1981

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EFFECTS OF GLUCOCORTICOID TREATMENT ON THE PROPERTIES OF SINGLE MOTOR UNITS IN CAT HINDLIMB MUSCLES

by

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B.S. SUNY at Buffalo, 1974

Thesis

submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Department of Physiology at the Medical College of Virginia.

Virginia Commonwealth University

Richmond, Virginia

May, 1981

This thesis by Andrew James Robinson is accepted in its present form as satisfying the thesis requirement for the degree of Doctor of Philosophy.

Date: 27 April 81

Approved: [redacted]

Advisor, Chairman of Graduate Committee

April 27 1981 [redacted]

April 27, 1981 [redacted]

April 27, 1981 [redacted]

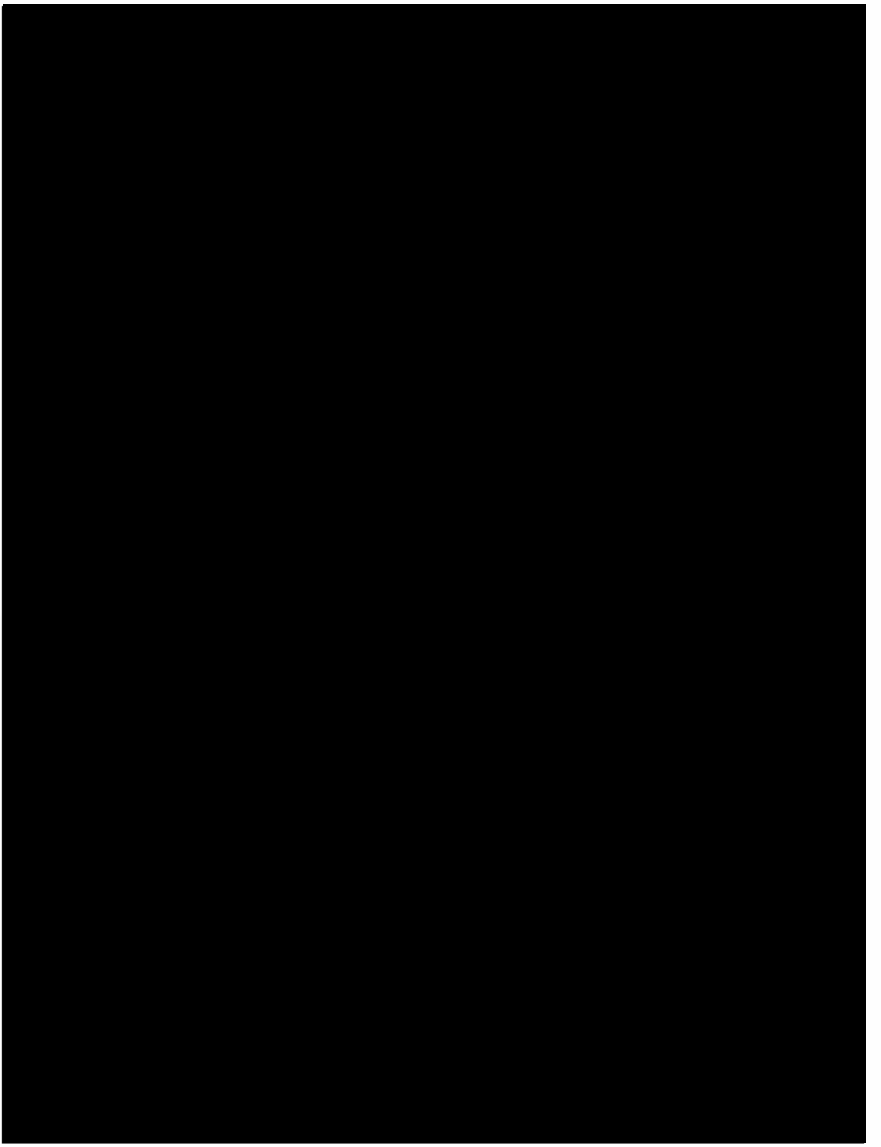
April 27 1981 [redacted]

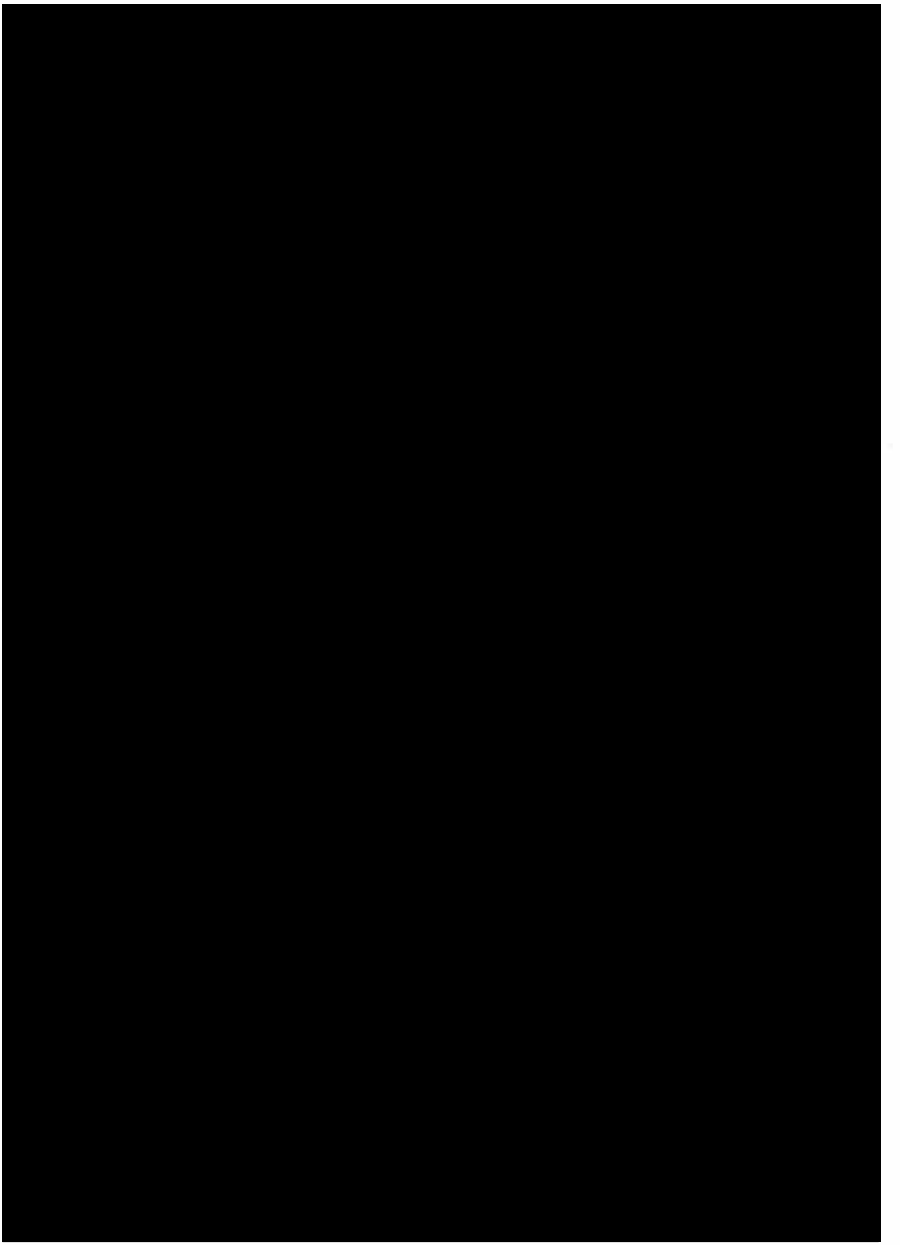
April 27, 1981 [redacted]

APPROVED: [redacted]

Chairman, MCV Graduate Council, Dean, School of Basic Sciences

Curriculum Vitae





Acknowledgements

I would like to express my appreciation to my friend and advisor, Dr. H. Peter Clamann for his guidance, assistance and patience over the past three years.

A special thanks to my classmates Linda Watkins, Phil Stein, and John McCaffie for their friendship and encouragement through all the hard times.

Also, my gratitude to Joan Moody and Laniel Dubose for their help in performing these studies.

Finally, I would like to acknowledge the generosity of the United Nuclear Corporation whose financial support of this student and project allowed this program to be completed.

To:

my wife, Sandra, for her unending support and sacrifice through the past four years

my little one, Tracy, whose presence has brought new meaning to my life

my parents for their lessons on perseverance and hard work and for their continual encouragement

Table of Contents

ABSTRACT

EFFECTS OF GLUCOCORTICOID TREATMENT ON THE PROPERTIES OF SINGLE MOTOR UNITS IN CAT HINDLIMB MUSCLES

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The administration of glucocorticoids has been shown to induce atrophy and weakness in whole skeletal muscle. These effects frequently appear to be more pronounced in pale muscle than in red muscle. The present study was undertaken to examine the effects of steroid treatment on the contractile, electrical, and fatigue properties of single motor units in pale and red cat hindlimb muscles. Single motor units in the medial gastrocnemius (MG) and soleus muscles were isolated using techniques developed by McPhedran, et al., (1965). The properties of a large number of motor units were examined in normal animals and were compared to those of single units from the muscles of steroid-treated (3-4 mg. of triamcinolone acetonide per kg. per day for 10 -16 days) animals. Motor units examined in both control and experimental animals were classified by type according to criteria developed by Burke, et al., (1973,1974). The results show that steroids produce alterations in the strength- and speed-related properties of motor units which are more pronounced in fast-twitch than in slow-twitch units. The mean maximum tetanic tension ($\overline{P_{max}}$) for types FF (fast-twitch, readily fatiguable) and FR (fast-twitch, fatigue-resistant) units in MG were reduced by 63% and 71% respectively. The $\overline{P_{max}}$ for type S units in MG was reduced by 25% as a result of steroid treatment but was unchanged for type S units in

soleus. The mean maximum rate of rise of tetanic tension ($\overline{dP/dt}$) for the three classes of motor units in MG was reduced in a pattern similar to that for tetanic tension. The $\overline{dP/dt}$ for types FF,FR, and S units in MG were reduced by 79%, 54%, and 25% respectively. The $\overline{dP/dt}$ for soleus units was increased by 73% as compared to control soleus units. Steroid treatment lengthened the mean twitch contraction time ($\overline{CT}$) of FF units and shortened the $\overline{CT}$ for type S units in both MG and soleus. The integrated EMG signals elicited from FF and FR units were unchanged following steroid treatment but were significantly increased for type S units in both pale and red muscles. Steroid administration did not markedly alter the susceptibility to fatigue of the three classes of units in MG or in soleus units. There were however fewer MG units with intermediate fatigue indeces in steroid-treated animals. These finding suggest that those units most frequently activated in muscular contractions are the least susceptible to steroid-induced changes in their contractile properties and vice versa. Since the overall frequency of activation of motor units is determined by cell size, these results imply that the Size Principle (Hennemann, et al., 1965) can be extended to not only explain the recruitment order of motor units in muscular contractions but also to account for the patterns of motor unit involvement in steroid-induced myopathy.

ABBREVIATIONS

MG: medial gastrocnemius muscle from control animals

MG_s: medial gastrocnemius muscle from steroid-treated animals

$soleus_s$: soleus muscle form steroid-treated animals

Motor unit types:

1) type FF: fast-twitch, readily fatiguable motor units
2) type FR: fast-twitch, fatigue-resistant motor units
3) type FI: fast-twitch, intermediate fatigue-resistant units
4) type S: slow twitch, fatigue-resistant units

Motor unit variables:

1) CV: axonal conduction velocity
2) CT: twitch contraction time
3) TT: twitch tension
4) P_{max}: maximum tetanic tension
5) dP/dt: maximum rate of rise of tetanic tension
6) EMG: electromyogram
7) IEMG: time integral of the full-wave rectified EMG signal
8) $\overline{(variable)}$: average value of variable

Dorsal and ventral root designations

1) L_6VR: sixth lumbar ventral root
2) L_7VR: seventh lumbar ventral root
3) S_1VR: first sacral ventral root
4) L_7DR: seventh lumbar dorsal root
5) S_1DR: first sacral dorsal root

INTRODUCTION

A. On the nature of skeletal muscle

For over two hundred years, it has been known that mammalian skeletal muscle is not a homogeneous tissue. In 1678, Stefano Lorenzini commented on the different types of muscles in animals and suggested that on the basis of their differences in color, they may be referred to as red and white muscles (Ciaccio, 1898; Denny-Brown, 1929). During the late 1800's, many workers attempted to explain this difference in muscle color on physiological and morphological grounds. Ranvier (1873, 1874, 1880) observed that the color and histological features of whole muscles were in many cases correlated with their speeds of contraction. Red muscles were found to contract slowly and white muscles contracted rapidly. He also noted that the blood supply in red muscles was more extensive than that in white muscles. During this same period, microscopic studies by Grützner (1884) and Knoll (1891) showed that the fibers comprising a single muscle were not uniform in character. The fibers of small diameter were darker and more granular in appearance than fibers of large diameter in the same muscle. On this basis, Knoll (1891) divided muscle fibers into two types; protoplasmareiche (rich in protoplasm) and protoplasmaarme (poor in protoplasm). Some muscles appeared to be composed entirely of either red or white fibers whereas in other muscles the fibers were mixed. Knoll was one of the first investigators to recognize the relationship between muscle fiber composition and muscle use. He found that muscles which were highly active (eg. ocular muscles and muscles of respiration and mastication) were composed primarily of small dark fibers. In contrast, muscles used less frequently were made up mainly of large, pale fibers. Thus, he commented on the

fact that in birds of flight, the breast muscles were red and leg muscles were white, in contrast to domestic fowl where the pectoral muscles were white and the leg muscles were red. Table I in Appendix A outlines the general morphological and functional properties of red and white muscles.

In the early part of this century, Bullard (1919) performed studies which provided the first indication that skeletal muscle was composed of not two but at least three types of muscle fibers. Using Sudan III stain for lipids, he showed that skeletal muscles consisted of light, intermediate and dark fibers and that the sizes and proportion of these fibers varied in different muscles. With the advent of improved histochemical techniques for the localization of muscle fiber oxidative and glycolytic enzymes, a large number of studies were performed, the results of which supported the hypothesis that skeletal muscles were composed of three fiber types. These techniques also enabled researchers to make direct correlations of the functional activity of individual fibers with a number of their morphological features. Table 2 in Appendix A displays several of the commonly used tripartite muscle fiber classification schemes developed by a variety of workers and shows the relationships between muscle fiber type and their structural and physiological properties.

The basic functional component of skeletal muscle is the motor unit. As defined by Sherrington and coworkers (Creed, et al., 1932), the motor unit consists of the cell body and axon of an alpha motoneuron and all of the muscle fibers innervated by the peripheral terminations of the axon. Based upon the histochemical evidence for three discrete fiber types and data obtained from experiments dealing with the contractile properties of single motor units, Henneman and Olson (1965) suggested that all muscle

fibers of one motor unit were of the same histochemically identified type and thus, that the motor units of skeletal muscle could also be divided into three types. In the late 1960's, Edström and Kugelberg (1968) using a method of glycogen depletion were able to successfully identify all of the muscle fibers belonging to an individual motor unit. They showed that all of the fibers of one unit displayed identical histochemical profiles, a result which directly supported Henneman's earlier hypothesis. Burke and coworkers (1967, 1971, 1973, 1974a, 1974b) have performed extensive studies on the mechanical and fatigue properties of single motor units in cat hindlimb muscles and have repeatedly shown that the histochemical profiles of motor units correlate well with their physiological properties. Based upon several physiological properties (contraction speed, shape of unfused tetanic responses, and sensitivity to fatigue), these workers were able to demonstrate that, in general, motor units fall into three major classes. The majority of motor units were found to be either 1.) fast-twitch and readily fatiguable (type FF), 2.) fast-twitch and fatigue-resistant (type FR) or 3.) slow-twitch and fatigue-resistant (type S). This scheme of motor unit classification was shown to be completely compatible with a tripartite muscle fiber classification system based upon the histochemical demonstration of myofibrillar ATPase (as an indication of contraction speed) and of the enzymes of intermediary metabolism (as an indication of susceptibility to fatigue) developed by Peter, et. al., (1972). Types FF, FR, and S units were found to be identical to muscle fiber types FG (fast twitch, high glycolytic activity), FOG (fast-twitch, high oxidative and glycolytic activity)and SO (slow twitch, high oxidative activity). Table 2 in Appendix A shows the relationship between motor unit and muscle fiber classification schemes

and the structural and physiological properties of identified motor units.

An important point to note in the consideration of nature of mammalian skeletal muscle is that the overall fiber compositions and physiological properties of skeletal muscle are not immutable. During the 1950's J.C. Eccles and associates had performed many studies to determine the pattern of monosynaptic connections to anterior horn cells by afferent nerve fibers entering the lumbar regions of the cat spinal cord (Buller and Pope 1977). Following these studies, Eccles designed a series of experiments to evaluate the extent to which the normal monosynaptic connections were modified by various surgical procedures performed on newborn kittens. In one of these studies, the nerves to two antagonistic hindlimb muscles (one slow twitch and one fast twitch muscle) were cut. The proximal cut end of one nerve was then sutured to the distal cut end of the opposite muscle nerve and vice versa. Following a period of about 10 months, the effects of this procedure (cross reinnervation) on the patterns of monosynaptic input of afferent fibers to motoneurons were reexamined in experiments identical to those performed in normal animals. No apparent changes had resulted from this procedure. At the conclusion of some of these experiments, Eccles decided to determine whether the muscles which had been acutely denervated had become successfully reinnervated. While stimulating the cross-reinnervated muscle nerves, Eccles noticed that the apparent speeds of contraction of the antagonistic muscles had changed. The originally fast-twitch muscles contracted much more slowly while the contraction speed of the initially slow twitch muscles was much faster. Quite by accident, Eccles became the first to demonstrate the marked plasticity of mammalian skeletal muscle (for personal account see Buller and Pope, 1977). The re-

port on this remarkable finding was first published in 1960 (Buller et. al. 1960).

Since this original finding, the plasticity of skeletal muscle has been the subject of study of a large number of investigators using a wide range of experimental interventions. The procedures which have been shown to produce either complete or partial alterations in the physiological properties and fiber composition of pale and red muscles include: (1)Cross-reinnervation (Barany and Close, 1971; Buller and Kean, 1973; Buller et al. 1969, 1971; Close, 1972; Cohen, 1978; Eccles et al. 1962; Fex and Jirmanová, 1969; Fex, 1969; Fex and Sonesson, 1970; Goth and Yellin, 1971; Hoh, 1975; Karpati and Engel, 1967; Lewis, et al. 1977; Luff, 1974, 1975; McArdle and Sansone, 1977; Mommaerts, 1968; Mommearts, et al. 1969, 1977; Prewitt and Salafsky, 1967; Robbins, et al. 1969; Romanul and VanderMuelen, 1966, 1967; Sreter et.al. 1975; Weeds et al. 1974; Yellin, 1967, 1975), (2)Tenotomy (Buller and Lewis, 1965; Engel, et al. 1965; Nelson, 1969; Vrbóva, 1962, 1963), (3)Immobilization (Booth and Kelso, 1973; Edström, 1970; Fischbach and Robbins,1969; Goldspink, 1977), (4) Denervation (Bajusz, 1964; Engel, et al.,1966; Gutmann, et al., 1972; Karpati and Engel, 1968; Lewis, 1972; Lomo, et al., 1974; Nachimias and Padykula, 1958; Syrovy, et al., 1971,1972), (5) Electrical stimulation (Al-Amood, et al., 1973; Heilmann and Pette, 1979; Lomo, et al., 1974; Munsat, et al., 1976; Peckham, et al.,1973; Pette, et al.,1973; Riley and Allin, 1973; Romanul, et al., 1974; Rubenstein, et al., 1978; Salmons and Sreter,1976; Salmons and Vrbova,1967,1969; Sreter, et al., 1974), (6) Dorsal root transection (Buller, et al., 1960; Riley and Allin, 1973), (7) Spinal cord transection (Caccia, et al., 1978; Davis and Montgomery, 1977; Hoh and Dunlop, 1975), (8) Exercise (for

review see Howald and Poortmans, 1975).

Alterations in the contractile and metabolic characteristics and histochemical profiles of mammalian skeletal muscle have also been seen during a variety of abnormal metabolic, nutritional, hormonal, and neural states. These include, for example, Cushing's syndrome, rheumatoid arthritis, spinal cord trauma, Parkinson's disease and a number of forms of muscular dystrophy (Brooke and Kaiser, 1974; see also Dubowitz and Brooke, 1973)

In general, the experimental interventions and clinical disorders which result in a "tonic" activation of pale muscle produce changes in these muscles such that their physiological and metabolic profiles resemble those of red muscle. In contrast, those procedures or disorders which dramatically reduce the overall activation or use of red muscles result in a shift of their contractile and metabolic properties toward those of a classically pale muscle.

Two hypotheses have been proposed to account for the plasticity of mammalian skeletal muscle. As a result of their original findings, Buller, et.al. (1960) suggested that the properties of muscle fibers were determined by either (1) a specific chemical agent (a neurotrophic factor) synthesized and released by the particular nerves innervating muscle fibers or (2) by the particular neural activity pattern impinging upon different groups of muscle fibers. To date, the dispute concerning the underlying mechanism which regulates the mechanical, biochemical and histochemical properties of muscle fibers and therefore the overall fiber composition and physiological properties of whole skeletal muscles has not been resolved. No specific neurotrophic factor contained in nerve terminals has been isolated which can account for the plasticity of skeletal muscle. In contrast, neural activity patterns have repeatedly been shown to be crit-

ical in determining the properties of the fibers in skeletal muscle. If one can tentatively accept the neural activity pattern hypothesis to account for skeletal muscle plasticity, the important question then becomes, "How are the neural impulse activity patterns to skeletal muscle different and how are they controlled?" This question will be addressed in the following section.

B. Role of skeletal muscle as a contractile machine

One of the major roles of skeletal muscle is the production of force. The generation of tension during muscular contraction may be viewed as the end result of a sequence of events beginning with the activation of a group of motoneurons lying within the spinal cord followed by transmission of nerve impulses along the axons to the muscle, transduction of nerve signals into muscle signals and subsequent activation of contractile processes within muscle fibers (Kukulka, 1978). The properties of any of the links in this chain of events are critical in determining the exact form of the tension produced during muscular contraction. It has been known for a long time that the muscle force produced during reflex and voluntary contractions is modulated by two primary mechanisms: muscle tension may be altered by the activation of a greater or lesser number of individual motor units (recruitment) and/or by changing the rate of discharge of single units already active in contraction (rate coding). Both the manner in which motor units are recruited and the overall frequency of discharge of motor units are determined by the threshold levels of excitation of alpha motoneurons which in turn are correlated with neuronal cell body size. It has been repeatedly demonstrated that the excitability of motorneurons is inversely proportional to cell size. In general, during

reflex or voluntary contractions, small motoneurons which innervate small and slowly contracting muscle units are recruited first, followed by progressively larger motoneurons which supply relatively large and rapidly contracting muscle fibers. As a consequence of this "size principle" as proposed by Henneman, et.al., (1965), weak contractions are produced by primarily small, red and non-fatigueable motor units. More powerful contractions result from the activation of large pale and readily fatigueable units which are recruited in addition to those small units already active. Thus, during normal activities, predominantly red muscles which are generally associated with the control of posture receive neural activation on an almost continuous basis. In contrast, predominantly pale muscles, associated with powerful phasic movements are called into action relatively infrequently. For both types of muscle the critical factor controlling their activation patterns is the size of the nerve cells which innervate their muscle fibers.

C. The role of skeletal muscle as an amino acid reserve

The second major role played by skeletal muscle is to serve as the primary protein and amino acid reserve of an organism. At maturity, skeletal muscle constitutes the largest single tissue in the human body and represents over 40% of body weight. Muscle protein comprises approximately 20% of the weight of skeletal muscle as a whole. By virtue of its role as a protein and amino acid reserve, under normal conditions muscle is an important tissue in homeostatic maintainance of an organism's metabolic processes and fuel supply. Because skeletal muscle is responsive to a variety of hormonal and nutritional states, this tissue is vital in the regulation of the supply of nutrients to other body tissues. During

nutritional or hormonal imbalances, skeletal muscle becomes essential to the survival of the organism. The mobilization of amino acids from muscle protein reserves during glucocorticoid excess (eg. Cushing's syndrome), relative or absolute insulin deficiency (eg. diabetes) or starvation (eg. cachexia secondary to carcinoma) represents an adaptation to these conditions essential for the continued function of relatively more vital organs. Amino acids released from muscle protein stores during conditions of stress can supply precursors for gluconeogenesis in the liver (Exton, et al., 1970), and kidneys (Kress, et al.,1963), as substrates for oxidation in muscle (Goldberg and Odessey, 1972), for lipogenesis in fat depots (Rudman and Digirolamo, 1970) and for the synthesis of new protein necessary to maintain the functional integrity of these tissues.

Although skeletal muscle plays a key role in the body's metabolic processes, relatively little is known about the mechanisms which regulate muscle fiber protein synthesis and degradation both at the cellular and systems levels even though these processes are likely to be subject to precise regulation (Goldberg, et al.,1980).

D. Effects of glucocorticoids on skeletal muscle

It has been shown that one way in which muscle protein synthesis and degradation can be altered is by the administration of steroid hormones. A common condition associated with spontaneous or iatrogenic glucocorticoid excess is the marked atrophy and wasting of skeletal muscles (Mayer and Rosen,1976). Patients with Cushing's syndrome or those undergoing prolonged glucocorticoid therapy often show a marked decrease in muscle mass with concomitant muscle weakness (Pleasure, et al.,1970). A variety of experimental animals receiving natural or synthetic steroids have also

been reported to develop a similar myopathy (Ellis,1956; Faludi,et al., 1964; Goldberg and Goodman,1969; Smith, 1964). Early laboratory and clinical observations indicated however, that the administration of corticosteroids did not affect skeletal muscles in a uniform manner. "Pale, phasic" muscles appeared to be more affected by steroid treatment as revealed by their greater loss in muscle weight and size than the "dark, tonic" muscles within the same animal (Goldberg and Goodman,1969). With the development of histochemical techniques to identify the fiber composition of whole muscles, Smith (1964) noted that pale fibers underwent more pronounced structural changes and atrophy than did the dark fibers within the same muscle in steroid-treated rabbits. The results of several subsequent studies supported the hypothesis that pale fibers were selectively or preferentially affected by the catabolic action of steroid hormones. Walsh and coworkers (1971) examined striated muscle from glucocorticoid-treated rats by enzyme histochemical techniques and electron microscopy. They found that the fiber diameter of histochemically-identified type II (fast twitch) fibers of the medial gastrocnemius were reduced by 18 - 30% while the diameter of type I (slow twitch) fibers in the soleus were decreased by only 5 - 11%. Pleasure and coworkers (1970) have also described a mixed fiber atrophy, type II greater than type I, in two patients with Cushing's syndrome. Gardiner,et al.,(1978) have presented evidence which demonstrated that the selective fiber atrophy noted in other species secondary to the action of glucocorticoids also occurred in steroid-treated cats. Their study revealed that the type IIb(FG) fibers responded with similar degrees of atrophy in both fast-twitch,pale and slow-twitch, red muscles. The type I(SO) and type IIa(FOG) fibers however, appeared to atrophy more in fast-twitch (least used) than in slow-twitch

(most frequently used) muscles. This muscle fiber atrophy pattern has been found to be similar to that seen in cases of food deprivation (Goldberg and Goldspink,1975), pyramidal tract disease(Brooke and Engel, 1969), rheumatoid arthritis (Brooke and Kaplan, 1972), muscular dystrophies (brooke and Kaiser, 1974) and a variety of other disorders.

Electrophysiological evidence presented by Gruener and Stern(1972) serves to support the findings from histochemical studies. In dexamethasone-treated mice, these workers found that the membrane excitability of type II fibers in the pale, extensor digitorum longus(EDL) was diminished and that many of these fibers could not generate action potentials. In contrast, the resting membrane potential of type I fibers in the red soleus muscle from treated mice was not significantly different than that in soleus type I fibers in control animals.

Although the physiological basis of preferential type II fiber atrophy seen in cases of steroid hormone excess, starvation and in other conditions has not been defined, many workers have hypothesized that a common mechanism may be involved which would explain these similar patterns of fiber atrophy. It has been suggested that selective type II fiber atrophy may be accounted for by the degree of reliance of different fibers on specific metabolic pathways for energy production(Vignos and Greene,1973). That is, the susceptibility of fast-twitch muscle to glucocorticoid-induced atrophy is associated with the degree of dependence of pale muscle on glycolytic metabolism for its energy supply, thus implying an effect on anaerobic pathways. The results of the work of Gardiner and coworkers (1978) argue strongly against this hypothesis. The alterations in the activity of phosphofructokinase in the pale medial gastrocnemius and red soleus muscle and the finding that type IIa (FOG)

and type I (SO) fibers in the same muscles atrophied to a similar extent failed to support the notion that glycolytic pathways were the primary focus for the differential action of steroids between the two muscles.

As an alternative hypothesis, other workers (Goldberg and Goodman, 1969) have suggested that selective fiber atrophy may result from the differences in the activity pattern between type I and type II fibers in different muscles. In support of this hypothesis, these workers found that steroid-induced fiber catabolism was more pronounced in less active muscles. The susceptibility of a muscle to the catabolic actions of steroid appeared to be inversely related to the level of muscular work or activity. Denervation of muscle which results in an absolute reduction in activity, enhanced the catabolic effects of corticosteroids on skeletal muscle. On the other hand, muscular work decreased the catabolic response of this tissue to glucocorticoids. From these findings, Goldberg and Goodman (1969) speculated that physiologically more active muscles, those probably more important for survival, were spared from the catabolic effects of these hormones.

Despite the regular occurrence of skeletal muscle weakness and muscle fiber atrophy in steroid myopathy, the effects of chronic steroid treatment on the contractile properties of muscle has received only modest attention. Vignos and coworkers (1976) examined the contractile properties of whole muscles, the predominantly pale extensor digitorum longus(EDL) and dark soleus in rabbits in vitro in order to determine if the differential catabolic effects of steroids were dependent on the fiber composition of muscle. They found that the contractile properties of soleus were unchanged by steroid treatment whereas these same properties (twitch and tetanic tensions, half-relaxation time) were significantly

altered in EDL. Gardiner, et al.,(1978) have examined the contractile properties of whole skeletal muscle in situ in steroid-treated cats. These workers have shown that although the ability to generate tetanic tension(kg) was reduced in both pale and red muscles, the tetanic tension capabilities of these muscles were unchanged when expressed in terms of either kg/gm of muscle or kg/gm of contractile protein. This same finding made in a subsequent study on the same two muscles in steroid-treated rats together with data on the effects of glucocorticoids on the intrinsic speeds of fast and slow muscles, suggested to these workers that no changes occur in the quality or functional integrity of the remaining contractile protein in steroid-atrophied muscle(Gardiner and Edgerton, 1979). The results of a more recent study (Gardiner, et al.,1980) suggest that fast muscles are functionally more suited as protein sources during catabolic conditions because they maintain a relatively higher proportion of their contractile capabilities following steroid treatment.

The profiles of the physiological properties of whole pale and red muscles in steroid-treated animals cannot reveal much information about the effects of glucocorticoids on the properties of the basic functional elements of skeletal muscle, single motor units. The information which is available on the properties of single motor units in steroid-treated muscle is therefore largely indirect. The present study was initiated to examine the responses of motor units of identified type following steroid treatment and to determine the extent to which their mechanical, electrical and fatigue properties are affected.

Several lines of evidence suggest that the dual functions of skeletal muscle as a contractile machine and as an amino acid reserve may be regulated by the same control mechanism and that the plasticity of this tissue in both roles may be linked by a single unifying principle. First, is has been clearly demonstrated that regular muscular activity is required to maintain normal muscle protein turnover(Goldspink, 1976). Conversely, normal muscle protein content is necessary for the routine production of muscular tension. Second, much experimental evidence shows that the pattern of neural activation imposed upon a muscle may alter its protein composition (Salmons and Sreter, 1976), metabolic properties and mechanical characteristics (Mommearts, 1974; Romanul, et al., 1974; Rubenstein, et al., 1978; Sreter, et al., 1974). Third, under conditions which result in the mobilization of amino acids from skeletal muscle stores, muscle fibers innervated by motoneurons of large diameter (least active motoneurons) often appear to undergo either a selective or preferential atrophy (Brooke and Kaiser,1970; Gruener and Stern,1972; Walsh, et al.. 1971).

These three lines of evidence indicate that the overall neural activity pattern impinging upon skeletal muscle fibers is of major importance in the regulation of muscle fiber protein metabolism. Since it is now generally accepted that the size of motoneuron cell bodies determine their excitability thresholds and thus their overall frequency of discharge, these observations suggest that the "Size Principle" can be extended, not only to explain the order in which motor units are recruited in muscular tension development but also to account for the patterns of muscle protein degradation and fiber atrophy seen in a variety

of metabolic and neuromuscular disorders. The present experiments were designed to obtain further evidence concerning the validity of this hypothesis.

To test this hypothesis, a series of initial experiments were performed on the muscles of normal adult cats in order to characterize the mechanical, electrical, and fatigue properties of single motor units. In another series of studies, muscle fiber atrophy and protein catabolism was induced by treating animals with the potent glucocorticoid, triamcinolone acetonide. The properties of single motor units in the muscles of steroid-treated animals were then examined and compared to the properties of motor units from control muscles. If the proposed hypothesis is correct, the properties of the least used motor units (type FF) should be more strongly affected than those of units used frequently in muscular contraction under conditions which produce a net catabolic effect on muscle protein.

METHODS

The present study was performed in two stages. In a series of control experiments, adult male cats weighing from 2.70 to 4.75 kg were anesthetized with either sodium pentobarbital (35 mg/kg IP) or alpha choralose (70 mg/kg IV) after ether preanesthesia. The mechanical, electrical and fatigue properties of identified single motor units were then examined in either the medial gastrocnemius or soleus muscles. The details of the individual acute experiments are described below. Similar studies were performed on a series of animals following a period of steroid administration. Adult male cats designated for the experimental group were conditioned for a period of approximately thirty days in the Medical College of Virginia animal care facility. After the conditioning period, these animals (2.90 to 4.38 kg) were given daily injections of triamcinolone acetonide (Sigma Chemical Co.), 3-4 mg/kg of body weight in a 0.9% NaCl solution for a period of 10-16 days. Food and water were provided ad libitum. On the day of the last steroid injection, the animals were weighted and anesthetized with alpha chloralose (70 mg/kg IV) in preparation for the acute study.

A. Surgical procedures: A tracheal cannula was inserted to maintain the animal's airway and to allow continuous monitoring of expired CO_2 levels. The femoral nerve to the left hindlimb was cut or crushed. The animal was then mounted in a standard spinal frame (David Kopf Instruments) and immobilized with hip pins and a clamp on the spinous process of the third lumbar vertebra. The left hindlimb was fixed by a clamp placed distal to the ankle and a drill passed through the femoral condyles and secured to the frame. By careful dissection in the pop-

lileal fossa, the nerve to the muscle to be examined (MG or soleus) was freed from adjacent nerves and surrounding connective tissues. All other nerves to the left hindlimb were cut or crushed so that only the muscle under study received an intact innervation. The tendon of the muscle to be examined was freed from adjacent tissues and attached to an isometric force transducer (Grass Instruments FT-03) with braided silk suture. The force transducer was mounted on a rack-and-pinion block (Narashige) which allowed it to be moved back and forth to apply a 100 gm preload to the muscle (Burke, et al., 1973). The tension transducer was oriented along the natural direction of pull of each muscle. Care was taken to preserve the normal blood supply to the muscle. A mineral oil pool was then formed from the skin of the hindlimb and maintained between 37° and 39° C with radiant heat. So as not to interfer with the accurate recording of motor unit mechanical properties, the temperature of a muscle adjacent to that examined was also monitored and maintained at an average temperature of 37-38° C.

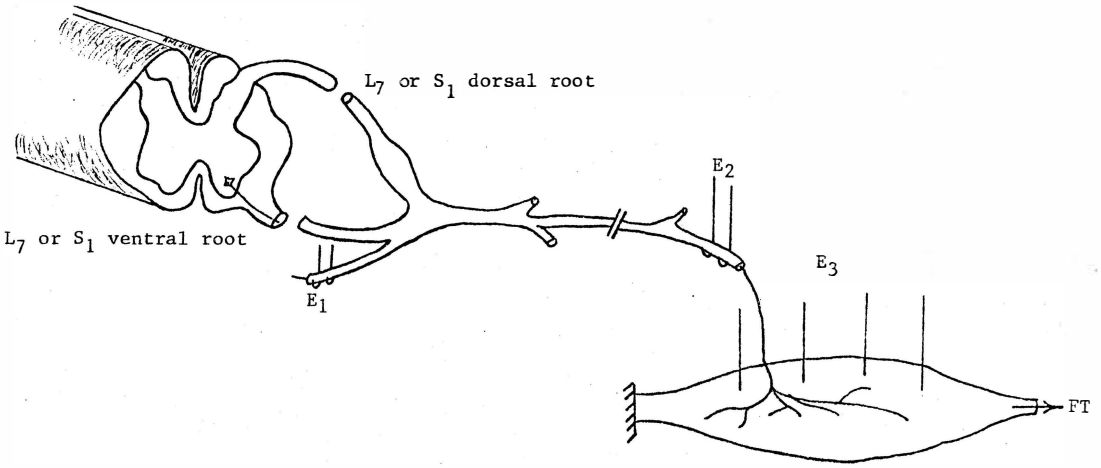
A laminectomy was performed between the fourth and seventh lumbar vertebrae. The spinal dura was cut and reflected to expose the spinal roots (L_7 DR and S_1 DR, respectively) which were cut on the left near their entrance through the intervertebral foramen, and were reflected medially. The corresponding ventral roots (L_7 VR and S_1 VR) were cut near their origin from the spinal cord and were reflected laterally. A small glass dissection table fabricated from a dental mirror was positioned near the cut ends of the ventral roots. A mineral oil pool was formed from the skin of the back to cover all exposed neural tissues. The animals core temperature was monitored with a rectal thermometer and maintained with a heating pad and radiant heat between 36° and 40° C.

B. Recording and Stimulating Procedures: Single motor units were isolated according to methods developed by McPhedran, et al., (1965). The scheme of the experiment is shown in Figure 1. A pair of silver hook electrodes was placed on the muscle nerve to allow antidromic activation of axons to the muscle examined. Fine ventral root filaments dissected free from the ventral root bundle were placed on a second pair of silver hook electrodes. This pair of electrodes was used to record antidromically elicited action potentials. When a ventral root filament was found to contain only one active axon, this same pair of electrodes was used to orthodromically stimulate the isolated single muscle unit.

Four stainless steel 27 guage needles were inserted at equal intervals along the long axis of the muscle and served as EMG electrodes. The EMG of single motor units was recorded from that pair of electrodes which gave the largest signal. The single unit EMG was differentially amplified (Princeton Applied Research Model 113) and was displayed on one channel of a storage oscilloscope. Simultaneously, the EMG signal was fed into a full-wave rectifier and gated integrator (Clamann, et al., 1974). The output of this device, a waveform whose amplitude is proportional to the area under the full wave rectified EMG signal, was displayed on a second channel of the same oscilloscope. The tension developed by single motor units was monitored by the isometric force transducer, the output of which was differentially amplified and also displayed on the storage oscilloscope.

A Grass model 588 stimulator, in conjunction with Grass model PSIU-6 stimulus isolation units, was used to deliver constant current pulses of 0.1 msec duration to either the ventral root filament or the peripheral muscle nerve.

Figure 1. Scheme of experiment: E_1 - electrodes placed upon a small ventral root filament; E_2 - electrodes on the muscle nerve; E_3 - EMG electrodes; FT - force transducer



Several tests were used to insure that the ventral root filament contained no more than one alpha motor axon to the muscle studied. Stimuli of graded strength were applied to the muscle nerve. The neurogram recorded from the ventral root filament had to be all-or-nothing in nature. The filament was then stimulated in turn with graded impulses while the twitch tension and EMG wave forms were recorded from the muscle. Both the twitch response and EMG waveforms had to be all-or-none in nature and invariant in shape to insure the isolation of only one single motor unit. Such care was necessary to avoid stimulation to more than one axon of similar electrical threshold in the same ventral root filament.

C. Data Collection: The following data were recorded for motor units examined in both control and steroid-treated animals:

1. ventral root from which motor unit axon was isolated (L_7 or S_1).
2. latency of antridromically activated action potential.
3. twitch tension (TT) both before and after potentiation by means of a brief, high frequency (100/sec) stimulus train.
4. twitch contraction time (CT): measured from the onset of EMG waveform to the peak of the tension record.
5. amplitude of integral of full-wave rectified EMG signal elicited by single shocks (IEMG).
6. tetanic tension: recorded at stimulation frequencies ranging from 10/sec to 200/sec; maximum tetanic tension (P_{max}) was usually produced at frequencies of >150/sec.
7. shape of unfused tetanus (sag test, Burke et al., 1973): occurence of maximum tension early in the course of an unfused tetanus and the subsequent decline to a somewhat lower steady tension.

8. apparent fusion frequency.
9. maximum rate of rise of tetanic tension (dP/dt): recorded at a stimulus rate of 200/sec.
10. fatigue ratio (see below for details).
11. fatigue index (Burke, et al., 1973): the ratio of the 120th tetanus tension (after 2 min of stimulation) to the tension output during the first tetanus in a stimulation sequence of 40/sec for 300 msec each second for a period of two minutes. Fatigue indices of >0.75 indicate marked fatigue resistance. Fatigue indices of <0.25 indicate marked susceptibility to fatigue.

The EMG waveform, the integral of the full-wave rectified EMG signal, output of the force transducer and a trigger pulse produced by the stimulator were recorded on magnetic tape with a 4-channel Vetter model B tape recorder. Data analysis was done either on-line or later by displaying waveforms on a storage oscilloscope.

A fatigue criterion developed in this laboratory was used to evaluate the relationship between the electrical and mechanical responses of single units during a sustained, fatiguing contraction (for rationale, see below). One storage oscilloscope was used to display the raw EMG and force produced by a single motor unit during continuous stimulation at 80/sec. Another storage oscilloscope was prepared with horizontal and vertical amplifiers. Tetanic tension was displayed on the horizontal axis and the integral of the rectified EMG signal on the vertical axis. For each stimulus delivered, a pulse intensified the oscilloscope beam at the peak of the EMG integral. When an individual unit was tetanized at 80/sec, a series of dots was produced on the

second oscilloscope which began at the upper left corner, rapidly moved toward the upper right corner and then more gradually traced a curve toward the lower left corner. Figure 2 shows three sample records using this procedure (IEMG-fatigue test) for units of differing fatiguability. Figure 3 illustrates the manner in which these records were analyzed. A horizontal line (BC) was drawn at the maximum value recorded for IEMG. A vertical line (CD) was constructed through the point of maximum force. Vertical (AB) and horizontal (AD) lines were drawn through point A which was the zero point for force and IEMG. Area ABCD was determined by planimetry, and was taken as 100% area. The shaded area underlying the return portion of the curve was also determined by planimetry. The fatigue ratio was then calculated by dividing the area under the curve (shaded) by the total area (ABCD). Readily fatiguable units would have large fatigue ratios near 1.00 and fatigue resistant units would have low fatigue ratios near zero.

Intermittently during the course of these experiments, the twitch and tetanic properties of single units were retested to determine the extent of deterioration in the muscle preparation. Although twitch and tetanic tensions of readily fatiguable units were frequently reduced during these tests, these same parameters in fatigue-resistant units were found to be essentially identical to those during initial testing.

At the conclusion of each experiment, the axonal conduction distance was measured by laying a string along the conduction path from the cut end of the ventral root to the stimulating electrode on the muscle nerve.

Two-tailed t-tests were used to determine whether the mean values of variables for motor units of identified type were significantly

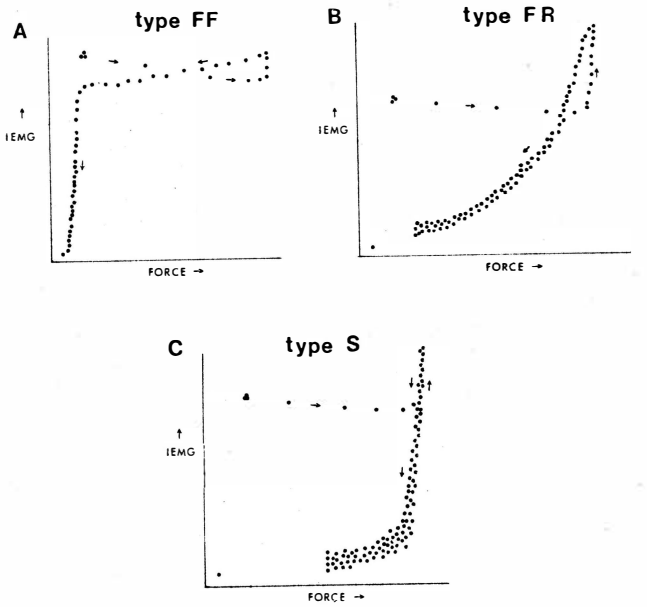


Figure 2. Sample records of IEMG-fatigue tests records reconstructed from the original data: (A) for a readily fatiguable motor unit, (B) for a moderately fatigue-resistant unit, and (C) for a very fatigue-resistant unit. Arrows indicate the direction of movement of the series of dots produced during 80/sec. stimulation.

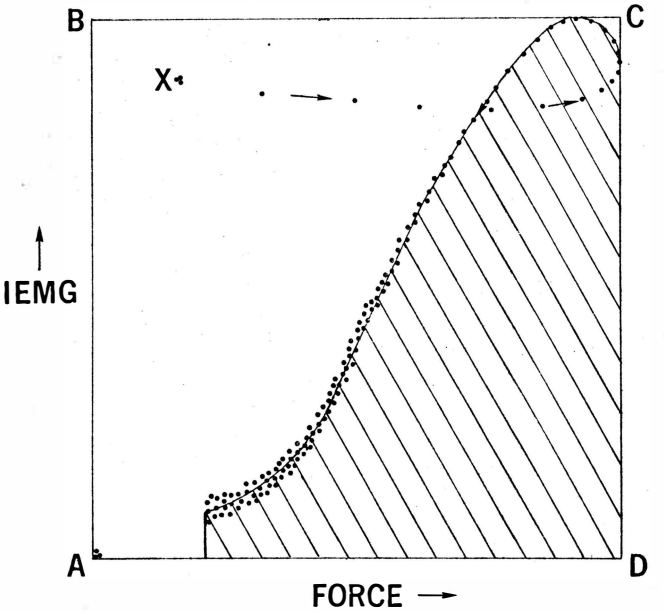


Figure 3. Illustration of the manner in which IEMG-fatigue test records were analyzed. Area ABCD and shaded area determined by planimetry. Fatigue ratio calculated by dividing shaded area by area ABCD. Point A = zero point for IEMG and force; Point X = response to stimulus rate of 0.8/sec.

different between control and experimental groups. These test were performed using the Statistical Analysis System computer program available at this institution for data analysis.

D. Rationale for the experimental procedures

The soleus and medial gastrocnemius muscles of the cat were chosen for study for several reasons. Both muscles, which form part of the triceps surae muscle group, serve to extend the ankle and have a common insertion on the calcaneus through the Achilles tendon. Their histochemically-identified fiber compositions and functional characteristics, however, are quite different. The cat soleus is a muscle which is made up of purely type SO (slow-twitch, oxidative) muscle fibers. This homogeneous muscle is very fatigue-resistant, produces relatively low levels of force and is active on an almost continual basis during normal postural and locomotive activities. In contrast, the cat medial gastrocnemius is a heterogeneous muscle composed of three histochemically and functionally different types of muscle fibers. Ariano and coworkers (1973) have shown that cat MG is made up of 61% type FG (fast-twitch, glycolytie) fibers, 14% type FOG (fast-twitch, oxidative-glycolytic) fibers and 25% type SO (slow-twitch, oxidate) fibers. As a whole, MG is readily fatiguable, is capable of producing powerful and rapid contractions, and is, in general, activated most during running or jumping activities in normal cats.

In addition, these two muscles were selected for study because the overwhelming majority of investigations designed to examine the mechanical and fatigue properties of single motor units have been performed on these muscles. From the results of his single motor unit studies, Burke has estimated that MG contains 45% type FF (fast-twitch, readily fatigue-

able) motor units, 25% type FR (fast-twitch, fatigue-resistant) units and 25% type S (slow-twitch, fatigue-resistant) units. Five percent of the fast-twitch units in MG have intermediate susceptibility to fatigue (fatigue index $0.25<FI<0.75$) and have been classified as FI.

In previous studies, two techniques have been employed to isolate single motor units in mammalian skeletal muscle: 1) the direct intracellular stimulation of alpha motoneuron cell bodies using microelectrodes (Devanandan, et al., 1965; Burke, et al., 1973) and 2) the stimulation of fine filaments dissected from the ventral root bundles (McPhedran, et al., 1965; Wuerker, et al., 1965). The latter technique was chosen for this study for three reasons. First, it was found that it was extremely difficult to maintain a microelectrode intracellularly for the necessary length of time (30-45 min) when muscle contractions were elicited. Second, in our hands, the ventral root filament approach allowed us to examine a greater number of motor units in individual experiments. Third, two tests were used to evaluate motor unit fatiguability. The isolation technique employed in this study allowed us to perform one of these fatigue tests, set the filament aside (for up to several hours) and then perform the second fatigue test upon the same motor unit.

Preliminary control studies were performed on sodium pentobarbital-anesthetized animals because this anesthetic has been used in the majority of studies examining the properties of single motor units in experimental animals. Although this anesthetic can be rapidly administered and minimizes surgical preparation time, frequent supplemental doses were required during the course of our control experiments to maintain the level of anesthesia. To minimize this problem, we have also used the

longer-lasting anesthetic agent alpha-chloralose in control studies. Since no statistically significant differences were found between the properties of single motor units in the control populations using the two anesthetics (See Results), alpha-chloralose was used exclusively as the anesthetic for steroid-treated animals.

For both control and experimental studies, many of the technical details and stimulation procedures were identical to those employed in previous single motor unit studies. Muscles were examined under a preload of approximately 100 grams. Burke (1967) has shown that this preload to triceps surae muscles maximizes the twitch tension attained by motor units without markedly affecting their tetanic tension capabilities. Several reports (Burke, et al., 1971, 1973, 1976; Olson and Swett, 1971; Stephens ans Stuart, 1975) have shown that the contractile mechanism of motor units in medial gastrocnemius adapts itself to previous activity. Brief high frequency stimulation both lengthens the twitch contraction times and augments the twitch tensions on MG units. In contrast, similar tetanic trains applied to soleus motor units generally shortens their twitch contraction times and reduces their twitch tensions (Burke, et al., 1974; Olson and Swett, 1971). With these findings in mind, twitch parameters of units examined in the present study were recorded both before and after brief, high frequency (100/sec) tetanization. In a few units, the twitch amplitude was so small prior to tetanization that twitch parameters could not be accurately determined. In some of these cases, signal averaging of twitch responses was performed using a PDP - 8E laboratory computer (Digital Equipment Corp.) to obtain accurate information. Because the stimulus history may alter the mechanical responses of motor units, all tests were performed in the sequence shown under data collection.

A number of studies presented in the literature have reported on the nature of the relationship between surface electromyogram and muscle force during voluntary contractions in human limb muscles (Basmajian, 1967; Bigland and Lippold, 1965; Chaffin, et al., 1980; Clamann and Broecker, 1979; DeJong and Freud, 1966; Edwards and Lippold, 1956; Gottlieb and Agarwal, 1971; Inman, et al., 1952; Kuroda, et al., 1970; Lippold, 1951; Mason and Munro, 1970; Stephens and Taylor, 1970; Zuniga and Simmons, 1964). Since the surface EMG represents the summated electrical activity of all active motor units, it is important to know the electrical properties of individual motor units (Milner-Brown and Stein, 1973). Curiously, however, only a few studies have been directed toward evaluating the electrical properties of identified motor unit types. To obtain more information regarding the relationship between single unit electrical signals and their mechanical responses, EMG waveforms elicitied during single unit stimulation were monitored and processed as described previously.

The tetanic tensions of MG and soleus motor units were recorded in response to stimulation over a broad range of frequencies. During this testing, both the apparent frequency at which tetanic tension appeared completely fused and the shape of the unfused tetanic responses were noted. Although most units showed complete fusion of tetanus at stimulus frequencies of 80-90/sec, previous studies have shown that higher stimulus frequencies are required to produce maximal tetanic tensions (Olson and Swett, 1971; Wuerker, et al., 1965). Wuerker and coworkers (1965) demonstrated that the tetanic responses of MG units stimulated at 100/sec were on the average 18% lower than those obtained at stimulus frequencies of 150-200/sec. In addition, this augmentation of tetanic

tension was more pronounced in fast-contracting units in MG. For this reason, the maximum tetanic tensions were recorded for single units in MG at stimulus rates in excess of 150/sec and for soleus units at stimulus frequencies of at least 100/sec. These same stimulus frequencies were used to produce tension records from which the maximum rate of rise of tetanic tension for individual units could be determined.

In several previous studies (McPhedran, et al., 1965; Olson and Swett, 1971; Wuerker, et al., 1965) motor units in the cat MG and soleus muscles have been stimulated with long tetanic trains in order to assess their susceptibility to fatigue. In each of these reports, workers have noted that continuous tetanic activation of motor units produces a decline in unit force which is often accompanied by a gradual diminuation in the amplitude of the EMG. No attempts were made to systematically examine this phenomenon. This reduction in EMG amplitude was attributed to a progressive failure of transmission at the neuromuscular junctions. This apparent neuromuscular fatigue was seen in only a few units during the intermittent stimulation procedure employed by Burke and coworkers (1971, 1973) to index motor unit fatiguability. To systematically examine the relationship between motor unit force and EMG elicited during fatiguing contractions, we have developed the IEMG-fatigue test. The stimulus rate of 80/sec used for this test was chosen for three reasons. First, this stimulus rate was near or above the fusion frequency for almost all motor units examined. Second, it was high enough to induce fatigue in all three types of motor units (stimulation of type S units in MG and soleus at rates near their fusion frequencies failed to produce significant fatigue during trains lasting five minutes and

longer). Finally, this particular frequency of stimulation did not result in any overlap or summation of EMG signals as their duration increased during the course of the test.

A stimulus paradigm identical to that employed by Burke and co-workers (1971, 1973) was also applied to isolated single units to assess their susceptibility to fatigue. As opposed to the continuous stimulation pattern used during the IEMG - fatigue test, this stimulus paradigm activates the motor unit on an intermittent basis in an attempt to minimize failure of the neuromuscular junctions. The fatigue index, determined from the results of this test (see Data Collection) in conjunction with information regarding the shape of the unfused tetanus (sag property) were used to categorize motor units by type according to criteria established in previous studies (Burke, et al., 1971, 1973; Burke and Tsairis, 1974). This particular motor unit classification scheme was chosen to facilitate data analysis and subsequent comparison of our control data with those obtained on the same two muscles in other laboratories (Burke, et al., 1971, 1973, 1974; Burke and Tsairis, 1974; Hammarberg and Kellerth, 1975; Proske and Waite, 1974, 1974). In addition, this method of motor unit classification which divides the majority of units into types FF (fast-twitch, readily fatiguable), FR (fast-twitch, fatigue-resistant) and S (slow-twitch, very fatigue-resistant) is essentially interchangeable with another commonly used scheme for motor unit categorization based upon a combination of physiological and histochemical findings. Types FF, FR, and S motor units appear identical to types FG (fast-twitch, glycolytic), FOG (fast-twitch, oxidative-glycolytic) and SO (slow-twitch, oxidative) units, respectively.

A difficulty in these experiments was that of performing two fatigue tests upon the same motor unit. How does one know if the unit has recovered prior to the second fatigue test? To minimize this problem, one fatigue test (usually the IEMG - fatigue test) was performed on an isolated unit. The filament containing the axon to the muscle unit was then set aside for a period of several hours. At the end of this period, the filament was returned to the stimulating electrodes and the second fatigue test (usually Burke's test) was performed. This measure appeared to be effective since motor units which were resistant to fatigue in the first test maintained their fatigue resistance in the second test in nearly every case.

RESULTS

A. Properties of single motor units in the control populations

The primary objective of the control studies was to investigate a variety of mechanical, electrical and fatigue response characteristics of muscle units in the pale, heterogeneous medial gastrocnemius (MG) and the red, homogeneous soleus muscles. These studies enabled us to assess the distributions of such properties within the population of units making up these two muscles. The properties of 163 motor units in MG and 39 units in soleus were examined in 31 untreated adult cats. Eighty-six MG and 20 soleus units were characterized in pentobarbital-anesthetized animals and 77 MG and 19 soleus units were examined in chloralose-anesthetized animals. Motor units were isolated by the dissection of ventral root filaments from the L_6, L_7, and S_1 ventral root bundles. In the entire MG control group, 5 units were isolated from the L_6VR, 79 from the L_7VR and 63 from the S_1VR. Of the 39 soleus motor units studied, 34 units were isolated from L_7VR filaments and 5 were found in S_1VR filaments. When the properties of individual MG muscle units originating from different ventral roots (L_7VR and S_1VR only) were compared, it was found that the mean twitch tension ($\overline{TT}$) and mean tetanic tension ($\overline{P_{max}}$) of L_7 units (14.4 gm and 41.7 gm respectively) were significantly greater ($p < .002$) than these same parameters in S_1 units ($\overline{TT}$= 7.4 gm and $\overline{P_{max}}$= 25.4 gm). No significant differences were found in the muscle unit properties of either L_7 or S_1 units between the two anesthetic subpopulations. As a result, the properties of MG units in the anesthetic subgroups were pooled to form an MG motor unit control group (MG_c). Almost every motor unit examined in the soleus control experiments was isolated from L_7 ventral roots. No significant differences were found between the properties of these units

examined in chloralose- and pentobarbital-anesthetized animals. For this reason, the soleus motor unit properties from the anesthetic subgroups were pooled to form a soleus motor unit control population($soleus_c$).

Individual motor units from each control group were classified by type according to criteria developed in previous studies (Burke, et al., 1971,1973.1974). Of the medial gastrocnemius motor units, 59 (36%) were classified as type FF, 38 (23%) were FR, 38 (23%) were type S, 21 (13%) were type FI and 7 (4%) defied classification. All 39 soleus motor units could be clearly categorized as type S units. Table 1 shows the average values and standard deviations of MG and soleus motor unit properties in the control populations. The frequency distributions of several of these properties are displayed in Figure 4. for MG units and Figure 5. for soleus units.

Several of the variables used to characterize single motor units in MG and soleus experiments were determined in ways identical to those described in previous studies (Burke,et al., 1973,1974). These variables included: 1) twitch tension, 2) twitch contraction time, 3) maximum tetanic tension, 4) presence or absence of "sag" in unfused tetanic responces, 5) presence or absence of potentiation of twitch responses and 6) fatigue index. The similarity of the average values and distributions of these properties to those reported from a variety of other laboratories (Bagust, 1974; Burke,1967,1978; Burke,et al.,1971,1973,1974; Burke and Edgerton,1975; Burke and Tsairis,1974; Devanandan, et al., 1965; Goslow, et al.,1977; Hammarberg and Kellerth,1975; Jami and Petit,1975; McPhedran, et al., 1965; Mosher, et al., 1972; Olson and Swett,1971; Proske and Waite,1974; Reinking,et al., 1973; Stephens, et al., 1973; Stephens and Stuart, 1975; Wuerker,et al., 1965) indicates that our control populations

TABLE 1. Mechanical, electrical and fatigue properties of single motor units in control animals.

		Medial gastrocnemius				Soleus
		all units	FF	FR	S	all units
N (%)		(100)	(36)	(23)	(28)	(100)
MECHANICAL PROPERTIES						
Contraction time (msec)	$\bar{X}$	37.7	31.0	33.2	61.4	78.8
	SD	13.2	3.5	5.1	12.3	9.6
	(N)	(75)	(26)	(27)	(14)	(19)
Twitch tension (gm)	$\bar{X}$	11.0	19.4	6.3	0.9	2.3
	SD	13.4	15.7	5.0	0.7	1.5
	(N)	(160)	(59)	(38)	(35)	(39)
Max. tetanic tension (gm)	$\bar{X}$	34.0	54.1	30.0	5.2	10.8
	SD	31.6	34.4	21.0	3.6	5.6
	(N)	(162)	(58)	(38)	(38)	(39)
Twitch-tetanus ratio	$\bar{X}$	0.28	0.36	0.21	0.19	0.21
	SD	0.14	0.12	0.08	0.09	0.06
	(N)	(159)	(58)	(38)	(35)	(39)
dP/dt (gm/msec)	$\bar{X}$	0.90	1.92	0.50	0.08	0.11
	SD	0.87	0.81	0.20	0.08	0.05
	(N)	(28)	(9)	(13)	(5)	(19)
ELECTRICAL PROPERTIES						
$\int$EMG dt $(v\text{-}sec)\cdot 10^{-6}$	$\bar{X}$	0.59	0.92	0.43	0.18	0.51
	SD	0.74	0.98	0.35	0.18	0.30
	(N)	(163)	(59)	(38)	(38)	(39)
FATIGUE PROPERTIES						
Fatigue ratio	$\bar{X}$	0.42	0.66	0.32	0.17	0.11
	SD	0.23	0.13	0.13	0.10	0.05
	(N)	(73)	(24)	(27)	(14)	(19)
Fatigue index	$\bar{X}$	0.54	0.13	0.88	0.97	0.96
	SD	0.38	0.09	0.11	0.06	0.05
	(N)	(160)	(59)	(37)	(38)	(39)
OTHER PROPERTIES						
Conduction velocity (m/sec)	$\bar{X}$	104.4	105.9	110.4	95.0	79.3
	SD	39.1	7.1	5.3	8.9	8.9
	(N)	(163)	(59)	(38)	(38)	(39)

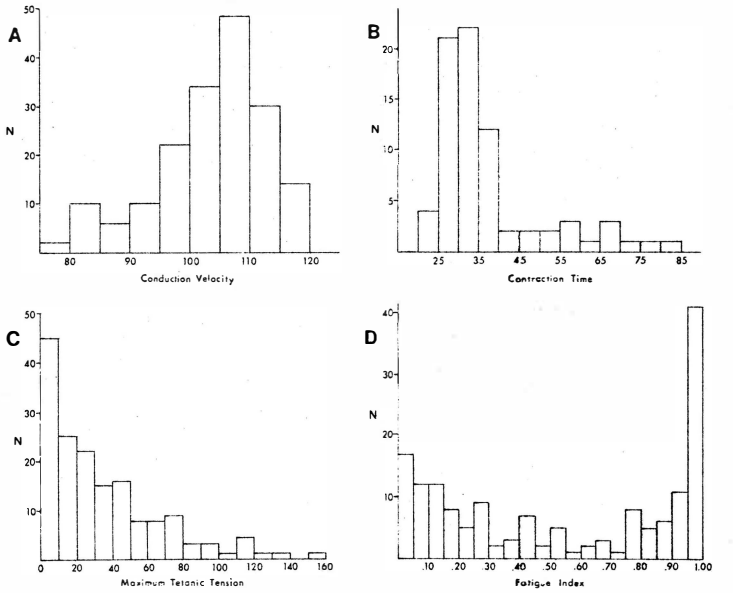


Figure 4. Frequency distributions of A) conduction velocity (m/sec, N = 163), (B) twitch contraction time (msec., N = 75), (C) maximum tetanic tension (grams, N= 162), (D) fatigue index (N= 160) for medial gastrocnemius motor units in the control population.

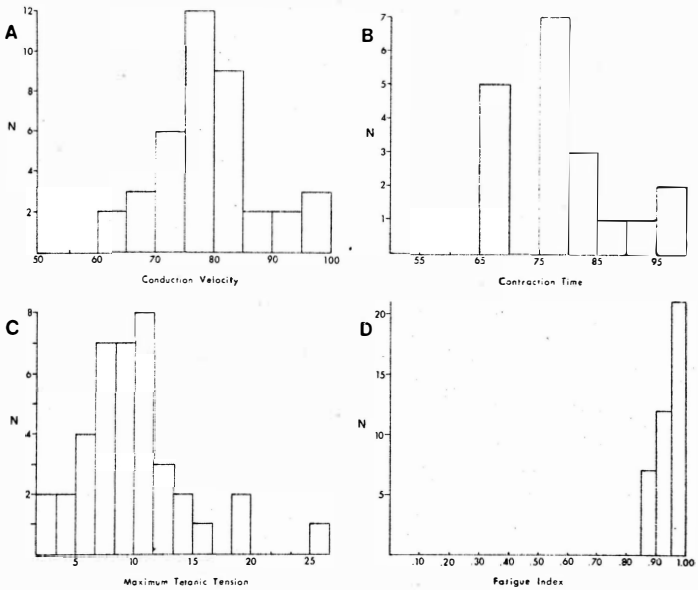


Figure 5. Frequency distributions of (A) conduction velocity (m/sec, N= 39), (B) twitch contraction time (msec.,N= 19), (C) maximum tetanic tension (grams, N= 39), and (D) fatigue index (N= 39) for soleus motor units in the control populations.

of soleus and/or MG units was representative of the muscle as a whole.

1. Mechanical properties of single units in the control populations

The mean values for maximum twitch and tetanic tensions were highest for type FF units, intermediate for type FR units and lowest for type S units (Table 1.) As a group, the twitch and tetanic tensions of identified FF units were significantly higher($p<.0002$) than those for either FR or S units. These properties for FR units were also significantly greater($p<.0001$) than those for type S units in MG. Type S units in soleus were found to generate significantly more($p<.0001$) twitch and tetanic force than type S units in MG.

A strong positive correlation was found between twitch tension and P_{max} for all units in both muscles(MG: $r=0.92, p<.0001$; soleus: $r=0.86, p<.0001$). This strong positive correlation between unit twitch and tetanic tension persisted when these properties were correlated for the separate motor unit types in MG (FF: $r=0.90, p<.0001$; FR: $r=0.90, p<.0001$; S: $r=0.70, p<.0001$). Therefore, twitch to tetanus ratios were calculated for each identified motor unit type. Average twitch-tetanus ratios were very similar for all fatigue resistant units being 0.21 for MG type FR units, 0.19 for MG type S units and 0.21 for soleus type S units. The ratio for MG type FF units (0.36) was much higher.

During the course of these experiments, it became clear that the maximum rate of rise of tetanic tension(dP/dt) was an important variable in characterizing the speed-related properties of single motor units. This value was determined from maximum tetanic tension records of 28 motor units in MG and 19 units in soleus. The average dP/dt values for types FF, FR and S units in MG were 1.92 gm/msec, 0.50 gm/msec, and 0.08 gm/msec respectively. An average dP/dt value of 0.11 gm/msec was found for

soleus type S units which was similar to that for MG type S units. Of interest is the fact that the frequency distribution of dP/dt values for all MG units almost perfectly divided motor units into three non-overlapping groups. See Figure 6. dP/dt was the only mechanical variable which had this property.

Not unexpectedly, there was a strong positive correlation of dP/dt with twitch tension($r=0.92, p<.0001$) and with tetanic tension($r=0.92, p<.0001$) for MG motor units. Just as was seen for the correlation of twitch with tetanic tension of MG units, the correlations of dP/dt with twitch and tetanic tensions remained high when determined for the separate classes of MG motor units. See Table 2. The correlations of dP/dt with motor unit twitch and tetanic tensions appear better for type S units in MG than for the same correlations for soleus type S units. See Table 2.

Two other parameters were routinely determined to estimate motor unit contraction speed: twitch contraction time(CT) and the apparent fusion frequency. The latter variable provided only a rough indication of the speed of contraction but was frequently found to useful in motor unit classification when a motor unit's twitch tension was so small that contraction time could not be accurately measured. Contraction times were determined for 75 MG and 19 soleus units(chloralose-anesthetized animals only) in a manner similar to that employed in previous studies(Burke, et al., 1971, 1973, 1974; Reinking, et al., 1975; Stephens and Stuart, 1975). The twitch contraction times recorded for motor units from both muscles in pentobarbital-anesthetized animals were measured differently and therefore have not been presented here. In general, the average contraction times of types FF(31.0 msec) and FR(32.2 msec) units were shorter than the mean contraction times of type S units in both MG (61.4 msec) and

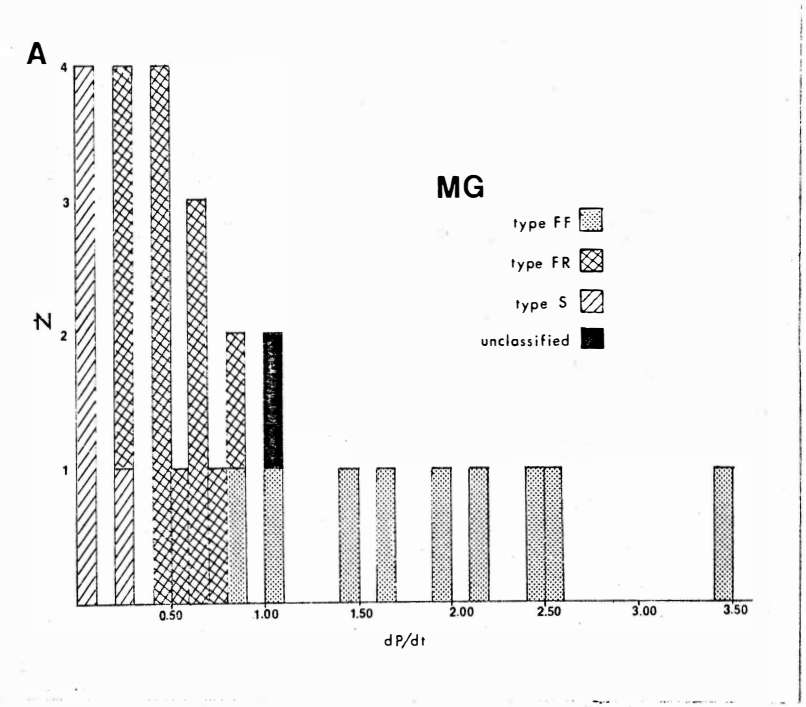


Figure 6. Frequency distribution of maximum rate of rise of tetanic tension (dP/dt in grams/msec.) for medial gastrocnemius motor units in the control population.

TABLE 2. Correlations of dP/dt with twitch and tetanic tensions of single motor units in the control populations.

PROPERTIES		Medial gastrocnemius all units	Medial gastrocnemius FF	Medial gastrocnemius FR	Medial gastrocnemius S	Soleus all units
dP/dt - TT	r	0.92	0.80	0.88	0.99	0.89
	p<	.0001	.0096	.0001	.0005	.0001
	(N)	(28)	(9)	(13)	(5)	(19)
dP/dt - P_{max}	r	0.92	0.82	0.77	0.98	0.83
	p<	.0001	.0073	.0019	.0030	.0001
	(N)	(28)	(9)	(13)	(5)	(19)

soleus(78.8 msec). With but one exception, the contraction times of type S units in both muscles were >45 msec. All type FF and FR units had contraction times of <45 msec. The apparent fusion frequencies of FF and FR units were always $\geq$50/sec. All type S units from both muscles showed an apparent fusion of tetanus at frequencies of 40/sec or less.

There was a strong negative correlation ($r=0.86$, $p<.0001$) of contraction time(CT) with fusion frequency for all units examined in MG. The correlation of CT with fusion frequency was poor for type FF and FR units in MG and for soleus S units but was moderate ($r=-0.58$, $p<.03$) for MG type S units.

One of the parameters used to characterize motor units was the presence or absence of what has been called the "sag" property in unfused tetanic responses. Of the units systematically examined for this property, 119 of 159 units in MG (75%) exhibited "sag". Eighty-six of these units (54%) were subsequently classified as type FF or FR units, 3 (3%) were type S units and 20 (17%) were units which did not clearly fit into the standard tripartite scheme. Of the remaining 40 units without "sag", 35 (88%) were classified as type S and 5 (13%) were unclassifiable. The "sag" property was absent in all 39 soleus units studied.

Although the extent to which brief tetanic trains applied to motor units augmented twitch responses was not systematically evaluated, the presence or absence of post-tetanic potentiation of twitch responses was recorded. Ninety-six percent(156 of 162) of MG units exhibited potentiation of twitch tensions following tetanization. In only 6 MG units(1 type FF and 5 type S units) did brief tetanization fail to augment twitch responses. In contrast, only 13%(5 of 39) of the twitch responses monitored in soleus units were augmented by tetanization.

For the slowly conducting, type S motor units in soleus, there existed an approximate linear relationship between axonal conduction velocity(CV) and maximum tetanic tension(P_{max}). See Figures 7A and 7B. Similar findings for the relationship between CV and P_{max} for soleus units have been previously reported by McPhedran, et al.,(1965). Results from the present study reveal that the good correlation of CV with P_{max} seen for soleus units(r=0.73, Figure 7A.)also exists for the slowly conducting type S units in MG (r=0.93, Figure 7C.) confirming the findings of Wuerker,et al.,(1965). Of additional interest was the existence of a strong positive correlation (r=0.81) between CV and P_{max} for units exhibiting marked resistance to fatigue(types FR and S units as a group). The technique of linear regression by the method of least squares was used to determine the equations which best described the relationship between CV and P_{max} for 1) the fatigue-resistant units(FR and S units) and 2) for type S units in MG alone. The equation obtained for type S units in MG (P_{max}= 0.34 CV-27.3) was very similar to that obtained for all fatigue-resistant units (P_{max}=0.42 CV -34.2). It is important to note that pooling of conduction velocity and force values from several experiments may obscure the relationship between these variables (McPhedran, et al., 1965; Wuerker,et al., 1965). For this reason the graphs shown in Figures 7A-7D were plotted from data obtained in individual experiments where the number of motor units examined was relatively large.

Figures 7E and 7F show plots of axonal conduction velocity versus another motor unit variable, the maximum rate of rise of tetanic tension (dP/dt) for MG and soleus units in the control groups. Although the data used to produce these plots was obtained from several different experiments, there is a clear similarity between the form of these graphs

Figure 7. Relationship between maximum tetanic tension(P_{max} in gm) and axonal conduction velocity (CV in m/sec) for soleus units (figures 7A and 7B) and for MG units (figures 7C and 7D) from control animals. Data for figures 7A - D from individual experiments. Relationship between axonal conduction velocity (CV) and the maximum rate of rise of tetanic tension(dP/dt in grams/msec) for control soleus(7E) and MG (7F) motor units. Key: filled circles=type S units; filled triangles=type FR units; open triangles= type FF units; open squares= unclassified units.

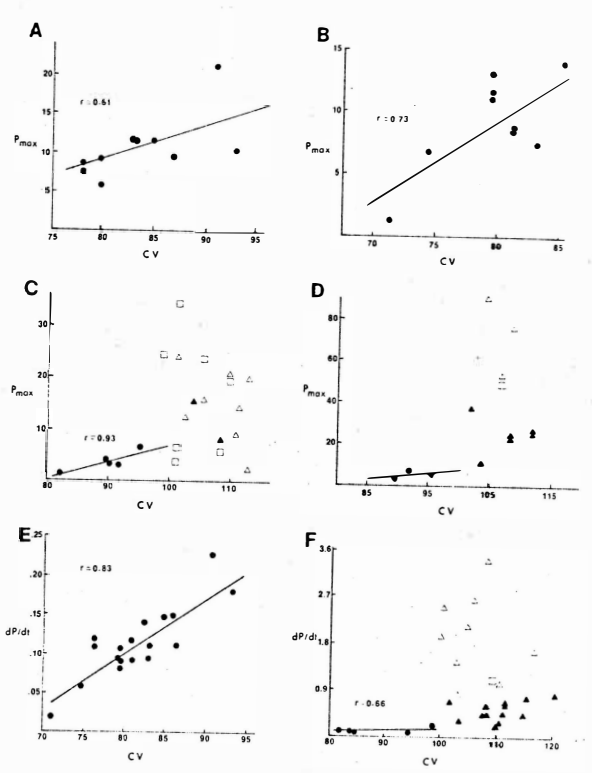

A
r = 0.61
Pmax
CV
B
r = 0.73
Pmax
CV
C
r = 0.93
Pmax
CV
D
Pmax
CV
E
r = 0.83
dP/dt
CV
F
r = 0.66
dP/dt
CV

Figure 7.

and those shown for the relationship between unit tetanic tension and conduction velocity(Figures 7A - 7D). Since motor unit tetanic tension was linearly related to dP/dt, the similarity between these plots was not unexpected.

2. Electrical properties of single units in the control populations.

The time-integral of the full-wave rectified EMG (IEMG) elicited by a single 0.1 msec. duration stimulus was recorded for 163 motor units in the MG control group and 39 units in the soleus control group. The average values of IEMG for FF, FR, and S units in MG were 0.92μv-sec, 0.43 μv-sec, and 0.18 μv-sec respectively. In comparison, the average IEMG value recorded for soleus units was 0.51 μv-sec,markedly higher than the mean IEMG determined for either type FR or type S units in MG. The mean IEMG for all units originating in the L_7VR(0.77 μv-sec, n=79) was significantly higher($p < .0012$)than the average IEMG determined for those units originating from the S_1VR(0.33 μv-sec, n=63). When MG motor units from these ventral roots were classified by type, it was found that the mean IEMG values for types FF,FR, and S units originating from the L_7VR were significantly higher($p < .035$ in each case) than the average IEMG values for the same unit types originating from the S_1VR. The frequency distribution of IEMG values for units in the control populations is shown in Figure 8.

3. Fatigue properties of single units in the control populations.

Two tests were employed in the present studies to examine the susceptibility to fatigue of single motor units(see Methods for details).

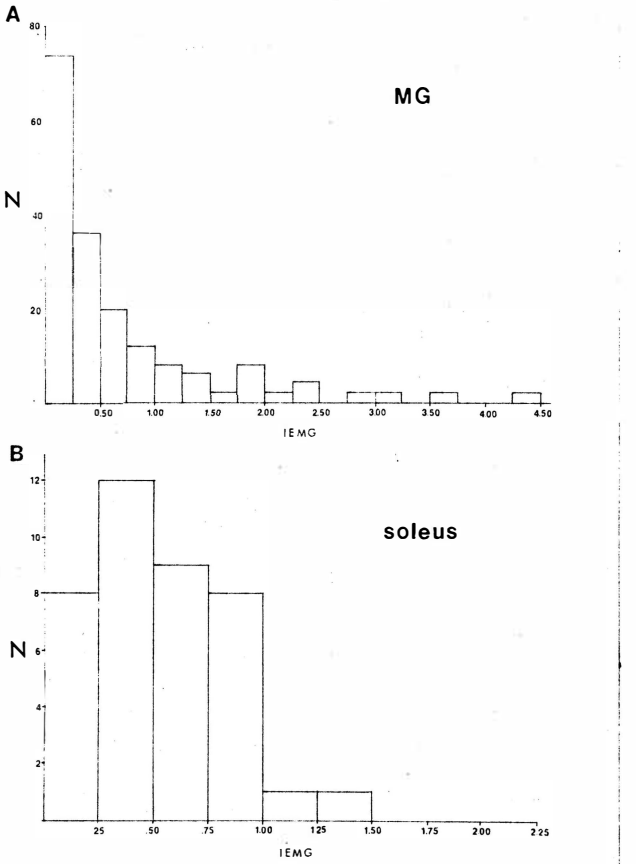


Figure 8. Histograms for IEMG recorded from single motor units in the medial gastrocnemius(A) and soleus (B) muscles of control animals. IEMG in volts-seconds x 10^{-6}.

Generally, motor units were subjected to the IEMG-fatigue test after which the fatigue index was determined. The typical records obtained for these two tests performed upon a readily fatiguable type FF motor unit in MG are shown in Figure 9. Figure 9A shows the raw EMG signal (upper trace) and tetanic force(lower trace) recorded from an FF unit during continuous 80/sec stimulation. Figure 9B. shows the IEMG-force plot recorded simultaneously. Note that the peak-to-peak amplitude of the raw EMG signal declined prior to the peak in the tetanic tension record. Following the development of peak tension, the raw EMG amplitude fell gradually while the motor unit tension dropped rapidly. The level portion of the IEMG-force curve indicates that during the first few seconds of stimulation, the reduction in EMG amplitude was accompanied by an increase in the duration of individual EMG waveforms. After five to ten seconds of stimulation, the IEMG amplitude declined more rapidly than motor unit force. The fatigue ratio determined for this unit from Figure 9B. was 0.77. Figure 9C. shows the EMG and force records recorded from the same unit during stimulation for 330 msec each second at a frequency of 40/sec. The fatigue index determined from figure 9C for this unit was 0.02.

Figure 10. shows typical records of the two fatigue tests for a fatigue-resitant, type FR motor unit in MG. For the first 15 seconds of stimulation at 80/sec, the peak-to-peak amplitude of the raw EMG signal and the tetanic force fell gradually(figure 10A). During this period, the reduction in EMG amplitude was accompanied by a marked increase in EMG duration which resulted in a sharp rise in the IEMG shown in figure, 10B. After approximately 15 seconds of stimulation, both force and EMG declined more rapidly. The IEMG- force plot recorded during this period traced a concave curve back toward the zero point for IEMG and force.

Figure 9. Sample records of IEMG-fatigue test (A and B) and fatigue index test(C) for a type FF motor unit. A) EMG (upper trace) and force(lower trace) recorded in response to 80/sec stimulation. B) IEMG - force plot recorded during 80/sec stimulation. C) EMG (upper trace) and Force(lower trace) recorded in response to stimulation of 40/sec for 330 msec each second for a period of 2 minutes. Fatigue ratio determined from figure 9B. was o.77. Fatigue index determined from figure 9C. was 0.02. Calibrations: A) EMG= 1 mv/div., force=15 gm/div. time=5 sec/div.,B) IEMG = .45 mv-sec/ div., force = 30 grams/ div.; C) EMG = 1 mv/div., force=15 grams/div., sweep = 15 sec./ div.

Figure 9.

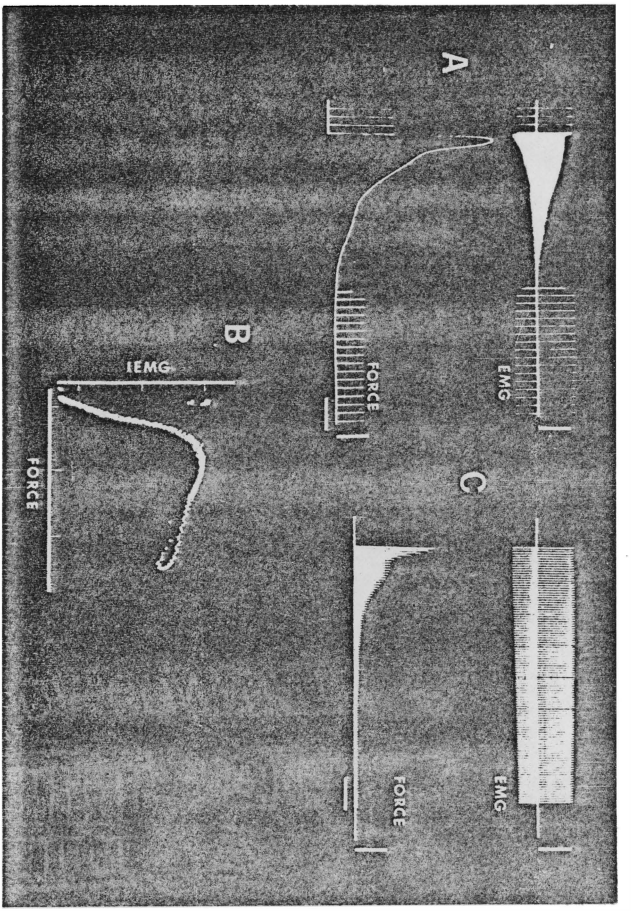
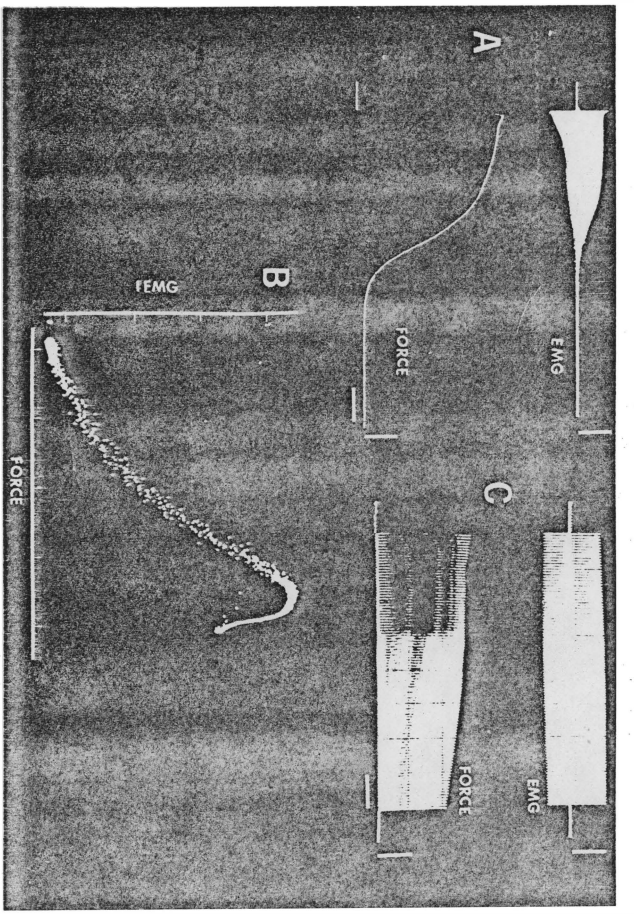


Figure 10. Sample records of IEMG-fatigue test(A and B) and fatigue index test (C) for a type FR unit. Stimulation patterns in A), B) and C) as described for Figure 9. Calibrations: A) EMG = 1mv/div, force= 30 gm/div. B) IEMG =.23 mv-sec/ div., force= 7.3 grams/div. C) EMG = 1 mv/div., force= 15 gm/div., sweep= 15 sec/div.

Figure 10.



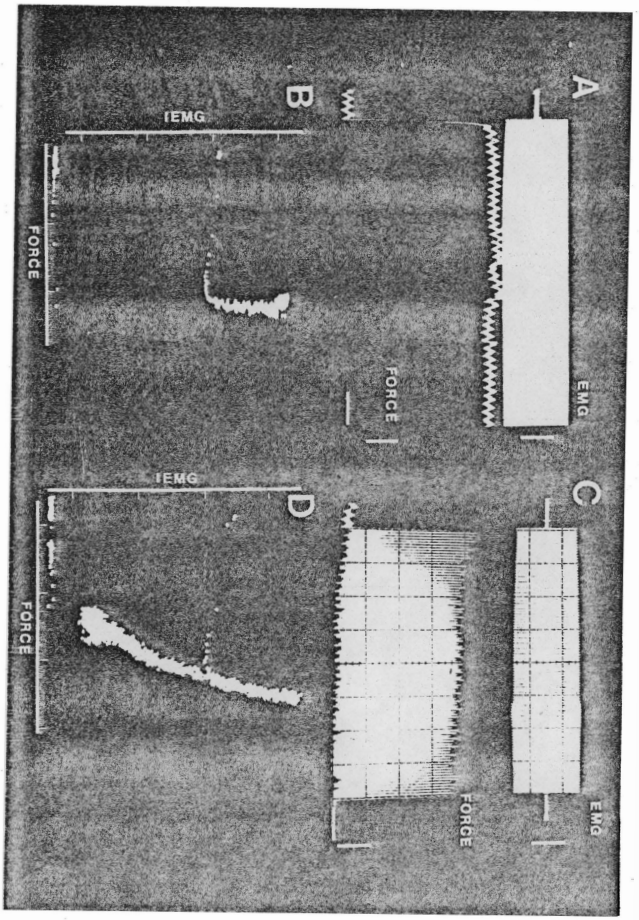
The fatigue ratio determined from the record shown in Figure 10B was 0.41. Figure 10C. shows the electrical and mechanical responses of this unit to the second fatigue test. The fatigue index determined from this record was 0.79.

Figure 11 shows the typical fatigue records for a very fatigue-resistant, type S unit in MG. Figure 11A. shows the EMG and force produced by this unit in response to stimulation at a rate of 30/sec. Little or no change in either EMG or force occurred during the three minutes of stimulation shown here. Figure 11B. shows the IEMG-force plot simultaneously recorded during the continuous 30/sec stimulation. This record shows that while there was no change in EMG peak-to-peak amplitude, there was an initial increase in the duration of the EMG signal as reflected by the transient rise in the IEMG. Once the IEMG and force reached maximum values, no further force of IEMG reductions could be produced. These records were typical for S type units stimulated at or near their fusion frequencies. Figure 11D. shows the IEMG-force plot for the same unit stimulated at a rate of 80/sec. Stimulation at this frequency produced an initial augmentation in the EMG duration which was similar to that seen in response to stimulation at 30/sec. The 80/sec. stimulation however was sufficient to result in an eventual reduction in IEMG and force as reflected by the falling segment of the IEMG-force plot. The fatigue ratio for this unit was 0.24 and the fatigue index determined from the record in Figure 11C. was 1.00. The shapes of IEMG-force plots for soleus type S units were in all respects similar to those obtained for type S units in MG.

Fatigue ratios were determined for 73 MG units and 19 soleus units in the control animals. The mean fatigue ratio for FF units(0.66) was

Figure 11. Sample records of IEMG - fatigue test (D) and fatigue index test(C) for a type S unit. A) EMG (upper trace) and force recorded in response to continuous stimulation at 30/sec. B) IEMG-force plot recorded during 30/sec stimulation. D) IEMG-force plot for same unit stimulated at 80/sec. Calibrations: A) EMG= 250uv/div., force=2gm/div. 15sec/div. B) IEMG= .11 mv-sec/div force= 6 grams/div C) EMG and force as in (A)., D) IEMG and force as in (B).

Figure 11.



significantly higher($p<.0001$) than that for either FR units(0.32) or S units(0.17) in MG. The average fatigue ratio for soleus units(0.17) was identical to that for type S units in the medial gastrocnemius. Figure 12 displays the frequency distributions of fatigue ratios for the motor units examined in both control muscles.

Fatigue indices were determined for 160 MG and 39 soleus units in the control populations. The frequency distributions for fatigue indices are shown in Figures 4. and 5.

As expected, there was a very good negative correlation($r=0.79$, $p<.0001$) of fatigue ratio with faigue index for the 72 motor units on which both of these parameters were determined. These two variable were not significantly correlated in the MG motor unit subpopulations or in the soleus motor unit control group.

4. Relationships between mechanical, electrical and fatigue properties of single motor units in the control populations

The variables used in the present study to characterize motor unit force (twitch and tetanic tensions) were moderately correlated with the amplitudes of the integrated electrical signals elicited by single shocks. The correlation coefficient determined for the varibles IEMG and tetanic tension($r=0.62, p<.0001$) was comparable to that determined for the relationship between IEMG and twitch tension($r=0.55, p<.0001$). The IEMG values recorded for individual MG units were also correlated fairly well with dP/dt values form the same units. No significant correlations were found between either IEMG and motor unit tension or IEMG and dP/dt for the units examined in soleus.

Table 3. shows the correlation coefficients determined for the relationship between the two fatigue properties and a number of differ-

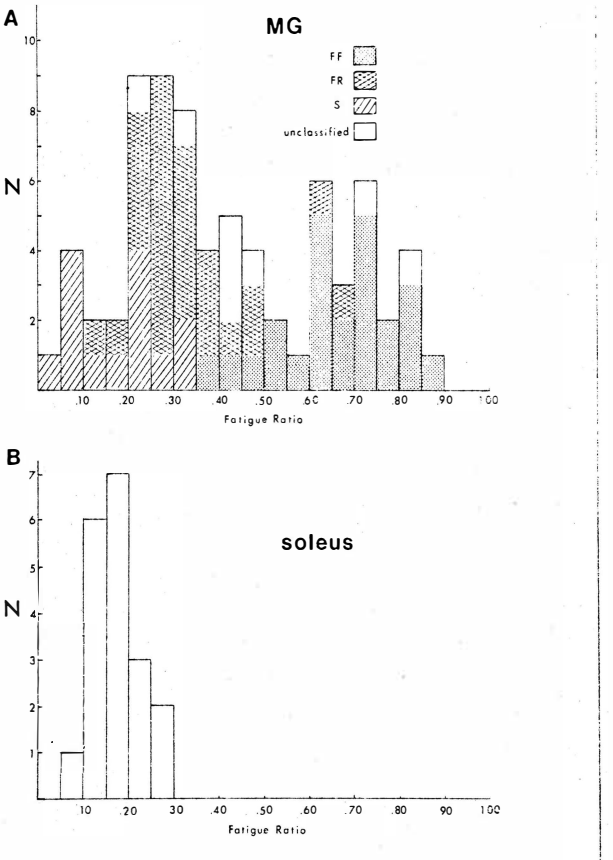


Figure 12. Histograms of fatigue ratios for medial gastrocnemius motor units (A) and soleus motor units (B) in the control populations.

TABLE 3. Correlations of fatigue properties with mechanical properties of single motor units in the control populations.

	MG			SOLEUS		
VARIABLES	r	p<	N	r	p<	N
CT - Fatigue Ratio	-0.57	.0001	71		NS	
CT - Fatigue Index	0.47	.0001	144		NS	
TT - Fatigue Ratio	0.67	.0001	73		NS	
TT - Fatigue Index	-0.54	.0001	157		NS	
P_{max}- Fatigue Ratio	0.71	.0001	72	0.50	.0305	19
P_{max}- Fatigue Index	-0.54	.0001	159		NS	
dP/dt-Fatigue Ratio	0.87	.0001	24		NS	
dP/dt-Fatigue Index	-0.83	.0001	28		NS	

NS: indicates correlation was not significant

ent mechanical properties recorded for MG and soleus motor units in the control groups. As shown in Table 3., motor unit contractile speed was moderately correlated with unit fatiguability and indicated that in general, the slower a unit's speed of contraction, the higher its resistance to fatigue. The correlation coefficients calculated for the relationship of unit twitch and tetanic tension with unit fatiguability indicated that motor units which were capable of producing large forces were generally more susceptible to fatigue than those which could generate only small forces. Both of these results are in agreement with the findings from a variety of other laboratories(Burke, 1967; Burke, et. al.,1971,1973.1974; Reinking, et.al.,1975; Stephens, et.al.,1973). In addition to these findings, when MG motor unit fatiguability was correlated with parameter which depended on both unit strength and speed (dP/dt), the correlation was much greater than that for either unit force or unit speed separately.

B. Properties of single motor units in the experimental populations

The properties of 95 MG and 31 soleus motor units were examined in 10 steroid-treated adult male cats. In the preliminary stages of this study, four animals were given a daily dose of triamcinolone acetonide of 4 mg/ kg of body weight. Three of these animals died prior to or during the surgical preparation period and as a result no data was collected from these animals. Nine animals were therefore subsequently treated with a daily dose of the steroid of 3 mg/ kg of body weight. The mean body weight of all 10 steroid-treated animals was 3.57 kg(range 2.80 to 4.02 kg). The average reduction in body

weight as a result of steroid administration was 7.25%.

Of the 95 MG units examined from steroid-treated animals (MG_s), 74 units were isolated from the L_7VR and 21 were found in the S_1VR. In the soleus unit population from steroid-treated animals($soleus_s$), 21 units were isolated from the L_7VR and 10 were from the S_1VR.

Individual motor units from the experimental groups were classified by type using the same criteria employed for control unit classification. In MG_s, 30 units (32%) were categorized as type FF, 21 units (22%) were type FR, 34 units (36%) were type S and 10 units (11%) did not clearly fall into one of the three major classes. All31 soleus units studied in the experimental animals were clearly type S. Table 4. shows the average values and standard deviations of MG and soleus unit properties in the experimental populations. Frequency distributions of several of these properties are shown in Figures 13 and 14. Since the general form of the fatigue index histograms for steroid-treated units were similar to those for control units and all fast-twitch units exhibited the "sag" property, the motor unit classification scheme appeared to remain valid for units in the experimental groups.

1. Mechanical properties of single motor units in the experimental populations.

In steroid-treated cats, the average twitch tensions of all three major types of MG motor units were significantly lower than those in control animals(Figure 15). This reduction was more pronounced in fast-twitch than in slow-twitch units. The mean twitch tensions of FF and FR units were reduced by 64% and 66% respectively as compared to a 41% drop in the mean twitch tension of type S units in MG_s. Although the mean twitch tension of soleus units was also diminished(↓23%), this reduction

TABLE 4. Mechanical, electrical and fatigue properties of single motor units in steroid-treated animals.

		Medial gastrocnemius				Soleus
		all units	FF	FR	S	all units
N (%)		(100)	(32)	(22)	(36)	(100)
MECHANICAL PROPERTIES						
Contraction time (msec)	$\bar{X}$	42.3	38.3	34.8	50.1	68.4
	SD	9.7	5.1	4.3	10.7	6.9
	(N)	(95)	(30)	(21)	(34)	(31)
Twitch tension (gm)	$\bar{X}$	3.6	7.1	2.1	0.6	1.7
	SD	3.9	3.8	1.8	0.4	0.7
	(N)	(95)	(30)	(21)	(34)	(31)
Max. tetanic tension (gm)	$\bar{X}$	11.2	19.9	8.7	3.8	12.5
	SD	9.3	9.7	4.2	3.3	5.1
	(N)	(94)	(30)	(21)	(34)	(31)
Twitch-tetanus ratio	$\bar{X}$	0.26	0.35	0.22	0.16	0.14
	SD	0.14	0.13	0.12	0.06	0.04
	(N)	(93)	(30)	(21)	(33)	(31)
dP/dt (gm/msec)	$\bar{X}$	0.23	0.40	0.23	0.06	0.19
	SD	0.23	0.24	0.20	0.05	0.09
	(N)	(93)	(30)	(21)	(33)	(31)
ELECTRICAL PROPERTIES						
$\int$EMG dt $(v\text{-}sec)\cdot 10^{-6}$	$\bar{X}$	0.61	1.16	0.43	0.29	1.01
	SD	0.58	0.70	0.33	0.18	0.48
	(N)	(95)	(30)	(21)	(34)	(31)
FATIGUE PROPERTIES						
Fatigue ratio	$\bar{X}$	0.37	0.71	0.23	0.12	0.16
	SD	0.27	0.10	0.08	0.06	0.05
	(N)	(89)	(29)	(21)	(30)	(31)
Fatigue index	$\bar{X}$	0.61	0.10	0.85	0.97	0.95
	SD	0.42	0.10	0.22	0.05	0.04
	(N)	(84)	(26)	(21)	(31)	(31)
OTHER PROPERTIES						
Conduction velocity (m/sec)	$\bar{X}$	101.4	104.8	107.2	94.2	71.9
	SD	11.3	8.7	6.9	12.1	7.7
	(N)	(95)	(30)	(21)	(34)	(31)

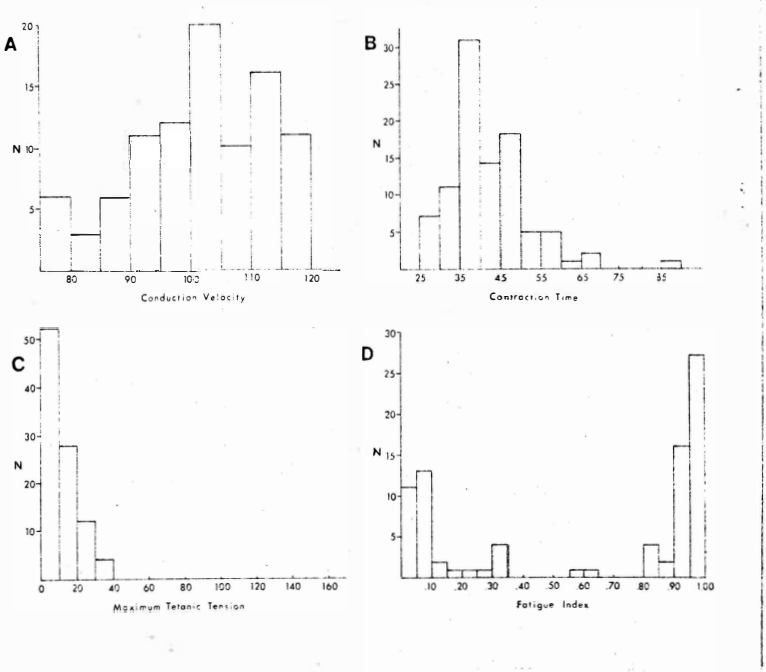


Figure 13. Frequency distributions of (A) conduction velocity (m/sec, N=95), (B) twitch contraction time (msec., N= 95), (C) maximum tetanic tension (grams, N=94), and (D) fatigue index (N= 84) for medial gastrocnemius motor units in the experimental population.

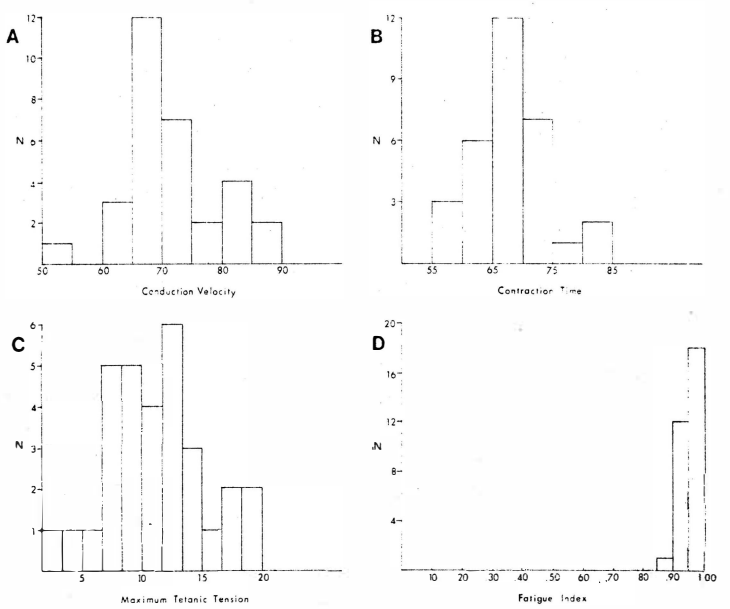


Figure 14. Frequency distributions of (A) conduction velocity (m/sec, N=31), (B) twitch contraction time (msec., N=31), (C) maximum tetanic tension (grams, N=31), and (D) fatigue index (N= 31) for soleus motor units in the experimental population.

was not found to be statistically significant.

The mean tetanic tensions of FF and FR units in MG of treated animals were also significantly lower than those in control animals, On the average, FF and FR units could generate only about 1/3 of the tetanic force produced by control units of the same type(Figure 15.). In contrast, the average tetanic tensions of type S units in MG_s was not significantly lower than that for MG type S units in control animals. Similarly, there was no significant effect of steroid treatment on the average tetanic tension of soleus units.

Although steroid treatment markedly reduced the absolute tension generating capabilities of MG units, it did not alter the relationship between motor unit twitch and tetanic tensions. The strong positive correlations between TT and P_{max} found for all MG and soleus units in the control groups were also seen for MG and soleus units in the experimental animals (MG_s: r=0.89, p<.0001; $soleus_s$: r=0.76, p<.0001). Just as was noted for MG control motor units, the strong positive correlation of TT with P_{max} persisted for the separate classes of MG motor units (FF: r=0.81, p<.0001; FR: r=0.77, p<.0001; S: r= 0.84, p<.0001) in steroid-treated animals.

The average dP/dt values for the three subpopulations of MG_s units were reduced in a pattern similar to that for average P_{max} values(Figure 15). Average dP/dt values for FF and FR units were 21% and 46% respectively of control dP/dt values for the same unit types. The average dP/dt for type S units in MG_s was not significantly altered as a result of steroid treatment. For soleus units in steroid-treated animals, the average dP/dt was 73% greater than the mean dP/dt value determined for control soleus units. In contrast to control studies, the range of dP/dt values for type FF and type FR units in MG_s were nearly identical. The range of dP/dt values for type S units in MG_s was similar to that for type S units in control MG

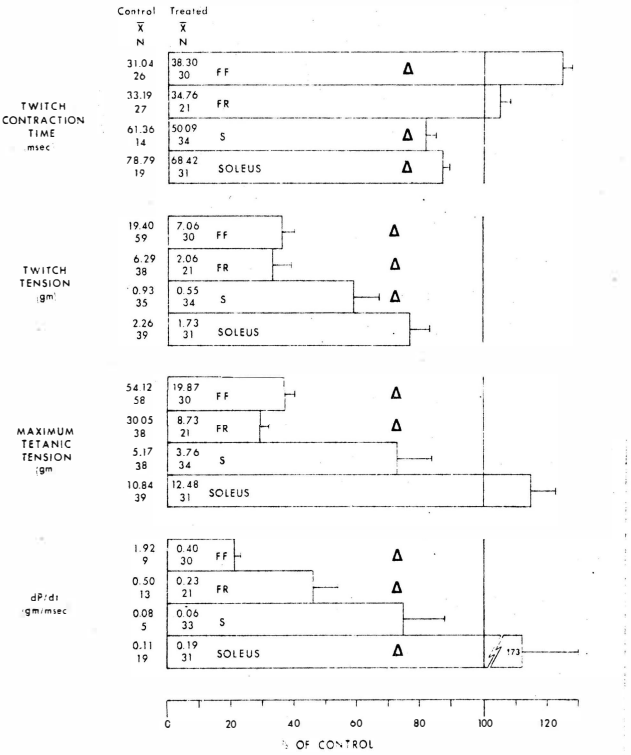


Figure 15. Strength- and speed- related contractile properties of single motor units is steroid-treated animals expressed as percentages± standard error of control mean values. Actual control means are presented to the left of bars. Δ indicates significant difference ($p<.001$) between control and experimental means.

and soleus muscles but was narrower than that found for soleus units in animals from the experimental group.

Table 5 shows the correlation coefficients for the relationships between dP/dt and motor unit tensions for units in the experimental populations. The relationships between these speed and force variable were similar to those found for MG and soleus control units.

The average twitch contraction time of type FF units in MG_s (38.8msec) was significantly longer than the mean contraction time for FF units in MG_c (31.0 msec). For FR units in MG_s the mean contraction time was not significantly different from the mean CT of FR units in control animals. Type S units in both MG_s and $soleus_s$ had mean CT values(50.1 msec and 68.4 msec respectively) which were significantly shorter than those seen for type S units in control muscles(61.4 and 78.8 msec respectively). With but three exceptions, the contraction times of FF and FR units were <45msec. The contraction times of soleus units in steroid-treated animals were in all cases >45 msec. In contrast to the findings from control studies, 24% of type S units in MG_s had contraction times of <45 msec. 50 of 51 fast-twitch units(FF and FR units) showed apparent fusion of tetanus at stimulation frequencies of 50/sec or greater, a finding similar to that in control studies. Of the 65 type S units examined in steroid-treated muscles, 64 had fusion frequencies of 35/sec or less.

All units isolated in steroid-treated animals were systematically examined for the presence or absence of the "sag"property. All units in MG_s classified as type FF or FR units exhibited sag in unfused tetanic responses. Of the 34 type S units in MG_s, 5 units exhibited sag. This property was absent in all but one soleus unit in animals from the experimental group.

TABLE 5. Correlations of dP/dt with twitch and tetanic tensions of single motor units in the experimental populations.

PROPERTIES		Medial gastrocnemius all units	Medial gastrocnemius FF	Medial gastrocnemius FR	Medial gastrocnemius S	Soleus all units
dP/dt - TT	r	0.81	0.63	0.93	0.85	0.66
	p<	.0001	.0002	.0001	.0001	.0001
	(N)	(93)	(30)	(21)	(33)	(31)
dP/dt - P_{max}	r	0.88	0.85	0.77	0.90	0.92
	p<	.0001	.0001	.0001	.0001	.0001
	(N)	(92)	(30)	(21)	(33)	(31)

All 21 type FR units and 28 of 30 FF units in MG_s showed an augmentation of twitch responses following brief tetanic stimulation. 9 of 34 type S units in MG_s did not exhibit post-tetanic potentiation of twitch responses. In soleus units from experimental animals, only one unit's twitch response was augmented by tetanization.

The approximate linear relationship between axonal conduction velocity and maximum tetanic tension for soleus type S units in control animals persisted for soleus units in steroid-treated animals(Figure 16A.) Similarly, glucocorticoid treatment did not markedly alter the linear relationship between conduction velocity and dP/dt for type S units in $soleus_s$ (Figure 16C.). A comparison of the graphs of conduction velocity versus P_{max} and conduction velocity versus dP/dt for MG units in steroid-treated animals(Figures 16B. and 16D.) with those for the same variables in control MG muscles reveals that the administration of steroids did not alter the general relationship between these variables for the three major classes of MG motor units.

2. Electrical properties of single motor units in experimental populations.

The time integral of full-wave rectified EMG signals(IEMG) elicited in response to a single stimulus was recorded for each motor unit examined in the muscles of steroid-treated animals. The average values of IEMG for FF, FR and S units in MG_s and for soleus units expressed as a percentage of the control mean are presented in Figure 17. The $\overline{IEMG}$ of FF units was larger than that for FR or S units in MG_s. As compared to control values, the average IEMG of type S units in MG_s and $soleus_s$ were significantly increased. Figure 18 shows the frequency

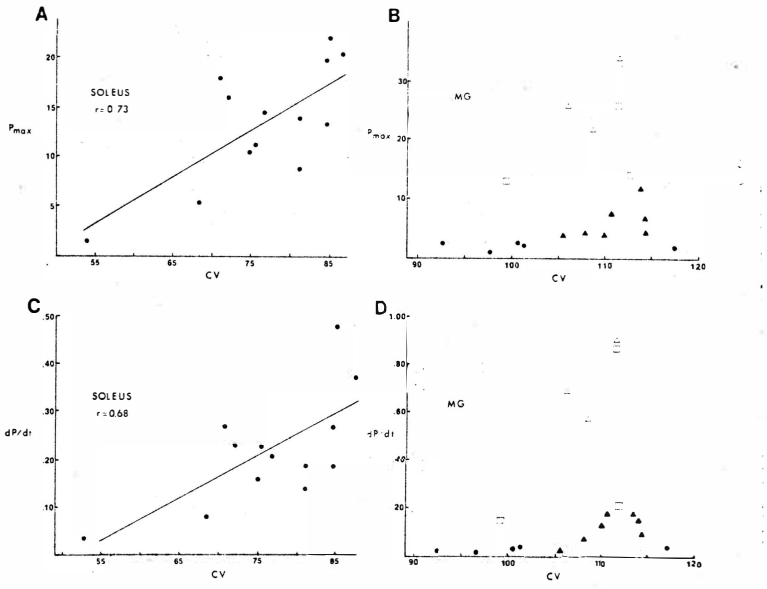


Figure 16. Relationship between maximum tetanic tension(P_{max} in grams) and axonal conduction velocity(CV in m/sec) for soleus units (16A) and for MG units(16B) from experimental animals. Relationship between axonal conduction velocity (CV) and maximum rate of rise of tetanic tension(dP/dt in grams/msec) for soleus(16C) and MG (16D) motor units in steroid-treated animals. Data for (A) and (C) from same experiment. Data for (B) and (D) from same experiment. Symbols as in Figure 7.

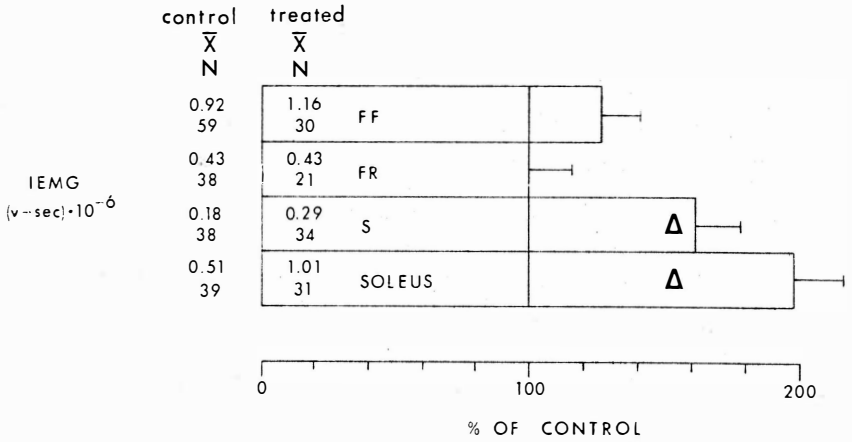


Figure 17. Electrical properties of identified motor units in steroid-treated animals expressed as percentages ± standard error of control mean values. Actual control means are presented to left of bars. Δ indicates significant difference ($p<.001$) between control and experimental means.

distribution of IEMG values for the units examined in steroid-treated muscles.

3. Fatigue properties of single motor units in experimental populations

Fatigue ratios and fatigue indices were determined for individual units in the muscles of treated animals in a manner identical to that for control units. Figure 19 shows the fatigue-related properties of motor units from MG_s and $soleus_s$ expressed as percentages of control values. Overall, there was no apparent effect upon the fatiguability of the motor units of either muscle as a result of steroid treatment. The only significant difference between control and experimental units was an increase in the resistance to fatigue of type FR units as reflected by a significant reduction in the mean FR fatigue ratio. The frequency distributions of the fatigue related variable are shown in Figures 13 and 20 for MG_s units and Figures 14 and 20 for $soleus_s$ units. A comparison of the fatigue index histograms for MG units in the control and experimental populations reveals that although the general forms of these histograms are similar, fewer units were found in steroid-treated MG whose fatigue indeces fell into the intermediate range ($0.25<FI<0.75$).

4. Relationships between mechanical, electrical and fatigue properties of single motor units in the experimental population.

The correlations of IEMG with unit force parameters in the experimental group were similar to those found for motor units in the MG control population. The correlation coefficients determined for the relationship

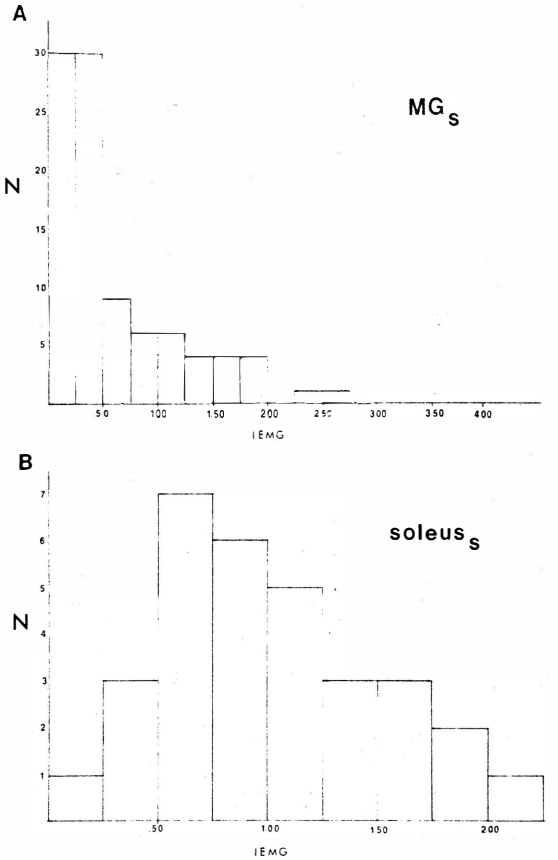


Figure 18. Frequency distribution of IEMG for single motor units in medial gastrocnemius (A) and soleus (B) of steroid-treated animals. IEMG in volts-seconds x 10^{-6}.

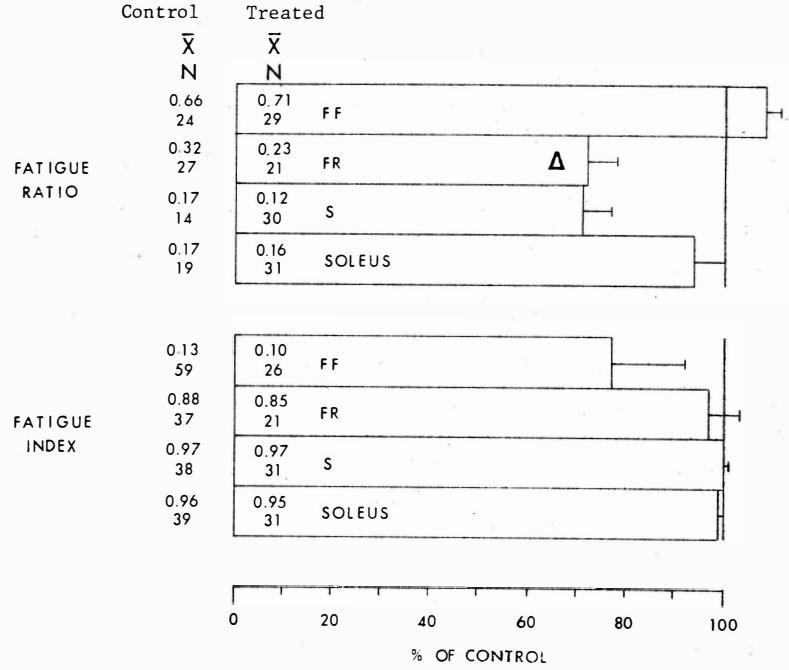


Figure 19. Fatigue-related properties of single motor units in medial gastrocnemius and soleus muscles of steroid-treated animals expressed as percentages ±standard error of control mean values. Actual means are presented to the left of bars. Δ indicates significant difference ($p<.001$) between control and experimental means.

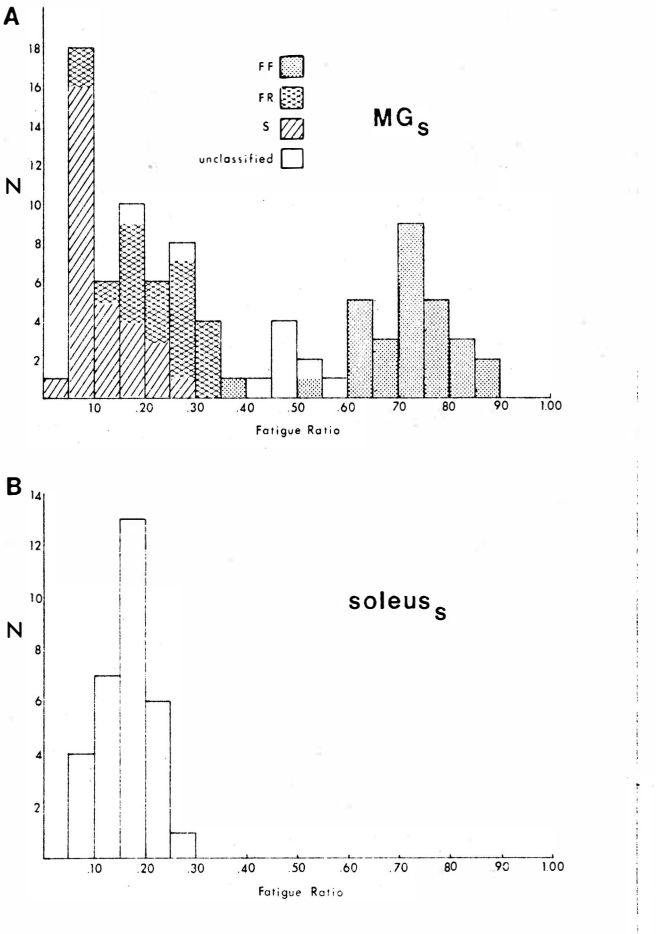


Figure 20. Histograms of fatigue ratios for medial gastrocnemius(A) and soleus (B) motor units in steroid-treated animals.

between IEMG and P_{max} in MG_S ($r= 0.73$, $p<.0001$) was slightly greater than that determined for the relationship between IEMG and twitch tension ($r= 0.66$, $p<.0001$). The IEMG values recorded for individual units in MG_S were also correlated fairly well with dP/dt values recorded from these same units ($r=.065$, $p<.0001$). Although no significant correlations were found between IEMG and tension parameters in soleus control units, the correlation of IEMG with P_{max} ($r= 0.65$, $p<.0001$), with twitch tension ($r= 0.61$, $p< .0002$) and with dP/dt ($r=0.63$, $p<.0002$) for soleus units in steroid-treated animals were all moderately good.

Table 6 shows the correlation coefficients determined for the relationships between the fatigue parameters and a variety of mechanical properties recorded for MG and soleus motor units in the experimental groups. The correlations between the fatigue variables and the tension variables for units in MG_S were similar to those calculated for MG control units(Table 3). In contrast, weaker correlations were found for the relationships between the fatigue variables and speed variables for units in MG_S than in MG control units.

TABLE 6. Correlations of fatigue properties with mechanical properties of single motor units in the experimental populations.

	MG			SOLEUS		
VARIABLES	r	p<	N	r	p<	N
CT - Fatigue Ratio	-0.34	.0012	89		NS	
CT - Fatigue Index	0.29	.0078	84	0.37	.0381	31
TT - Fatigue Ratio	0.69	.0001	89	0.42	.0172	31
TT - Fatigue Index	-0.74	.0001	84		NS	
P_{max}- Fatigue Ratio	0.68	.0001	89	0.54	.0016	31
P_{max}- Fatigue Index	-0.69	.0001	84		NS	
dP/dt-Fatigue Ratio	0.54	.0001	88	0.44	.0131	31
dP/dt-Fatigue Index	-0.60	.0001	83		NS	

NS: indicates correlation was not significant

DISCUSSION

Previous studies have shown that glucocorticoid administration produces more pronounced effects on the contractile properties of pale muscles than on red muscles from the same animal (Gardiner and Edgerton, 1979; Gardiner, et al.,1978,1980; Vignos, et al,1976). The results of the present study have demonstrated that steroid treatment produces preferential alterations in the strength- and speed-related properties of isolated single motor units in pale muscles which can account for the observed changes in the mechanical properties of these muscles in steroid-induced myopathy.

A. The Effect of Steroid Administration on the Contractile Properties of Single Motor Units.

The average twitch tensions generated by all three major classes of motor units in the pale MG were significantly reduced as a result of steroid administration (Figure 15). This reduction in twitch tension was greater in fast-twitch units than in slow-twitch units from the same muscle. The average reduction in twitch tension for types FF and FR units was 64% and 66%, respectively. By comparison, the mean twitch tension of MG type S units was decreased by 41%.

The maximum tetanic tensions produced by identified MG motor units were also reduced a a consequence of glucocorticoid treatment (Figure 15). The mean tetanic tensions generated by type FF and FR units in steroid-treated MG muscles were decreased by 63% and 71% respectively. The average tetanic tension produced by type S units in MG_S was reduced by only 25%, a change which was not statistically significant.

In contrast, steroid administration had no apparent effect upon the strength-related properties of single motor units in the red soleus

muscle. Neither the mean twitch nor mean tetanic tension of type S units in treated soleus muscles were significantly different from those in control animals (Figure 15).

The finding that the average twitch and tetanic tensions of type S units in MG were reduced and that these same properties were unaltered in soleus type S units may be accounted for in one of several ways. Gardiner, et al.,(1978) have shown that slow-twitch type SO muscle fibers atrophy more in pale muscles than in red muscles from the same steroid-treated animal. These workers have suggested that the atrophic response of SO fibers may be dependent upon some general characteristic of the parent muscle(i.e., speed of contraction) in which these fibers are contained. An alternative hypothesis to account for the more pronounced involvement of SO fibers inpale muscles is that although these SO fibers from red and pale muscles have identical histochemical profiles, there may be as yet unidentified fundamental differences among similar fiber types in different muscles. Several lines of evidence tend to support the latter contention. Spamer and Pette(1977) have shown that the levels of glycolytic and oxidative enzymes in SO fibers from the red soleus in rabbits are lower than those of SO fibers within the pale psoas muscle. It has also been suggested that SO fibers within different muscles may have different protein turnover rates(Li and Goldberg,1976) which could account for their varying susceptibility to the catabolic action of glucocorticoids. The findings presented here, as well as those from other studies have consistently demonstrated that the mechanical properties of type S motor units in red and pale muscles are quite different. Type S units in predominantly red muscles produce larger tetanic tensions, contract more slowly, and have, on the average, fibers of larger diameter

than do type S units in primarily pale muscles. In addition, tetanic stimulation augments the twitch responses of type S units in pale muscles with little or no effect upon the twitch responses of type S units in red muscle. Since it is known that the impulse activity pattern imposed upon skeletal muscle fibers can determine their protein turnover rates, metabolic characteristics and mechanical properties, the differential effects of steroids on the strength-related properties of type S motor units in pale and red muscles may reflect differences in the overall activity patterns to these units between different muscles.

Although twitch and tetanic tensions of single motor units were decreased as a result of steroid treatment, the average twitch-tetanus ratios for the three classes of units in MG_S were unchanged with respect to those in control animals. The positive linear relationship between twitch and tetanic tension demonstrated for control MG units was also found for MG units following steroid treatment. These findings may indicate that while some muscle protein was undoubtedly lost as a result of steroid treatment, there were no changes in the tension generating capabilities of the remaining muscle tissue. This suggestion has been previously proposed by workers examining the effects of glucocorticoids on the strength-related properties of whole muscles. Vignos, et al.,(1976) have shown that although the absolute twitch and tetanic tensions(in grams) of rabbit pale muscle are reduced following glucocorticoid treatment, the normalized twitch and tetanic tensions(expressed as grams per cm of cross-sectional area of the whole muscle) were unchanged. They interpreted this finding as an indication that although chronic steroid administration significantly reduced pale muscle mass, the strength of the remaining fibers was maintained at normal levels. Gardiner and coworkers have examined the

strength-related properties of pale(MG) and red (soleus) muscles in steroid-treated rats(Gardiner and Edgerton,1979) and cats(Gardiner,et al., 1978). In each of these studies, when twitch and tetanic tensions for each muscle were normalized(expressed as either grams per gram of contractile protein or grams per gram of remaining muscle), there were no significant alterations in these values as compared to those in control animals. Since the specific tension capabilities of the contractile material in pale red muscles were maintained, these workers suggested that the muscle weakness generally associated with chronic steroid treatment was not due to qualitative changes in the contractile proteins, but might be ascribed to alterations in muscle mass, changes in the excitation-contraction coupling machinery or to a combination of these factors.

The changes in the strength-related properties of identified motor units as a consequence of sterid administration indicate that catabolic steroids exert a preferential effect upon fast-twitch as compared to slow-twitch units. Additional evidence in support of this hypothesis is derived from data on the speed-related properties of identified units in control and treated animals. The average dP/dt values obtained for the three subpopulations of MG_S units were found to be lower than for those for the same motor unit types in control animals (Figure 15). This reduction in dP/dt was greatest in type FF units(79%) and intermediate in type FR units (54%) and least in type S units (25%). In contrast, the average dP/dt value for soleus type S units was dramatically increased (73%). The pattern of the reduction in dP/dt for the three classes of units in MG_S (FF>FR>S) was similar to that found for the reduction in $\overline{P_{max}}$ values in MG_S. Close (1964) has shown that the maximum rate of rise of isometric tension for whole muscles correlates well with muscle shortening velocity

determined under isotonic conditions. In addition, Drachman and Johnson(1973) have demonstrated that this index of contraction speed (dP/dt) in rat muscles is a good predictor of a muscle's actomyosin ATPase activity. If these findings can be extended to motor units, the differential alteration in dP/dt values of the three motor unit types suggests that catabolic steroids exert differential effects on the myofibrillar ATPase activity of FF, FR, and S motor units. The pattern of changes in the twitch contraction times of FF, FR, and S units in steroid-treated muscles tend to support this contension. The twitch contraction times of type FF units in MG_S were significantly longer than those of FF units in control muscles (Figure 15). This increase in twitch time to peak tension could be accounted for by a reduction in myofibrillar ATPase activity as reflected by the decrease in $\overline{dP/dt}$ values for FF units. In contrast, type S units in $soleus_S$ had shorter twitch contraction times than were observed for soleus units in control animals. The $\overline{dP/dt}$ value for $soleus_S$ units was significantly higher than that for control soleus units and may reflect an actual enhancement of myofibrillar ATPase activity within the SO fibers of steroid-treated muscles.

The relationships between the maximum rate of rise of tetanic tension (dP/dt) and the absolute maximum tetanic tensions of identified units in both MG and soleus were not apparently altered as a result of the steroid-treatment used in this study. This finding may indicate that glucocorticoids do not alter the force-velocity relationships within the remaining functional contractile material.

B. The Effect of Steroid Administration on the Electrical Properties of Single Motor Units.

Since the summated electrical activity of skeletal muscle during reflex and voluntary contractions is frequently used as an index of muscular force, it seemed desirable to examine the relationship between single unit EMG and force in the muscles of both control and steroid-treated animals. In addition, it has been suggested that the changes observed in the contractile properties of whole muscles in response to steroid treatment may reflect alterations in the electrical properties of their fibers (Gardiner, et al., 1979; Gruener and Stern, 1972). In the present study, no significant differences were found between the average integrated electrical activity ($\overline{IEMG}$) of MG fast-twitch units in steroid-treated animals and those in control animals. In contrast, the EMG signals for type S units in both MG_S and $soleus_S$ appeared to have larger peak-to-peak amplitudes, longer durations and generally more complex waveforms than those in control animals, although no systematic measurements were made on these signals. These results are of interest in view of the findings from other laboratories concerning the effects of glucocorticoids on muscle fiber electrical properties. Gruener and Stern, (1972) have reported that dexamethasone treatment in mice reduces the resting membrane potential of the pale extensor digitorum longus fibers and renders this muscle less electrically excitable. In spite of this effect, the total amplitude of the action potentials elicited in EDL fibers was constant in both control and treated muscle fibers. The steroid regimen employed in that study had no apparent affect upon the electrical properties of the red muscle fibers in soleus. Grossie and

Albuquerque(1978) examined the effects of triamcinolone acetonide (29 mg/kg/day for 4 days) on the characteristics of action potentials in the rat EDL muscle. Although these characteristics (action potential overshoot, amplitude and rate of depolarization) were reduced after one day of treatment, they were returned to normal by the fourth day of treatment. Prabhu and Oester (1971) have shown that the EMG signals recorded from the rat tibialis anterior muscle were essentially normal following an eight-week period of cortisone administration(10 mg/kg/day). It is apparent from the results of these studies as well as those from the present work that the administration of glucocorticoids does not exert a significant effect upon the electrical properties of pale muscle fibers and, thus, it is unlikely that the alterations in the contractile properties of steroid-treated fast-twitch motor unit fibers can be accounted for by a change in these properties. The finding that the $\overline{IEMG}$ values of type S units in both MG_s and $soleus_s$ were markedly increased was unexpected in view of the work of Gruener and Stern(1972) who found no apparent changes in the electrical properties of type I fibers in soleus muscles from sterid-treated mice. The increase in IEMG values from type S motor units may reflect a slowing of action potential propagation along type I fibers in steroid-treated animals but the overall significance of such an effect is as yet unclear.

C. The Effect of Steroid Administration on the Fatigue Properties of Single Motor Units

Gardiner, et al., (1978) have performed the only other study to date which examined the effects of glucocorticoid administration (4 mg/kg/day of triamcinolone acetonide for 10 - 14 days) on the susceptibility to fatigue of cat pale and red hindlimb muscles.

Using a stimulus paradigm originally employed by Burke, et al., (1973) to induce fatigue, these workers showed that as a whole, the pale MG muscles in the cat hindlimb were less resistant to fatigue (decreased fatigue indices) as the result of steroid treatment. The susceptibility to fatigue of the red soleus muscles however, which are normally very fatigue-resistant, was not significantly different than that found in untreated animals. The results of the present study have shown that the fatigue properties of single motor units in both MG and soleus were essentially unaltered following glucocorticoid treatment. The only significant alteration noted was a reduction in the mean fatigue ratio of type FR units. Since the electrical responses of type FR units were unaltered in steroid-treated muscles, this finding suggests that FR units in MG_s were on the average more resistant to contractile fatigue than those in control animals. Because the daily steroid dose used in the present study was 25% lower than that used by Gardiner and coworkers (1978), the possibility exists that the amount of steroid administered was insufficient to result in changes in the oxidative capacity of the majority of muscle fibers which would result in alterations in their resistance to fatigue.

It was clear by a comparison of the frequency distributions of fatigue indices for MG units in the control (Figure 4) and experimental (Figure 13) groups, that there were far fewer units in MG_s whose fatigue indices fell into the intermediate range ($0.25<FI<0.75$). This finding suggests that units which would normally fall into this range had either lost or gained resistance to fatigue. Some workers have suggested that units with an intermediate resistance to fatigue actually represent units in a "transition" state (Johnson, et al., 1978; Saltin and

Henrikkson, 1977). The paucity in the number of units having intermediate fatigue indices may indicate that steroids either hasten or prevent the conversion of these units into either type FF or type FR units.

D. Site of Action of Glucocorticoids

Where in the electro-chemical mechanical process of muscle contraction do steroids exert their effects to produce the observed alterations in the contractile properties of skeletal muslce. In theory, steroids may act at one or a combination of points in the sequence of events which ultimately lead to the production of muscular force. The list of sites and/or processes which have been suggested to be affected by corticosteroids includes:1)neuromuscular transmission, 2) action potential generation or propagation along the muscle fibers, 3) sarcoplasmic reticulum function -changes in the activation of SR, the rate of release of Ca^{++} and/or the rate of Ca^{++} reuptake, 4) the myofibrillar regulatory proteins, 5) myosin molecules, 6) myosin ATPase activity changes, 7) alterations in oxidative and glycolytic enzyme activity, and 8) changes in the muscle connective tissue matrix.

During the course of the present study, there were no indications that neuromuscular transmission was impaired as a result of the glucocorticoid regimen used. In no instance did we note the failure of a stimulus to the motor axon to produce both electrical and mechanical responses in isolated single units. The finding that the integrated EMG signals elicited from motor units in the pale MG_s were not significantly different from those in control MG units, even though the

speed- and strength-related properties of these units were dramatically altered would suggest that the steroid administration employed in the present study did not alter the electrical properties of these fibers.

The patterns of changes in the dP/dt values for MG units in the experimental animals suggests that steroids exert a differential effect on myofibrillar ATPase activity. However, this finding does not rule out the possibility that steroids can alter sarcoplasmic reticulum function and/or the myofibrillar proteins as well.

Since there has been shown a clear correlation between muscle oxidative enzyme activity and endurance capacity (Holloszy,1973), the data concerning motor unit fatiguability obtained in this study suggest that oxidative enzyme activity of motor units examined here was not altered by the particular steroid treatment program employed.

The results of this study do not allow specualtion concerning the effects of steroids on the properties of the connective tissue matrix of skeletal muscles.

E. The Relationship between Motor Unit Use and Susceptibility to Glucocorticoids.

The results of the present study concerning the speed- and strength-related properties of single motor units have clearly shown that the pattern of preferential atrophy of the three types of muscle fibers commonly seen in steroid-induced myopathy (Gardiner, et al., 1980) is accompanied by a preferential effect on the contractile properties of the three corresponding classes of motor units. The steroid-induced changes are, in general, greatest in type FF units, intermediate in

type FR units and least in type S units in mixed muscles. In addition, the contractile properties of type S units in pale muscles undergo more pronounced changes following steroid treatment than do type S units in red muscles. How then is this preferential susceptibility of motor units to the catabolic action of steroids regulated?

Goldberg and Goodman(1969) have provided evidence which indicates that muscle activity may play a key role in the resistance of muscles to atrophic or catabolic influences. These workers showed that the administration of large doses of cortisone to rats produced a marked atrophy and wasting of pale muscle (plantaris) and had little or no effect on the size of red muscle (soleus). Denervation which produces an absolute reduction in muscle activity increased the sensitivity of both pale and red muscle to the catabolic actions of cortisone. When the overall activity to plantaris was increased by tenotomy of the synergistic gastrocnemius muscle, the plantaris muscle became less sensitive to steroid-induced atrophy. These workers concluded that the catabolic actions of steroids were more pronounced in less active muscles, and thus demonstrated a relationship between muscle use and muscle sensitivity to the action of glucocorticoids.

It has been known for quite some time that there is also a relationship between muscle type and muscle use. Slow, red muscles have the lowest thresholds to reflex activation and are used most in a variety of postural and locomotive activities. Muscles composed primarilly of pale fibers are used rather infrequently and tend to be active only in more powerful movements(Adrian and Bronk,1928,1929; Creed,et al., 1932). This principle has also been extended to pale and red motor units. Functionally, motor units are brought into activity

in order of increasing size(Clamann and Henneman,1976; Henneman, et al., 1964,1974). Thus, the most fatigue resistant, red type S units are the most used and the most frequently activated. Pale, readily fatiguable type FF units are used only occasionally in more powerful movements and are thus activated much less frequently. It is now generally agreed that this "Size Principle" governs motor unit recruitment and thus the overall frequency of activation of motor units in animals and in man(Burke, et al.,1975; Desmedt and Godaux,1977; Freund, et al., 1975; Henneman,et al., 1965). The present study has shown that the units which are normally the least active(type FF) undergo the most pronounced changes in their contractile properties as a result of steroid treatment. In contrast, those motor units most frequently activated (type S) during normal activities were the least susceptible to the catabolic action of steroids. In view of these findings, we propose that the "Size Principle" can be extended to explain not only the order in which motor units are recruited in the development of muscular tension, but also to account for the patterns of muscle fiber atrophy and motor unit involvement seen in steroid-induced myopathy.

If this hypothesis is correct, a question which then remains is, "how does the overall pattern of neural activation of motor units control their susceptibility to steroid-induced dysfunction?" Changes in the frequency of activation of skeletal muscles by a variety of experimental means have repeatedly been shown to alter their contractile properties, histochemical profiles, and biochemical characteristics. These findings suggest that changes in the neural activation patterns to skeletal muscle may be associated with alterations in the processes of protein synthesis or with the regulation of gene expression in skeletal muscle

fibers. Recent studies by Metafora,et al.,(1980a,1980b) have provided the first direct evidence demonstrating the link between neural activation and protein synthesis in skelatal muscles. These workers have shown that following eight days of denervation, the function of the protein synthesizing machinery in the rat MG fibers is reduced. They suggested that the reduction in ribosomal biosynthetic activity was altered as a consequence of a decreased number of ribosomes, or a reduction in the amount of activity translated mRNA(Metafora,et al., 1980a). In a companion paper, these workers (Metafora, et al., 1980b) found that following denervation, there were marked changes in many of the mRNA sequences in muscle fibers. This result lends strong support to the hypothesis thatmotoneurons are capable of controlling gene expression in muscle fibers.

Studies from the laboratory of Almon (Almon and DuBois,1980; DuBois and Almon,1980) have examined the hypothesis that selective muscle atrophy, regardless of the cause, is due to an increase in the sensitivity of particular muscles to circulating levels of steroids. These workers induced muscle atrophy in the rat gastrocnemius muscle by denervation or immobilization of the rat hindlimb. In both experimental situations, the number of glucocorticoid receptors in atrophic muscles were increased as compared to control muscles while there was no apparent change in the affinity of these receptors for steroids. The results of this study may indicate that the reduction in muscle activation associated with denervation or immobilization actually enhances the synthesis of steroid receptor protein, thus increasing the susceptibility of this tissue to atrophy, even though the overall synthesis of protein is reduced (Goldberg,1969; Metafora,

et al., 1980). From these findings one may then speculate with respect to the question posed above, that the neural activation of skeletal muscle regulates the susceptibility to atrophy of this tissue by controlling the synthesis of glucocorticoid receptor proteins in muscle fibers , and hence the number of steroid receptors within these fibers.

F. Summary

1) This study represents the first attempt to examine the effects of glucocorticoids on the mechanical, electrical, and fatigue properties of single motor units in pale and red hindlimb muscles of the cat.

2) Catabolic steroids exert profound effects on the strength- and speed-related properties of motor units in pale muscles. The changes in these properties are more pronounced in the two types of fast-twitch units than in the slow-twitch units within the same muscle. On average, types FF and FR units (in steroid-treated pale muscle) can generate only about 1/3 of the tetanic tension of control fast-twitch units. The average rate of rise of tetanic tension for FF and FR units were reduced by 79% and 54% respectively as a result of steroid treatment. In contrast, the mean maximum tetanic tension and mean rate of rise of tetanic tension for MG type S units were reduced by only 25% following steroid treatement.

3) Steroid administration appears to have more pronounced effects on the contractile properties of slow-twitch units in pale muscle than on the same properties of slow-twitch units in red muscle.

The mean twitch tension of type S units in MG was reduced by 41% as compared to a 23% decrease in the mean twitch tension of type S units in soleus.

4) The particular dose and duration of steroid treatment employed in this study did not impair the electrical activity or the fatigueresistance of the three major classes of motor units within pale muscles.

5) These findings imply that units which are used the most during normal activities are the least susceptible to steroid- induced changes. Conversely, those units used only occasionally are the most severely affected by the actions of steroid administration.

6) Since the overall neural activation and thus the degree of use of motor units can be accounted for by the "Size Principle", the data derived from this study imply that this principle can be extended to account for the preferential involvement of fast-twitch motor units in steroid-induced myopathy.

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APPENDIX A

TABLE 1.

A. General Morphlogical and Physiological Properties of Red and White Muscles

CHARACTERISTIC	RED MUSCLE	WHITE MUSCLE
1. Appearance	opaque, granular, large proportion of sarcoplasm, distinct longitudinal striations, less distinct cross striations	translucent, small proportions sarcoplasm, distinct cross striations
2. Fiber diameter	small	large
3. Mitochondrial density	high	low
4. Sarcoplasmic reticulum	poorly developed	well developed
5. Z lines	wide	narrow
6. M lines	not discrete	discrete
7. H zones	poorly defined	well defined
8. T-tubules	poorly developed	well developed
9. Capillary density	high	low
10. Contraction time	slow	fast
11. Fusion frequency	low	high
12. Fatiguability	fatigue resistant	readily fatigable
13. Resting membrane potential	low(50 -70 mv)	high (80-90mv)

B. General Biochemical Properties of Red and White Muscle

PROPERTY	RED MUSCLE	WHITE MUSCLE
1. Na^+ Content	high	low
2. K^+ COntent	low	high
3. Ca^{++} assoc. with SR	low	high
4. Ca^{++} assoc. with mitochondria	high	low

TABLE 1 continued:

PROPERTY	RED MUSCLE	WHITE MUSCLE
5. Total protein	12%< white	12% > red
6. ATPase activity	low	high
7. Myoglobin content	high	low
8. Glycogen content	low	high
9. Creatine phosphate	moderate	high
10. Myofibrillar protein	same	same
11. Lipid content	high	low
12. Fatty acids	high	low
13. Triglycerides	high	low
14. Oxidative capacity	high	low
15. Glycolytic capacity	low	high
16. Phosphorylase activity	low	high

APPENDIX A continued:

TABLE 2. Tripartite muscle fiber and motor unit classificationschemes: Physiological, morphological, biochemical and enzymatic properties.

Fiber Classification			
(Henneman and Olson, 1965)	A	B	C
(Romanul, 1964)	I	II	III
(Brooke and Kaiser,1970)	IIb	IIa	I
(Guth and Yellin,1971)	αβ	α	β
(Barnard, et. al., 1971)	fast-twitch white	fast-twitch red	slow-twitch red
(Peter, et.al.,1972)	FG	FOG	SO
	(fast-twitch glycolytic)	(fast-twitch oxidative-glycolytic	(slow-twitch oxidative)
Motor Unit Classification			
(Burke, et.al., 1973)	FF (fast-twitch, fatiguable)	FR (fast-twitch, fatigue-resistant)	S (slow-twitch, fatigue-resistant)
Physiological properties			
Conduction velocity	fast	fast	slow
Resistance to fatigue	low	high	high
Twitch speed	fast	fast	slow
Tetanic tension	high	intermed.	low
sag property	present	present	present or absent
post-tetanic potentiation	present	present	present or absent
fusion frequency	high	high	low

APPENDIX A Table 2 continued

Morphological properties			
Color	white(pale)	red(dark)	red(dark)
Mitochondrial content	small	intermed.	large
Capillary density	sparse	rich	rich
Muscle fiber diameter	large	variable	small
Z lines	narrow	broad	intermed.
# fiber/motor unit	large	intermed.	small
Biochemical properties			
Myoglobin content	low	high	high
Glycogen content	intermed.	high	low
Neutral fat	low	low	high
Enzymatic properties			
Myofibrillar ATPase activity	high	high	low
Mitochondrial ATPase activity	low	high	intermed.
Oxidative enzyme activity	low	intermed. or high	intermed or high
Glycolytic enzyme activity	high	intermed.	low