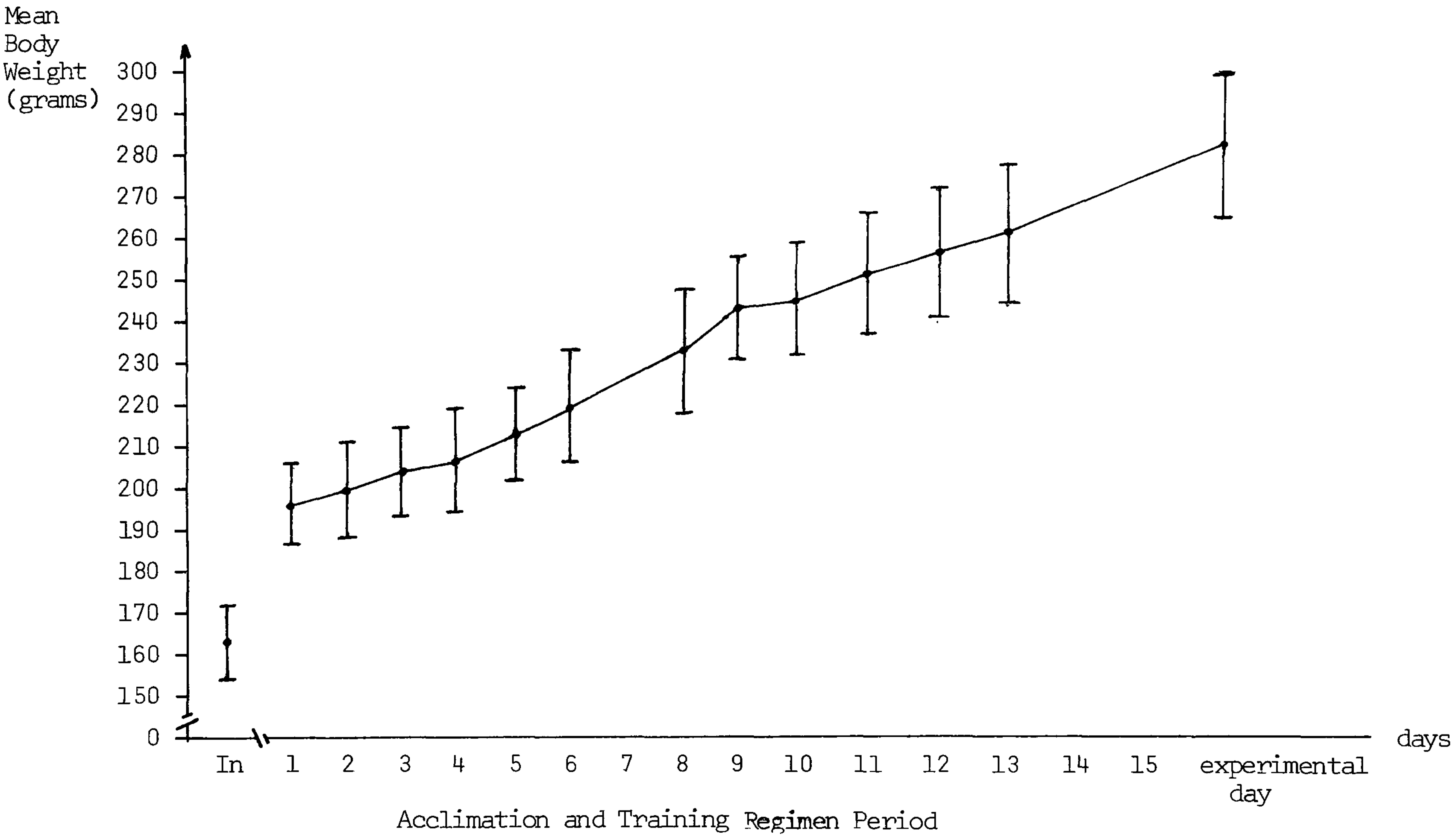

Figure 6:- Mean Body Weight (±SD) of Experimental Animals during Acclimation and Training Regimen

APPENDIX C

FREQUENCY DATA OF PAS STAINING INTENSITY OF MUSCLE FIBERS

Table 10

PAS Staining Intensity of Rat Plantaris Muscle Fibers following Treatments

Identification			Protocol					PAS Staining Intensity											
								SO				FOG				FG			
Group	Anim. #	Anim. wt.g.	Speed m/min	Grade % inc	Dist. m.	Work/Rest interval	Work Time sec.	D	M	L	N	D	M	L	N	D	M	L	N
G-0	3	297	35	0	122.5	30 / 30	210			5	10	37	46	79	36	73	21	2	
G-0	11	276	35	0	122.5	30 / 30	210			15	27	43	49	43	58	23	4	6	
G-0	33	293	35	0	122.5	30 / 30	210			18	42	52	32	51	111	9	20	12	
G-0	38	266	35	0	122.5	30 / 30	210			1	31		3	78	82		11	7	
G-0	41	304	35	0	122.5	30 / 30	210			1	38		8	123	82		3	7	
G-0	47	251	35	0	122.5	30 / 30	210				19		23	52	93		30	25	
N-6		281.2			122.5		210			7	28	22	27	71	77	18	15	10	
					Total of Fibers (275)				(35)				(197)				(43)		
G-20	6	284	35	20	122.5	30 / 30	210			5	20		19	70	75		27	36	
G-20	10	294	35	20	122.5	30 / 30	210		2	8	2		25	55	88		27	25	
G-20	13	260	35	20	122.5	30 / 30	210			23	26		20	29	152		12	40	11
G-20	23	280	35	20	122.5	30 / 30	210		1	16	35		8	63	124		33	26	
G-20	32	271	35	20	122.5	30 / 30	210			11	15			43	183		9	48	3
G-20	45	270	35	20	122.5	30 / 30	210		1	5	13		19	30	120		21	12	
N-6		276.5			122.5		210		1	11	19		15	48	120		22	31	2
					Total of Fibers (273)				(31)				(187)				(55)		

Key: SO - Slow-Oxidative FOG - Fast-Oxidative-Glycolytic FG - Fast-Glycolytic
D - Dark M - Moderate L - Light N - Negative

PAS Staining Intensity of Rat Plantaris Muscle Fibers following Treatments

Identification		Protocol						PAS Staining Intensity											
Group	Anim. #	Anim. wt.g.	Speed m/min	Grade % inc	Dist. m.	Work/Rest interval	Work Time sec.	SO				FOG				FG			
								D	M	L	N	D	M	L	N	D	M	L	N
G-40	2	289	35	40	122.5	30 / 30	210		11	17	16	2	2	51	189		1	9	4
G-40	17	270	35	40	122.5	30 / 30	210			20	24			21	179		5	33	12
G-40	21	280	35	40	122.5	30 / 30	210			9	12		7	14	243			2	28
G-40	25	257	35	40	122.5	30 / 30	210				38			4	289			1	12
G-40	49	288	35	40	122.5	30 / 30	210				41			23	226			7	4
G-40	50	282	35	40	122.5	30 / 30	210		24	1			4	24	143		5	8	34
N-6		277.7			122.5		210		6	8	22		2	23	212		2	10	16
					Total of Fibers		(301)		(36)				(237)				(28)		
S-25	7	286	25	0	471	contin.	1130				33	43	25	18	29	28	2		
S-25	14	289	25	0	471	contin.	1130			5	47		7	75	113	10	51	23	2
S-25	34	312	25	0	471	contin.	1130				12		1	51	76		3	41	6
S-25	29	278	25	0	471	contin.	1130				17		2	38	212			24	46
S-25	42	292	25	0	471	contin.	1130				47		9	114	65			50	4
S-25	48	268	25	0	471	contin.	1130				40	50	21	15	98	14	5		
N-6		287.5			471		1130			1	33	16	11	52	99	9	10	23	10
					Total of Fibers		(264)		(34)				(178)				(52)		

<u>Key</u>: SO - Slow-Oxidative FOG - Fast-Oxidative-Glycolytic FG - Fast-Glycolytic
D - Dark M - Moderate L - Light N - Negative
contin. - Continuous run

PAS Staining Intensity of Rat Plantaris Muscle Fibers following Treatments

Identification		Protocol						PAS Staining Intensity											
								SO				FOG				FG			
Group	Anim. #	Anim. wt.g.	Speed m/min	Grade % inc	Dist. m.	Work/Rest interval	Work Time sec.	D	M	L	N	D	M	L	N	D	M	L	N
S-35	5	279	35	0	471	contin.	807			1	13		4	49	29	6	39		
S-35	20	272	35	0	471	contin.	807			3	23			45	99		49	3	
S-35	22	264	35	0	471	contin.	807				30			62	59		51		
S-35	26	269	35	0	471	contin.	807			5	19			43	99		82	2	
S-35	36	298	35	0	471	contin.	807				20		5	43	50	6	56		
S-35	44	246	35	0	471	contin.	807				35		11	49	111	11	74	5	
N-6		271.3			471		807			2	23		3	49	75	4	59	2	
					Total of Fibers		(217)		(25)				(127)				(65)		
S-45	9	287	45	0	471	contin.	628			26	25			43	148			5	19
S-45	12	299	45	0	471	contin.	628			2	52			17	147		7	38	29
S-45	24	283	45	0	471	contin.	628			5	47			30	161			51	6
S-45	30	280	45	0	471	contin.	628			1	26			50	243		2	55	6
S-45	46	275	45	0	471	contin.	628			7	23	39	14	38	57	34	26	2	
N-5		284.8			471		628			8	35	8	3	36	151	7	7	30	12
					Total of Fibers		(297)		(43)				(198)				(56)		

Key: SO - Slow-Oxidative FOG - Fast-Oxidative-Glycolytic FG - Fast-Glycolytic
D - Dark M - Moderate L - Light N - Negative
contin. - Continuous run

PAS Staining Intensity of Rat Plantaris Muscle Fibers following Treatments

Identification			Protocol					PAS Staining Intensity											
Group	Anim. #	Anim. wt.g.	Speed m/min	Grade % inc	Dist. m.	Work/Rest interval	Work Time sec.	SO				FOG				FG			
								D	M	L	N	D	M	L	N	D	M	L	N
S-55	4	290	55	0	357.5	contin.	390			4	22			18	117		2	10	
S-55	16	308	55	0	574.8	contin.	627				24				167				54
S-55	19	306	55	0	682.9	contin.	745				35			6	233		1	1	60
S-55	28	254	55	0	394.9	contin.	431				30		4	24	260		5	16	11
S-55	37	287	55	0	405.2	contin.	442			1	38			2	222				40
S-55	40	320	55	0	410.7	contin.	448			9	25	5	14	49	227	9	20	27	
N-6		294.2			471		514			2	29	1	3	17	204	2	5	9	28
					Total of Fibers (300)				(31)				(225)				(44)		
Ex-C	8		0	0	0	0	0		13	14		91	108	53		1	7	1	
Ex-C	15		0	0	0	0	0	1	41	8		11	169	53		13	22		
Ex-C	18		0	0	0	0	0		8	11		68	89	18		2	31		
Ex-C	27	283	0	0	0	0	0	1	15	6		62	78	19		6	27		
Ex-C	31	255	0	0	0	0	0	2	9	19		65	91	9		22			
Ex-C	43	293	0	0	0	0	0			10		52	32	32		20	16		
N-6		277			0		0	1	14	11		58	95	31		11	17		
					Total of Fibers (238)				(26)				(184)				(28)		

Key: SO - Slow-Oxidative FOG - Fast-Oxidative-Glycolytic FG - Fast-Glycolytic
D - Dark M - Moderate L - Light N - Negative
contin. - Continuous run
Ex-C - Exercised-Control

APPENDIX D

PERCENTAGE PAS STAINING INTENSITY OF FIBER TYPES

TABLE 11

PERCENTAGE PAS STAINING INTENSITY OF FIBER TYPES

	PAS Staining Intensity											
	SO				FOG				FG			
Group	D	M	L	N	D	M	L	N	D	M	L	N
EX-C	3.8	53.9	42.3		31.5	51.6	16.9		39.3	60.7		
G-0			20.0	80.0	11.2	13.7	36.0	39.1	41.9	34.9	23.2	
G-20		3.2	35.5	61.3		8.0	25.7	66.3		40.0	56.4	3.6
G-40		16.7	22.2	61.1		0.8	9.7	89.5		7.1	35.7	57.1
S-25			2.9	97.1	9.0	6.2	29.2	55.6	17.3	19.2	44.2	19.2
S-35			8.0	92.0		2.4	38.6	59.0	6.2	90.8	3.0	
S-45			18.6	81.4	4.0	1.5	18.2	76.3	12.5	12.5	53.6	21.4
S-55			6.5	93.5	0.4	1.3	7.6	90.7	4.5	11.4	20.5	63.6