EFFECTS OF LONG TERM PHYSICAL TRAINING UPON THE HISTOCHEMISTRY AND MORPHOLOGY OF THE VENTRAL MOTOR NEURONS

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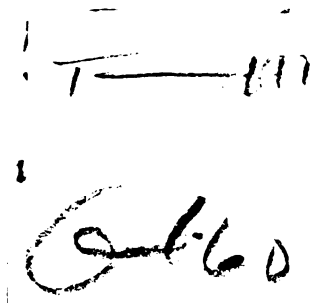

ABSTRACT

EFFECTS OF LONG TERM PHYSICAL TRAINING UPON THE HISTOCHEMISTRY AND MORPHOLOGY OF THE VENTRAL MOTOR NEURONS

by LeRoy B. Gerchman

The influence exerted by ventral motor neurons upon red and white skeletal muscle fiber types is becoming more apparent. For example, cross-innervation and reinnervation studies demonstrate the capability of nerves to change the physiological and histochemical profiles of fiber types within a muscle. Physiological studies have shown that axons innervating white muscle fibers conduct at a faster rate than those of red muscle fibers. Henneman et al. (1965) indicate strongly that the dimensions of motor neurons are directly related to their susceptibility to discharge, therefore to theis activity and possibly to the red and white motor units within a skeletal muscle. It was, therefore, believed to be of interest to examine the histochemical and morphological changes which occur in the ventral motor neuron with a long term exercise program designed to produce changes in the proportions of red to white muscle fibers in skeletal muscle.

A number of investigations demonstrate that the morphology of motor neurons changes with increased functional activity. Few studies, however, deal with the influence of physical activity upon the histochemistry of the motor neuron. In both morphological and histochemical studies there is often a lack of distinction between the effects of a sudden exhaustive increase in activity and the effects of continued muscular training.

The purpose of this study is to determine changes in morphology as well as to estimate alteration in enzyme activity in the ventral motor neuron following long term physical exercise programs.

Sixty, male Sprague-Dawley rats, 100 days of age were randomly placed into three groups. The first group was housed in sedentary cages and comprised the sedentary control group. The second group was housed in sedentary cages but was subjected daily to a period of forced exercise. The third group was housed in spontaneous exercise cages and was subjected daily to two periods of forced exercise. A period of forced exercise consisted of thirty minutes of swimming with weights attached to the animals' tails. Each weight equaled three per cent of the animal's body weight in the second group and four per cent of the animal's body weight in the third group. The experimental procedures were carried out for a period of fifty-two days.

At the time of sacrifice the lumbar intumescence of the spinal cord was immediately removed. Fresh frozen sections were taken for cholinesterase, acid phosphatase, malate, and glucose-6-phosphate dehydrogenase techniques. Formalin-fixed, paraffin-embedded sections were taken for staining with toluidine blue and luxol fast blue-neutral red.

Areas of cell bodies, nuclei, and nucleoli of ventral motor neurons were obtained from projected images of ventral horns with the aid of a compensating planimeter. Histochemically the motor neurons were graded according to intensity and the percentages of dark, medium and light staining cells were recorded for the three groups.

Approximately 2000 ventral motor neurons were evaluated for each enzyme and for area measurements without knowledge of the experimental groups from which they came.

Cholinesterase, glucose-6-phosphate dehydrogenase, and acid phosphatase showed marked activity changes with increased functional activity. Alterations in morphology and staining properties of ventral motor neurons were also observed at both experimental levels of increased functional activity.

EFFECTS OF LONG TERM PHYSICAL TRAINING UPON THE HISTOCHEMISTRY AND MORPHOLOGY OF THE VENTRAL MOTOR NEURONS

By

LeRoy B. Gerchman

A THESIS

Submitted to
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Department of Anatomy

1968

DEDICATED TO:

MY WIFE, VINNY:

For her love, understanding, and encouragement.

OUR PARENTS:

The most wonderful people in the world, who have contributed so much in so many ways.

OUR CHILDREN:

Who make it all worthwhile.

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A special word of thanks is extended to Dr. W. W. Heusner and Dr. W. VanHuss, Department of Health, Physical Education and Recreation, for providing animal quarters, feed, cages and all the exercise equipment required in this study.

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
General Comments	1
Development of Problem	1
Specific Aims of Research	3
Significance of Study	4
REVIEW OF PERTINENT LITERATURE	6
Red and White Skeletal Muscle	6
Anatomical Differences	6
Physiological Differences	7
Biochemical Differences	8
Neural Influence Upon Red and White Muscle	9
The Motor Unit	13
Motor Neurons and Fiber Types	15
Influence of Exercise	19
Functional Activity and the Motor Neuron	21
Morphological Effects	21
Histochemical Effects	26
MATERIALS AND METHODS	31
Experimental Animals	31
Experimental Groups	31
Experimental Treatment	32
Tail Weights	32
Swimming	33
Environment and Food	34
Sacrifice Procedures	35
Histochemical Procedures	36
Freezing	36
Cutting	36
Incubation	37
Histochemical Techniques	37
Cholinesterase	37
Acid Phosphatase	38
Malic Acid Dehydrogenase	39
Glucose-6-Phosphate Dehydrogenase	39

Page

Histological techniques 40
Measurements of Neurons 40
Evaluation and Analysis 41
Histochemical 41
Reliability Data 42
Measurement Data 43

RESULTS 44

Histochemical 44
Cholinesterase 44
Glucose-6-Phosphate Dehydrogenase 48
Acid Phosphate Dehydrogenase 51
Malic Acid Dehydrogenase 54
Reliability Data 56
Morphological 57
Nissl Staining 57
Measurements of Neurons 58
Body Weights 59

DISCUSSION 61

Sedentary-Forced Group 62
Voluntary-Forced Group 64
Relationship to Muscular Results 67
Limitations of This Study and Suggestions for Future Studies 71

SUMMARY AND CONCLUSIONS 73

Summary 73
Conclusions 75

LITERATURE CITED 77

APPENDIX 99

LIST OF TABLES

TABLE		Page
1.	Cholinesterase: Mean percentages of dark, medium, and light staining neurons in three experimental groups	46
2.	Frequency distribution of subjective ratings for cholinesterase activity in treatment groups	47
3	Glucose-6-phosphate dehydrogenase: Mean percentages of dark, medium, and light staining neurons in three experimental groups	49
4.	Frequency distribution of subjective ratings for G-6-PD activity in treatment groups	50
5.	Acid phosphatase: Mean percentages of dark and light staining cells in three experimental groups	52
6.	Frequency distribution of subjective ratings for A.Pase activity in treatment groups	53
7.	Malic acid dehydrogenase: Mean percentages of dark, medium, and light staining cells in three experimental groups	55
8.	Frequency distribution of subjective ratings for MDH activity in treatment groups	55
9.	Correlation from reliability data	57
10.	Mean areas of ventral motor neurons in square micra	58
11.	Body weights and per cent correlation between body weights and mean neuronal areas	60

LIST OF FIGURES

Figures		Page
1.	Cholinesterase activity in ventral motor neurons of a sedentary animal	90
2.	Cholinesterase activity predominant in the neurons of the sedentary-forced group of animals	90
3.	Ventral motor neurons of the voluntary-forced group of animals incubated for cholinesterase	90
4.	G-6-PD activity in ventral motor neurons of a sedentary animal	92
5.	Activity of G-6-PD in motor neurons of the sedentary-forced group showing increased numbers of light staining cells . . .	92
6.	G-6-PD activity in the ventral horn of a voluntary-forced animal	92
7.	Acid phosphatase activity in two neurons of the sedentary group of animals	94
8.	Increased acid phosphatase activity in neurons of the sedentary-forced group	94
9.	Diminished acid phosphatase activity in motor neurons of the voluntary-forced group of animals	94
10.	Nissl staining in ventral motor neurons of a sedentary animal	96
11.	Chromophobic ventral motor neurons in the sedentary-forced group	96
12.	Severe chromophilic reactions in neurons of the voluntary-forced group of animals . .	96

Figures Page

13. CHE activity in a medium staining cell from a sedentary-forced animal 98

14. CHE activity in a ventral motor neuron of a voluntary-forced animal 98

15. MDH activity in two motor neurons from the sedentary group of animals 98

16. Acid phosphatase in a neuron from the sedentary group 98

17. Toluidine blue stained neuron from the sedentary-forced group 98

18. Toluidine blue stained neuron from the voluntary-forced group 98

19. BuChE in the ventral horn from a voluntary-forced animal 98

LIST OF APPENDICES

Appendix		Page
A.	Histochemical Evaluation Data	99
B.	One Way Analysis of Variance Tables	110
C.	Neuron Areas and Body Weights	116

LIST OF ABBREVIATIONS

AChE	Acetycholinesterase
A.Pase	Acid phosphatase
BuChE	Pseudocholinesterase
CHE	Cholinesterase
DPN	Diphosphopyridine nucleotide
E-600	Di-ethyl-P-nitro-phenyl phosphate
G-6-PD	Glucose-6-phosphate dehydrogenase
MDH	Malic acid dehydro-genase
NBT	Nitro blue tetrozolium salt
TPN	Triphosphopyridine nucleotide

INTRODUCTION

General Comments

The present investigation represents the neural aspects of a complementary neuromuscular study concerned with the influence of physical exercise programs upon neuromuscular histochemistry and morphology. The intimate relationship between nerve and muscle lends itself readily to a coinvestigation of this type in which the same experimental animals can be utilized and the results of each study interpreted in a framework of the motor unit rather than individually. A complete treatment of the muscular aspects of this problem is presented in a doctoral thesis submitted to the Department of Health, Physical Education, and Recreation (Edgerton, 1968).

Development of Problem

That the intact motor neuron exerts a "trophic" influence upon the muscle it supplies was probably first observed clinically following disturbances of innervation. Denervation studies have now clearly demonstrated a strong dependance of muscle upon an intact nerve supply (Nachmias et al., 1958; Sheves et al., 1956; Romanul et al., 1965; Humoller et al., 1951; Hogan et al., 1964). More recent

evidence obtained from cross-innervation and reinnervation studies (Close, 1965; Buller et al., 1960b; Romanul et al., 1966; Yellin, 1967) suggest not only a general dependency between nerve and muscle but a very specific relationship between a neuron and the muscle fiber type which it innervates.

Mammalian skeletal muscle can be differentiated into red and white types based upon anatomical, physiological and biochemical properties. The red and white fibers represent the extremes of a continuum with a number of intermediate types as demonstrated by histochemical techniques (Romanul, 1964). The close relationship which exists between a neuron and the specific muscle fiber types it innervates suggests that neurons supplying opposite types would exhibit distinguishing characteristics. Physiological evidence has revealed the existence of different kinds of alpha motor neurons based upon their speed of conduction, the size of their spike potentials, and their susceptibility to discharge (Granit et al., 1956). From physiological evidence it has been postulated that neurons innervating red muscle fibers are smaller in size and more active, in terms of discharge and conduction, than those supplying white fibers (Somjen et al., 1964; Henneman et al., 1965).

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of the biochemical and kinetic properties of muscle fiber types. It was therefore the author's contention that non-pathological alterations in the proportions of red to white fibers in skeletal muscle would be reflected in the enzyme activity and morphological features of the neurons supplying those muscles.

Heretofore, it has not been demonstrated that an exercise program would provide a non-pathological means of altering the proportions of red to white fiber types in skeletal muscle. However, the results of certain quantitative biochemical studies (Halloszy, 1967; Peter et al., 1968) suggest that a possible shift in the proportions of fiber types rather than an overall change in muscle metabolism may occur with exercise. Other authors have suggested that the transformation of fiber types probably occurs as the result of changing functional demands upon nerve and muscle (Guth et al., 1968). It was therefore believed that alterations in muscle fiber types would occur, with an exercise program, and that such changes would be reflected in the motor neurons of the spinal cord, therefore, the entire motor unit.

Specific Aims of Research

In view of the influence exerted by motor neurons upon red and white fiber types it was believed to be of interest to examine the ventral motor neuron following long

term physical exercise programs designed to produce alterations in the proportion of red to white fiber types in skeletal muscles supplied by those neurons.

The specific aims of this research were first to investigate the influence of long term physical exercise programs upon the ventral motor neuron. Histological and histochemical techniques were utilized in examining the following parameters (1) the sizes of cell bodies, nuclei and nucleoli (2) the staining of Nissl substance within motor neurons (3) the enzyme activity of motor neurons as demonstrated by histochemical techniques. Secondly, the findings were to be correlated with the changes in muscle fiber types obtained by Edgerton (1968) from skeletal muscle of the same experimental animals.

Significance of Study

In view of the above discussion it is believed that this study will contribute to the existing body of knowledge concerned with the effects of increased functional activity upon motor neurons. In regard to the convincing evidence on the delicate neural control of the kinetic and metabolic properties of muscle fiber types this study should further emphasize the intimate relationships between ventral motor neurons and fiber types within skeletal muscle. It should also contribute much needed evidence regarding the adaptability of the motor neuron,

as an integral part of the motor unit, to meet the demands of increased functional activity.

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REVIEW OF PERTINENT LITERATURE

Red and White Skeletal Muscle

Mammalian skeletal muscle fibers can be differentiated into red and white fiber types. Differences between the two fiber types can be observed anatomically, physiologically, and biochemically. The following paragraphs are intended to highlight the major differences on all four levels of investigation.

Anatomical

That skeletal muscles of animals varied in color from a deep red to a pale pink color has been known as early as 1678 (Denny-Brown, 1929a). Early morphological studies revealed that two major categories of fibers exist in varying proportions in the skeletal muscles of mammals. One fiber type had a smaller diameter and a more granular sarcoplasm and was described as a dark or red fiber. The other, a light or white fiber, was larger in diameter with a less granular sarcoplasm (Bullard, 1912; Denny-Brown, 1929a). Increased quantities of myoglobin and cytochromes have been shown to be responsible for the red color of the smaller fibers (Hawrowitz and Harden, 1954). Most mammalian muscles are "mixed" in that they contain both red and

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white fibers (Needham, 1926). The term "red" or "white," therefore, does not necessarily imply complete homogeneity of the component fibers.

Increased capillary to fiber ratios have been demonstrated in red muscle fibers and have been related to the increased oxidative metabolism of red fibers (Romanul, 1965). Recently, an increase in the capillary to fiber ratio of both red and white fibers has been demonstrated in exercised rats (Carrow et al., 1967).

Electron microscopic studies have revealed a greater number of mitochondria in red than in white fibers with a predominantly peripheral distribution of mitochondria in the red fibers (Gauthier and Padykula, 1966; Gauthier, 1968).

The increased vascularity and increased numbers of mitochondria in red fibers is consistent with the increased oxidative metabolism of these fibers discussed in a later section.

Physiological

Early investigators described the contractions of red and white skeletal muscles and demonstrated that red muscles contracted more slowly than did the white. In an attempt to relate histologic structure with functional activity, Denny-Brown (1929a) described the contractions of red and white muscles and considered the quota of red

and white fibers in relation to the physiological properties of the whole muscle. Close (1964, 1965, 1967) studied the contraction characteristics of skeletal muscle and reported that red fibers contract at a slower rate than white fibers. From the same work he concluded that slower red fibers are associated with the sustained contractions generally associated with postural muscles while white fibers are responsible for rapid forceful contractions.

Biochemical

Data from both histochemical and quantitative biochemical sources show marked differences in the various metabolic pathways of red and white muscle fibers. Red muscle fibers have intense activity of enzymes related to oxidative and lipid metabolism and represent a strong dependence upon aerobic pathways. White muscle fibers demonstrate higher activities of enzymes concerned with anaerobic glycolysis (Dubowitz et al., 1960; Dawson et al., 1964; Romanul, 1964; Beatty et al., 1966). Beatty et al. (1963) have also shown intense concentrations of glycogen and phosphorylase in white fibers which is consistent with a system dependent upon glycolytic metabolism and an immediately available energy reserve. The basic biochemical differences between red and white fibers allowed enzyme histochemistry to become a very valuable tool in the differentiation of fiber types. Until recently the main

histochemical methods for differentiating fiber types were those of Sudan III and IV (Bullard, 1912; Denny-Brown, 1929a). With the descriptions of differences in succinic dehydrogenase found in the different fiber types (Padykula, 1952; Thimann and Padykula, 1955) the methods of differentiating fiber types shifted toward enzyme histochemistry. Since these early studies a number of enzyme techniques have been employed to differentiate muscle fiber types (Stein and Padykula, 1962; Romanul, 1964).

Neural Influence Upon Red and White Muscle

A number of investigations have also demonstrated the strong dependence of muscle upon an intact nerve supply to maintain normal contraction characteristics as well as normal histochemical and biochemical differentiation (Humoller et al., 1951; Humoller et al., 1952; Sheves et al., 1956; Nachmias et al., 1958; Hogan et al., 1964; Close, 1965; Romanul et al., 1965; Guth et al., 1967). It has been speculated that perhaps differentiation of muscle fibers into red and white types, as defined by metabolic criteria is under the control of motor neurons since both innervation and metabolic differentiation occur early in fetal development (Beatty et al., 1967).

Utilizing the technique of surgical cross-union of nerves Buller, Eccles, and Eccles, (1960b) and Close (1965) demonstrated that a slow contracting muscle,

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reinnervated by the motor nerve to a fast muscle, shows a marked increase in its speed of contraction. Conversely, the reinnervation of a fast muscle by a nerve to a slow muscle reduces the speed of its contraction. Control experiments with self union and regeneration demonstrated that these changes were not merely a result of nerve section and regeneration but were the result of a newly acquired innervation by a motor nerve that normally would innervate muscle having a different speed of contraction. From these studies Buller, Eccles and Eccles (1960b) postulated that the changed speeds of contractions were produced through the influences of two types of alpha motor neurons which normally innervated fast and slow muscles respectively.

Since studies of enzymes in normal skeletal muscle have shown that muscles with fast contraction characteristics have a preponderance of white fiber types and slow contracting muscles have a preponderance of red fiber types (Dubowitz and Pearse, 1960; Romanul, 1964; Dawson and Romanul, 1964), the question arose as to whether surgical cross-union of motor nerves would produce corresponding changes in the histochemical profiles of fibers within the physiologically altered muscles. Romanul and Van Der Meulen (1966) investigated the physiological as well as the histochemical alterations produced in muscle as a result of cross-union of motor nerves. The physiological alterations

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were in agreement with those of Buller et al. (1960b) and Close (1965) and were accompanied by corresponding histochemical changes as confirmed by Yellin (1967a). The capability of the motor neuron to alter the physiological and histochemical profiles of red and white muscle has, therefore, been clearly demonstrated by motor nerve cross-union studies.

The exact mechanism by which ventral motor neurons are able to affect alterations in the physiological and biochemical properties of muscle fiber types is unknown. One proposal has suggested the sequence or the quantity of impulses per unit time delivered to the muscle fiber as the controlling factor. Cross-union of motor nerves between red and white muscle can reverse the contraction characteristics of the newly innervated muscles (Buller, Eccles, and Eccles, 1960b; Buller and Lewis, 1965; Close, R., 1965). Reversal of contraction characteristics was not observed when cross-union was performed in spinalized cats (Buller, Eccles and Eccles, 1960a). Conversely, continuous stimulation of the popliteal nerve consistently resulted in a slowing of contractions in the anterior tibialis muscle (Salmons and Vrbova, 1967). Together these studies indicate the importance of an impulse conduction mechanism in altering muscle fiber types.

A second mechanism by which motor neurons are believed to influence muscle fibers is through the release

of some chemical factor from the neuron into the muscle fiber, the "ooze" theory. However no such chemical agent has yet been identified. There are certain observations, however, which can readily be explained by such a mechanism. For example, at birth all muscles contract slowly; only shortly after birth do the contraction times of white muscle shorten (Denny-Brown, 1929a; Buller, Eccles and Eccles, 1960a; Buller and Lewis, 1965). This shortening of contraction times can be reversed by transection of the peripheral motor nerve but not by transection of the spinal cord and deafferentation, which silences the ventral motor neurons. Therefore, the differentiating process appears to be related to a neuronal control but not to nerve impulses (Buller, Eccles, and Eccles, 1960a).

Activation of glycogen-resynthesis in muscle occurs after direct stimulation of its motor nerve (Gutmann et al., 1954). After stimulation, sectioning of the nerve produces an impairment of glycogen-resynthesis in the muscle which takes place at a time related to the length of the peripheral stump (Gutmann et al., 1956). Substances from the motor neuron released into muscle fibers apparently take part in the activation of this process. That substances move proximodistally in the axons of motor nerves has been well established (Sawyer, 1946; Weiss et al., 1948; Hebb et al., 1956; Friede, 1959; Weiss, 1967a; Weiss, 1967b). Nerve stimulation or physiological activity induces the

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transport of substances within axons (Vodicka, 1956). That substances continue to be transported for a time in the proximal and distal portions of a severed nerve has also been demonstrated (Hebb et al., 1956; Friede, 1959). It is therefore apparent from these studies that a neural agent is liberated from the neuron into the muscle which influences at least the metabolic process of glycogen synthesis.

It is not possible to explain all observations solely in terms of electrical activity of nerves, nor can they all be accounted for by some hypothetical neurotrophic substance. Furthermore, it is probable that neither represents the complete mechanism and that the two are probably closely interrelated in producing an influence in skeletal muscle. In any case the important concept is that the neuron is capable of regulating many characteristics of muscle fiber types.

The Motor Unit

The previous discussion indicates that the preferential energy metabolism of fiber types is determined by nerve supply. A basic concept which must be discussed in regard to this idea is the homogeneity of the motor unit. Are all the muscle fibers, supplied by a specific motor neuron, of the same histochemical type (homogeneous) or do they represent both red and white histochemical types

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(heterogeneous)? If the energy metabolism of fiber types is governed by the neurons which supply them it would seem reasonable that all the fibers governed by a specific neuron would be of the same histochemical type.

Romanul and Van Der Meulen (1966) and Yellin (1967a, b) have presented histochemical evidence which strongly suggest homogeneity of the motor unit. With few exceptions the muscle fibers of a motor unit are widely distributed in a muscle and motor units overlap considerably. Histochemically red and white fiber types are seen to be randomly interspersed between one another. Following regeneration of a motor nerve to a muscle, type groups or islands of enzymatically similar muscle fibers are found. Romanul and Van Der Meulen (1966) interpret this finding to mean that during normal development axons follow direct pathways within a nerve and arrive at the muscle fibers in close time relationship with other axons. As collaterals form they are unable to innervate near by fibers which have already been innervated by adjacent axons and therefore grow out until they reach fibers which have not been innervated. Regenerating axons, because of the tortuous course through the area of trauma, arrive at muscle fibers at widely varying times. The first axon to arrive can innervate adjacent fibers which have not yet been innervated. Subsequent axons act similarly. The result being that an axon supplies the muscle fibers in a

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specific area and all the fibers supplied by that axon are histochemically similar suggesting a homogeneous motor unit. It was of interest to this author to learn that type groups or islands of enzymatically similar muscle fibers are a normal feature in the skeletal muscles of the pig (Merkel, 1968). In view of the above discussion it would seem that this animal may exhibit distinctive features in the developmental innervation of its skeletal muscle.

Motor Neurons and Fiber Types

Evidence presented in previous discussions suggests that red and white motor units are, to a large extent, dependent upon their respective neurons for biochemical and physiological differentiation. The question then arises as to what characteristics can be attributed to the specific neurons supplying red, white or intermediate motor units of skeletal muscle. Granit, Henatsch, and Steg (1956), in a survey of 100 extensor alpha motoneurons of the cat responding to stretch of the gastrocnemius-soleus muscles, demonstrated that 53% of the motoneurons discharged only at the beginning of the stretch while the remainder discharged throughout the duration of the stretch. The phasic alpha motoneurons were also distinguished from the tonic by their larger axonal spike potentials. Granit, Phillips, Skoglund and Steg (1957) have shown that activation by the

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crossed extensor and pinna-twist reflexes gives the same discriminatory response between phasic and tonic and motoneurons. The phasic motoneurons were shown to exhibit a higher frequency of discharge (30-60/sec) than the tonic (10-20/sec). Eccles, Eccles and Lundberg (1958) characterized motoneurons by duration of after potentials as well as by the conduction velocities of their axons. They demonstrated that axons which conduct rapidly and have shorter after-hyperpolarization were characteristics of neurons innervating white or fast muscles while axons which displayed slow conduction velocity and a prolonged after-hyperpolarization innervated red or slow muscles. It was concluded that, in general, the tonic and phasic types of motor neurons activate slow and fast muscle respectively. The after-hyperpolarization determines the frequency of discharge (Eccles, 1936; Eccles, 1953; Pitts, 1943). This is supported by the findings that slowly discharging tonic motor neurons have a longer after-hyperpolarization than the rapidly discharging phasic motor neurons (Eccles et al., 1957a, b; Eccles et al, 1958). The tonic motor neurons innervate slowly contracting red motor units, which could therefore result in a fused tetanus at a relatively low frequency of discharge. Phasic motor neurons have high frequencies of discharge and are matched to the relatively fast-white muscles which they innervate and are therefore capable of producing an optimal tetanic

fusion. Any serious mismatching would result in inefficiency (Eccles et al., 1958). This emphasizes the importance of the neuron in differentiating the fibers which it innervates into fast or slow types in order to insure a proper match. This would also imply a homogeneity of the motor unit.

The properties of motor units in a red muscle, the soleus, versus a mixed pale muscle, the medial head of the gastrocnemius were investigated (Wuerker, McPhedran, and Henneman, 1965; McPhedran, Wuerker, and Henneman, 1965). An attempt was made to define these types of neurons in regard to their speed of contraction which would correspond to the type A, B, and C fibers of the gastrocnemius as described histochemically by Stein and Padykula (1962). The axons supplying type A and type B motor units were outlined with considerable clarity however the axons of type C motor units were not clearly defined. The results however, demonstrated that the average conduction velocity of the axons supplying the red muscle was considerably less than that of the axons supplying pale muscle.

Histologically and histochemically the ventral motor neurons specifically associated with red or white motor units have not been identified. Physiological evidence, however, implies a size relationship and a possible biochemical relationship of the motor neuron to its motor unit.

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In regard to size, a direct proportionality exists between the axon diameter and the conduction velocity of myelinated nerve fibers (Hursh, 1939; Rushton, 1951). The amplitudes of nerve impulses from peripheral axons are also directly related to their diameter (Gasser, 1941). If it is assumed that the diameters of axons are related to the sizes of their cell bodies, as scattered histological evidence indicates (Henneman, Somjen, Carpenter, 1965) we may then expect that axons displaying a large impulse and a rapid speed on conduction, characteristic of white (fast) motor units arise from a large ventral motor neuron. In the same respect impulses of low aptitude and slow conduction velocity would be expected to arise from a neuron of smaller dimensions (Somjen et al., 1964). It would then seem reasonable to assume that the white motor units are innervated by large motor neurons in contrast to small neurons supplying red motor units. It is also of interest to note that gamma motor neurons whose axons are small (1 to 8 microns) innervate intrafusal muscle fibers which are predominantly red histochemically (Yellin, 1968). Their tendency to fire continuously even in the resting limb of an anesthetized animal (Henneman, Somjen, and Carpenter, 1965) would then appear to be a functional expression of their small size.

In regard to activity Henneman (1957) demonstrated that the excitability of ventral motor neurons varied

inversely with their size, the reflex threshold of individual cells increasing in parallel with the amplitude of impulses recorded from their axons. In the light of the previous discussion it can be concluded that smaller ventral motor neurons, demonstrated to innervate red motor units, are more susceptible to discharge than are the larger motor neurons associated with white motor units. This concept is in agreement with the findings that slow muscles are activated in the lowest threshold range of the postural reflexes while fast muscles are in the upper range of threshold (Denny-Brown, 1929b). Vrbova (1963) also demonstrated that the soleus, a red muscle, is almost continuously activated, according to electromyographic recordings, whereas a white muscle is activated during periods of reflex activity or voluntary movement. It has also been shown that in the post-tetanic potentiated state (Granit et al., 1956), extensor motor neurons fall into two categories in their responses to a constant stretch: (i) a tonic group which fires for a long duration in a maintained stretch and (ii) a phasic group which only fires one or two spikes on the rising phase of the stretch. Analysis of spike size demonstrate that the tonic group tends to have smaller spike potentials than the phasic group and concludes that tonic motor neurons tend to group themselves among the smaller ventral motor neurons and the phasic among the larger (Granit et al., 1956; Granit et al., 1957).

The results, therefore, of predominantly physiological studies would suggest not only small neurons innervating red motor units, but also more active neurons. Increased functional activity in a neuron is suggestive of an increased energy metabolism and possible increased activities of enzymes associated with the transmission of impulses along the axons or neuromuscular transmission.

In summary physiological evidence demonstrates that red motor units are innervated by neurons which display in general the following characteristics with respect to neurons of white motor units: (1) a lower threshold of excitability; (2) a long duration of tonic activity in response to a maintained stretch reflex; (3) smaller spike potentials; (4) a slower speed of conduction; (5) a longer after-hyperpolarization time; (6) conduct fewer impulses per second. Analysis of conduction velocity and spike potentials suggests that these neurons are smaller in size and their tonic character suggests neurons of increased functional as well as biochemical activity.

Influence of Exercise

It had not been demonstrated that alterations in the proportions of red to white fiber types could occur with a physical training program, however, quantitative biochemical studies demonstrated changes in oxidative and glycolytic enzymes of exercised muscle (Halloszy, 1967;

Peter et al., 1968) which strongly suggested this possibility. Vrbova (1963) stated that a muscle becomes slower with continuous use which indicates possible changes in fiber types with increased functional activity. It has also been suggested that transformations of fiber types probably occurs throughout life as the result of changing physiological demands upon nerve and muscle (Guth et al., 1968). The fact that all muscle fibers are slow at birth and gradually differentiate into fast types is also suggestive of an influence by functional activity.

A number of investigators have demonstrated morphological changes in ventral motor neurons with exercise Eve, 1896; Hyden, 1943; Wendt, 1951; Edstrom, 1957). However, few if any have investigated this problem in relation to possible alterations in the proportions of red to white fibers in skeletal muscles following long term exercise programs.

Functional Activity and the Motor Neuron Morphological Effects

The literature dealing with the influence of increased functional activity upon the morphology of motor neurons indicates that motor neurons can become altered in response to increased activity.

A wide variety of experimental procedures have been utilized to increase the activity of motor neurons. Included among these are electrical stimulation, chemical

irritation, and exercise all of which have been used at varying levels of intensity and duration.

It is believed that the confusion and contradiction apparent in this area of investigation is, in part, the result of diversity in experimental procedures.

Whether the activity of nerve cells is associated with recognizable changes in their structure was first investigated by Hodge (1889, 1892, 1894). A number of experiments consisted of stimulating posterior root ganglion cells through the brachial or sciatic plexus in frogs or cats for one to ten hours. A few experiments were devoted to ascertaining if the effects of fatigue could be removed by varying periods of rest. The ganglia of birds were also compared before and after a hard days work. The results of these varied experiments suggested that the nucleus decreases markedly in size and becomes dark and jagged with functional activity. The cell body was reported to become slightly shrunken and vacuolated. It was also observed that the nerve cells recovered with rest. A number of early investigators have reported that neurons enlarge as a result of increased activity whereas shrinkage occurs with exhaustion. (Vas, 1892; Mann, 1894; Lugaro, 1895). Others have found that shrinkage is the only effect of activity with respect to volume changes (Hodge, 1889; Legendre and Pieron, 1908). Negative findings have also been reported (Eve, 1896; Kocher, 1916; and Hyden, 1943).

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More recently hypertrophy of nerve fibers was reported in response to increased work load produced by surgical elimination of synergistic muscles or the contralateral limb (Wedeles, 1949). Wendt (1951) demonstrated enlarged nerve cell nuclei following similar denervations. Dimensions and staining properties were markedly altered in motor neurons of exercised guinea pigs sacrificed immediately after a thirty-minute running period (Edstrom, 1957). Swelling of the cell body, nucleus, and nucleolus was demonstrated in the neurons of these animals. However, a prolonged exercise program over a twenty-nine day period produced only a slightly enlarged darkly staining nucleolus.

Most authors in the above discussion of exercise and neuron dimensions have also reported changes in the staining intensities of nerve cells with functional activity. Comprehensive studies in regard to the stainability of Nissl substance with increased activity were reported by Dolley (1909a, 1909b, 1911b). Various experimental procedures were used in these studies, e.g. dogs running on a track, administration of drugs, and others. Nerve cell changes were divided into six stages with activity, fatigue, and exhaustion and a close relationship was shown between Nissl substance and the nucleolus. Since these early reports a number of investigations have been conducted upon the stainability of Nissl substance of neurons with acute pathological and physiological states of

neuronal activity (Dolley, 1911a, 1913, 1917; Einarson, 1935; Hyden, 1943; Casperson, 1950; Einarson et al., 1955; Vraa-Jensen, 1957; Aleinikova et al., 1966).

Einarson (1933) concluded from developmental studies of neurons that chromatin material was first visible around the nucleolus. It then migrated toward the periphery of the nucleus and diffused gradually through the nuclear membrane to form the Nissl substance. Continual reformation could be observed during normal activity of normal nerve cells as well as during stages of recovery following artificial stimulation or severe injury to axons. These conclusions have been corroborated through the comprehensive research of Hyden (1947). Extensive biochemical research has led to a more complete understanding of the above findings (Einarson, 1957; White et al., 1964; Watson, 1965). It is now widely accepted that RNA plays an important role in the synthesis of proteins, including enzymes and that the synthesis of RNA is under the control of DNA of nuclear chaomatin. During development of the neuron the essential nuclear component (RNA) of Nissl substance is formed in or near the nucleolus inside the nucleus; it migrates toward the periphery of the nucleus, and then passes through the nuclear membrane into the cytoplasm. Subsequently, it is incorporated as an essential constituent of the complex cytoplasmic nucleoprotein elements, e.g. the Nissl substance (Einarson, 1933; Casperson, 1950;

Einarson, 1957). Nissl substance is formed not only during development, which has been studied by Hyden (1943) and more recently by Vraa-Jensen (1957) but also in the adult organism during life. The consumption and reformation are particularly conspicuous in conditions of increased activity, pathological stress and regeneration of the neuron (Einarson and Krogh, 1955). Likewise, corresponding changes in protein production in the cell is apparent during growth and function (Casperson, 1950).

Based upon the works of several investigators Einarson (1957) described the stainability of Nissl substance at various levels of functional activity. A chromoneutral condition represented the staining profile of nerve cells during a state of normal activity. A moderate chromophilia (hyperchromasia) characterized cells when their activity began to increase; moderate chromophobia, gradually becoming more marked was characteristic of cells in increasing activity of longer duration. Extreme chromophobia represents cells in a state of severe functional stress, fatigue and exhaustion. Chromophilia was believed to be characteristic of cells in a state of prolonged active inhibition, or cells whose activity was diminished or abolished for some time. In pathological conditions extreme chromophobia may proceed to total dissolution of the cell.

Edstrom (1957) was one of the first to compare the immediate effects of acute exhaustive exercise with that of a prolonged exercise program. One group of guinea pigs was exercised to exhaustion by running and immediately sacrificed, while a second group was exercised for thirty-two hours in intervals during a twenty-nine day period. Ventral motor neurons of the acute exercise group showed a severe chromophobic reaction in Nissl staining but the nucleolus remained deeply stained. Neurons of the prolonged exercise group showed intense staining of Nissl substance with a light staining nucleolus.

During functional activity it has been shown that the stainability of neurons is related to a balance between the rate of RNA production by the nucleus and its rate of breakdown in the cytoplasm. Alterations in this balance can result in chromophobic or chromophilic staining reactions (Vraa-Jensen, 1956).

Histochemical Effects

The role of acetyl choline and acetylcholinesterase in neuronal transmission and conduction mechanisms has been subject to numerous investigations since the experiments of Dale (1914). Pharmacological evidence indicated the role of AChE to be the removal by hydrolysis of released transmitter agent, acetylcholine, for repeated activity, (Eccles, 1957).

Since the demonstration of a histochemical technique for cholinesterase activity (koelle and Friedenwald, 1949) the distribution of cholinesterases have been studied in a variety of situations and structures in order to correlate structure with function. However, these investigations have not employed the use of physical activity in studying the cholinesterase distribution in ventral motor neurons. It is generally accepted that cholinesterases are synthesized in the cell bodies of cholinergic neurons and are transported along the axons to their sites of activity (Feldberg, 1957). For example, Sawyer (1946) found that after section of the sciatic nerve in guinea-pigs, the concentration of acetylcholinesterase in the central stump increased by about 300% whereas the concentration in the distal end fell to 40%, indicating a constant production by the ce-l body and conduction along the axon.

In the mammalian spinal cord granules attached to the surface of the ventral motor neurons stain deeply with acetycholinesterase methods and are associated with synaptic vesicles (Adams, 1965). There is also intense staining throughout the perikaryon (Roessmann and Friede, 1967).

A number of isozymes of acetylcholinesterase in nervous tissue have been demonstrated (Bernsohn, Barron, and Hess, 1962). However, the significance of the multiple nature of acetylcholinesterase has not been illucidated.

Although the biological functions of pseudocholinesterase are unknown, they have been implicated in certain lipid metabolic pathways (Clitherow, Mitchard, and Harper, 1963). Biochemical studies indicate that butyryl cholinesterase (BuChE) activity resides predominantly in white matter (Ord et al., 1952; Cavanagh et al., 1954). More specifically it has been found to be active in glia cells, capillary endothelium and smooth muscle of blood vessels rather than neurons of the central nervous system (Koelle, 1952, 1954; Brightman et al., 1959).

Acid phosphatase, a hydrolytic enzyme associated with lysosomes, has been correlated with phagocytosis, intracellular digestion, secretion, and necrobiosis (DeDuve, 1959). This enzyme has also been associated with the localization of the complex neuronal lipofuscin pigment which is actively or passively formed in the cell (Anderson and Song, 1962). Acid phosphatase activity has been related to nucleic acid sites in nerve cells and it has been suggested to play an important role in an enzyme system acting upon Nissl substance to release phosphate groups for cell maintenance and function (LaVelle et al., 1954). It has been shown, for example, that under stressful conditions increased acid phosphatase in neurons is closely related to the disappearance of stainable Nissl bodies (Bodian and Mellors, 1944; Smith and Luttrell, 1947). Acid phosphatase activity has been reported to be greater

at the axon hillock (Smith, 1948). The axon hillock has been suggested to be a region of "physiological chromatolysis" where there is a continuous local breakdown of RNA (LaVelle et al., 1954).

Quantitative biochemical studies have not demonstrated an increase in acid phosphatase in chromatolytic neurons. For example, an increase or decrease in the amount of this enzyme in chromatolytic cells of regenerating axons could not be shown (Fieschi et al., 1959). Acid phosphatase rich lysomes can become enlarged under conditions of stress (Strause, 1967). This may provide a reason for an apparent increase in acid phosphatase activity observed in chromatolytic neurons rather than an actual increase in the quantity of this enzyme.

Dehydrogenase activity has been shown to be high in neurons of the central nervous system (Lowry, 1957; Adams, 1965; Thomas and Pearse, 1961). Malic acid dehydrogenase is an enzyme of the Krebs' cycle and shows a greater activity in nerve cell bodies than does glucose-6-phosphate dehydrogenase, an enzyme of the pentose shunt. A relationship of glucose-6-phosphate dehydrogenase to Nissl staining has not been demonstrated in nerve cells, however, the pentose shunt is an important pathway for the production of ribose sugars which are incorporated into RNA. In growing tumors, pyroninophilia of the rapidly growing cells is accompanied by increased glucose-6-phosphate

dehydrogenase activity (Pearse, 1958; Pearse, 1961). However, an increase in glucose-6-phosphate dehydrogenase activity can occur without an increase in RNA production as it does in the macula densa of kidneys in hypertensive animals (Pearse, 1958; Hess and Pearse, 1959). The pentose phosphate shunt also produces reduced triphosphopyridine nucleotides required in lipid synthesis.

Few studies have employed the use of exercise to determine the influence of increased functional activity upon enzyme histochemistry of the ventral motor neuron. That dramatic histochemical alterations can occur in ventral motor neurons of guinea-pigs, forced to run for 30 and 60 minutes and sacrificed immediately, was demonstrated with adenosine triphosphatase techniques (Wawrzyniak, 1963). The dramatic results obtained were those of a rapid exhaustive type of exercise rather than a long term exercise program.

MATERIALS AND METHODS

Experimental Animals

Seventy-two male Sprague-Dawley rats 100 days of age were obtained for this investigation. The animals were immediately housed in sedentary cages for seven days to provide an environmental adjustment period. Sixty animals were selected from this group on the basis of weight. Animals demonstrating extremely high or low body weights were eliminated. The selected animals were then placed into groups of three according to similarity of body weights and each animal of a trio was randomly assigned to one of three experimental groups. The animals were grouped in this manner in order to minimize extreme differences in weight as a possible source of variance. The end result was twenty trios each of which contained a representative from each of three experimental groups.

Experimental Groups

Group A (Sedentary). The animals of this group were housed in sedentary cages (24 x 18 x 18 cm.) for the entire treatment period, but were removed from their cages once each week for body weight determinations.

Group B (Sedentary-forced). This group of rats was housed in sedentary cages in the same manner as in Group A. However, they were forced to swim one thirty-minute period per day with a lead weight equal to three per cent of their body weights attached to their tails.

Group C (Voluntary-forced). The animals of Group C were housed in cages of the same dimensions as in Groups A and B. However, these cages were equipped with voluntary exercise wheels (35 cm. in diameter and 13 cm. wide) which allowed the rats to run at will. Revolutions of the voluntary wheels were mechanically recorded and activity records for each animal were taken. In addition, this group was forced to swim two thirty-minute bouts per day, with a weight equal to four per cent of their body weights attached to their tails.

Experimental Treatment

Tail Weights

The lead weights attached to the rats' tails were adjusted weekly in accordance with their body weights. The weights were attached at the tip of their tails rather than at the base. It was observed that their tails, which are used to a great extent for balance and support against the sides of the tank, were immobilized to a greater extent by placing the weight at the tip. This treatment forced the use of their legs to a much greater extent in

maintaining balance and swimming. The weights were attached by means of miniature plastic clothespins which facilitated attachment and removal. They also provided a secure attachment of the weight during the swimming period. Trauma to the tip of the tail by the clothespins was prevented by protection with adhesive tape.

Swimming

The sedentary-forced group (B) of animals swam thirty minutes each day between the hours of nine o'clock a.m. and twelve o'clock noon. This treatment was continued six days a week over a period of 52 days.

The voluntary-forced group (C) received the same experimental treatment as Group B with an additional thirty-minute swim per day between the hours of five and eight p.m. A minimum of five hours of rest therefore intervened between exercise bouts.

Previous experience has shown that a training period of approximately 50 days is sufficient to produce alterations in skeletal muscle of rats and that continued training for longer periods of time becomes detrimental to the animal.

In order to facilitate sacrifice and tissue processing procedures two triplicates (6 animals) were sacrificed on ten consecutive days. It was therefore necessary to initiate into the swimming program an additional two

triplicates on ten consecutive days in a sequence corresponding to the sequence of sacrifice. This permitted the duration of treatment to be constant for all animals.

The rats were swum in individual cylindrical tanks eleven inches in diameter and thirty inches deep. Following each swim the animals were dried with towels and returned to their respective cages. During a swimming period the rats were observed closely. If an animal remained submerged for more than ten seconds it was removed from the tank for a brief rest period and the weight was removed its tail. The animal was then returned to the tank with no weight attached to its tail.

The sedentary-forced group of animals demonstrated considerably more difficulty in tolerating the swimming program. Four rats died during the swimming treatment, three of which belonged to the sedentary-forced (B) group. The fourth rat belonged to the voluntary-forced group (C). The exercise program imposed upon the sedentary-forced group (B), therefore, did not appear to be well suited for adaptation by these animals to the training program. The increased difficulty in swimming displayed by these animals continued throughout the exercise program.

Environment and Food

The ambient temperature of the animal quarters ranged from 21° C to 25° C. The water temperature of the

swimming tanks ranged from 32° C to 34° C. The water in the tanks was renewed at each swim period. The animal rooms were automatically regulated to maintain twleve hours of light (eight a.m. to eight p.m.) and twelve hours of darkness (eight p.m. to eight a.m.).

All animals were fed ad libitum with commercial block feed. (Wayne)

Sacrifice Procedures

Six animals per day (two triplicates) were sacrificed on ten consecutive days. On each day following their final treatment each rat from the group of six animals was anesthetized intraperitoneally with two ccs. of 1% Pentobarbital. The lumbar intumescence of the spinal cord was removed and trimmed of its spinal nerves in two to five minutes. The removed portion of the cord was then divided at the fifth lumbar segment. The upper portion was fixed in ten per cent formalin for histological procedures and the distal portion was rapidly frozen for histochemical analyses. All sections were taken from the region of the 4th, 5th and 6th lumbar segments which contribute to the formation of the sciatic nerve in the rat (Green, 1955).

Histochemical Procedures

Freezing

The spinal cord block was mounted, in gum tragacanth, on a metal chuck. Only the chuck was submerged into isopentane which was cooled to a viscous fluid by liquid nitrogen. Complete submersion was observed to crack this tissue. Approximately forty-five seconds were required to completely freeze the block of tissue when only the chuck was submerged. No more than one to two minutes elapsed between the time of removal and the transformation of the tissue to the frozen state.

Cutting

Immediately after freezing, the block was placed into an Ames Lab Tek cryostat in which the temperature of the tissue was allowed to equilibrate to that of the cryostat (between minus fifteen and minus twenty degrees centigrade). Sections of cord used for cholinesterase and pseudocholinesterase procedures were cut at twenty micra. Those for all other procedures were sectioned at ten micra. These thicknesses were observed to give the best results in preliminary staining trials of the spinal cord. Two to five sections, not less than 20 microns apart, were cut from each block for histochemical procedures. The sections were placed on cover slips and fan dried while incubation media were being prepared.

Incubation

Freshly prepared incubation media were prepared daily. All sections to be stained each day for a specific enzyme were incubated together in the same medium for the same length of time. Comparisons and evaluations were made only within the group of animals processed on the same day. Incubation times for cholinesterase and pseudocholinesterase were five hours, but those for all other enzymes were thirty minutes. All sections were mounted on slides with glycerine jelly.

Control sections were incubated periodically for each procedure to determine the specificity of the reactions. In some cases the substrate was excluded from the medium while in others the coenzymes, diphosphopyridine nucleotide (DPN) or triphosphopyridine nucleotide (TPN), were deleted in procedures which normally require them for satisfactory reactions. Control sections for cholinesterases were also preincubated for one hour in E-600 (diethyl-P-nitrophenyl phosphate) at 10-7 molar concentration. E-600 has been shown to be a powerful cholinesterase inhibitor at the above concentration (Pearse, 1961).

Histochemical Techniques

Cholinesterase (CHE)

Demonstration of cholinesterase activity in ventral motor neurons was accomplished by the acetylthiocholine

method (Koelle and Friedenwald, 1949). This procedure employs acetylthiocholine iodine as the substrate and is hydrolyzed by both acetycholinesterase (AChE) and pseudocholinesterase at a pH of 6. Parallel sections were incubated in the same basic medium but with butyrylthiocholine iodine as the substituted substrate, which is hydrolyzed specifically by pseudocholinesterase. In both reactions the copper and sulfate present in the medium combine with the released thiocholine to produce a copper thiocholine sulfate complex which is converted to copper sulfide by passage of the section through dilute ammonium sulfide. The brown deposits of copper sulfide mark the sites of cholinesterase activity. The difference in staining intensity between the two procedures provides an estimation of acetylcholinesterase (AChE) activity.

Acid Phosphatase (A.Pase)

The procedure used to demonstrate acid phosphatase activity was that of Barka (1960). The method is based upon the hydrolysis of alpha naphyl phosphate (substrate) by acid phosphatase to liberate naphthol at an optimal pH of 5. This compound then couples with one or more azo groups on each molecule of freshly diazotized pararosanilin to form an azo dye produce which is highly insoluble and resistant to ordinary organic solvents (Davis and Ornstein, 1959). Barka's hexazonium pararosanilin

technique has been demonstrated to be particularly suitable for the demonstration of acid phosphatase in neural tissue (Anderson and Song, 1962).

Malic Acid Dehydrogenase

The procedure for malic acid dehydrogenase consisted of incubation of the tissue in a medium composed of one ml. of one molar malic acid, ten mg. of diphosphopyridine nucleotide (DPN), fifteen mg. of nitro blue tetrazolium salt (NBT), and 10 ml. of 0.2M Tris buffer at a pH of 7.4. The final pH was adjusted to 7.4 with ten normal sodium hydroxide.

The oxidation of malic acid by malic acid dehydrogenase converts DPN to its reduced form. In the oxidation of reduced DPN the terazolium salt (NBT) becomes reduced and is converted to an insoluble colored formazan product which marks the sites of MDH activity.

Glucose-6-phosphate dehydrogenase (G-6-PD)

Glucose-6-phosphate dehydrogenase catalyzes the reaction from glucose-6-phosphate to 6-phosphogluconolactone, a reaction characteristic of the pentose phosphate shunt. This reaction is accompanied by the reduction of the coenzyme triphosphopyridine nucleotide (TPN). The reoxidation of this compound results in a reduction of the

tetrazolium salt (NBT) to a colored, insoluble, formazan product.

Both dehydrogenase techniques are those of Hess et al. (1959) as modified by Barka et al. (1963).

Histological Techniques

The proximal portion of spinal cord was fixed in 10% formalin and embedded in paraffin. Sections were cut at seven microns and stained for Nissl substance with Luxol fast blue and neutral red (Lockard and Reers, 1962) and with toluidine blue O (Stenram, 1954). All sections were carried through the staining procedures together and were stained and destained for the same lengths of time. A subjective indication of the degree of chromophilia in the three groups was obtained from these sections.

Measurements of Neurons

Photographs of right and left ventral horns from each animal were taken at 100X magnification using a Zeiss photomicroscope. Negatives obtained from this procedure were mounted in slide holders and the images of ventral horns were projected onto sixteen by twenty-four inch sheets of paper. The projected images of the neuron cell body, nucleus, and nucleolus was then outlined. Areas of the traced cellular features were measured with a compensating planimeter. The average of three consecutive readings was taken as the final area for each cell feature.

A stage micrometer was also photographed and the negative projected under the same conditions to determine the magnification of the traced neurons for conversion to square microns. Approximately 2000 ventral motor neurons were measured without knowledge of the experimental group from which they came. The mean areas for each experimental group were then computed and converted to square microns.

Evaluation and Analysis

Histochemical

Histochemically the motor neurons, in two to five sections of spinal cord per animal, were graded into dark, medium and light staining cells according to intensity of reaction. All ratings were made without knowledge of the treatment groups. The percentages of dark, medium and light staining cells were computed for each animal as well as the mean percentages for each treatment group (Appendix A). Acid phosphatase reactions permitted grading of motor neurons into only dark and light categories. Approximately 2000 ventral motor neurons were rated for each enzyme studied.

The resulting data were analyzed using a one-way analysis of variance (Guenther, 1964). Differences in the percentages of dark, medium and light staining cells were examined among the three treatment groups. When the F-ratio was significant the Duncan multiple range test was

used to determine which means differed (Bliss, 1967).

One-way analysis of variance performed on the percentages of dark, medium and light staining cells in these treatment groups involves an inherent interdependence of data. For example, determining percentage of dark staining cells of one group limits the percentages of medium and light staining cells of that group. However, there is independence of data within each test.

Reliability Data

One month following the initial evaluation a second determination of the percentage of dark staining neurons was performed on approximately one third of the animals selected randomly from each group (Appendix A). A linear correlation was employed to determine the correlation between the percentages of dark staining neurons obtained on the first and second determinations.

In addition to the evaluation stated above, the animals of each group were rated from one through four based upon an overall impression of enzyme activity in the motor neurons of the sections examined (Appendix A). This evaluation served to indicate whether or not changes in the percentages of dark, medium and light neurons were reflected in an overall subjective impression of their staining intensity. It was apparent that a change in enzyme activity could occur in all ventral motor neurons

without alteration in percentages of dark, medium and light cells. These subjective ratings were tested using a contingency chi-square to determine significant trends.

Measurement Data

Data involving area measurements (Appendix C) was analyzed using a one-way analysis of variance (Guenther, 1964). Differences were examined in regard to areas of cell bodies, nuclei, and nucleoli among the three experimental groups. When the F-ratio was significant the Duncan multiple range test was utilized to determine which means differed (Bliss, 1967). One-way analysis of variance tables for both histochemical and area data are recorded in Appendix B.

RESULTS

Histochemical Results

Cholinesterase

The ventral motor neurons of all experimental groups showed an intense staining reaction at the periphery of the cell bodies (Figs. 1, 2, 3). The staining reaction throughout the remaining cell body was less intense but uniform. Less cholinesterase activity was observed in the nucleus than in the surrounding perikaryon, however, the nucleolus exhibited a very strong reaction (Figs. 13, 14).

The neurons of the ventral horn differed from one another in staining intensity of their cytoplasm. On the basis of these differences the neurons were categorized into dark, medium and light staining cells. The staining reaction of the perikaryon was the most obviously affected feature of neurons in the treatment groups for all enzymes studied.

The ventral horns of the sedentary group (A) of animals demonstrated a preponderance of moderate to heavy staining cells (Fig. 1). The nuclei showed little enzyme activity while the nucleoli were heavily stained.

The sedentary-forced group (B) showed a marked decrease in cholinesterase activity in the perikaryon as well as in the nucleoli of these neurons. The intense cholinesterase activity at the periphery of the cells appeared to be little affected in these animals and therefore appeared even more pronounced in cells showing decreased activity in the perikaryon (Fig. 2).

The ventral motor neurons of the voluntary-forced group demonstrated staining reactions similar to those of the sedentary group with the exception that the dark staining neurons displayed an even greater intensity in the perikaryon and nucleolus than those of the sedentary group.

Table 1 demonstrates the changes in percentages of dark, medium and light staining cells in the three experimental groups. Each group representing the evaluation of six to seven hundred ventral motor neurons. The lower portion of Table 1 shows the F-ratios obtained in each category, from a one way analysis of variance and secondly which groups differed significantly using the Duncan multiple range test.

The most marked alteration in activity occurred in the sedentary-forced (B) group (Table 1). The percentages of dark and medium staining cells were significantly lower than in the sedentary group while the percentage of light intensity cells was significantly higher than in the

sedentary group. No significant difference in the proportions of cell intensity categories was observed between the sedentary and voluntary-forced groups. However, in subjective evaluations the neurons and neuropil of the voluntary-forced group appeared more intensely stained than the sedentary group which is reflected in a frequency distribution of subjective ratings shown in Table 2.

Table 1

CHOLINESTERASE

Mean percentages of dark, medium and light staining neurons in three experimental groups

Group	% Dark Cells	% Medium Cells	% Light Cells
A - sed.	30.98	51.57	17.45
B - sed. forced	8.02	35.84	56.13
C - vol. forced	49.23	35.01	16.35

	F	.05	Between Groups	
% D	35.75	s*	A - B	s*
% M	11.67	s*	B - C	s*
% L	56.00	s*	A - C	ns*

s* - significant
ns* - non-significant

Subjectively the motor neurons from each animal were collectively given a rating from one through four based upon an overall impression of their staining intensity (Appendix A). The intensities were rated as follows:

1. light
2. moderate
3. intense
4. very intense

The following table represents the distribution of these ratings in the three treatment groups.

Table 2

Frequency distribution of subjective ratings for cholinesterase activity in treatment groups

Intensity Rating	Group A Sed.	Group B Sed.-Forced	Group C Vol.-Forced
1	1	9	0
2	6	2	1
3	5	1	9
4	0	0	2

The subjective data for cholinesterase activity does not satisfy the basic assumption for a contingency-chi-square test (not more than 20% of the cells may have a frequency of less than five). The frequency of distribution, however, is in an expected direction based upon the percentage data presented previously.

Pseudocholinesterase activity in the ventral horns and ventral motor neurons in all three groups was observed to be very slight. Distinguishing individual neurons within the equally light staining neuropil was difficult (Fig. 19). The paucity of pseudocholinesterase activity in the ventral motor neurons suggested that the intense enzyme activity, described in the experimental groups of animals as cholinesterase activity, was predominantly that of acetylcholinesterase. The reactivity demonstrated by acetylthiocholine procedures in this study will, therefore, be referred to as acetylcholinesterase (AChE) activity.

Glucose-6-Phosphate Dehydrogenase (G-6-PD)

The activity of G-6-PD was observed to be considerably stronger in the ventral motor neurons than in the surrounding neuropil in all treatment groups. The intensity of staining throughout the perikaryon was characteristically uniform. Absence of activity in the nucleus and nucleolus was evident in all neurons. Different staining intensities between the neurons permitted their classification into three intensity groups (dark, medium and light). The mean percentages of cells in each category per animal is given in Appendix A.

Table 3 demonstrates the mean percentages of dark, medium, and light staining neurons representing G-6-PD activity in each treatment group. Five to six hundred

neurons were evaluated for each group. The F-ratio and significance between groups are recorded at the bottom of the table.

Table 3

GLUCOSE - 6 - PHOSPHATE DEHYDROGENASE

Mean percentages of dark, medium and light staining neurons in three experimental groups

Group	% Dark Cells	% Medium Cells	% Light Cells
A - sed.	34.94	50.87	14.16
B - sed. forced	17.19	30.52	59.60
C - vol. forced	26.92	54.15	16.93

	F	.05	Between Groups	
% D	5.20	s*	A - B	s*
% M	7.84	s*	B - C	s*
% L	30.49	s*	A - C	ns*

*s - significant
*ns - non-significant

The overall picture observed with the percentage data (Table 3) is very similar to that observed in Table 1 for cholinesterase. The sedentary-forced group again

exhibits a marked decrease in the percentages of dark and medium staining neurons and an increased percentage of light staining cells. The voluntary-forced animals do not differ significantly from the sedentary animals. The differences in G-6-PD activity of the three groups is illustrated in Figs. 4, 5 and 6.

Subjectively, a rating (one through four) was assigned to the neurons of each animal based upon an overall impression of reactivity (Appendix A). The intensities were rated in a manner similar to that used for cholinesterase:

1. light
2. moderate
3. intense
4. very intense

Table 4 below illustrates the frequency distribution of the assigned ratings for G-6-PD in the three experimental groups. The distribution is similar to that in Table 2 for cholinesterase.

Table 4

Frequency distribution of subjective ratings for G-6-PD activity in treatment groups

Intensity Rating	Group A Sed.	Group B Sed.-Forced	Group C Vol.-Forced
1	1	5	0
2	7	8	7
3	8	1	7
4	1	0	23

Significant at .05 level.

Collapsing this data by combining the intensity ratings 1 with 2 and 3 with 4 permitted a contingency chi-square to be used to determine significance. A significant difference was determined between the distribution of ratings in the treatment groups. The sedentary-forced group demonstrates a significant decrease in G-6-PD activity.

A slight increase in G-6-PD activity in the voluntary-forced group is indicated by the frequency distributions of the sedentary and voluntary-forced groups.

Acid Phosphatase (A.Pase)

Acid phosphatase activity was observed in all ventral motor neurons examined. Generally, these showed discrete staining throughout their cytoplasm. Increased staining at specific sites in the cytoplasm was not a consistent feature of the sedentary and voluntary-forced groups but many cells of the sedentary-forced group demonstrated an increased perinuclear staining (Fig. 8). Nuclear and nucleolar activity was absent in all neurons.

The variation in staining intensity between neurons was small but permitted their classification into two intensity categories designated dark and light. Approximately eight hundred neurons were evaluated and the mean percentages of dark and light neurons were obtained for each group. The results of this evaluation are given in Table 5. The F-ratios and the significance between groups are

indicated in the lower part of the table.

Table 5

ACID PHOSPHATASE

Mean percentages of dark and light staining cells in three experimental groups

Group	% Dark Cells	% Light Cells
A - sed.	25.93	74.11
B - sed. forced	40.43	59.51
C - vol. forced	23.71	76.27

	F	.05	Between Groups	
% D	5.17	s*	A - B	s*
% L	4.16	s*	B - C	s*
			A - C	ns*

*s - significant
*ns - non-significant

The results of this evaluation show an increased acid phosphatase activity in the neurons of the sedentary-forced group of animals. This is in contrast to the results of CHE and G-6-PD reactivity observed in this group. However, it is again the sedentary-forced group which has demonstrated the most marked alteration in

enzyme activity. The voluntary-forced group shows no significant change in the percentages of dark and light neurons from the sedentary animals.

The reactivity of acid phosphatase was subjectively rated as in the evaluation for CHE and G-6-PD. Intensity ratings of one through three were employed:

1. light 2. moderate 3. heavy

The frequency distribution within the three experimental groups is shown in Table 6.

Table 6

Frequency distribution of subjective ratings for A.Pase activity in treatment groups

Intensity Rating	Group A Sed.	Group B Sed.-Forced	Group C Vol.-Forced
1	8	1	8
2	8	9	8
3	3	5	1

Significant at .05 level

The distribution was collapsed by combining intensity ratings 2 and 3 in order to utilize a contingency chi-square. A significant difference was obtained among the frequency distributions of the treatment groups. The results of this evaluation are in agreement with the percentage data (Table 5) showing a significant increase in

A.Pase activity in the sedentary-forced group with no significant difference between the sedentary and voluntary-forced groups.

Malic Acid Dehydrogenase (MDH)

Malic acid dehydrogenase activity was observed to be uniform throughout the cytoplasm. Many neurons of the voluntary-forced group of animals showed an increased staining intensity at or near axonal and dendritic origins. Nuclei and nucleoli demonstrated no enzyme activity (Fig. 15).

Table 7 illustrates the percentages of dark, medium and light staining cells obtained in each experimental group of animals and the F-ratio obtained for each intensity category. No significant differences were obtained between the experimental groups.

Table 8 illustrates the frequency distribution of subjective ratings assigned to the neurons of each animal. The overall intensities were rated as follows:

1. light
2. moderate
3. intense
4. very intense

Table 7

MALIC ACID DEHYDROGENASE

Mean percentages of dark, medium and light staining cells in three experimental groups

Group	% Dark Cells	% Medium Cells	% Light Cells
A - sed.	12.94	51.58	34.44
B - sed. forced	20.67	53.14	26.16
C - vol. forced	30.29	50.95	23.90

	F	.05
% D	1.72	ns*
% M	.07	ns*
% L	1.11	ns*

*ns - non-significant

Table 8

Frequency distribution of subjective ratings for MDH activity in treatment groups

Intensity Ratings	Group A Sed.	Group B Sed.-Forced	Group C Vol.-Forced
1	9	4	2
2	8	7	5
3	3	5	9
4	0	1	3

Significant at .05 level

A contingency chi-square performed on this data, after collapsing 1 with 2 and 3 with 4 intensity categories, showed a significant difference between the distributions in the treatment groups. A trend toward greater activity is observed in groups B and C. The subjective ratings assigned to the general reactivity in these groups may have been influenced by the increased MDH activity evident in the neuropil immediately surrounding the neurons of the exercise groups. On the other hand, a trend toward increased MDG activity in neurons of both sedentary-forced and voluntary-forced groups could conceivably occur without an accompanying change in the relative proportions of dark, medium and light staining neurons in these groups.

Reliability Data

The percentage of dark staining neurons in each treatment group was determined in two separate evaluations. This was done for each enzyme and on approximately one-third of the animals of each group (Appendix A). The correlation between the two sets of data for each enzyme is recorded in Table 9.

Table 9

Correlation from reliability data

Enzymes	Correlation
CHE	80
G-6-PD	93
A.Pase	87
MDH	98

Morphological Results

Nissl Staining

Luxolfast blue and toluidine blue staining of ventral motor neurons showed almost identical results. Neurons of the sedentary group of animals demonstrated moderate to heavy staining with an intensely staining nucleolus. Those of the sedentary-forced group demonstrated a distinct chromophobic reaction with an indistinct and lightly staining nucleolus. The Nissl substance was less coarse than that of the sedentary group. Neurons of the voluntary-forced group of animals showed a more intense staining of Nissl substance which generally obscured the nucleus (Figs. 10, 11, 12, 17, 18). A deeply stained nucleolus, however, could be observed in these cells. The intensity of staining in the voluntary-forced group was generally more intense than that observed in the sedentary group of animals.

Measurements of Neurons

The mean areas of ventral motor neurons, their nuclei and nucleoli in each of the treatment groups are given in Table 10. The mean areas for each cell feature per animal is recorded in Appendix C.

Approximately two thousand ventral motor neurons were measured. The F-ratios for each cell feature are given at the bottom of the table.

Table 10

Mean areas of ventral motor neurons in Square Microns

Cell Feature	Group A Sed.	Group B Sed.-Forced	Group C Vol.-Forced
Cell Body	271.2398	244.2521	234.8018
Nucleus	63.5164	66.6968	59.2456
Nucleolus	12.1762	17.5374	10.9041

	F	.05	Between Groups (Nucleoli)	
Cell Body	1.88	ns*	A - B	s*
Nucleus	.50	ns*	B - C	s*
Nucleolus	5.55	s*	A - C	ns*

*s - significant
*ns - non-significant

The results recorded in Table 10 do not show a significant difference between the mean areas of cell bodies in the three treatment groups. The mean for group B, however, is less than that of A and the mean area of group C is less than that of group B. Although the differences are not significant at the 0.05 level, a decrease in the mean areas of groups B and C is apparent, and was the only feature that paralleled the increasing levels of functional activity.

The differences between the mean areas of the nuclei are not significant at the 0.05 level. The nucleolus, however, shows a marked increase in area in the sedentary-forced group of animals, while the nucleolar area of the voluntary-forced group of animals does not differ significantly from that of the sedentary group.

Body Weights

The final body weights for each animal are recorded in Appendix C. The mean body weights for each group are given in Table 11. A significant decrease in body weights occurred in the sedentary-forced group and an even greater decrease in the voluntary-forced group.

The percent correlation between final body weights and mean neuronal areas is very low in all three groups.

Table 11

Mean body weights and per cent correlation between final body weights and mean neuronal areas

	Group A Sed.	Group B Sed.-Forced	Group C Vol.-Forced
Mean Body Weight*	424.30	371.52	333.63
% Correlation	.07	31	13

* A>B>C - significant at .05 level

DISCUSSION

With one exception the intracellular distribution of AChE described in this study is in agreement with other histochemical and quantitative biochemical studies. Giacobini (1959) analyzing microdissected parts of motor neurons found no activity in the nucleoli of the cells. Intense staining of the nucleolus for AChE in neurons of all experimental groups was evident in this study and is in agreement with intense activity of AChE in ventral motor neuron nucleoli reported by Roessmann and Friede (1967). The reason for the disparity between the quantitative findings of Giacobini and those of histochemical studies is not clear.

The neurons displayed an intense reactivity at the periphery of the cell bodies, with a lesser activity in the perikaryon. This was consistent with the internal and external fraction of neuronal AChE (with respect to the cell membrane) described by Koelle et al., (1956) and Koelle (1962). It has been demonstrated that anti-cholinesterase agents inhibit only the external fraction in sympathetic ganglion cells and was therefore termed "functional AChE." The internal fraction, probably associated with the endoplasmic reticulum (Fukuda and Koelle, 1959; Toschi, 1959)

could be inactivated without immediate apparent effects and was called "reserve AChE" (McIsaac and Koelle, 1959).

Sedentary-Forced Group

The decreased AChE activity exhibited in the sedentary-forced group of animals was apparently the result of a reduction in the "reserve AChE." The decreased activity was probably the result of diminished production of the enzyme by the cell or a rapid depletion due to transport along the axon to the motor end plates with increased muscular activity. In contrast, the cell bodies of the voluntary-forced group, in which the exercise program was more severe, exhibited increased AChE activity. A diminished production, rather than depletion by utilization, would therefore appear to be an important factor in the decreased AChE activity in the neurons of the sedentary-forced group of animals.

Nissl substance of the cell bodies of the sedentary-forced group exhibited a chromophobic reaction with the nucleus containing an enlarged pale staining nucleolus. During continued functional activity RNA is used up, the breakdown exceeding synthesis which presents a typical chromophobic appearance of the nerve cells (Vraa-Jensen, 1957). However, chromophobia may also indicate a condition of increased protein production. Regenerating neurons demonstrate an increase in organic cell material during a

period when the amount of RNA remains unchanged and the neurons present a typical chromatolytic appearance (Brattgard, Edstrom and Hyden, 1957). The chromatalytic appearance suggesting a transformation of Nissl substance to a more active form rather than a reduction in amount. The reduced AChE and G-6-PD activity in the sedentary-forced group of this study would suggest a diminished amount of functioning RNA.

Edstrom (1957) studied the stainability of Nissl substance in the ventral motor neurons of guinea pigs exhausted after a single running exercise period. He also studied the neurons of animals after a prolonged exercise program. The neurons of Edstrom's exhausted animals showed a chromophobic reaction in their Nissl staining very similar to those of the sedentary-forced group of the present study with the exception that the nucleoli of the exhausted animals were deeply staining and apparently unaffected. The nucleoli of the sedentary-forced group in this study were markedly enlarged and pale staining. That the neurons of exhausted animals resembled very much the neurons of the sedentary-forced group in this study also suggests a poorly adapted state of the cells to the increased activity.

An increased acid phosphatase activity was observed in the neurons of the sedentary-forced group. This finding substantiates the close relationship of acid phosphatase and the breakdown of Nissl substance (LaVelle et al., 1954),

since the neurons of this group show a chromophobic response. The decreased levels of glucose-6-phosphate dehydrogenase activity is suggestive of a decreased RNA production. This enzyme belongs to the pentose phosphate shunt which is an important pathway for the production of ribose sugars, necessary for the synthesis of RNA. No significant change was observed in malic acid dehydrogenase activity in this group of animals from those of the sedentary group. Shapot (1957) suggested that energy produced in a nerve cell is utilized for structural metabolism (renewal of cell structures and protein production) and for specialized functional metabolism. It is possible that during increased functional activity most of the energy produced by the cell would be used to satisfy the functional demands of the neuron at the expense of protein synthesis at least until a metabolic adaptation to the increased activity could take place.

Voluntary-Forced Group

Although the animals of this group were subjected to a more severe and more continuous exercise program for the same time period, their motor neurons showed a metabolic response quite different from those of the sedentary-forced group. The ventral motor neurons demonstrated an overall increase in AChE activity above that of the sedentary animals as well as increased glucose-6-phosphate dehydrogenase activity. A chromophilic response was

observed in the Nissl substance of these cells as well as an intensely staining nucleus and nucleolus. Diminished acid phosphatase activity corresponding to the chromophilic response was also observed.

The neurons of guinea pigs (Edstrom, 1957) subjected to a prolonged exercise treatment by treadmill running (exercised thirty-two hours in intervals during twenty-nine days) showed a staining of Nissl substance comparable to that of sedentary animals. The nucleoli of these neurons were enlarged and pale staining. The voluntary-forced group of the present study showed a more intense staining of Nissl substance than their sedentary control counterparts and the nucleoli were deeply stained. The increased basophilia in the cytoplasm of these ventral motor neurons as well as the enzymatic changes are consistent with neurons adapted to an increased level of activity.

It is the author's opinion that the opposite metabolic responses obtained between the sedentary-forced and voluntary-forced groups is a result of the different exercise programs to which these animals were subjected. The sedentary-forced group was vigorously exercised for a thirty-minute period per day and then forced to rest for the remaining twenty-three and one half hours. It would appear that each exercise period begins an adaptive phase by the neuron which then regresses during the resting

stage, preventing complete metabolic adaptation by the neuron.

The voluntary-forced group of animals, however, were exercised for two thirty-minute periods per day, and exercised at will in cages equipped with voluntary exercise wheels as demonstrated by activity records obtained from voluntary wheel revolution data. Under this more continuous type of activity the neurons were able to adapt even though the exercise program was more vigorous than that of the sedentary-forced group. In short, the neurons adapt with greater difficulty to the type of exercise programs imposed upon the sedentary-forced (B) group of animals, than that imposed upon the voluntary-forced (C) group.

Although the muscle fibers of the plantaris (Edgerton, 1968) showed a moderate adaptation by a significant increase in the proportion of red to white fibers, the neurons demonstrated a poorly adapted state by their enzymatic activity and chromophilic reactions. The sedentary-forced group of animals also demonstrated increasing difficulty in coping with their exercise program. This was demonstrated by their inability to keep up with the swimming pace for a thirty-minute period even though they swam with less weight (3% of their body weights) than the voluntary-forced group (4% of their body weights). It is also interesting to note that three of the four animals that died while swimming belonged to this sedentary-forced

group. The increased difficulty in swimming displayed by these animals was reflected in the ventral motor neurons rather than the skeletal msucle.

The exercise program to which the sedentary-forced group was subjected appeared to be detrimental to the ventral motor neurons as was reflected in the exercising capabilities of the animals. These results should be considered in treatment of muscle myopathies with exercise programs and in the design of experiments related to the effects of exercise treatments on pathological conditions such as muscular dystrophy.

Relationships to Muscular Results

Edgerton (1968) demonstrated an increase in the proportion of red to white muscle fibers in the plantaris muscle of these same experimental animals. A significant increase in this proportion occurred in the sedentary-forced group with an even greater increase in the voluntary-forced group. The results of a number of physiological studies concerned with neuromuscular relationships (see review of pertinent literature) suggest that ventral motor neurons innervating red motor units are smaller in size and functionally more active. In regard to size changes, the mean area of ventral motor neurons decreased in the sedentary-forced group and decreased to an even greater extent in the voluntary-forced group. Although these changes were

not significant at the 0.05 level, there was a consistent decrease in mean areas with increasing levels of activity. This was the only morphological feature studied which paralleled the increasing levels of functional activity and proportions of red to white fiber types as reported by Edgerton (1968). In judging the biological significance of this data, it must be remembered that the neurons occupied the region of the fourth, fifth and sixth lumbar segments of the spinal cord and innervated a large number of muscles in addition to the plantaris. Certainly, all of the muscles of the extremity would not be expected to be exercised to the same degree nor to demonstrate the same alterations as shown in the plantaris. However, results from the plantaris muscle suggest similar trends in other mixed skeletal muscle though not necessarily to the same degree. Muscles which were already predominantly red to begin with would not be expected to show this trend. For example, no alterations in the proportions of intermediate to red fibers were observed in the soleus muscles of the exercised groups (Edgerton, 1968).

Another factor which should be considered is the size of the motor unit. It has been shown, for example, that the average motor unit of the medial gastrocnemius of man contains approximately 1400 muscle fibers (Fernstein et al., 1955). An alteration of ten motor units from white to red represents a change in the intensity of staining of

14,000 muscle fibers in the medial gastrocnemius. Assuming a corresponding change in neuronal areas, the same alteration would be reflected in the areas of only ten motor neurons of the 4th, 5th, and 6th lumbar segments. It therefore seems reasonable that significant alterations of motor units should be more readily reflected in the proportions of red to white fibers of specific muscle rather than in the areas of neurons in the lumbar spinal cord.

Although the mean areas of neurons in the experimental groups suggest a decreasing size of the neuron with increasing levels of activity and increasing proportions of red to white fibers in the plantaris muscle, definite conclusions in this regard await more specific biochemical and morphological studies of neurons innervating specific muscles.

The ventral motor neurons innervating red motor units have been shown to be much more active in terms of excitability, susceptibility to discharge, and have been demonstrated to fire almost continuously in the resting extremity of an anesthetized animal (Wuerker, et al., 1965; McPhedran et al., 1965; Henneman et al., 1965). It might, therefore, be anticipated that an exercise program which increased the proportions of red muscle fiber types in skeletal muscle would be reflected in an increased proportion of neurons demonstrating intense activity of

enzymes related to energy production and function of the neuron. This concept was not immediately reflected in the histochemical findings of the sedentary-forced group of animals. Indeed AChE, an enzyme related to the function of alpha motor neurons, and glucose-6-phosphate dehydrogenase, related to energy metabolism and to ribose production for RNA synthesis, demonstrated a marked decrease in activity. The proportion of cells showing intense activity of these enzymes diminished greatly, and a general chromophobia was observed in the neurons of this exercised group. A possible explanation for these unexpected findings might be found in the fact that the motor neurons were functioning at an increased level of activity during the exercise periods, but were unable to adapt to the brief bouts of forced exercise separated by long periods of complete rest. The decreased proportions of enzymatically active cells was an unexpected manifestation of chronically increased activity and, in terms of functional activity, was consistent with the increased proportions of red to white muscle fiber types as indicated by the findings of Edgerton (1968).

Ventral motor neurons of the voluntary-forced group demonstrated increased proportions of active cells showing increased enzyme activity corresponding to a prolonged increased functioning and an accompanying increased metabolism.

Limitations of This Study and Suggestions For Future Studies

The functional significance of cell size in the nervous system has been emphasized and more specifically that of the ventral motor neurons has been related to the varied types of motor units in skeletal muscle (Henneman et al., 1965). This is based upon physiological evidence only in that the spike potential and speed of conduction are proportional to axonal diameter and the assumption that axonal diameter is directly related to the size of the neuron from which it arises. It is the author's opinion that investigations on the morphology of ventral motor neurons supplying red versus white skeletal muscle would be extremely valuable to further research in this area. It might then be possible to substantiate with morphological evidence the conclusions drawn from physiological data. Studies such as this would also provide a precise localization of neurons in the spinal cord which innervate specific muscles and would provide a more sound basis for correlating changes in ventral motor neurons with changes in muscle fiber types under various pathological and physiological conditions.

Similarily, a thorough histochemical investigation of neurons as related to the muscle fiber types they supply would be extremely valuable for future studies in this area of research as well as autoradiographic studies on RNA and protein turnover in these cells.

There is an absence of histochemical and morphological evidence for the existence of "red and white alpha motor neurons." An attempt, therefore, to relate changes in ventral motor neurons to alterations in the proportions of muscle fiber types is somewhat premature. However, the results of this study suggests changes in ventral motor neurons which are consistent with the current concepts of red and white motor units and the increased proportion of red to white fibers in the skeletal muscle of chronically exercised animals. It is the author's present ambition to investigate specifically the morphological and histochemical features of neurons supplying predominantly red or predominantly white muscles.

In considering the results of exercise on ventral motor neurons it is quite apparent that different intensities as well as different types of exercise programs may vary the morphological and histochemical features of motor neurons and that more studies of this kind would add greatly to an understanding of the effects of chronic exercise.

It became apparent, during the course of this study, that physiological analysis of the motor nerves, in regard to speed of conduction and after-hyperpolarization, would have provided a wider scope of information on which to interpret morphological and histochemical results.

SUMMARY AND CONCLUSIONS

Summary

Ventral motor neurons of sedentary and chronically exercised adult male rats were studied histochemically using CHE, BuChE, A.Pase, G-6-PD and MDH techniques. The mean areas of ventral motor neurons and the stainability of Nissl substance were also considered.

Sixty male Sprague-Dawley rats, 100 days of age, were placed into three groups. The control group (A) was housed in sedentary cages. The animals of a sedentary-forced group (B) were placed into sedentary cages but were exercised by swimming thirty minutes per day with a weight equalling three per cent of their body weights attached to the animals' tails. A voluntary-forced group (C) was housed in cages equipped with voluntary exercise wheels and received two thirty-minute forced exercise swims per day. The animals of this group swam with weights equal to four per cent of their body weights. The duration of the exercise treatment was fifty-two days.

Fresh frozen sections from the fourth, fifth and sixth lumbar segments were obtained for CHE, BuChE, A.Pase, MDH, and G-6-PD techniques. Formalin fixed, paraffin embedded sections were also taken for toluidine blue and

luxol fast blue neutral red techniques.

Histochemically the motor neurons were graded according to intensity and the percentages of neurons falling into each intensity category were recorded for each animal of the experimental groups. A subjective rating of overall intensity of activity in the neurons of each animal was also recorded. Measurements of neuron areas were obtained using a compensating planimeter upon projected negatives of ventral horns.

Approximately 2000 ventral motor neurons were evaluated for each enzyme and for area measurements without knowledge of the experimental groups from which they came.

The sedentary-forced group of animals demonstrated a diminished CHE, G-6-PD activities, and all animals of this group showed some degree of chromophobia in their cell bodies. An increased acid phosphatase activity was observed in these neurons. The animals of this group also exhibited increasing difficulty in coping with the exercise program.

The voluntary-forced group showed intense activity of CHE and G-6-PD in their neurons along with a deminished acid phosphatase activity. A chromophilic reaction was also observed in the neurons of this group.

BuChE, an enzyme of unknown function, showed very little activity in the ventral motor neurons of all three groups.

The mean areas of ventral motor neurons for the treatment groups decreased with increased levels of activity, however, the differences observed were not significant at the 0.05 level. The nucleolus of the sedentary-forced group exhibited a significant increase in size while that of the voluntary-forced group showed no significant change from the sedentary control animals.

The results of the present study are discussed in regard to the nature of the exercise programs and in the light of alterations in the proportions of red to white fiber types in skeletal muscle with chronic exercise.

Conclusions

The following conclusions were drawn of the results of this study.

1. That an exercise program consisting of short bouts of swimming, to which the sedentary-forced group of animals was subjected, presents a metabolic picture of neurons poorly adapted to the increased activity of the exercise periods.

2. Rats subjected to such an exercise program exhibit increasing difficulty in swimming a thirty-minute period and therefore become poorly adapted to this treatment.

3. Metabolically, neurons and the skeletal muscles they innervate do not appear to adjust simultaneously to an

exercise program such as imposed upon the sedentary-forced animals.

4. A more continuous and intensive exercise treatment results in neurons which appear to be metabolically better equipped to meet the demands of increased functional activity. Animals subjected to this treatment were also better adapted physically to the swimming periods.

5. That the increased proportions of cells showing intense enzyme activity and the decrease in the mean areas of cells in the voluntary-forced group of animals appears to be consistent with the increased proportions of red to white fiber types in exercised muscle, suggested by the results of Edgerton (1968). It is also consistent with the physiologically derived data that motor neurons supplying red fibers are smaller in size and functionally more active.

6. That another similar study should be done, but prefaced by studies on the histochemistry and morphology of neurons specifically related to red and white muscles and their specific location in the spinal cord of the rat. From such studies more definite conclusions could be drawn concerning the adaptability of the motor neuron, as an integral part of the motor unit, to physiological and pathological changes in functional activity.

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FIGURE 1

Cholinesterase activity in ventral motor neurons (arrows) of a sedentary animal. Increased activity is seen at the periphery of the cells and intense activity throughout the perikaryon. (340 X).

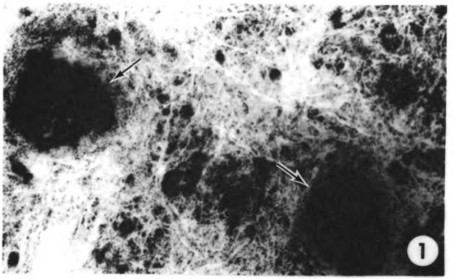
FIGURE 2

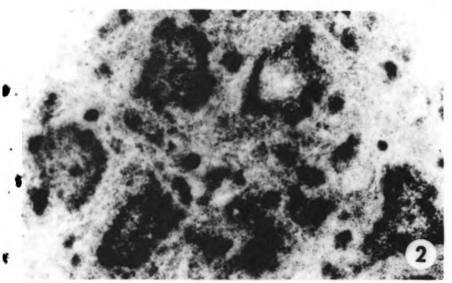
Cholinesterase activity predominant in the neurons of the sedentary-forced group of animals. Diminished activity is seen in the perikaryon while intense activity remains at the periphery. (340 X).

FIGURE 3

Ventral motor neurons of the voluntary-forced group of animals incubated for cholinesterase. Intense reactivity in both the perikaryon and periphery of the cell bodies is noted. (340 X).

PLATE I





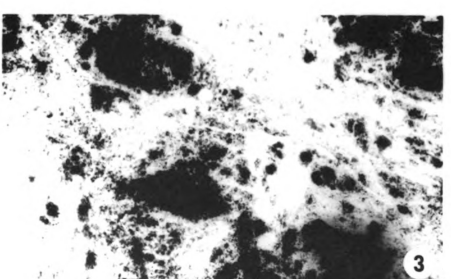


FIGURE 4

G-6-PD activity in the ventral motor neurons of a sedentary animal. Dark and medium staining cells are prevalent. (290 X).

FIGURE 5

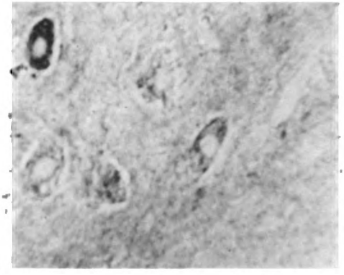
Activity of G-6-PD in the motor neurons of the voluntary-forced group showing increased numbers of light staining cells. One dark (upper left corner) and three light neurons (middle left) are seen in this figure. (290 X).

FIGURE 6

G-6PD activity in the ventral horn of a voluntary-forced animal. Increased numbers of dark and medium intensity cells are evident. Absence of nuclear and nucleolar activity was evident in cells of all groups. (290 X).

PLATE II

4



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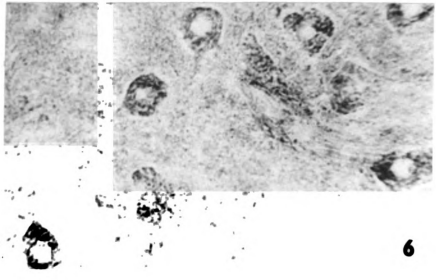

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FIGURE 7

Acid phosphatase activity in two neurons of the sedentary group of animals. Nuclear and nucleolar activity was absent from the cells of all groups. (320 X).

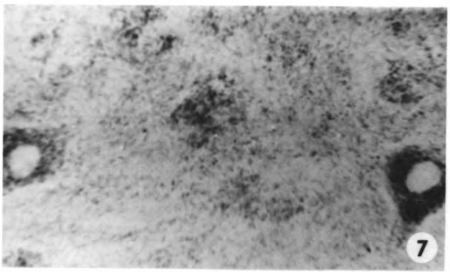
FIGURE 8

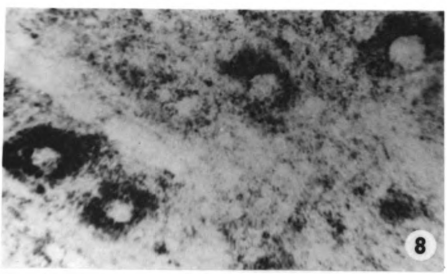
Increased acid phosphatase activity in neurons of the sedentary-forced group of animals. Increased staining intensity surrounding the nucleus is very evident in the neuron at the middle left margin of this figure. (320 X).

FIGURE 9

Diminished acid phosphatase activity in motor neurons of the voluntary-forced group of animals. Moderate and light staining cells are seen in this figure. (320 X).

PLATE III





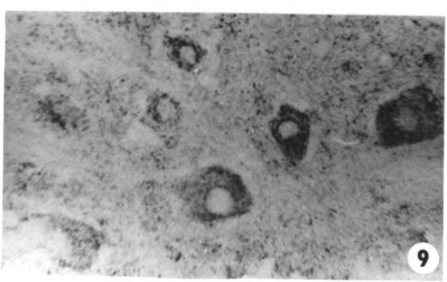


FIGURE 10

Nissl staining in ventral motor neurons of a sedentary animal. A deeply staining nucleolus and moderately stained Nissl substance were evident in these neurons. (toluidine blue - 320 X).

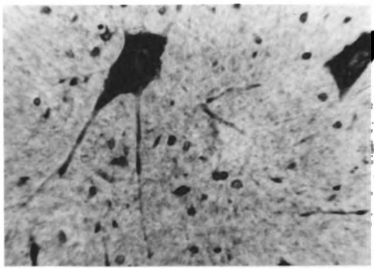
FIGURE 11

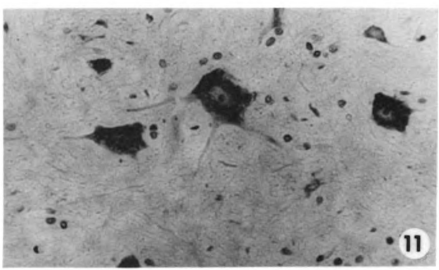
Chromophobic ventral motor neurons in the sedentary-forced group of animals. These neurons represent the most chromophobic reactions observed, however, a paler staining Nissl substance and a light staining nucleolus were constant features in neurons of this group. (toluidine blue - 320 X).

FIGURE 12

A severe chromophilic reaction in neurons of the voluntary-forced group of animals. Deeply stained nucleoli and intense staining of Nissl substance, often obscuring nucleus, are observed in these neurons. (toluidine blue - 320 X).

PLATE IV





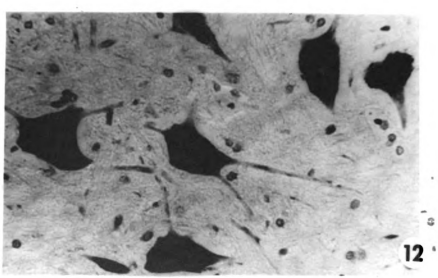


FIGURE 13

CHE activity in a medium staining cell from a sedentary-forced animal. Nucleolar activity is seen and moderate staining throughout the perikaryon. (710 X).

FIGURE 14

CHE activity in a ventral motor neuron of a voluntary-forced animal. Intense nucleolar staining is clearly demonstrated. Less activity is observed in the nucleus with greater activity in the remainder of the cell body. (710 X).

FIGURE 15

MDH activity in two motor neurons from the sedentary group of animals. Absence of nuclear and nucleolar activity and intense cytoplasmic activity was observed in all treatment groups. (460 X).

FIGURE 16

Acid phosphatase in a neuron from the sedentary group. An increased activity is observed at the base of a neuronal process. A complete absence of nuclear staining is seen. (520 X).

FIGURE 17

Toluidine blue stained neuron from the sedentary-forced group. A mild chromophobic reaction and a pale staining nucleolus can be seen. A distinct nuclear outline is also evident. (540 X).

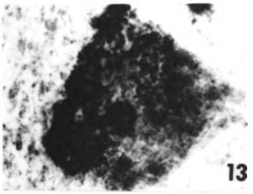
FIGURE 18

Toluidine blue stained neuron from the voluntary-forced group. A somewhat obscured nucleus and intensely staining Nissl substance and nucleolus can be seen. (540 X).

FIGURE 19

BuChE in the ventral horn from a voluntary-forced animal. A uniformly slight distribution is observed throughout the ventral horn with increased activity in blook vessels. (90 X).

PLATE V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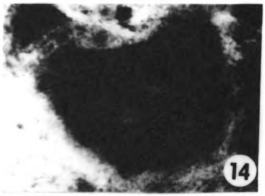
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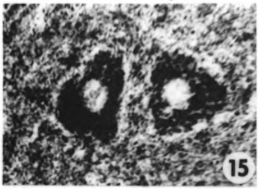
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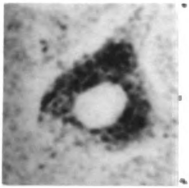
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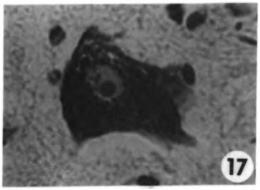
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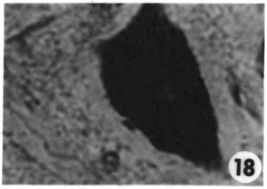
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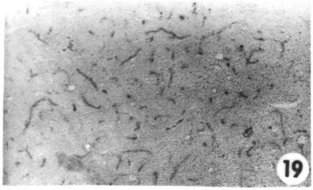
19

APPENDIX A

CHOLINESTERASE

Number of dark (D), medium (M) and light (L) staining cells per animal and their sum.

Group A					Group B					Group C				
A.N.*	D	M	L	Sum	A.N.*	D	M	L	Sum	A.N.*	D	M	L	Sum
6	7	12	7	26	29	1	6	10	17	21	37	12	1	50
34	7	11	8	25	55	1	5	9	15	5	40	15	2	57
48	16	14	5	35	45	9	18	12	39	43	7	5	2	14
14	16	14	5	35	28	10	19	13	42	51	10	7	2	19
44	23	22	4	49	25	1	8	14	23	31	30	20	7	57
42	24	23	6	53	29	1	10	15	26	41	31	21	7	59
12	11	35	9	55	35	2	23	25	50	17	32	22	15	69
8	9	37	7	53	3	4	26	27	57	56	29	18	13	60
15	16	28	12	56	22	3	15	56	74	9	24	18	17	55
52	19	29	13	61	19	2	13	56	71	50	22	17	13	52
59	15	54	14	83	38	3	23	44	70	16	21	36	16	73
4	16	56	15	87	11	5	26	46	77	1	20	34	14	68

*A.N. - Animal Number

CHOLINESTERASE

Per cent of dark (D), medium (M) and light (L) staining cells per animal and subjective rating (S.R.) of each animal.

Group A					Group B					Group C				
A.N.*	%D	%M	%L	S.R.	A.N.*	%D	%M	%L	S.R.	A.N.*	%D	%M	%L	S.R.
6	26.9	46.1	26.9	2	29	5.9	35.3	58.82	1	21	74.0	24.0	2.0	4
34	28.0	44.0	28.0	2	55	6.7	33.3	60.0	1	5	70.2	26.3	3.5	4
48	45.7	40.0	14.3	3	45	23.1	46.1	30.8	2	43	50.0	35.7	14.3	3
14	45.7	40.0	14.3	3	28	23.8	45.2	30.9	3	51	52.6	36.8	10.5	3
44	46.9	44.9	8.2	3	25	4.3	34.8	60.9	1	31	52.6	35.1	12.3	3
42	45.3	43.4	11.3	3	29	3.9	38.5	57.7	1	41	52.5	35.6	11.9	3
12	20.0	63.6	16.4	1	35	4.0	46.0	50.0	1	17	46.4	31.9	21.7	3
8	17.0	69.8	13.2	2	3	7.0	45.6	47.4	1	56	48.3	30.0	21.7	3
15	28.6	50.0	21.4	2	22	4.1	20.3	75.7	1	9	43.6	32.7	30.9	3
52	31.1	47.5	21.3	3	9	2.8	18.3	78.9	1	50	42.3	32.7	25.0	3
59	18.1	65.1	16.9	2	38	4.3	32.8	62.8	1	16	28.8	49.3	21.9	2
4	18.4	64.4	17.2	2	11	6.5	33.8	59.7	2	1	29.4	50.0	20.6	3

*A.N. - animal number

GLUCOSE - 6 - PHOSPHATE DEHYDROGENASE

Number of dark (D), medium (M) and light (L) staining cells per animal and their sum.

Group A					Group B					Group C				
A.N.*	D	M	L	Sum	A.N.*	D	M	L	Sum	A.N.*	D	M	L	Sum
6	4	5	2	11	55	4	10	12	26	21	8	18	0	26
34	15	16	0	21	23	9	8	17	34	5	0	11	8	19
20	23	4	0	27	28	3	11	19	33	51	14	10	21	26
48	3	15	5	23	25	15	25	8	48	39	8	19	0	28
14	10	13	5	28	29	2	9	31	42	43	4	13	3	20
44	15	23	10	48	35	0	11	23	34	31	13	15	6	34
42	39	21	0	60	3	3	17	16	36	41	30	24	2	56
12	11	31	12	54	24	0	0	32	32	17	23	20	15	58
8	17	17	4	38	40	0	5	21	26	56	23	15	3	41
37	15	35	1	51	53	6	9	13	38	57	0	13	3	16
30	12	18	3	33	36	7	14	17	38	2	7	27	7	41
26	3	4	5	48	60	0	2	19	21	33	22	23	0	45
13	14	26	2	42	46	0	1	15	16	49	14	35	7	56
10	0	13	6	19	22	3	20	12	35	32	3	13	4	20
27	1	13	8	22						47	1	15	7	23
15	14	21	0	35						9	4	17	23	44
52	7	4	6	17						50	3	11	3	17

*A.N. - Animal number

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Per cent of dark (D), medium (M) and light (L) staining cells per animal and subjective rating (S.R.) for each animal

Group A					Group B					Group C				
A.N.*	%D	%M	%L	S.R.	A.N.*	%D	%M	%L	S.R.	A.N.*	%D	%M	%L	S.R.
6	36.4	45.4	18.2	2	55	15.4	38.5	46.1	2	21	30.8	69.2	0.0	3
34	71.4	28.6	0.0	4	23	26.5	23.5	50.0	2	5	0.0	57.9	42.1	2
20	85.2	14.8	0.0	3	28	9.1	33.3	57.6	1	51	53.8	38.5	7.7	3
48	13.0	65.2	21.7	3	25	31.2	52.1	16.7	2	39	28.6	67.9	0.0	2
14	35.7	46.4	17.8	3	29	4.8	21.4	73.8	2	43	20.0	65.0	15.0	4
44	31.2	47.9	20.8	3	35	0.0	32.4	67.6	1	31	38.2	14.1	17.6	3
42	65.0	35.0	0.0	3	3	8.3	47.2	44.4	2	41	53.6	42.9	3.6	4
12	20.4	57.4	22.2	2	24	0.0	0.0	100	1	17	39.7	34.5	25.9	4
8	44.7	44.7	10.5	3	40	0.0	19.2	80.8	2	56	56.1	36.6	7.3	3
37	29.4	68.6	2.0	3	53	15.8	50.0	34.2	3	57	0.0	81.3	18.8	2
30	36.4	54.5	9.1	3	36	18.4	36.8	44.7	2	2	17.1	65.9	17.1	2
26	6.2	83.3	10.4	1	60	0.0	9.5	90.5	1	33	48.9	51.1	0.0	3
13	33.3	61.9	4.8	2	46	0.0	6.2	93.8	1	49	25.0	62.5	12.5	3
10	0.0	68.4	31.6	2	22	8.6	57.1	34.3	2	32	15.0	55.0	20.0	3
27	4.5	59.1	36.4	2						47	4.3	65.2	30.4	2
15	40.0	60.0	0.0	2						9	9.1	38.6	52.3	2
52	41.2	23.5	35.3	2						50	17.6	64.7	17.6	2

*A.N. - Animal Number

ACID PHOSPHATASE

Dark (D) and light (L) staining cells per animal and their sum

Group A				Group B				Group C			
A.N.*	D	L	Sum	A.N.*	D	L	Sum	A.N.*	D	L	Sum
34	46	29	75	55	23	29	52	51	11	36	47
54	6	30	36	23	13	19	32	39	9	26	35
20	6	40	46	28	13	22	35	43	5	45	50
48	8	37	45	45	17	50	67	31	18	47	65
14	4	26	30	25	13	14	47	41	19	57	76
44	15	27	43	29	35	21	56	17	10	44	54
42	25	22	47	35	34	57	91	56	32	37	69
12	47	42	89	3	14	40	54	57	24	30	54
8	23	39	62	24	29	12	41	2	11	54	65
37	14	57	71	53	42	30	72	33	16	52	68
30	14	52	66	60	12	40	52	49	26	60	86
26	19	66	85	46	23	34	57	32	17	56	73
13	8	52	60	22	9	49	58	47	9	53	62
10	6	37	43	38	16	39	55	9	4	26	30
27	15	53	68	11	12	34	46	50	12	41	53
15	15	34	49					16	19	52	71
52	5	38	43					1	6	49	55
59	9	53	62								
4	14	56	70								

A.N.* - Animal Number

ACID PHOSPHATASE

Per cent of dark (D) and light (L) staining cells
per animal and subjective rating (S.R.) of each animal

Group A				Group B				Group C			
A.N.*	%D	%L	S.R.	A.N.*	%D	%L	S.R.	A.N.*	%D	%L	S.R.
34	61.3	38.7	3	55	44.2	55.2	3	51	23.4	76.6	2
54	20.0	80.0	2	23	40.6	59.4	3	39	25.7	74.2	2
20	13.0	86.9	2	28	37.1	62.8	2	43	10.0	90.0	1
48	17.8	82.2	1	45	25.4	74.6	3	31	27.7	72.3	3
14	13.3	86.6	1	25	70.2	29.8	3	41	25.0	75.0	2
44	34.8	66.2	2	29	62.5	37.5	2	17	18.5	81.5	2
42	53.2	46.8	3	35	37.4	62.7	2	56	46.4	53.6	2
12	52.8	47.2	3	3	25.9	74.1	2	57	44.4	55.5	1
8	37.1	62.9	2	24	70.7	29.3	3	2	16.9	83.1	1
37	19.7	80.3	2	53	58.3	41.7	2	33	23.5	76.5	2
3	21.2	78.8	1	60	23.1	76.9	2	49	30.2	69.8	2
26	23.3	77.6	1	46	40.3	59.6	1	32	23.3	76.7	1
13	13.3	86.6	1	22	15.5	84.5	2	47	14.5	85.5	1
10	13.9	86.0	1	38	29.1	70.9	2	9	13.3	86.7	1
27	22.0	77.9	2	11	26.1	73.9	2	50	22.6	77.3	2
15	30.6	69.4	1					16	26.8	73.2	1
52	11.6	88.4	1					1	10.9	89.1	1
59	14.5	85.5	2								
4	20.0	80.0	2								

*A.N. - animal number

MALIC ACID DEHYDROGENASE

Number of dark (D), medium (M) and light (L) staining cells per animal and their sum

Group A					Group B					Group C				
A.N.*	D	M	L	Sum	A.N.*	D	M	L	Sum	A.N.*	D	M	L	Sum
6	3	25	13	41	55	24	18	1	43	21	19	33	6	58
34	27	12	11	50	23	2	17	3	22	5	1	18	13	32
54	0	7	13	20	28	0	3	27	30	51	0	1	25	26
20	2	12	5	19	45	11	22	0	33	39	11	7	2	20
48	4	15	18	37	25	7	11	1	19	43	16	6	2	24
14	6	12	6	24	29	8	31	5	44	31	8	28	13	49
44	11	31	15	57	35	0	30	14	44	41	23	33	4	60
42	13	29	7	49	3	0	9	23	32	17	60	29	21	56
12	0	8	36	44	24	31	21	1	53	56	0	26	18	44
8	0	9	17	26	40	10	23	5	38	57	36	9	0	45
37	14	31	2	47	53	7	21	18	46	2	42	14	0	56
30	3	49	16	68	36	3	15	13	31	33	16	31	7	54
26	0	22	19	41	60	0	31	17	48	49	9	24	16	49
13	3	25	14	42	46	15	41	1	57	32	1	32	10	43
10	0	22	16	38	22	0	25	20	45	47	2	47	12	61
27	7	37	18	52	38	6	34	10	50	9	2	35	7	44
15	0	39	11	50	11	34	34	0	68	50	0	27	22	49
52	0	20	18	38						16	10	30	14	54
59	0	15	21	36						1	17	35	1	53
4	35	34	0	69										

*A.N. - Animal Number

MALIC ACID DEHYDROGENASE

Per cent of dark (D), medium (M) and light (L) staining cells per animal and subjective rating (S.R.) for each animal

Group A					Group B					Group C				
A.N.*	%D	%M	%L	S.R.	A.N.*	%D	%M	%L	S.R.	A.N.*	%D	%M	%L	S.R.
6	7.3	61.0	31.7	1	55	55.8	41.9	2.3	3	21	32.5	66.0	10.3	3
34	54.0	24.0	22.0	2	23	9.1	77.3	13.6	2	5	3.1	56.3	40.6	3
54	0.0	35.0	65.0	2	28	0.0	10.0	90.0	1	51	0.0	3.8	96.2	1
20	10.5	63.2	26.3	2	45	33.3	66.5	0.0	2	39	55.0	35.0	10.0	3
48	10.8	40.5	48.4	2	25	36.8	57.9	5.3	4	43	66.7	25.0	8.3	3
14	25.0	50.0	25.0	1	29	18.2	70.5	11.4	3	31	16.3	57.1	26.5	3
44	19.3	54.4	26.3	3	35	0.0	68.2	31.8	2	41	38.3	55.0	6.7	4
42	26.5	59.2	14.3	3	3	0.0	28.1	71.9	1	17	10.7	51.8	37.5	2
12	0.0	18.2	81.9	1	24	58.5	39.6	1.9	3	56	0.0	59.1	40.9	2
8	0.0	24.6	55.4	1	40	26.5	39.5	34.0	3	57	80.0	20.0	0.0	4
37	29.8	66.0	4.3	1	53	15.2	45.7	39.1	2	2	75.0	25.0	0.0	4
30	4.4	2.1	23.5	2	36	9.7	48.4	41.9	2	33	29.6	57.4	13.0	3
26	0.0	53.7	46.3	1	60	0.0	64.6	35.4	1	49	18.3	49.0	32.7	3
13	7.1	59.5	33.3	2	46	26.3	72.0	1.8	2	32	2.3	74.4	23.3	2
10	0.0	57.9	42.1	1	22	0.0	55.6	44.4	1	47	3.2	77.0	19.7	2
27	13.5	71.2	15.4	3	38	12.0	68.0	20.0	2	9	4.5	79.5	15.9	2
15	0.0	78.0	22.0	1	11	50.0	50.0	0.0	3	50	0.0	55.1	44.9	1
52	0.0	52.6	47.4	2						16	18.5	55.5	25.9	3
59	0.0	41.7	58.3	1						1	32.1	66.0	1.8	3
4	50.7	49.3	0.0	2										

*A.N. - animal number

RELIABILITY DATA

Per cent of dark staining cells obtained in the first versus second evaluations of randomly selected animals

ACID PHOSPHATASE

Group A		Group B		Group C	
20.00	17.80	40.62	50.00	23.40	26.40
13.04	12.20	37.14	31.50	25.71	24.30
17.77	46.00	25.37	20.30	10.00	9.70
34.80	29.50	70.21	65.20	27.69	25.70
53.19	62.20	62.50	66.00	25.00	21.20
52.80	43.50	37.36	31.50	18.52	28.30

CHOLINESTERASE

28.00	23.56	6.66	11.80	20.17	62.00
45.71	50.00	23.07	18.90	50.00	50.00
46.93	32.70	4.34	13.60	52.63	54.00
16.98	16.60	7.01	13.20	48.33	49.30
31.14	32.40	2.81	4.80	41.30	44.50

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

0.00	0.00	0.00	0.00	15.00	17.80
71.42	62.50	15.38	17.40	0.00	8.00
85.18	54.70	9.09	6.25	28.57	27.70
13.04	25.60	4.76	2.00	20.00	27.50
65.00	47.50	31.25	24.50	53.57	58.10
31.25	33.30	0.00	6.45	38.23	40.50
20.37	26.70			39.65	43.50

RELIABILITY DATA Cont'd.

MALIC ACID DEHYDROGENASE

4.41	13.00	26.50	33.40	75.00	68.00
50.72	52.00	50.00	51.00	32.07	33.40
0.00	4.10	9.67	15.20	0.00	1.70
7.14	13.20	0.00	1.70	18.36	16.20
0.00	2.10	0.00	2.33	4.50	11.03
10.52	11.60			55.00	55.40

APPENDIX B

CHOLINESTERASE

One way analysis of variance tables.

Per cent dark staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	10,207.4422	5,103.7211	35.75
Within levels	33	4,710.2879	142.7359	
Total	35	14,917.7301		

Per cent medium staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	2,089.7027	1,044.8513	11.67
Within levels	33	2,955.1256	89.5492	
Total	35	5,044.8283		

Per cent light staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	12,320.4341	6,160.2170	56.00
Within levels	33	3,630.0694	110.0021	
Total	35	15,950.5035		

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

One way analysis of variance tables.

Per cent dark staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	4,541.4018	2,270.7009	5.20
Within levels	45	19,635.3543	436.3412	
Total	47	24,176.7561		

Per cent medium staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	4,887.9158	2,443.9579	7.84
Within levels	45	14,032.9766	311.8439	
Total	47	18,920.8924		

Percent light staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	19,313.7756	9,656.8878	30.49
Within levels	45	14,252.1292	316.7139	
Total	47	33,565.9048		

ACID PHOSPHATASE

One way analysis of variance tables

Per cent dark staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	3,188.1121	1,594.0560	5.17
Within levels	31	9,870.8276	308.4633	
Total	33	13,058.9397		

Per cent light staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	2,623.4490	1,311.7245	4.16
Within levels	31	10,096.6931	315.5216	
Total	33	12,720.1421		

Body Weight and Treatment Groups

One way analysis of variance

Source of Variance	Df.	SS	MSS	F
Among levels	2	80,975.12	40,487.56	40.30
Within levels	53	53,234.87	1,004.43	
Total	55	134,209.99		

MALIC ACID DEHYDROGENASE

One way analysis of variance tables

Per cent dark staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	1,586.4628	793.2314	1.72
Within levels	53	24,355.8290	459.5439	
Total	55	25,942.2918		

Per cent medium staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	45,2163	22.6081	.07
Within levels	53	17,815.6582	336.1440	
Total	55	17,860.8745		

Per cent light staining cells:

Source of Variance	Df.	SS	MSS	F
Among levels	2	1,199.2786	599.6393	1.11
Within levels	53	28,592.2361	539.4761	
Total	55	29,791.5147		

VENTRAL MOTOR NEURON AREAS

One way analysis of variance tables

Cell body:

Source of Variance	Df.	SS	MSS	F
Among levels	2	0.0155	0.0077	1.88
Within levels	51	0.2119	0.0041	
Total	53	0.2274		

Nucleus:

Source of Variance	Df.	SS	MSS	F
Among levels	2	0.0005	0.0002	0.50
Within levels	51	0.0206	0.0004	
Total	53	0.0211		

Nucleolus:

Source of Variance	Df.	SS	MSS	F
Among levels	2	632.0284	316.0142	5.55
Within levels	48	2,729.1034	56.8563	
Total	50	3,361.1318		

APPENDIX C

MEASUREMENTS

Mean Areas of ventral motor neurons per animal in square centimeters

Cell Body			Nucleus			Nucleolus		
A	B	C	A	B	C	A	B	C
.2390	.1966	.2231	.0573	.0552	.0643	.0100	.0127	.0133
.4240	.4100	.1937	.1290	.0972	.0511	.0258	.0290	
.2412	.1895	.2245	.0720	.0471	.0461	.0131	.0110	.0093
.3064	.2445	.1530	.0759	.0846	.0252	.0151	.0161	.0100
.1955	.2241	.1834	.0594	.0447	.0576	.0102	.0129	.0069
.4497	.2997	.2902	.0731	.0927	.0868	.0079	.0167	.0055
.2986	.2040	.2550	.0910	.0534	.0777	.0144	.0182	.0117
.2868	.3007	.2872	.0456	.0831	.0719	.0107	.0581	.0095
.2695	.2071	.2701	.0418	.0525	.0692	.0100	.0132	.0079
.2382	.2870	.3606	.0544	.0726	.0912	.0121	.0264	.0132
.2897	.3160	.2356	.0562	.1430	.0584	.0111	.0274	.0093
.3238	.2826	.3337	.0907	.0708	.0760	.0164	.0186	.0126
.2464	.1911	.3124	.0756	.0490	.0806	.0121	.0104	.0167
.2888	.3510	.2445	.0716	.0590	.0754	.0139	.0260	.0087
.2994	.2695	.2933	.0622	.0746	.0756	.0091	.0156	.0117
.2163	.2645	.1836	.0528	.0963	.0327	.0221	.0120	
.4212	.2610	.2611	.0959	.0664	.0624		.0135	.0157
.3391		.3467	.0543		.0704	.0137		.0137

MEANS PER GROUP

in^2	.2985	.2688	.2584	.0699	.0734	.0652	.0134	.0193	.0120
u^2	271.24	244.25	234.80	63.52	66.70	59.25	12.18	17.54	10.90

BODY WEIGHTS (grams)

A		B		C	
Animal Number	Body Weight	Animal Number	Body Weight	Animal Number	Body Weight
6	400	7	Died	21	352
34	452	55	364	5	316
54	435	23	280	51	326
20	459	28	369	39	304
48	420	45	386	43	353
14	449	18	Died	58	Died
44	436	25	419	31	318
42	423	29	300	41	322
12	412	35	325	17	342
8	428	3	376	56	309
37	413	24	383	57	315
30	473	40	384	2	348
26	487	53	382	33	371
13	419	36	399	49	370
10	421	60	357	32	358
27	378	46	394	47	321
15	325	22	400	9	312
52	420	19	Died	50	348
59	438	38	394	16	329
4	398	11	404	1	325