



National Library
of Canada

Bibliothèque nationale
du Canada

Acquisitions and
Bibliographic Services Branch

Direction des acquisitions et
des services bibliographiques

395 Wellington Street
Ottawa, Ontario
K1A 0N4

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file - Votre référence

Our file - Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

The Role of the Motor Neuron in the Maintenance
of Contractile Properties and Myosin Expression
in Skeletal Muscle of the Mouse

by

George Desypris

A thesis submitted to the Faculty of Graduate Studies
and Research in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy



National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your list / Votre référence

Our list / Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-85842-7

Canada



UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

To my beloved children, Alexandra and Christopher
and my dearest friend, Annette

Thank you Joan for standing by my side and encouraging me through all the rougher times. You helped me to focus my thoughts in times when I could not see the bright light at the end of this convoluted tunnel. Rest assured that when the day comes that you embark on your own hazardous journey, I will be by your side.

To the late Dr. Graham Mainwood.

I feel priveleged to have been taught by you. Your effortless and lucid way of conveying the most complex biophysical concepts made the learning experience a most pleasant one. Many of us whom you taught look upon you as our inspirational father. When you left us you also left behind a void in the universe of science that may never be filled. You are the greatest scientist I have known for you were genuinely interested in pursuit of the truth and not the self-glorification with which so many "scientists" these days seem to be obsessed. Hopefully you have charmed enough of us so that collectively we may fill the void you left.

Acknowledgements

No words can accurately express my gratitude to Suzanne, Camille and Marie-Andree Boutin for their unending support and encouragement throughout the course of my studies. Thank you for giving me the chance to realise my aspirations.

To my supervisor David Parry, I am grateful for having had the opportunity to carry out my fledgling developmental stages under your guidance. You have seen my emergence from an uncommitted mesenchymal stem cell, through the myoblast stage, and now, hopefully I have fulfilled the criteria to be classified as a mature skeletal myofiber.

I would also like to thank Dr. Heather Davis for co-supervising me during the early stages of the project.

Many thanks to Dr. Tony Krantis for his most valuable advise on matters of academia as well as general politics in the sometimes turbulent world of science.

Much of the work in this thesis was carried out in the Department of Anatomy at McGill University. I would like to thank Dr. Dennis G. Osmond, Chairman, for allowing me the use of his computer facilities as well as the permission to carry out experiments in the Department of Anatomy.

As well I would like to thank Dr. Mike Lalli for looking the other way in regards to my multiple parking infractions. I am sure you saved me well over a couple of months in tuition fees. Also thank you for letting me stay in Heathers office for the extended period that I did.

Many thanks to Sylvie Lachapelle for her valuable technical expertise.

In addition to the intellectual stimulation the warm friendship and encouragement by Dwayne Rahal and Dajan O'Donnell will always be remembered.

A special thank you to my friends Luc Major and Stavros Georganos for providing me with a place to stay during my sporadic visits to Ottawa.

I am also gratefull to Audrey Innis and Karen Halse for their help during the course of my studies.

Table of Contents

<u>Abstract</u>	pp. 1-3
<u>Introduction</u>	pp. 4-46
Short Note on Nomenclature.....	pp. 5-6
Mammalian Skeletal Muscle and Classification Systems.....	pp. 7-13
E-C Coupling and Mechanical Activation of Skeletal Muscle.....	pp. 13-19
The Motor Unit and Myotrophic Interactions.....	pp. 20-29
Models of Disuse.....	pp. 29-32
Isolating Activity from Myotrophic Influences.....	pp. 32-37
Chronic Stimulation Paradigms and the Role of Activity in Transforming Skeletal Muscle Properties.....	pp. 37-44
Experimental Approach.....	pp. 44-46
<u>Materials and Methods</u>	pp. 47-64
Experimental Groups.....	pg. 47
Denervations.....	pg. 48
Preparation of TTX cuffs.....	pp. 48
Preparation of colchicine cuffs.....	pg. 49
Implantation of TTX cuffs.....	pp. 49-51
Implantation of colchicine cuffs.....	pg. 52-53
Contractile Parameters.....	pp. 54-55
Measurement of the Maximum Velocity of Shortening using the Slack Test.....	pp. 55-57
Histological Processing of Muscle.....	pg. 57
Immunohistochemistry.....	pp. 58-59

Polyacrylamide Gel Electrophoresis:.....	pp. 60-61
Preparation of Muscle Extract.....	pp. 60
Myosin Heavy-Chain Separation.....	pg. 61
Myosin Light-Chain Separation.....	pg. 61
Statistical Analysis.....	pg. 61
<u>Results</u>	pp. 65-98
Viability of Cuffing Procedure.....	pp. 65-67
Muscle Weights.....	pg. 67-68
Twitch and Tetanic tensions and Pt/Po.....	pg. 68-69
In-vivo Twitch temporal Parameters.....	pg. 69-70
In-vitro Twitch temporal Parameters and Vus.....	pg. 70-71
Myosin heavy-chain composition of EDL and SOL muscles from various experimental groups.....	pp. 71-73
Myosin Light-Chain composition of EDL and SOL muscles	pg. 73-74
Experiment with Peripheral Nerve Extract.....	pp. 74-75
<u>Discussion</u>	pp. 100-124
<u>References</u>	pp. 125-144
<u>List of Abbreviations</u>	pp. 145-146

Tables and Figures

Table I. Muscle wet weights of EDL and SOL muscles from various experimental groups.....	pg. 76
Table II. Isometric force measurements from EDL muscles	pg. 77
Table III. Isometric force measurements from SOL muscles	pg. 78
Table IV. Twitch Temporal Parameters for EDL muscles (in- vivo).....	pg. 79
Table V. Twitch Temporal Parameters for SOL muscles (in- vivo).....	pg. 80

Table VI. Isometric and Isotonic parameters for EDL muscles (in-vitro).....	pg. 81
Table VII. Isometric and Isotonic parameters for SOL muscles (in-vitro).....	pg. 82
Table VIII. Isometric and Isotonic parameters for EDL and SOL muscles of Dystrophic (dy2j/dy2j) mice..	pg. 83
Table IX. % Fibers showing +ve Immunoreactivity to anti I, IIA and IIB myosin in EDL muscles.....	pg. 84
Table X. % Fibers showing +ve Immunoreactivity to anti I, IIA and IIB myosin in SOL muscles.....	pg. 85
Figure 1. E-C Coupling Scheme.....	pg. 14
Figure 2. Developmental Profile for Mammalian Skeletal Muscle Fibers.....	pg. 21
Figure 3. Schematic of mini-osmotic pump setup.....	pg. 62
Figure 4. Twitch temporal parameters.....	pg. 63
Figure 5. Determination of Vus using the slack test.	pg. 64
Figure 6. Retrograde labelling of Untreated and Colchicine cuffed sciatic nerves with horseradish peroxidase (HRP).....	pg. 66
Figure 7. Results from 4 Vus experiments.....	pg. 86
Figure 8. Immunohistochemistry of normal EDL muscle.	pg. 87
Figure 9. Immunohistochemistry of normal SOL muscle.	pg. 88
Figure 10. 6% PAGE of normal and 6 week denervated SOL and EDL muscles.....	pg. 89
Figure 11. Immunohistochemistry of 6 week denervated SOL muscle.....	pg. 90
Figure 12. Immunohistochemistry and 6% PAGE of 6 week TTX-treated EDL muscles.....	pg. 91-92
Figure 13. Immunohistochemistry of 6 week TTX-treated EDL muscle showing co-existence of type I and IIA myosin	pg. 93
Figure 14. Immunohistochemistry of 6 week denervated SOL muscle showing co-existence of type I and IIA myosin	pg. 94

Figure 15. 6% PAGE of normal EDL, SOL and dystrophic SOL muscles
..... pg. 95

Figure 16. Immunohistochemistry of 6 week denervated EDL muscle
showing co-existence of type IIA and IIB myosin
..... pg. 96

Figure 17. 2-dimensional PAGE profile for EDL muscles.
a) control, 10 days denervated and
6 weeks denervated..... pg. 97
b) four 14 day TTX-treated..... pg. 98

Figure 18. 2-dimensional PAGE profile for SOL muscles
..... pg. 99

Figure 19. Proposed Scheme for Activity Induced Modulation of
Na⁺ channels and ACh receptors..... pg. 121

ABSTRACT

This study was undertaken to further elucidate the relative contributions of activity and myotrophic influences in maintaining certain contractile and histochemical properties of skeletal muscle.

Four month old male C57BL mice were subjected to unilateral hindlimb denervation for either 10 days or 6 weeks. The denervation-induced changes in contractile properties as well as fiber-type distribution and myosin isoforms of the soleus (SOL) and extensor digitorum longus (EDL) muscles were investigated. To determine whether the denervation-induced alterations seen were due to withdrawal of putative myotrophic influences or activity, colchicine containing cuffs wrapped around the sciatic nerve were used to block fast axoplasmic transport thus restricting the access of myotrophic substances to the muscles, and tetrodotoxin (TTX) cuffs were used to block action potential propagation and thus activation of the muscles.

When stimulated directly, denervated SOL and EDL muscles contracted slower than their innervated counterparts. This was reflected in an increase in both the time to peak tension (TTP) and time to half-relaxation ($1/2RT$) of the isometric twitch. In the case of denervated EDL muscles a decrease in the velocity of unloaded shortening (V_{us}) was also seen for both denervated groups. EDL muscles that were paralysed by

TTX-treatment for 14 days also showed a reduction in the Vus. For both denervated and TTX-treated groups the reduction in Vus was accompanied by a loss of myosin light-chain 3f (LC3f). No change in velocity from control SOL was noted in any of the SOL experimental groups.

Using 6% PAGE a loss of IIB myosin heavy chain and an increase in IIA myosin heavy chain was seen in the case of EDL muscles denervated for 6 weeks. This was corroborated by a decrease in the proportion of fibers expressing IIB myosin and an increase in the proportion of fibers expressing IIA myosin that was seen using immunohistochemistry with monoclonal antibodies directed against the various myosin heavy chain isoforms. Aside from 6 weeks of denervation, only paralysis with TTX was effective in precipitating a decrease in the proportion of fibers containing IIB myosin heavy chain in EDL muscles. An increase in fibers expressing type I myosin was seen in 6 weeks denervated mouse SOL muscles. Paralysis with TTX resulted in an increase in the proportion of fibers expressing type I myosin in SOL muscles. Also, treatment with TTX led to the appearance of fibers expressing type I myosin in EDL muscles.

It is concluded that loss of activity is the main precipitating factor for the alterations seen in contractile and histochemical properties of denervated EDL and SOL muscles. Furthermore, the early reduction in Vus seen in EDL muscles denervated for 10 days or treated for 14 days with TTX

and which was accompanied by a loss of myosin LC3f argues that loss of activity is an important stimulus for velocity transformation and that velocity characteristics can be significantly altered in a relatively short period of time. The results are also consistent with the idea that LC3f is an important regulator of Vus.

INTRODUCTION

It has long been appreciated that the motorneuron plays a crucial role in the maintenance of many of the physiological properties of mature skeletal muscle. The clearest example of this influence has been derived from the results of denervation experiments where the total withdrawal of neural influence can be achieved with relative ease and reliability. Thus the observed denervation-induced alterations in muscle structure and function have been largely attributed to the loss of neuronal influence. While it has been relatively easy to define the denervation-induced changes in muscle, what has remained less obvious is the actual role of the motorneuron "per se" in maintaining normal muscle physiology. The idea that a motorneuron's influence may be directed by either some event(s) associated with activation of the muscle fiber (activity), or by nutritive factors that are synthesised by the motorneuron and somehow supplied to the muscle fiber (trophic) is by no means a new notion and has gained a great deal of experimental support. In fact, to date the bulk of evidence would suggest that both activity and trophic influences act in concert to maintain the stability of muscle structure and function.

Because of this, the better understanding of the relative contributions of activity and trophic input to skeletal

muscle function may have beneficial offshoots in terms of the better understanding and development of more effective therapeutic strategies for disorders involving the motorneuron-skeletal muscle axis. As well, this knowledge may have useful applications in the area of sports and rehabilitative medicine.

The main purpose of this thesis is to explain the denervation-induced changes in the contractile speed related properties of muscle in terms of i) loss of activity (disuse), ii) loss of trophic factors or a combination of i) and ii). Also the nature of the alterations leading to the changes in contractile properties will be investigated.

Short note on Nomenclature

Before describing the normal physiology of nerve-muscle interaction it will be necessary to first define the nomenclature in use with respect to trophic interactions as it has undergone drastic revision over the past few years. To begin, the term trophic is derived from the Greek word "trophein" literally meaning nourishment.

In his rigorous synopsis of the field of trophic interactions Gutmann (1976) stated that neurotrophic relations should be restricted to those long-term maintenance functions that occur independent of nerve impulses. Through the vast amount of work that has been done on trophic systems

it has become clear that these interactions occur in a bi-directional way such that for example a neuron may exercise trophic control over some property of its target cell and similarly the target cell could sustain some physiological properties of the neuron to which it is connected (Smith and Kreutzberg, 1976). In order to more clearly define the directionality of the process the terms anterograde exeterotrophic influences (neuron to target cell) and retrograde exeterotrophic influences (target cell to neuron) were introduced as general terms. More specifically in the case of the motoneuron:skeletal muscle system the terms myotrophic and neuronotrophic are the correlates of anterograde and retrograde exeterotrophic influences respectively (Fernandez and Donoso 1987).

In order to avoid confusion these terms will be used exclusively throughout the text even though some of the papers cited may have used older nomenclature to describe the same phenomena.

Before delving into the issue of neuronal control of muscle physiology it is important to first review some normal physiological properties of skeletal muscle.

Mammalian Skeletal Muscle and Classification Systems

A whole muscle is composed of many muscle fibers that run approximately parallel to the long axis of the muscle. Skeletal muscle fibers are long, approximately cylindrical cells having diameters ranging anywhere from 10 to 100 μm depending on the species. The plasma membrane of the muscle fiber is similar to that of other cells and is called the sarcolemma. Within each muscle fiber are many parallel cylindrical units of approximately 1 μm in diameter called the myofibrils. The myofibrils are in turn made up of short cylindrical units (1 μ in diameter: 1.5-3.5 μ in length) stacked end to end, called sarcomeres. These sarcomeres are the functional contractile units of skeletal muscle. They are largely composed of the contractile proteins actin and myosin. Surrounding the myofibrils is an elaborate system of connected tubules and vesicles known as the sarcoplasmic reticulum (SR). Calcium released from the SR initiates contraction, while its sequestration back into the SR results in relaxation. The processes of contraction, release of calcium and uptake of calcium are all energy dependent and rely on the availability of ATP which can be generated by either oxidative or glycolytic metabolism. Not all muscle fibers are identical. Thus differences in the properties of skeletal muscle fibers can be attributed to the heterogeneity of contractile protein composition, differences in SR function and metabolic factors

related to ATP formation.

It was earlier described by Ranvier (1874) that the slow-contracting or slow-twitch muscles are red and contract slowly while the fast-contracting or fast-twitch muscles are white and fatigue readily. In the 1950's and 60's many investigators attempted to classify skeletal muscle fibers according to morphological (Porter and Palade 1957; Bennett 1960), biochemical (Lawrie 1952; Szent-Gjorgyi 1953) and physiological (Buller, Eccles and Eccles 1960; Close 1964) criteria. It was noted that red muscle is rich in mitochondria and has a less extensive sarcoplasmic reticulum system than white muscle. Also the myosin adenosine triphosphatase (ATPase) activity of red muscle was found to be less than that of white muscle and physiological measurements confirmed the existence of slow (red) and fast (white) muscle. However histochemical studies revealed that the division into red and white was far too simplistic. Stein and Padykula (1962) described the existence of three fiber types (A, B and C) based on differences in the cytochemical distribution of the mitochondrial enzyme of oxidative metabolism, succinate dehydrogenase (SDH). Based on differences in pH lability of the myosin ATPase system two fiber types were initially described. One type is alkali-labile (type I) while the other is acid-labile (type II) (Padykula and Herman 1955). The type II fibers can be further subdivided into two groups (IIA, IIB) on the basis of their pH lability and their inhibition by

sulfhydryl blocking agents (Brooke and Kaiser 1970).

More recently with the use of electrophoretic (Carraro and Catani 1983) and immunohistochemical (Gauthier and Lowey 1979; Billeter, Heizmann, Howald, and Jenny 1981; Pierobon-Bormioli, Sartore, Dalla Libera, Vitadello and Schiaffino 1981) techniques the myosin isoform composition has been rigorously determined for the various fiber types. Muscle fibers can also be classified based on the mode of generation of ATP whether by enzymes in the glycolytic or oxidative pathways of carbohydrate metabolism (Dubowitz 1960; Stein and Padykula 1962; Pette 1985; Pette and Spamer 1986).

With the advent of the glycogen depletion technique (Edstrom and Kugelberg 1968) it became possible to identify constituent muscle fibers of a motor unit by artificially stimulating the unit to fatigue thereby depleting it of its stores of glycogen and then identifying the pale fibers in a cross-section that is stained for glycogen. This invaluable technique has made possible the direct correlation of contractile properties with histochemical fiber types. In addition the uniformity of muscle fiber properties within a given motor unit has been stringently demonstrated using this approach (Burke, Levine, Tsairis and Zajac 1973; Kugelberg and Lindegren 1979; Nemeth, Pette and Vrbova 1981). It was thus shown that the slower contracting (slow-twitch) fibers are also the type I fibers (Burke and Tsairis 1974) that can be visualized histochemically by their pale appearance in the

myosin ATPase stain following alkaline pre-incubation (Brooke and Kaiser 1970). Their myosin heavy chain has been shown to be distinct from that of the fast forms and they rely predominantly on mitochondrial oxidative metabolism for the generation of ATP. Slow muscles utilise ATP at a much lower rate compared to fast muscle. Also, delivery of oxygen to slow muscle is enhanced by virtue of the high density of capillaries/cross-sectional area of muscle (Armstrong and Laughlin 1983). Since slow muscles rely on oxidative metabolism for the generation of ATP and because oxygen delivery is facilitated by the short effective diffusion distances, the supply of ATP is not likely to be compromised even under extreme usage. Indeed, slow muscles are extremely resistant to fatigue (Kugelberg and Edstrom 1968; Burke, Levine, Tsairis and Zajac 1973) and their high endurance is ideally suited to their physiological role as postural muscles.

Fast muscle differs from slow muscle in many respects. To begin with it contracts faster. This is reflected in a higher activity of myofibrillar ATPase and consequently its faster speed of shortening compared to slow muscle (Close 1964; Trayer and Perry 1966; Barany 1967). The myosin heavy chain composition is not the only determinant of speed of shortening. Differences in myosin light chain composition have also been directly correlated to the differences in speed of shortening (Sweeney, Kushmerick, Mabuchi, Sreter and Gergely

1988).

By virtue of myofibrillar ATPase staining following acid pre-incubation (Brooke and Kaiser 1970) or with paraformaldehyde fixation followed by alkaline pre-incubation (Guth and Samaha 1970), two types of fast fibers (IIA and IIB) can be identified in the mouse. The existence of IIA and IIB fibers has been confirmed using immunohistochemical techniques with monoclonal antibodies against the myosin heavy chains. Recently a third subgroup of fast fibers, the IIX fiber was discovered on the basis of negative staining with a monoclonal antibody that recognises type I, IIA and IIB myosin heavy chains (Schiaffino et al 1986). While electrophoretic separation of the IIX isoform by denaturing gels was initially unsuccessful (Carraro and Catani 1983), Bar and Pette (1988) were able to demonstrate a fourth myosin heavy chain (presumably IIX) using gradient gel electrophoresis. At this time very little is known about the physiological properties of IIX fibers but they appear to be intermediate between type IIA and IIB fibers and may represent a transitional fiber type (Ausoni, Gorza, Schiaffino, Gunderson, and Lomo 1990).

Much more is known about the physiology of type IIA and IIB fibers. There are species differences, but for example in the mouse, IIA fibers are generally the smallest in diameter, depending on the muscle studied. IIB fibers are the largest, while type I fibers are generally intermediate in diameter.

IIA fibers are capable of generating ATP from both glycolytic and oxidative enzyme pathways and are fairly resistant to fatigue even though their utilisation rate of ATP when active is much greater than that of slow muscle. The fact that IIA motor units are phasically active may allow for an adequate amount of recovery time which is probably sufficient in length for adequate H^+ buffering to occur. This could explain their high resistance to fatigue. They are however more susceptible to fatigue than type I units.

Type IIB fibers rely predominantly on glycolytic metabolism for the generation of ATP and thus are prone to fatigue as buildup of lactic acid may follow extensive usage. Normally however, IIB motor units are relatively quiescent as they are only recruited for short duration strength manoeuvres and thus fatigue is not an imminent threat.

A key aspect of this thesis is concerned with changes in contractile properties of skeletal muscle following denervation, paralysis by TTX cuffing and blockage of fast axoplasmic transport with colchicine. One of the contractile parameters, namely the time course of the isometric twitch is dependent on the type of contractile proteins (Examples: myosin, actin) as well as factors involving Ca^{++} kinetics (sarcoplasmic reticulum, parvalbumin). Excitation-contraction (EC) coupling represents the link between muscle action potential generation and the release of Ca^{2+} by the

sarcoplasmic reticulum (SR) and therefore should be reviewed because alterations in muscle twitch time course may in part be due to changes in Ca^{2+} handling. Toward the end of the section on EC coupling I will review some of the work that has examined the link between SR function and muscle contractile properties.

Excitation-Contraction (EC) Coupling and Mechanical Activation of Skeletal Muscle

The events subsequent to the initiation of the muscle action potential and leading to mechanical activation of the muscle fiber have been studied in great depth. I will briefly review what is known to date without delving into the controversies since even at this moment certain details are not entirely resolved.

Depolarisation of the surface membrane of the muscle fiber is not in itself sufficient to elicit muscle contraction. This has been shown in experiments where the radial invaginations of the muscle membrane (t-tubules) were disconnected from the surface membrane and although an action potential could be faithfully propagated along the entire muscle fiber surface, no contraction was seen (Eisenberg and Gage 1967). It was subsequently shown that the action potential must penetrate the surface of the muscle fiber. This is accomplished via the extensive t-tubular network.

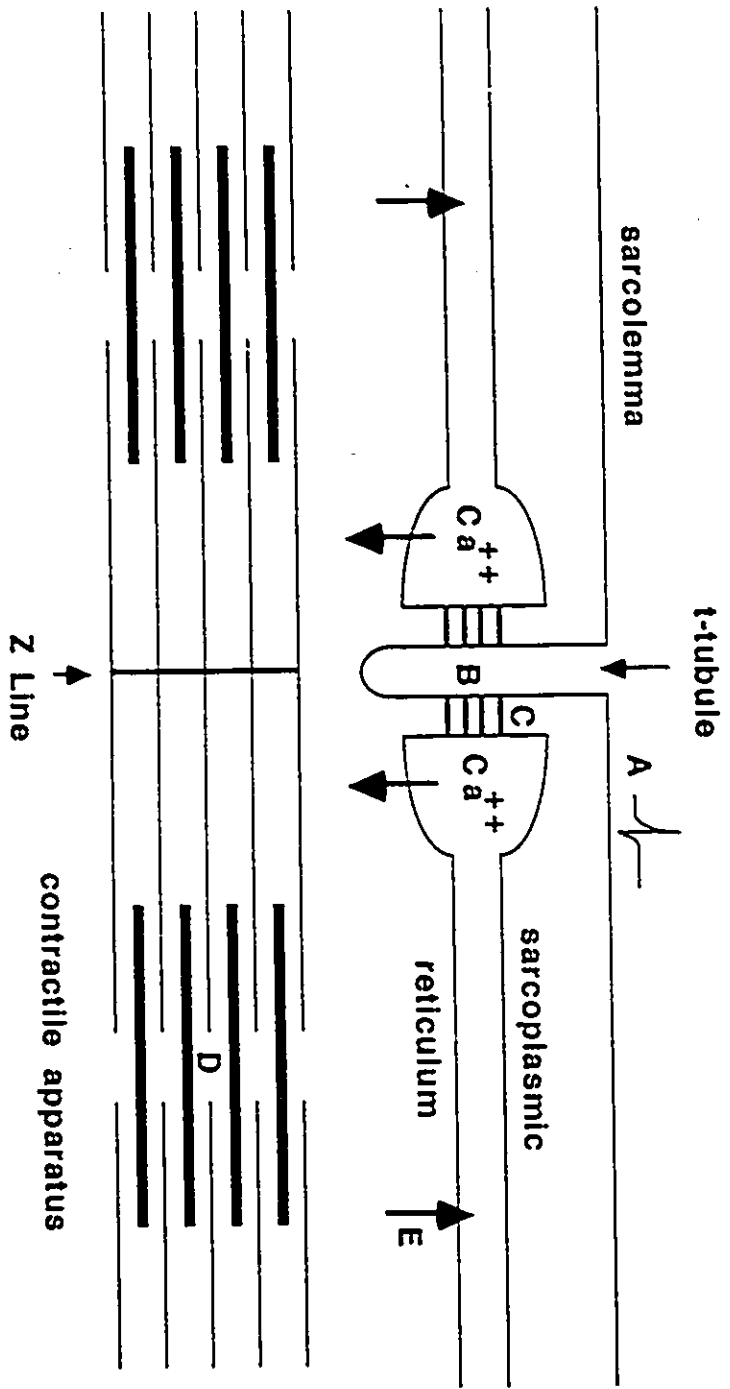


Figure 1

These specialised invaginations of the surface membrane penetrate deep into the muscle fiber. Their close proximity with the terminal cisternae of the sarcoplasmic reticulum (SR) (Franzini-Armstrong 1973) has been taken as physical evidence that electrical events occurring in t-tubules may affect the permeability of the SR to Ca^{++} . It has long been known that Ca^{++} is necessary to activate the contractile apparatus (Winegrad 1968) and its storage and release from the SR has been observed with the aid of Ca^{++} sensitive dyes such as aequorin (Ridgeway and Ashley 1967) and azo-1 (Vergara and Delay 1986). It is now believed that depolarisation of t-tubules leads to charge movement in the triadic junction (Chandler, Rakowski, and Schneider 1976). Since pharmacological blockade of this charge movement is an effective way of preventing contraction it has been suggested that the charge movement may underly the activation of Ca^{++} release channels in the SR (Rios and Brum 1987). There is now substantial evidence that the receptor responsible for the charge movements (dihydropyridine, DHP receptor) is physically linked to the SR Ca^{++} release channel (ryanodine receptor) (Campbell, Leung and Sharp 1988). Also, it was shown that in mice with muscular dysgenesis, a mutation that selectively disrupts E-C coupling, there is a decrease in the number of DHP receptor binding sites (Pincon-Raymond, Rieger, Fosset, and Lazdunski 1985). Recently a complementary DNA (cDNA) probe to the skeletal muscle DHP receptor was

constructed and inserted into myotubes from dysgenic mice. It was found that E-C coupling and slow calcium channel currents were fully restored (Tanabe, Beam, Powell and Numa 1988). As is the case for skeletal muscle (Armstrong, Bezanilla and Horowicz 1972) no dependence on external calcium was noted. Since its cloning, rapid advances in the understanding of the structure-function relationship of the DHP receptor with respect to the role it plays in E-C coupling have ensued. It was recently discovered that a cytoplasmic structural domain of the receptor is functionally linked to E-C coupling. Indeed, differences in that domain between the skeletal- and cardiac muscle variety of DHP receptor resulted in changes in the calcium dependency of E-C coupling in myotubes injected with an expression plasmid containing either the skeletal- or cardiac form of DHP receptor (Tanabe, Beam, Adams, Niidome and Numa 1990). Thus the proposed scheme of events underlying E-C coupling are thought to be; i) Surface action potential (Fig. 1A) is propagated into the muscle interior via t-tubules (Fig. 1B), ii) Depolarisation of t-tubule:triadic junction leads to charge movements in triadic membrane (Fig. 1C), iii) These DHP receptor mediated gating currents underly activation of Ca^{++} -release channels in SR membrane (Fig. 1D), iv) Ca^{++} flows down its electrochemical gradient, v) Contractile apparatus is activated (Fig. 1E) (for review see Endo 1977).

The high intra-SR Ca^{++} levels are needed to support contraction and are maintained through the action of an

ATP-dependent SR Ca^{++} pump (Ca^{++} ATPase) (Fig. 1F) (Martonosi and Feretos 1964).

Early in ontogeny the levels of Ca^{++} -ATPase in skeletal muscle are virtually indistinguishable from non-muscle tissue. A few weeks following development a greater than 20 fold increase in the amount of Ca^{++} -ATPase was seen. In addition there were parallel changes in Ca^{++} transport and Ca^{++} -sensitive ATPase activity (Fanburg, Drachman, Moll and Roth 1968; Sarzala, Zubrycka and Michulak 1975; Martonosi, Roufa, Boland, Reyes and Tillack 1977; Zubrycka, Michulak, KoskKosicka and Sarzala 1979).

Equally important as its function to sequester Ca^{++} the pump also ensures that intra-fiber levels of Ca^{++} are kept low. It is known that persistently high levels of intra-fiber Ca^{++} can cause cell damage (Publicover, Duncan and Smith 1978) most likely via activation of the cytosolic proteolytic calcium-activated neutral protease (Reddy, Etlinger, Rabinowitz, Fischman and Zak 1975; McGowan, Shafiq and Stracher 1976). There are several reports of increased intracellular levels of Ca^{++} in dystrophic muscle fibers (Pearce 1966; Bodensteiner and Engel 1978). Also it was recently shown that in the mouse model of dystrophy (mdx) the elevated rate of protein degradation could be explained by the higher intracellular levels of Ca^{++} that were seen (Turner, Westwood, Regen and Steinhardt 1988).

Alterations in Ca^{++} homeostasis may also result from a

constructed and inserted into myotubes from dysgenic mice. It was found that E-C coupling and slow calcium channel currents were fully restored (Tanabe, Beam, Powell and Numa 1988). As is the case for skeletal muscle (Armstrong, Bezanilla and Horowicz 1972) no dependence on external calcium was noted. Since its cloning, rapid advances in the understanding of the structure-function relationship of the DHP receptor with respect to the role it plays in E-C coupling have ensued. It was recently discovered that a cytoplasmic structural domain of the receptor is functionally linked to E-C coupling. Indeed, differences in that domain between the skeletal- and cardiac muscle variety of DHP receptor resulted in changes in the calcium dependency of E-C coupling in myotubes injected with an expression plasmid containing either the skeletal- or cardiac form of DHP receptor (Tanabe, Beam, Adams, Niidome and Numa 1990). Thus the proposed scheme of events underlying E-C coupling are thought to be; i) Surface action potential (Fig. 1A) is propagated into the muscle interior via t-tubules (Fig. 1B), ii) Depolarisation of t-tubule:triadic junction leads to charge movements in triadic membrane (Fig. 1C), iii) These DHP receptor mediated gating currents underly activation of Ca^{++} -release channels in SR membrane (Fig. 1D), iv) Ca^{++} flows down its electrochemical gradient, v) Contractile apparatus is activated (Fig. 1E) (for review see Endo 1977).

The high intra-SR Ca^{++} levels are needed to support contraction and are maintained through the action of an

ATP-dependent SR Ca^{++} pump (Ca^{++} ATPase) (Fig. 1F) (Martonosi and Feretos 1964).

Early in ontogeny the levels of Ca^{++} -ATPase in skeletal muscle are virtually indistinguishable from non-muscle tissue. A few weeks following development a greater than 20 fold increase in the amount of Ca^{++} -ATPase was seen. In addition there were parallel changes in Ca^{++} transport and Ca^{++} -sensitive ATPase activity (Fanburg, Drachman, Moll and Roth 1968; Sarzala, Zubrycka and Michulak 1975; Martonosi, Roufa, Boland, Reyes and Tillack 1977; Zubrycka, Michulak, KoskKosicka and Sarzala 1979).

Equally important as its function to sequester Ca^{++} the pump also ensures that intra-fiber levels of Ca^{++} are kept low. It is known that persistently high levels of intra-fiber Ca^{++} can cause cell damage (Publicover, Duncan and Smith 1978) most likely via activation of the cytosolic proteolytic calcium-activated neutral protease (Reddy, Etlinger, Rabinowitz, Fischman and Zak 1975; McGowan, Shafiq and Stracher 1976). There are several reports of increased intracellular levels of Ca^{++} in dystrophic muscle fibers (Pearce 1966; Bodensteiner and Engel 1978). Also it was recently shown that in the mouse model of dystrophy (mdx) the elevated rate of protein degradation could be explained by the higher intracellular levels of Ca^{++} that were seen (Turner, Westwood, Regen and Steinhardt 1988).

Alterations in Ca^{++} homeostasis may also result from a

dysfunction in the Ca^{++} release channel. Indeed, the high intra-fiber levels of Ca^{++} seen in patients with congenital malignant hyperthermia are thought to be due to an alteration in Ca^{++} homeostasis secondary to a defect in the SR Ca^{++} release channel. Experimental support for this was recently obtained when the gene that codes for the Ca^{++} release channel was mapped in close proximity to genetic markers for malignant hyperthermia (MacLennan, Duff, Zorzato, Fujii, Phillips, Korneluk, Frodis, Britt and Worton 1990).

In addition to the well known contribution of contractile proteins, the speed of muscular contraction is also correlated to the developmental maturity of the SR (Jolesz and Sreter 1981) as well as the concentration of the cytosolic Ca^{++} buffer, parvalbumin (Heizmann, Berchtold and Rowlerson 1982).

Thus the density of SR and Ca^{++} -ATPase activity of microsomes derived from fast-twitch muscle is greater than that from slow-twitch muscle (Martonosi 1972; Beringer 1976). In general, alterations in Ca^{++} homeostasis do not in themselves have a profound effect on isotonic properties (maximum velocity of shortening) of skeletal muscle. The maximum velocity of shortening of skeletal muscle is more dependent on the activity of myofibrillar ATPase (Barany 1967). In other words, velocity measurements usually provide information about contractile protein (myosin, actin, etc.) function. Calcium handling becomes important when one

considers the single twitch contractile properties. In this case the development and loss of tension are a complex function of Ca^{++} release, sequestration, buffering and myofibrillar ATPase activity (Biscoe and Taylor 1967; Stein, Gordon and Shriver 1982).

At the moment there is much evidence suggesting that muscle activity or something directly related to activity such as Ca^{++} transients can influence the level of expression of Ca^{++} -ATPase in the SR. The observation that cross-innervation of fast-twitch with slow-twitch muscles resulted in changes in the amount and composition of SR membranes (Mommaerts, Buller and Seraydanian 1969; Sreter, Luff and Gergely 1975; Jolesz and Sreter 1981) along with the demonstration that chronic stimulation of fast-twitch rabbit muscle with the frequency pattern common to a slow nerve resulted in a switch of SR properties to resemble slow-twitch SR (Heilmann and Pette 1979; Heilmann, Muller and Pette 1981) are in support of activity as being the important variable in conferring SR properties at least in terms of Ca^{++} transport.

The demonstration that Ca^{++} bound to parvalbumin could be removed by the SR led to the suggestion that parvalbumin may act as a soluble relaxing factor (Gerday and Gillis 1976). Consistent with this view are the observations that fast-twitch muscles contain much higher cytosolic levels of parvalbumin than slow-twitch muscles (Heizmann, Berchtold and Rowleron 1982). Also, along with a conversion of contractile

properties following cross-reinnervation of a fast with a slow muscle there was a marked decrease in the content of parvalbumin in cross-reinnervated fast-twitch muscle (Muntener et al 1985). The finding that the in-vivo administration of nerve extract could prevent the denervation induced loss of parvalbumin in a fast-twitch muscle is supportive evidence for myotrophic regulation of parvalbumin expression in skeletal muscle (Davis, Bressler and Jasch 1988).

Skeletal muscle is among the most flexible and adaptive organs in the body. The normal phenotype of mature skeletal muscle is in large part modulated through neuronal influences. Mature skeletal muscle is organised into units (motor units) that are functionally distinct. The homogeneity of the properties of the constituent muscle fibers comprising a motor unit reflects in part an adaptive response of the muscle fiber genome to neuronal modulation. I will now focus more on work that has examined nerve-skeletal muscle interactions.

The Motor Unit and Myotrophic Interactions

The concept of a motor unit was introduced by Sherrington in the mid 1920's. A motor unit is simply a single alpha motorneuron and all the muscle fibers that it innervates (Liddell and Sherrington 1925). In normal healthy adult animals each muscle fiber receives innervation from a single

Developmental Profile for Mammalian Skeletal Muscle Fibers

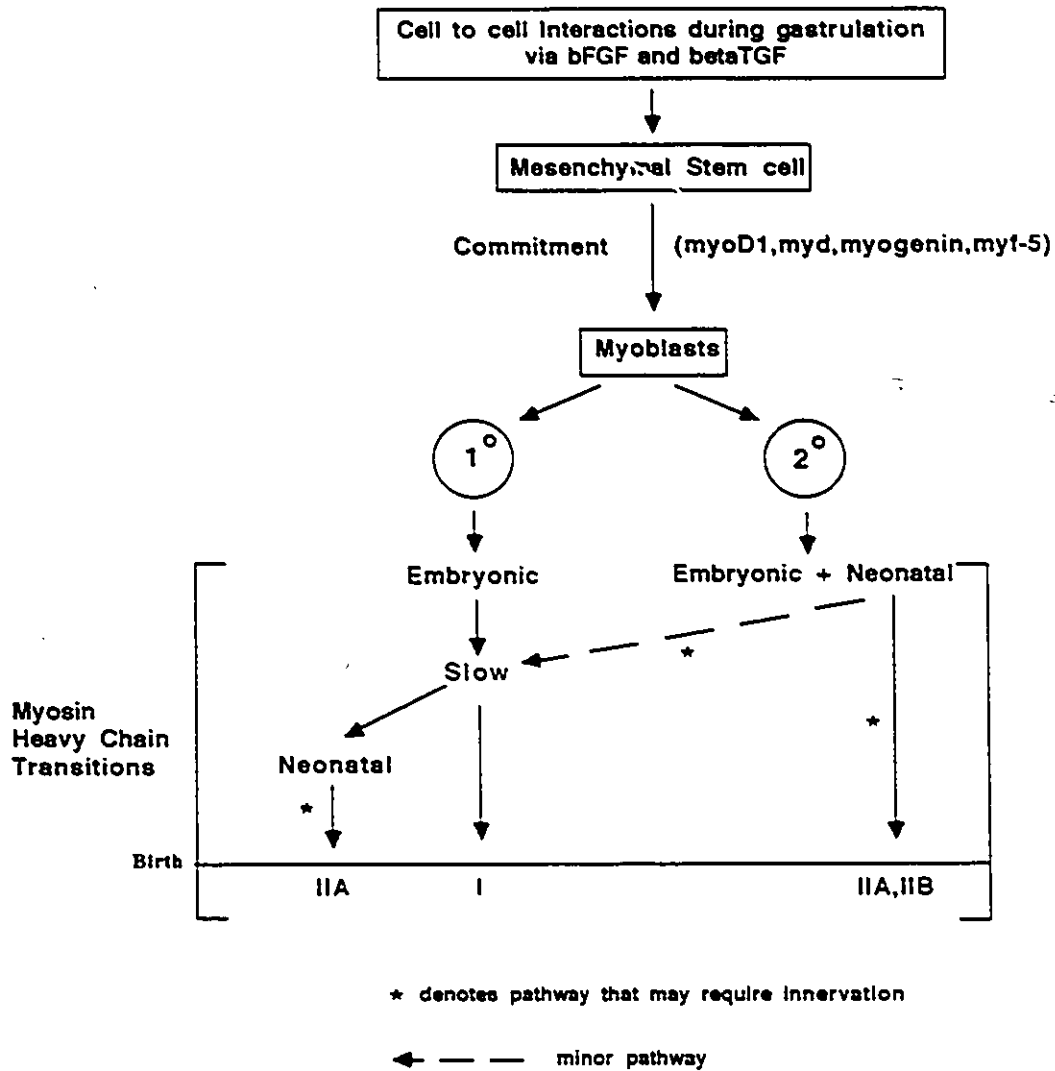


Figure 2

alpha motorneuron. This however is not the case in neonatal animals where extensive innervation of muscle fibers by more than one motorneuron can be observed (Redfern 1970; Brown, Jansen, and Van Essen 1976). The withdrawal of polyneuronal innervation in neonates coincides with the increase in locomotor activity at that time (Navarette and Vrbova 1983).

Early in development a superfluous number of motorneurons exist. Through the process of competition some of the neurons withdraw synaptic contact and ultimately die leaving behind a healthy complement of motor neurons most of which will usually survive throughout most of the lifespan of the individual (Hamburger 1975; Oppenheim and Wu Chuway 1983). The one to one matching of motorneuron to muscle fiber that is achieved upon withdrawal of polyneuronal innervation results in a functional unit whose physiological properties are a reflection of the interaction of anterograde as well as retrograde influences. Although both anterograde and retrograde influences have been demonstrated (Czeh, Gallego, Kudo and Kuno 1978), most of the work to date has uncovered anterograde influences in the case of the motorneuron:muscle unit.

While the motorneuron may play a key modulatory role in the adult animal, it is now clear that neuronal influences are minimal during early muscle development (refer to Figure 2). Embryologically muscle cells are derived from mesenchymal cells as a result of cell-cell interactions. There is now

evidence that these interactions may be mediated through basic fibroblast growth factor and β transforming growth factor (Slack, Darlington, Heath and Godsave 1987; Kimelman and Kirschner 1987). The events leading to commitment of cells to the myogenic lineage remain sketchy. However recent work has uncovered a family of genes whose products are capable of transforming fibroblasts to the myogenic lineage in-vitro. Thus the products of the MyoD1 (Davis, Weintraub and Lassar 1987), myd (Pinney, Pearson-White, Konieczny, Latham and Emerson 1988) myogenin and Myf-5 (Wright, Sasson and Lin 1989; Edmondson and Olsen 1989) genes were able to transform cells from the mouse fibroblast cell line C3H10T1/2 into myoblasts. It is possible that the function of these genes in-vivo is to commit mesenchymal precursor cells to the myogenic lineage. Are all myoblasts then identical? Whole muscles are composed of different fiber types. This would suggest that either distinct precursor myoblasts exist that subsequently develop into given fiber types, or that all myoblasts are the same and the resulting adult fibers are a reflection of environmental influences (nerve, hormones) on the myoblast genome. It would appear that the former possibility is the more correct one. The idea that distinct classes of precursor myoblasts are committed to the development of specific fiber types has received much attention (Miller and Stockdale 1987, Hoh, Hughes, Hale and Fitzsimons 1988). In a thorough study of the developing EDL muscle in mice Ontell and Kozeka (1984) found

that two stages of myogenesis could be distinguished. At 12 days in utero, a time prior to the entry of the blood and nervous supply, the developing EDL muscle was solely comprised of primary myotubes and mononucleated cells. There was a delay of at least two days before the appearance of secondary myotubes that surrounded the primary fibers and at birth the only undifferentiated mononucleated cells present were satellite cells. The question as to whether primary and secondary myoblasts develop into the distinct fiber types that are seen in the mature animal has been the subject of some debate. The early experiments supported the idea that primary myoblasts are committed to becoming type I (slow-twitch) fibers while secondary myoblasts are destined to become type II (fast-twitch) fibers (Kelly and Rubinstein 1980). However, some recent work has shown that this generalisation does not hold true. For example, the existence of at least five different myoblasts containing slow (SM1 and/or SM2), fast (embryonic fast), and fast + slow myosin heavy chains has been described in chick embryos (Stockdale and Miller 1987). Also recent work has shown that almost all primary myotubes in rat embryos contain both fast (embryonic) and slow myosin heavy chain isoforms (Dhoot 1986; Narusawa et al 1987). It is also clear that some primary myoblasts must develop into type II fibers since there are muscles which are exclusively comprised of fast type II fibers (mouse EDL, tibialis anterior). In a recent study by Condon et al (1990a) the development of muscle

fiber types in the rat was rigorously determined. Using monoclonal antibodies against slow and fast (embryonic, neonatal) myosin they were able to show that primary fibers first express embryonic myosin and then begin to express slow myosin by approximately embryonic day 18 (E18). Following E18 the fate of primary myoblasts with respect to fiber type can differ. For example, while some primary fibers retained their slow phenotype others became fast (neonatal). Secondary myotubes were found to initially express neonatal as well as embryonic myosin and most of these fibers retained their fast phenotype. However it was noted that in a small subset of secondary fibers a neonatal to slow transition occurred. These fibers were found in the soleus and the adjacent "slow" regions of the other superficial posterior muscles.

The question arises as to what stimuli are important for the transformations in phenotype that are seen throughout the course of development. In this regard there is evidence that thyroid hormone status may influence the development of fast phenotype. Thus when pregnant rats were administered propylthiouracil to induce hypothyroidism in the pups, the resulting offspring failed to express fast myosin in their gastrocnemius muscles. Instead large amounts of neonatal myosin were observed (Butler-Browne, Herlicoviez and Whalen 1984; Narusawa, Fitzsimons, Izumo, Nadal-Ginard, Rubinstein and Kelly 1987). While it has been generally accepted that innervation is not required for normal development to proceed

(Law and Stockdale 1986; Vivarelli, Brown, Whalen and Cossu 1988) this may not be entirely true in the case of slow fibers. In an elegant study by Condon et al (1990b) they injected the pre-synaptic neurotoxin beta-bungarotoxin in rat fetuses to destroy all ingrowing motorneurons. The injection was done at a time just prior to nerve-muscle contact in order to rigorously exclude any contribution by the motorneuron. It was found that aside from atrophic changes, the fiber-type distribution of muscles examined 4-6 days following injections was the same as age-matched untreated embryos. The only dependence on innervation was seen for slow fibers, as a reduction in their number was observed in injected embryos. It has been suggested that muscle activity is important for the maintenance of slow myosin synthesis (Phillips, Everett and Bennett 1986). This is probably not true of all slow fibers since slow myosin synthesis was unaffected in the aneural SOL muscle (Condon et al 1990b). Also, it would appear that in order for normal post-natal development to occur, an intact nerve supply is necessary. Thus the abnormal post-natal development in contractile (Redenbach and Bressler 1988) and histochemical (Redenbach, Ovalle and Bressler 1988) properties of neonatally denervated mouse EDL muscles would argue that once synaptic contacts are made they must be maintained in order for development to proceed normally.

The role of the motorneuron in maintaining muscle

integrity has been studied in great depth. The simplest experimental paradigm designed to test possible trophic interactions between muscle and nerve has been to surgically denervate a muscle thus depriving it of all neuronal influence while at the same time removing any neuronotrophic influence that the muscle may have on the motorneuron. Following such a procedure profound morphological changes occur both in muscle and nerve. Denervated muscle undergoes changes in membrane properties. There is a decrease in the normal resting membrane potential (Thesleff 1963; Albuquerque, Schuh, and Kaufman 1971) an increased extrajunctional sensitivity to acetylcholine (ACh) that is due to a spread in the distribution of ACh receptors to the non-endplate region of the muscle fiber and the appearance of TTX resistant action potentials (Axelsson and Thesleff 1959; Albuquerque and McIssac 1970). Also there is a loss of junctional AChE (Guth, Albers, and Brown 1964) as well as a profound change in the metabolic properties of muscle such that eventually the muscle is placed in a catabolic state which ultimately leads to complete muscle degeneration and atrophy (Gutmann 1964; Goldberg 1972).

Although it is clear that the denervation-induced changes in muscle properties are due to a loss of neuronal influence, the question as to whether the changes are due to a withdrawal of activity or a loss of myotrophic influence remains less obvious. Numerous experiments by many

investigators have been conducted in an attempt to clarify this issue. Amongst the earliest work that addressed the neuronal influence of nerve on muscle are the classic experiments of Buller, Eccles and Eccles (1960). They found that by transposing the nerve from a fast-contracting (fast-twitch) muscle to a slow-contracting (slow-twitch) muscle and vice-versa, a change in the contractile properties of the reinnervated muscles occurred in the direction of the muscle originally innervated by that nerve. Thus the previously slow muscle reinnervated by a fast nerve now contracted faster while the fast muscle reinnervated by the slow nerve contracted slower. The slowing or speeding-up was later found to occur with the same time course as the switch in the myosin isoforms that were synthesised by the muscle (Barany and Close 1971; Sreter, Luff and Gergely 1975) suggesting that myosin conversion may underly the observed changes in contractile speed. Thus it was shown that indeed some as yet undefined aspect intrinsic to the nervous supply was instrumental in determining muscle phenotype. The original postulate to account for this influence was that the neuronal control was exercised via: i) the neuronal activity pattern imposed on the muscle and hence some aspect of muscle activity per se, or ii) special neuronally derived myotrophic substances which are synthesised in the motor nerve and supplied to the muscle fiber in an activity-independent manner. Unfortunately the situation is not as clear-cut as

the above two possibilities would suggest. The idea that both activity and non-activity related factors act in concert should not be ignored (Salmons and Sreter 1976, Goldring, Kuno, Nunez and Weakley 1981). Also the effects of circulating hormones (Gambke, Lyons, Haselgrove, Kelly and Rubinstein 1983; Butler-Browne, Herlicoviez and Whalen 1984) or mechanical factors related to unloading or loading of muscle (Goldspink 1977; Jaenicke, Martindale, Loughna, Chang, Williams and Goldspink 1991) cannot be ruled out.

To address the relative roles of activity versus myotrophic influences in determining muscle phenotype experiments aimed at isolating activity from myotrophic influences were devised. The validity of these approaches rests on the effectiveness in blocking on the one hand activity while leaving myotrophic factors unaffected (disuse models) and on the other hand eliminating myotrophic communication without affecting the normal usage of the muscle.

Models of Disuse

Strategies that have been devised with the purpose of selectively eliminating muscular activity while allowing normal myotrophic communication to continue are called models of disuse. There are many models of disuse. They all have their shortcomings but with the advent of newer more selective pharmacological approaches a pure disuse model can

be achieved. Some of the earlier attempts at producing disuse included cast immobilisation (Fischbach and Robbins 1969, 1971; Maier, Crockett, Simpson, Saubert, and Edgerton 1976), tenotomy (Eccles 1944; Lomo and Rosenthal 1972), hindlimb suspension (Jaspers and Tischler 1984; Winiarski, Roy, Alford, Chiang, and Edgerton 1985; Michel and Gardiner 1990), cordotomy and spinal isolation with de-afferentation (Tower 1935; Eccles 1944; Roy, Sacks, Baldwin, Short, and Edgerton 1984). The problem with all the above models with the exception of the latter is that a residual amount of activity remained and thus any interpretation of the data could not exclude the permissive participation of activity. Indeed, Michel and Gardiner (1990) concluded that for fast hindlimb extensor muscles in the rat, the hindlimb suspension model more closely resembled a model of normal usage than of disuse. In the case of the spinal isolation model although paralysis was complete the problems lay in the technically difficult nature of the procedure and post-surgical complications that arose. In addition the effects of immobilisation differed depending on the position of the immobilised limb (flexed vs extended) (Thomsen and Luco 1944; Tabary, Tabary, Tardieu, Tardieu and Goldspink 1972; Goldspink 1977). Thus the well documented effects of stretch on muscle growth could explain the opposite effects of immobilisation on SOL and EDL muscles that had been immobilised in different positions.

Despite the shortcomings of these crude models of disuse

the consistent observation that some denervation-like changes could be brought about suggested that activity played some as yet unquantifiable role in maintaining normal muscle function. Recently the introduction of pharmacological techniques that interfere with neuromuscular transmission has provided a more selective tool that can reliably produce paralysis without affecting the anterograde transport of putative myotrophic substances. Two pharmacological approaches have been employed utilising drugs that act presynaptically by either blocking the release of transmitter or affecting impulse conduction. Thus botulinum toxin (Thesleff 1960; Drachmann 1967; Lomo and Westgaard 1975; Bambrick and Gordon 1987) or β -bungarotoxin (Hofmann and Thesleff 1972) have been used to affect the release of ACh while lidocaine (Robert and Oester 1970; Lomo and Rosenthal 1972) and tetrodotoxin (TTX) (Lavoie, Collier, and Tenenhouse 1976; Cseh, Gallego, Kudo and Kuno 1978; Lapointe and Gardiner 1984; St.Pierre and Gardiner 1985; Spector 1985; Arancio, Cangiano, Magherini and Pasino 1988) have been applied to the nerve resulting in a blockage of impulse conduction. The demonstration that local anesthetics also block axoplasmic transport (Bisby 1975) has made them a less favorable choice especially concerning experiments in which the aim is to dissect activity-related from trophic phenomena. Fortunately TTX does not suffer from the same drawback (Lavoie, Collier and Tenenhouse 1977; Czeh et al 1978) and thus the lack of

effect on the myotrophic component of neuronal influence has made TTX inactivation a more popular model of pure disuse.

While these models have produced denervation-like changes that are more extensive than the cruder immobilisation models in which variable effects were found, most did not exactly mimic the effects of denervation (but see Bambrick and Gordon 1987). This uncertainty coupled with the demonstrated effects of nerve-derived extracts on muscle both in-vitro as well as in-vivo raised the serious possibility that some nerve-derived factor(s) not associated with activation of the muscle were also important in maintaining the normal physiological properties of muscle.

Isolating Activity from Myotrophic Influences

It has been known for some time now that proteinaceous substances that are synthesised in the motorneuronal cell body can access the motor nerve terminal via a microtubular axonal transport system. The initial work in this area sought to explain how remote parts of the axon (nerve terminals) could sustain metabolic control when the source of constituents (enzymes of metabolism) can be as far as a meter distant. It was reported in 1948 by Weiss and Hiscoe that substances moved by bulk centrifugal flow. It is now well recognized that axoplasmic flow can occur at different rates for different substances and in both directions (Lasek 1968;

Ochs 1974). In addition to substances required for the upkeep of the nerve terminal and axon, axoplasmic flow is also thought to convey the putative myotrophic substances that act in concert with activity to maintain the normal physiological properties of skeletal muscle. The earliest experiments devised to address this possibility were the variable nerve stump length studies (Luco and Eyzaguirre 1955; Albuquerque, Schuh, and Kaufmann 1971; Harris and Thesleff 1972). Muscles were denervated but the nerve stump attached to the muscle varied in length. Thus in all the denervated muscles activity ceased at the instant of transection however the supply of myotrophic factors would be greater in the case of muscles that had the longer nerve stumps attached to them. In these studies it was found that the time to onset of some of the denervation-induced changes was directly proportional to nerve stump length. In other words muscles with the shorter nerve stump showed denervation-like changes (increased sensitivity to ACh, TTX resistant action potentials, decreased resting membrane potential) earlier than the muscles that had a longer nerve stump attached to them (Luco and Eyzaguirre 1955; Albuquerque, Schuh and Kauffman 1971; Harris and Thesleff 1972). The interpretation of these results led to the suggestion that some factor(s) intrinsic to the nerve was being supplied to the muscle in an activity-independent fashion and furthermore that the motorneuronal axonal milieu contained this factor(s) and hence longer nerve stumps would

contain more of the factor(s) than shorter nerve stumps accounting for the delay in onset of the denervation-like changes. This interpretation was challenged by Jones and Vrbova (1974) who found that placing a piece of silk thread on the surface of a muscle resulted in increased sensitivity to ACh in muscle fibers immediately beneath the thread. They proposed that an inflammatory response to products of nerve degeneration was responsible for inducing increased ACh sensitivity in denervated muscles. This idea has received support from other groups (Lomo and Westgaard 1975; Cangiano and Lutzemberger 1977). However the experiments of Tiedt, Albuquerque and Guth (1977) demonstrated that products of nerve degeneration have no effect on the membrane properties of innervated muscle fibers. In an almost identical experimental paradigm to that of Cangiano and Lutzemberger (1977) they partially denervated the hindlimbs of rats by sectioning one of the major spinal nerves (L4 or L5) thereby creating a situation whereby innervated fibers in EDL and SOL muscles were immediately adjacent to denervated ones. They found that only the denervated muscle fibers exhibited an increase in ACh sensitivity and reduced membrane potential. In light of this information it would appear that products of nerve degeneration need not be invoked as candidates for provoking denervation-like changes in membrane properties.

While the variable nerve stump length paradigm is an appropriate model to study early denervation-induced changes

unfortunately it is impossible to address the later changes such as atrophy, biochemical alterations and changes in contractile proteins since the supply of myotrophic substances would be exhausted prior to the onset of any of these changes, even with the longest possible nerve stump. To shed more light on this issue it has become necessary to resort to pharmacological tools. The alkaloids colchicine and vinblastine are known to disrupt the axonal microtubular assembly and thus inhibit fast axoplasmic transport (Borisy and Taylor 1967; Ochs and Ranish 1969) without affecting the conduction of nerve impulses (Albuquerque, Warnick, Sansone, and Onur 1974). Their toxicity however is very high and the earlier systemic administration of these drugs showed direct effects on the muscle hence making it impossible to distinguish a neuronal component (Markand and D'Agostino 1971). Recently attempts were made to restrict the influence of these drugs on the nerve. The use of colchicine or vinblastine impregnated silastic cuffs wrapped around the sciatic nerve has gained popularity as a means of blocking axoplasmic transport without affecting the muscle directly. Even in these cases a certain amount of controversy does exist. Some investigators have shown effects on the side contralateral to the treated limb (Cangiano 1973). It would appear that the dosage is an important variable that should be stringently controlled. Indeed, studies utilising low dosages have shown success in blocking axoplasmic transport

without having a direct effect on the muscle (Kauffman, Warnick and Albuquerque 1974; Warnick, Albuquerque and Guth 1977).

Using the colchicine impregnated silastic cuff paradigm to block axoplasmic transport it was found that certain membrane properties of muscle (increased extrajunctional sensitivity to ACh, decrease in resting membrane potential) were more profoundly affected than in disuse paradigms thereby supporting the possibility of myotrophic regulation of membrane properties. However the atrophy and strength related properties (twitch and tetanic tension) were not affected to a great extent (Warnick, Albuquerque and Guth 1977). This would suggest that myotrophic influences play a minor role in maintaining the size and strength of muscle. However it was recently shown that the in-vivo administration of a partially purified sciatic nerve extract was effective in preventing up to 75% of the denervation atrophy in one week denervated EDL muscles of the rat (Davis and Kiernan 1981). Whether this effect could be maintained over a longer period of time (6-8 weeks) as in the silastic cuff paradigms is unknown however at least in the early stages of denervation atrophy it would appear that loss of myotrophic substances may be a contributing factor to the loss of bulk. Further evidence for in-vivo myotrophic regulation is that the denervation induced loss of cytosolic parvalbumin (Muntener, Berchtold and Heizmann 1985), an intracellular Ca^{++} buffer, was shown to be

completely prevented by the daily administration of a sciatic nerve extract (Davis, Bressler and Jasch 1988).

Chronic Stimulation Paradigms and the Role of Activity in Transforming Skeletal Muscle Properties

Chronic stimulation experiments have been carried out in order to test the hypothesis that the activity to a muscle is important in determining its contractile and metabolic properties. As previously mentioned, the various motor unit types have their characteristic patterns of activation. This was described in early experiments on cats by Eccles, Eccles and Lundberg (1958). They observed that motor neurons supplying slow-red muscles are of the tonic variety while those supplying fast-pale muscles are phasically active. Although the absolute frequency patterns may differ across species, in general the division into phasically and tonically active units holds true. In a human study it was found that motor unit firing patterns formed a continuum. The slowest motor units could be driven tonically at a rate of about 10-30 Hz while the fastest motor units could only be activated phasically at higher frequencies (Grimby, Hannerz and Hedmann 1979). In the rat slow units were found to be tonically active with an average firing frequency of 5-10 Hz while fast (fatigue-resistant) units were phasically active as were fast (fatiguable) units, however the aggregate activity of the former greatly exceeded that of the latter (Hennig and Lomo

1985).

The earliest experiments aimed at elucidating the role of activity in determining muscle phenotype utilised stimulation protocols that were structured according to the experimentally derived characteristic patterns of activation in fast and slow muscles. It was found that when for instance the extensor digitorum longus (EDL) muscle, a fast muscle, was chronically stimulated at a frequency of 10 Hz, the frequency of activation typical of slow motor units (Hennig and Lomo 1985), its metabolic and contractile apparatus switched to resemble that of a slow muscle (Salmons and Vrbova 1967, 1969). The first parameter to change was the metabolic profile to a more oxidative one (Pette, Muller, Leisner and Vrbova 1976). The muscle contracted more slowly but the early slowing was not attributable to changes in myosin isozymes but likely a reflection of the alterations seen in the calcium sequestering capability of the sarcoplasmic reticulum (Salmons and Sreter 1976; Heilmann and Pette 1979). After longer periods of stimulation the isotonic contractile characteristics (shortening velocity) also changed. This suggested that the time course for transformation of the SR is distinct from that of contractile proteins (Buller and Pope 1977). The myosin ATPase activity of stimulated fast-twitch muscle declined progressively until it reached the level of a slow-twitch muscle (Sreter, Romanul, Salmons and Gergely 1974). Also, changes in myosin light chains from fast to slow

type were seen following long periods of stimulation (Sreter, Salmons, Romanul and Gergely 1973). While transformation from a fast to a slow muscle was more or less complete, the opposite could not be achieved to the same extent. Thus stimulation of SOL with intermittent volleys of 100 Hz did not lead to a complete transformation from slow to fast. For example, while 10 Hz stimulation of EDL resulted in slowing that even surpassed that of a normally slow muscle (Salmons and Sreter 1976), the speeding up obtained by intermittent pulses of 100 Hz was in between the two extremes. This relative resistance of SOL muscle to complete transformation has also been observed following cross-reinnervation with a foreign nerve that normally supplies a fast muscle (Dum, O'Donovan, Toop and Burke 1985; Foehring, Sybert and Munson 1987; Gillespie, Gordon and Murphy 1987; Gordon, Thomas, Stein and Erdebil 1988). Recently Ausoni et al (1990) examined the effects of different patterns of stimulation on denervated rat EDL and SOL muscles. Their stimulation paradigm incorporated the characteristic firing frequencies for the slow and fast motor units that was recently reported by Lomo's group (Hennig and Lomo 1985). Ausoni et al examined the fiber type composition using monoclonal antibodies directed against the various myosin heavy chains. In addition they measured the velocity of shortening of these muscles. Their results of incomplete transformation of the velocity characteristics was reconciled by the fact that in the case of EDL muscles

stimulated at frequencies characteristic of a slow muscle there was no slow myosin expressed while in the case of SOL muscles stimulated at frequencies characteristic of a fast muscle type IIX myosin was the predominant isoform expressed whereas no type IIB myosin was seen. To complicate matters further some investigators have shown compelling evidence that total active time may supercede the role of activity pattern per se in determining muscle contractile speed (Hudlicka, Tyler, Srihari, Heilig and Pette 1982; Sreter, Pinter, Jolesz and Mabuchi 1982; Eerbeek, Kernell and Verhey 1984; Donselaar, Eerbeek, Kernell and Verhey 1987) while activity pattern may be important for imparting strength characteristics (Kernell, Eerbeek, Verhey and Donselaar 1987). Since these studies were only done on fast skeletal muscle it is therefore unknown if similar manipulation of slow muscle could result in complete transformation to fast phenotype.

Collectively these observations would suggest that although the activity pattern is important for maintaining some of the physiological properties of skeletal muscle, it is not in itself sufficient to explain the plasticity of skeletal muscle myosin heavy-chain expression. A built-in adaptive range in muscle fiber transformation may also be in operation (Westgaard and Lomo 1988). Since muscle fibers are derived from distinct lineages (Stockdale, Miller, Schafer and Crow 1986) their adaptive range may simply reflect the inherent plasticity of the lineage in which they were derived. Thus

primary myoblasts may develop into type I or type IIA fibers while secondary myoblasts may give rise to type IIA or IIB fibers. The plasticity windows of these fibers would depend on their lineage. For example a type IIA fiber derived from a primary myoblast could not express type IIB myosin but may under the appropriate circumstances express type I myosin. Similarly a type IIA fiber derived from a secondary myoblast could not express type I myosin but may acquire a type IIB phenotype. The possibility of myotrophic regulation of these processes should not be ignored. Indeed while denervated fast or slow muscle became slower contracting, slow muscle that had been rendered quiescent by cast immobilisation (Unsworth, Witzmann, and Fitts 1982), cord transection (Salmons and Vrbova 1969) or paralysis with TTX containing cuffs (Spector 1985), became faster contracting.

It would therefore appear that some nerve directed influence unrelated to muscle activation may be required for a muscle to express fast myosin. Perhaps increasing activity modulates the interaction of the factor with the genome. This could explain the seeming paradox that while tonic stimulation of a fast muscle leads to slowing, complete cessation of activity by denervation also leads to slowing (Eccles, Eccles and Kozak 1962; Lewis 1972). In both cases the slowing is correlated to the synthesis of slow myosin. In this paradigm the activity during stimulation would be of sufficient magnitude to fully negate the actions of the

factor thus leading to an expression of slow myosin. Similarly, in denervated muscle the physical lack of factor would be analogous to an ineffective factor.

There are however problems with this explanation and the overall picture appears to be far more complex. For instance, during development the transition between embryonic, neonatal, and adult forms of myosin appears to be largely nerve-independent and probably endogenously preprogrammed since neonatal denervation does not alter this ordered sequence (Butler-Browne, Bugaisky, Cuenoud, Schwartz and Whalen 1982; Gambke et al 1983). While most developmental work has shown that slow myosin expression is largely nerve-independent (McLennan 1983; Dhoot 1986; Ecob-Prince, Jenkison, Butler-Browne and Whalen 1986; Law and Stockdale 1986) a more recent study has shown that the expression of slow myosin heavy chain is dependent on innervation during a critical embryonic time window (Harris, Fitzsimons and McEwen 1989). Also in the case of the myosin light chains (LC's) it was shown that while the expression of LC1f mRNA is nerve-independent, an intact nerve is necessary in order to express LC3f mRNA (Barton, Harris and Buckingham 1989). As mentioned previously attention has focused on the importance of thyroid hormone in initiating the developmental transition from neonatal to adult fast myosin isoforms (Gambke, Lyons, Haselgrove, Kelly and Rubinstein 1983). In mature animals the complexity of thyroid hormone action is illustrated by its

differential effects on myosin heavy chain gene expression. Whereas hypothyroidism resulted in the repression of fast IIA mRNA in rat soleus muscle the same treatment had no effect on the levels of IIA mRNA in some fast muscles (masseter, diaphragm, EDL) and resulted in the expression of IIA mRNA in the fast tensor fascial latae (TFL) a muscle that does not normally express this isoform of myosin. In the case of IIB mRNA a repression was seen in masseter and diaphragm while no effect was observed in EDL and TFL. When the animals were made hyperthyroid by exogenous T3 administration IIA mRNA levels were enhanced in soleus and repressed in diaphragm, masseter and EDL, while IIB mRNA levels were unaffected in the fast muscles. Interestingly the soleus of hyperthyroid rats which does not normally express IIB myosin was found to contain IIB mRNA (Izumo, Nadal-Ginard and Mahdavi 1986). More recently it was shown in adult rat fast-twitch muscle that the effects of neural activity on myosin heavy chain mRNA isoforms were antagonised by thyroid hormone. Thus, thyroid hormone attenuated the stimulation induced increase in type I myosin heavy chain mRNA. Also the thyroid hormone induced increase in type IIB mRNA was abolished by increased neuromuscular activity (Kirschbaum, Kucher, Kelly and Pette 1990). Clearly the regulation of myosin heavy chain expression is complex. It would appear that many factors are interacting at various levels and that ultimately their integrated influence will determine phenotype.

The basic aim of this thesis is to address this issue in terms of the slowing in contractile properties that has been reported in denervated muscle. The basic questions being posed are:

1) What are the effects of denervation on the fiber type and myosin isozyme composition and contractile properties of mouse EDL and soleus muscles?

2) Can the denervation induced changes in fiber type and myosin isozyme composition and or contractile properties be precipitated by disuse or loss of myotrophic influences?

Experimental Approach

Adult male C57BL mice 4 months of age were used throughout the study. In order to examine the effects of denervation on the various muscle fiber types it became necessary to choose representative muscles that contained substantial proportions of the fiber type(s) in question. For that purpose the mouse soleus and EDL muscles were used. The soleus (SOL) muscle is composed of approximately 40% type I (slow) and 60% type IIA (fast) fibers and thus is a good model to examine plasticity of type I and IIA fibers. On the other hand the EDL muscle is comprised of roughly 70% type IIB (fast), 10% type IIA, and 20% type IIX (fast) fibers and thus serves as a good model to study plasticity of IIB fibers.

To examine the effects of denervation on the parameters

being tested a short period of denervation (10 days) corresponding to a time when changes in myosin isozymes would not be anticipated was compared to a longer period of denervation (6 weeks) in which changes in contractile proteins are known to occur. By comparing the differences in contractile properties between 6 week and 10 day denervated muscles with those seen in contractile proteins it may be possible to determine to what extent changes in contractile function result from alterations in contractile protein content.

To determine the proportion of fiber types serial cryo-sections of either EDL or SOL muscles were incubated with monoclonal antibodies directed against the various myosin heavy chain isoforms and subsequently visualized following a secondary incubation with an HRP-conjugated antibody. The total number of fibers was counted as well as the individual fiber types. Furthermore, high salt extracts of the remaining muscle were run on 6% polyacrylamide gels (PAGE) to visualise the myosin heavy chain (MHC) pattern while 2-dimensional PAGE was also carried out to permit characterization of the myosin light-chain pattern.

The isometric twitch contractile parameters (time to peak tension, time to half-relaxation) were obtained on in-vivo as well as in-vitro preparations. Since changes in isometric temporal parameters are not necessarily attributable to alterations in contractile proteins a more accurate measure of

the crossbridge cycling rate was obtained by measuring the maximum velocity of unloaded shortening. These latter measurements were carried out in-vitro at room temperature because of the technical difficulty of performing these experiments in-vivo. The isometric data were also recorded under these conditions for the purpose of comparison.

In order to examine the role of activity versus myotrophic influences in precipitating the denervation induced changes animals were treated for 14 days with TTX cuffs and 6 weeks with either TTX or colchicine cuffs wrapped around the sciatic nerve to block activity or myotrophic influences respectively.

MATERIALS AND METHODS

The animals used in this study were 4 month old male C57BL mice from a colony maintained at the University of Ottawa. In a few experiments 4 month old male dystrophic mice (C57BL/dy2j/dy2j) mice were used.

In procedures requiring anesthesia, chloral hydrate (50 mg/kg) or Sodium Pentobarbital (75 mg/kg) were administered intraperitoneally.

Experimental Groups

C57BL mice were divided into four experimental groups.

- 1) Unoperated
- 2) Unilateral hindlimb denervation (sciactomy) - 10 days and 6 weeks duration.
- 3) Colchicine cuff - 6 weeks duration.
- 4) Tetrodotoxin cuff - 14 days and 6 weeks duration.

A group of dystrophic (C57BL/dy2j/dy2j) mice were also used. Reports of defects in axoplasmic transport in peripheral nerves of dystrophic mice (Jablecki and Brimijoin 1974; Brimijoin and Schreiber 1982) prompted me to investigate whether there was any similarity between the observations seen in the experimentally treated animals with dystrophic ones.

The muscles investigated were the extensor digitorum longus

(EDL), and soleus (SOL).

Surgical Procedures

Denervations

Under anesthesia, a 1 cm. segment of the sciatic nerve was surgically removed from the upper thigh region. The wound was closed with 6-0 silk sutures and the animal was monitored throughout the course of the study (10 days or 6 weeks) for lack of limb withdrawal to foot pinching and the absence of toe extension upon tail suspension.

TTX cuffs

Cuffs of approximately 5 mm in length were prepared with Dow Corning silastic tubing (0.062" I.D., 0.095" O.D.) to which was connected a silastic lead tube (0.020" I.D., 0.037" O.D.) that was destined to be attached to the mini-osmotic pump (Alzet mini-osmotic pump; model 2002). The lead tube was bonded to the cuff with silastic adhesive.

The cuffs were stored in isopropanol until use.

Colchicine Cuffs

Colchicine cuffs were made by mixing colchicine HCl (0.1% w/w) with silastic polymer (Dow Corning). The mixture was spread evenly over the narrow portion of a Pasteur pipette and allowed to vulcanise in the dark in order to prevent photolysis of the colchicine. The vulcanised colchicine:silastic polymer was removed from the pipette and cuffs of approximately 0.5 cm in length weighing about 10 mg each were cut. They were stored in a light proof container and gas sterilised prior to use.

Implantation of TTX cuffs

All surgical instruments were autoclaved prior to use (260°C for 30 min.). The miniosmotic pumps and accessories were sterilised by the manufacturer.

Surgery was performed in a laminar flow fume hood. The operating surface was wiped clean prior to and after use with distilled water followed by 1% bleach followed by 90% ethanol.

Animals were anaesthetised and the back and the right hindlimb were shaved with a razor blade. The exposed skin was washed with Proviiodine and allowed to dry. The animal was then transferred to the fume hood.

The sciatic nerve was surgically exposed with great care

taken to avoid vascular injury as well as trauma to the nerve itself. A 1.0 cm. segment of the sciatic nerve was cleared of fascial tissue in order to facilitate the placement of the cuff. A 3.0 cm. longitudinal incision was then made in the back and the skin was lifted and cleared of adhering fascia. A tunnel running from the back to the hindlimb was fashioned by running a pair of straight forceps from the back incision to the hindlimb incision via the skin/muscle interface. This was done to allow passage of the catheter connecting the cuff to the mini-osmotic pump. Next the cuff was rinsed in sterile saline to wash off the isopropanol. The cuff was wrapped around the sciatic nerve and two ligatures (8-0 silk or nylon) were used to seal the ends of the cuff. (Fig. 3, pg. 62).

The sealed cuff did not compress the nerve and it was necessary to tie the catheter to the hip musculature in order to prevent slippage of the cuff. The catheter was led via the subcutaneous tunnel to the back where the filled mini-osmotic pump (TTX 100-120 $\mu\text{g}/\text{ml}$ in saline, pumping rate: 0.44 ± 0.02 $\mu\text{l}/\text{hr}$) was positioned by strapping it on the back via a ligature passing through the back musculature. The dosage chosen was the same as that used by St.Pierre and Gardiner (1985). A syringe was used to fill the dead space in the cuff-catheter with sterile saline. The end of the catheter was then attached to the pump and the wounds were closed with 6-0 silk sutures and dabbed with Proviiodine solution.

The animals were housed in individual cages. By approximately 36 hours the onset of paralysis could be detected, the delay being due to the dead space within the catheter, and was usually manifest as a weakened toe extension reflex upon tail suspension. By 48 hours a total lack of toe extension upon tail suspension could be observed. Animals who showed a weakened toe extension reflex shortly (6-8 hours) following surgery were considered to have suffered nerve injury associated with either the surgical manipulation of the nerve or induced by mechanical trauma from the cuff and were not included in the study. Two time periods were chosen, a short one (14 days) and a longer one (6 weeks) in order to compare early and later effects as in the denervated group. Amongst the commercially available mini-osmotic pumps the only suitable one for this study was the 2 weeks-pumping duration model. It would have been preferable to have used the 4 week model for the 6 week group but it was too large and could not be surgically implanted without causing serious discomfort to the animal. The duration of the study (6 weeks) therefore necessitated that pumps be replaced on two occasions. This was done under anesthesia. A longitudinal incision was made in the back and the spent pump was removed and replaced by a freshly filled one.

Implantation of Colchicine Cuffs

Essentially the same surgical procedure was used as in the implantation of the TTX cuffs with the omission of the back incision and tunnel to the thigh incision. The cuffs were left on for 6 weeks during which the animals were tested periodically (daily for first week then once every 3 days until 6 weeks) for the toe extension and limb withdrawal reflex on both sides. Animals displaying a weakened reflex were not included in the study. In order to test the efficacy of the colchicine cuffs in blocking fast axoplasmic flow the following experiment was performed.

It is known that when horseradish peroxidase (HRP) is injected into skeletal muscle it can be endocytosed by motorneurons and transported by fast axoplasmic transport to the soma. This technique has been successfully used to label the motorneurons supplying skeletal muscles (Burke, Strick, Kanda, Kim and Walmsley 1977; McHanwell and Biscoe 1981). I therefore reasoned that if colchicine was effective in obliterating fast axoplasmic flow, then if HRP is injected into musculature innervated by the colchicine treated nerve there should be an accumulation of HRP in the region of the colchicine-containing cuff while the proximal portion of the nerve should be free of HRP.

One week following the application of a colchicine cuff (0.1%) around the sciatic nerve the gastrocnemius and tibialis

anterior muscles from both treated and contralateral untreated limbs were injected with 5 μ l of a 10% HRP saline solution. The next day (20 hours later) the mouse was anesthetised and perfused via the ascending aorta with 0.9% saline (50 mls.) followed by 4% paraformaldehyde in 100 mM Phosphate buffer (100 mls.) followed by 10% sucrose in 100 mM Phosphate buffer (50 mls). The cuffed and contralateral sciatic nerves were dissected and allowed to equilibrate in 10% sucrose for one hour. The nerves were then pre-incubated for 20 minutes in 0.05% diaminobenzidine, 25 mM PBS/0.1% Triton-X. Hydrogen peroxide (30%) was then added to a final concentration of 0.1%. After 10 minutes the nerves were rinsed in PBS and photographed.

Contractile Parameters

The in-situ measurement of isometric contractile parameters were obtained using a similar experimental apparatus as Parry and Desypris (1983). The animal was anesthetised and the muscle under investigation (EDL or SOL) was surgically isolated. A stainless steel wire with looped ends was attached to the distal tendon for subsequent attachment to the force transducer. The animal was then transferred to the tension measuring apparatus. The prepared limb was positioned in the bath and clamped in place with a pair of Dumont #5 stainless steel forceps. The leg was sealed in the bath with cotton wadding soaked in a 5% aqueous Agar solution. The bath was then filled with Ringers solution of the following composition (in mM): 121 NaCl, 4.75 KCl, 25 NaHCO₃, 0.5 KH₂PO₄, 1.5 CaCl₂, 0.23 MgCl₂, and 11.1 dextrose, and bubbled with 95% O₂ /5%CO₂. Bath temperature was maintained at 37° C by a thermostatically controlled heating coil that was located at the base of the bath. The distal end of the wire was then attached to the force transducer (Kulite Precision Load Cell, Durham Instruments, Pickering Ontario) whose output after suitable amplification was displayed on a Hitachi V-134 storage oscilloscope and immediately analysed. The compliance of the transducer was 1.12 μm/gm. and the unloaded natural

frequency was 2.25 KHz. The SOL or EDL muscles were stimulated either directly (single pulse, 100 μ sec duration) or indirectly (single pulse, 50 μ sec duration). Twitch tension (Pt), time to peak (TTP) and time to 1/2 relaxation (1/2 RT) were recorded (Fig. 4, pg. 63). All measurements were taken at optimal muscle length. For tetanic stimulation trains of pulses were delivered at a frequency that yielded the highest tension. Maximal tetanic tension (P_o) was recorded.

For the TTX-treated group it was necessary to ensure that complete paralysis had occurred. Thus if tetanic stimulation of the sciatic nerve proximal to the cuff yielded any tension the animal was not included in the study. To assess the extent of functional innervation the ratio of tetanic tension obtained by distal nerve stimulation to that obtained by stimulating the muscle directly was determined. In the case of animals that had received colchicine-containing cuffs tetanic stimulation proximal and distal to the cuff was compared and the animal was included in the study only if the two tensions were the same.

Measurement of Velocity of Unloaded Shortening (V_{us}) using the Slack Test

The slack test method (Edman 1979) was used to measure V_{us} . Animals were anaesthetised and the EDL or SOL muscles were surgically removed. Both EDL and SOL muscles were prepared for velocity measurements. However, in preliminary

experiments it was noted that following velocity measurements on the first muscle the condition of the second muscle had deteriorated to a great extent if both muscles were removed at the same time. Therefore in subsequent experiments, after removal of the first muscle the animal was maintained under anesthesia until the second muscle was removed at which time the animal was then sacrificed.

One tendon was attached to a short (approx. 1.25 cm long) stainless steel wire with looped ends while the other tendon was attached to the end of the arm of a lever. The apparatus operating the lever was a Cambridge Series 300 Dual Servo. The muscle was positioned in a perspex bath (volume of approx. 250 ml) filled with Ringers solution of the same composition as previously mentioned. Bath temperature (20 - 21°C) was room temperature. Stimulation was direct with platinum wire electrodes. Twitch and tetanic tensions as well as twitch TTP and $1/2RT$ were determined at optimal length. The muscle was then tetanised (EDL 50-120 Hz, SOL 40-80 Hz) and while maximally activated the lever was displaced so as to slacken the muscle. Tension dropped to zero and the force-time trace was monitored until sufficient force redevelopment had occurred to allow accurate placement of the line tangent to the force redevelopment curve. Usually 4-5 releases were done per muscle. Velocity was calculated by taking the slope of the regression line obtained by plotting the release amplitudes (normalised to muscle length) against

the latency or time delay between the end of release and the start of force redevelopment (Fig. 5, pg. 64).

Histological Processing of Muscle

Muscles were removed, blotted gently with a Kimwipe tissue and weighed on a Sartorius balance to the nearest 0.1 mg. The muscles were placed on a block of cork in an upright position alongside the tibialis anterior muscle such that their long axis was perpendicular to the flat surface of the cork. The muscles were covered with OCT embedding compound and quickly immersed in liquid nitrogen cooled isopentane for approximately 10 seconds. The blocks were then stored at -70°C for future processing. A cryostat was used to take cross-sections of the muscles. Serial sections of approximately 15μ thickness were taken from the mid-belly region and used for either immunohistochemical staining with monoclonal antibodies against the myosin heavy chains or staining with Hematoxylin and Eosin.

Immunohistochemistry

Monoclonal antibodies (mAb's) against type I, IIA, and IIB myosin (generous gift of Dr. S. Schiaffino, Padua, Italy) were used in a modification of the technique of Sternberger 1979 (see below).

1) Incubate in primary (1°) mAb at room temperature for 20 - 24 hours.

Plastic petri dishes were used as incubation chambers with a water moistened filter paper at the base. Two wooden rods (2 mm thickness) were used to support the slide.

<u>Myosin Heavy-Chain</u>	<u>1° mAb</u>	<u>Dilution</u>
I	BA-D5	1:400 - 1:200
IIA	SC-71	1:300 - 1:200
IIB	BF-F3	1:100 - 1:50
I, IIA, IIB	BF-35	1:50

All antibodies (1° as well as 2°) were diluted in a 25 mM phosphate buffered saline (PBS)/0.1% BSA. The phosphate buffer was made by mixing 25 mM K₂ HPO₄ /0.9%NaCl with 25 mM KH₂ PO₄ /0.9% NaCl to a final pH of 7.2.

- 2) Remove slides from incubation chamber and rinse in 0.1% Nonidet/PBS for 20 minutes.
- 3) Remove from rinse and dry the spaces between sections with a Kimwipe tissue.
- 4) Incubate for 3 hours at room temperature in 2° Ab (rabbit anti- mouse IgG, heavy and light chain:HRP conjugated) diluted 1:50 as above.
- 5) Repeat steps 2) and 3)
- 6) Incubate in 3'3 diaminobenzidine(DAB) 0.1% (w/v)/0.03% H₂O₂ for approximately 30 seconds. Remove slides every 10 seconds and rinse in distilled water. Use light microscope to observe level of stain intensity. To prepare DAB/H₂O₂ dissolve 100 mg. DAB in 100 mls 25 mM PBS and add 100 µl of 30% H₂O₂ . This must be made immediately prior to use.
- 7) Tap water wash: 5 Min.
- 8) 95% ethanol: 2 x 1 min.
- 9) 100% ethanol: 2 x 1 min.
- 10) Xylene or Xylene Substitute: 2 x 2 min.
- 11) Add a drop of Permount on section and place cover slip on top .

Polyacrylamide Gel Electrophoresis

Preparation of Muscle Extract

Muscle extracts were prepared by homogenising the muscle in a low-salt buffer (20 mM KCl, 2 mM $K_2 HPO_4$, 1 mM EGTA, pH 6.8). The homogenate was centrifuged for 45 sec. in an Eppendorf centrifuge and the supernatant containing the soluble (non-myosin) fraction was discarded. High-salt buffer (40 mM Na Pyrophosphate, 1 mM $MgCl_2$, 1 mM EGTA, pH 9.5) was then added to solubilise the actomyosin. Extraction was carried out on ice for a minimum of 15 minutes. The crude extract was then centrifuged in an Eppendorf centrifuge for 10 minutes. The supernatant (muscle extract) was kept and immediately assayed for its protein content using the Bio-Rad microassay. The muscle extract was then diluted in Sample buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol, 5% mercaptoethanol, 0.001% bromophenol blue, pH 6.8) to a final protein concentration of 0.5 - 1.0 mg/ml and boiled for 2 minutes. Samples were stored at $-20^{\circ} C$ until use.

In the case of some muscles extraction was carried out with Guba Straub solution (0.3 M NaCl, 0.1 M $NaH_2 PO_4 H_2 O$, 0.05 M $Na_2 HPO_4$, 1mM EDTA, 0.2 mM ATP, pH 6.5).

Isolation of Myosin Heavy Chains (MHC's)

The MHC's were separated on 6% polyacrylamide gels (10% glycerol) and visualised by silver or Coomassie blue staining. Order of migration from fastest to slowest for the MHC's was type I > type IIB > type IIA (Carraro and Catani 1983).

Running conditions for the gels were, one hour at 100V for the stacking gel (4% no glycerol) and 4 hours at 200V for the resolving gel at constant voltage.

Myosin Light-Chains

Myosin light-chains were separated by 2-dimensional gel electrophoresis using the Bio-Rad mini-gel electrophoresis setup. The first dimension gel (9.2 M Urea, 3% Acrylamide/Piperazide Diacrylamide (29:1) was prepared on the day prior to the run. Samples (1 µg) were loaded onto tube gels and were either run at 300 V for 20 hours or 750 V for 3 1/2 hours. The second dimension was carried out on 12% polyacrylamide slab gels.

Statistical Analysis

All values represent means \pm standard errors (n). Statistical significance versus control groups was obtained by unpaired Students t-test.

Schematic of Mini-Osmotic Pump Setup

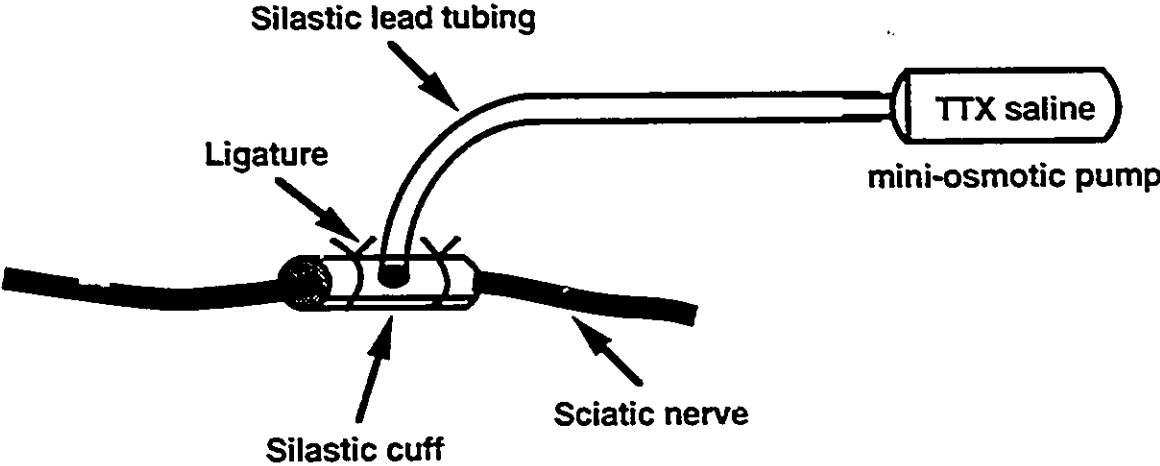
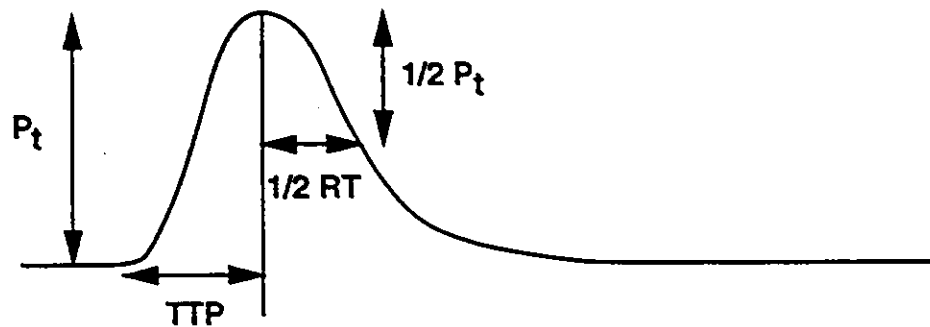


Figure 3.

Twitch Temporal Parameters



TTP: influenced by nature of contractile proteins as well as SR function.

**$1/2 RT$: good correlative marker for SR function.
- higher $1/2 RT$'s are associated with lower SR Ca^{++} uptake rates and vice-versa.**

Figure 4

Figure 5.

A. Schematic of apparatus setup for the determination of V_{us} .

B. Illustration of determining V_{us} . Length steps standardised to muscle length (a,b,c) are plotted against the latency to force redevelopment (a',b',c'). The slope of this line is V_{us} in muscle lengths/sec.

The MacIntosh application 'Cricket Graph' was used to determine the slopes by linear regression.

Determination of V_{us} using the Slack Test

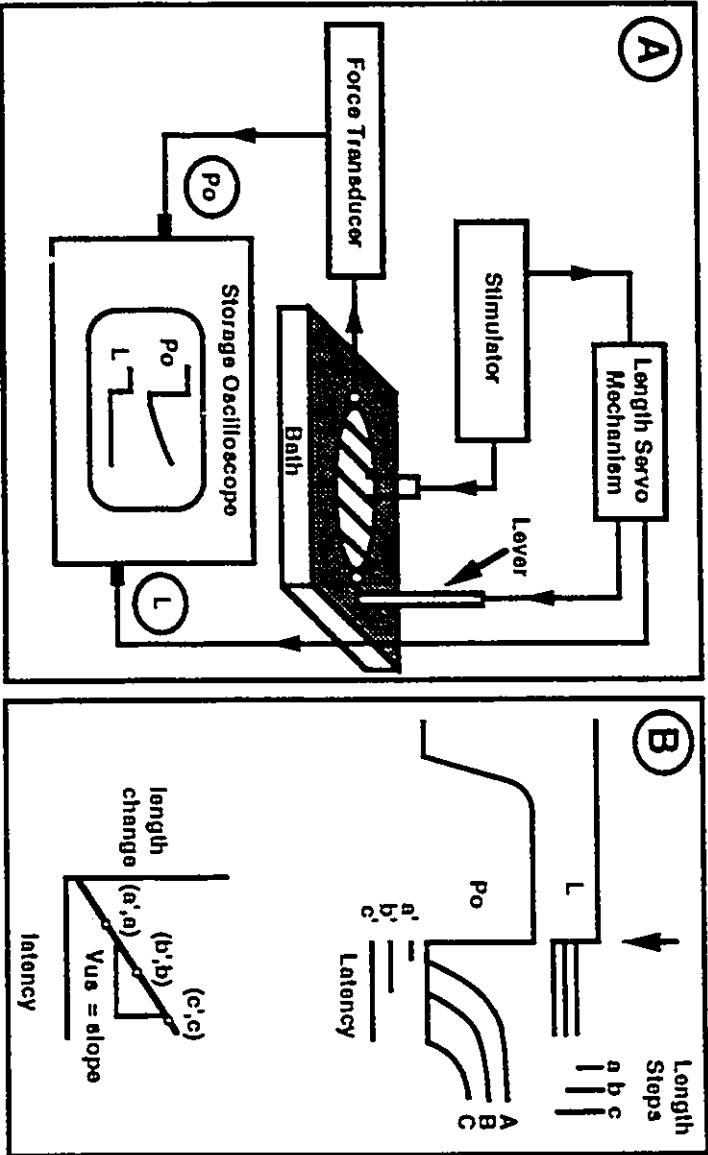


Figure 5

RESULTS

Viability of Cuffing Procedures

Colchicine Cuffs

The effectiveness of colchicine in blocking fast axoplasmic transport is illustrated in the results from an experiment where HRP was injected into the hindlimb musculature as previously described in Materials and Methods (pg. 52-53). Figure 6 shows the result from such an experiment. The untreated sciatic nerve was heavily stained for HRP along the entire length (Fig. 6A). Note the transition from heavy labeling to background levels in the colchicine-treated sciatic nerve (Fig. 6B, arrow). Combined these observations support the idea that colchicine was effective in blocking fast axoplasmic transport. A possible criticism arising from the short duration that the colchicine-containing cuff was applied prior to HRP injection is that the supply of colchicine may have been exhausted prior to the time that the physiological measurements were made. This was not the case. After 6 weeks each cuff was observed under low magnification (5X) and was found to contain many grains of colchicine. The assumption must therefore be made that the remaining colchicine is still active. In this regard others have demonstrated effects on skeletal muscle following chronic treatment with colchicine for 3 weeks (Albuquerque, Warnick,



Figure 6. Retrograde labelling of untreated and colchicine treated sciatic nerves with horseradish peroxidase (HRP).

A) segment of untreated sciatic nerve

B) segment of colchicine treated sciatic nerve that includes the portion that was surrounded by the colchicine-containing cuff (arrow denotes hypothetical area of blockage of fast axoplasmic transport).

Bottom: peripheral end of sciatic nerve.

Sansone and Onur 1974) or one month (Ramirez 1984).

Out of 14 animals that received colchicine cuffs 10 satisfied the physiological criteria (see Materials and Methods pgs. 52, 56) necessary to be included in the study.

TTX-cuffs

Out of the 18 animals that received TTX-cuffs for the 6 week period only 4 satisfied the physiological criteria necessary to be included in the study (Materials and Methods pgs. 51, 56).

The main complications that arose were related to the extreme invasiveness of the surgical procedure and the subsequent stress, infection and general deterioration in health that ensued. Most of the 14 animals that were not included in the study died prior to the end of the 6 weeks period. In two animals that survived the 6 weeks, nerve damage precluded their being included in the study.

Out of the 8 animals that received TTX-cuffs for the 14 day period, 6 satisfied the physiological criteria necessary to be included in the study.

Muscle Weight

Table I shows the effect of various perturbations on the muscle mass of EDL and SOL muscles. The early rate of atrophy of denervated SOL was greater than denervated EDL as

indicated by the larger proportional decrease in mass at 10 days following denervation. From 10 days to 6 weeks of denervation EDL and SOL muscles atrophied to the same extent, however the overall atrophy of SOL muscles was greater.

Blockage of fast axoplasmic transport with colchicine cuffs resulted in a slight but significant decrease in SOL wet weight. A significant decrease in both EDL and SOL mass was seen when the muscle weights of colchicine-treated groups were compared to their respective contralateral muscles. TTX-treatment for 14 days resulted in a significant decrease in wet weight of both EDL and SOL muscles. There was no significant difference in the muscle wet weights between the 14 day and 6 week TTX-treated muscles (Table I).

Twitch and Tetanic tensions and Pt/Po

Of all the experimental groups only 6 week denervated EDL and SOL muscles showed a significant reduction in twitch tension (Pt) (Tables II, III). Although the twitch tensions of 10 day denervated EDL and SOL muscles showed a trend to higher values than control the difference was not statistically significant when compared to the whole group. However a significant increase in twitch tension ($p < .05$) was seen for 10 day denervated SOL and EDL muscles when compared to the contralateral control muscles (Tables II, III). When normalised to the mass of the muscle in the case of the EDL

muscle only the TTX-treated group showed a significant difference, an increase of about 50% (Table II). However if the comparison is made with the contralateral muscle an increase in Pt was also seen in the 10 day denervated group. In the case of SOL muscles TTX-treatment was as effective as either 10 days or 6 weeks denervation in causing an increase in Pt. Colchicine had no effect on absolute or normalised values of Pt or Po in either EDL or SOL muscles. A significant reduction in tetanic tension was seen for 10 day and 6 week denervated as well as 6 week TTX-treated EDL and SOL muscles (Tables II and III). Only 6 week denervated SOL showed a reduced Po while 10 day denervated but not 6 week denervated EDL muscles produced less tetanic tension when normalised for muscle mass.

The values of Pt/Po were significantly higher than control in all experimental groups.

In-vivo twitch temporal parameters

Tables IV and V are data obtained from in-vivo stimulation experiments at 37°C. In the case of EDL muscles an increase in both TTP and 1/2 RT was seen in all experimental groups.

After 6 weeks denervation the TTP and 1/2 RT were prolonged in denervated SOL muscles. However only the TTP was affected by TTX-treatment and colchicine had no effect on either TTP or 1/2 RT (Table V).

Interestingly the 1/2 RT of 10 day denervated EDL muscles was considerably more prolonged than that of any other experimental group. This may however be artefactual since aftercontractions were seen in two of the 10 day denervated muscles, as previously reported by Lewis (1972). The most likely explanation for the longer 1/2 RT therefore is that the aftercontractions were obscured in the relaxation phase of the twitch of the remaining muscles thus leading to an overestimation of the 1/2 RT. The fact that aftercontractions were not seen in the isometric twitches recorded in-vitro at room temperature of 10 day denervated EDL is curious. It is possible that either the mechanism underlying this phenomenon is temperature sensitive, or that the aftercontractions were once again obscured in the relaxation phase of the twitch. The finding that none of the 7 animals showed obvious aftercontractions would tend to favor the former possibility.

In-vitro twitch temporal parameters and Vus

Tables VI and VII are the results from in-vitro stimulation of EDL and SOL muscles at room temperature. Only the denervated groups and the TTX-treated group showed prolonged TTP and 1/2 RT. In contrast to the colchicine treated EDL in the in-vivo group which showed prolonged TTP and 1/2 RT there was no effect seen in the in-vitro group.

A group of dystrophic mice were also examined (Table VIII)

to see if there was any similarity between the contractile properties of muscles from these mice and those of the experimental groups. The rationale for this was that there is evidence for motorneuronal impairment in mice of the dy2j strain and thus a parallel response with one of the experimental groups may aid in pinpointing the defect as an activity or myotrophic imbalance. When compared to control animals an increase in 1/2 RT was observed for the dy2j SOL muscles.

The maximum velocities of shortening are also found in Tables VI, VII and VIII. The Vus of normal EDL was roughly twice that of normal SOL. A reduction in Vus was seen for both EDL denervated groups. 14 days of treatment with TTX resulted in a reduced Vus in EDL muscles that was identical to that of the denervated groups. Fig 7 shows results taken from 4 velocity experiments from a normal, 10 day denervated, 14 day TTX-treated and 6 weeks denervated EDL muscle.

Although the absolute value of Vus for the colchicine treated group was higher than control this difference was not statistically significant. In the case of the SOL muscle there was no significant difference in Vus in any of the groups. However, the dy2j SOL showed a significant increase in Vus and although statistically insignificant, the colchicine treated SOL showed a trend to higher values. Interestingly 6% PAGE revealed that dy2j SOL contained significant amounts of fast IIB myosin heavy chain (Fig. 15) thus providing a possible

basis for the higher Vus seen in these muscles. No increase in IIB myosin was seen in any of the other experimental groups.

Myosin Heavy-Chain composition of EDL and SOL muscles from various Experimental Groups

The proportion of fibers showing positive immunoreactivity to the various myosin heavy chains in EDL and SOL muscles are listed in Tables IX and X. In normal EDL muscles (Fig. 8) 13.2% of the fibers reacted positive to anti type IIA myosin heavy chain (type IIA fibers) while 64% of the fibers reacted positive to anti type IIB myosin heavy chain (type IIB fibers). The remaining 22.8% are most likely type IIX fibers. This value is close to that reported from Schiaffino's laboratory for the rat (Schiaffino, Saggin, Viel, Ausoni, Sartore, Gorza 1986) as well as observations in Dr. Parry's laboratory (Parry and Zardini 1990). For normal SOL muscles 32.6% of the fibers reacted positive to anti type I myosin heavy chain (type I fibers), while 67.4% were type IIA fibers. Most of the SOL muscles did not contain any type IIB fibers. Therefore the average of 1.7% (Table X) is virtually meaningless. Myosin immunohistochemistry of a normal SOL is shown in Figure 9. Figure 10 shows the myosin heavy-chain profile of normal and denervated EDL and SOL muscles that were run on 6% PAGE.

Denervation resulted in a significant increase in fibers that expressed type I myosin in SOL muscles only (Fig. 11 and Table X). However TTX-treatment caused an increase in fibers expressing type I myosin in both EDL (Fig. 12) and SOL muscles (Tables IX, X). It would appear that in the TTX-treated SOL muscles, type IIA fibers are undergoing conversion to type I fibers since many of the fibers that contain type I myosin also contained type IIA myosin (Fig. 13). This is also supported by the observation of varying degrees of staining intensity. This was due to the different proportions of myosin heavy chains contained in transforming fibers. While denervation and TTX-treatment both caused a significant increase in the proportion of fibers expressing type IIA myosin in EDL muscles the opposite effect namely a decrease in the proportion of fibers expressing IIA myosin was seen in all experimentally manipulated solei. The co-existence of type I and IIA myosin that was seen in fibers of 6 week denervated SOL muscles (Fig. 14) is suggestive evidence of a conversion of type IIA to type I fibers in these muscles. Six weeks of denervation as well as TTX-treatment caused a significant reduction in fibers expressing type IIB myosin in EDL muscles (Table IX). The loss of IIB myosin was also evident on muscle extracts that were run on 6% polyacrylamide gels (Fig. 10). The very low incidence of co-existence of type IIB and IIA myosin in fibers of denervated EDL muscles (Fig. 16) would suggest that IIB fibers are being converted

to IIA fibers via an intermediate step or alternatively, that the rate of conversion from IIB to IIA fibers may be faster than the rate of conversion of IIA to type I fibers.

No change in the proportion of fibers expressing IIB myosin was seen in any of the C57 SOL muscles. Interestingly, dy2j SOL muscles contained significantly higher levels of IIB myosin than normal SOL muscles (Fig. 15). Desypris (1986) also noted the presence of fibers in dy2j SOL muscles that reacted positive with anti-IIB myosin antibody.

Myosin Light-Chain composition of EDL and SOL muscles

Figures 17a and 18 show the myosin light-chain patterns for normal and denervated EDL and SOL muscles. In EDL muscles a reduction of LC3f was seen following 10 days of denervation. By 6 weeks of denervation LC3f was completely absent. A complete loss of LC3f was also noted in 14 day TTX-treated EDL muscles (Fig. 17b). Interestingly dystrophic EDL did not contain any LC3f. In the case of SOL muscles a reduction in LC2f was noted following 6 weeks of denervation.

Experiments with Peripheral Nerve Extract

In previous experiments by Davis (1985) it was found that the intraperitoneal administration of a peripheral nerve extract derived from rat sciatic nerves prevented part of the

denervation-induced atrophy in hindlimb muscles of the mouse. I sought to determine if the loss of IIB myosin in denervated EDL was due to withdrawal of myotrophic input.

In four animals that had been denervated for 6 weeks, nerve extract that was prepared according to the protocol of Davis and Kiernan (1981), was injected intraperitoneally daily at a dosage of 50 mg/animal for 2 weeks in order to see if there was a reversal of the loss of IIB myosin in 6 weeks denervated EDL muscles.

Immunohistochemistry of denervated injected EDL muscles showed a loss of IIB fibers that was indistinguishable from 6 week denervated EDL. In addition the spleens of the injected animals weighed over twice that of age matched control spleens indicating the an immune response had been mounted against the extract. These results underline the inadequacy of such a long-term in-vivo repletion model. Further experiments using immunosuppressive drugs or immunologically incompetent mice (nude mice) may have provided the necessary data; however the colchicine experiments indicated that a myotrophic component to IIB myosin synthesis was not tenable and thus these experiments were not justified.

Table I

Groups	Muscle wet weight (mg)		Animal weight (g)
	EDL	SOL	
Control	10.8 ± 0.3 (32)	10.4 ± 0.2 (34)	
10 days Denervated	8.1 ± 0.7 (8) * 0.84 ± 0.03	5.8 ± 0.4 (8) * 0.61 ± 0.03	28.2 ± 2.7 (11)
14 days TTX	7.1 ± 0.6 (6) * 0.62 ± 0.01	5.5 ± 0.4 (6) * 0.52 ± 0.03	32.5 ± 4.1 (6)
6 weeks Denervated	5.6 ± 0.2 (5) * 0.58 ± 0.01	4.1 ± 0.4 (7) * 0.41 ± 0.03	29.3 ± 2.5 (8)
6 weeks Colchicine	9.2 ± 1.1 (9) * 0.80 ± 0.05	9.0 ± 0.9 (9) * 0.83 ± 0.03	29.1 ± 2.8 (10)
6 weeks TTX	7.4 ± 1.1 (4) * 0.65 ± 0.06	6.1 ± 0.4 (4) * 0.59 ± 0.04	28.8 ± 1.7 (4)

Controls are pooled contralateral muscles.

* Significantly different from respective contralateral muscles

All values represent means ± standard error

Ratios represent experimental values compared to contralateral controls.

Table II

Isometric Force Measurements from EDL Muscles (in-vivo)

Groups	P1 (gms)	P1 (gms/mg)	Po (gms)	Po (gms/mg)	P/Po
Control	6.85 ± 0.30 (21)	0.67 ± 0.03 (21)	35.49 ± 1.31 (21)	3.44 ± 0.13 (21)	0.19 ± 0.004 (21)
10 days Denervated	7.54 ± 0.97 (4) 1.48 ± .18 *	0.82 ± 0.08 (4) 1.55 ± .25 *	17.54 ± 1.91 (4) .59 ± .03 *	1.91 ± 0.16 (4) .01 ± .08 *	0.43 ± 0.006 (4) 2.54 ± .20 *
6 weeks Denervated	4.50 ± 0.65 (5) .65 ± .06 *	0.79 ± 0.10 (5) 1.12 ± .09	15.20 ± 2.06 (5) .36 ± .05 *	2.67 ± 0.31 (5) .01 ± .11	0.36 ± 0.05 (5) 2.07 ± .08 *
6 weeks Colchicine	7.57 ± 0.71 (7) 1.06 ± .06	0.85 ± 0.07 (7) 1.24 ± .04	34.50 ± 5.60 (7) .91 ± .13	3.67 ± 0.31 (7) 1.04 ± .10	0.25 ± 0.04 (7) 1.29 ± .20 *
6 weeks TTX	7.39 ± 0.69 (4) .91 ± .06	1.00 ± 0.08 (4) 1.37 ± .09 *	23.11 ± 1.73 (4) .59 ± .05 *	3.15 ± 0.27 (4) .04 ± .09	0.32 ± 0.006 (4) 1.59 ± .09 *

Smaller case values represent ratios of experimental values compared to contralateral controls.

* Significantly different from contralateral muscles

Bath temperature (37 °C)

All values are means ± standard error

Table III

Isometric Force Measurements from SOL Muscles (in-vivo)

Groups	Pt (gms)	Pt (gms/mg)	Po (gms)	Po (gms/mg)	PV/Po
Control	3.30 ± 0.19 (20)	0.32 ± 0.02 (20)	20.80 ± 0.86 (19)	2.05 ± 0.08 (19)	0.16 ± 0.005 (19)
10 days Denervated	3.94 ± 0.40 (4) 1.55 ± .08 *	0.59 ± 0.04 (4) 2.35 ± .17 *	11.54 ± 1.16 (4) .72 ± .08 *	1.74 ± 0.17 (4) 1.09 ± .13	0.34 ± 0.03 (4) 2.15 ± .12 *
6 weeks Denervated	2.05 ± 0.95 (3) .59 ± .18 *	0.68 ± 0.09 (3) 2.18 ± .22 *	5.06 ± 2.16 (3) .29 ± .03 *	1.27 ± 0.48 (3) .69 ± .15 *	0.38 ± 0.04 (3) 2.47 ± .10 *
6 weeks Colchicine	3.41 ± 0.42 (7) 1.03 ± .13	0.39 ± 0.05 (7) 1.32 ± .15	17.86 ± 3.02 (7) .75 ± .15	1.94 ± 0.21 (7) 1.11 ± .13	0.21 ± 0.03 (7) 1.47 ± .20 *
6 weeks TTX	3.17 ± 0.49 (4) .60 ± .12	0.53 ± 0.09 (4) 1.56 ± .24 *	12.71 ± 2.28 (4) .58 ± .12 *	2.34 ± 0.64 (4) 1.03 ± .20	0.26 ± 0.03 (4) 1.66 ± .21 *

Smaller case numbers are ratios of experimental values compared to contralateral controls.
 * significantly different from contralateral muscles
 All values are means ± standard error
 Bath temperature (37°C)

Table IV

Twitch Temporal Parameters for EDL Muscles (in-vivo)

Groups	TTP (msec)	1/2 RT (msec)
Control	8.3 ± 1.0 (21)	9.7 ± 1.7 (21)
10 days Denervated	14.0 ± 0.8 (4) p<.001 1.63 ± .09 *	20.0 ± 0 (2) N.B. p<.001 2.00 ± 0 *
6 weeks Denervated	12.2 ± 1.0 (5) p<.001 1.53 ± .08 *	14.9 ± 2.5 (5) p<.001 1.57 ± .05 *
6 weeks Colchicine	12.0 ± 2.8 (7) p<.001 1.61 ± .41 *	12.9 ± 3.5 (7) p<.005 1.32 ± .27 *
6 weeks TTX	10.0 ± 1.0 (4) p<.02 1.22 ± .06 *	15.7 ± 1.1 (4) p<.001 1.73 ± .11 *

N.B. aftercontractions obscured measurement of
1/2 RT in two animals

Smaller case numbers are ratios of experimental values compared to
contralateral controls.

* Significantly different from contralateral muscles

All values are means ± standard error.

Bath temperature (37°C)

Table V

Twitch Temporal Parameters for SOL Muscles (in-vivo)

<u>Groups</u>	<u>TTP (msec)</u>	<u>1/2 RT (msec)</u>
Control	14.4 ± 1.4 (21)	17.8 ± 2.6 (21)
10 days Denervated	23.8 ± 4.7 (4) p<.001 1.68 ± .26 *	31.3 ± 1.9 (4) p<.001 1.67 ± .18 *
6 weeks Denervated	32.7 ± 3.2 (3) p<.001 2.37 ± .21 *	46.0 ± 7.0 (3) p<.001 2.61 ± .43 *
6 weeks Colchicine	14.3 ± 1.9 (7) 1.04 ± .15	20.0 ± 4.5 (7) 1.22 ± .34
6 weeks TTX	17.3 ± 1.7 (4) p<.001 1.19 ± .08 *	20.0 ± 1.6 (4) 1.16 ± .20

Smaller case numbers are ratios from experimental values compared to contralateral control values.

* Significantly different from contralateral muscles

All values are means ± standard error.

Bath temperature (37°C)

Table VI

Isometric and Isotonic Parameters for EDL Muscles			
Groups	TTP (msec)	1/2 RT (msec)	V_{US}(ML/sec)
Control	21.4 ± 2.0 (11)	23.0 ± 3.7 (10)	7.14 ± 0.59 (11)
10 days Denervated	28.0 ± 2.4 (7) p<.05	39.0 ± 2.4 (7) p<.001	4.45 ± 0.29 (6) p<.005
14 days TTX	29.3 ± 0.9 (6) p<.001	45.0 ± 6.2 (6) p<.001	4.35 ± 1.15 (6) p<.001
6 weeks Denervated	32.6 ± 2.4 (3) p<.001	45.7 ± 3.2 (3) p<.001	4.56 ± 0.33 (3) p<.001
6 weeks Colchicine	20.8 ± 2.2 (3)	22.9 ± 2.4 (3)	9.41 ± 3.67 (3)

all measurements were carried out
in-vitro at room temperature (20-21 °C)

Table VII

Isometric and Isotonic Parameters for SOL Muscles

Groups	TTP (msec)	1/2 RT (msec)	V _{US} (ML/sec)
Control	44.2 ± 3.9 (11)	49.7 ± 7.2 (10)	3.43 ± 0.71 (12)
10 days Denervated	65.1 ± 2.9 (7) P<.001	89.7 ± 4.7 (7) P<.001	3.24 ± 0.39 (7)
14 days TTX	71.7 ± 6.7 (6) P<.001	119.8 ± 10.3 (6) P<.001	3.50 ± 0.72 (6)
6 weeks Denervated	79.3 ± 7.9 (7) P<.001	130.0 ± 29.3 (7) P<.001	3.67 ± 0.45 (7)
6 weeks Colchicine	————	————	4.53 ± 0.92 (3)

all measurements were carried out
in-vitro at room temperature (20-21 °C)

Table VIII

Isometric and Isotonic Parameters for EDL and SOL Muscles of Dystrophic (dy2j/dy2j) Mice			
	EDL	SOL	
TTP (msec)	23.6 ± 2.3 (3)	40.0 ± 4.1 (3)	
1/2 RT (msec)	32.0 ± 5.7 (3)	54.9 ± 0.1 (3)	* p<.01
V_{us} (ML/sec)	5.24 ± 0.91 (3)	5.63 ± 0.92 (3)	* p<.05

* Value significantly different than control in TableVII

Table IX

**% Fibers Showing +ve Immunoreactivity to
anti-I, IIA or IIB myosin in EDL Muscles**

Groups	anti-I	anti-IIA	anti-IIB
Control	0	13.2 ± 1.4 (5)	64.0 ± 1.5 (5)
6 weeks Denervated	0	36.1 ± 1.9 (5) p<.001	16.9 ± 1.9 (5) p<.001
6 weeks Colchicine	0	12.5 ± 1.8 (6)	70.3 ± 2.6 (6)
6 weeks TTX	5.3 ± 0.3 (3) p<.001	19.9 ± 0.5 (3) p<.001	28.9 ± 6.3 (3) p<.001

Table X

% Fibers Showing +ve Immunoreactivity to
anti-I, IIA or IIB myosin in SOL Muscles

Groups	anti-I	anti-IIA	anti-IIB
Control	32.6 + 1.8 (5)	67.4 + 1.8 (5)	1.7 + 1.5 (5)
6 weeks Denervated	86.3 + 7.2 (3) p<.001	47.6 + 1.3 (3) p<.001	0
6 weeks Colchicine	38.1 + 2.5 (6)	55.5 + 1.2 (6) p<.001	1.5 + 1.1 (6)
6 weeks TTX	48.0 + 1.9 (3) p<.001	54.8 + 3.8 (3) p<.02	0.6 + 0.05 (3)

Figure 7. Results from 4 Vus experiments

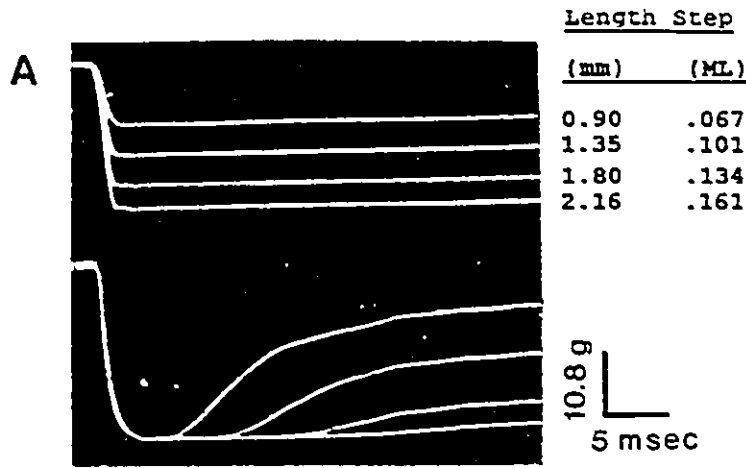
**A) The force-time trace corresponding to 4 release amplitudes.
(normal EDL)**

B) Release amplitude vs latency from A) vel: 6.87 ML/sec

C) same as B) for 10 day denervated EDL, vel: 4.48 ML/sec

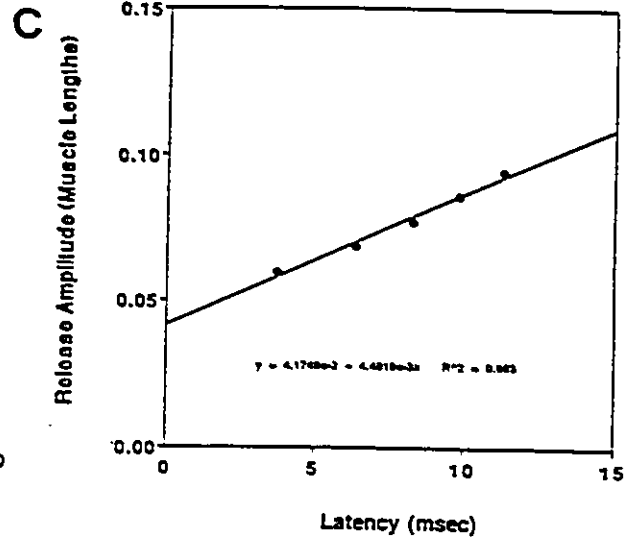
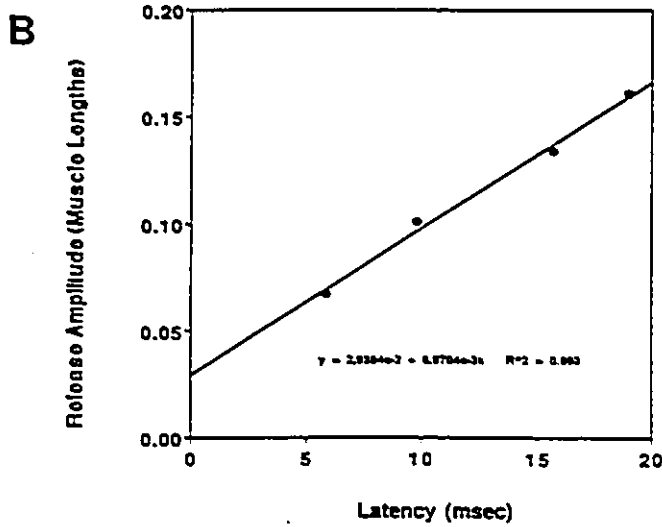
D) same as B) for 14 day TTX treated EDL, vel:4.05 ML/sec

E) same as B) for 6 week denervated EDL, vel: 4.89 ML/sec



Normal EDL

10 day Denervated EDL 3



14 day TTX-treated EDL

6 weeks Denervated EDL 1

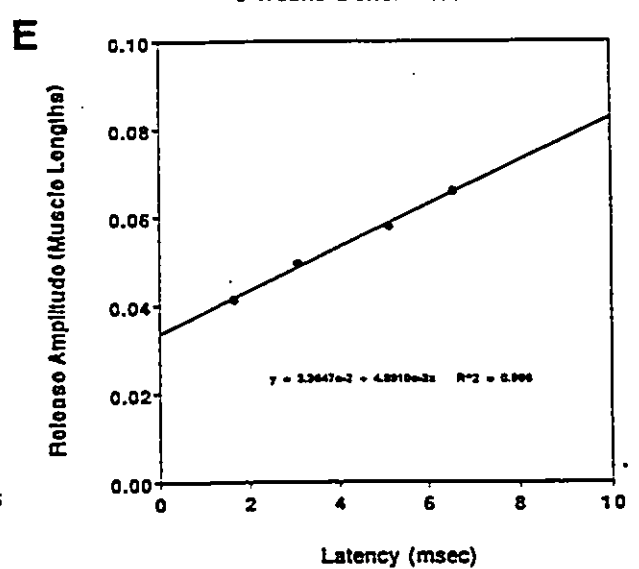
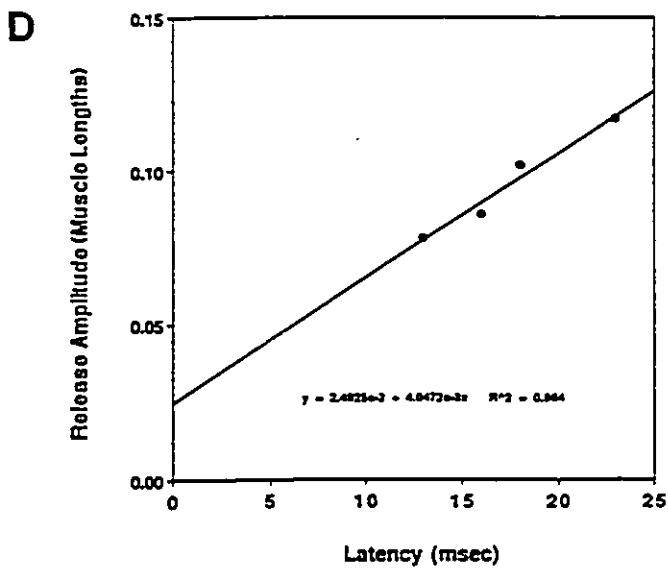


Figure 8. Immunohistochemistry of normal EDL muscle.

A) anti-type IIA myosin

B) anti-type IIB myosin

Magnification: 174X

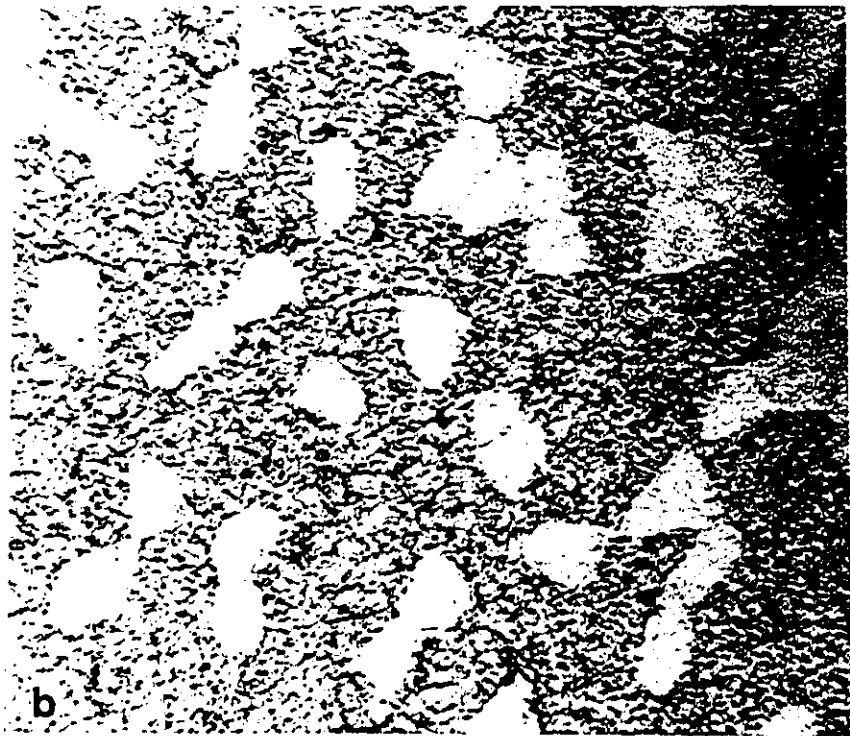
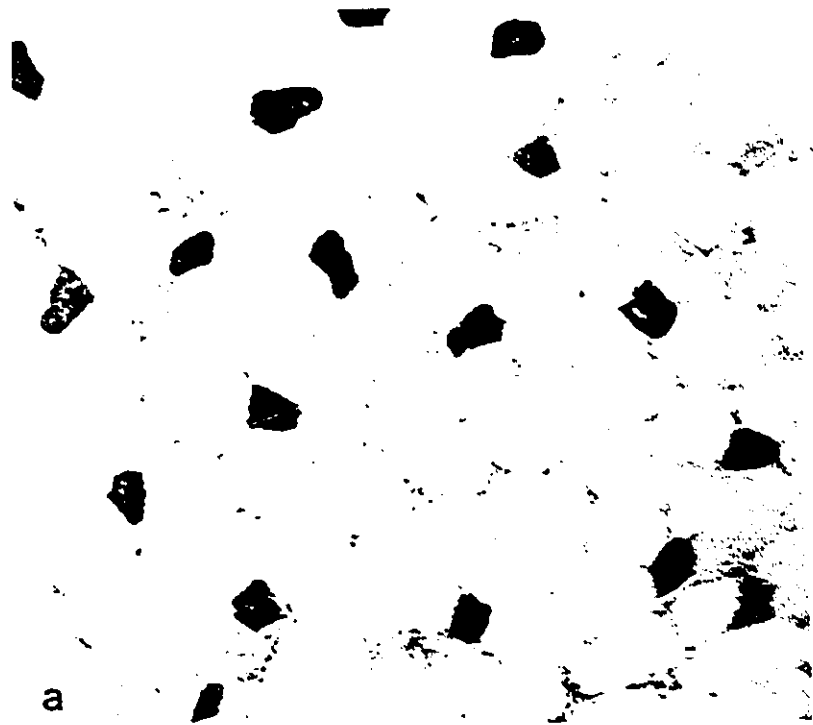
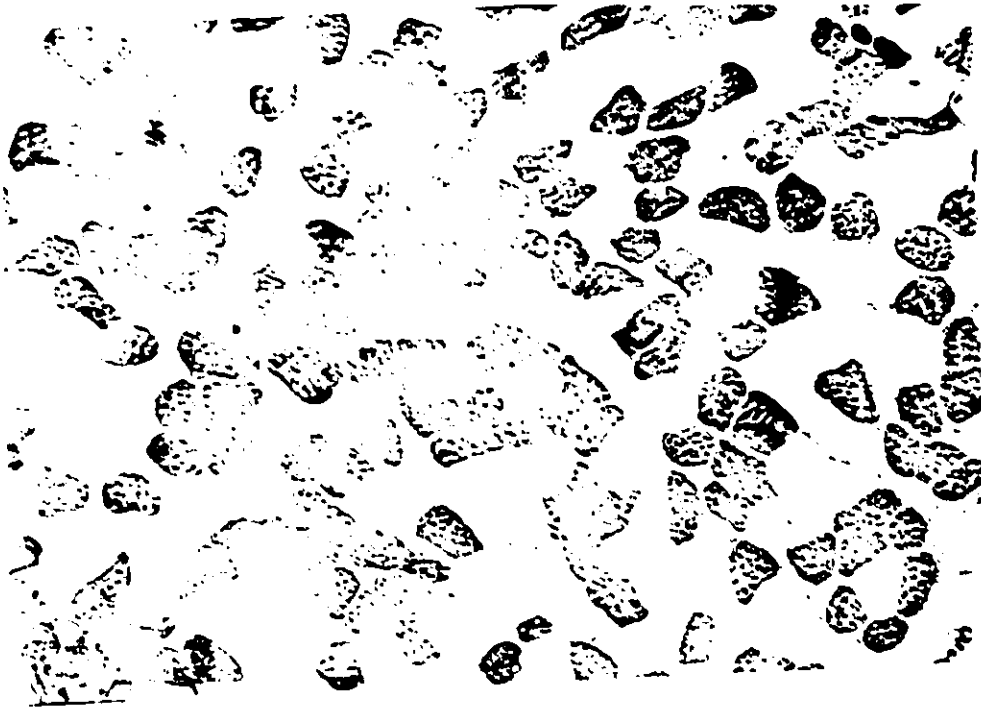


Figure 9. Immunohistochemistry of normal SOL muscle.

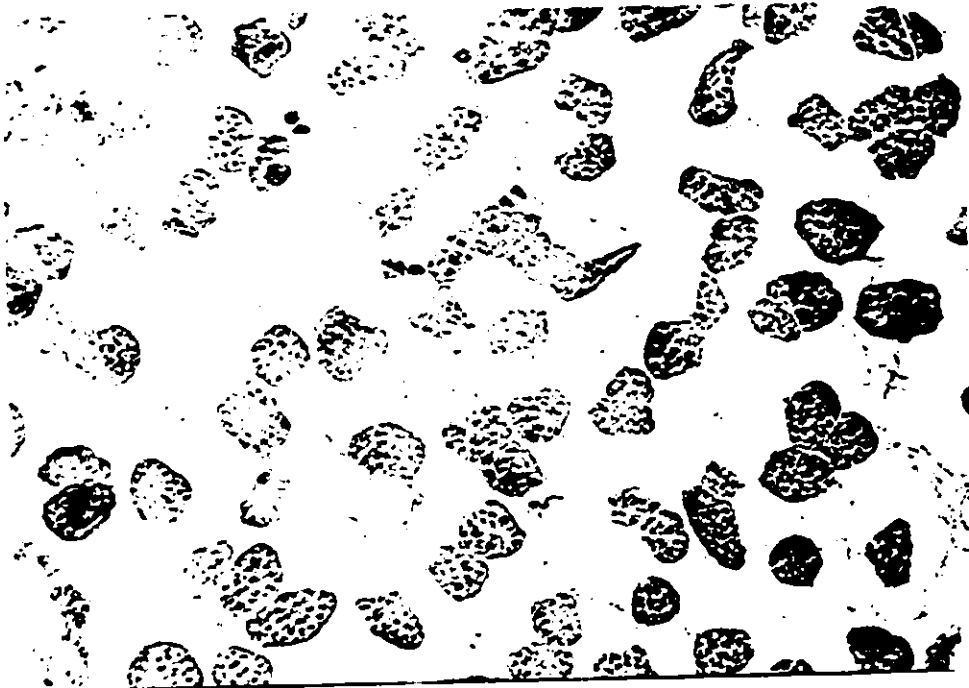
A) anti-type IIA myosin

B) anti-type I myosin

Magnification: 87X



A



B

Figure 10. 6% PAGE of normal and 6 week denervated SOL and EDL muscles.

lane 1: normal SOL

lane 2: normal EDL

lane 3: 6 week denervated SOL

lane 4: 6 week denervated EDL

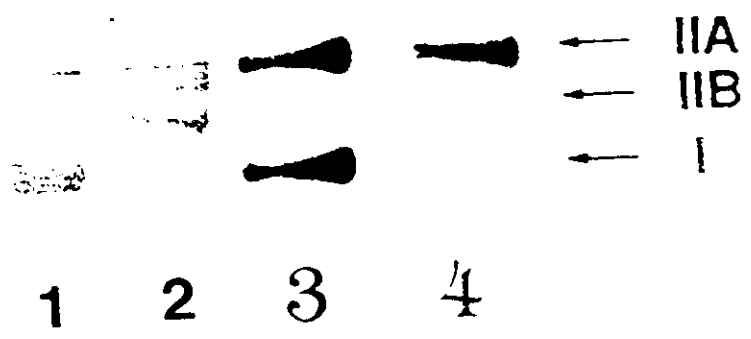


Figure 11. Immunohistochemistry of 6 week denervated SOL muscle.

A) anti-type IIA myosin

B) anti-type I myosin

Magnification: 174X

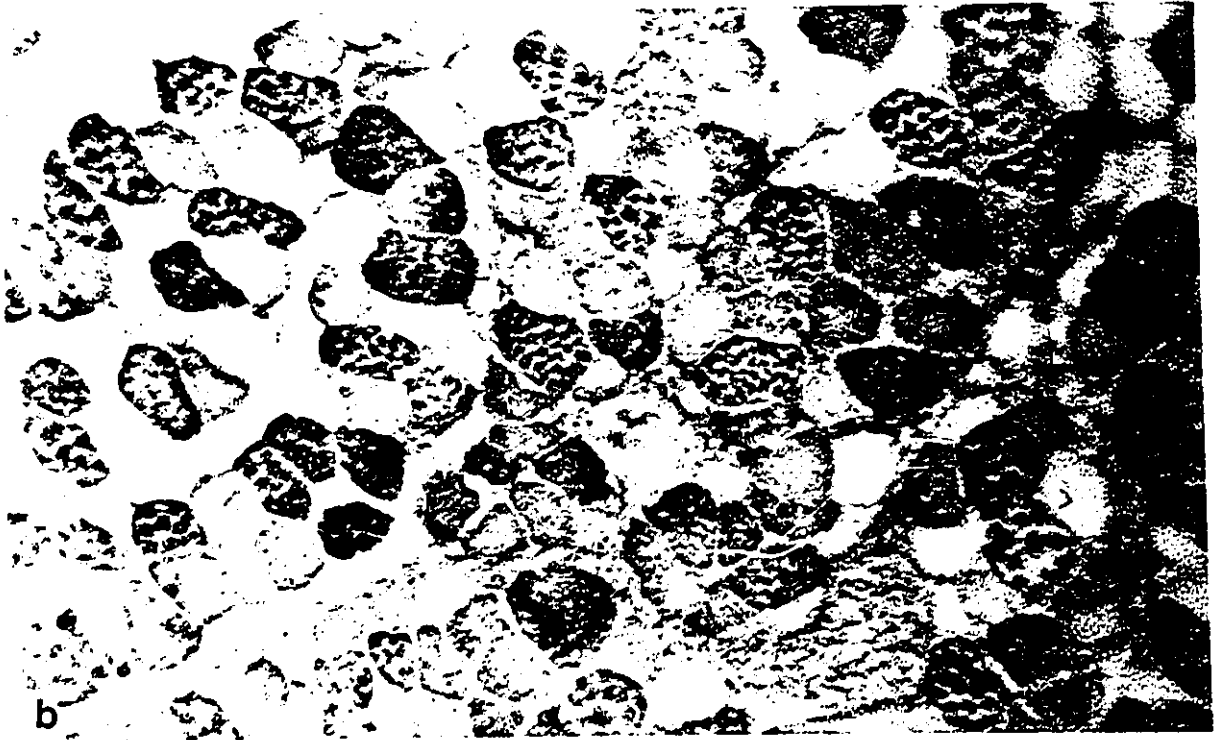
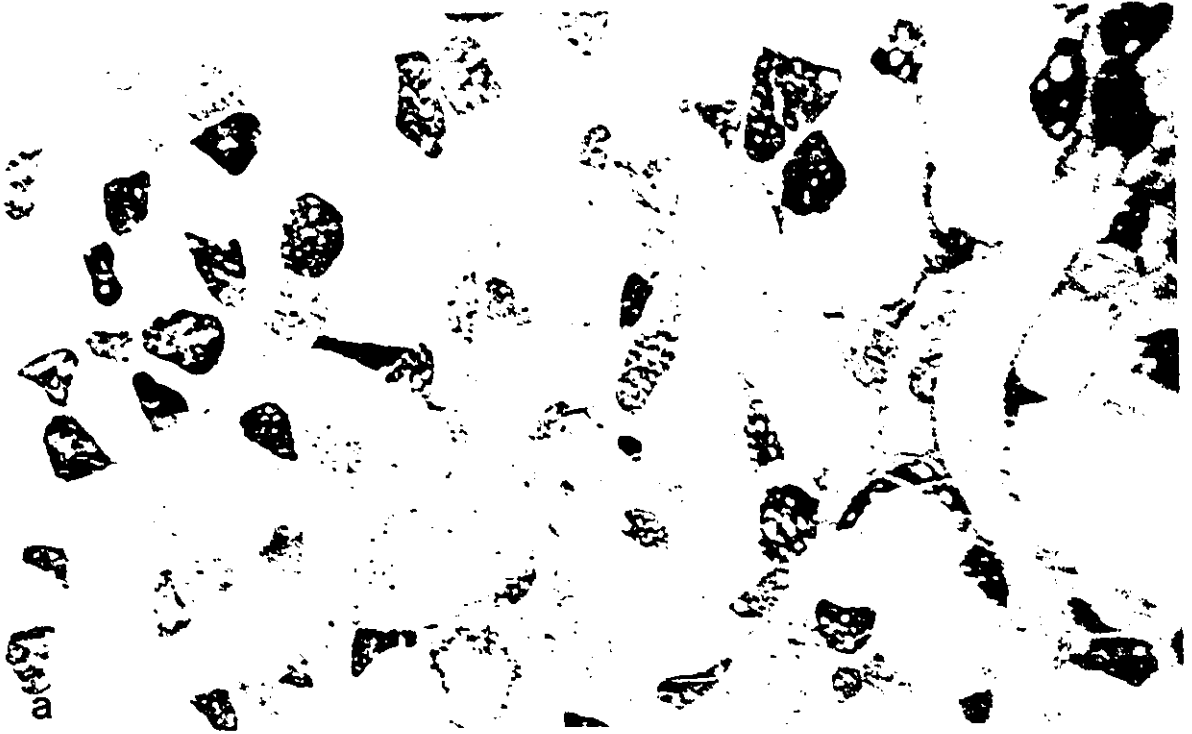
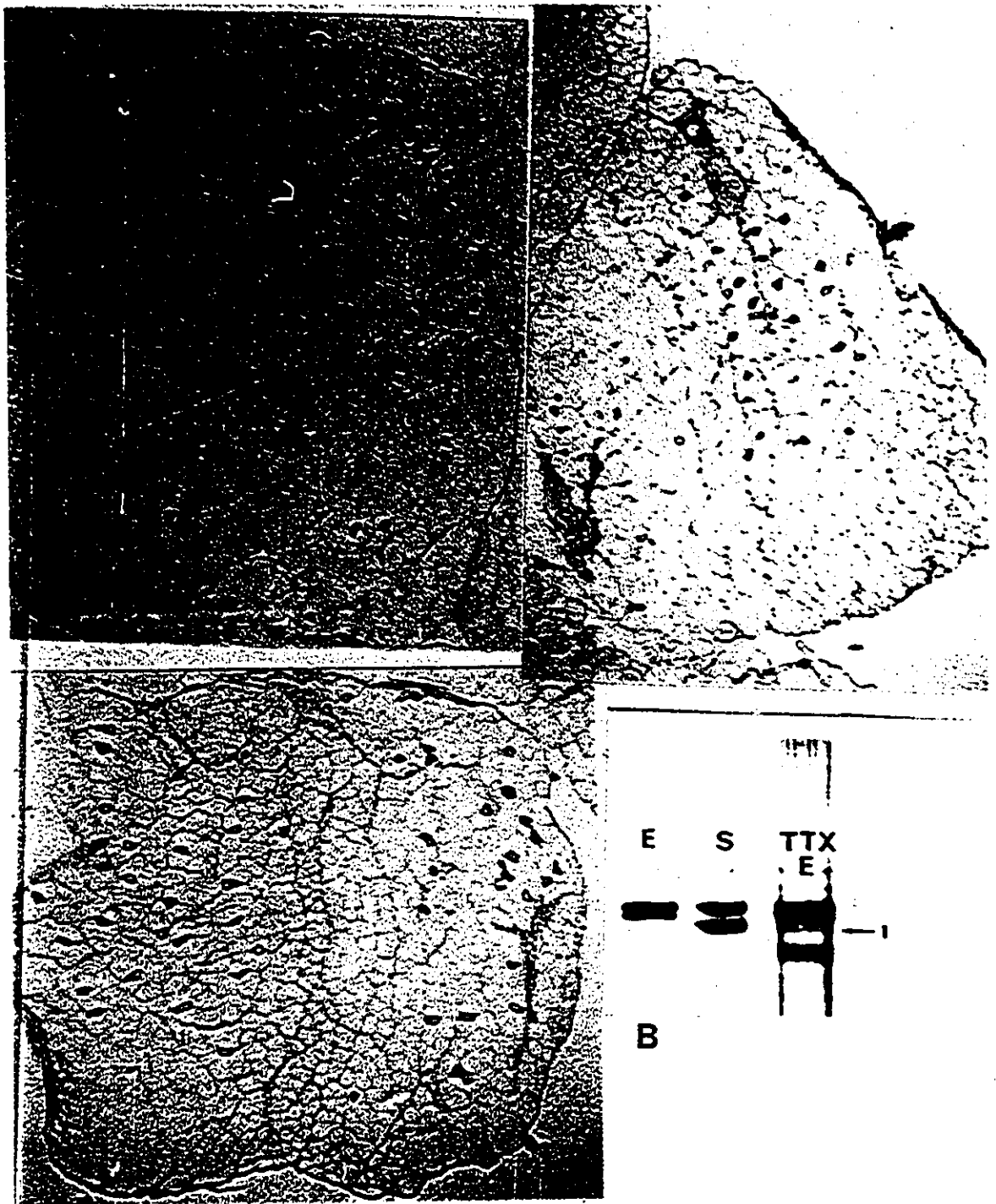


Figure 12. Immunohistochemistry and 6% SDS PAGE of six week TTX-treated EDL muscles.

- A) Three TTX-treated EDL muscles stained with anti-type I myosin.
- B) 6% PAGE of E:EDL, S:SOL, TTX E: TTX-treated EDL, showing the presence of type I myosin heavy-chain.
- C) Contralateral EDL stained with anti-type I myosin showing the absence of type I fibers
Magnification: 87X



A



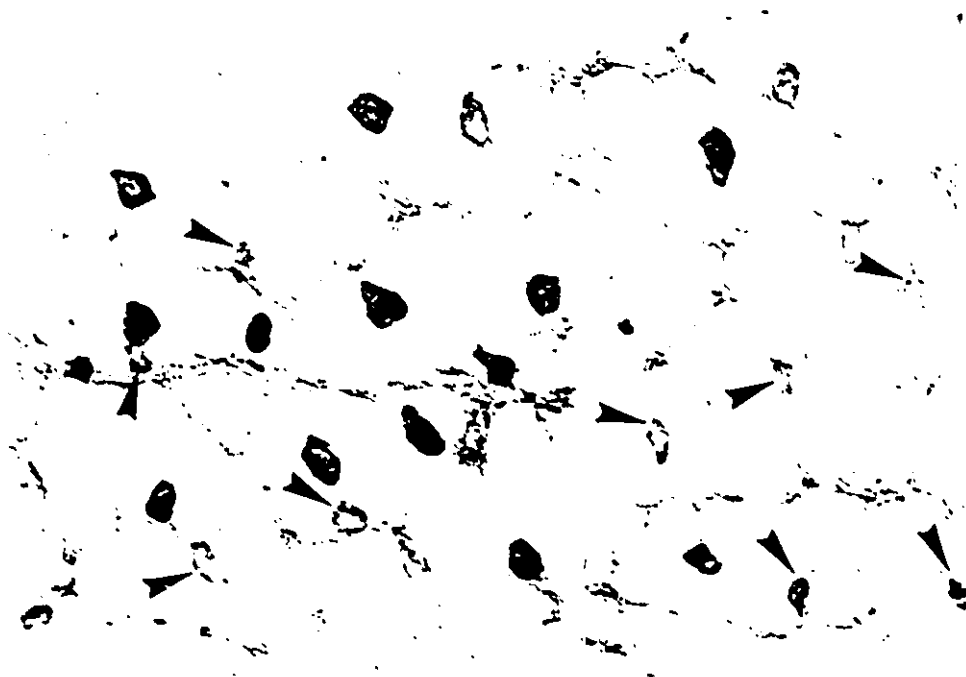
Figure 13. Immunohistochemistry of 6 week TTX-treated EDL muscle.

A) anti-type I myosin

B) anti-type IIA myosin

Arrows point to fibers that contain both type I and IIA myosin.

Magnification: 87X



A



B

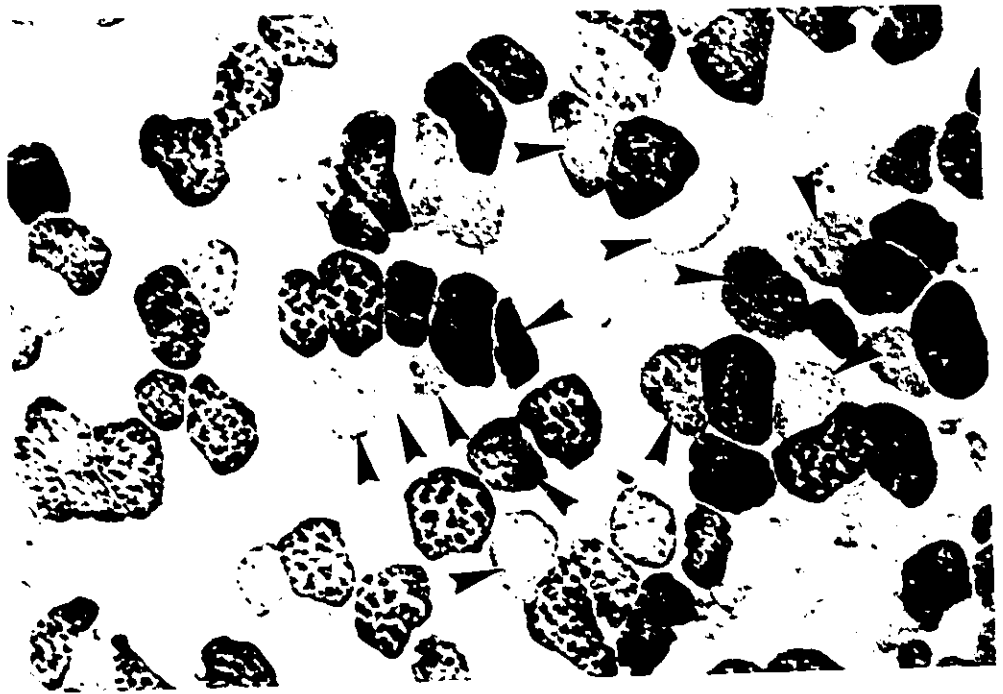
Figure 14. Immunohistochemistry of 6 week denervated SOL muscle.

A) anti-type I myosin

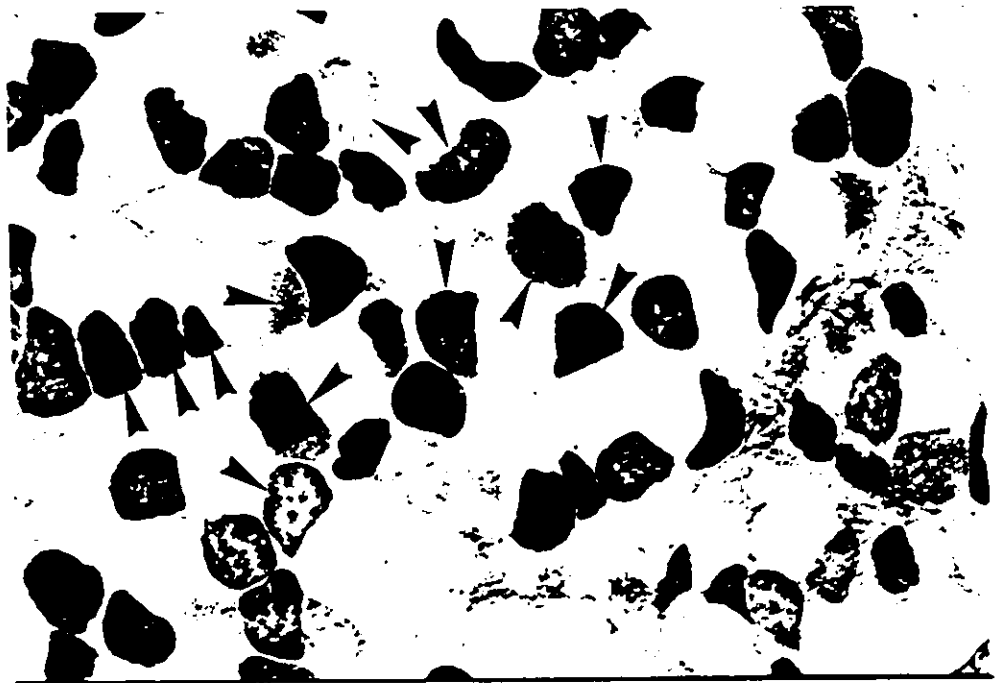
B) anti-type IIA myosin

Arrows point to fibers that contain both type I and IIA myosin.

Magnification: 157X



A



B

Figure 15. 6% PAGE of normal EDL, SOL and dystrophic SOL muscles showing the presence of IIB myosin in dy2j SOL.

Lanes 1,5: normal SOL

Lanes 2,4: normal EDL

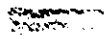
Lanes 3,6: dystrophic SOL

Arrowheads point to IIB myosin heavy chain band in dystrophic SOL.

1



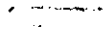
2



3



4



5



6



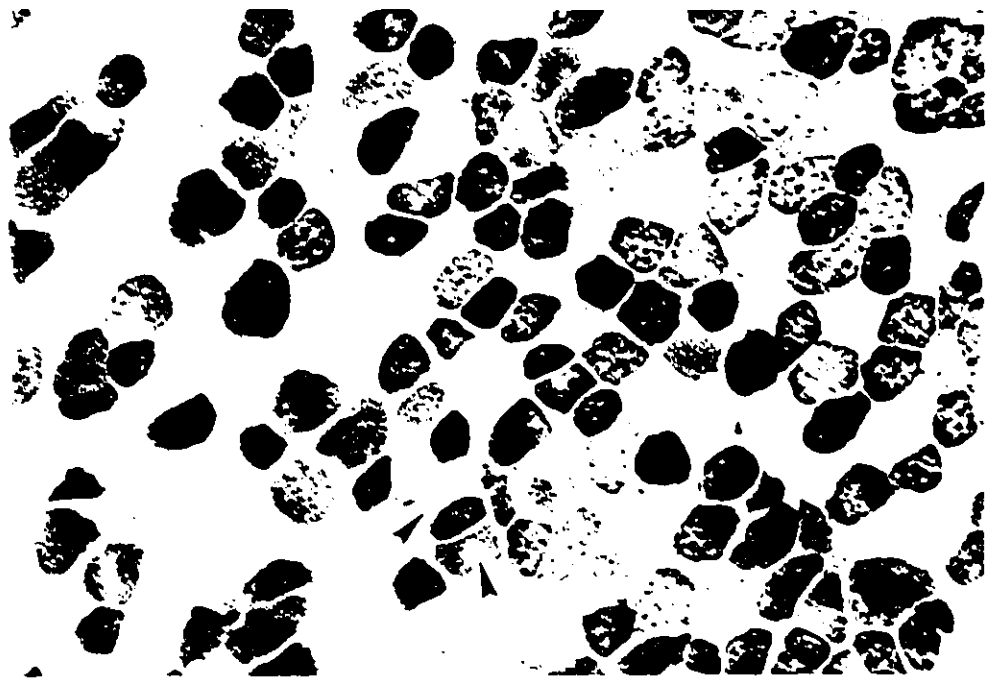
Figure 16. Immunohistochemistry of 6 week denervated EDL muscle.

A) anti-type IIA myosin

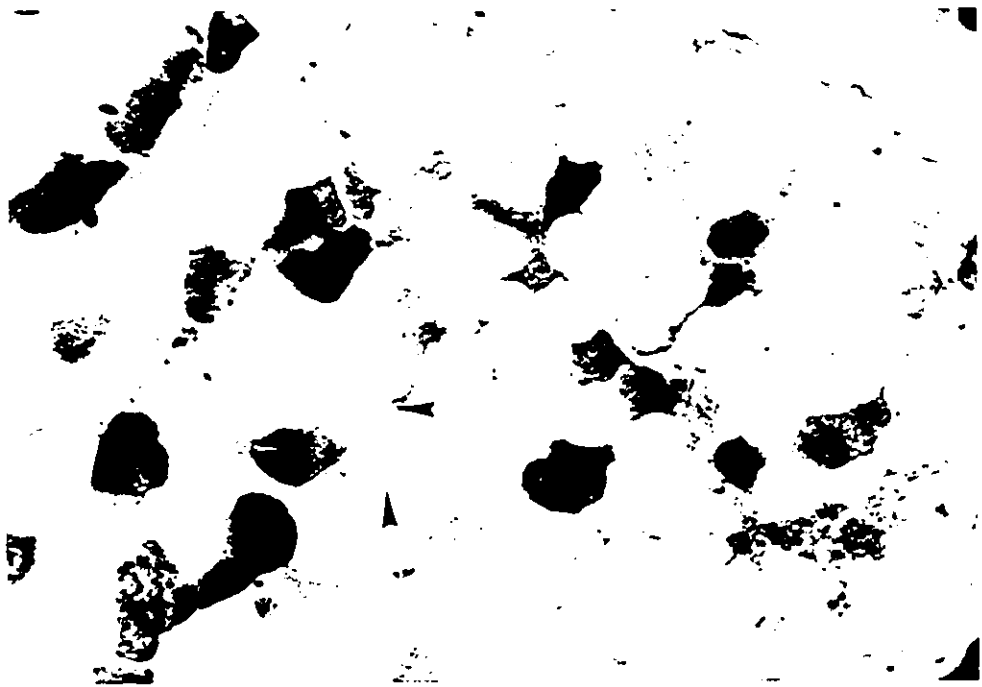
B) anti-type IIB myosin

Arrows point to fibers that contain both type IIA and IIB myosin.

Magnification: 157X



A



B

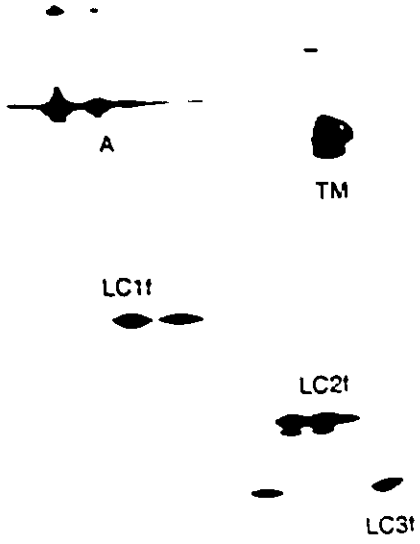
Figure 17. a) 2-dimensional PAGE profile for control, 10 days denervated, and 6 weeks denervated EDL muscles.

b) 2-dimensional PAGE profile for four 14 day TTX-treated EDL muscles.

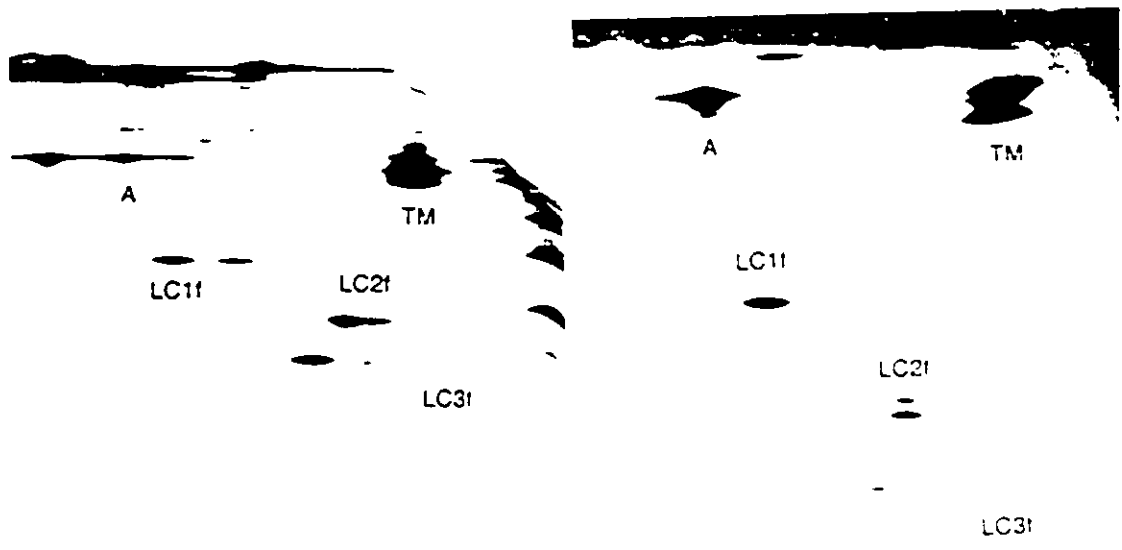
A: Actin, TM: Tropomyosin, LC: Myosin Light Chain
f: fast, s: slow

a.

EDL



Control



10 days Denervated

6 weeks Denervated

b

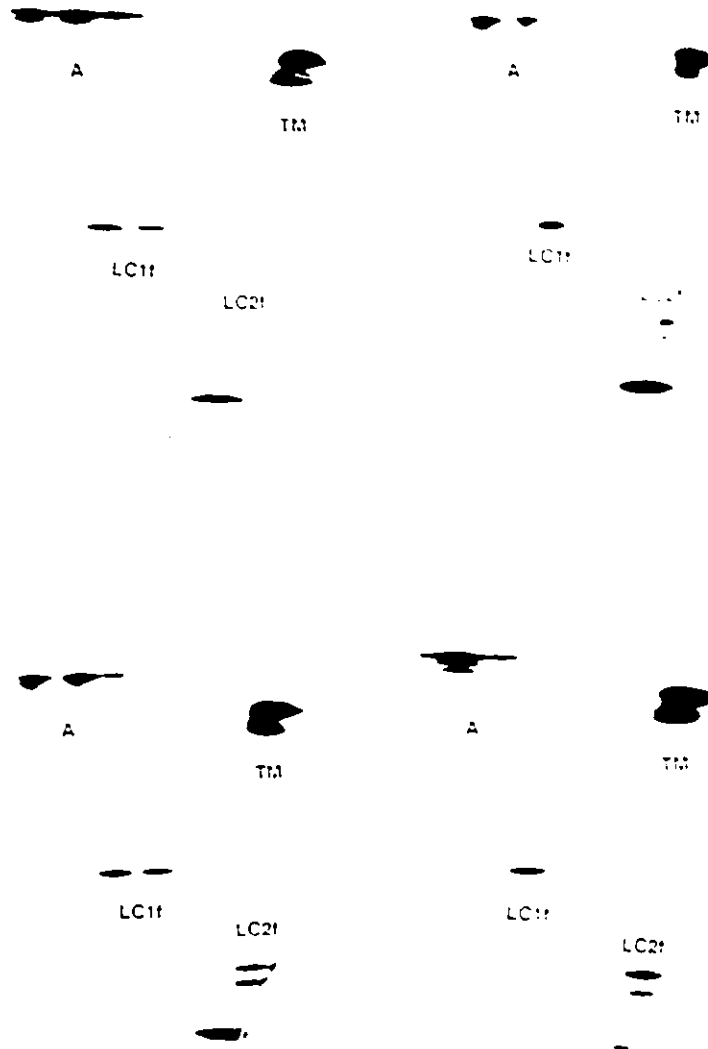
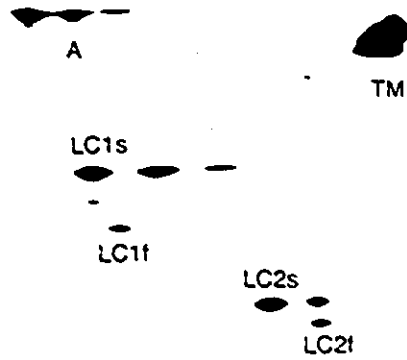


Figure 18. 2-dimensional PAGE profile for SOL muscles

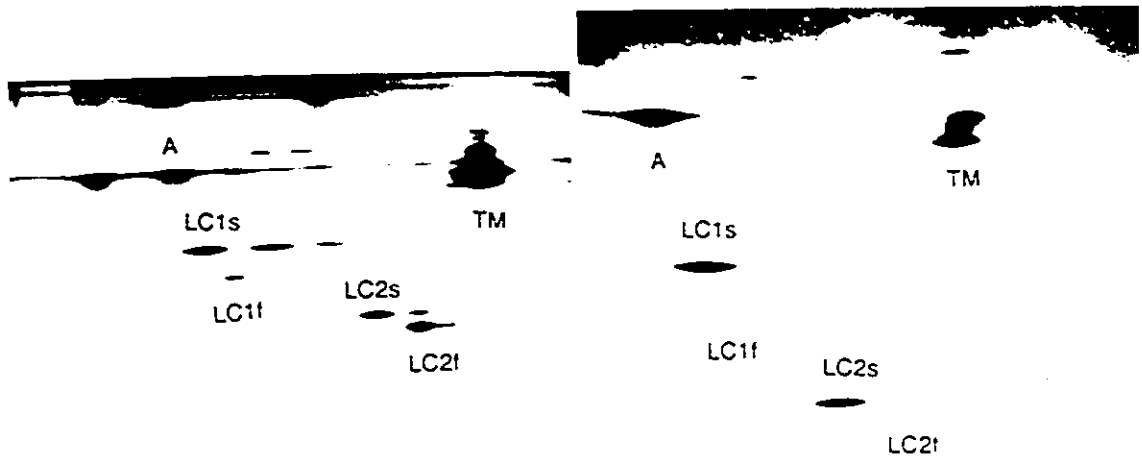
A: Actin, TM: Tropomyosin, LC: Myosin Light Chain

f: fast, s: slow

SOL



Control



10 days Denervated

6 weeks Denervated

DISCUSSION

In this study the effect of denervation on the contractile and histochemical properties of mouse EDL and SOL muscles was examined. An attempt was made to dissociate the neuronal influences responsible for maintaining the normal physiological properties of muscle. This was done by selectively eliminating either activity or myotrophic influences. The application of TTX via a mini-osmotic pump that was connected to a silastic cuff surrounding the sciatic nerve resulted in paralysis of the hindlimb and hence disuse of that limb. Colchicine impregnated silastic cuffs surrounding the sciatic nerve resulted in blockage of fast axoplasmically transported material thus depriving the hindlimb musculature of putative myotrophic influences.

The effects of disuse and loss of myotrophic influences on the contractile and histochemical properties of mouse EDL and SOL muscles were investigated and the following novel findings emerged.

- 1) Following 6 weeks of denervation there was a loss of IIB myosin in the EDL muscles which was also observed in the 6 week TTX treated group but not in the colchicine treated group thereby suggesting that muscle activation is a partial requisite for IIB myosin expression.
- 2) Denervation of SOL muscles resulted in a virtually complete conversion of type IIA to type I fibers. Colchicine had no

effect while TTX treatment resulted in a significant increase in type I fibers thus suggesting that inactivity was partially responsible for the denervation-induced conversion.

3) Paralysis for 6 weeks with TTX resulted in an increase in the proportion of fibers expressing type I myosin heavy-chain in EDL whereas neither denervation nor colchicine caused any such changes.

4) After 10 days of denervation there was a reduction in the maximum velocity of shortening of EDL muscles. This reduction in velocity was not paralleled by any changes in myosin heavy-chain composition however a loss of myosin LC3f was noted and this could account for the reduction in velocity that was seen.

5) Following 14 days of paralysis with TTX the EDL muscles showed a reduction in maximum velocity of shortening that was indistinguishable from the 10 day denervated group. A loss in myosin LC3f was also evident. These findings are consistent with the idea that activity may modulate myosin LC3f expression. In addition the lowering in maximum velocity of shortening that was seen in denervated and TTX-treated EDL muscles may reflect altered crossbridge cycling rates secondary to changes in LC3f content.

Reports of the effects of denervation on muscle size are extensive. Early atrophy can be somewhat variable depending on the amount of passive stretch that the denervated muscle is experiencing. In some cases denervation-induced stretch

hypertrophy has been reported in the first few weeks following denervation (Sola and Martin 1953, Goldspink 1977). In this study progressive atrophy was seen for both EDL and SOL muscles (Table I). By 10 days post-denervation the EDL weighed 84% of its normal weight while the SOL muscle had atrophied to 61% of control. After 6 weeks of denervation the SOL muscle weighed 41% of its original mass while the EDL was less severely affected and weighed 58% of control. There are many reports in the literature of differential atrophy of slow versus fast muscles. In most cases slow muscles were more affected in terms of loss of bulk (St. Pierre and Gardiner 1985, Al-Amood and Lewis 1989). The results of this study are also supportive of differential atrophy. However, while the 6 week denervated SOL muscle lost more mass than the 6 week denervated EDL, the proportion of atrophy attributable to disuse was similar in both EDL and SOL muscles as indicated by the results from the TTX treated group. Colchicine treatment also resulted in a loss of muscle mass in both EDL and SOL muscles (Table I). However this was considerably less than that induced by inactivity thus suggesting that most of the denervation induced atrophy in EDL and SOL muscles is related to disuse. It is of considerable interest that the amount of atrophy in 6 week denervated SOL (59% of original mass) can largely be explained by a summated effect of loss of activity (TTX group: 41%) and myotrophic influences (colchicine group: 17%). This is not

true in the case of EDL muscles that were denervated for 6 weeks. In this case the combined effects of inactivity (35% atrophy) and loss of myotrophic influence (20% atrophy), if additive, should result in an atrophy of approximately 55%. In fact 6 week denervated EDL muscles atrophied by 42% of their original mass. Thus, while the denervation-induced atrophy of SOL could be reconciled by combined disuse and loss of myotrophic influences, in the case of EDL muscles the degree of denervation induced atrophy was less than one would expect from combining disuse and loss of myotrophic influences. Some other factors must play a role. The disparate response of EDL and SOL muscles to denervation may be due to differences in the amount of passive tension that the muscles are experiencing. It is known that immobilized muscles that are fixed at longer lengths experience less atrophy (Goldspink 1977). Also in previous studies the differential responses to denervation were attributed to differences in resting length (Sola, Christensen and Martin 1973; Williams and Goldspink 1976). In this study I observed that the mice tended to drag their denervated hindlimb. This behaviour would cause the EDL to be stretched while the SOL would be in an unloaded position. Indeed, the amount of atrophy in 10 day denervated EDL (16%) was considerably less than that observed for the 10 day denervated SOL (39%). It is of interest that the amount of atrophy after 14 days of disuse was the same as that following 6 weeks of disuse. It would therefore appear that the

mechanisms underlying disuse atrophy are rapidly set in motion and the longer term steady state is achieved in a short period of time.

It is difficult to make comparisons on the atrophy and contractile data with the TTX or colchicine data since no other study has addressed this issue using the mouse model. However studies in the rat have provided comparable results. Thus Spector(1985) found a 53% decrease in wet weight of 4 week TTX inactivated SOL muscles and St.Pierre and Gardiner (1985) noted a 47% decrease in wet weight of SOL after 14 days of TTX inactivation. In this study the 6 week TTX inactivated SOL weighed 59% of control while the 6 week TTX inactivated EDL weighed 65% of control. Unfortunately only a limited number of studies utilising colchicine to selectively block axoplasmic transport have also looked at atrophy or contractile properties of muscles treated in such a way. Out of the work that has been done, a slight but insignificant reduction in muscle mass has been noted and a reduction or no change in maximal twitch or tetanic tension as well as an increased twitch to tetanus ratio have also been reported (Hofmann and Thesleff 1972, Cangiano 1973, Albuquerque, Warnick, Sansone and Onur 1974, Warnick, Albuquerque and Guth 1977). In this study both colchicine treated EDL and SOL muscles showed an increase in the twitch to tetanus ratio to values intermediate to that of the TTX inactivated and 6 week denervated groups. Also, as previously described, both

colchicine treated EDL and SOL muscles showed a small reduction in muscle mass. It would therefore appear that in the long run myotrophic influences are not important in maintaining the dynamic properties of EDL and SOL muscles. This is further substantiated by the lack of change in twitch and tetanic tensions as well as Vus of colchicine treated EDL and SOL muscles. (Tables II, III, VI, VII).

Changes in the contractile properties as well as the fiber-type composition of denervated muscle have been reported by many investigators. The response to denervation however appears to be dependent on the species being studied. For example in the cat the soleus muscle contracts more slowly following long periods of denervation (Lewis, 1972) while in the rat the soleus muscle shows an increase in velocity of shortening (Gutmann, Melichna and Syrový 1972, Spector 1985, Al-Amood and Lewis 1989) as well as myosin ATPase activity (Gutmann et al 1972). A conversion of type I to type II fibers has also been reported (Spector 1985, Al-Amood and Lewis 1989).

Figures 9 and 11 show the histochemical profile for normal and denervated SOL muscles of mice used in this study. Contrary to results obtained in the rat SOL by other groups, an increase in the proportion of fibers containing myosin heavy chain I was noted. This is illustrated quantitatively in Table X. It would appear that type IIA fibers are undergoing conversion to type I fibers since there were many

fibers that contained both type IIA and type I myosin heavy chain (Fig. 14). This was further supported by the observation that in a separate group of animals (n=4) which were denervated for 5 months the percentage of fibers expressing type IIA and I myosin was 3.3% and 96.7% respectively. It is not clear why the mouse SOL responded differently to denervation than what has been previously reported for the rat SOL muscle. Both muscles have a mixed fiber-type composition consisting of varying proportions of type I and IIA fibers.

To explain the disparity between results in the rat and guinea-pig Al-Amood and Lewis (1989) advanced the notion that the opposite responses to denervation may simply reflect differences in the extent of post-denervation fibrillation that the muscles experience. The rationale for this stems from the classic electrostimulation experiments that showed that while tonic stimulation of a slow muscle resulted in a preservation of its slow contractile properties, in the case of fast muscles the same electrostimulation protocol converted their contractile properties to resemble that of a slow muscle. Hence in animals whose denervated muscles fibrillate for long periods following denervation (ie:mimicking tonic stimulation) there would be slowing (eg:cat, guinea pig) while in animals (eg: rat) whose denervation-induced fibrillation occurs only transiently there would be a speeding-up (Lewis, Robinson and Tufft 1988). There was evidence (visual) of

fibrillation in both the 10 day and 6 weeks denervated groups. However none of the 6 weeks denervated EDL muscles had any type I fibers. This may however not be surprising in light of the results obtained by Ausoni et al (1990) where chronic tonic stimulation of denervated rat EDL at 20 Hz resulted in a conversion of most fibers to IIX and IIA. No type I fibers were seen. Indeed, in this study, in 6 week denervated EDL the sum of all immunohistochemically labelled fibers accounted for only 53% of the total number. The rest are most likely type IIX fibers. Parry and Zardini (1990), using immunohistochemical techniques found that roughly 1/3 of the fibers in normal EDL are type IIX. Indeed, in the present study the sum of all immunohistochemically labelled fibers was 77.2% for normal EDL thus leaving 22.8% (presumably type IIX) unaccounted for. Therefore the suggestion that the unlabelled fibers in 6 week denervated EDL are type IIX is reasonable (Gorza 1990) and in light of Ausoni's results would be anticipated in a theoretical context if fibrillation were to persist for that long. Similarly the increase in type I fibers of 6 week denervated SOL may also be attributable to fibrillation. Although attractive unfortunately this postulate does not lend itself easily to verification. In a separate group of animals (n=3), 6 weeks following denervation there were no type IIX fibers seen in denervated EDL or tibialis anterior muscles. The major difficulty in interpreting this result arises from the nature of the antibody used to classify

type IIX fibers. Type IIX fibers are identified based on negative staining. In other words the antibody (BF-35) used to identify type IIX fibers recognizes type I, IIA, and IIB myosin heavy chain but not type IIX. Herein lies the difficulty. Some denervated muscle fibers are undergoing conversion. These hybrid fibers which may contain type IIX myosin heavy chain will not be detected immunohistochemically since they will also contain one of the other myosin heavy chains and thus will appear positive with BF-35. Since in the mouse it is not possible to visualize IIX myosin heavy chain using the electrophoretic approach (Parry and Zardini 1990) the question of conversion to IIA myosin through IIX myosin heavy chain will remain unresolved until alternate ways of detecting IIX myosin heavy chain are developed. It remains curious however that only 53% of fibers in 6 week denervated EDL could be accounted for by using antibodies to type I, IIA, and IIB myosin, while all the fibers stained positive with BF-35. Perhaps fibers with a low staining intensity to the antibodies against type IIA or IIB myosin escaped visual detection. This seems unlikely since labelling with BF-35 was intense for all fibers in 6 week denervated EDL. Also 6% PAGE confirms the loss of IIB and increase in IIA myosin heavy chain (Fig. 10). An attractive possibility is the existence of a novel or altered form of myosin heavy chain that is also recognized by BF-35. This possibility warrants further investigation. Perhaps longer periods of denervation (many

months) will yield the necessary information since the incidence of co-existence (hybrid fibers) of myosin heavy chains would be greatly diminished.

Interestingly EDL muscles from the 6 week TTX treated group contained a significant population of type I fibers (5.3%) (also see Fig. 12). Curiously, aside from denervation, only TTX treatment was effective in precipitating an increase in type I fibers in the SOL muscle. TTX inactivated solei also had longer TTP's. This is probably a reflection of the increased contribution of slow fibers to the isometric twitch kinetics. Also, the slight but significant increase in TTP that was seen in the TTX inactivated EDL may be explained by the presence of type I myosin containing fibers that were observed in these muscles. Colchicine had no effect on the type I fiber population of either EDL or SOL muscles. It would therefore appear that inactivity per se can stimulate the conversion to type I fibers, although it seems that at least in the case of the SOL muscle this is quantitatively a minor component accounting for only about 1/4 of the conversion.

In contrast to the SOL muscle the EDL muscle does not show species specificity in its response to denervation. To date all studies addressing contractile properties of denervated EDL have reported a profound slowing both in terms of the isometric twitch as well as the isotonic velocity of shortening. The slowing cannot be explained on the basis of alterations in the fiber-type composition of denervated EDL.

Few investigators have reported the presence of substantial amounts of type I fibers in denervated EDL. In this study there were no type I fibers in the 6 week denervated EDL, however there was a dramatic decrease in the proportion of fibers expressing type IIB myosin as well as myosin heavy chain and an increase in the proportion of fibers expressing type IIA myosin and myosin heavy chain (Figs. 8,10,16). This change in fiber type distribution has also been observed in the denervated rat EDL by Ausoni et al (1990) and cannot be attributed to selective loss of fibers since the number of fibers in denervated EDL and SOL muscles did not differ from control. Surprisingly only a very small number of fibers containing both type IIA and IIB myosin were seen in 6 week denervated EDL (Fig. 16). This observation is consistent with the notion that IIB myosin heavy chain is replaced by IIX myosin heavy chain before the appearance of IIA myosin heavy chain. However, another possibility is that the turnover rate from IIB to IIA fibers is faster than the turnover rate from IIA to type I fibers. Since the TTX treated group also showed a substantial loss of IIB fibers it would appear that inactivity is the major precipitating factor for the conversion. Colchicine had no effect on the fiber type distribution of EDL muscles and thus no myotrophic component is indicated.

A major component of this thesis addresses the denervation-induced slowing that has been reported

extensively in the literature. Changes in calcium kinetics as well as myosin isozyme composition have been implicated as factors contributing to the increase in twitch duration that is seen following denervation. In this study the isometric twitch showed prolongations in both the TTP as well as 1/2RT phases in both 10 day and 6 week denervated EDL and SOL muscles. The early slowing seen in the 10 day denervated group could not be reconciled on the basis of changes in myosin heavy chain composition since immunohistochemical staining showed identical fiber type profiles as with control animals. Also, 6% SDS polyacrylamide gels did not reveal the presence of slow myosin heavy chain in the case of 10 day denervated EDL muscles. Since the isometric twitch TTP is a complex function whose time course is dependent on contractile protein function as well as Ca^{++} kinetics, a more accurate physiological marker of contractile protein function was chosen in order to study this process in isolation. Thus the maximum velocity of unloaded shortening was determined. Surprisingly a reduction in velocity was seen in the 10 day denervated EDL but not SOL muscles. At first this finding would seem discordant considering the immunohistochemical and PAGE results. However, recent reports have lent support to the notion that not only is the myosin heavy-chain composition important in influencing the speed of contraction but that the myosin light-chain pattern particularly the LC3f/LC1f ratio can also influence the velocity of shortening. From

studies on single fibers it was shown that the fibers with the fastest velocities also had the highest LC3f/LC1f ratios (Greaser, Moss and Reiser 1988). In this study 2-dimensional PAGE analysis of the light-chain patterns revealed a clear reduction and in some cases a virtual absence of LC3f in 10 day denervated EDL (Fig. 17a). No increase in slow light chains was seen in either 10 day denervated EDL or SOL muscles. Therefore the initial slowing of twitch kinetics in denervated EDL could be due to a loss of LC3f. Since no alteration in light chain pattern or myosin heavy-chain was seen in 10 day denervated SOL muscles it would appear that in this case the prolonged twitch kinetics may be secondary to altered Ca^{++} handling. Since the rate of relaxation correlates well with the Ca^{++} pump activity (Stein, Gordon and Shriver 1982) the $1/2RT$ is a reliable marker for SR function. Indeed, muscles with longer $1/2RT$'s are also known to contain SR with lower Ca^{++} pumping rates and similarly muscles whose SR has a high Ca^{++} pumping rate have lower $1/2RT$'s. The $1/2RT$'s of both 10 day denervated EDL and SOL muscles were prolonged thus suggesting that in the case of the EDL muscles both changes in contractile proteins as well as SR function contribute to the early slowing while in the case of the SOL muscle the early changes in twitch temporal parameters are solely due to altered SR function since no change in velocity was seen.

After 6 weeks of denervation the isometric and isotonic

parameters of EDL muscles were slowed while in the case of the SOL muscle, only the isometric parameters showed a further slowing. No reduction in Vus was seen despite a dramatic increase in the proportion of fibers containing slow myosin heavy-chain. The finding that Vus for 6 week denervated EDL was not different from that of 10 day denervated EDL is not surprising as there was no accumulation of slow heavy- or light-chain in 6 week denervated EDL. The results of this study would therefore suggest that in the mouse the loss of LC3f is the major contributor to the decrease in velocity since at both time groups studied there was no accumulation of slow light-chains, but the decrease in LC3f was extensive both at 10 days and 6 weeks of denervation. Interestingly the Vus of the dystrophic EDL group was indistinguishable from the denervated group and the dystrophic EDL muscles were also devoid of LC3f. Since maximum velocity of shortening of EDL muscles was reduced after only 10 days of denervation it would appear that muscle velocity can be altered following perturbations of very short duration. Hence the loss of LC3f that was seen following 14 days of disuse would argue that activation is important for its expression. In addition, since the Vus of 14 day TTX inactivated EDL muscles was the same as that of 10 day and 6 weeks denervated EDL muscles it would appear that activity (or activity pattern) is the important mediator of plasticity at least in terms of velocity modulation. The case of IIB myosin expression is also

interesting in this regard. It would seem from the results of this study that disuse (TTX treatment) and denervation (fibrillation ?) were equally potent stimuli for its loss. IIB myosin expression may therefore require a precise pattern of activation for its expression and this may in part account for the relative difficulty in artificially effecting a slow to fast compared to a fast to slow transformation in muscle phenotype (Ausoni et al 1990). Of potential significance with respect to IIB myosin expression are the results obtained from the dystrophic SOL muscles. In these muscles a significant amount of IIB myosin heavy chain was seen (Fig. 15). Also many fibers staining for IIB myosin were visualized immunohistochemically (Desypris 1986). This is odd since SOL muscles from control animals rarely contain any type IIB fibers (Parry and Desypris; personal observations). A possible explanation for this curious observation may lie in the fact that the hindlimb muscles of dystrophic mice are spontaneously active. This arises from the presence of regions of amyelination in the lumbar spinal roots (Bradley and Jenkison 1974). In these areas bare axons are in close apposition thus favoring ephaptic transmission of signals originating from the periphery or centrally (Rasminsky 1978). EMG recordings from dystrophic hindlimb muscles show bursts of activity in anesthetised animals (Parry and Desypris 1983). It is therefore reasonable to suggest that some motor units in the dystrophic SOL muscle may be activated in a phasic manner

thereby providing an activity pattern favorable for the expression of IIB myosin heavy chain.

The finding of an accumulation of slow myosin heavy-chain in the denervated SOL was unexpected and as mentioned previously may represent a species difference in the response to denervation of slow muscle fibers. In other species denervated SOL becomes faster contracting and an increase in fast myosin heavy-chain has been extensively documented. The results of Vus measurements on denervated SOL were somewhat confusing. In spite of a dramatic increase in slow myosin heavy-chain there was no corresponding decrease in Vus. Even in one animal whose soleus had been denervated for 4 months the Vus was 3.29 ML/sec. The most likely explanation for this lack of slowing is that at 6 weeks of denervation there still remains a substantial population of fibers that contain both fast and slow myosin and are presumably undergoing conversion from fast to slow. Indeed co-existence of type I and IIA myosin heavy chain was seen in 6 week denervated soleus muscles (Fig. 14). A fiber that contains both slow and fast myosin will contract faster than one that is homogeneous for slow myosin. Since the slack test method of determining velocity will measure the velocity of the fastest fibers, despite the increase in slow myosin that was seen in denervated SOL, a faster Vus will be anticipated because of the presence of hybrid fibers. A more appropriate way of measuring velocity in this case would have been the variable

load method where the velocity at fixed loads is determined and the unloaded velocity (V_{max}) is estimated by extrapolation. Using this technique there is no velocity bias and a weighted average of the contribution by all the crossbridges would be obtained. Recently Asmussen and Marechal (1989) compared the values of V_{us} and V_{max} on the soleus muscles from mice, rats, and guinea-pigs. They found that V_{us} was equal to V_{max} only in the guinea-pig soleus, which is exclusively composed of type I fibers. As the fiber-type distribution became more heterogeneous, the disparity between V_{us} and V_{max} increased. Their value for V_{us} of mouse SOL was 6.11 fiber lengths (FL)/sec. At first this value seems to be considerably higher than my value of 3.42 muscle lengths (ML)/sec (Table VII). The discrepancy can be resolved by the fact that their measure of fiber length was obtained on an average of a few superficial fibers (ave. length, 6.84 mm) while I determined the actual muscle length (distance from lever attachment to wire loop attachment, 11.0 mm). Since muscle fiber length can be considerably less than whole muscle length owing to the fact that the geometrical arrangement of muscle fibers is not parallel to the long axis of the muscle, when velocity is normalised to fiber length a higher value would be anticipated. They reported a fiber length to muscle length ratio of 0.68. If this ratio is applied to my value of 3.42 ML/sec then the velocity becomes 5.03 FL/sec, a value that is close to their value of 6.11

FL/sec. With respect to not having measured V_{max} in my experiments unfortunately I was not technically equipped to carry out these measurements and therefore I am unable to provide these data.

Despite the lack of direct evidence showing a reduction in V_{us} of 6 week denervated SOL other data would suggest that 6 week denervated SOL was indeed slower. In this regard the further increase in TTP that was seen in 6 week denervated SOL muscles compared to 10 days denervated (Tables V, VII) is likely a reflection of the increase in slow myosin containing fibers. In denervated EDL muscles the TTP of 10 day denervated muscles did not differ significantly from that of 6 week denervated muscles, nor was there any slow myosin present. However in 6 week TTX inactivated EDL and SOL muscles there was an increase in TTP as well as an increase in slow myosin. The increase in slow myosin containing fibers in TTX inactivated EDL is of considerable interest since no such observation was made in any of the other experimental groups. The co-existence of type IIA and type I myosin in TTX inactivated EDL (Fig. 13) is strongly suggestive of a conversion of IIA to type I fibers especially since control EDL contained no type I fibers. The observation that TTX treatment and not denervation caused an increase in type I fibers in EDL muscles is strong evidence in favor of myotrophic regulation of slow myosin synthesis. In this case activity may negate the actions of the myotrophic factor in

the intact system thus accounting for the lack of expression of type I myosin in normal EDL. The picture however becomes more complex when one considers the case of the SOL muscle. In this case denervation was a more potent stimulus for IIA to I conversion. Nonetheless a significant proportion of the IIA to I conversion in SOL could also be precipitated by TTX-induced paralysis thus indicating that the SOL muscle has the capacity for myotrophic regulation of type I myosin expression. It appears unlikely however that this form of myotrophic regulation plays a significant role in-vivo since treatment with colchicine cuffs did not result in a reduction in the proportion of fibers containing type I myosin. Interestingly an apparent loss of type I myosin was seen in dy2j SOL muscles (Fig. 15). This is particularly relevant since neuronal involvement in murine dystrophy is well documented (McComas, Sica and Campbell 1971, Bradley and Jaros 1973, Parry 1977).

Activity vs Myotrophic Influences in Regulating Contractile Properties of Skeletal Muscle

Ever since the experiments by Eccles et al in 1960 the original tenet that the physiological properties of skeletal muscle are sustained through the combined influences of activation and myotrophic substances supplied by alpha motorneurons has been placed under intense scrutiny. To date

the bulk of evidence would support the postulate that in terms of functionality ie: contractile and metabolic characteristics, the pattern of activation appears to play a greater role than myotrophic influences since these parameters can be effectively manipulated simply by artificially changing the activation history that is experienced by a muscle when it has been deprived of its innervation.

The results of this thesis do not alter this contention significantly and any evidence for myotrophic regulation is small and of unclear functional significance. Thus the increase in slow myosin containing fibers of TTX inactivated EDL and SOL muscles should not be construed to mean that, physiologically, type I fiber phenotype is myotrophically regulated. Rather this may simply represent the recapitulation of an earlier developmental stage. In this regard it was shown by Hoh, Hughes, Hugh and Pozgaj (1989) that following denervation of the rat tibialis anterior muscle, there was an increase in the number of type I fibers. The total number of type I fibers was found to correspond to the number of primary fibers that were seen during development. It should also be pointed out that the model used in this study to abolish myotrophic influences rests on the assumption that all myotrophic influences are conveyed via fast axoplasmic transport. While this is generally a valid assumption (Younkin, Brett, Davey and Younkin 1978) the possibility of

neuronal factors conveyed by other means cannot be rigorously excluded.

From a teleological point of view it is not surprising that activity may play a more important role in regulating the type of contractile machinery or metabolic apparatus of skeletal muscle. After all, the overall physiological demands of a muscle will depend on how often it is activated, the load characteristics imposed on it as well as the type of movement performed. It would therefore make good sense from a homeostatic standpoint that the apparatus needed to achieve such an end be regulated in a large part by the variables it influences. Indeed as it turns out, muscle tends to adapt itself to altered activity patterns and load demands in such a way as to handle the perturbation in a more efficient manner. It is not clear how activity may modulate gene expression in terms of myosin isoforms. However there is experimental evidence that activity acts via calcium, calmodulin and cyclic nucleotides to regulate the biosynthesis of TTX-sensitive Na^+ channels (Sherman, Chrivia and Catterall 1985) and ACh receptor (Betz and Changeux 1974) in muscle cells. Also it was shown that in-vitro myoblast differentiation was facilitated by cAMP (Curtis and Zalin 1981). Sreter's group have observed that changes in myosin heavy chain expression are preceded by a transient increase in intracellular Ca^{2+} levels (Sreter, Lopez, Alamo, Mabuchi and Gergely 1987). Thus a Ca^{2+} mediated pathway perhaps

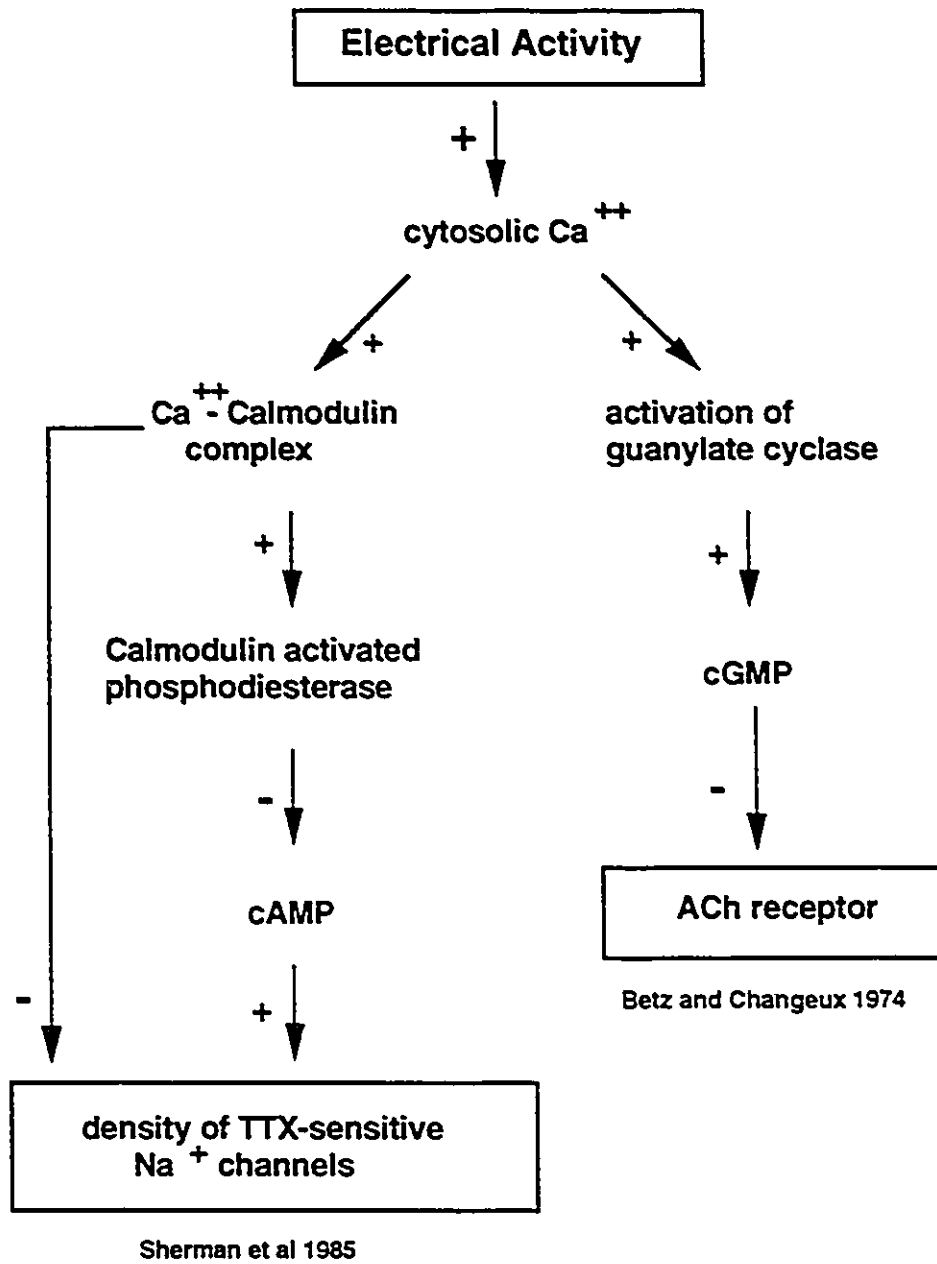


Figure 19.

involving cyclic nucleotides may exist for the modulation of myosin heavy chain expression. Fig. 19 depicts two hypothetical schemes of how activity may regulate the density of TTX-sensitive Na⁺ channels and endplate ACh receptors. Clearly more work is necessary in order to determine how this scheme relates to modulation of myosin heavy chain expression.

One may ask, what possible role could myotrophic influences play in the normal regulation of muscle phenotype? It is known for example that some of the denervation induced alterations in membrane properties can be reproduced by blocking fast axoplasmic transport. Perhaps the post-synaptic membrane is in part physiologically regulated by myotrophic influences. An attractive possibility is that some of these substances may play a role in target recognition subsequent to injury. Thus alpha motorneurons would recognise muscle fibers and reinnervate them while Ia sensory afferents would recognise for example muscle spindles and would target them. This indeed does occur. The mechanism could involve an retrograde recognition system in fine tuning with an anterograde repair system. In this regard it would be of considerable didactic value to examine the specificity of reinnervation of a mixed target area whose innervation has been deprived of fast axoplasmic transport.

Evidence for trophic mediation in regeneration of the CNS is extensive (Hefti 1986, Korsching 1986, Cuello, Pioro, Maysinger, Garofalo and Tagari 1988). It is therefore not

unreasonable to propose a parallel role in the case of PNS regeneration. There is some evidence that both PNS and CNS regenerative responses may in part be enhanced by the administration of the monoganglioside GM1 (Gorio, Marini and Zanoni 1983, Cuello, Garofalo, Kenigsberg and Maysinger 1989). A common mechanism may involve the participation of a trophic factor such as nerve growth factor (NGF). In that regard it has been shown that GM1 potentiates the ameliorative effects of NGF in CNS regeneration (Cuello et al 1989). Perhaps a PNS equivalent nerve-derived factor may interact with GM1 to mediate the enhanced sprouting that is seen following administration of GM1 to a denervated in-vivo preparation.

Unfortunately at this stage any discussion of myotrophic mediators can only be speculative. More work must be done in order to be able to ascribe a clear causal involvement of myotrophic factors in skeletal muscle function.

To the extent that the knowledge of myotrophic regulation of skeletal muscle properties remains fragmentary, the opposite is true of the role of activity in determining muscle phenotype. Effective transformation of skeletal muscle contractile properties can be achieved artificially by altering the pattern of activation experienced by the muscle. The results of this thesis would suggest that a very short period of time is necessary to transform the velocity characteristics of fast muscle. Also from the TTX experiments it appears that activity is the important variable for

velocity control. This may have implications in the field of sports medicine where the maximal speed of shortening could be rapidly altered by changing activity protocols. It would be of considerable value to determine the optimal stimulation paradigm for maximal LC3f expression as well as the temporal constraints embodied therein. One could easily envisage training tasks in which activation of desired muscle groups could be temporally entrained to the optimal stimulation paradigm. An athlete may thus be able to achieve maximal muscle speed within a very short period of time.

Electrostimulation has been of benefit in therapeutic circumstances. In cases of proximal peripheral nerve injury where months may pass before the regenerating neurons reach their targets it would be of considerable benefit to maintain the contractile properties of the muscles concerned. Also electrostimulation at the optimal stimulation paradigm has been found to be useful in sparing some of the disuse atrophy that would normally occur, thus preserving strength.

Just because very little evidence exists for myotrophic regulation of skeletal muscle properties it does not mean that its potential role in normal muscle homeostasis should be downplayed. Thus research in this area should not be considered futile. Indeed many of the neuromuscular disorders whose etiology eludes us now may ultimately turn out to represent a primary disruption in the neuronomyotrophic axis.

REFERENCES

Al-Amood W.S. and D.M. Lewis. A comparison of the effects of denervation on the mechanical properties of rat and guinea-pig skeletal muscle.

J. Physiol. 414: 1-16, 1989.

Albuquerque E.X., Warnick J.E., Tasse J.R. and F.M. Sansone. Effects of vinblastine and colchicine on neural regulation of the fast and slow skeletal muscles of the rat.

Expt. Neurol. 37: 607-634, 1972.

Albuquerque E.X., Schuh F.T. and F.C. Kaufman. Early membrane depolarisation of the fast mammalian muscle after denervation.

Pflugers Arch. 328: 36-50, 1971.

Albuquerque E.X., Warnick J.E., Sansone F.M. and R. Onur. The effects of vinblastine and colchicine on neuronal regulation of muscle.

Ann. N.Y. Acad. Sci. 228:224-243, 1974.

Albuquerque E.X. and R.J. McIssac. Fast and slow mammalian muscles after denervation.

Expt. Neurol. 26: 183-202, 1970.

Arancio O., Cangiano A., Magherini P.C. and E. Pasino. Effects of reinnervation with normal and TTX inactive nerves on resting membrane potential of rat skeletal muscle.

Neurosci. Lett. 88: 179-183, 1988.

Armstrong C.M., Bezanilla F.M. and P. Horowicz. Twitches in the presence of ethylene glycol bis (β -aminoethyl ether)-N,N'-tetraacetic acid.

Biochim. Biophys. ACTA 267: 605-608, 1972.

Armstrong R.B. and M.H. Laughlin. Blood flows within and among rat muscles as a function of time during high speed treadmill exercise.

J. Physiol. 344: 189-208, 1983.

Asmussen G. and G. Marechal. Maximal shortening velocities, isomyosins and fiber types in soleus muscle of mice, rats and guinea-pigs.

J. Physiol. 416: 245-254, 1989.

Ausoni S., Gorza L., Schiaffino S., Gunderson K. and T. Lomo. Expression of myosin heavy chain isoforms in stimulated fast and slow rat muscles.

J. of Neurosci. 10(1): 153-160, 1990.

Axelsson J. and S. Thesleff. A study of supersensitivity in denervated muscles after denervation.
J. Physiol. 144: 178-193, 1959

Bambrick L. and T. Gordon. Acetylcholine receptors and sodium channels in denervated and botulinum-toxin-treated adult rat muscle.
J. Physiol. 382: 69-86, 1987.

Bar A. and D. Pette. Three fast myosin heavy chains in adult rat skeletal muscle.
FEBS Lett. 235: (1,2) pp. 153-155, 1988.

Barany M. and R.J. Close. The transformation of myosin in cross-reinnervated rat muscles.
J. Physiol. 213: 455-474, 1971.

Barany M. ATPase activity of myosin correlated with speed of muscle shortening.
J. Gen. Physiol. 50 (suppl pt. 2), 197-218, 1967.

Barton P.J.R., Harris A.J. and M.E. Buckingham. Myosin light chain gene expression in developing and denervated fetal muscle in the mouse.
Development 107: 819-824, 1989.

Bennett H.S. The structure of striated muscle as seen by the electron microscope in: The Structure and Function of Muscle, vol. I G.H. Bourne ed., Acad. Press, N.Y. p. 137-181, 1960

Betz H. and J.P. Changeaux. Regulation of muscle ACh receptor synthesis in-vitro by cyclic nucleotide derivatives.
Nature 278: 749-752, 1979.

Billeter R., Heizmann C.W., Howald H. and E. Jenny. Analysis of myosin light and heavy chains in single human skeletal muscle fibers.
Eur. J. Biochem. 116: 389-395, 1981.

Bisby M.A. Inhibition of axonal transport in nerves chronically treated with local anesthetics.
Exp. Neurol. 47: 481-489, 1975.

Biscoe T.J. and A. Taylor. The effect of fast and slow muscle in determining the form of the muscle twitch.
Med. and Biol. Engineering 5: 473-479, 1967.

Bodensteiner J.B. and A.G. Engel. Intracellular calcium accumulation in duchenne dystrophy and other myopathies: A study of 567,000 muscle fibers in 114 biopsies.
Neurology 28: 439-446, 1978.

Borisy G.G. and E.W. Taylor. The mechanism of action of colchicine.

J. Cell Biol. 34: 525-533, 1967.

Bradley W.G. and E. Jaros. Axoplasmic flow in axonal neuropathies II: Axoplasmic flow in mice with motor neuron disease and muscular dystrophy.

Brain 96: 247-258, 1973.

Bradley W.G. and M. Jenkison. Abnormalities of peripheral nerve in murine muscular dystrophy.

J. Neurol. Sci. 18: 227, 1974.

Brimijoin S. and P. Schreiber. Reduced axonal transport of 10S AChE in dystrophic mice.

Muscle and Nerve 5: 405-410, 1982.

Brooke M.H. and K.K. Kaiser. Three myosin ATPase systems: The nature of their pH lability and sulfhydryl dependence.

J. Histochem. Cytochem. 18: 670-672, 1970.

Brown M.C., Jansen J.K. and D. VanEssen. Polyneuronal innervation of skeletal muscle in newborn rats and its elimination during maturation.

J. Physiol. 261: 387-422, 1976.

Buller A.J., Eccles J.C. and R.M. Eccles. Interactions between motoneurons and muscles in respect of their characteristic speeds of their responses.

J. Physiol. 150: 417-439, 1960.

Buller A.J. and R. Pope. Plasticity in mammalian skeletal muscle.

Philosoph. Trans. R. Soc. Ser. B. 278: 295-305, 1977.

Burke R.E., Levine D.N., Tsairis P. and F.E. Zajac. Physiological types and histochemical profiles in motor units of the cat gastrocnemius.

J. Physiol. 234: 723-748, 1973.

Burke R.E., Strick P.L., Kanda K., Kim C.C. and B. Walmsley. Anatomy of medial gastrocnemius and soleus motor nuclei in the cat spinal cord.

J. Neurophysiol. 40: 667-680, 1977.

Burke R.E. and P. Tsairis. The correlation of physiological properties with histochemical characteristics in single muscle units.

Ann. N.Y. Acad. Sci. 228: 145-159, 1974.

Butler-Browne G.S., Bugaisky L.B., Cuenoud S., Schwartz K. and R.G. Whalen. Denervation of newborn rat muscles does not block the appearance of adult fast myosin heavy chain. Nature 299: 830-833, 1982.

Butler-Browne G.S., Herlicoviez D. and R.G. Whalen. Effects of hypothyroidism on myosin isozyme transitions in developing rat muscle. FEBS Lett. 166: 71-75, 1984.

Cangiano A. Acetylcholine supersensitivity: the role of trophic factors. Brain Res. 58: 255-259, 1973.

Cangiano A. and L. Lutzemberger. Partial denervation affects both denervated and innervated fibers in the mammalian skeletal muscle. Science 196: 542-545, 1977.

Carraro U. and C. Catani. A sensitive SDS-PAGE method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. Biochem. Biophys. Res. Comm. 116: 793-802, 1983.

Chandler W.K., Rakowski R.F. and M.F. Schneider. A non-linear voltage dependent charge movement in frog skeletal muscle. J. Physiol. 254: 245-283, 1976.

Close R. Dynamic properties of fast and slow skeletal muscles of the rat during development. J. Physiol. 173: 74-95, 1964.

Condon K., Silberstein L., Blau H.M. and W.J. Thompson. Development of muscle fiber types in the prenatal rat hindlimb. Develop. Biol. 138: 256-274, 1990a.

Condon K., Silberstein L., Blau H.M. and W.J. Thompson. Differentiation of fiber types in aneural musculature of the prenatal rat hindlimb. Develop. Biol. 138: 275-295, 1990b.

Crow M.T. and F.A. Stockdale. Myosin expression and specialization among earliest muscle fibers of the developing avian limb. Dev. Biol. 113: 238-254,

Curtis D.H. and R. Zalin. Regulation of muscle differentiation: stimulation of myoblast fusion in-vitro by catecholamines. Science 214: 1355-1357, 1981.

Czeh G., Gallego R., Kudo N. and M. Kuno. Evidence for the maintenance of motorneuron properties by muscle activity. J. Physiol. 281: 239-252, 1978.

Davis H.L. Myotrophic effects on denervation atrophy of hindlimb muscles of mice with systemic administration of nerve extract. Brain Res. 343: 176-179, 1985.

Davis H.L., Bressler B.H. and L.G. Jasch. Myotrophic effects of denervated fast-twitch muscles of mice: Correlation of physiological, biochemical and morphological findings. Expt. Neurol. 99: 474-489, 1988.

Davis H.L. and J.A. Kiernan. The effect of nerve extract on atrophy of denervated or immobilised muscle. Exp. Neurol. 72: 582-591, 1981.

Davis R.L., Weintraub H. and A.B. Lassar. Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell 51: 987-1000, 1987.

Desypris G. Characteristics of reinnervation in control (C57BL/6J) and dystrophic (dy2j/dy2j) soleus muscles. Masters Thesis, 1986.

Dhoot G.K. Selective synthesis and degradation of slow skeletal muscle myosin heavy chain in developing muscle fibers. Muscle and Nerve 9: 155-164, 1986.

Donselaar Y., Eerbeek O., Kernell D. and B.A. Verhey. Fiber sizes and histochemical staining characteristics in normal and chronically stimulated fast muscle of the cat. J. Physiol. 382: 237-254, 1987.

Drachmann D.B. Is ACh the trophic neuromuscular transmitter? Arch. Neurol. 17: 206-218, 1967.

Dubowicz V. Reciprocal relationship of phosphorylase and oxidative enzymes in skeletal muscle. Nature 185: 701-704, 1960.

Dum R.P., O'Donovan M.J., Toop J. and R.E. Burke. Cross-reinnervated motor units in cat muscle I: Flexor digitorum longus reinnervated by soleus motoneurons. J. Neurophysiol. 54: 818-836, 1985.

Ebashi S. EC coupling. Ann. Rev. Physiol. 38: 293-313, 1976.

Eccles J.C., Eccles R.M. and W. Kozak. Further investigations on the influence of motoneurons on the speed of muscle contraction.

J. Physiol. 163:324-339, 1962.

Eccles J.C., Eccles R.M. and A. Lundberg. The action potentials of the alpha motoneurons supplying fast and slow muscles.

J. Physiol. 142: 275-291, 1958.

Eccles J.C. Investigations on muscle atrophies arising from disuse and tenotomy.

J. Physiol. 103: 253-266, 1944.

Ecob M.S., Butler-Browne G.S. and R.G. Whalen. The adult fast isozyme of myosin is present in a nerve-muscle tissue culture system.

Differentiation 25: 84-87, 1983.

Edman K.A.P. The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibers.

J. Physiol. 291: 143-159, 1979.

Edmondson D.G. and E.N. Olson. A gene with homology to the myc similarity region of MyoD1 is expressed during myogenesis and is sufficient to activate the muscle differentiation programme.

Genes and Dev. 3: 628-640, 1989.

Edstrom L. and E. Kugelberg. Histochemical composition distribution of fibers and fatiguability of single motor units.

J. Neuro. Neurosurg. Psychiat. 31: 424-433, 1968.

Eerbeek O., Kernell D. and B.A. Verhey. Effects of fast and slow patterns of tonic long-term stimulation on contractile properties of fast muscle in the cat.

J. Physiol. 352: 73-90, 1984.

Eisenberg R.S. and P.W. Gage. Frog skeletal muscle fibers. Changes in electrical properties after disruption of T-tubular system.

Science 158: 1700-1701, 1967.

Endo M. Calcium release from the sarcoplasmic reticulum.

Physiol. Rev. 57: 71-108, 1977.

Fambrough D.M. Acetylcholine receptor: revised estimates of extrajunctional receptor density in denervated rat diaphragm.

J. Gen. Physiol. 64: 468-472, 1974.

Fanburg B.L., Drachman D.B., Moll D. and S.I. Roth. Calcium transport in isolated sarcoplasmic reticulum during muscle maturation.

Nature 218: 962-964, 1968.

Fatt P. and B. Katz. Spontaneous subthreshold activity at motor nerve endings.

J. Physiol. 117: 109, 1952.

Fernandez H.L. and J.A. Donoso. Nerve muscle cell trophic communication. Chapter 1, CRC Press, Boca Raton, 1987.

Fischback G.D. and N. Robbins. Changes in contractile properties of disused soleus muscles.

J. Physiol. 201: 305-320, 1969.

Fischback G.D. and N. Robbins. Effect of chronic disuse of rat soleus neuromuscular junctions on post-synaptic membrane.

J. Neurophysiol. 34: 562-569, 1971.

Foehring R.C., Sybert G.W. and J.B. Munson. Motor unit properties following cross-reinnervation of cat lateral gastrocnemius and soleus muscles with medial gastrocnemius nerve. II: Influence of muscle on motoneurons.

J. Neurophysiol. 57: 1227-1245, 1987.

Franzini-Armstrong C. Membrane systems in muscle fibers In: The structure and function of muscle. ed: G.H. Bourne 2: 531-619, 1973.

Gambke B., Lyons G., Haselgrove J., Kelly A.M. and N.A. Rubinstein. Thyroidal and neural control of myosin specialization in developing rat fast and slow muscles.

FEBS Lett. 156: 335-340, 1983.

Gauthier G.F. and S. Lowey. Distribution of myosin isoenzymes among skeletal muscle fiber types

J. Cell Biol. 81: 10-25, 1979.

Gerday V. and J.M. Gillis. The possible role of parvalbumins in the control of contraction.

J. Physiol. 258: 96P-97P, 1976.

Gillespie M.J., Gordon T. and P.R. Murphy. Motor units and histochemistry in rat lateral gastrocnemius and soleus muscles: Evidence for dissociation of physiological and histochemical properties after reinnervation.

J. Neurophysiol. 57: 921-937, 1987.

Goldberg A.L. Mechanisms of growth and atrophy of skeletal muscle. In: Muscle Biology ed. R.G. Cassens, vol.1:81-118 New York:Dekker, 1972.

Goldring J.M., Kuno M., Nunez R. and J.N. Weakley. Do identical activity patterns in fast and slow motor axons exert the same influence on the twitch time of cat skeletal muscle? J. Physiol. 321: 211-223, 1981.

Goldspink D. The influence of immobilisation and stretch on protein turnover of rat skeletal muscle. J. Physiol. 264: 267-282, 1977.

Gordon T., Thomas C.K., Stein R.B. and S. Erdebil. Comparison of physiological and histochemical properties of motor units after cross-reinnervation of antagonistic muscles in the cat hindlimb. J. of Neurophysiol. 60: #1, 365-378, 1988.

Gorza L. Identification of a novel type 2 population in mammalian skeletal muscle by combined use of histochemical myosin ATPase and anti-myosin monoclonal antibodies. J. of Histochem. Cytochem. 38: 257-265, 1990.

Greaser M.L., Moss R.L. and P.J. Reiser. Variations in contractile properties of rabbit single muscle fibers in relation to troponin T isoforms and myosin light chains. J. Physiol. 406: 85-98, 1988.

Grimby L., Hannerz J. and B. Hedman. Contraction time and voluntary discharge properties of individual short toe extensor motor units in man. J. Physiol. 289: 191-201, 1979.

Guth L., Albers R.W. and W.C. Brown. Quantitative changes in cholinesterase activity of denervated muscle fibers and sole plates. Expt. Neurol. 10: 236-250, 1964.

Guth L. and F.J. Samaha. Procedure for the histochemical demonstration of actomyosin ATPase. Exp. Neurol. 28: 365-367, 1970.

Gutmann E. Neurotrophic Relations. Ann. Rev. Physiol. 38: 177-216, 1976.

Gutmann E. In "The Denervated Muscle". Ed. E. Gutmann, Czechoslovak Acad. Sci. 1962.

Gutmann E. Neurotrophic relations in the regeneration process. Prog. Brain Res. 13: 72-112, 1964.

Gutmann E., Melichna J. and I. Syrový. Contraction properties and ATPase activity in fast and slow muscle of the rat during denervation.

Exp. Neurol. 36: 488-497, 1972.

Hamburger V. Cell death in the development of the lateral motor column of the chick embryo.

J. Comp. Neurol. 160: 535-546, 1975.

Harris A.J., Fitzsimons R.B. and J.C. McEwan. Neural control of the sequence of expression of myosin heavy-chain isoforms in foetal mammalian muscles.

Development 107: 751-769, 1989.

Harris J.B. and S. Thesleff. Nerve stump length and membrane changes in denervated skeletal muscle.

Nature New Biol. 236: 60-61, 1972.

Hartzell H.C. and D. Fambrough. ACh Receptors: Distribution and extrajunctional density in rat diaphragm after denervation correlated with ACh sensitivity.

J. Gen. Physiol. 60: 248-262, 1972.

Heilmann L., Muller W. and D. Pette. Correlation between ultrastructural and functional changes in sarcoplasmic reticulum during chronic stimulation of fast muscle.

J. Membr. Biol. 59: 143-149, 1981.

Heilmann L. and D. Pette. Molecular transformations in sarcoplasmic reticulum of fast-twitch muscle by electrostimulation.

Eur. J. Biochem. 93: 437-446, 1979.

Heizmann C.W., Berchtold M.W. and A.M. Rowlerson. Correlation of parvalbumin concentration with relaxation speed in mammalian muscles.

PNAS (USA) 79: 7243-7247, 1982.

Hennig R. and T. Lomo. Firing patterns of motor units in normal rats.

Nature 314: 164-166, 1985.

Hinckley R.E. and L.S. Green. Effects of halothane and colchicine on microtubules and electrical activity of rabbit vagus nerves.

J. Neurobiol. 2: 97-105, 1971.

Hofmann W.W. and S. Thesleff. Studies on the trophic influence of nerve on skeletal muscle.

Eur. J. Pharm. 20: 256-260, 1972.

Hoh J.F.Y., Hughes S., Hale P.T. and R.B. Fitzsimons. Immunocytochemical and electrophoretic analyses of changes in myosin gene expression in cat limb fast and slow muscles during post natal development.

J. Muscle Res. Cell Motil. 9: 30-47, 1988.

Hoh J.F.Y., Hughes S., Hugh G. and I. Pozgaj. Three hierarchies in skeletal muscle fiber classification: allotype, isotype and phenotype. in Cellular and Molecular Biology of Muscle Development. eds. L.H. Kedes and F.E. Stockdale, A.R. Liss N.Y. 1989.

Hudlicka O., Tyler L.R., Srihari T., Heilig A. and D. Pette. The effect of different patterns of long-term stimulation on contractile properties and myosin light-chains in rabbit fast muscles.

Pflugers Arch. 393: 164-170, 1982.

Izumo S., Nadal-Ginard B. and V. Mahdavi. All members of the MNC multigene family respond to thyroid hormone in a highly tissue specific manner.

Science 231: 597-600, 1986.

Jablecki C. and S. Brimijoin. Reduced axonal transport of ChAT in dystrophic mice.

Nature 250: 151-154, 1974.

Jaenicke T., Martindale J., Loughna P.T., Chang K.C., Williams P. and G. Goldspink. Mechanical signals involved in the expression of the slow type genes and the determination of muscle fiber-phenotype in the mammalian soleus.

J. of Muscle Res. and Cell Motil. 12: 80, 1991.

Jaspers S.R. and M.E. Tischler. Atrophy and growth failure of rat hindlimb muscles in tail-cast suspension.

J. Appl. Physiol. 57: 1472-1479, 1984.

Jolesz F. and F.A. Sreter. Development, innervation and activity-pattern induced changes in skeletal muscle.

Ann. Rev. Physiol. 43: 531-552, 1981.

Jones R. and G. Vrbova. Two factors responsible for the development of denervation supersensitivity.

J. Physiol. 236: 517-538, 1974.

Kauffman F.C., Warnick J.E. and E.X. Albuquerque. Uptake of ³H colchicine from silastic implants by mammalian nerves and muscles.

Expt. Neurol. 44: 404-416, 1974.

Kelly A.M. and N.A. Rubinstein. Why are fetal muscles slow? Nature 288: 266-269, 1980.

Kernell D., Eerbeek O., Verhey B.A. and Y. Donselaar. Effects of physiological amounts of high and low-rate chronic stimulation on fast-twitch muscle of the cat hindlimb.

J. Neurophys. 58: 598-613, 1987.

Kimelman D. and M. Kirschner. Synergistic induction of mesoderm by FGF and TGF- β and the identification of an mRNA coding for FGF in the early xenopus embryo.

Cell 51: 869-877, 1987.

Kugelberg E. and B. Lindegren. Transmission and contraction fatigue of rat motor units in relation to SDH activity of motor unit fibers.

J. Physiol. 288: 285-300, 1979.

Lapointe M.A. and P.F. Gardiner. Effects of TTX induced disuse on properties of developing rat gastrocnemius muscle.

Can. J. Physiol. Pharmacol. 62: 1106-1111, 1984.

Lasek R. Axoplasmic transport in cat dorsal root ganglion cells as studied with ^3H Leucine.

Brain Res. 7: 360-377, 1968.

Lavoie P.A., Collier B. and A. Tenenhouse. Comparison of alpha bungarotoxin binding of skeletal muscles after inactivity or denervation.

Nature 260: 349-350, 1976.

Lavoie P.A., Collier B. and A. Tenenhouse. Role of skeletal muscle activity in the control of muscle acetylcholine sensitivity.

Exp. Neurol. 54: 148-171, 1977.

Lawrie R.A. Biochemical differences in red and white muscle.

Nature 170: 122-123, 1952.

Lewis D.M. The effect of denervation on the mechanical and electrical responses of fast and slow mammalian twitch muscle.

J. Physiol. 222: 51-75, 1972.

Lewis D.M., Robinson A.J. and N.R. Tufft. Fibrillation in the denervated skeletal muscle of the anesthetised rat and guinea-pig.

J. Physiol. 403: 69P, 1988.

Liddell E.G.T. and C.S. Sherrington. Recruitment and some other factors of reflex inhibition.

Proc. Royal Soc. B97: 488-518, 1925.

Lomo T. and J. Rosenthal. Control of ACh sensitivity by muscle activity in the rat.

J. Physiol. 221: 493-513, 1972.

Lomo T. and R.H. Westgaard. Further studies on the control of ACh sensitivity by muscle activity in the rat.
J. Physiol. 252: 603-626, 1975.

Luco J.V. and C. Eyzaguirre. Fibrillation and hypersensitivity to ACh in denervated muscle: effect of length of degenerating nerve fibers.
J. Neurophysiol. 18: 65-73, 1955.

Maclagan J. and G. Vrbova. The importance of peripheral changes in determining the sensitivity of striated muscle to depolarising drugs.
J. Physiol. 184: 618-630, 1966.

MacLennan D.H., Duff C., Zorzato F., Fujii J., Philips M., Korneluk R.G., Fordis W., Britt B.A. and R.G. Worton. Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia.
Nature. 343: 559-561, 1990.

McHanwell S. and T.J. Biscoe. The sizes of motoneurons supplying hindlimb muscles in the mouse.
Phil. Trans. R. Soc. Lond. (B)293: 477-508, 1981.

Maier A., Crockett J.L., Simpson D.R., Saubert C.W. and V.R. Edgerton. Properties of immobilised guinea pig hindlimb muscles.
Am. J. Physiol. 231: 1520-1526, 1976.

Markand O.N. and A.N. D'Agostino. Ultrastructural changes in skeletal muscle induced by colchicine.
Arch. Neurol. 24: 72-82, 1971.

Martonosi A. Biochemical and clinical aspects of sarcoplasmic reticulum function.
Curr. Top. Membr. Transp. 3: 83-197, 1972

Martonosi A. and R. Feretos. SRI, The uptake of calcium by SR fragments.
J. Biol. Chem. 239: 648-658, 1964.

Martonosi A., Roufa D., Boland R., Reyes E. and T.W. Tillack. Development of sarcoplasmic reticulum in cultured chicken muscles.
J. Biol. Chem. 252: 318-332, 1977.

McComas A.J., Sica R.E.P. and M.J. Campbell. Sick motoneurons. A unifying concept of muscle disease.
Lancet 1: 321-325, 1971.

McGowan E.B., Shafiq S.A. and A. Stracher. Delayed degeneration of dystrophic and normal muscle cell cultures treated with pepstatin, leupeptin and antipain.
Exp. Neurol. 50: 649-657, 1976.

Michel R.W. and P.F Gardiner. To what extent is hindlimb suspension a model of disuse.
Muscle and Nerve 13: 646-653, 1990.

Miller J.B. and F.E. Stockdale. Deveopmental regulation of the multiple myogenic cell lineages of the avian embryo.
J. Cell Biol. 103: 2197-2208, 1987.

Mommaerts W.F.H.M., Buller A.J. and K. Seraydarian. The modification of some biochemical properties of muscle by cross-innervation.
P.N.A.S. USA 64: 128-133, 1969.

Muntener M., Berchtold M.W. and C.W. Heizmann. Parvalbumin in cross-reinnervated and denervated muscles.
Muscle and Nerve 8: 132-137, 1985.

Narusawa M., Fitzsimons R.B., Izumo S., Nadal-Ginard B., N.A. Rubinstein and A.M. Kelly. Slow myosin in developing rat skeletal muscle.
J. of Cell Biol. 104: 447-459, 1987.

Navarette R. and G. Vrbova. Changes of activity patterns in slow and fast muscles during postnatal development.
Dev. Brain Res. 8: 11-19, 1983.

Nemeth P., Pette D. and G. Vrbova. Comparison of enzyme activities among single muscle fibers within defined motor units.
J. Physiol. 311: 489-495, 1981.

Ochs S. Axoplasmic transport energy metabolism and mechanisms. In, The Peripheral Nervous System. (Hubbard J.I. ed.) Plenum Press, N.Y. pp. 47-67, 1974.

Ochs S. and N. Ranish. Characteristics of the fast transport system in mammalian nerve fibers.
J. Neurobiol. 1: 247-261, 1969.

Ontell M. and K. Kozeka. The organogenesis of murine striated muscle: A cytoarchitectural study.
Am J. Anat. 171: 133-148, 1984.

Oppenheim R.W. and WuChuway. Aspects of naturally occurring motoneuron death in the chick spinal cord during embryonic development In: Somatic and autonomic nerve-muscle interactions. Elsevier/North Holland, Amsterdam, 57-109, 1983.

Padykula H.A. and E. Herman. The specificity of the histochemical method for adenosine triphosphatase. J. Histochem. Cytochem. 3: 170-195, 1955.

Parry D.J. A study of functional denervation in fast and slow muscles of dystrophic mice of various ages. Exp. Neurol. 55: 556-566, 1977.

Parry D.J. and G. Desypris. Slowing of the twitch of dystrophic mouse is partially due to altered activity pattern. Muscle and Nerve 6: 397-407, 1983.

Parry D.J. and D. Zardini. Characteristics of IIX fibers in mouse muscles. In "The Dynamic State of Muscle Fibers" ed. D.Pette. DeGruyter, Berlin. 343-354, 1990.

Pearce G.W. Electron microscopy in the study of muscular dystrophy. Ann. N.Y. Acad. Sci. 138: 138-150, 1966.

Pette D., Muller W., Leisner E. and G. Vrbova. Time dependent effects on contractile properties, fiber population, myosin light chains, and enzymes of energy metabolism in intermittently and continuously stimulated fast-twitch muscles of the rabbit. Pflugers Arch. Eur. J. Physiol. 364: 103-112, 1976.

Pette D. Metabolic heterogeneity of muscle fibers. J. Exp. Biol. 115: 179-189, 1985.

Pette D. and C. Spamer. Metabolic properties of muscle fibers. Fed. Proc. 45: 2910-2914, 1986.

Phillips W.D., Everett A.W. and M.R. Bennett. The role of innervation in the establishment of the topographic distribution of 1° myotube types during development. J. Neurocytol. 15: 397-405, 1986.

Pierobon-Bormioli S., Sartore S., Dalla Libera L., Vitadello M. and S. Schiaffino. Fast isozymes and fiber types in mammalian skeletal muscle. J. Histochem. Cytochem. 29: 1179-1188, 1981.

Pincon-Raymond M., Rieger F., Fosset M. and M. Lazdunski. Abnormal transverse tubular system and abnormal amount of receptors for Ca^{++} channel inhibition of the dihydropyridine family in skeletal muscle from mice with embryonic muscular dysgenesis. Develop. Biol. 112: 458-466, 1985.

Pinney D.F., Pearson-White S.H., Konieczny S.F., Latham K.E. and C.P. Emerson. Myogenic lineage determination and differentiation: evidence for a regulatory gene pathway. Cell 53: 781-793, 1988.

Porter K.R. and G.E. Palade. Studies on the endoplasmic reticulum III. It's function and distribution in striated skeletal muscle. J. Biophys. Biochem. Cytol. 3: 269-300, 1957.

Publicover S.J., Duncan C.J. and J.L. Smith. The use of A23187 to demonstrate the role of intracellular calcium in causing ultrastructural damage in mammalian muscle. J. Neuropathol. Exp. Neurol. 37: 544-557, 1978.

Ramirez B. Axonal transport blockade and denervation have qualitatively different effects on skeletal muscle metabolism. J. of Neurobiol. 15: 119-126, 1984.

Ranvier L. De quelques traits relatifs a l'histologie et a la physiologie des muscles stries. Archs. Physiol. Norm. Pathol. 6: 1-15, 1874.

Rasminsky M. Ectopic generation of impulses and cross-talk in spinal roots of dystrophic mice. Ann. Neurol. 3: 351, 1978.

Reddy M.K., Etlinger J.D., Rabinowitz M., Fischman D.A. and R.Zak. Removal of Z-lines and α -actinin from isolated myofibrils by a calcium activated neutral protease. J. Biol. Chem. 250: 4278-4284, 1975.

Redenbach D.M. and B.H. Bressler. Alterations in contractile properties of EDL muscle from C57BL/6J mice following denervation at 1 day of age. Expt. Neurol. 100: 542-555, 1988.

Redenbach D.M., Ovalle W.K. and B.H. Bressler. Effect of neonatal denervation on the distribution of fiber types in a mouse fast-twitch skeletal muscle. Histochem. 89: 333-342, 1988.

Redfern P.A. Neuromuscular transmission in newborn rats. J. Physiol. 209: 701-709, 1970.

Reiser P.J., Moss R.L., Giulian G.G. and M.L. Greaser. Shortening velocity in single fibers from adult rabbit soleus muscles is correlated with myosin heavy chain composition. Journal of Biological Chemistry 260: 9077-9080, 1985.

Ridgeway E.B. and C.C. Ashley. Calcium transients in single muscle fibers.

Biochem. Biophys. Res. Comm. 29. 229-234, 1967.

Robert E.O. and Y.T. Oester. Absence of supersensitivity to acetylcholine in innervated muscle subjected to prolonged pharmacologic block.

J. Pharm. Exp. Ther. 174: 133-140, 1970.

Roy R.R., Sacks R.D., Baldwin K.M., Short M. and V.R. Edgerton. Interrelationship of CT, Vmax and myosin ATPase after spinal transection.

J. Appl. Physiol. 56: 1594-1601, 1984.

St.Pierre D. and P.F. Gardiner. Effect of disuse on mammalian fast-twitch muscle: joint fixation compared with neurally applied tetrodotoxin.

Expt. Neurol. 90: 635-651, 1985.

Salmons S. and F.A. Sreter. Significance of impulse activity in the transformation of skeletal muscle type.

Nature 263: 30-34, 1976.

Salmons S. and G. Vrbova. Changes in the speed of mammalian fast muscle following long term stimulation.

J. Physiol. 192: 39-50, 1967.

Salmons S. and G. Vrbova. The influence of activity on some contractile characteristics of mammalian fast and slow muscles.

J. Physiol. 201: 535-549, 1969.

Sarzala M., Zubrycka E. and M. Michalak. Changes in the structure, composition and function of SR membrane during development.

Eur. J. Biochem. 57: 25-34, 1975.

Schiaffino S.L., Saggin L., Viel A., Ansoni S., Sartore S. and L. Gorza. Muscle fiber types identified by mAbs to myosin heavy chains. In: Biochemical Aspects of Physical Exercise, Eds. G. Benzi, L. Packer, N. Siliprandi, pp. 27-34, Elsevier, Amsterdam 1986.

Slack J.M.W., Darlington B.G., Heath J.K. and S.F. Godsave. Mesoderm induction in early Xenopus embryos by heparin-binding growth factors.

Science 326: 197-200, 1987.

Smith B.H. and G.W. Kreutzberg. Neuron-target cell interactions.

Neurosci. Res. Prog. Bull. 14: 222, 1976.

Sola O.M., Christensen D.L. and A.W. Martin. Hypertrophy and hyperplasia of adult chicken anterior latissimus dorsi muscles following stretch with and without denervation.
Exp. Neurol. 41: 76-100, 1973.

Sola O.M. and A.W. Martin. Denervation hypertrophy and atrophy of the hemidiaphragm of the rat.
Am. J. of Physiol. 172: 324-332, 1953.

Spector S. Effects of elimination of activity on contractile and histochemical properties of rat soleus muscle.
J. Neurosci. 5: 2177-2188, 1985.

Sreter F.A., Lopez J.R., Alamo L., Mabuchi K. and J. Gergely. Changes in intracellular ionized calcium concentration associated with muscle fiber type transformation.
Am. J. Physiol. 253: C296-C300, 1987.

Sreter F.A., Luff A.R. and J. Gergely. Effect of cross-reinnervation on physiological parameters and on properties of myosin and SR of fast and slow muscles of the rabbit.
J. Gen. Physiol. 66: 811-821, 1975.

Sreter F.A., Pinter K., Jolesz F. and K. Mabuchi. Fast to slow transformation of fast muscles in response to long-term phasic stimulation.
Exp. Neurol. 75: 95-102, 1982.

Sreter F.A., Romanul F.C.A., Salmons S. and J. Gergely. The effect of changed activity pattern on some biochemical characteristics of muscle.
Exploratory Concepts in Muscular Dystrophy. ed. T. Milhorat
PP. 338-343, 1974.

Sreter F.A., Salmons S., Romanul F.C.A. and J. Gergely. Synthesis by fast muscle of myosin light-chains characteristic of slow muscle in response to long-term stimulation.
Nature New Biol. 241: 17-19, 1973.

Stein R.B., Gordon T. and J. Shriver. Temperature dependence of mammalian muscle contraction and ATPase activities.
Biophys. J. 40: 97-107, 1982.

Stockdale F.E., Miller J.B., Schafer D.A. and M.T. Crow. Myosins, myotubes and myoblasts origins of fast and slow muscle fibers. in Molecular Biology of Muscle Development pg. 213-223, Alan R. Liss Inc., 1986.

Sweeney H.L., Kushmerick M.J., Mabuchi K., Sreter F.A. and J. Gergely. Myosin alkali light chain and heavy chain variations correlate with altered shortening velocity of isolated skeletal muscle fibers.

J. Biol. Chem. 263: #18, 9034-9039, 1988.

Szent-Gjorgyi A. Chemical physiology of contraction in, Body and Heart muscles.

Acad. Press. N.Y. 1953.

Tabary J.C., Tabary C., Tardieu C., Tardieu G. and G. Goldspink. Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts.

J. Physiol. 224: 231-244, 1972.

Tanabe T., Beam K.G., Powell J.A. and S. Numa. Restoration of EC-coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA.

Nature 336: 134-139, 1988.

Tanabe T., Beam K.G., Adams B.A., Niidome T. and S. Numa. Regions of the skeletal muscle dihydropyridine receptor critical for excitation coupling.

Nature 346: 567-572, 1990.

Thesleff S. Spontaneous electrical activity in denervated rat skeletal muscle In: The effect of use and disuse on neuromuscular functions. Eds: E. Gutmann and P. Hnik: 41-51, Czechoslovak Acad. Prague, Czechoslovakia, 1963.

Thesleff S. Supersensitivity of skeletal muscle produced by botulinum toxin.

J. Physiol. 151: 598-607, 1960.

Tiedt T.N., Albuquerque E.X. and L. Guth. Degenerating nerve fiber products do not alter physiological properties of adjacent innervated skeletal muscle fibers.

Science 198: 839-841, 1977.

Tower S.S. Atrophy and degeneration in skeletal muscle.

Am. J. Anat. 56: 1-43, 1935.

Trayer I.P. and S.V. Perry. The myosin of developing skeletal muscle.

Biochem, Z. 345: 87-100, 1966.

Turner P.R., Westwood T., Regen C.M. and R.A. Steinhardt. Increased protein degradation results from elevated free calcium levels found in muscle from mdx mice.

Nature 335: 735-738, 1988.

Unsworth B.R., Witzmann F.A. and F.H. Fitts. A comparison of rat myosin from fast and slow skeletal muscle and the effect of disuse.

J. Biol. Chem. 257: 15129-15136, 1982.

Vergara J. and M. Delay. A transmission delay and the effect of temperature at the triadic junction of skeletal muscle.

Proc. R. Soc. Lond. B 229: 97-110, 1986.

Vivarelli E., Brown W.E., Whalen R.G. and G. Cossu. The expression of slow myosin during mammalian somitogenesis and limb bud differentiation.

J. Cell Biol. 107: 2191-2197, 1988.

Warnick J.E., Albuquerque E.X. and L. Guth. The demonstration of neurotrophic function by application of colchicine or vinblastine to the peripheral nerve.

Expt. Neurol. 57: 622-636, 1977.

Weiss P. and H. Hiscoe. Experiments on the mechanism of nerve growth.

J. Exp. Zool. 107: 315-395, 1948.

Westgaard R.H. and T. Lomo. Control of contractile properties within adaptive ranges by patterns of impulse activity in the rat.

J. Neurosci. 8: 4415-4426, 1988.

Williams P.E. and G. Goldspink. The effect of denervation and dystrophy on the adaptation of sarcomere number in young and adult mice.

J. Anat. 122: 455-465, 1976.

Winegrad S. Intracellular calcium movements of frog skeletal muscle during recovery from tetanus.

J. Gen. Physiol. 51: 65-83, 1968.

Winiarski A.M., Roy R.R., Alford E.K., Chiang P. and V.R. Edgerton. Hindlimb suspension effects on mechanical properties of rat skeletal muscle.

Physiologist 28: 316, 1985.

Wright E.N., Sasson D.A. and V.K. Lin. Myogenin, a factor regulating myogenesis has a domain homologous to MyoD.

Cell 56: 607-617, 1987.

Younkin S.G., Brett R.S., Davey B. and L.H. Younkin. Substances moved by axonal transport and released by nerve stimulation have an innervation-like effect on muscle.

Science 200: 1292-1295, 1978.

Zubrycka E., Michalak M., KoskKosicka D. and M.G. Sarzala.
Properties of microsomal subfractions isolated from developing
rabbit skeletal muscle.

Eur. J. Biochem. 93: 113-121, 1979.

List of Abbreviations

Chemicals

TTX: tetrodotoxin
HRP: horseradish peroxidase
PBS: potassium phosphate buffer
EDTA: ethylenediaminetetracetic acid
BSA: bovine serum albumin
IgG: immunoglobulin G
NaCl: sodium chloride
KCl: potassium chloride
NaHCO₃: sodium bicarbonate
KH₂PO₄: potassium phosphate monobasic
CaCl₂: calcium chloride
MgCl₂: magnesium chloride
NaH₂PO₄:H₂O: sodium phosphate monobasic
Na₂HPO₄: sodium phosphate dibasic
SDS: sodium dodecyl sulphate
ATP: adenosine triphosphate
Tris: tris(hydroxymethyl)aminomethane
DAB: 3'3-diaminobenzidine

Contractile Parameters

TTP: time to peak tension
1/2 RT: time to half-relaxation
Pt: absolute twitch tension

Po: absolute tetanic tension
Pt: twitch tension/ mg wet weight
Po: tetanic tension/ mg wet weight
FIR: functional innervation ratio

Miscellaneous

SR: sarcoplasmic reticulum
SOL: soleus
EDL: extensor digitorum longus
mg: milligrams
kg: kilograms
cm: centimeters
I.D.: inside diameter
O.D.: outside diameter
w/w: weight/weight
w/v: weight/volume
°C: degrees centigrade
V: volts
μg: micrograms
ml: millilitre
mM: millimolar
mmol: millimole
L: litre
Hz: hertz
mm: millimeter