A COMPARISON OF THE EFFECTS OF INTERVAL AND CONTINUOUS TRAINING PROGRAMS ON THE PROPORTION OF RED, WHITE AND INTERMEDIATE MUSCLE FIBERS OF THE MEDIAL GASTROCNEMIUS OF RATS

PETER GEORGE SELIG


CENTRE FOR NFLD. STUDIES
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MEMORIAL UNIVERSITY
OF NEWFOUNDLAND

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BY

© PETER GEORGE SELIG, B.Sc.

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ABSTRACT

Previous studies have shown that the proportions of red and white fibers in a muscle can be altered following continuous and interval training. Succinic dehydrogenase and reduced nicotinamide adenine dinucleotide diaphorase activity was studied histochemically to analyse the changes taking place in the fiber proportions.

Male albino rats were subjected to interval and continuous training programs at 60% and 50% levels of maximal activity over a seven week period. The intervals were 110 seconds in length. The number of intervals swum and the length of the continuous swim periods was determined every sixth training day by swimming the animals to exhaustion and then taking 60% and 50% of the maximum swim times to exhaustion. This gave the number of intervals and the length of the continuous swim periods to be used during the next five training days.

After seven weeks of training the medial gastrocnemius of the left leg was excised and stained to show succinic dehydrogenase and reduced nicotinamide adenine dinucleotide diaphorase activity. Microphotographs were taken of cross-sections of the medial red and peripheral white regions of

the gastrocnemius. The proportion of red, white and intermediate fibers in each region was determined.

It was found that a significant increase in red fibers and a significant decrease in white fibers occured in both regions when the exercise groups were compared to the control group. No significant difference was found in the fiber proportions when the 60% and 50% continuous trained groups were compared nor when the 60% and 50% interval groups were compared.

In comparing the combined interval and the combined continuous groups it was found that interval training caused a significantly greater increase in red fibers and decrease in white fibers than the continuous training. This would indicate that interval training was the better method of training for developing the aerobic capacity of the muscle at the cellular level.

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ABBREVIATIONS

I TREATMENT GROUPS

(A)	CON	-	CONTROL GROUP
(B)	60C	-	60% CONTINUOUS GROUP
(C)	60I	-	60% INTERVAL GROUP
(D)	50C	-	50% CONTINUOUS GROUP
(E)	50I	-	50% INTERVAL GROUP

II BIOCHEMICAL ABBREVIATIONS

(A)	MYOSIN ATPase	-	MYOSIN ADENINE TRIPHOSPHATSE
(B)	NBT	-	NITRO BLUE TETRAZOLIUM
(C)	NADH	-	REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE
(D)	NADH-D	-	REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE DIAPHORASE
(E)	SDH	-	SUCCINIC DEHYDROGENASE

III OTHERS

(A)	INTER.	-	INTERMEDIATE

CHAPTER I

INTRODUCTION

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INTRODUCTION

It has long been known that repeated physical overloading, as employed in athletic training programs, can produce elevations in the performance capability of the trained individual. Controversy has arisen as to whether physical overloading is most effective when the work is accomplished continuously or intermittently.

Astrand (2) and Christensen (25) have found that a greater maximal oxygen uptake may be accomplished using an intermittent type training program. Since the initial findings of these researchers many people have carried out research comparing intermittent and continuous training (1,52,93). Several authors are convinced that intermittent training results in the best possible improvement in endurance capabilities (32,93). In contrast other studies have failed to demonstrate any differences in the two types of training especially when the total work output was identical (65,87).

The extent to which alterations within skeletal muscle itself contribute to such training effects has been a matter of speculation. Much is known of the biochemical make-up of muscle and its functioning under normal conditions and data on the biochemical response of muscle to chronic exercise indicates that increased functioning at the molecular level can occur. As early as 1928 Whipple (136) identified differences in the myoglobin concentration of muscles in hunting and sedentary dogs. In the years following this discovery much research has been carried out dealing with the effects of regularly performed, vigorous exercise on the normal biochemical and histochemical patterns in skeletal muscle. Adaptations that interact to produce an increase in the performance capacity of the muscle have been identified by many people (74,86,88).

Numerous researchers have studied these biochemical and histochemical adaptations in the various muscle fiber types of the body. Since Edgerton's (40) discovery that muscle fiber types can be altered in a nonpathologic state, namely exercise, researchers have identified

alterations in the major muscle fiber types in various muscles of the body following continuous training programs (3,4,9). Other researchers have attempted to study muscle fiber proportions in exercised muscle following isometric and weight lifting programs for rats (88,98). Some researchers have also studied the muscle fiber populations in muscles of well trained athletes (29,45,130). Very little research has been undertaken comparing the effects of continuous and intermittent training on the fiber populations of exercised muscle.

The need for a study of this nature stems from the controversy that has arisen between the proponents of continuous training and those of interval training. The majority of investigators today believe that interval training is the best method for developing the endurance capacity of the athelete (2,25,52,93). Other researchers (39,70,79) believe there is no difference between the two when equal workloads are utilized.

The effect of both these training techniques has been studied in the whole organism (2,25,32) as well as

at the cellular level (8,70,74,79). Increases in the aerobic capacity have been found in both cases with interval training providing the greater increase. The effect of continuous training at the cellular level has been studied a great deal and all studies indicate an increased aerobic metabolism in the cell (8,74,96,97). Studies of the cellular changes following interval training are not as comprehensive as those for continuous training. Very little research has been completed comparing the biochemical, anatomical and histochemical changes taking place in the muscle cells following interval and continuous training programs of equal total work-loads, thus, the need for this study.

The effect of different overload stresses, of varying degrees of intensity, on the structural-functional relationship of the muscle is still lacking. This was the reasoning behind the use of two levels of intensity in the design of this research.

Purpose

The purpose of this study was to determine the effects of interval and continuous training programs on

1. There is no significant difference in the mean percentage of red muscle fibers in the medial gastrocnemius of rats following interval and continuous training programs.

2. There is no significant difference in the mean percentage of white muscle fibers in the medial gastrocnemius of rats following interval and continuous training programs.

3. There is no significant difference in the mean percentage of red muscle fibers of the medial gastrocnemius in the 60% continuous and 50% continuous groups following a continuous training program.

4. There is no significant difference in the mean percentage of red muscle fibers of the medial gastrocnemius in the 60% interval and 50% interval groups following an interval training program.

Statistical Hypotheses

1. $\mu RC = \mu RI$

2. $\mu WC = \mu WI$

3. $\mu 60C = \mu 50C$

4. $\mu 60I = \mu 60I$

the various fiber populations of the medial head of the gastrocnemius. It has been shown that continuous training programs alter the proportion of red, white and intermediate fibers of the exercised muscle causing a shift from white to red in the fiber spectrum. This would indicate an increased aerobic metabolism in the muscle. The purpose of this research was to determine if interval or continuous training caused a greater change in the fiber proportions of the medial head of the gastrocnemius.

Hypotheses

In undertaking research of this nature a number of hypotheses were developed. The major aim of this research was to analyse the changes occuring in muscle fiber ratios following interval and continuous training. In the design of this research two levels of intensity, 60% and 50% of maximum swim time to exhaustion, were employed. Because of this a comparison of the two levels of intensity for each training technique was undertaken as part of this research.

Limitations of the study

1. The major limitation of this study was the problem of equating the work loads for the exercise groups.

2. Exercise programs which involve swimming white rats are inherently limited by the lack of control of the intensity of muscular exercise.

3. The histochemical methods used to determine the activity of the enzymes are quantitatively limited. The use of quantitative biochemical parameters would give a more accurate and complete picture of the changes taking place in the muscle.

4. The amount of time available for experimentation limited the number of animals used in each of the experimental groups.

Definition of Terms

1. Adaptation

This was defined as an alteration in the normal "set" of the muscle which in some manner improves the performance of its primary function which is to do

external work.

2. Continuous Training

This was defined as a system of conditioning in which the animals were subjected to one continuous work bout with no rest periods.

3. Continuous Swim Time to Exhaustion

This has been defined as the length of time that the animals swim continuously to a point where they stay submerged for ten seconds or longer.

4. Continuous Swim Time

This was the length of time that the continuous swim group swam during the training periods. It represented 60% and 50% of the continuous swim time to exhaustion depending on what group the animal belonged to.

5. Endurance Capacity

This was the ability of the animal to complete the work bout without having to be taken from the water before the work bout was completed. An increase in the endurance capacity was defined as an increase in the amount of swim time completed during each work period.

6. Exhaustion

In this study exhaustion was defined by the animal staying submerged for ten seconds or longer during the continuous training program. In the interval training

program exhaustion was when the animal ceased coordinated movement or could not rise above a determined depth in the swimming tank.

7. Intermediate Muscle Fibers

These muscle fibers stained intermediately in intensity to the red and white muscle fibers. They stained intermediately in intensity for both succinic dehydrogenase and reduced nicotinamide adenine dinucleotide diaphorase activity.

8. Interval Swim Time

This was the length of time that the interval groups had to swim during the training period. It represented 60% and 50% of the interval swim time to exhaustion depending on the groups' level of intensity. It was divided into 110 second intervals which were separated by 120 second rest periods.

9. Interval Swim Time to Exhaustion

This was defined as the length of time that the animals swam using alternating 110 work bouts and 120 second rest periods. This continued until the animal ceased coordinated movement or could not rise above a determined depth in the swimming tank. The total time of

swimming was compiled to give the interval swim time to exhaustion.

10. Interval Training

This was defined as a system of conditioning in which the animals were subjected to short but regularly repeated periods of work stress interspersed with adequate periods of relief.

11. Red Muscle Fibers

These muscle fibers stained intensely for both succinic dehydrogenase and reduced nicotinamide adenine dinucleotide diaphorase.

12. Red Region

This is a region of the medial gastrocnemius having a high percentage of red muscle fibers. It is found centrally in the muscle when compared with the peripheral white region.

13. Training Day

This is defined as a day on which the animals actually performed the required work bouts.

14. White Region

This is a region of the medial gastrocnemius having a high percentage of white muscle fibers. It is found

peripheral to the central red region of the muscle.

15. Work Bout

A work bout was defined as one continuous bout of work with no rest periods. In the interval program it was considered as one interval and in the continuous program it was considered as the total work period.

16. Work Period

This has been defined as the total time needed to complete the number of work bouts necessary to meet the total swim time required during a specific training day.

CHAPTER II

RELATED LITERATURE

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RELATED LITERATURE

Nomenclature of Fiber Types

Over the years with advances in enzyme histochemistry and physiological and biochemical testing procedures, there has been a wide range of classifications applied to muscle fibers. The classical white and red fiber types are no longer used as researchers have identified at least three major types of muscle fibers (38,126).

Stein and Padykula (126) identified A,B and C type fibers in the rat gastrocnemius based on staining for the oxidative enzyme succinic dehydrogenase. Dubowitz and Pearse (38) in studying individual muscle fibers suggested two subdivisions. Type I fibers were rich in dehydrogenases and low in phosphorylase while Type II fibers were rich in phosphorylase and poor in dehydrogenases.

Romanul (119) added greatly to the complexity of fiber type nomenclature by finding eight fiber types which formed a spectrum. He classified them into three categories with Type I being low in oxidative metabolism but high in

glycolytic activity, Type II was high in oxidative metabolism but low in glycolytic activity, while Type III was intermediate to the two.

The classification utilized in this paper was proposed by Padykula and Gauthier (109). They proposed that the muscle fibers be classified as red, white and intermediate fibers according to their staining intensity for succinic dehydrogenase.

Some researchers have correlated the twitch times of the muscle fibers with the enzyme staining. Barnard et al. (8) studied myosin adenosine triphosphatase (myosin ATPase) activity and twitch times and came up with the three categories of fast-twitch red, fast-twitch white and slow-twitch intermediate. Peter et al. (114) investigated this even further and came up with three categories that link the metabolism and the twitch times of the fibers. They proposed that muscle fibers be classified as fast-twitch oxidative-glycolytic, fast-twitch glycolytic and slow-twitch oxidative. The fast-twitch oxidative-glycolytic are the most enduring fiber as they are high in both oxidative and glycolytic metabolism. The fast-twitch

glycolytic is the least enduring fiber as it is low in oxidative capacity and high in glycolytic capacity. The intermediate fiber stands between the two in its endurance capability.

Perhaps the most functional classification proposed so far was that by Burke et al. (21). They proposed that fibers be classified according to their fatigue resistance. The fibers that are virtually fatigue resistant are high in both oxidative and glycolytic activity while those that are fast fatigueing are those fibers which have mainly glycolytic metabolism. The fiber depending solely on aerobic metabolism falls between the other two fibers in its fatigue resistance.

An understanding of the relationship of the many fiber types must be understood in order to understand the morphology and metabolism of the fiber types. Table I presents the relationship of a number of fiber type nomenclature proposals to the red, white and intermediate proposal of Padykula and Gauthier (109).

TABLE I

VARIOUS NOMENCLATURE USED IN TYPING MUSCLE FIBERS.

REFERENCE	TYPE OF FIBER*		
	RED	WHITE	INTERMEDIATE
Dubowitz and Pearse (38)	II	III	I
Stein and Padykula (126)	C	A	B
Brooke and Kaiser (20)	IIA	IIB	I
Ashmore and Doerr (1)	α -red	α -white	β -red
Barnard et. al. (8)	Fast Twitch Red	Fast Twitch White	Slow Twitch Intermediate
Burke et. al. (21)	Fatigue Resistant	Fast Fatigue	Slow Fatigue
Peter et. al. (114)	Fast Oxidative Glycolytic	Fast Twitch Glycolytic	Slow Twitch Oxidative
Romanul (119)	II	I	III

*The fiber type nomenclature used in this research was the typing proposed by Gauthier and Padykula (47).

Morphology of Muscle Fibers

Skeletal muscle fibers can be classified into different fiber types morphologically. Various degrees of redness and paleness of different skeletal muscles have been a common observation for years, although the meaning of this was not recognized for some time (35). The relationship between the ultrastructural and cytochemical features of the three fiber types, namely red, white and intermediate, and the color of the muscle can be easily demonstrated (55).

At the cellular level one can find a number of structural differences. The red muscle fibers, in most cases, have a smaller diameter than the white or intermediate fibers (108). Also the white fibers seem to have a greater amount of connective tissue (11). Differences exist in the number and quality of the mitochondria associated with the specific types of muscle fibers. The mitochondral content of the muscle fibers is inversely related to the diameter of the muscle fiber (56). The red fibers, having a small diameter, are richly supplied with mitochondria (108). The white fibers have fewer mitochondria than either the

red or intermediate fibers (102). The mitochondria in the red muscle fibers form interfibrillar rows and are paired at the I-bands while large spherical mitochondria form easily seen aggregations at the periphery of the fibers (56). The white fibers differ in that they consist almost entirely of filamentous profiles which reflect paired mitochondria at the I-bands. The peripheral regions are noticably free of spherical aggregations (56,57). The intermediate fibers have many characteristics of the red fibers but the subsarcolemmal aggregations and the interfibrillar rows are less conspicious (55,57,108). The cristae and the mitochondral granules are more elaborate in the red muscle fibers than in the white muscle fibers (56,15). This would indicate a higher level of aerobic metabolism in the red fibers.

The width of the Z-line in the three types of muscle fibers varies greatly. The Z-lines are widest in the red fibers with the white fiber Z-lines being about one-half the width of the red fiber Z-lines (55,56). The width of the Z-line in the intermediate fiber is found between the width of the Z-lines of the red and white fibers.

Ultrastructural differences also occur at the neuromuscular junctions. In the red muscle fibers the axonal endings are elliptical and contain only moderate numbers of acetylcholine vesicles while in the white fibers the axonal endings are flat and elongated and the vesicles are closely packed. (107, 138). The intermediate fibers have properties that resemble both the red and white neuromuscular junctions (56,106). The axonal ending is wider than the red fiber and at times longer than the white fiber (107).

Physiological Characteristics of Muscle Fibers.

The major physiological difference between the three muscle fiber types is the twitch times of the respective fibers. Barany (6,7) and his co-workers reported that the specific activity of myosin ATPase is correlated with the contraction time of the muscle. After staining for myosin ATPase Edgerton and Simpson (42) proposed that the intermediate fiber is slow-twitch and the red and white fibers are fast-twitch. Barnard et. al. (8) studied twitch times and myosin ATPase activity in skeletal

Differences also exist in the structure of the sarcoplasmic reticulum. The distribution of this membrane system is the same for all three fibers but at the region of the H-band the structures differ. In the red fibers an elaborate open network of narrow tubules extends across the myofibrils in the region of the H-band. The white fibers have a more compact arrangement which consists of broad parallel tubules or flattened sacs (56,57).

Capillarization varies greatly with the three types of muscle fibers. In 1965 researchers linked the capillarization of muscle fibers with the metabolic needs of that particular muscle fiber (100,118). Romanul found that there was a higher population of capillaries in red muscles because of the dependence of the red fibers on the blood carried entities for aerobic metabolism (118). It was also found that the white fibers have lesser capillarization and this was related to their dependence on anaerobic metabolism for energy (117,118). The capillarization of the intermediate fibers varied but was always found to be at levels between that of the red and white fibers.

muscle and came to the same conclusion as Edgerton and Simpson (42).

In a more physiological relevant 'in vitro' test, Fiehn and Peter (51) studied fragmented sarcoplasmic reticulum (FSR) to measure the calcium concentrating ability of the FSR. They found that the calcium concentrating ability of the fast-twitch red and the fast-twitch white muscle was identical and much faster than the rate with FSR from slow-twitch intermediate muscle. This would indicate faster relaxation times for the fast-twitch muscle (112). This assay for calcium concentrating ability of FSR correlates well with the known relaxation times of the three fiber types (8).

In studies of quantitative differences in the specific activity of magnesium (Mg^{++}) activated actomyosin ATPase at ph 9.4, it was found that fast-twitch white was highest in activity and fast-twitch red is intermediate in activity (114). This would correspond to the intensity of staining of the same fibers for myofibrillar ATPase (8).

These studies seem to adequately demonstrate the theory that there are both fast-twitch red and white fibers and that the intermediate muscle fiber is slow-twitch.

This assumption proves that the old theory of fast-twitch white and slow-twitch red can no longer be used in the physiological analysis of the three types of muscle fibers.

Metabolism of Muscle Fibers

The observation of variations in the metabolic needs of different muscle fibers is very important in interpreting histochemical and biochemical studies. The classic postulate regarding the metabolic and functional differences between red and white muscle states that white muscle, capable of rapid but brief contractions, primarily utilizes glycolysis for energy production while red muscle which can contract for long periods of time, relies chiefly on oxidative mechanisms (127).

In the biochemical analysis of the three muscle fiber types it has been found that the glycogen content of white muscle is higher than that of the red fibers (14). Histochemical studies have revealed a reciprocal relationship between phosphorylase and glycogen synthetase in individual fibers. The red fibers stain deeply for glycogen synthetase while the white fibers stain deeply

for phosphorylase (17,73). More recently higher glycogen levels have been histochemically demonstrated in the red fibers of the guinea pig gastrocnemius (59). These researchers emphasized the relatively high amount of glycogen poor, intermediate fibers in the "red" muscles of various species. This would seem to indicate a higher activity of glycolytic metabolism in the red fibers of the "red" muscle. Short et. al. (125) also found a slightly higher glycogen concentration in red muscle strips of rats. Results of "in vitro" studies of skeletal muscle show that the glycogen level of white muscle fibers decreases more precipitously than that of red muscle during incubation (14,19). This would indicate higher glycogen levels in white muscle. Researchers have also noted a greater occurance of uridine diphosphoglucose synthetase in red fibers (73). This synthetase is one of the catalysts in the synthesis of glycogen from glucose and would indicate that glycogenolysis and glycolysis is greater in red than in white muscles (124). These findings would add support to the proposal of Peter and his co-workers (114) who proposed that red muscle fibers be

termed fast-twitch oxidative-glycolytic because of the high levels of oxidative and glycolytic metabolism.

In general, glycolysis is thought to be the major metabolic pathway for the functioning of white muscle fibers. Glycogenolysis plus glycolysis as measured by chemical lactate and pyruvate productions is at least two times higher in white than in red muscles (18,36). The activity of glycolytic enzymes, with the exception of hexokinase activity is higher in white muscles. Researchers have found that hexokinase activity is higher in red muscle fibers than in the expected white fibers (22,115).

Red fibers have been shown to have lower amounts of lactate dehydrogenase and alpha glycerophosphate dehydrogenase than white muscles (16,34). According to Blanchaer at al. (16) descrepancies can arise in the identification of these two enzymes by histochemical and biochemical methods. White fibers when measured biochemically have higher levels of these two enzymes but histochemical studies have shown the opposite to be true (16.134). The descrepancy arises from the low

of white muscle. George (58), knowing that lipase activity is known to be in the mitochondria, suggested that lipase activity is an index of the extent of fat utilization and the capacity of muscle to sustain activity. Lehninger (90) has stated that red muscle fibers have a higher fatty acid uptake and that this could be correlated with the number of mitochondria in the respective muscle fibers.

Dawson and Romanul (34) found that phosphorylase activity was inversely proportional to esterase activity. Esterase activity was found to be the reciprocal of enzyme activity in white fibers thus showing the dependence of red fibers on fatty acid metabolism. Citric acid cycle enzymes were also found to be reciprocal to the enzyme activity in white fibers (34). Romanul (119) offers the explanation that the frequency of contractions of muscle fibers appear to relate better with their relative proportion of carbohydrate and lipid metabolism than their general oxidative metabolism.

Red muscle fibers have higher glucose-6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase

studies of Hess and Pearse (73) and Engel (46). Peter (115) and his co-workers verify this further. They found that hexokinase activity is greater in red than in white muscle and this would indicate a greater conversion of glucose to glycogen.

Present evidence indicates that fatty acids are substrates for muscle metabolism (26,53). Dubowitz and Pearse (38) stated that because of a high oxidative capacity there could be a strong oxidation of fatty acids by the red muscle fibers via the citric acid cycle. Many researchers have demonstrated, histologically and analytically, a sharp difference in the distribution of lipids in red and white muscle (54,94). Vallyathan (133) considers red muscle fibers to be fat utilizing as opposed to the white glycogen utilizing fibers. The red muscle fibers contain lipid inclusions where white muscle fibers do not and also they show a higher corbon dioxide output when incubated in acetoacetate (133). Red muscles also have a higher lipase activity. George and Talesara (58) have reported that the lipase activity in the red pigeon pectoral muscle is almost three times greater than that

classification and quantitative measurement of succinic dehydrogenase in red and white muscle. The succinic dehydrogenase activity is higher in red muscle fibers than in white muscle fibers. Other citric acid cycle enzymes are found in higher quantities in the red muscle than in the white or intermediate muscle fibers (14,104). The oxygen consumption by the red muscles is significantly higher in the red fibers but the per cent oxygen uptake by carbohydrate oxidation appears similar for the different fiber types (24).

Beatty (14) found more acetoacetate was converted to beta hydroxybutyrate by red muscle fiber groups than white fiber groups. This again is evidence of a higher activity in red muscle since beta hydroxybutyrate dehydrogenase is reported to be attached to the respiratory enzyme assembly of the mitochondral membranes.

Higher glucose uptake has been noted in red muscle fibers (18). It was also reported in this study that the synthesis of glucose into glycogen and its breakdown into carbon dioxide was higher and that the breakdown into lactate and pyruvate was lower in red than in white muscles. This fact is supported by the histochemical

diaphorase levels in the white muscle fibers which seem to give the erroneus impression of the two diaphorases being inactive (49,50).

Lactate dehydrogenase is found in skeletal muscle as five different isoenzymes (15). The heart or Type H isoenzyme and the muscle or Type M isoenzyme are the two major ones to consider. The type H lactate isoenzyme is found mainly in the red muscle where it restrains lactate formation and favors the oxidative degradation of pyruvate. In contrast the Type M lactate isoenzyme is predominant in white muscles where it will rapidly catalyze the formation of considerable amounts of lactate (116).

Evidence that citric acid cycle activity is higher in red than in white muscle is overwhelming. Dubowitz and Pearse (38) and Engel (46) have shown by histochemical staining that the activity of the citric acid cycle is elevated in red muscle fibers. Analytical techniques have also proven that red muscle depends heavily on the citric acid cycle as a means of producing energy (37,102, 103,104). Beatty (12) and his researchers have shown a direct correlation between the histochemical

activity than does white muscle (34). This would be expected because red muscle has a higher rate of protein synthesis and therefore needs a larger supply of ribose for ribonucleic acid (RNA) synthesis. Beatty et al. (13) showed that less than 0.5% of the glucose uptake was metabolized via this pathway.

Calcium content of the three fiber types has also been observed. The calcium pool connected with the sarcoplasmic reticulum is higher in white muscle fibers, whereas the amount of mitochondral calcium is higher and the sarcoplasmic calcium almost negligable in red muscle (110). This would indicate faster contraction and relaxation times by white muscle.

Myoglobin content of red muscle is two to five times greater than that in white muscle (34). Holloszy (74) suggests that this may facilitate oxygen utilization in muscle by enhancing oxygen transport through the cytoplasm to the mitochondria.

There are many more biochemical differences between red, white and intermediate muscle fibers that are beyond the scope of this paper. The major importance of this biochemical section is to stress the differences in the

metabolic needs of the cell rather than to take into consideration all the biochemical differences that may exist between the major muscle fiber types.

Response of Muscle Fibers to Exercise

Regularly performed exercise induces a variety of adaptations that interact to produce an increase in the performance capacity of the muscle fibers. The nature of the adaptive responses varies with the type of activity (88). Endurance training, such as long distance running and swimming will result in an increase in aerobic metabolism. These changes can be easily studied through biochemical and histochemical analyses.

One of the reasons for increased aerobic metabolism could be increases in myoglobin. Pattengale and Holloszy (111) have shown that myoglobin increases of up to 80% have been found in rats subjected to a fifteen week training program. Whipple (136) was probably the first to indicate that exercise may increase myoglobin in muscle after finding that the muscles of an active hunting dog had a higher myoglobin content than the muscle of sedentary dogs. Lawrie (89) demonstrated increases in

muscle myoglobin concentration in rats subjected to a swimming exercise program. It has been shown that myoglobin increases the rate of oxygen transport through fluid layers and that it may also facilitate oxygen utilization in muscle by enhancing oxygen transport through the cytoplasm to the mitochondria (74).

Morgan et al. (97) have shown through electronmicroscope studies that exercised muscles show increased numbers of mitochondria as well as increases in the size of the mitochondria. This would verify the doubling of the concentration of cytochrome c and the approximately 60% increase in the protein content of the mitochondral fraction of exercised skeletal muscle (75,96,105).

Two fold increases have been found in the respiratory chain enzymes involved in the oxidation of reduced nicotinamide adenine dinucleotide (NADH) and succinate in the leg muscles of rats subjected to endurance training (75). SDH, NADH-dehydrogenase, NADH cytochrome c reductase, succinate oxidase and cytochrome O per gram of muscle tissue doubled in the same animals. There was

also an increase in mitochondral coupling factor one which is closely associated with the respiratory chain in the cristae of the mitochondria (105). The coupling factor helps to catalyze the oxidative phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) coupled to electron transport. The increases in this factor run parallel to increases in the components of the respiratory chain (105).

Coinciding with the increase in respiratory enzymes is the increased ability of the exercised muscle to oxidize a variety of substrates (74). The mitochondria of exercised muscle has a high level of respiratory control and tightly coupled oxidative phosphorylation with either pyruvate or fatty acids as a substrate (75,96).

Increases in the mitochondral enzymes have been noticed by many researchers (5,41,75) but not all mitochondral enzymes increase in concentration. Holloszy et al. (76,105) have found that mitochondral alpha-glycerophosphate dehydrogenase, creatin phosphokinase and adenylate kinase do not change their concentration but their activity per milligram of mitochondral

compliment of mitochondria.

The activity of the glycolytic enzymes is varied. Hexokinase activity increases progressively with regular, repeated bouts of work. In rodents it was found that as the mitochondral content and the respiratory enzymes increased in the muscle fibers the hexokinase activity also increased (5,77,115). Baldwin et al. (5) showed that in rats subjected to a long term running program there was a marked increase in hexokinase activity averaging approximately 170% in fast-twitch red muscles and 30% in fast-twitch white muscle. In addition, exhaustive exercising of a trained animal is accompanied by a rapid decrease in hexokinase activity which thereafter rises to supranormal levels within forty-eight hours (10). The cause of this decrease is not as yet known.

Except for the large increase in hexokinase activity in the fast-twitch red muscle, the exercise induced adaptive changes in the levels of activity of the glycolytic enzymes in the muscle fiber types are rather small. Glycogen phosphorylase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase,

protein is greatly reduced because of the increase in the mitochondral protein (105).

The concentration of lactate in skeletal muscle is lower in trained than in the untrained muscle during exercise (26,122,123). It appears that at a given rate of pyruvate and NADH formation, less pyruvate is reduced to lactate and the removal of pyruvate and oxidation of NADH by alternate pathways is more rapid in muscle that has adapted to endurance exercise. The total activity of lactate dehydrogenase has also come under investigation. In preliminary experiments separation of the various isoenzymes of lactate dehydrogenase have been attempted. These investigations have indicaated a shift of the isoenzyme pattern from the muscle type to the heart type isoenzyme (82). Such a shift, coupled with the increased concentration of mitochondria, is consistant with the role of the lactate dehydrogenase system in controlling the flux of pyruvate to lactate for storage or export from the muscle or to the mitochondria for oxidation (112). Increases in the heart type isoenzyme would facilitate the oxidation of pyruvate and lactate by the increased

lactate dehydrogenase and cytoplasmic alphaglycerophosphate dehydrogenase levels decrease approximately 20% in fast-twitch red muscles in response to exercise (5). Following training programs, increases from 18-50% have been found for these same enzymes in the slow-twitch intermediate fibers. In white muscle the only change, other than hexokinase, was a 15% decrease in lactate dehydrogenase (5,74). This indicates that the muscle fibers are adapting to endurance type activity.

Complete glycogen depletion in the leg muscles of man is not usually seen after intense distance running (27, 30,31). Selective glycogen depletion in human muscle fibers has been found during bicycle exercise (65). Slow-twitch fibers were the first to be depleted of their glycogen at work intensities demanding 60-80% of an individual's maximum oxygen uptake. Fast twitch fibers also became glycogen depleted when the exercise was continued to exhaustion. Costill et al. (30) studied the selective depletion following a running program. The most important finding from their study of prolonged running was the selective marked glycogen depletion from

the slow twitch fibers with only a very minor reduction occuring in the fast twitch fibers.

Histochemical changes in muscle fibers also exist, following a training program. Edgerton (41) subjected rats to a fifty-two day training program and found through staining for the oxidative enzymes that there was an increase in the number of red muscle fibers in the plantaris of the exercised rats. In the soleus there was no alteration in the proportion of fibers classified as intermediate and high in malate dehydrogenase, NADH-D and SDH activity (41). This is due to the fact that high oxidative activity is a characteristic of this muscle. Corresponding to the increase in the number of red fibers in the plantaris was a decrease in the number of white fibers having low oxidative activity (41).

It has also been found that there is a significant increase in the number of red fibers of the peripheral white and central red regions of the medial head of the gastrocnemius following exercise (9). The number of intermediate fibers in the untrained and trained animals remained the same while the number of white fibers decreased.

Baldwin et al. (5) studied the gastrocnemius of rats subjected to an eight week running program. The percentage of red fibers increased while the percentage of white fibers decreased in the exercised animals.

Histochemical studies of myosin ATPase have been used to differentiate skeletal muscle fiber types. The use of myosin ATPase staining can be misleading. Edgerton and Simpson (42) used myosin ATPase and identified the light staining fibers as intermediate fibers and the dark staining fibers as red and white fibers. Barnard et al. (8) have suggested that the dark staining fibers be referred to as fast-twitch red and fast-twitch white fibers while the light staining fibers be called slow-twitch intermediate fibers. Using the identification method of Barnard and his co-workers Bagby et al. (3) studied myosin ATPase activity in rats subjected to sprint and endurance training. They found little or no increase in the percentage of fast-twitch fibers in the gastrocnemius of exercised rats. Other studies have shown that the ratio of slow-twitch to fast-twitch muscle fibers remains unchanged as a result of four to six

months of training in eleven to thirteen year olds and adult males (48,63).

Changes in the fiber diameter of red and white muscles have also been studied. Carrow et al. (23) subjected rats to a running program of thirty minutes per day. Following this, increases in the fiber size of the red and white fibers of the gastrocnemius was noted. The red fibers had a more pronounced increase than the white fibers. In another study increases of 110% were found in the diameter of the red fibers and 21% in the diameter of white fibers of the rat gastrocnemius following a six week training program (92). Increases in fiber size can be very misleading. Man-i et al. (92) found a great deal of variability in the hypertrophy of exercised muscle. He suggested that an optimal training program for hypertrophy could not be determined as the type and duration of the training are the major determinats of the hypertrophy. Edgerton (40) suggests that a more plausable explanation involves the differential responses of red and white muscles to exercise. Often one type of exercise will produce changes

in one type of fiber while another type of exercise will predominatly affect another type of fiber.

Investigations with skeletal muscle have revealed that there is a rapid synthesis of protein during the process of hypertrophy (61,69,71). Helander (71) provided the first information that changes in muscular components other than sarcoplasm might account for the increased muscular size. He found a 15% increase in myofibril protein in the gastrocnemius muscle of guinea pigs after a four-month running program. Goldberg (61) has shown that the protein formed during compensatory hypertrophy in the soleus and the plantaris muscles of rats after sectioning of the gastrocnemius tendon is evenly distributed among all the muscle fractions such as sarcoplasm, myofilaments and mitochondria.

Gordon and his co-workers (69) have studied the protein concentration of the various muscle fibers following exercise. They have suggested that the amount of work in a training program dictates the changes that will take place in the fiber. They have found that highly repetitive low resistance type exercise results

in sarcoplasmic hypertrophy and an increase in cytoplasmic protein. ~~In high~~ resistance low repetition exercise there was an increase in structural protein. These findings suggest that a muscle will increase its capacity to maintain its metabolic ability by increasing its cytoplasmic proteins. It will increase its capacity to work against high loads with less repetition by increasing its structural proteins.

Besides the type of training the particular sport that one is participating in also has an effect on the fiber ratio in muscles. Numerous authors have attempted to study fast-twitch/slow-twitch fiber ratios in atheletes trained for various sports. Thorstensson et al. (130) analysed the fiber ratios of the vastus lateralis in members of various Swedish national teams. They found that the percentage of fast-twitch fibers was lower in endurance trained atheletes and that the fast to slow twitch muscle fiber area ratio was higher in sprinting and jumping members of the track and field team. Costill et al. (28) also studied track and field atheletes and found a predominence of fast-twitch fibers in the

sprinters and jumpers and a predominence of slow-twitch fibers in the endurance trained atheletes. Edstrom and Ekblom (45) studied the fiber populations in weight lifters and found a high percentage of fast-twitch fibers in the muscles that were analysed. Whether these findings are due to hereditary factors or adaptation is uncertain but reports of higher fast to slow twitch fiber ratios following strength training (131) would favor the latter alternative.

Muscle weight has also been found to increase after chronic exercise (40) but just as frequently researchers have found the weight of the exercised muscle was unchanged (62) or even decreased (23). Carrow (23) studied the muscle weights of the tibialis anterior, soleus and the gastrocnemius in exercised rats and found that the muscles weighed less than the unexercised muscles.

Goldspink (62) found that exercised muscles did not necessarily wigh more than the control muscles even though the fibers were larger. His conclusion was that the descrepancy was brought about by a reduction of the extracellular components of the exercised muscle. He also found that the number of myofibrils per fiber increased

in high resistance type exercise. Goldspink also proposed that the hypertrophy of the fibers brings about a consolidation of the tissue, and further hypertrophy of the fibers results in the hypertrophy of the muscles as a whole, thus there may be no increase in the muscle weight following exercise.

Gordon (67) has come up with the proposal that one type of fiber may become larger while the other becomes smaller when a muscle is exposed to strenuous training, since the ratio of body weight to muscle weight is not altered.

Worsfield and Peter (137) studied the concentrating ability of fragmented sarcoplasmic reticulum from the skeletal muscle of trained animals. In the muscle fibers they found that the rates of calcium concentration by the fragmented sarcoplasmic reticulum from the fast-twitch red and the fast-twitch white fibers are identical and much faster than the rate for slow-twitch intermediate. They found no changes in the calcium concentrating ability of non-exercised and exercised skeletal muscle.

Barnard et al. (9) found that the yield of fragmented

sarcoplasmic reticulum protein is unchanged in exercised muscle. This shows that exercise does not cause a proliferation of the intracellular membrane systems of the muscle.

Studies on contraction kinetics have also been carried out by different researchers (9,112). These studies on control and endurance trained muscle show no significant differences in contraction time (time-to-peak tension), half relaxation time, twitch or tetanic tension, twitch-tetanus ratio or the maximum rate of tension development (9). The similar twitch and tetanic tensions in trained and control muscle demonstrate that the gross strength of the muscle had not increased (112). This also confirms the lack of muscle hypertrophy with endurance type training.

Mai et al. (91) studied the capillarization of exercised muscle and found that the capillary to fiber ratio increases in endurance trained muscle. This would create an increased blood flow and substrate supply to the exercising muscle thus increasing its endurance capacity.

As can be seen the increase in the endurance capacity of the muscles of exercised animals encompasses a wide variety of biochemical, morphological and histochemical changes in the "set" of the muscle. The overall effect is a more efficient functioning of the oxidative mechanisms of the body following endurance type training.

Interval versus Continuous Training

Over the years much research has been completed studying the effects of interval and continuous training on the total organism. The scope of this paper does not allow for an in depth study of all the literature completed on these two subjects. The research that was chosen was selected to show that a controversy does exist between the proponents of interval and continuous training. An annotated bibliography listing different research dealing with interval and continuous training at the total organism level may be found in Appendix F.

The goals of endurance training are:

1. To enhance the capacity of the central circulation, and

which are more darkly stained for oxidative enzymes are the fibers which show the most profound loss of glycogen (65). This suggests a selective recruitment of the most highly oxidative fibers in intermittent and continuous training.

Changes at the cellular level for interval training and continuous training aimed at developing the aerobic capacity of the subject are the same. Increases in mitochondral protein and oxidative enzymes activity have been implicated in both training methods as an adaptation in muscle cells that helps to develop the aerobic capacity of the muscle. Green and Houston (70) studied the succinic dehydrogenase and cytochrome oxidase activity in rats subjected to interval and continuous training. They found significant increases in the level of these two enzymes in the trained animals. These researchers also found no significant differences in the activity of these enzymes when the interval and continuous groups were compared.

Overall, most practitioners feel that interval training is the best type of endurance training. This is attributed to the fact that a greater work load can

changes in performance of the intact organism is a difficult problem because of the many intervening variables. Gross physiological parameters such as maximal oxygen uptake and performance times have been used to evaluate an individual's performance but even these parameters have rarely been correlated with observed boichemical and structural alterations of muscle.

Numerous researchers have found biochemical and structural alterations in muscle following continuous training programs (9,40,74,75). At the high intensity level very little work has been completed analysing the functional overload of muscle at the cellular level. Houston and Green (79) compared muscle weights of animals exercised continuously and intermittently at 75% and 50% of their maximum. No significant difference was found in the muscle weights of all four groups when compared with the control group.

In intermittent as well as continuous training, a definite loss of glycogen from fast-twitch and slow-twitch muscle fibers indicates that both are continuously involved in the exercise. Within both fiber types, those

Exercise physiologists have also found that higher work rates of short duration could be repeated with relatively low lactic acid levels (2,25,93). They attributed their findings to the alternate depletion and reconstitution of the muscle myoglobin. In addition to low lactic acid levels, there is little change in the arterial blood glucose during short intermittant work (83).

In contrast to this, other studies comparing continuous and intermittant work have failed to show any difference between the two (39,70,87). This is especially true when the total work output is the same. A large number of successful endurance atheletes (cross-country skiers, runners and bicyclists) have almost exclusively used continuous training in their efforts to attain high levels of maximum oxygen uptake. This fact may indicate that continuous training can be as good as intermittent training in conditioning for endurance events. In light of these findings, interval training does not appear to have an advantage over continuous training in enhancing endurance.

Linking a given response at the cellular level with

2. to enhance the capacity of the musculature to consume oxygen. Saltin et al. (121) believe that the stimulus sought for this improvement is related to the magnitude and duration of the stress imposed during a training session. Interval training for events which are largely aerobic can be developed around work intervals ranging from thirty seconds to five minutes. The duration of the rest periods depends on the intensity of each work bout. The higher the intensity of the work bout the longer the length of the rest periods.

Astrand et al. (2) found that a work load that could be tolerated for an hour when done intermittentantly resulted in exhaustion in nine minutes when done continuously. The effect of various combinations of work and rest intervals ranging from five to one hundred and eighty seconds have been studied by Christensen et al. (25), Margaria et al. (93) and Fox et al. (52). They have found that running and cycling with repeated extremely high work rates for short periods, alternated with rest pauses, allows an individual to accomplish a considerable quantity of work for a prolonged period of time without fatigue.

be accomplished over a given period of time and also this work can be done at higher intensities than the continuous workloads. Others feel that continuous training is more beneficial and that there is no difference in the final results of the two training methods. This is very debatable and will always be one of the major controversies of endurance training.

CHAPTER III

METHODOLOGY

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METHODOLOGY

Twenty male Sprague Dawley rats were divided into one control and four exercise groups. Two of the exercise groups were subjected to continuous training programs at fifty and sixty percent of their maximum swim time to exhaustion. The other two exercise groups were subjected to interval training programs at fifty and sixty percent of the total number of intervals performed before exhaustion. All the animals were sacrificed following the training program and the peripheral white and central red regions of the medial gastrocnemius were studied for SDH and NADH-D activity. The alterations in the fiber type ratios were determined and between group comparisons were made with the control and the exercise groups, and between the exercise groups, at both the fifty percent level and the sixty percent level of intensity.

Sample

Thirty, one hundred day old male albino rats of the

Sprague-Dawley strain were chosen for this research. Their weight range was between 268 - 285 grams. The top 20 animals, according to swim time to exhaustion were chosen to complete the research. Four rats were randomly chosen for the control group and sixteen animals were placed into matched exercise groups according to their swim times to exhaustion with four percent of their body weight attached to their tails.

Treatment Groups

The five treatment groups used in this investigation were as follows:

1. Sedentary Control Group

These rats were housed in sedentary cages measuring 45 cm. in length, 24 cm. in width and 20 cm. deep for the duration of the experiment. They were removed from their cages every sixth training day for body weight determinations.

2. 60% Continuous Group

Each of these animals was forced to swim continually for a time equaling 60% of its' swim time to exhaustion. A weight equaling 4% of the animals body weight was

Treatment Procedures

All of the rats used in this research were housed in cages measuring 45 cm. in length, 24 cm. in width and 20 cm. deep. There were four animals housed in each cage.

The rats swam in large commercial garbage containers measuring 55 cm. in diameter and having a depth of 60 cm. There were two of these containers used during the experimentation. These containers were subdivided into four equal compartments using 3 mm. thick plexiglass dividers. During the rest periods between the intervals the rats were placed in a plastic container measuring 45 cm. in length, 24 cm. in width and 20 cm. deep. At the end of each interval the weights were removed from the animal's tail and each animal was dried off with a towel.

There was a total of thirty animals in the initial group. Four of these animals were randomly chosen for the sedentary control group. The remaining twenty-six animals participated in the pre-training period. During the first two days of the pre-training period each

attached to the tail for the duration of the swim.

3. 60% Interval Group

These animals swam 110 second intervals with a weight equaling 10% of their body weight attached to their tails. Between intervals they were given a 120 second rest period. During this rest period they were placed in an empty animal cage. These animals swam a total time equaling 60% of their maximum swim time to exhaustion for intervals of the same length.

4. 50% Continuous Group

This group followed the same swimming pattern as Group Two except these animals swam 50% of their maximum swim time to exhaustion.

5. 50% Interval Group

These animals followed the same swimming pattern as Group Three except this group swam for only 50% of their maximum interval swim time to exhaustion.

animal swam 15 minutes with no weight attached to its tail. During the following three days each of the exercise animals swam for a period of ten minutes with a weight equaling 4% of their body weight attached to their tail. On the sixth day and eighth day each animal swam to exhaustion with a weight equaling 4% of their body weight attached to their tail. The mean swim time to exhaustion was then determined for each animal. The top eight swim times were equally divided between the two 60% groups and the next eight swim times were equally divided between the two 50% groups. The remaining animals were not used in the experimentation. A T-test was performed on these groups and there was no significant difference at the .01 level of confidence between the 60% continuous and interval groups nor between the 50% continuous and interval groups (Appendix C).

The continuous swimming groups swam work bouts representing 60% and 50% of their continuous swim time to exhaustion. These animals swam with 4% of their body weight attached to their tail. Each of these animals swam to exhaustion every sixth training day with 4% of

their body weight attached to their tail. The length of the continuous swim for the following five training days was then determined from the swim time to exhaustion.

The interval groups swam a total time in intervals equal to 60% and 50% of their maximum swim time to exhaustion for a work period made up of these same intervals. The length of the interval was determined by taking 50% of the mean swim time to exhaustion for both groups with 10% of the animal's body weight attached to its' tail. The length of time determined for each interval was 110 seconds (Appendix C). This was determined on the first two days of the training program. Each animal was given a 120 second rest period between each interval. During the following three days of the first training week each animal of both the 60% and 50% interval groups swam two intervals per day. Following week one, two, three, five and six these animals swam to exhaustion with 10% of their body weight attached to their tail. They swam to exhaustion using 110 second intervals and 120 second rest periods. The total interval swim time to exhaustion was determined and the interval

swim time to be completed during each of the next five training days was calculated. Following weeks four and seven the interval groups swam continuously to exhaustion with 4% of their body weight attached to their tails. This was done to provide some comparison with the continuous groups in swim time to exhaustion. The interval swim time was not determined for week five because of the determination of the continuous swim time to exhaustion. The animals were thus required to swim one more interval per work period in week five than was swam in week four. This was done to compensate for the training effect taking place during week four.

On every sixth training day the animals were weighed and the weights to be attached to the animals tail were adjusted. The weights used were commercial nuts and washers. They were attached to the animals' tail through the use of small clothespins. The weights were attached to the clothespins by a thin piece of wire. Adhesive tape was placed between the prongs of the clothespins to reduce the trauma. The weights were attached approximately 10 cm. from the tips of the animals' tails.

Exhaustion was determined in the continuous group by the animal staying below the surface of the water for over ten seconds. McArdle (95) has found a correlation of .9 between exhaustion after submersion for ten seconds and the total swim time to exhaustion. In the case of the interval groups exhaustion was determined by uncoordinated movement of the animal or the animal being unable to rise above a level 20 cm. below the surface of the water. This method was used instead of the ten second method because the animals swimming with the heavier weight on their tail tended to swim with their noses a few centimeters below the surface of the water.

The animals swam five days at the training rate and on the sixth day they swam to exhaustion. The seventh day was a rest day for the animals to recover from the swim to exhaustion on the day before. Two rest days were given between the end of the pre-training period and the training period. The pretraining period lasted eight days while the training period lasted forty-two training days. The animals swam between the hours of 0900 and 1200 hours on each training day.

Each animal was coded through the use of a color system. The tails were marked with two colors, one color representing the group the animal was in, and the second color distinguished each animal from the other members of the group.

All animals were fed ad libitum with commercial rat feed. The air temperature in the animal quarters ranged from 21°C. - 25°C. The water temperature for the swimming program ranged from 32°C. - 34°C.

Sacrifice Procedures

Each animal was sacrificed following the training program. The animals were sacrificed by placing one animal at a time in a large beaker that contained a large peice of cotton soaked in ether. A lid was placed over the beaker to prevent the escape of the ether. One group per day was sacrificed for slide preparation.

The left gastrocnemius was excised by cutting the muscle at its origins and insertion. The medial head of the gastrocnemius was separated from the whole gastrocnemius. A cross-section approximately 5 mm. thick was ablated from the belly of the medial head. These pieces

of tissue were then attached to a cutting block with Ames Lab Tek.

The cutting block was quenched for approximately twenty seconds with carbon dioxide gas.

Tissue sections were cut at 10 μ at a temperature of -20°C. A Slee rotary arm cryostat was used for the sectioning of the tissue. As the sections were cut they were individually placed on microscope slides and air dried.

Histochemical Procedures

Both the SDH and the NADH-D procedures used Nitro Blue Tetrazolium (2,2'-di-p-nitrophenyl-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride) as an electron acceptor. The method for studying SDH activity was that used by Nachlas et al. (99). The Novikoff et al. (101) procedure was used in studying the NADH-D activity.

Thirty minute incubation times were used for the enzyme histochemical procedures. Permount was used as a mounting medium in both procedures.

Control sections were incubated with each of the

enzymes to determine the specificity of the enzyme reaction. Controlled incubating solutions excluded the substrate in the case of the SDH procedure and also the substrate in the case of NADH-D procedure.

Black and white photomicrographs were taken using Kodak Plus Pan-x 35 mm. film. One second exposure times were used in all cases. These photomicrographs were taken at a power of 100X and blown up to give a magnification of 350X. The areas to be photographed were randomly chosen from the peripheral white and the central red portions of the medial head of the gastrocnemius.

Methods of Tissue Analysis

Specific regions of the muscle were chosen to be analysed because the concentration of the fiber types varies intramuscularly as well as intermuscularly. Both the red and white regions of the medial head were analysed. This was done to give an overall view of the changes taking place in the medial head of the gastrocnemius.

The method of randomly selecting the slides to be analysed from each group was carried out in the following manner for both staining techniques. Twelve slides were

perpared from the muscle tissue of each animal. Six slides were stained for SDH activity and the remaining six were stained for NADH-D activity. Each slide was coded to enable the investigator to know which animal the tissue came from. Four slides were then chosen from those prepared for each animal. These slides were then placed in a group with those from the other animals of that group. From this collection of sixteen slides, eight were randomly chosen to be photographed and analysed. This gave a total of eight slides being analysed for each group per staining technique. The red and white regions were analysed from each slide.

Areas of the peripheral white and the central red regions of the medial gastrocnemius were randomly chosen and photographed. All the fibers on or partially on each of the photomicrographs were analysed. The fibers were rated according to their staining intensity as light, dark and intermediate. This method applied to both the SDH and the NADH-D staining techniques. The red fibers stained darkly, the white fibers stained lightly and the intermediate fibers stained intermediate to the red and white fibers.

Besides the intensity of staining, the pattern of the staining was also analysed. In examples drawn from electron photomicrographs it was demonstrated that red muscle fibers have subsarcolemmal aggregations of numerous mitochondria (97).

These mitochondria have more dense cristae than those found in white fibers. Peripherally located mitochondrial aggregations result in a dark subsarcolemmal staining pattern with oxidative enzymes such as SDH and NADH-D. Also, such a fiber would stain more intensely throughout than would a white fiber. The intermediate fiber would also stain more intensely throughout than the white fibers because their mitochondrial patterns resemble those of the red fibers. The difference is that intermediate fibers lack the subsarcolemmal aggregations that are characteristic of the red fibers. The intensity of the staining throughout the intermediate fiber is not as deep because the interfibrillar rows of mitrochondria are not as prevalent in the intermediate fibers as in the red fibers (42). Examples of the fiber typing used in this research may be found in Figures I and II.

FIGURES I AND II

Cross sections of a portion of the red region of the medial gastrocnemius stained for SDH and NADH-D activity respectively. The microphotographs show the three major fiber types analysed in this research. The fiber ratings of light (L), dark (D) and intermediate (I) are shown. Notice the subsarcolemmal aggregations of mitochondria in the dark staining red fibers. Also notice that the intermediate muscle fibers are stained more intensely throughout than the white muscle fibers and that they lack the subsarcolemmal aggregations of mitochondria seen in the red fibers. The light fibers also lack the subsarcolemmal aggregations of mitochondria and are not as intensely stained throughout as are the intermediate fibers. (500X)

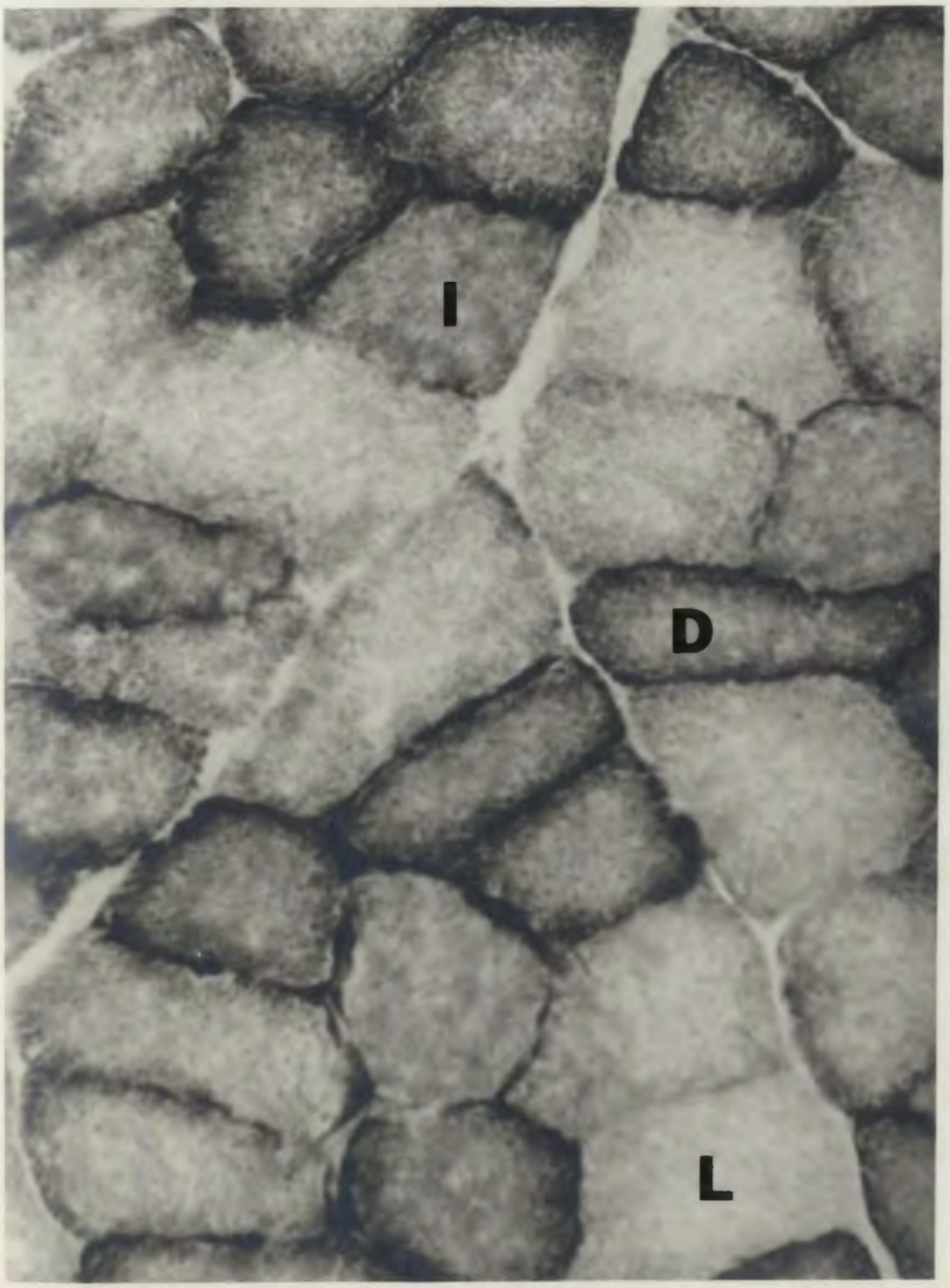

Figure I

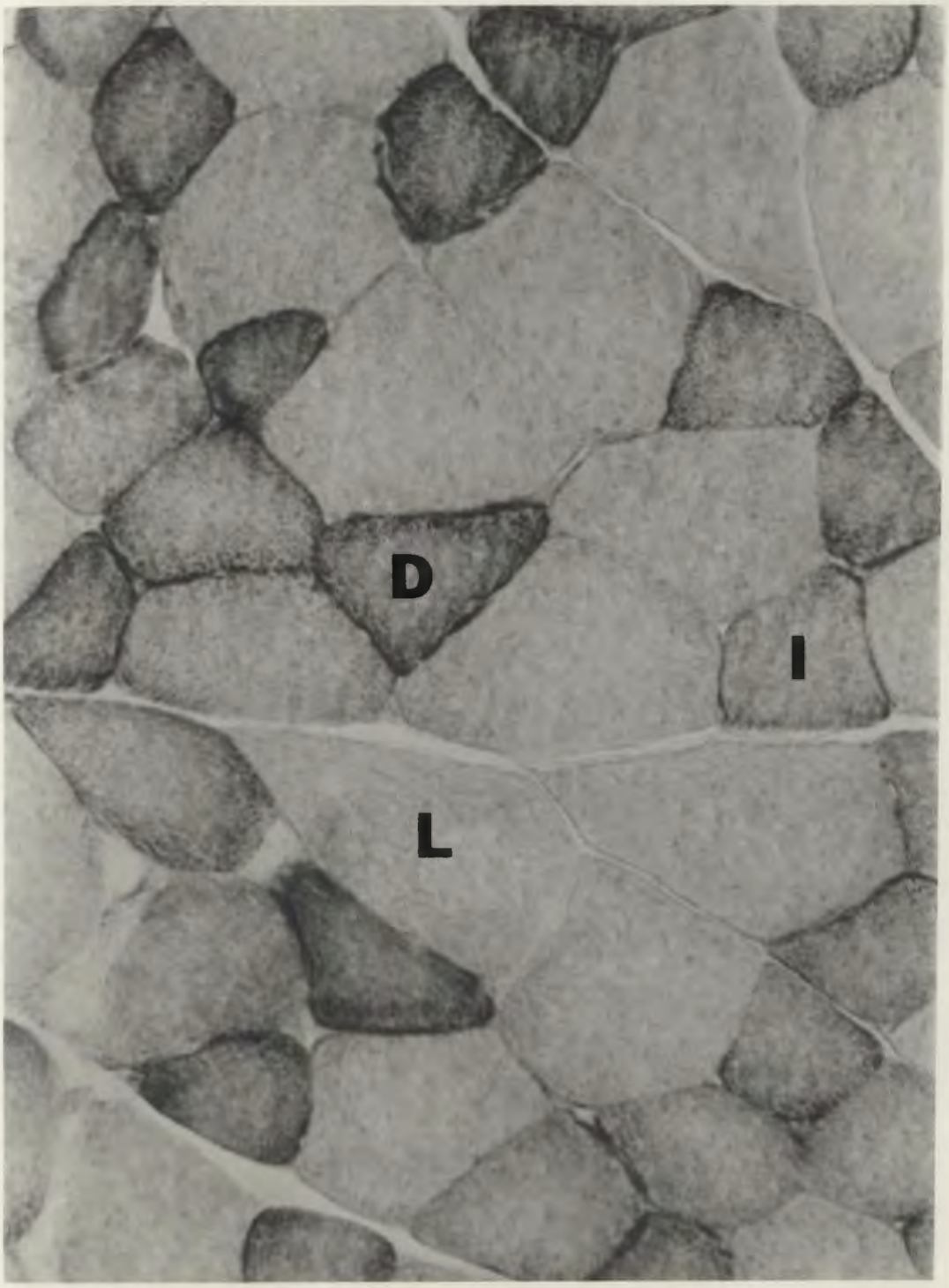

Figure II

Statistical Methods

The grouping of the animals for this research was done according to swim time to exhaustion. The two 60% groups were from the top eight swim times and the 50% groups were grouped from the next eith swim times. A T-test was used to determine if both groups were equal at each level of intensity.

At the end of the test period a T-test for correlated samples (135) was used to determine if a significant increase in the swim time to exhaustion did occur in each group.

In analysing the fiber types, the percentages of the types of fibers in each area were analysed using a one-way analysis of variance (60). The mean percentage differences between the five treatment groups were examined for red, intermediate and white fibers with separate one-way analyses of variance. When the F-ratio was significant, the Tukey method of multiple comparisons was used to determine which means differed (60). Also if the F-ratio was significant a Scheffe method of multiple comparisons was used to determine if there was a significant difference

in the combined 60% and 50% continuous group and the combined 60% and 50% interval group.

Due to the nature of the fiber typing data, consistancy in typing the data was determined in two ways. In both cases ten photographs were randomly chosed from groups stained for NADH-D and from groups stained for SDH. In testing the objectivity of the typing procedure an impartial observer was instructed in identifying the three fiber types. The results were then correlated with the original tabulation of the researcher. In testing to determine the reliability of the fiber typing, ten microphotographs stained for SDH activity and ten stained for NADH-D activity were analysed by the researcher ten days following the initial analysis. The results were then correlated with the initial analysis of the researcher.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

Analysis of Data

In the course of this research two types of data were gathered. The fiber analysis data dealt with the analysis of the muscle fibers in the selected regions of the gastrocnemius while the performance data was the swim-times and weights of the animals gathered throughout the investigation. The performance data will not be discussed in this section as it does not pertain to the analysis being undertaken in this investigation. The performance data has been made available in Appendix D.

Analysis of Fiber Types

In analysing the changes taking place in the mean percentages of fibers the analysis was divided into two sections. The first section deals with the central red region of the medial head while the second section deals with the analysis of the peripheral white region. In both cases the three fiber types will be analysed individually.

Red Regions

In the analysis of the red region it was found that an overall significant increase did occur in the mean percentage of red fibers while a significant decrease occurred in the mean percentage of white fibers. There was no significant change found in the mean percentage of intermediate fibers (Table II). As can be seen in Table II the difference in the mean percentage of fibers rated by staining for SDH and NADH-D was small with the greatest difference being 2.11% which occurred with the intermediate fibers of the 60% continuous level.

TABLE II

MEAN FIBER DISTRIBUTION IN THE CENTRAL

RED REGION OF THE MEDIAL GASTROCNEMIUS

STAIN	FIBER TYPE	CONTROL	60% CONTINUOUS	60% INTERVAL	50% CONTINUOUS	50% INTERVAL
SDH	RED	41.47	55.84*	58.68*	54.23*	61.44*
NADH	RED	40.68	54.98*	58.18*	55.54*	61.15*
SDH	WHITE	43.97	31.45*	27.49*	31.93*	24.28*
NADH	WHITE	45.03	30.21*	27.64*	30.86*	25.36*
SDH	INTER-MEDIATE	14.56	12.71	13.83	13.84	14.29
NADH	INTER-MEDIATE	14.28	14.82	14.17	13.60	13.46

* Significant at α .05 as determined by TUKEY ANALYSIS (Appendix E)

a. Red Fibers

As was mentioned earlier a significant increase did occur in the mean percentage of red fibers at all levels for both staining techniques. The increase in the mean percentage of red fibers in this region ranged from 14.37% for the 60% continuous group stained for SDH activity, to 20.47% in the 50% interval group stained for NADH-D activity. In all cases the increase was significant at the .05 level of confidence. (Table III)

TABLE III

F-TEST VALUES AND SCHEFFÉ ANALYSIS RATIOS FOR RED FIBERS OF THE RED REGION

STAIN	F-TEST value (a)	SCHEFFÉ ANALYSIS value (b)*
SDA	73.52	5.46
NADH-D	35.45	3.38

(a) Significant at α .05 = 2.65

(b) Significant above 2.91

* $\frac{\Psi}{\overline{\sigma\psi}}$

Since the F-value was significant a Tukey analysis was performed on the data. (Appendix E) Ten different comparisions were found from the data. No significant difference was found between the 60% continuous groups and the 50% continuous groups nor between the 60% interval group and the 50% interval group stained for NADH-D. A significant difference was found between the 60% interval and 50% interval groups stained for SDH activity. Individual comparisions between the continuous groups and the interval groups were also calculated and may be found in Appendix E.

For the purpose of the analysis of the combined continuous and the combined interval groups a Scheffé Analysis was employed. In both tissues stained for SDH and NADH-D activity a significant difference was found in favor of the interval training groups. The combined interval training groups caused a greater increase in red fibers than the combined continuous training groups.

(b) White Fibers

In the red region the mean percentage of white fibers decreased significantly following the training programs. The decrease in the mean percentage of white

fibers ranged from 12.04% in the 50% continuous group stained for SDH activity to 19.69% in the 50% interval group stained for SDH activity. In both tissues stained for SDH activity and tissues stained for NADH-D activity the decreases were significant in all groups at the .05 level of confidence (Table II).

TABLE IV

F-TEST VALUES AND SCHEFFÉ ANALYSIS RATIOS

FOR WHITE FIBERS OF THE RED REGION

STAIN	F-TEST value (a)	SCHEFFÉ ANALYSIS value (b)*
SDH	47.15	5.38
NADH-D	32.81	3.00

(a) Significant at α .05 = 2.65

(b) Significant above 2.91

* $\frac{\Psi}{\overline{\sigma\Psi}}$

A Tukey analysis was then performed to determine where the significant decreases were found. As before ten comparisions were determined. In the comparison of the 60% and 50% continuous groups no significant difference in the mean percentage decrease in white fibers was found between the two groups. The same applied to the 60% and 50% interval groups. The individual interval and continuous comparisons are available in Appendix E. A Scheffé analysis of the combined interval and the combined continuous groups showed that interval training caused a greater decrease in the mean percentage of white fibers than continuous training in the red region of the gastrocnemius. This occurred in both tissues stained for SDH and tissues stained for NADH-D activity.

(c) Intermediate Fibers

The change in the mean percentage of intermediate fibers analysed in this region was insignificant. The range of mean percentages in fibers stained for SDH activity was 1.85% while the range for intermediate fibers stained for NADH-D activity was 1.36%. The

TABLE V

MEAN FIBER DISTRIBUTION IN THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS

STAIN	FIBER TYPE	CONTROL	60% CONTINUOUS	60% INTERVAL	50% CONTINUOUS	50% INTERVAL
SDH	RED	23.40	43.22*	51.00*	48.36*	50.62*
NADH	RED	28.42	47.70*	52.23*	47.35*	48.18*
SDH	WHITE	68.15	47.01*	40.13*	42.58*	40.43*
NADH	WHITE	63.32	42.55*	39.03*	43.78*	42.95*
SDH	INTER-MEDIATE	8.47	9.77	8.86	9.06	8.94
NADH	INTER-MEDIATE	8.25	9.75	8.74	8.86	8.86

* Significant at = .05 as determined by TUKEY ANALYSIS (Appendix E)

lowest mean percentage of intermediate fibers for both staining techniques was 12.71% while the highest was 14.82% (Table II).

White Region

In analysing the peripheral white region of the medial head of the gastrocnemius it was found that a significant increase in the mean percentage of red fibers occurred in all training groups. While a significant increase occurred in red fibers, a significant decrease in the mean percentage of white fibers occurred in this region. As in the red region of the medial head no significant change was found in the mean percentage of intermediate fibers (Table V). As can be seen the difference in the mean percentages of fibers in the same groups stained for both SDH and NADH-D activity had a maximum difference of 5.02% and that was in the case of the red fibers of the control group. In this case a difference of approximately the same size could be found between the two staining techniques in the corresponding mean percentage of white fibers (Table V). This was due to the nonsignificant change occuring in the mean percentage of intermediate fibers.

(a) Red Fibers

The mean percentage of red fibers increased significantly in the white region of the medial gastrocnemius for both staining techniques. The increase in the mean percentages of red fibers stained for SDH activity ranged from 19.82% for the 60% continuous group to 27.6% for the 60% interval group. In the case of the fibers stained for NADH-D activity the range was from 18.93% for the 50% continuous group to 23.81% for the 60% interval group (Table V). In both cases the 60% interval group provided the greatest increase in the mean percentage of red fibers.

TABLE VI

F-TEST VALUES AND SCHEFFÉ ANALYSIS RATIOS FOR RED FIBERS OF THE WHITE REGION

STAIN	F-TEST value (a)	SCHEFFÉ ANALYSIS value (b)*
SDH	69.87*	3.62
NADH-D	23.44	1.39

(a) Significant at $\alpha.05 = 2.65$

(b) Significant above 2.91.

* $\frac{\Psi}{\sigma\Psi}$

In both cases the F-test values were significant and Tukey analyses were performed. In analysing the results of the Tukey analysis it was found that there was no significant difference between the 60% and 50% continuous groups in the increase in red fibers. Also the differences in the increases in red fibers caused in the 60% interval group and the 50% interval group was found to be non-significant (Appendix E).

The Scheffé analysis comparing the combined interval and the combined continuous groups showed a significant difference between the combined groups stained for SDH activity. The interval training caused a greater increase in the mean percentage of red fibers in this region than did the continuous training. In those fibers stained for NADH-D activity no significant difference was found between the increase in red fibers caused by interval training and the increase caused by continuous training.

(b) White Fibers

As in the red region the mean percentage of fibers in the peripheral white region decreased significantly.

The decrease in white fibers stained for SDH activity ranged from 21.14% for the 60% continuous group to 28.02% for the 60% interval group. In the fibers stained for NADH-D activity the smallest decrease was 19.54% in the 50% continuous group and the greatest decrease was 24.29% in the 60% interval group (Table V). The greatest decrease in white fibers corresponds with the 60% interval group having the greatest increase in red fibers in this region.

TABLE VII

F-TEST VALUES AND SCHEFFÉ ANALYSIS RATIOS FOR WHITE FIBERS OF THE WHITE REGION

STAIN	F-TEST value (a)	SCHEFFÉ ANALYSIS value (b)*
SDH	50.00*	2.27
NADH-D	39.53*	1.41

(a) Significant at $\alpha .05 = 2.65$

(b) Significant above 2.91

* $\frac{\Psi}{\sigma\Psi}$

Since the F-test values were significant a Tukey analysis was performed to dtermine where the significant differences occurred. In both staining techniques there was no significant differences between the 60% and 50% continuous groups nor the 60% and 50% interval groups (Appendix E).

To compare the combined continuous and the combined interval groups a Scheffé analysis was performed. In both the tissues stained for SDH activity and the tissues stained for NADH-D activity there was no significant difference in the decrease of white fibers caused by interval and continuous training. The Scheffé value for fibers stained for SDH activity was almost significant with a calculated value of 2.72. The required value for significance was 2.91.

(c) Intermediate Fibers

In the peripheral white region of the medial head the change in intermediate fibers, like the red region, was insignificant. The mean percentage range in fibers stained for SDH was 1.3% while the mean percentage range

for NADH-D activity was 1.5%. The lowest mean percentage for both staining techniques was 8.25% while the highest was 9.75% (Table V). Both of these occurred in fibers stained for NADH-D activity.

Objectivity and Reliability of the Subjective Analysis Procedure

In an analysis of this nature the reliability and objectivity of the subjective evaluation of muscle fiber types is very important. Objectivity of the subjective analysis was determined by calculating a correlation between the initial fiber count of the researchers and a second fiber count of the randomly chosen microphotographs by an impartial observer. Reliability was determined by calculating a correlation between the initial fiber count of the researcher and a second fiber count on randomly chosen microphotographs completed by the researcher.

TABLE VIII

OBJECTIVITY AND RELIABILITY CORRELATIONS OF FIBER ANALYSIS PROCEDURE

Fiber Type	Stain	Correlation for Reliability r=	Correlation for Objectivity r=
Red	SDH	.985	.978
White	SDH	.994	.959
Intermediate	SDH	.857	.730
Red	NADH-D	.991	.986
White	NADH-D	.982	.942
Interdediate	NADH-D	.790	.979

As can be seen the range in the correlations is from .994 to .857 for the reliability testing and from .986 to .730 for the objectivity testing. This would indicate a high level of reliability and objectivity in the subjective analysis procedure employed in this analysis. In all cases but one (intermediate fibers stained for NADH-D activity) the correlations for reliability are higher than those for objectivity. The correlations of intermediate fibers are lower because of the lower number of intermediate fibers analysed (Appendix E).

The swim times to exhaustion of the exercise groups were also determined every sixth training day. In all of the animals the greatest increase in swim time to exhaustion occurred in the first four weeks of the training program. Following the end of the fourth training week the majority of swim times to exhaustion levelled off or decreased with the result that the only significant increase in the mean swim time to exhaustion occurred in the 50% interval group (Appendix D). Numerous authors have also found non-significant increases in the swim time to exhaustion of trained animals (95, 131, 132). These authors feel that the results are due to an increase in the body weight of the animals and thus an increase in the proportionate load attached to the animal. McArdle and Montoye (95) found that the duration of the swim time to exhaustion was significantly lower in heavier rats when weights were attached according to same percentage of their body weight. This could be an explanation for the non-significant increase in the swim time to exhaustion of three of the exercise groups. The increase in performance was hidden by the increase

Like the previous hypothesis, this hypothesis was accepted as no significant difference was found between the two groups following the interval training program.

Discussion

In analysing the results of this research a brief discussion of the performance data will be given along with a more detailed discussion of the muscle fiber type data.

Performance Data

Throughout the length of the training program the weights of all the animals were recorded every sixth training day. At the end of the training program the mean weight of the control group was as much as 63 grams greater than that of one of the exercise groups. This would be in agreement with the research of Barnard, Edgerton and Peter (9) who also found that the weight gain in the control animals was greater than that in the exercised animals

This hypothesis was also rejected because a significant difference in the mean percentage of white muscle fibers was found following the interval and continuous training programs. The interval training caused a greater decrease in the mean percentage of white muscle fibers than did continuous training.

Hypothesis 3.

There is no significant difference in the mean percentage of red muscle fibers of the medial gastrocnemius in the 60% continuous and 50% continuous groups following a continuous training program.

This hypothesis was accepted as no significant difference in the mean percentage of red fibers was found between the 50% and 60% groups following completion of a continuous training program.

Hypothesis 4

There is no significant difference in the mean percentage of red muscle fibers of the medial gastrocnemius in the 60% and 50% interval groups following an interval training program.

Summary of Data

In summarizing the data the four major hypotheses are taken into consideration.

Hypothesis 1

There is no significant difference in the mean percentage of red muscle fibers in the medial gastrocnemius of rats following interval and continuous training.

This hypothesis was rejected because it was found that there was a significant difference in the mean percentage of red muscle fibers in the medial gastrocnemius of rats following interval and continuous training. It was found that interval training caused a more significant increase in red muscle fibers than did continuous training.

Hypothesis 2

There is no significant difference in the mean percentage of white muscle fibers in the medial gastrocnemius of rats following interval and continuous training programs.

in body weight of the exercised animals and also by the increased proportionate absolute loads brought about by the increase in body weight.

Fiber Typing Data

It is well established that oxidative enzymes such as SDH and NADH-D are associated with mitochondria. Since the number and size of mitochondria as well as the density of the mitochondrial cristae are some of the characteristics which aid in typing muscle fibers it has become an accepted fact that the proportion of red and white muscle fibers can be modified by an alteration in the mitochondrial population which in turn can alter the intensity of enzyme activity as demonstrated biochemically (9,40,63). According to Holloszy (74,75) increases in mitochondrial enzyme activity and oxidative capacity in skeletal muscle are apparantly due to an increase in mitochondrial protein. Holloszy (74) has found a 60% increase in the protein content of the mitochondrial fraction of skeletal muscle. Electronmicroscopic studies attribute the increase in mitochondrial protein to increases in the number and size of the mitochondria (86,87).

The increase in the intensity of staining as noted in this research would thus indicate an increase in the size and number of mitochondria in the stained muscle fibers.

In analysing the changes in the metabolic profiles of the muscle fibers, an increase in the mean percentage of the highly oxidative red muscle fibers has been identified following endurance type training. Previous histochemical investigations by Edgerton (40) and Barnard et al. (9) have identified increases in red muscle fibers following endurance training programs. These studies have also indicated an increase in the aerobic capacity of the subject as identified by increased swim times to exhaustion. Like Barnard and his co-workers (9) the research undertaken in this paper studied the central red and peripheral white regions of the medial gastrocnemius of rats following endurance type training programs. A significant increase in the mean percentage of red fibers was found in both regions following both continuous and interval training. Coinciding with the increase in red fibers was a significant decrease in white fibers

Investigations of aerobic enzyme activity following endurance type interval training are few in number. Green and Houston (70) attempted to compare interval and continuous training programs through an enzymatic analysis of oxidative enzymes. After 50 exercise bouts little difference in the fiber populations of the aerobically stressed interval and continuous groups was found. Also no significant difference was found in muscle fiber percentage between the 75% and 50% of maximum levels of intensity for the continuous trained animals. The results of this research is in opposition to the first finding of the research of Green and Houston (70). In this study it was found that interval training stressing the aerobic capacity of the muscle caused a more significant alteration in the mean percentage of red muscle fibers than did continuous training of the same intensity. This may be due to the fact that swimming stressed the aerobic pathways of the animal more than the running program employed by Green and Houston. In agreement with their second finding, no significant difference was found in the mean

and no significant change in the mean percentage of intermediate fibers. Again this is in agreement with the work of other researchers (9,40,41).

In view of the significant increase in the mean percentage of red fibers, it is evident that an increase in the aerobic metabolism of the medial gastrocnemius also occurred. It has become accepted that red muscle fibers have a greater capacity for the oxidation of fatty acids than do white fibers (54,94). Also red fibers contain relatively large amounts of triglycerides whereas white muscle fibers contain very minimal amounts (133). This would indicate an increased aerobic metabolism in the medial gastrocnemius due to the increased oxidation of fatty acids brought about by the increase in red fibers in the gastrocnemius.

Numerous authors have used a continuous training regime and identified increased aerobic metabolism in muscle fibers (9,41,75). Holloszy (74) has identified an almost 2-fold increase in aerobic enzymes following continuous training and this has been verified by the research of Gollnick and his co-workers (63).

percentage increase in red muscle fibers between the 60% and 50% levels of intensity for both continuous and interval training. This can be accounted for because of the small difference in the severity of exercise used in this research for both training techniques.

Peter and Barnard (113) attempted to correlate exercise-induced increases in cytochrome concentration with the increased endurance of skeletal muscles. They found that an increase in the aerobic capacity of the muscle is associated with an increase in the performance capacity of the individual muscle. A very poor correlation was observed between cytochrome concentration and running time to exhaustion. In this study a significant increase was found in the aerobic enzymes studied following the two training programs but in only one group was there a significant increase in the mean swim time to exhaustion (Appendix D). Jeffress and Peter (80) have stated that it is almost impossible to correlate the biochemical changes occurring in the muscle cell with the increase in the aerobic capacity of the total organism. This is due to the many intermediary steps that occur in the aerobic process. For this reason

the performance and fiber typing data were not correlated in this research.

Overall the findings of this research agree with the many authors who feel that interval training is the best type of training for developing the endurance capacity of an athelete (2,52,93). However it must be remembered that this research was done on rats rather than atheletes and one can only assume that these same changes occur in man. Because of the increase in the mean percentage of red muscle fibers in the medial gastrocnemius, the muscle is capable of sustaining a level of activity for longer periods of time and the muscle is capable of using the substrates supplied for aerobic purposes more efficiently.

CHAPTER V

SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

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SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

The central red region and the peripheral white region of the medial gastrocnemius were analysed in male albino rats following interval and continuous training programs. Succinic dehydrogenase and reduced nicotinamide adenine dinucleotide diaphorase were analysed histochemically in all the test groups.

A total of five groups with four animals in each group was used in this research. The groups were classified as control, 60% and 50% continuous training and 60% and 50% interval training. The training groups were exercised at 60% and 50% of their maximum swim time to exhaustion using either continuous or interval training. The continuous training groups swam with a weight equaling 4% of their body weight attached th their tails. The interval group swam 110 second intervals with a weight equaling 10% of their body weight attached to their tails. Rest periods between the intervals were

120 seconds in length. Swim times to exhaustion were determined every sixth training day and the animals were also weighed on these days. Following this the length of the work bouts and the absolute load to be attached to the animal were determined for the following five training days. The training program lasted a total of 50 days, 8 days for pre-training and 42 days for the actual training program.

Each group of animals was housed in sedentary cages and fed commercial rat food. The temperature of the water for the training ranged from 32°C. to 34°C.

The right medial gastrocnemius was excised from each of the test animals. Small blocks were cut from each muscle and frozen immediately with carbon dioxide gas. The tissues were sectioned at a thickness of ten microns and stained for SDH and NADH-D activity.

Both the central "red" region and the peripheral "white" region of the medial gastrocnemius were analysed for alterations in the proportion of red, white and intermediate muscle fibers. The red, white and intermediate fibers were identified as high, low and intermediate

activity fibers according to the intensity of staining.

In all the exercise groups a significant increase in red muscle fibers and a significant decrease in white muscle fibers was found when compared with the control group. No significant change occurred in the mean percentage of intermediate fibers in the trained animals when compared with the control animals.

The 60% and 50% levels of intensity were compared for each training technique. No significant difference in the mean percentage of fiber types was found between the 60% and 50% continuous groups nor the 60% and 50% interval groups.

The major purpose of this research was to determine which type of training caused the greatest alteration in the mean percentage of red and white fibers in the two regions of the gastrocnemius that were analysed. It was found that the alteration in fiber types caused by interval training was significantly greater than that caused by continuous training.

The red muscle fibers stain intensely for aerobic enzymes and this staining intensity correlates well with

an increase in the energy forming mitochondria in the muscle cell. The increase in red muscle fibers would thus mean an increase in the mitochondrial population of the muscle which would bring about a higher aerobic capacity of the muscle. This would supply further support to the theory that interval training promotes a greater development in the aerobic capacity of muscle than does continuous training. Although this study was undertaken using rats as subjects, it is thought that these same changes take place in human skeletal muscle and thus interval training can be assumed to cause a greater development in the aerobic capacity (at the cellular level) of an athelete than does continuous type training.

Conclusions

The results of this investigation have led to the following conclusions.

1. Exercise treatment as used in this study significantly alters the proportion of red and white muscle fibers in the peripheral "white" and central "red" regions of the

medial gastrocnemius of rats.

2. Exercise treatment of this nature does not cause any alteration in the proportion of intermediate fibers in the peripheral "white" and central "red" regions of the medial gastrocnemius of rats.

3. Interval training causes a greater alteration in the proportion of red and white fibers than does continuous training. Thus interval training causes a greater increase in red fibers and a greater decrease in white fibers than does continuous training. In light of this the proposed hypothesis may be rejected.

4. Exercise treatment of this nature, using 60% and 50% levels of intensity, does not create any significant difference in the mean fiber percentages between the two treatment levels.

Reccomendations

1. A similar study of this nature should be conducted where the oxygen consumption of the animals is measured so as to equate the workloads more accurately.

2. A quantitative biochemical analysis should be carried out following the same type of training programs. This would give a more accurate analysis of the activity of the aerobic enzymes of the muscle.

3. A study similar to this should be conducted making use of a motorized treadmill rather than using swimming programs. This would help greatly in equating the work-loads of the animals.

4. A study similar to this should be conducted using interval work periods having lengths of 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 seconds. This would aid in giving an accurate analysis of the length of interval which best develops the aerobic capacity of the exercising animal.

5. Recently a method has been described to dissect out and identify single fast-twitch and slow-twitch fibers from freeze-dried muscle biopsy material (47). This method makes it possible to perform biochemical analyses on pure samples of fast and slow-twitch fibers. By using this method a more accurate analysis of the

biochemical changes occuring in the muscle fiber can be undertaken because in previous biochemical analyses a mixed group of fibers were analysed rather than a pure sample.

6. A study similar to this should be carried out using human subjects. Muscle biopsy samples would be analysed prior to training and following the training program to see if an alteration in muscle fiber types did occur.

7. A study similar to this using at least 10 animals per group should be conducted. This would add greater statistical validity to the analysis.

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APPENDIX A

A COMPARISON OF THE EFFECTS OF INTERVAL AND CONTINUOUS TRAINING PROGRAMS ON THE PROPORTION OF RED, WHITE AND INTERMEDIATE MUSCLE FIBERS OF THE MEDIAL GASTROCNEMIUS OF RATS

BY

© PETER GEORGE SELIG, B.Sc.

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OF THE REQUIREMENTS FOR THE DEGREE OF
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ST. JOHN'S NEWFOUNDLAND

APPENDIX B

MICROPHOTOGRAPHS

The following figures are microphotographs of a cross section of either the central red or the peripheral white region of the medial gastrocnemius. The photographs are at a magnification of approximately 350 X. The intensity of staining and the staining pattern of the muscle fibers should be noticed. Also to be taken into consideration should be the population of red, white and intermediate fibers.

The microphotographs will be identified according to the test groups (CON, 60C, 50C, 60I, 50I), enzyme staining used (SDH or NADH-D) and the region (red or white) of the medial gastrocnemius from which the sample was photographed.

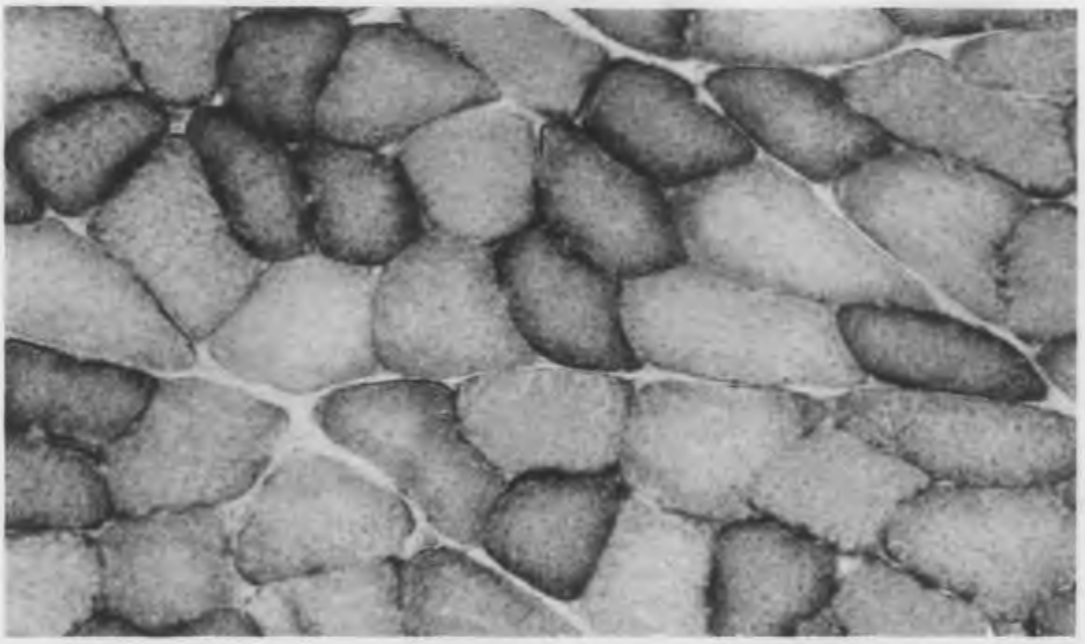


FIGURE III CON, SDH, RED

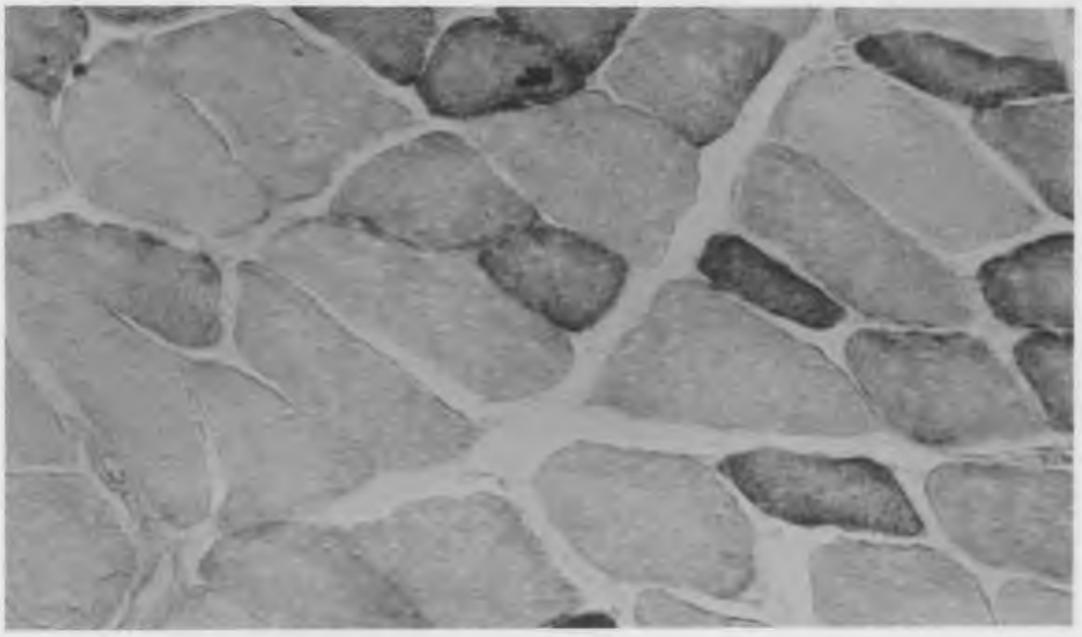


FIGURE IV CON, SDH, WHITE

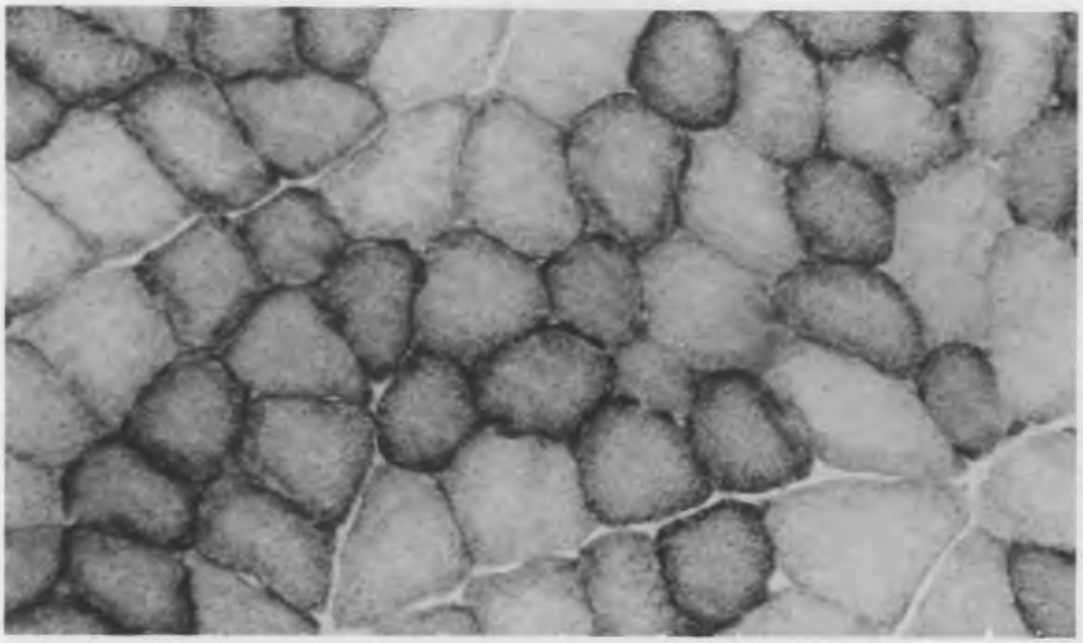


FIGURE VII 60C, SDH, RED

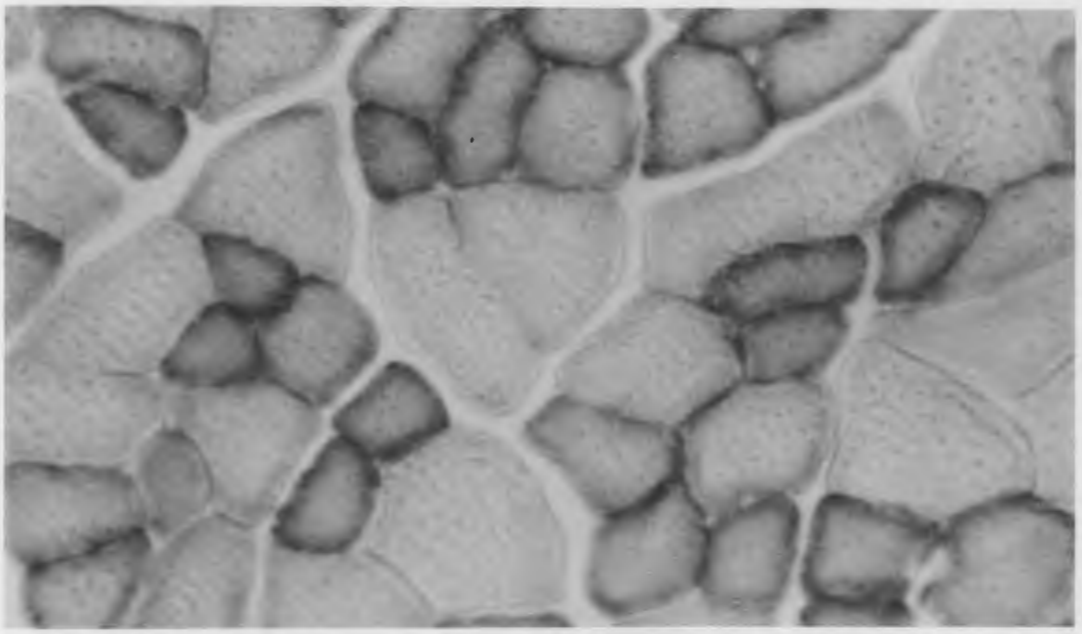


FIGURE VIII 60C, SDH, WHITE

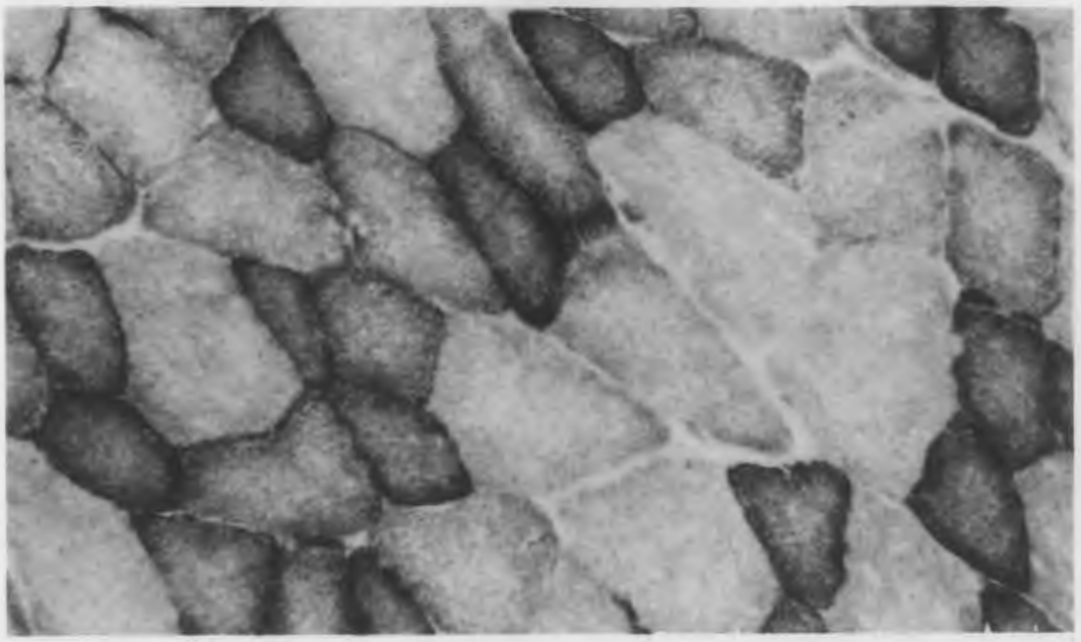


FIGURE V CON, NADH-D, RED

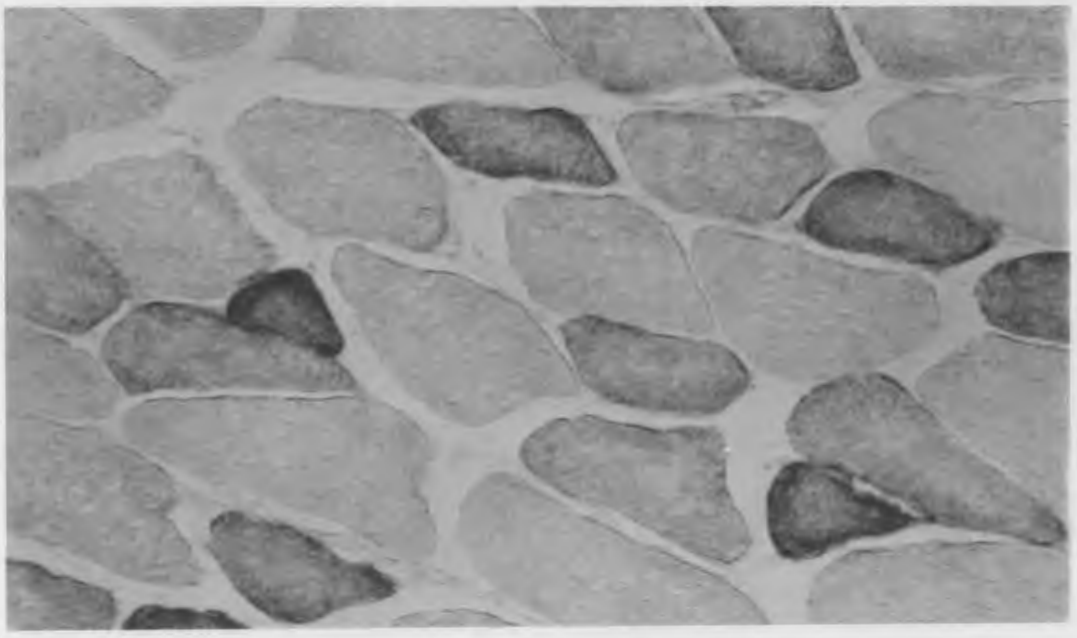


FIGURE VI CON, NADH-D, WHITE

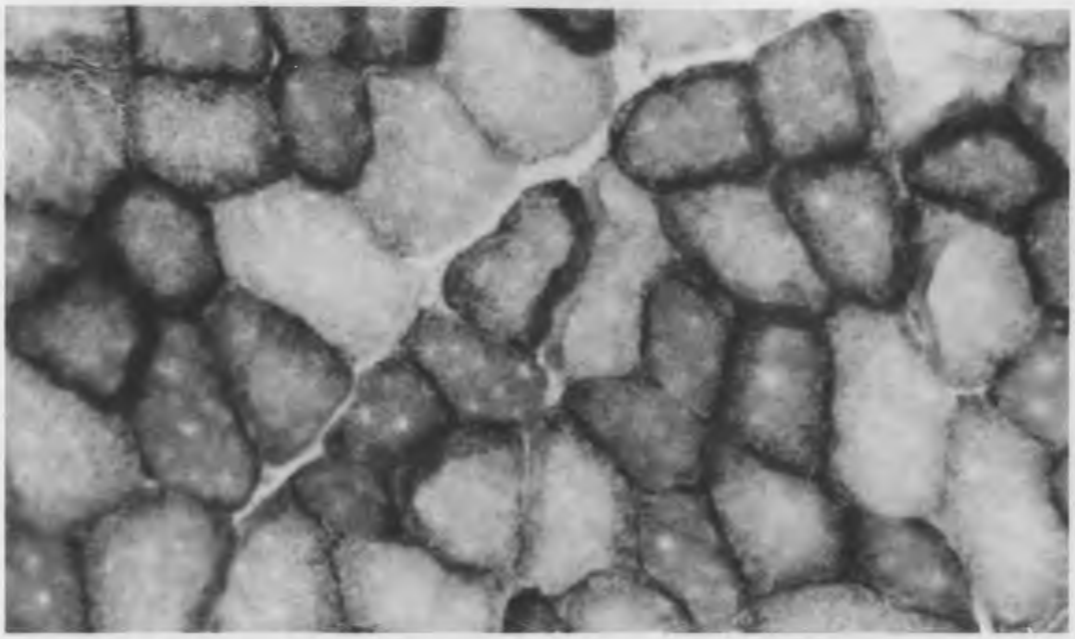


FIGURE IX 60C, NADH-D, RED

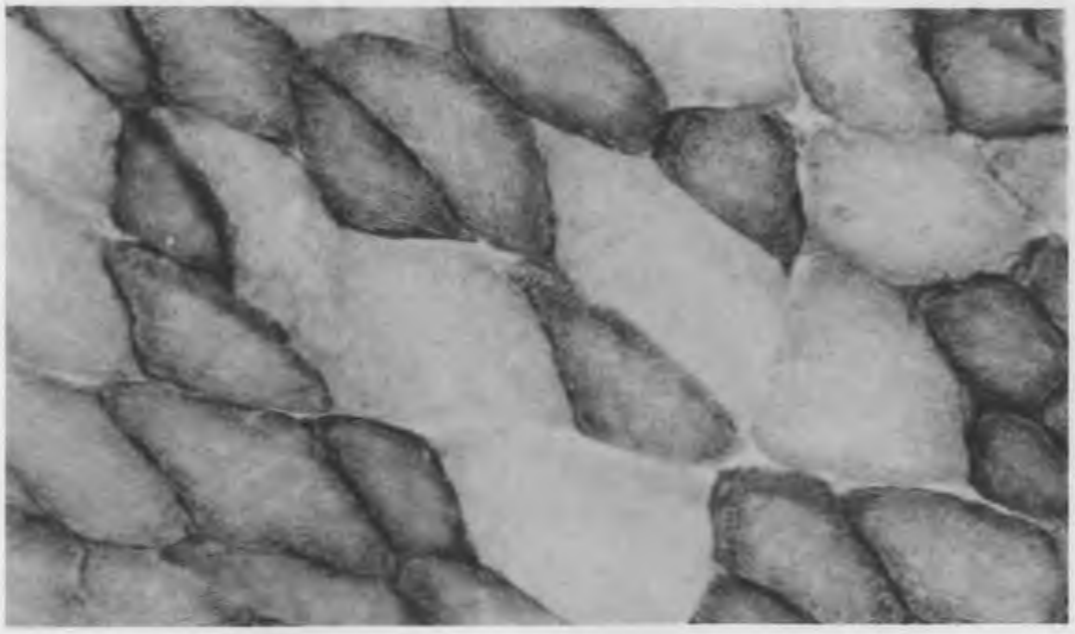


FIGURE X 60C, NADH-D, WHITE

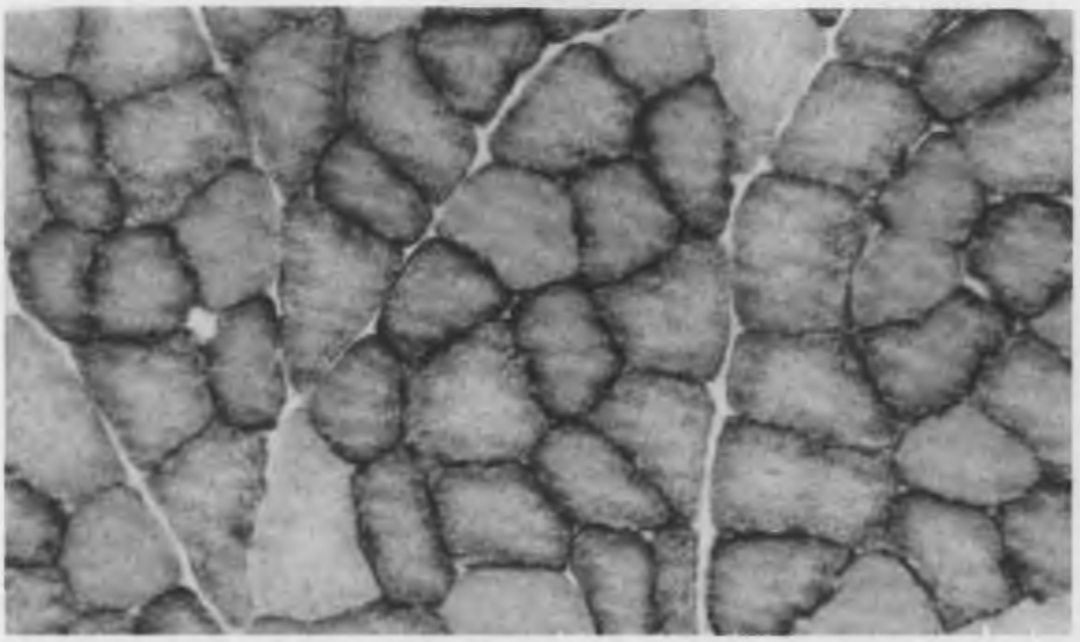


FIGURE XI 60I, SDH, RED

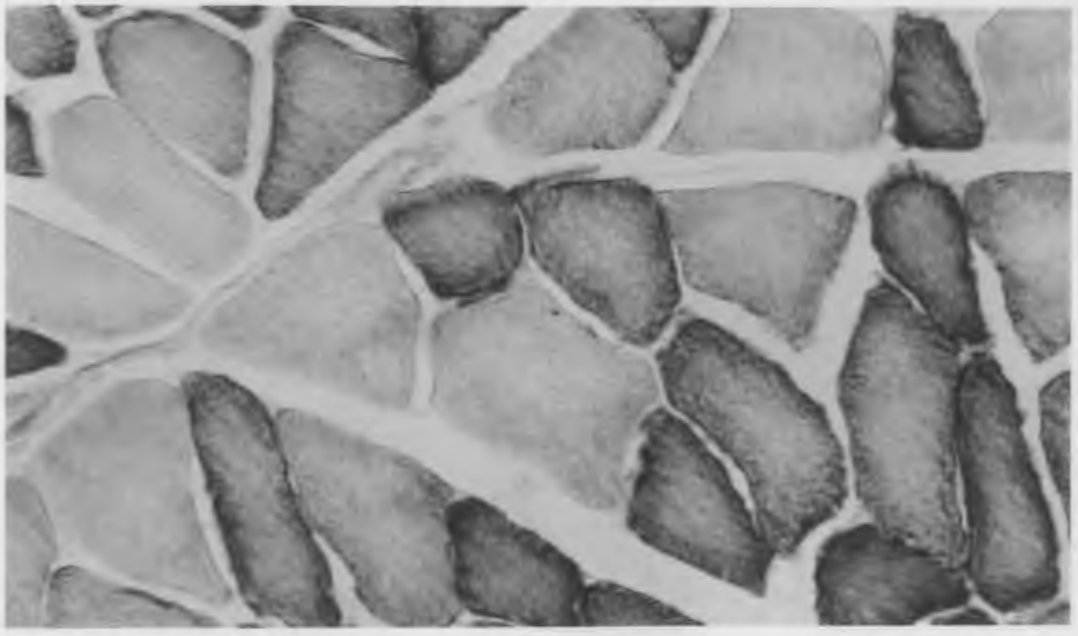


FIGURE XII 60I, SDH, WHITE

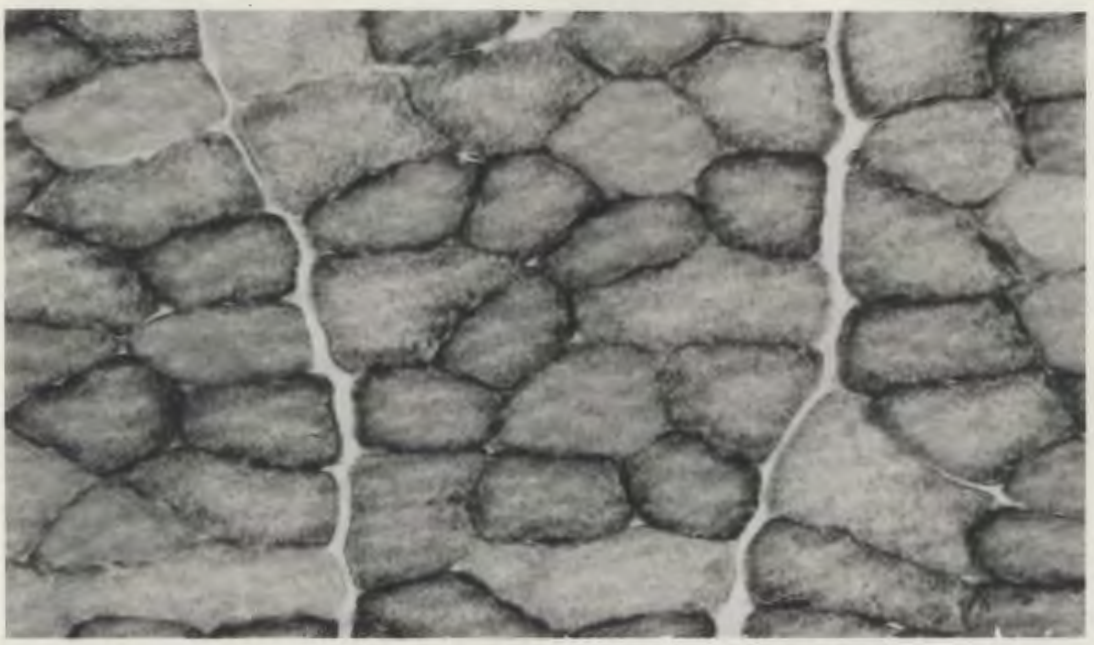


FIGURE XV 50C, SDH, RED

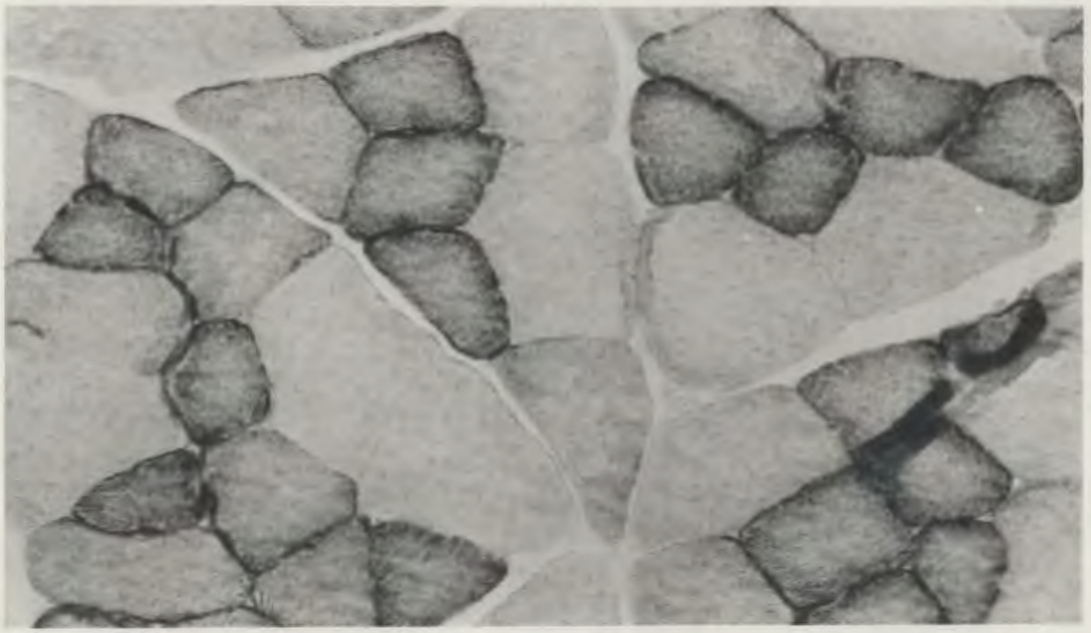


FIGURE XVI 50C, NADH-D, WHITE

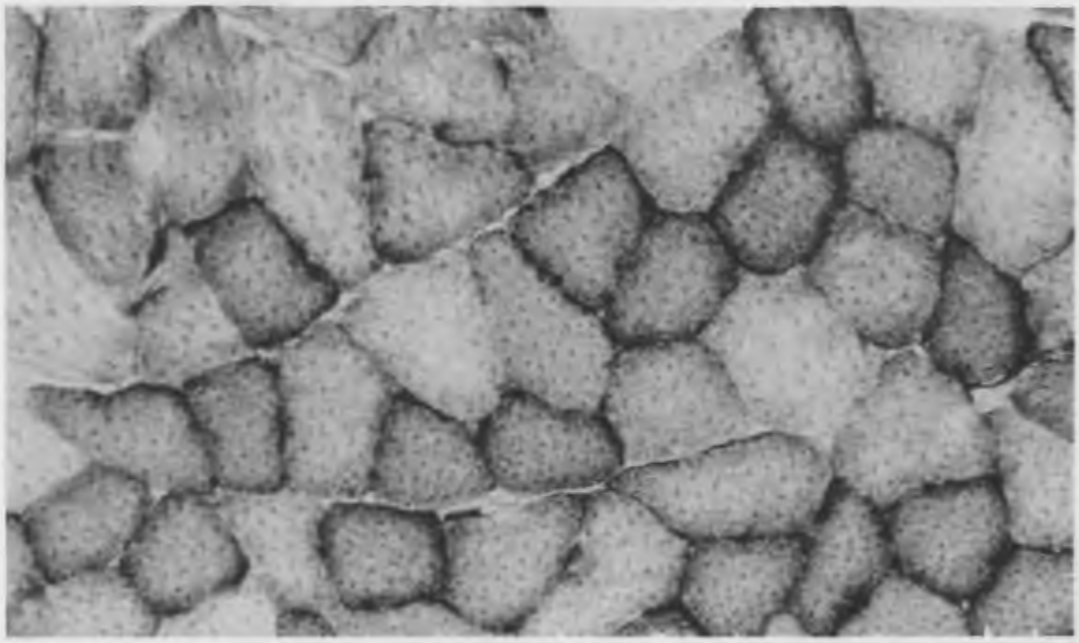


FIGURE XIII 60I, NADH-D, RED

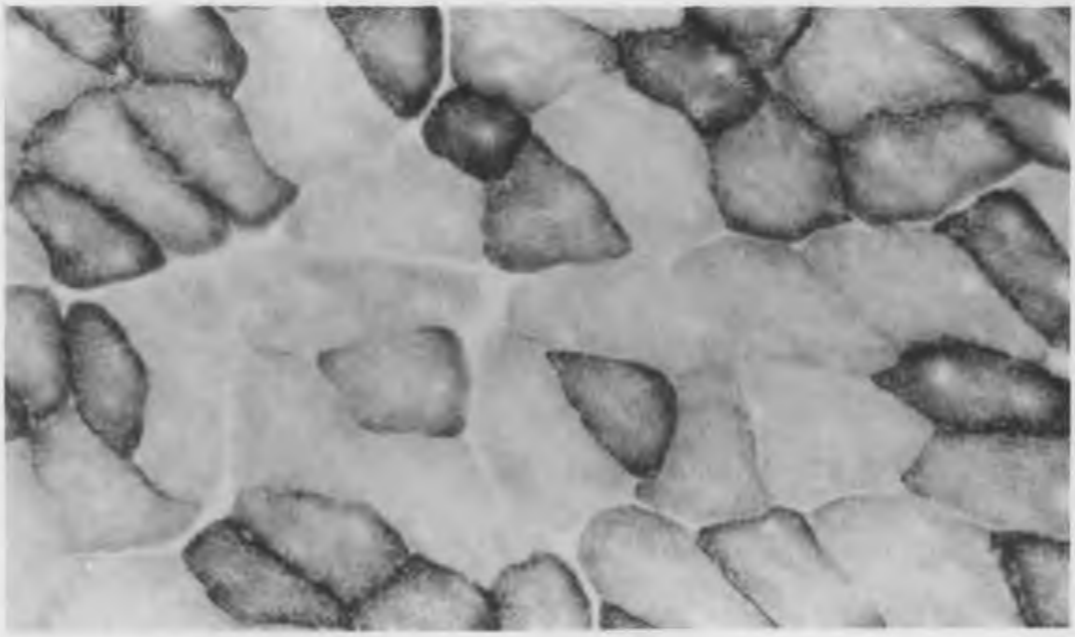


FIGURE XIV 60I, NADH-D, WHITE

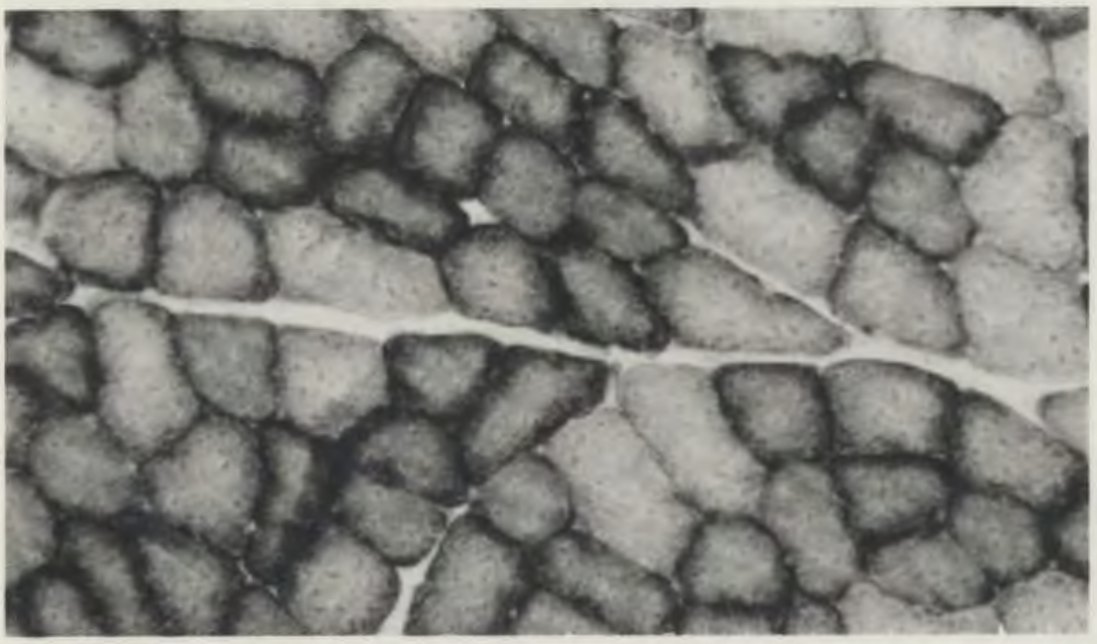
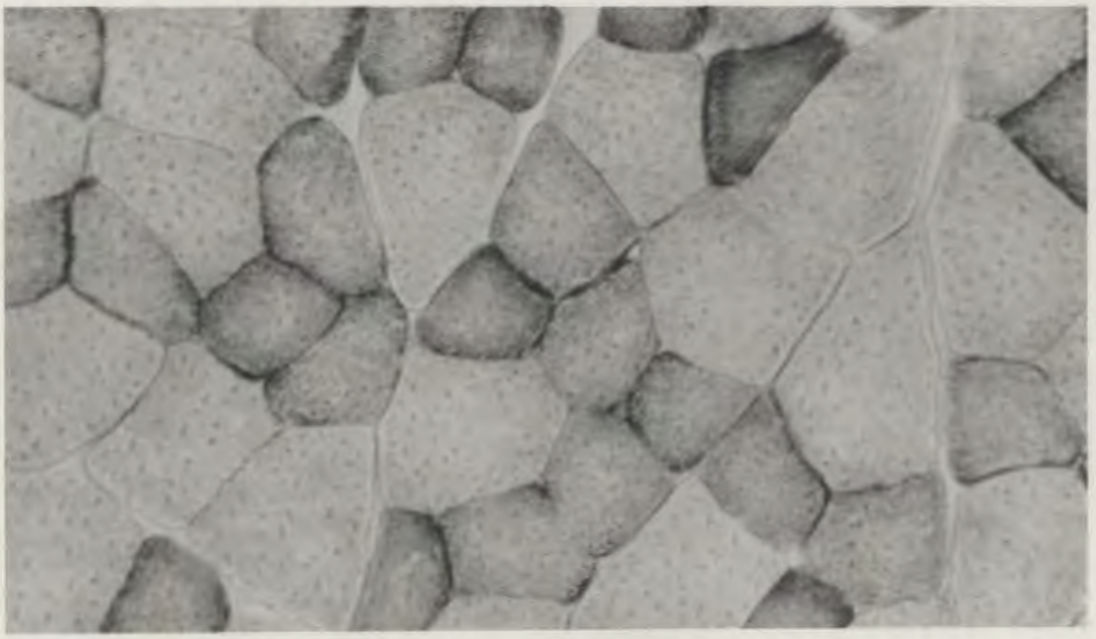


FIGURE XIX 50I, SDH, RED



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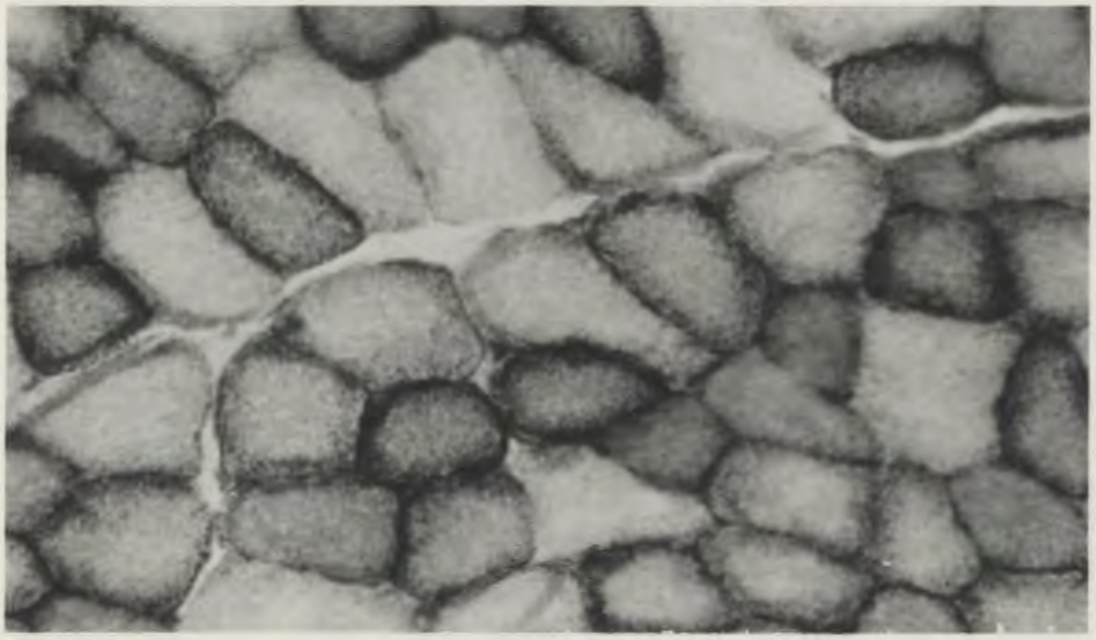
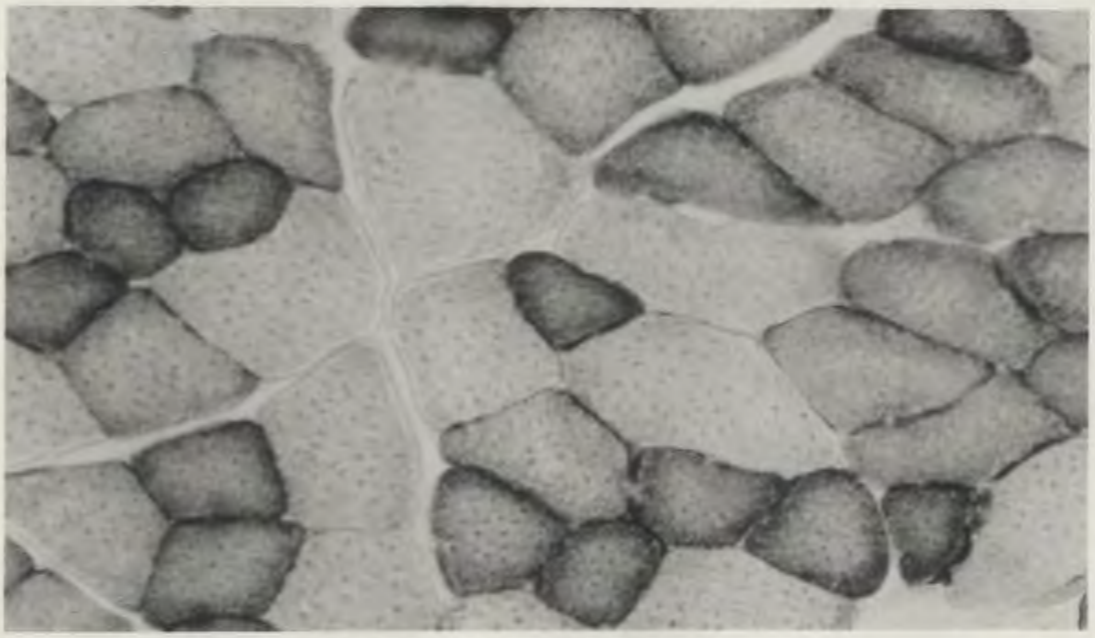


FIGURE XVII 50C, NADH-D, RED



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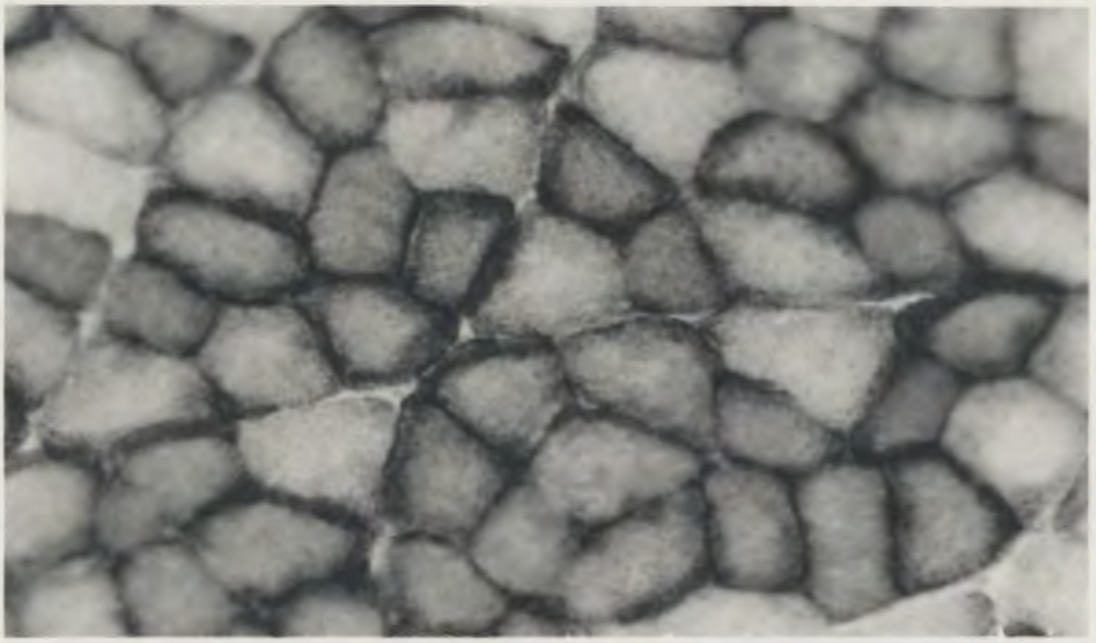

FIGURE XXI 50I, NADH-D, RED

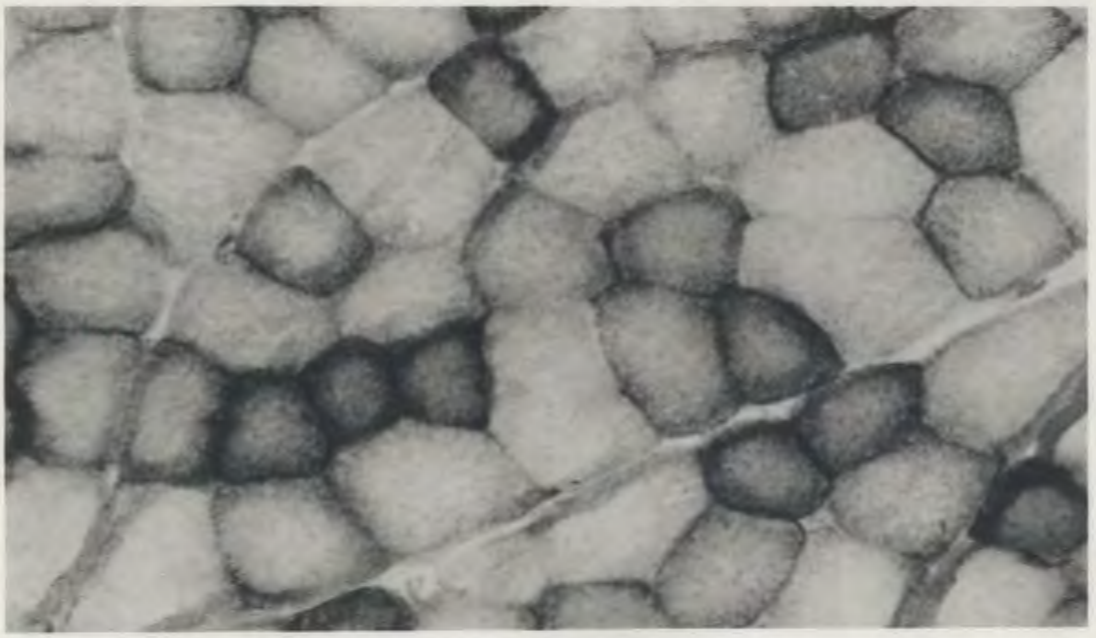

FIGURE XXII 50I, NADH-D, WHITE

APPENDIX C

PRETRAINING DATA

TABLE IX

SWIM TIMES TO EXHAUSTION IN SECONDS FOR THE PURPOSE OF THE INITIAL GROUPING OF ANIMALS*

ANIMAL NUMBER	SWIM TIME TO EXHAUSTION	SWIM TIME TO EXHAUSTION	MEAN TIME	ANIMAL NUMBER	SWIM TIME TO EXHAUSTION	SWIM TIME TO EXHAUSTION	MEAN TIME
1	1170	1479	1324.5	16	785	1005	895
2	520	368	444	17	852	1038	945
3	1890	1895	1892.5	18	control	control	control
4	417	295	356	19	308	561	434.5
5	2387	1869	2128	20	control	control	control
6	720	701	710.5	21	1501	1222	1361.5
7	1565	1634	1599.5	22	1527	1563	1545
8	395	476	435.5	23	522	403	462.5
9	control	control	control	24	620	914	767
10	960	1220	1090	25	437	489	463
11	647	917	782	26	2187	1536	1861.5
12	2371	1937	2154	27	912	723	817.5
13	2353	1879	2116	28	1545	1022	1283.5
14	control	control	control	29	1650	1222	1436
15	2354	1770	2062	30	1270	1455	1362.5

* 4% of body weight attached to the tail of the animal

TABLE X

T-TEST SHOWING EQUALITY IN SWIM TIME TO EXHAUSTION OF THE 60% CONTINUOUS AND 60% INTERVAL GROUPS

	X_1	X_2
ΣX	7653.5	7704.5
ΣX^2	14860141	15027294
$(\Sigma X)^2$	58576062	59359320
$\bar{X}$	1913.38	1926.13

S^2 = 403590 T = 0.28*

SX_1-X_2 = 499.215 O.O.F. = 6

*Significant at α0.05 = 1.94

TABLE XI

T-TEST SHOWING EQUALITY IN SWIM TIME TO EXHAUSTION OF THE 50% CONTINUOUS AND 50% INTERVAL GROUP

	X_1	X_2
ΣX	4939.5	4760
ΣX^2	6265688.5	5800828.8
$(\Sigma X)^2$	24398660	22657600
$\bar{X}$	1234.88	1191

S^2 = 302452.4 T = 0.115*

SX_1-X_2 = 388.88 O.O.F. = 6

*Significant at α0.05 = 1.94

TABLE XII

SWIM TIMES TO EXHAUSTION IN SECONDS FOR THE PURPOSE OF DETERMINING THE LENGTH OF THE INTERVALS TO BE USED IN THE INTERVAL TRAINING PROGRAM*

ANIMAL	SWIM TIME TO EXHAUSTION	SWIM TIME TO EXHAUSTION	MEAN
5	245	248	246.5
7	200	208	204
10	206	210	208
13	238	235	236.5
17	209	211	210
21	220	214	217
26	224	216	220
30	229	234	231.5
MEAN	221.38	222	221.69

* 10% of body weight attached to the tail

PERFORMANCE DATA

A. ANIMAL WEIGHTS

TABLE XIII

WEIGHT IN GRAMS OF CONTROL GROUP ANIMALS

AS MEASURED EVERY 6TH TRAINING DAY

ANIMAL NUMBER	PRE TRAINING	INITIAL TRAINING	WEEK I	WEEK II	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII
9	262	272	312	355	372	389	409	425	446
14	268	289	331	364	391	408	424	446	462
18	269	289	340	389	412	425	435	448	466
20	264	277	317	364	388	402	419	443	458
MEAN	265.75	281.75	325	368	390.75	406.	421.75	440.5	458

TABLE XIV

WEIGHT IN GRAMS OF 60% CONTINUOUS GROUP ANIMALS

AS DETERMINED EVERY 6TH TRAINING DAY

ANIMAL NUMBER	PRE TRAINING	INITIAL TRAINING	WEEK I	WEEK II	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII
3	268	284	312	337	357	360	364	381	398
12	271	283	315	333	349	357	367	384	392
15	275	290	302	332	364	371	378	389	401
22	260	291	305	328	349	362	366	382	389
MEAN	268.5	287	308.5	332.5	354.75	362.5	368.75	384	395

TABLE XV

WEIGHT IN GRAMS OF 60% INTERVAL GROUP ANIMALS

AS DETERMINED EVERY 6TH TRAINING DAY

ANIMAL NUMBER	PRE TRAINING	INITIAL TRAINING	WEEK I	WEEK II	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII
5	260	274	305	330	357	366	376	401	414
7	271	284	308	326	339	357	362	382	395
13	270	285	324	341	354	364	381	395	407
26	268	279	301	319	331	352	368	390	401
MEAN	267.25	280.5	309.5	329	345.25	359.75	371.75	392	404.25

APPENDIX D

TABLE XVI.

WEIGHT IN GRAMS OF 50% CONTINUOUS GROUP ANIMALS

AS DETERMINED EVERY 6TH TRAINING DAY

ANIMAL NUMBER	PRE TRAINING	INITIAL TRAINING	WEEK I	WEEK II	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII
1	277	289	316	348	370	382	395	412	425
16	271	290	327	343	329	351	364	382	398
28	260	282	303	332	346	361	381	396	409
29	255	266	294	318	334	356	372	391	418
MEAN	265.75	281.75	309	335.25	344.75	362.5	378	395.25	412.5

TABLE XVII

WEIGHT IN GRAMS OF 50% INTERVAL GROUP ANIMALS

AS DETERMINED EVERY 6TH TRAINING DAY

ANIMAL NUMBER	PRE TRAINING	INITIAL TRAINING	WEEK I	WEEK II	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII
10	259	272	298	310	319	334	339	359	368
17	270	289	296	319	339	356	372	394	409
21	265	283	326	343	358	365	371	395	403
30	269	280	293	314	335	352	373	386	400
MEAN	265.75	281	303.25	321.5	337.75	351.75	363.75	383.5	395

B. SWIM TIMES OF EXERCISE GROUPS

TABLE XVIII

SWIM TIME TO EXHAUSTION OF THE 60% CONTINUOUS GROUP

AS DETERMINED EVERY 6TH TRAINING DAY

ANIMAL NUMBER	MEAN INITIAL	WEEK I	WEEK II	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII FINAL
3	1893.5	2234	2760	2918	3015	2760	2321	1802
12	2154	2549	2670	2805	2849	2529	2215	1795
15	2062	2173	2541	3059	3314	3491	3558	3461
22	1545	2132	2410	2614	2825	3010	3095	2918
MEAN	1913.63				3000.75			2494

TABLE XIX

T-TEST CORRELATED SAMPLE COMPARISON OF

INITIAL AND FINAL CONTINUOUS SWIM TIMES TO EXHAUSTION

OF THE 60% CONTINUOUS GROUP

ΣD	- 2322
ΣD^2	3863492
T	1.27*

*Significant at α 0.05 = 2.353

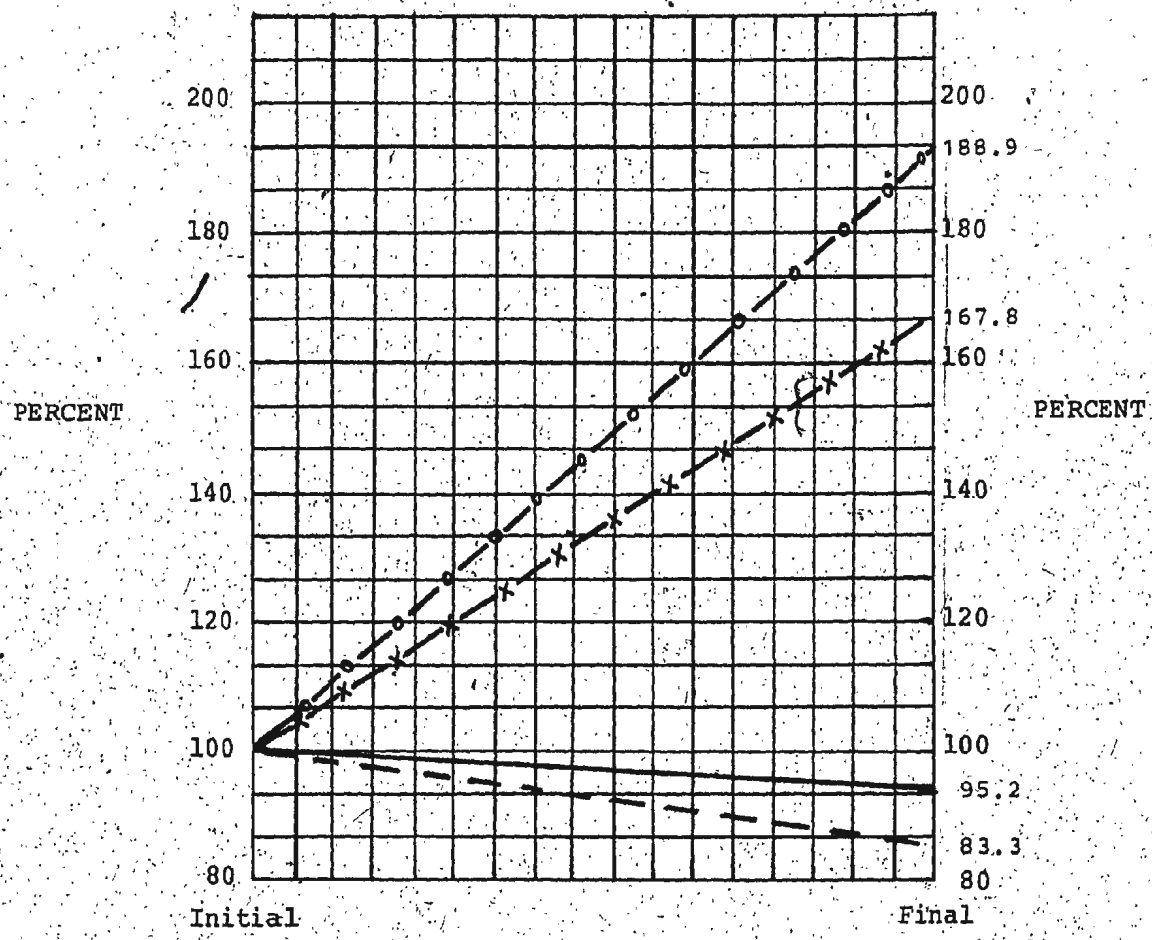

FIGURE XXIV Percent comparison of the initial and final continuous swim times to exhaustion of the 60% continuous group with the initial swim time represented as 100%

KEY

———— Animal 3

— — — — Animal 12

—X—X—X— Animal 15

—o—o—o— Animal 22

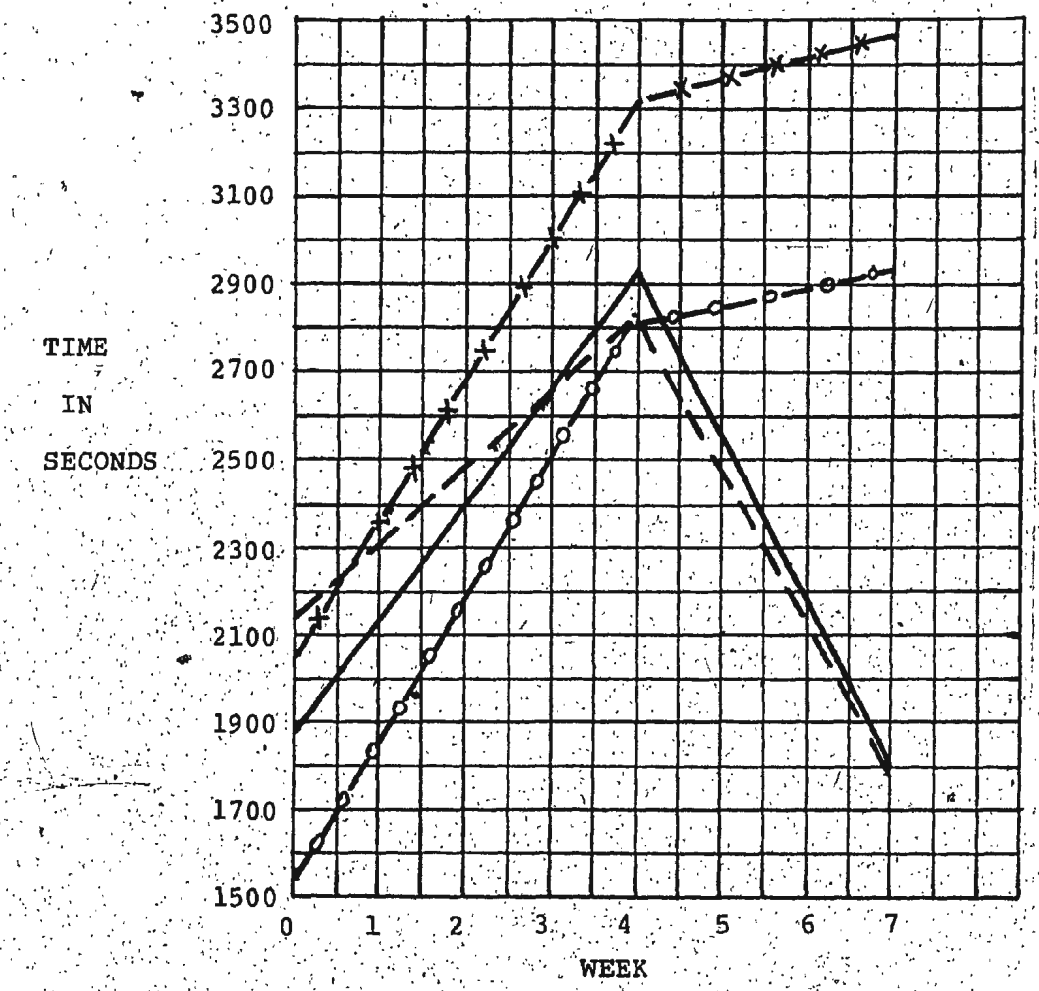

FIGURE XXIII Continuous swim times to exhaustion of the 60% continuous group as determined at the beginning, middle and end of the training program

KEY

——————— Animal 3
— — — — Animal 12
—x—x—x Animal 15
—o—o—o Animal 22

TABLE XX

SWIM TIME TO EXHAUSTION OF THE 60% INTERVAL GROUP

AS DETERMINED EVERY 6TH TRAINING DAY

ANIMAL NUMBER	MEAN INITIAL*	WEEK I	WEEK II	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII FINAL
5	2128	484	650	715	2408	1087	949	2314
7	1600	477	604	741	1841	980	870	1722
13	2116	490	636	745	2614	1159	1121	2701
26	1862.5	527	678	768	2208	1051	972	1916
MEAN	1926.63				2267.75			2163.25

* Continuous swim time to exhaustion with 4% of the body weight attached to the tail.

TABLE XXI

T-TEST CORRELATED SAMPLE COMPARISON OF

INITIAL AND FINAL CONTINUOUS SWIM TIMES TO EXHAUSTION

OF THE 60% INTERVAL GROUP

ΣD	- 947
ΣD^2	394621
T	1.99*

*Significant at α 0.05 = 2.353

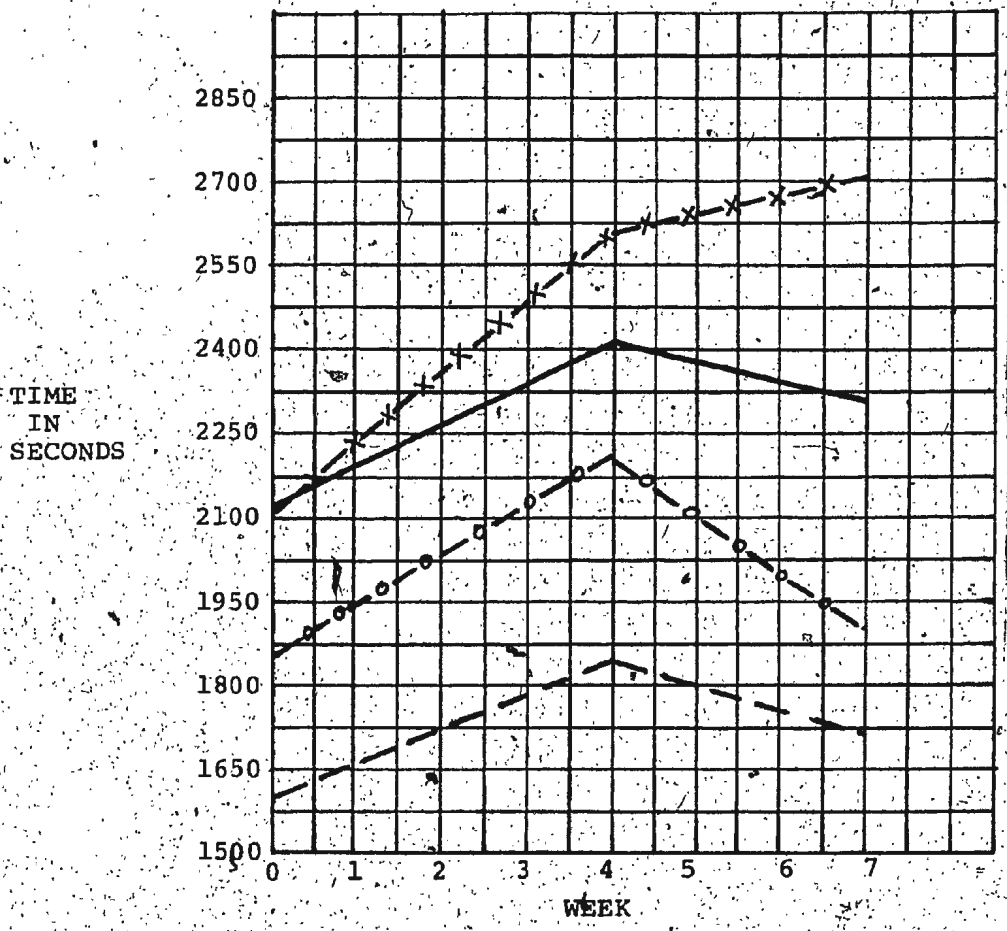

FIGURE XXV Continuous swim times to exhaustion of the 60% interval group as determined at the beginning, middle and end of the training program

KEY

———	Animal 5
— — — —	Animal 7
—X—X—X—	Animal 13
—o—o—o—	Animal 26

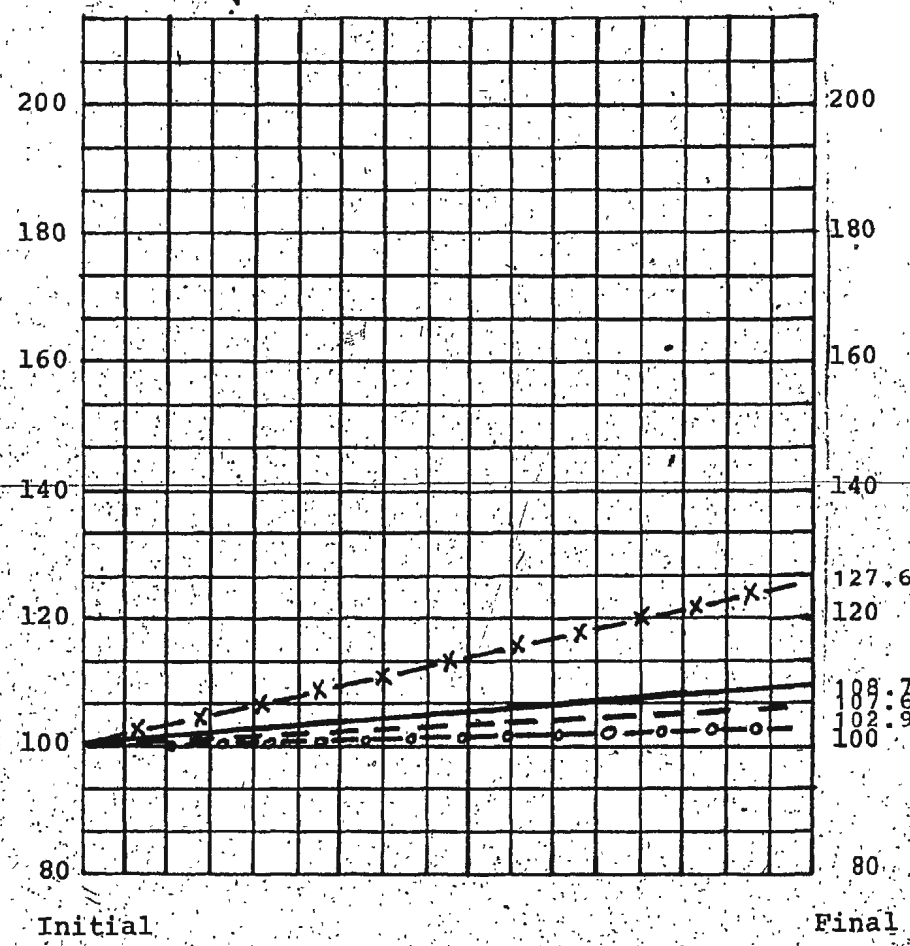

FIGURE XXVI Percent comparison of the initial and final continuous swim times to exhaustion of the 60% interval group with the initial swim time represented as 100%

KEY

————	Animal 5
— — — —	Animal 7
—X—X—X—	Animal 13
—o—o—o—	Animal 26

TABLE XXII

SWIM TIME TO EXHAUSTION OF THE 50% CONTINUOUS GROUP

AS DETERMINED EVERY 6TH TRAINING DAY

ANIMAL NUMBER	MEAN INITIAL	WEEK I	WEEK II	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII FINAL
1	1324.5	1712	1902	2028	2129	2217	2447	2537
16	895	1025	1214	1312	1342	1214	1095	902
28	1283.5	1587	1628	1809	1828	1622	1314	1112
29	1436	1689	1795	1825	2014	2159	3218	2448
MEAN	1234.75				1828.25			1749.75

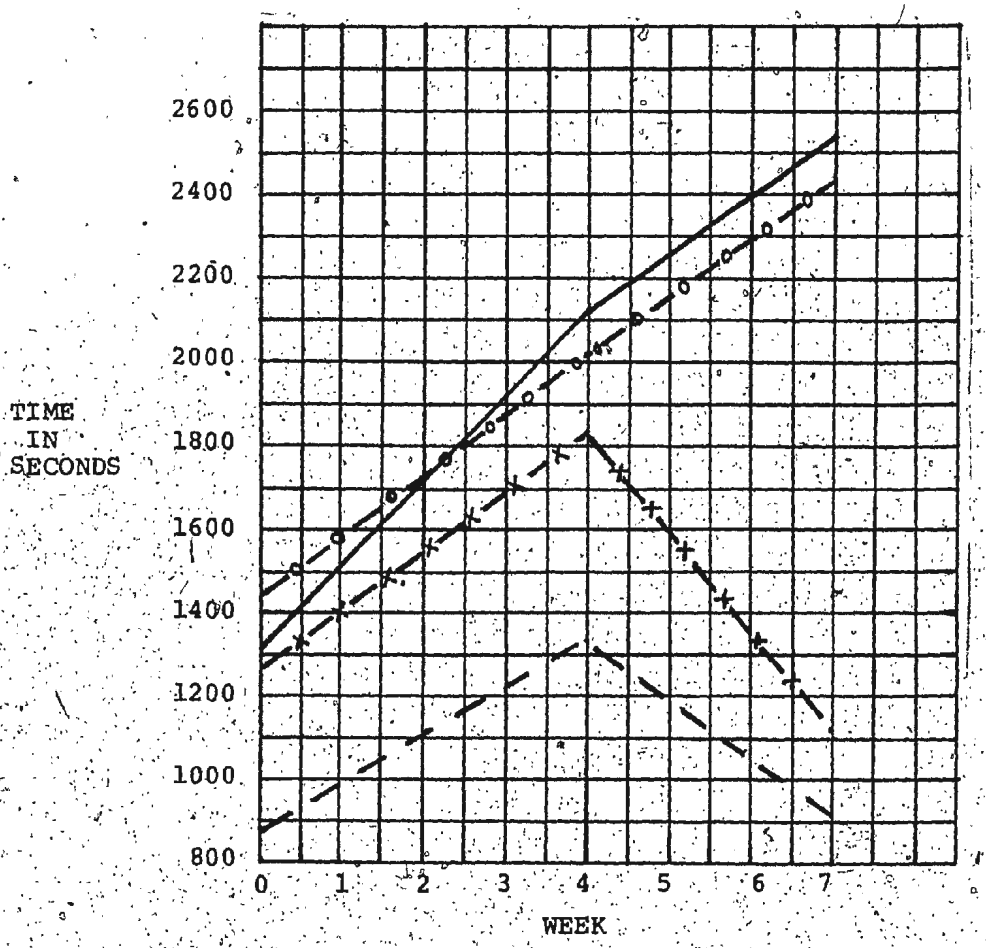

FIGURE XXVII Continuous swim times to exhaustion of the 50% continuous group as determined at the beginning, middle and end of the training program

KEY

———— Animal 1
— — — Animal 16
—X—X— Animal 28
—o—o— Animal 29

TABLE XXIII

T-TEST CORRELATED SAMPLE COMPARISON OF

INITIAL AND FINAL CONTINUOUS SWIM TIMES TO EXHAUSTION

OF THE 50% CONTINUOUS GROUP

ΣD	- 2060
ΣD^2	2522378
T	1.47*

* Significant at α 0.05 = 2.353

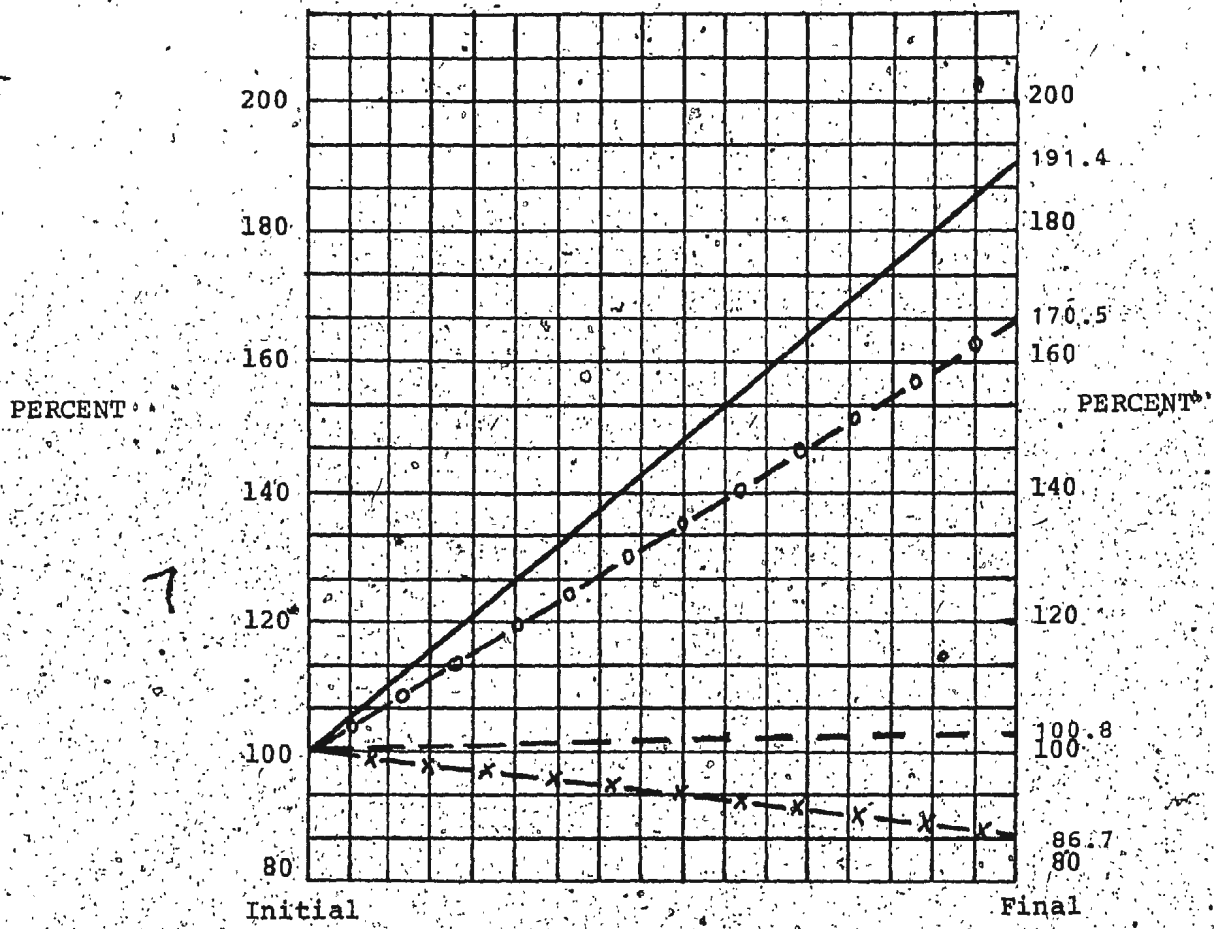

FIGURE XXVIII Percent comparison of the initial and final continuous swim times to exhaustion of 50% continuous group with the initial swim time represented as 100%

KEY

———— Animal 1

— — — — Animal 16

—X—X—X— Animal 28

—o—o—o— Animal 29

TABLE XXIV

SWIM TIME TO EXHAUSTION OF THE 50% INTERVAL GROUP

AS DETERMINED EVERY 6TH TRAINING DAY

ANIMAL NUMBER	MEAN INITIAL*	WEEK I	WEEK II	WEEK III	WEEK IV*	WEEK V	WEEK VI	WEEK VII* FINAL
10	1090	569	714	767	1720	1089	920	1422
17	945	538	703	792	1604	1139	1150	1703
21	1361.5	545	695	762	1656	1062	970	1508
30	1362.5	474	691	741	1748	1128	1178	1923
MEAN	1189.75				1682			1639

* Continuous swim time to exhaustion with 4% of the body weight attached to the tail

TABLE XXV

T-TEST CORRELATED SAMPLE COMPARISON OF

INITIAL AND FINAL CONTINUOUS SWIM TIMES TO EXHAUSTION

OF THE 50% INTERVAL GROUP

ΣD	- 1797
ΣD^2	1019997
T	3.37*

* Significant at α 0.05 = 2.353

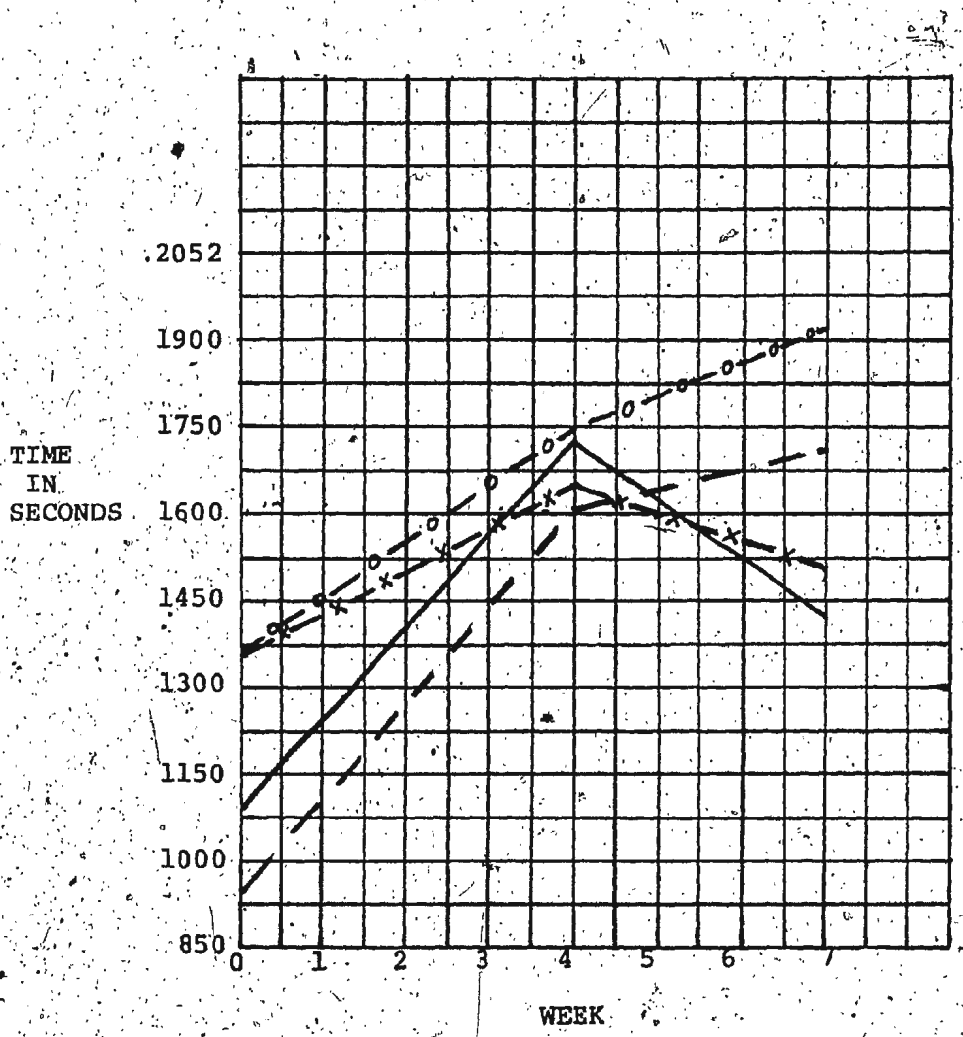

FIGURE XXIX Continuous swim time to exhaustion of the 50% interval group as determined at the beginning, middle and end of the training program

KEY

——————— Animal 10

— — — Animal 17

—X—X— Animal 21

—o—o— Animal 30

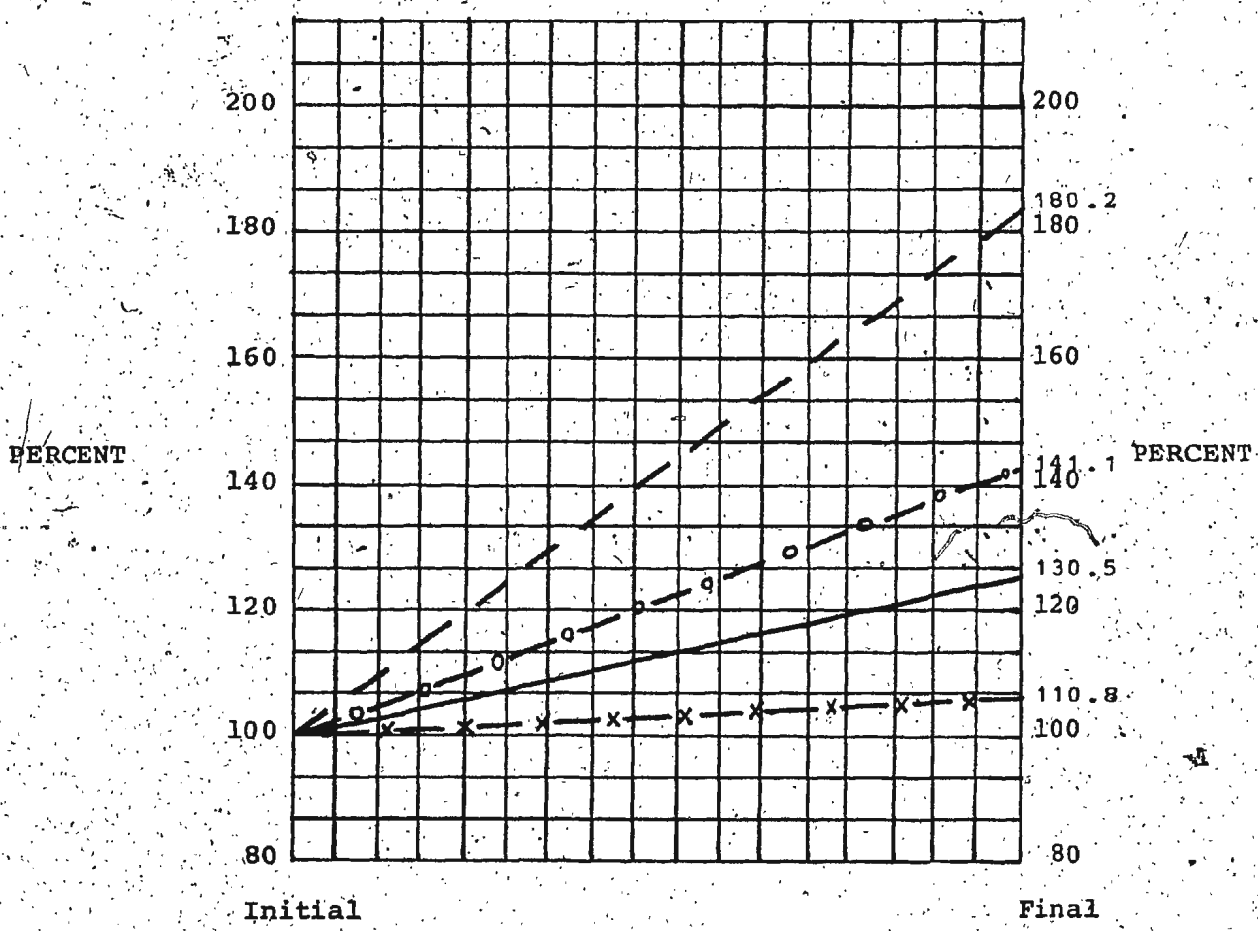

FIGURE XXX Percent comparison of the initial and final continuous swim times to exhaustion of the 50% interval group with the initial swim time represented as 100%

KEY

————	Animal	10
— — — —	Animal	17
—X—X—X—	Animal	21
—o—o—o—	Animal	30

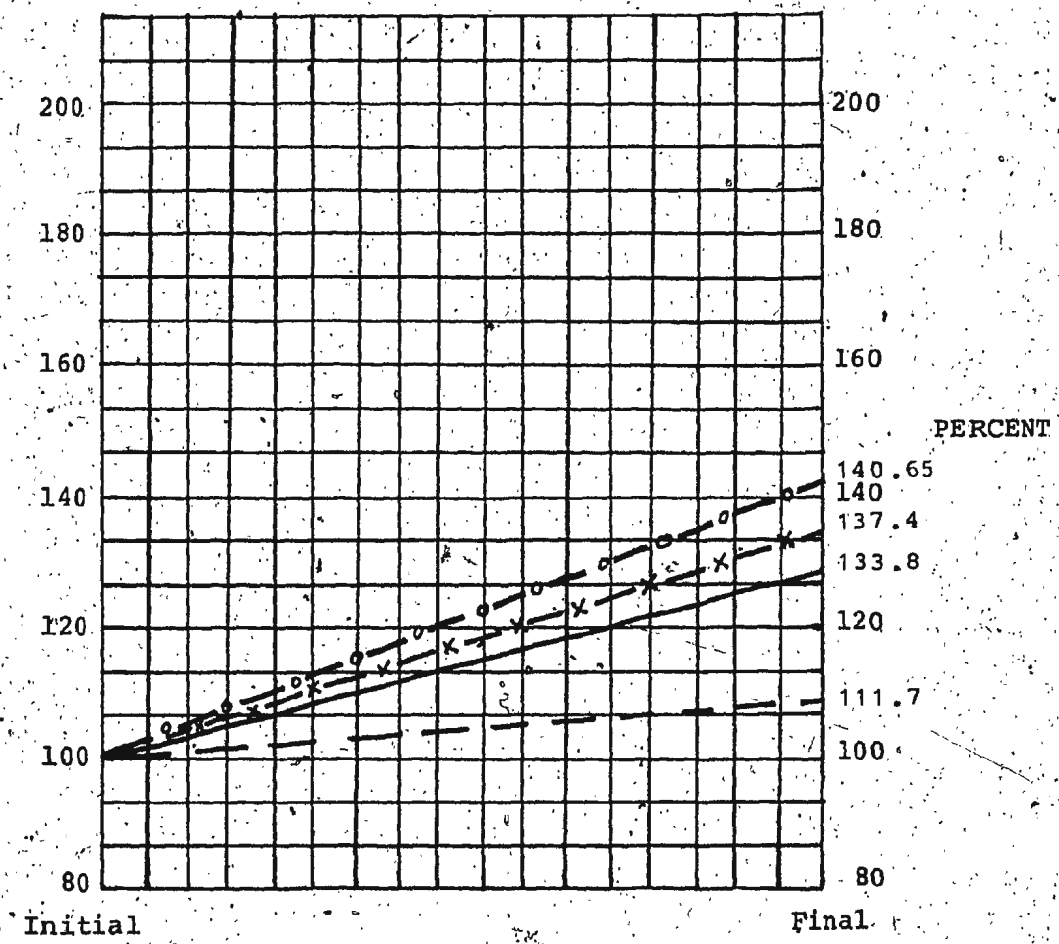

FIGURE XXXI Percent comparison of the initial and final mean continuous swim times to exhaustion of the four exercise groups with the initial swim time represented as 100%

KEY

———— 50% Continuous Group

— — — 60% Interval Group

—X—X— 50% Continuous Group

—0—0— 50% Interval Group

APPENDIX E

FIBER ANALYSIS DATA

In this appendix the data will be split into two major sections:

I Raw Fiber Analysis Data

II Statistical Analysis of Raw Data

I. RAW FIBER ANALYSIS DATA

A. RED REGION STAINED FOR SDH ACTIVITY

TABLE XXVI: FIBER ANALYSIS DATA OF THE SDH STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE CONTROL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
9(1)	22	41.5	8	15.09	23	43.39	53
9(2)	27	44.26	8	13.11	26	42.62	61
14(1)	17	40.48	6	14.29	19	45.24	42
14(2)	16	35.56	5	11.11	24	53.33	45
14(3)	27	48.21	5	8.93	24	42.86	56
18(1)	17	40.48	7	16.67	18	42.86	42
20(1)	16	37.21	8	18.60	19	44.19	43
20(2)	26	44.07	11	18.64	22	37.29	59
MEAN		41.47		14.56		43.97	

TABLE XXVII: FIBER ANALYSIS DATA OF THE SDH STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE 60% CONTINUOUS GROUP.

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
3(1)	28	59.57	7	14.89	12	25.53	47
3(2)	29	52.73	8	14.55	18	32.72	55
12(1)	30	54.55	6	10.91	19	24.55	55
15(1)	27	51.92	6	11.54	19	36.54	52
15(2)	29	59.18	4	8.16	16	32.65	49
22(1)	24	57.14	6	14.29	12	28.57	42
22(2)	26	56.52	6	13.04	14	30.43	46
22(3)	27	55.10	7	14.29	15	30.61	49
MEAN		55.84		12.71		31.45	

TABLE XXVIII: FIBER ANALYSIS DATA OF THE SDH STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE 60% INTERVAL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
5	17	56.67	5	16.67	8	26.67	30
7	19	59.38	4	12.50	9	28.13	32
13(1)	31	59.62	8	15.38	13	25.00	52
13(2)	29	60.42	6	12.50	13	27.08	48
13(3)	28	57.14	6	12.24	15	30.61	49
26(1)	28	60.87	7	15.22	11	23.91	46
26(2)	27	56.25	6	12.50	15	31.25	48
26(3)	26	59.09	6	13.64	12	27.27	44
Mean		58.68		13.83		27.49	

TABLE XXIX: FIBER ANALYSIS DATA OF THE SDH STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE 50% CONTINUOUS GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
1(1)	25	55.55	5	11.11	15	33.33	45
1(2)	24	54.54	6	13.63	14	31.82	44
16(1)	25	54.35	6	13.04	15	32.62	46
16(2)	23	56.09	5	12.20	13	31.7	41
28	20	52.63	6	15.79	12	31.58	38
29(1)	23	52.27	7	15.91	14	31.82	44
29(2)	28	53.85	8	15.38	16	30.77	52
29(3)	24	54.54	6	13.64	14	31.82	44
MEAN		54.23		13.84		31.93	

TABLE XXX: FIBER ANALYSIS DATA OF THE SDH STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE 50% INTERVAL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
10(1)	38	59.37	10	15.62	16	25.00	64
10(2)	42	61.76	9	13.23	17	25.00	68
17(1)	40	62.50	10	15.62	14	21.89	64
17(2)	45	62.50	11	15.29	16	22.22	72
17(3)	41	61.19	9	13.43	17	25.39	67
21(1)	40	62.50	8	12.50	16	25.00	64
21(2)	45	64.28	11	15.91	14	20.00	70
30(1)	27	57.44	6	12.76	14	29.78	47
MEAN		61.44		14.29		24.28	

B. RED REGION STAINED FOR NADH-D ACTIVITY

TABLE XXXI. FIBER ANALYSIS DATA OF THE NADH-D STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE CONTROL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
9(1)	17	45.94	3	8.10	17	45.94	37
9(2)	15	44.11	5	14.70	14	41.17	34
14	23	41.81	8	14.54	24	43.63	55
18(1)	16	34.04	6	12.76	25	53.19	47
18(2)	14	37.87	6	16.21	17	45.94	37
20(1)	18	41.86	8	18.60	17	39.53	43
20(2)	13	35.13	5	13.51	19	51.35	37
20(3)	17	44.73	5	15.80	15	39.47	34
MEAN		40.68		14.28		45.03	

TABLE XXXII: FIBER ANLYSIS DATA OF THE NADH-D STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE 60% CONTINUOUS GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
3(1)	23	53.49	6	13.95	14	32.56	43
3(2)	17	53.13	4	12.50	11	34.38	32
3(3)	19	52.78	6	16.67	11	30.55	36
12	22	55.00	6	15.00	12	30.00	40
15(1)	21	55.26	5	13.16	12	31.59	38
15(2)	23	53.49	7	16.28	13	30.23	43
22(1)	25	59.52	7	16.67	10	23.80	42
22(2)	24	57.14	6	14.29	12	28.57	42
MEAN		54.98		14.82		30.21	

TABLE XXXIII: FIBER ANALYSIS DATA OF THE NADH-D STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE 60% INTERVAL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
5(1)	25	59.52	6	14.28	11	26.19	42
5(2)	36	60.00	8	13.33	16	26.67	60
7	27	51.92	8	15.38	17	32.69	52
13(1)	23	58.97	6	15.38	10	25.64	39
13(2)	30	53.57	7	12.50	19	33.93	56
13(3)	27	60.00	6	13.33	12	26.67	45
26(1)	28	59.57	7	14.89	12	25.53	47
26(2)	26	61.90	6	14.29	10	23.81	42
MEAN		58.18		14.17		27.64	

TABLE XXXIV: FIBER ANALYSIS DATA OF THE NADH-D STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE 50% CONTINUOUS GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSES
1(1)	25	55.55	6	13.33	14	31.11	45
1(2)	26	55.32	6	12.77	15	31.91	47
16	21	58.33	5	13.89	10	27.78	36
28(1)	29	59.18	7	14.29	13	26.53	49
28(2)	25	51.02	7	14.29	17	34.69	49
29(1)	29	58.00	7	14.00	14	28.00	50
29(2)	25	58.13	5	11.63	13	30.23	43
29(3)	20	48.78	6	14.63	15	36.59	41
MEAN		55.54		13.60		30.86	

TABLE XXXV: FIBER ANALYSIS DATA OF THE NADH-D STAINED RED REGION OF THE MEDIAL GASTROCNEMIUS OF THE 50% INTERVAL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
10(1)	30	55.55	7	12.72	17	31.48	54
17(1)	24	60.00	6	15.00	10	25.00	40
17(2)	36	66.66	6	11.11	12	22.22	54
17(3)	31	65.96	6	12.77	10	21.27	47
21(1)	29	63.04	5	10.87	12	26.08	46
21(2)	21	56.75	7	18.91	9	24.32	37
30(1)	30	60.00	6	12.00	14	28.00	50
30(2)	30	61.22	7	14.29	12	24.49	49
MEAN		61.15		13.46		25.36	

C. WHITE REGION STAINED FOR SDH ACTIVITY

TABLE XXXVI: FIBER ANALYSIS DATA OF THE SDH STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE CONTROL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
9(1)	7	18.92	2	5.41	28	75.68	37
9(2)	9	20.93	3	6.98	31	72.09	43
14(1)	7	20.59	2	5.89	25	73.53	34
14(2)	10	25.64	3	7.69	26	66.67	39
14(3)	8	19.05	3	7.14	31	73.81	42
18	10	27.03	5	13.51	22	59.55	37
20(1)	9	25.00	4	11.11	23	63.89	36
20(2)	9	30.00	3	10.00	18	60.00	30
MEAN		23.40		8.47		68.15	

TABLE XXXVII: FIBER ANALYSIS DATA OF THE SDH STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE 60% CONTINUOUS GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
3(1)	18	38.30	3	6.38	26	55.32	47
3(2)	21	53.85	4	10.26	14	35.90	39
12	25	43.86	5	8.77	27	47.37	57
15(1)	18	40.00	5	11.11	22	48.89	45
15(2)	15	42.86	3	8.57	17	48.57	35
22(1)	22	44.90	4	8.16	23	46.94	49
22(2)	18	42.85	5	11.90	19	45.32	42
22(3)	18	39.13	6	13.04	22	47.82	46
MEAN		43.22		9.77		47.01	

TABLE XXXVIII: FIBER ANALYSIS DATA OF THE SDH STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE 60% INTERVAL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
5	13	48.15	3	11.11	11	40.74	27
7	22	51.16	4	9.30	17	39.53	43
13(1)	[redacted]	51.35	3	8.11	15	40.54	37
13(2)	17	50.00	3	8.82	14	41.18	34
13(3)	17	48.57	2	5.71	16	45.71	35
26(1)	21	52.50	4	10.00	15	37.50	40
26(2)	20	52.63	4	10.53	14	36.84	38
26(3)	22	53.66	3	7.32	16	39.02	41
MEAN		51.00		8.86		40.13	

TABLE XXXIX: FIBER ANALYSIS DATA OF THE SDH STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE 50% CONTINUOUS GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
1(1)	21	52.50	3	7.50	16	40.00	40
1(2)	21	48.83	3	6.98	19	44.19	43
16(1)	20	51.28	4	9.30	15	38.46	39
16(2)	23	53.49	4	10.26	16	37.21	43
28	18	41.86	5	11.63	20	46.51	43
29(1)	23	52.27	2	4.56	19	43.18	44
29(2)	19	42.22	5	11.11	21	46.67	45
29(3)	16	44.44	4	11.11	16	44.44	36
Mean		48.36		9.06		42.58	

TABLE XL: FIBER ANALYSIS DATA OF THE SDH STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE 50% INTERVAL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
10(1)	26	50.98	5	9.80	20	39.21	51
10(2)	23	53.48	4	9.30	16	37.20	43
17(1)	20	54.05	4	10.81	13	35.13	37
17(2)	26	50.00	5	9.61	21	40.38	52
17(3)	24	52.19	4	8.69	18	39.13	46
21(1)	22	52.38	4	9.52	16	38.09	42
21(2)	19	45.23	3	7.14	20	47.62	42
21(3)	21	46.66	3	6.66	21	46.66	45
MEAN		50.62		8.94		40.43	

D. WHITE REGION STAINED FOR NADH-D ACTIVITY

TABLE XLI: FIBER ANALYSIS DATA OF THE NADH-D STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE CONTROL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
9(1)	10	28.57	3	8.57	22	62.85	35
9(2)	11	27.50	2	5.00	27	67.50	40
14(1)	12	35.29	3	8.82	19	55.88	34
18(1)	11	27.50	4	10.00	25	62.50	40
18(2)	9	21.95	5	12.19	27	65.85	41
20(1)	12	34.28	3	8.57	20	57.14	35
20(2)	15	34.09	3	6.82	26	59.09	44
20(3)	6	18.16	2	6.06	25	75.75	33
MEAN		28.42		8.25		63.32	

TABLE XLII: FIBER ANALYSIS DATA OF THE NADH-D STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE 60% CONTINUOUS GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
3(1)	16	51.61	3	9.68	12	38.71	31
3(2)	18	45.00	4	10.00	18	45.00	40
3(3)	19	50.00	3	7.89	16	42.11	38
12	19	47.50	4	10.00	17	42.50	40
15(1)	17	43.59	3	7.69	19	48.72	39
15(2)	20	45.45	6	13.63	18	40.91	44
22(1)	15	50.00	3	10.00	12	40.00	30
22(2)	16	48.48	3	9.09	14	42.42	33
MEAN		47.70		9.75		42.55	

TABLE XLIII: FIBER ANALYSIS DATA OF THE NADH-D STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE 60% INTERVAL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
5(1)	27	54.00	4	8.00	19	38.00	50
5(2)	24	54.55	3	6.82	17	38.64	44
7	25	52.08	4	8.33	19	39.58	48
13(1)	22	51.16	5	11.62	16	37.21	43
13(2)	20	51.28	3	7.69	16	41.02	39
13(3)	24	53.33	4	8.89	17	37.78	45
26(1)	24	47.05	5	9.80	22	43.13	51
26(2)	31	54.38	5	8.77	21	36.84	57
MEAN		52.23		8.74		39.03	

TABLE XLIV: FIBER ANALYSIS DATA OF THE NADH-D STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE 50% CONTINUOUS GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
1(1)	15	40.54	3	8.11	19	51.35	37
1(1)	20	48.78	2	4.88	19	46.34	41
16	24	52.17	5	10.87	17	36.96	45
28(1)	16	43.24	3	8.11	18	48.65	37
28(2)	20	47.62	4	9.52	18	42.86	42
29(1)	17	51.51	3	9.09	13	39.39	33
29(2)	14	46.66	3	10.00	13	43.33	30
29(3)	14	48.28	2	10.34	12	41.38	29
MEAN		47.35		8.86		43.78	

TABLE XLV: FIBER ANALYSIS DATA OF THE NADH-D STAINED WHITE REGION OF THE MEDIAL GASTROCNEMIUS OF THE 50% INTERVAL GROUP

ANIMAL NUMBER	TOTAL RED FIBERS	% RED FIBERS	TOTAL INTER. FIBERS	% INTER. FIBERS	TOTAL WHITE FIBERS	% WHITE FIBERS	TOTAL FIBERS ANALYSED
10(1)	23	46.00	6	12.00	21	42.00	50
17(1)	24	44.44	5	9.25	25	46.30	54
17(2)	18	51.42	4	11.42	13	37.14	35
21(1)	24	43.64	3	5.45	28	50.91	55
21(2)	25	47.17	5	9.43	23	43.40	53
21(3)	29	51.79	4	7.14	23	41.07	56
30(1)	26	50.98	3	5.88	22	43.14	51
30(2)	29	50.00	6	10.34	23	39.66	58
MEAN		48.18		8.86		42.95	

II STATISTICAL ANALYSIS OF RAW DATA

In the Scheffé analysis the following simplified notation has been used:

$\hat{\psi}$ is represented by AA

$\hat{\sigma}^2\psi$ is represented by BB

$\frac{\hat{\psi}}{\sigma\psi}$ is represented by CC

The value of $\sqrt{(j-1)_{1-\alpha}F_{j-1,N-j}}$ is called the critical value.

II STATISTICAL ANALYSIS OF RAW DATA

A. STATISTICAL ANALYSIS OF RED FIBERS

TABLE XLVI: F-TEST ANALYSIS OF RED FIBERS OF THE RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between groups	4	234.98	6.71	73.52*
Within groups	35	1896.90	475.2	
Total	39	2131.88		

*Significant at α .05 = 2.65

TABLE XLVII: TUKEY ANALYSIS OF RED FIBERS OF THE RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

	CON	60C	60I	50C	50I
Con		15.90	19.04	14.12	22.09
60C	15.90		2.72	1.78	6.20
60I	19.03	2.72		4.92	3.05
50C	14.12	1.78	4.92		7.98
50I	22.09	6.20	3.05	7.98	

SCHEFFE ANALYSIS OF COMBINED CONTINUOUS VERSUS COMBINED INTERVAL MEAN PERCENTAGES OF RED MUSCLE FIBERS STAINED FOR SDH ACTIVITY IN THE RED REGION OF THE MEDIAL GASTROCNEMIUS

AA = -5.025, BB = 0.84, CC = 5.46*, Critical value = 2.91

* Significant if greater than 2.91

TABLE XLVIII: F-TEST ANALYSIS OF RED FIBERS OF THE RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	1925.61	481.4	35.45*
Within Groups	35	475.24	13.6	
Total	39	2400.85		

*Significant at α .05 = 2.65

TABLE XLIX: TUKEY ANALYSIS OF RED FIBERS OF THE RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

	CON	60C	60I	50C	50I
CON		10.98	13.43	11.41	15.71
60C	10.98		2.46	.43	4.74
60I	13.43	2.46		2.03	2.28
50C	11.41	.43	2.03		4.31
50I	15.71	4.74	2.28	4.31	

*Significant at α .05 = 4.07

SCHEFFE ANALYSIS OF COMBINED CONTINUOUS VERSUS COMBINED INTERVAL GROUP MEAN PERCENTAGES OF RED FIBERS STAINED FOR NADH-D ACTIVITY IN THE RED REGION OF THE MEDIAL GASTROCNEMIUS

AA = -4.405, BB = 1.70, CC = 3.38* Critical value = 2.91

*Significant if greater than 2.91

TABLE L: F-TEST ANALYSIS OF RED FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	4277.78	1069.45	69.87*
Within Groups	35	535.75	15.31	
Total	39	4813.53		

*Significant at α .05 = 2.65

TABLE LI: TUKEY ANALYSIS OF RED FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

	CON	60C	60I	50C	50I
CON		14.33	19.95	18.04	19.68
60C	14.33		5.62	3.72	5.35
60I	19.95	5.62		1.91	.28
50C	18.04	3.72	1.91		1.63
50I	19.68	5.35	.28	1.63	

*Significant at α .05 = 4.07

SHEFFE ANALYSIS OF COMBINED CONTINUOUS VERSUS COMBINED INTERVAL GROUP MEAN PERCENTAGES OF RED FIBERS STAINED FOR SDH ACTIVITY IN THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS

AA = -5.02, BB = 1.91, CC = 3.62*, Critical value = 2.91

*Significant if greater than 2.91

TABLE LII: F-TEST ANALYSIS OF RED FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

Source	DEGREES OF FREEDOM	SS	MS	F
Between groups	4	2799.43	699.86	23.44*
Within groups	35	1044.85	29.85	
Total	39	3844.28		

*Significant at α .05 = 2.65

TABLE LIII: TUKEY ANALYSIS OF RED FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

	CON	60C	60I	50C	50I
CON		17.37	21.46	17.06	17.81
60C	17.37		4.08	.32	.43
60I	21.46	4.08		4.40	3.65
50C	17.06	.32	4.40		.74
50I	17.81	.43	3.65	.74	

*Significant at α .05 = 4.07

SCHEFFE ANALYSIS OF COMBINED CONTINUOUS VERSUS COMBINED INTERVAL GROUP MEAN PERCENTAGES OF RED FIBERS STAINED FOR NADH-D ACTIVITY IN THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS

AA = -2.69, BB = 3.73, CC = 1.39*, Critical value 2.91

*Significant if greater than 2.91

B. STATISTICAL ANALYSIS OF WHITE FIBERS

TABLE LIV: F-TEST ANALYSIS OF WHITE FIBERS OF THE RED RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	1787.47	446.87	47.15*
Within Groups	35	331.68	9.48	
Total	39	2119.15		

*Significant at α .05 = 2.65

TABLE LV: TUKEY ANALYSIS OF WHITE FIBERS OF THE RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

	CON	60C	60I	50C	50I
CON		11.50	15.14	11.06	18.09
60C	11.50		3.64	.44	6.59
60I	15.14	3.64		4.08	2.95
50C	11.06	.44	4.08		7.03
50I	18.09	6.59	2.95	7.03	

*Significant at α .05 = 4.07

SCHEFFE ANALYSIS OF COMBINED CONTINUOUS VERSUS COMBINED INTERVAL GROUP MEAN PERCENTAGES OF WHITE MUSCLE FIBERS STAINED FOR SDH ACTIVITY IN THE RED REGION OF THE MEDIAL GASTROCNEMIUS

AA = 5.81, BB = 1.18, CC = 5.38*, Critical value = 5.38

*Significant if greater than 2.91

TABLE LVI: F-TEST ANALYSIS OF WHITE FIBERS OF THE RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	1897.50	474.37	32.81*
Within Groups	35	506.09	14.46	
Total	39	2403.59		

*Significant at α .05 = 2.65

TABLE LVII: TUKEY ANALYSIS OF WHITE FIBERS OF THE RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

	CON	60C	60I	50C	50I
CON		11.02	12.94	10.54	14.63
60C	11.02		1.90	.48	3.61
60I	12.94	1.90		2.40	1.70
50C	10.54	.48	2.40		4.09
50I	14.63	3.61	1.70	4.09	

*Significant at α .05 = 4.07

SCHEFFE ANALYSIS OF COMBINED CONTINUOUS VERSUS COMBINED INTERVAL GROUP MEAN PERCENTAGES OF WHITE MUSCLE FIBERS STAINED FOR NADH-D ACTIVITY IN THE RED REGION OF THE MEDIAL GASTROCNEMIUS

AA = 4.04, BB = 1.81, CC = 3.00*, Critical value = 2.91

*Significant if greater than 2.91

TABLE LVIII: F-TEST ANALYSIS OF WHITE FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	4441.00	1110.25	50.00*
Within Groups	35	777.17	22.21	
Total	39	5218.17		

*Significant at α .05 = 2.65

TABLE LVIX: TUKEY ANALYSIS OF WHITE FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

	CON	60C	60I	50C	50I
CON		12.69	16.82	15.35	16.64
60C	12.69		4.13	2.66	3.95
60I	16.82	4.13		1.47	.18
50C	15.35	2.66	1.47		1.29
50I	16.64	3.95	.18	1.29	

*Significant at α .05 = 4.07

SCHEFFE ANALYSIS OF COMBINED CONTINUOUS VERSUS COMBINED INTERVAL GROUP MEAN PERCENTAGES OF WHITE MUSCLE FIBERS STAINED FOR SDH ACTIVITY IN THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS

AA = 4.51, BB = 2.77, CC = 2.72*, Critical value = 2.91

*Significant if greater than 2.91

TABLE LX: F-TEST ANALYSIS OF WHITE FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	3001.49	750.37	39.53*
Within Groups	35	664.47	18.98	
Total	39	3665.96		

*Significant at α .05 = 2.65

TABLE LXI: TUKEY ANALYSIS OF WHITE FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

	CON	60C	60I	50C	50I
CON		13.48	15.77	12.69	13.22
60C	13.48		2.29	.80	.26
60I	15.77	2.29		3.08	2.55
50C	12.69	.80	3.08		.54
50I	13.22	.26	2.55	.54	

*Significant at α .05 = 4.07

SCHEFFE ANALYSIS OF COMBINED CONTINUOUS VERSUS COMBINED INTERVAL GROUP MEAN PERCENTAGES OF WHITE FIBERS STAINED FOR NADH-D ACTIVITY IN THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS

AA = 2.18, BB = 2.37, CC = 1.41*, Critical value = 2.91

*Significant if greater than 2.91

C. STATISTICAL ANALYSIS OF INTERMEDIATE FIBERS

TABLE LXII: F-TEST ANALYSIS OF INTERMEDIATE FIBERS OF THE RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	15.68	3.92	0.77*
Within Groups	35	178.12	5.09	
Total	39	193.80		

*Significant at α .05 = 2.65

TABLE LXIII: F-TEST ANALYSIS OF INTERMEDIATE FIBERS OF THE RED REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	9.60	2.40	0.39*
Within Groups	35	216.96	6.20	
Total	39	226.56		

*Significant at α .05 = 2.65

TABLE LXIV: F-TEST ANALYSIS OF INTERMEDIATE FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR SDH ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	7.26	1.81	0.38*
Within Groups	35	168.48	4.81	
Total	39	175.74		

*Significant at α .05 = 2.65

TABLE LXV: F-TEST ANALYSIS OF INTERMEDIATE FIBERS OF THE WHITE REGION OF THE MEDIAL GASTROCNEMIUS STAINED FOR NADH-D ACTIVITY

Source	Degrees of Freedom	SS	MS	F
Between Groups	4	9.31	2.33	0.57*
Within Groups	35	142.36	4.07	
Total	39	151.67		

*Significant at α .05 = 2.65

D. ANALYSIS OF OBJECTIVITY AND RELIABILITY

TABLE LXVI: FIBER ANALYSIS USED IN TESTING FOR OBJECTIVITY OF THE FIBER TYPING PROCEDURE USED IN ANALYSING SDH STAINED FIBERS

ANIMAL NUMBER	ANALYSIS 1 RED FIBERS	ANALYSIS 2 RED FIBERS	ANALYSIS 1 INTER. FIBERS	ANALYSIS 2 INTER. FIBERS	ANALYSIS 1 WHITE FIBERS	ANALYSIS 2 WHITE FIBERS
RED REGION						
14(1)	17	19	6	5	19	18
22(2)	26	27	6	5	14	14
13(3)	28	26	6	6	15	17
1(1)	25	24	5	5	15	16
30(1)	27	27	6	7	14	13
WHITE REGION						
20(1)	9	9	4	4	23	23
3(2)	21	21	4	3	14	15
26(3)	22	21	3	4	16	16
29(2)	19	19	5	5	21	21
10(1)	26	24	5	6	20	21
CORRELATION	.978		.730		.959	

TABLE LXVII: FIBER ANALYSIS USED IN TESTING FOR OBJECTIVITY OF FIBER TYPING PROCEDURE USED IN ANALYSING NADH-D STAINED FIBERS

ANIMAL NUMBER	ANALYSIS 1 RED FIBERS	ANALYSIS 2 RED FIBERS	ANALYSIS 1 INTER. FIBERS	ANALYSIS 2 INTER. FIBERS	ANALYSIS 1 WHITE FIBERS	ANALYSIS 2 WHITE FIBERS
RED REGION						
9(1)	17	18	3	3	17	16
15(1)	21	23	5	5	12	10
26(1)	28	28	7	7	12	12
16	21	20	5	6	10	10
17(2)	36	34	6	7	12	13
WHITE REGION						
18(2)	9	9	5	6	27	26
22(1)	15	15	3	3	12	12
7(1)	25	23	4	5	19	20
1(2)	20	20	2	3	19	18
21(2)	25	23	5	5	23	25
CORRELATION	.986		.942		.979	

TABLE LXVIII: FIBER ANALYSIS USED IN TESTING FOR RELIABILITY OF FIBER TYPING PROCEDURE USED IN ANALYSING SDH STAINED FIBERS

ANIMAL NUMBER	ANALYSIS 1 RED FIBERS	ANALYSIS 2 RED FIBERS	ANALYSIS 1 INTER. FIBERS	ANALYSIS 2 INTER. FIBERS	ANALYSIS 1 WHITE FIBERS	ANALYSIS 2 WHITE FIBERS
RED REGION						
14(2)	16	18	5	4	24	23
22(1)	24	23	6	6	12	13
13(2)	29	29	6	5	13	14
29(1)	23	22	7	7	14	15
30(1)	27	25	6	8	14	14
WHITE REGION						
14(1)	7	7	2	3	25	24
15(2)	15	14	3	3	17	18
7	22	21	4	4	17	18
1(2)	21	22	3	2	19	19
17(2)	26	25	5	6	21	21
CORRELATION	.985		.857		.994	

TABLE LXIX: FIBER ANALYSIS USED IN TESTING FOR RELIABILITY OF FIBER TYPING PROCEDURE USED IN ANALYSING NADH-D STAINED FIBERS

ANIMAL NUMBER	ANALYSIS 1 RED FIBERS	ANALYSIS 2 RED FIBERS	ANALYSIS 1 INTER. FIBERS	ANALYSIS 2 INTER. FIBERS	ANALYSIS 1 WHITE FIBERS	ANALYSIS 2 WHITE FIBERS
RED REGION						
18(1)	16	15	6	5	25	27
22(1)	25	25	7	6	10	11
5(1)	25	24	6	6	11	12
28(2)	25	26	7	6	17	17
21(1)	29	27	5	7	12	12
WHITE REGION						
20(3)	6	7	2	3	25	23
3(3)	19	18	3	3	16	17
26(1)	24	24	5	5	22	22
16(1)	24	23	6	6	17	17
30(2)	29	28	6	6	23	24
CORRELATION	.991		.790		.982	

APPENDIX F

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