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**LA THÈSE A ÉTÉ
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EFFECTS OF MUSCULAR DYSTROPHY
ON CONTRACTILE AND HISTOCHEMICAL PROPERTIES
OF MOUSE FAST AND SLOW MUSCLE

by

HARRY GLEN PARSLOW

A thesis submitted to the School of Graduate Studies of the University of Ottawa in partial fulfillment of the requirements for the degree of Master in Science in the Department of Physiology, Faculty of Health Sciences.

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ABSTRACT

As the result of an initial observation that fast muscle is slowed in murine muscular dystrophy it was decided to test and extend the hypothesis of Brust that fast muscle is slowed and slow muscle is spared. For this purpose the extensor digitorum longus was chosen as a representative fast muscle. The soleus was chosen as a slow muscle.

The present findings confirm that the extensor digitorum longus at 4 to 6 weeks (young) and at greater than 6 months of age (old) is slowed in both contraction time and one half relaxation time, and this is intensified with age. The old soleus muscle is slowed slightly in one half relaxation time but the young soleus is speeded in both contraction time and one half relaxation time.

Since Brust's hypothesis was for the most part confirmed, an in vivo physiological study was designed to determine whether the cause was due to extrinsic factors or to intrinsic property changes in the fibers themselves. Several properties characteristic of fast and slow muscles were examined for this purpose, as follows. The cooling potentiation/depression and post-tetanic potentiation/depression data showed that the extensor digitorum longus had undergone intrinsic changes towards properties characteristic of slow muscle and the old soleus had changed only slightly from the control. The young soleus speeded. The duration of active state plateau did not change in the dystrophic animals.

A further histochemical study was designed to determine whether a generalized effect on all fiber types or a selective sparing of the slow fibers was involved.

The 3 to 4 week old control soleus was found to contain 26% healthy Type I fibers and 72% healthy Type II oxidative fibers.

The dystrophic soleus suffered a 42% loss of healthy Type II oxidative fibers while only a 28% loss of Type I fibers was sustained, and this was interpreted as a partial sparing of the Type I fiber.

The results are discussed with respect to Brust's hypothesis and its implication regarding currently available information on histochemical/physiological correlations.

CHAPTER. I

INTRODUCTION

1. A brief Historical Review of the Human Muscular Dystrophies

It is known that the progressive muscular dystrophies are caused by defective genetic machinery. The major problem lies in the specific localization of the site of primary expression of the disease.

The first studies of human muscular disease began in the mid 19th century (see Rowland for review 1974). Duchenne dystrophy was originally described by the astute French neurologist of the same name in 1868 and Gowers provided the first detailed account in English in 1879.

In view of the fact that there are some subtle similarities between features of the dystrophies, their classification has historically caused some difficulty. (Reviewed by Walton & Nattrass 1954, Gardner-Medwin, 1974).

Essentially, the most common human dystrophies are-1. the severe sex-linked Duchenne dystrophy 2. the autosomal recessive Limb-Girdle (LG) type 3. the autosomal dominant facioscapulohumeral (FSH) dystrophy and 4. the autosomal dominant myotonic dystrophies.

The classic work of Erb (1883) which is still valid today enumerated the major histopathological criteria common to all dystrophies and which can therefore be used in diagnosis. These were: 1. Rounding of the outline of muscle fibers in transverse

section with hypertrophy of some and atrophy of others. 2. Random variation in fiber size and shape 3. Central migration of sarcolemmal nuclei in many fibers 4. Fiber splitting and 5. intensive infiltration with fat and connective tissue.

Several of these signs were present in the dystrophic mouse tissue which I examined in this study (fat infiltration generally not present) and several served as criteria for classification in my histochemical study. It is of interest that central nucleation is infrequent in Duchenne dystrophy but is very common in myotonic dystrophy and in mouse dystrophy (Pearce & Walton 1963).

It is particularly relevant to this study that the clinical symptoms in man include a selective initial involvement of certain muscles (Walton 1973). For instance, in Duchenne, LG and FSH dystrophy the muscles most often affected in the upper body are the pectorals, serrati, biceps, and brachioradiales while the deltoids are almost always relatively unaffected. In the lower body the quadriceps and or hamstrings and anterior tibial muscles become weak early but gastrocnemius remains strong until later.

Muscular dystrophy has classically been considered a primary myopathy ie a disease originating within the muscle fibers themselves. Since the first studies were made, Meryon (1852) observed that there was no evidence of abnormalities in the nervous system and concluded it must be a disease of the muscle fibers. Duchenne in 1872 confirmed this finding.

Recently, however, the myopathic hypothesis has been challenged with a neural hypothesis (Dubowitz 1969, McComas et al, 1971). The very fact that muscles are selectively involved suggested that a neural component may be to blame. McComas developed the "Sick Motoneurone Hypothesis" whereby motoneurons could exist in the healthy or dead states as well as in the previously overlooked "sick" state. Part of the hypothesis was based on the assumption that in denervation (a neural problem) one would expect the number of motor units to decrease while the average unit size would increase due to collateral sprouting of the remaining intact axons. A motor unit as described by Sherrington (1929) refers to a motoneuron its axon and all of the muscle fibers it innervates. Evidence has recently been provided which indicates that the motor unit is composed of muscle fibers of a single histochemical type. However, McComas reasoned that in a myopathy one could expect a random fiber involvement throughout the muscle cross-section. If this were so, one would expect to observe at first a decrease in mean motor unit size but no decrease in number until such time as the full motor unit were destroyed. Since he found in Duchenne dystrophy that the motor units were decreased but the remaining ones had a normal or slightly smaller number of fibers he postulated that the remaining motoneurons were "sick" since they were unable to incorporate some of the denervated fibers into their units. McComas addressed the possibility that a particular fiber type may be affected and suggested that entire motor units composed of another fiber type were still unaffected. It is thus possible that the disease is primarily caused by a neurological defect which then manifests itself secondarily on the muscle cells causing them to exhibit myopathic features.

Several criticisms have been voiced against this hypothesis.

For instance, dystrophy does not resemble motoneuron disease in that there is no fasciculation nor fiber type grouping and there are differences in serum enzymes, fiber histology (especially the hypertrophy) and in motor unit territory (Rowland 1974). In addition other authors (Ballantyne & Hansen 1974) have been unable to duplicate the finding of decreased motor units in patients with Duchenne dystrophy.

Despite these criticisms the neurogenic hypothesis has revolutionized thinking about dystrophy and is presently at the center of considerable debate.

The experiments discussed in this study have been done on mice and while mouse dystrophy is reportedly the most similar model to human dystrophy, they are not necessarily of the same etiology.

2. Animal Models

Several species of animals are currently being used in research into muscular dystrophy. Asmundson and Julian (1956) reported a progressive dystrophy in domestic chicken which were homozygous for the autosomal recessive gene. Likewise, Homburger et al (1962) discovered a hereditary myopathy in the Syrian hamster and several lines of this autosomal recessive trait now exist. There have also been findings dealing with turkey, mink and Peking duck. (Hadlow 1973 McComas 1977).

However, the animal model that most closely resembles human muscular dystrophy is the house mouse. In 1951 at the R.B. Jackson Memorial Laboratories in Maine a mutation was observed in inbred strain 129 mice which was characterized by progressive ataxia of the axial and limb muscles, atrophy and paralysis. This hereditary myopathy was termed "dystrophia muscularis" and was given the symbol "dy" so that the full description of a dystrophic mouse would be 129/ReJ dy/dy, (Michelson et al 1955). The defect was caused by a single autosomal recessive gene with a maximum expression of 21% due to slight obscuring. In their microscopic study it was revealed that the pathological involvement of muscle tissue was similar to that seen in human dystrophy as described by Erb with the exception of large amounts of fat infiltration. These mice are severely afflicted by the disease, they are unable to breed and have a short life-span unless special precautions are taken (Butler and Cosmos 1977).

In 1969 a second hereditary myopathy was discovered in mouse which is an allele of the dy variant (Meier and Southard 1969). According

to genetic nomenclature rules this was designated "dy^{2j}" and was transferred onto the inbred strain "C57 BL/6J" background. The defect is caused by an autosomal recessive trait and thus the full title for a dystrophic animal is C57 BL/6J dy^{2j}/dy^{2j}. This is the mouse model which was used throughout this study. Despite the fact that the two mutant genes are allelic the disease courses differ in the two types of mouse. The onset of clinical signs is slightly later in the dy^{2j} and both affected males and females can breed. For the purposes of this study it was found adequate to simply select the dystrophic progeny from the heterozygous (dy^{2j}/+) matings. The 2 muscles examined in this study were the extensor digitorum longus (EDL) and the soleus (SOL) which are fast and slow muscles respectively of the hindlimb.

Clinical signs of the disease become evident at about twenty one days of age. There is progressive muscular weakness, principally in the hindlimbs so that the animal eventually moves about by means of its forelimbs. The latter are nonetheless severely affected histopathologically (personal observations). The hindlimbs become rigidly extended laterally, there is a pronounced arching of the back, the fur thins and wasting is obvious as the disease progresses. I have found that these animals can sometimes live to a normal age.

A constant twitching of the hindlimb and pelvic musculature develops which is potentially of importance regarding some of the specific changes that take place within the muscle fibers themselves (refer to discussion).

According to Meier and Southard (1969), muscular dystrophy caused by both the dy and dy^{2j} alleles is similar to Erb's dystrophy in man in a number of clinical, histological and physiological parameters (all of which were not specified) and resembles Duchenne's dystrophy except that it is not sex-linked.

The use of animal models is an extremely important aspect of the work on muscular dystrophy. It has enabled knowledge to proceed at an accelerated rate in both quantity and quality. However, it must be stressed that even the best animal models do not necessarily represent the actual situation seen in man. The animal diseases are however true dystrophies and will undoubtedly prove useful in the long run.

3. Evidence of Fast Muscle Slowing and Slow Muscle Sparing in Mouse Muscular Dystrophy

Brust (1966) studied some aspects of the muscle mechanics of the ReJ129 mouse. He found that a. the percent reduction in weight of the slow SOL muscle is only $\frac{1}{2}$ of that in the fast gastrocnemius, b. dystrophy has no effect on the one half relaxation time ($\frac{1}{2}RT$) of SOL but this parameter doubles in fast gastrocnemius and c. the % reduction of twitch tension in the dystrophic SOL is relatively less than that of the gastrocnemius.

On the basis of these experiments Brust thought his hypothesis of fast muscle slowing and slow muscle sparing may be correct, and supporting evidence has since come from other studies as well.

It should be emphasized immediately however that the two parts of this hypothesis are not necessarily causally related, as will be discussed later. If indeed such a selective involvement exists it would provide valuable information about the etiology of murine muscular dystrophy.

Other data supporting the suggestion that slow muscles are relatively spared have been derived from morphological, electrophysiological, biochemical and mechanical studies as reviewed below.

On the basis of rather poor evidence with lactate dehydrogenase staining Fahimi and Roy (1966) suggested that the "white" fibers were primarily affected in the ReJ129 mouse.

Morphological evidence of Rowe and Goldspink (1968) and Goldspink and Rowe (1969) also furthered the hypothesis since they found SOL spared regarding: muscle weight loss, total fiber numbers, mean fiber

diameters and total fiber cross-sectional area.

An electron microscopic investigation by Shafiq et al (1969) showed that both fast and slow muscles had degenerated but this was very pronounced in some muscles such as gastrocnemius. They also found in the dystrophic extensor digitorum longus (EDL), a fast muscle, that increases in mitochondria and decreases in calcium ion uptake and in sarcoplasmic reticulum caused this muscle to resemble a slow muscle in characteristics.

Moreover, the electrophysiological evidence of Harris (1971) suggested that the resting membrane potential was reduced in both SOL and EDL muscles of the ReJ129 mouse but they were relatively more reduced in the EDL. Likewise, Harris and Montgomery (1975) found a normal action potential generation in dy^{2j} SOL fibers but not in the EDL.

Hoh and Radulovacki (1973) found qualitatively similar changes in the ReJ129 SOL and EDL electrolyte compositions but that SOL was changed relatively less than EDL.

Furthermore Curran and Parry (1975) found that the miniature end plate potentials remained normal in the dy^{2j} SOL but were reduced in the EDL.

In addition Parry (1977) reported that the SOL does show decrements in twitch tension and in functional innervation ratio but only at a later date than does the EDL.

It is of particular interest that there do indeed exist in humans several neuromuscular diseases which attack a specific fiber type selectively. For instance in nemaline myopathy and central core

disease there is a selective loss of Type I fibers (fiber types to be defined later) whereas Type II fiber atrophy is seen as muscle wasting in cachexia, disuse and chronic corticosteroid intoxication. (The effects of this last illness resemble closely some of the features seen in mouse dystrophy and it would be of interest to explore this further).

Since different fiber type (I and II) proportions exist in SOL and gastrocnemius and since it is thought that the different fiber types have different contraction speeds this is relevant to Brust's hypothesis.

In addition, Sica and McComas (1971) observed that patients with LG. and FSH dystrophies exhibit a slowing of both contraction time (CT) and one half relaxation time ($\frac{1}{2}$ RT) of most muscles studied. In addition, in myotonic dystrophy there was a statistically significant increase in the CT and $\frac{1}{2}$ RT of the muscles examined (McComas et al 1971). A similar finding was seen in patients with Duchenne dystrophy (McComas and Thomas 1968). Finally, when bundles of fibers were stimulated in human dystrophics the proportion of bundles having slow contractions is increased and could be correlated with an abnormal preponderance of Type I (slow) fibers (Buchthal et al 1971).

There is, however, other evidence which has completely disagreed with Brust's hypothesis or has straddled the issue.

Working on 1-12 week old ReJ129 muscles it was concluded by Douglas and Baskin (1971) that EDL and SOL are affected chiefly by a reduced ability to exert tension, both muscles being affected to a similar extent. However, other isometric properties such as the passive

length-tension properties and $\frac{1}{2}$ RT indicated the EDL was more severely affected throughout the course of the disease.

Brust has been criticized because it was difficult to duplicate his results since the large size of the gastrocnemius results in the need to trim it surgically which may have contributed to its rapid deterioration in vitro. When smaller EDL and SOL muscles were used no evidence was found for a special fatigue resistance of slow muscle nor that fast muscles were preferentially affected (Hofmann and Ruprecht 1973). However, in agreement with Brust a weaker contraction strength and a longer total twitch time was observed.

The percent reduction of muscle fibers was reported by Montgomery and Swenarchuk (1977) to be greater in dy^{2j} EDL than SOL but this developed a bit later. On the other hand they found the % reduction in the myelinated axons of dystrophic SOL nerves was greater at every age than in the plantaris, another fast muscle.

Finally, the histochemical findings have provided some very controversial evidence. I believe this may be due to the fact that histochemistry is still in its infancy in some ways and its application to disease states can magnify its shortcomings if not used carefully. The implications of this are dealt with in the discussion section.

In summary, if the two part hypothesis of Brust is correct then this could provide some evidence in favour of the neurogenic hypothesis as proposed by McComas et al (1971).

According to McComas entire motor units may be selectively destroyed if the disease is due to a failure at the motoneurone level.

Thus an observed slowing of fast muscle and sparing of slow muscle could be due to a preferential loss of some fast motor units.

However, as previously mentioned the two parts of this hypothesis are not necessarily related causally; there could well be two separate processes involved.

If there is no selective effect on the particular muscle fiber types it is possible that the fibers are lost in a "random, myopathic" fashion. It is also possible in this case however that whole motor units of all fiber types are being knocked out with equal frequency and this would still be consistent with the neurogenic hypothesis since it would imply a primary motoneurone failure.

4. Fast and Slow Muscle Properties

A. Dynamic Physiological Properties

i. Speed of Contraction - Mammalian limb muscles are typically classified as fast or slow depending on their speed of contraction. Ranvier (1874) first described a systematic investigation of the contractions of fast and slow limb muscles and this was extended by Fisher (1908). Ranvier showed that certain red muscles of the rabbit contract more slowly than white muscles but Paukul (1904) showed that slow twitch muscles such as the soleus are always red, but not all red muscles are slow. This was furthered by Denny-Brown (1929) and Hall-Cragg (1968) found that the thyroartenoideus of the rabbit which is a red muscle, contracts very rapidly.

The most obvious difference between fast and slow muscles is the time course of their isometric twitch contractions. In this respect I have considered contraction time (CT) the time from the first deviation of the baseline of the tension-time curve to the summit of the contraction. The half relaxation time ($\frac{1}{2}$ RT) is the time taken to decline from the summit of the curve to $\frac{1}{2}$ its total height. Thus a typical isometric contraction of a fast muscle has a shorter total duration, steeper rate of rise and rate of decline than does the slow muscle. As the temperature of the muscle bath is lowered from body temperature to room temperature (20-25°) both muscle types contract slower but the fast muscle remains relatively faster. The form of the isometric twitch contraction of skeletal muscle is dependent upon the properties of the series elastic component, time course of the active state (AS) and the force-velocity properties of the contractile material (Hill 1949).

While all of the factors controlling the time course of the contraction have not been fully investigated, there is considerable evidence that the activator is calcium ions (Ca^{+2}) released from the sarcoplasmic reticulum (SR). Moreover, it is presumed that the uptake of Ca^{+2} by the SR is intimately involved in the process of relaxation (Ebashi and Endo 1968). It has been shown that fragmented SR of fast muscle can take up Ca^{+2} at a greater rate than that of slow muscle which helps to explain the more rapid time course of the fast muscle (Fiehn and Peter 1971).

Since the time course of AS is different for fast and slow muscles it is likely that the speed of contraction and the rate of rise, duration and rate of decline of AS in a single fiber are coupled in some way (Close 1972).

It is thought that the form of the isometric twitch of a whole muscle can be modified by different admixtures of "fast" and "slow" contracting fibers. Biscoe and Taylor (1967) performed a computerized study which compared the effects of different proportions of these fibers constituting a muscle. They assumed equal twitch amplitudes for fast and slow fibers and that it was valid to add individual components linearly. They found it was only when the mixture contains less than 15% fast muscle fibers that the admixture is not obvious ie there is no inflection in the resultant curve. However curiously proportions as low as 5% slow fibers were detectable. Thus a number of factors enter into the resultant form of the isometric twitch.

The initial observation in this study of fast muscle slowing and little change in slow muscle time parameters served as the impetus

to further investigate the hypothesis of Brust as previously outlined.

If indeed Brust's hypothesis was correct it would be worthwhile to determine whether it was due to intrinsic or extrinsic fiber changes. For this purpose the following three physiological characteristics of fast and slow muscle would be examined: Cooling Potentiation/Depression (CP/CD), Post tetanic Potentiation/Depression (PTP/PTD) and Duration of Active State Plateau (DASP). These are particularly good representative properties of fast and slow muscles because the respective muscle responses occur in opposite directions for these properties. If the dystrophic muscles showed conversions in these properties then they could be considered genuine intrinsic property alterations, and not due to extrinsic factors.

ii. Cooling Potentiation/Cooling Depression (CP/CD)

Fast and slow muscles differ in their response to a change in temperature. Associated with these changes in isometric twitch tension is a slowing of CT and $\frac{1}{2}$ RT in both types of muscle. (Hill 1951, Truog et al 1964, Buller et al 1968, Close and Hoh 1968, Isaacson 1970, Hoh 1974, Ranatunga 1977).

Close and Hoh (1968) examined in vitro and later in vivo (1974) the maximum isometric twitch responses of rat EDL and SOL at temperatures ranging from 35°C to 20°C. They noted that as the temperature was decreased from near body temperature to room temperature there was a twitch potentiation which they termed "cooling potentiation" in the fast EDL by a factor of 1.7 times. In slow SOL, however this procedure

had the effect of slightly decreasing the twitch tension. These properties of fast and slow muscle are under neural control because studies of cross-innervated fast and slow muscle have shown a full interconversion of original fast muscle following innervation by a nerve originally supplying a slow muscle and a lesser conversion in original slow muscle (Buller et al 1968, Hoh 1974).

The cause of the temperature effects have been the subject of some speculation. Several investigators believe that it has to do with the degree of activation of these muscles (Hoh 1974, Ranatunga 1977). It has been proposed that at 37° fast muscle is incompletely activated whereas at room temperature it is more completely activated. This could imply that the lower temperature has an enhancing effect on the mechanisms responsible for activation, and could explain the cooling potentiation of fast muscle.

Slow muscle however, by virtue of its particular intrinsic properties would appear to be more fully activated at 37° than fast muscle since there is only a small tension change as the temperature is lowered.

It is known that fast muscle SR is both more abundant and more efficient at accumulating calcium than is the SR of slow muscle (Fiehn and Peter 1971). If the increase in temperature had the specific effect of enhancing the calcium pump activity, then this could explain the reduction in twitch amplitude seen in fast muscle at 37° . If the calcium ions are removed very rapidly this will reduce the tension generated because the contractile elements will be active for a lesser period of time. Conversely, the SR of slow muscle with its already

limited SR may not show such an effect because even if the calcium pump is enhanced at the higher temperature the rate-limiting step may be simply that too little SR is available to create a perceptible change. Consequently the rates of removal of calcium at the two temperatures may remain the same.

There have been several attempts to link the genesis of this temperature effect with the phenomenon of post tetanic potentiation and post tetanic depression. This effect is also explainable on the basis of changes in the degree of activation.

iii. Post-Tetanic Potentiation/Post-Tetanic Depression

Fast and slow muscles differ considerably in their responses to tetanic stimulation. Repetitive stimulation of mammalian fast muscle leads to a transitory increase in the peak of the isometric twitch tension at 37°C but only a small change at room temperature. This has been termed post-tetanic potentiation (PTP). Conversely, in slow muscle the response is one of no change or a transitory depression which has been termed post-tetanic depression (PTD).

(Brown & von Euler 1938, Bowman et al 1962, Standaert 1964, Close and Hoh 1968, Close and Hoh 1969, Kugelberg 1973, Burke et al 1974, Hoh 1974).

Close and Hoh (1968) have reviewed the rat fast muscle response and have elucidated some of the factors on which the nature of the potentiation is dependent. These are, the number of stimuli in the train, the frequency, the duration, and the time interval until the first post-tetanic twitch. They found for instance that short trains

of stimuli increase the peak tension with no change in the post-tetanic contraction time (CT), but after a prolonged stimulation there are marked changes in the twitch duration. They attributed the former case to an increase in the degree of activation of the individual muscle fibers and the latter, where the number of stimuli was in excess of that required to obtain full potentiation to an increase in the duration of the active state. The PTP is assumed to be maximal soon after the end of stimulus, decays at an exponential rate and returns to approximate pretetanic values in about 3 minutes (Buller et al 1971) but requires about 10 minutes for complete restitution (Brown and von Euler 1938). The tension has been found to settle at about 5% below pretetanus levels on the average.

Slow muscles, on the other hand show a transient depression for about 30 seconds at 37° after a tetanus of 57/sec for 2-4 seconds according to Brown and von Euler (1938) and in 1-3 minutes after a stimulus at 300/sec for 1 second according to Buller et al (1971), Smith (1974). Evidence that PTP and PTD are under neural control comes from the fact that following cross-innervation these properties can be interconverted in EDL but little conversion occurs in soleus.

Several authors have attempted to insure that the potentiation observed is a genuine potentiation of the muscle response itself and is not attributable to recruitment of muscle fibers through facilitation at the neuromuscular junction. Close and Hoh (1969) and Brown and von Euler (1938) noted that after complete curarization that the records for direct stimulation were identical to those obtained by indirect

stimulation prior to curarization. Moreover, Brown and von Euler denervated some preparations and obtained similar responses as when the nerve was intact. Hammond and Ridge (1978) have similar conclusions in their work on snake muscle.

Brown and von Euler noted in 1938 that tetani to soleus of very long duration eg. 20 sec. may actually lead to a potentiation of 160% pretetanic levels and may last for 10 minutes. Standaert (1963 and 1964) and Olson and Swett (1969) have studied this and have found that subsequent to conditioning by a high frequency stimulus, axons of the cat soleus nerve may respond to single stimuli with brief trains of action potentials. This was termed Post-tetanic Repetitive Activity and its occurrence, intensity and duration depends principally on the frequency and duration of the conditioning tetanus. It is generated in the motor nerve terminal and can be transmitted to the muscle to produce a potentiation.

The cause of PTP has been considered by several authors. Close and Hoh (1968), Hoh (1974) and Ranatunga (1977) suggest that fast muscle at 37° is incompletely activated during a twitch and that the after-effects of a tetanus is to induce a more complete activation.

Conversely, slow muscle would appear to be more completely activated at 37° since it does not exhibit PTP. These differences may be partially related to the structural nature of these muscles. If, for instance the fast muscle with its more abundant SR is able to effect relaxation rapidly due to an enhanced performance at 37°, this would limit the duration of the contraction and in so doing, only allow partial expression of its full potential. The effect of the tetanus

then could be to more fully saturate the contractile apparatus with activator (Ca^{+2}) and thereby to some degree mask the rapid re uptake of Ca^{+2} by the SR. This could explain the resultant potentiation which would decay at a rate proportional to the rate of removal of calcium ions from the contractile apparatus.

Presumably at room temperature there is a limited PTP because in the pre-tetanic state the Ca^{+2} re uptake mechanism is already slowed by the lower temperature and the twitch will effectively already be operating with a greater degree of activation. Consequently the tetanus is unable to further increase the twitch tension.

Since in slow muscle potentiation is not seen, the hypothesis, that slow muscle is more completely activated at 37° than is fast muscle is furthered if we assume that potentiation results from a greater activation of the muscle.

iv. Duration of Active State Plateau (DASP) - Skeletal muscle is thought to resemble a contractile component in series with an elastic component. The series elastic component is non-contractile and in order for mechanical force to be developed it must be transmitted through this element first. In an isometric response considerable time is required for the tension to reach its maximum value because the contractile component must shorten and "take up the slack" (Gabel et al 1970) before an external tension can be manifest (Hill 1949). Consequently the isometric response does not directly represent the pure activity of the contractile component divorced from the series elastic

component. Likewise, according to Hill this explains why the same tension is not developed in a twitch as in a tetanus. In tetanus the second stimulus utilizes the pre-stretched condition of the series elastic component in order to develop greater tension. Thus, at least in frog muscle, the initial physical state is the same as that of a tetanus except that its duration is shorter (MacPherson and Wilkie 1954).

A.V.Hill in 1949 defined the intensity of the active state (AS) as the tension which the contractile component would exert if it were neither lengthening nor shortening. (See Reviews Julian and Moss 1976, Brady 1968). This condition could occur for instance at the peak of the isometric twitch where both elastic and contractile elements would be briefly at unchanging length. Thus, the active state curve after a single stimulus is the time course of the isometric twitch response of the contractile material in the absence of the series elastic component. Hill also thought that the time course of the active state starts abruptly, is maintained for a time and then gradually declines.

Several ingenious attempts have been devised to measure various portions of the active state curve. Hill's original method of "quick-stretch" involved suddenly stretching the muscle a fixed amount after stimulation. This had the effect of rapidly stretching out the series elastic component and thereby enabling the observation of the slower development of tension due to the contractile component alone. By using a system of unfused tetani, Edman (1969) was also able to show that the active state is very rapidly engaged after stimulation.

Ritchie and Wilkie (1954) were able to measure the decay of active state by using Hill's theory that the point of maximum tension during the twitch coincided with the active state curve. They stretched frog muscle slightly more than the length at which measurements were to be made, stimulated the muscle and then released the muscle from its stretch so as to record the redevelopment of tension at its final length. They reasoned that the undamped series elastic component can revert suddenly to its unstretched length because of the quick release.

Bahler et al (1967) attempted to calculate the active state time course from its peak to the point where isometric twitch tension is reached, based on existing 3 component skeletal muscle models and theoretical models thereof and in so doing concluded the time course of A.S. is a function of muscle length.

Finally, MacPherson and Wilkie (1954) devised a technique to estimate the duration of the active state plateau (DASP) and this is the method used in the present paper. The principle of the method is that during the early part of the twitch the muscle is assumed to be fully active so it behaves as though it were tetanized. However, if the muscle is not-restimulated the tension curve soon declines. In a tetanic contraction the tension remains elevated until the stimulus is switched off or until the muscle fatigues. Therefore, if the tension curve for a twitch and a tetanus (even a pair of appropriately timed stimuli) are superimposed the point where the two curves separate is thought to represent the onset of decay of the A.S. of the twitch. In practice accuracy is improved by measuring the point of separation of the twitch first derivatives.

The A.S. of a muscle twitch is a fundamental property of the muscle fibers and directly reflects the contractile processes of the muscle whereas the twitch is a composite picture of all constituents. As a result, the time course of the A.S. while still somewhat of an abstraction has far reaching implications in muscle contraction. Close (1964) has suggested that the shape of the isometric twitch is greatly dependent on the A.S. time course. Wells (1964) and Close (1972) have indicated that the time course of decline of the A.S. curve is more rapid for fast muscles than for slow ones. Brust (1966) noted that mouse fast muscle such as gastrocnemius or flexor hallucis longus has a shorter DASP than does the slower soleus. These results would imply fundamental differences between these muscle types and once again the possibility must be explored that slow muscle is more fully activated than fast muscle. It is of interest that there is no evidence that mammalian muscle fibers are fully activated at any stage during a normal twitch (Close 1972).

The active state can be prolonged without affecting the other properties of the muscle by nitrate ions, adrenalin, caffeine or a second stimulus (Goffart and Ritchie 1952, Brust 1965, 1966) and this is manifest by an increased contraction time.

Since there is apparently a slowing of fast muscle in dystrophy (Sandow and Brust 1958, 1962, Brust 1966, Sabbadini and Baskin 1976) it was thought appropriate to examine the DASP of these muscles to determine whether the phenomenon might be explained on the basis of a change in the degree of activation.

B. Histochemical Properties of Muscle

i. Fiber Typing - Morphological and cytochemical studies have revealed several striking differences in muscle fibers. Early histological work by Grützner (1884) found most mammalian muscles to be made up of at least two kinds of fibers. Red fibers are usually dark and thin with many mitochondria and fat droplets whereas the white fibers are larger and clear with few mitochondria and fat droplets. Muscle fiber populations are rarely homogenous and structural differences were also noticed in the "protoplasmareiche" and "protoplasmaarme" fibers of Knoll (1891) and the "fibrillenstruktur" and "felderstruktur" of Krüger (1952).

Schiaffino et al (1970) in the fast rat EDL found most fibers were large, had few mitochondria and lesser myoglobin concentration and thicker Z lines than the slow SOL. In addition they found that these fibers were richly supplied with SR, contrasting greatly with the fibers of SOL. Specifically in the SOL they found discontinuous contacts between the SR and t system and dyads rather than the typical triads could occur. They suggested that this variable structural pattern is related to two distinct physiological parameters, contraction time (CT) and resistance to fatigue. Fisher and Peter (1971) studied fragmented SR in vitro and concluded that fast muscle SR takes up Ca^{+2} 4-11 times faster than slow muscle SR.

There are also differences in the specific activity of myosin ATPase in fast and slow muscles (Barany 1967). Fast and slow muscle myosins show several differences in their molecular structure (Sreter et al 1966).

The relatively recent adoption of muscle histochemical methods has permitted the examination of fiber types from an entirely new perspective. Unfortunately however, this field has been beset with sharp growing pains. One major stumbling block has been the widespread lack of agreement over the adoption of a single comprehensive nomenclature system. The literature now contains almost as many fiber type classifications as there are papers arguing the validity of each. In fact there are proposals for fiber types ranging from two to an unlimited number (Dubowitz 1960, Guth and Yellin 1971). This dilemma becomes comprehensible if one considers that the problem really results from the fact that the fiber typing system hinges on which properties the author considers representative of that particular fiber, a slightly circular argument. Many have tried with some success to classify fibers on the basis of colour, morphology, ultrastructure, metabolism, contractile properties, myosin ATPase activity and pH lability and oxidative and glycolytic enzymes. Romanul (1967) suggested 8 fibers types from a profile of histochemical reactions but this has not found much practical value. The fiber typing system used in this report is a modification of the system of Dubowitz (1960) and Engel (1962) who prefer the Type I and Type II system so as "to avoid names with controversial implications". The point is well taken since it avoids using the potentially erroneous physiological terms such as "fast" and "slow" to describe a histological entity.

The fibers are typed on the basis of the alkali stable myofibrillar ATPase (ATPase) reaction at pH 9.4 (see methods) and on the nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) reaction for oxidative capacity. These reactions are discussed in some detail in the methods section. Essentially however the reaction product for the ATPase stain results in the precipitation of cobaltous sulphide, a dark coloured material. Likewise, in the NADH-TR reaction NADH and tetrazolium are incubated with tissue and the NADH is oxidized while the tetrazolium is reduced resulting in a blue precipitate.

Thus, the Type I fiber stains negatively for ATPase and intermediately for NADH-TR. The Type II_{oxidative} (II_{ox}) fibers stain strongly positive for both reactions and the Type II_{glycolytic} (II_{glyc}) fibers stain intermediately for ATPase and negatively for NADH-TR in the mouse (see methods).

It is now generally accepted that there are 3 basic fiber types and according to Close (1972) "unequivocal identification of most fibers can be achieved by staining serial sections for myosin ATPase, phosphorylase, and oxidative enzymes". However, despite this effort to reduce entropy many different systems persist to describe these 3 fiber types. The following table has been included to illustrate several of the nomenclatures currently employed for fiber typing. This table is a composite survey from Close (1972) and McComas (1977) and the fiber type nomenclatures do not necessarily correspond exactly.

<u>System</u>			<u>Fiber Types</u>	
1. Present Classification		I	II _{ox}	II _{glyc}
2. Dubowitz & Pearse (1960)		I	II	II
3. Engel (1962)		I	II	II
4. Stein & Padykula (1962)		B	C	A
5. Romanul (1964)		III	II	I
6. Padykula & Gauthier (1966)	Intermed.		RED	White
7. Kugelberg & Edstrom (1968)		C	B	A
8. Yellin & Guth (1970)		b	a	ab
9. Brooke & Kaiser (1970)		I	IIA	IIB
10. Burke et al (1971)		S	FR	FF
11. Peter et al (1972)		S0	FOG	FG

It is important to realize that fiber types are not immutable and it is perhaps best to regard enzyme histochemistry as reflecting an instant in time under given conditions. As Engel (1974) more eloquently puts it "it should serve as a caution that histochemistry like nearly all other forms of tissue examination reflects the situation at but one brief shining moment in the life of a cell".

For instance, fiber types have been altered after nerve cross union, (Romanul and Van der Meulen 1967), exercise, (Barnard et al 1970), excision of synergists (Guth and Yellin 1971), tenotomy (Vrbova 1963) and during normal development (Kugelberg 1976). Guth and Yellin (1971) on the basis of fiber type transformations seen in normal and experimental conditons suggest that muscle cells are

dynamic and are undergoing continual adaptation throughout life to changing functional demands. Consequently, they feel that any attempt to classify fibers tends to mislead one into viewing their properties as stable characteristics. They are therefore in favour of a broad spectrum of fiber types.

There is considerable evidence that fiber types are under neural control (Guth review 1968). Eccles et al (1958) showed that slow muscle fibers are innervated by tonic motoneurons and fast muscles by phasic ones. Buller et al (1960) observed that mammalian limb muscles are slow at first and differentiate to fast or slow with maturation. They also noted that the cat soleus actually speeds up for several weeks prior to slowing down to its final slow nature. In neonatal kittens transection of the spinal cord resulted in no change in the developmental characteristics of fast muscle but soleus failed to differentiate to its final characteristics.

Finally, it has been very tempting teleologically to ascribe functional characteristics to the whole muscle and the fiber types themselves. Barnard et al (1970) suggest that the red (II_{ox}) muscles are involved in low intensity prolonged duration types of activity as in postural function. The white (II_{glyc}) muscles are thought to be primarily involved in high intensity short duration exercise. Dubowitz (1966) believes that the red muscles would thus depend on Krebs cycle intermediates and fats for fuel while white fibers derive energy from glycolysis. On the other hand, intermediate

(Type I) fibers are the low speed economical units suitable for sustained tonic activity (Close 1972).

ii. Some Problems Associated with the Histochemical

Stains Themselves

Inherent in any physiological-histochemical study are questions about the validity and the interrelationships of the methods used.

As muscle histochemistry made its debut there was a great deal of optimism that it represented a kind of microbiochemistry whereby stained sections could be assessed in at least a semi-quantitative fashion merely by microscopic examination. The attendant perils have recently been revealed as have the even more dangerous attempts to correlate histochemistry with physiology. Detailed subsequent studies have shown that caution must be observed not only when comparing muscles of different species but also different muscles of the same species. The severity of this situation prompted Engel (1970) to write "Classification of striated muscle into different types has always been somewhat confusing but recently has shown an alarming trend towards the incomprehensible".

The stains used in histochemistry have been criticized under a number of criteria. Brooke and Kaiser (1974) have reviewed the possibilities of false localization of the oxidative stain NADH-TR. Since the diaphorase is insoluble in water it is unlikely to diffuse away as can other enzymes such as lactate dehydrogenase (Fahimi and Roy, 1966). However, the tetrazolium of this reaction may bind

selectively to components such as the SR, t-tubules and mitochondria which can cause the reaction to occur in these loci where in fact the enzyme may not be present. More insidiously, if the tetrazolium doesn't bind to a particular tissue component that component cannot be demonstrated.

In addition, Engel (1974) has criticized the stain on the basis that it is quite readily altered in disease states and occasionally fiber type differentiation is difficult even in the normal animal.

The myofibrillar ATPase reaction has come under even closer scrutiny and Brooke and Kaiser (1974) have labelled the stain itself as "technically a difficult one". In addition, it is often complicated by a dirty deposition over the surface of the stain which is thought to be due to partial dissolution of the "intermyofibrillar" network composed of mitochondria, SR, triads, t-system etc. (Guth & Yellin, 1971). Guth (1973) has had reasonably good success in removing this artefact by adding calcium to the fixative solution. The ATPase enzyme is very fragile and is sensitive to pH alterations as well as reaction times.

In 1973 Guth concluded that since the phosphate liberated as a result of the ATPase activity of actomyosin and or mitochondria is selectively bound to the myofibrils, the localization and distribution of reaction product doesn't necessarily reveal the site of enzymatic activity. Furthermore the affinity for binding inorganic phosphate differs among the various muscle fiber types. For this reason the intensity of the histochemical myofibrillar ATPase reaction is not

necessarily indicative of the activity of this enzyme.

Thus while the histochemical reactions may not reflect the actual biochemical situation, perhaps the most important requirement of histochemistry is to demonstrate the fiber types in a consistent manner. In other words it is important if one wishes to examine several SOL muscles of mice of the same age and strain that the results be reproducible.

A major usefulness of these stains is simply to identify fiber types irrespective of their biochemical make-up. It will, however be shown in the following section that there are some muscle properties which appear to be accurately assessed by the histochemical reactions.

5. Muscle Physiology and Histochemistry:

Is There a Correlation?

Ultimately it would be best to examine single mammalian muscle fibers for correlation of histochemical and physiological properties but to date this has not been achieved to the best of my knowledge. Many indirect attempts have been made using whole muscles and more recently motor units thereof.

There is good evidence that the Type I fiber is a slow fiber because the adult guinea pig SOL which contracts very slowly is homogenously composed of this fiber type (Karpati and Engel 1968, Edgerton and Simpson 1969, Barnard et al 1971). They also concluded that the Type II_{glyc} and II_{ox} fibers were fast contracting, using muscles rich in these fiber types. The fast fibers contain myosin ATPase with a high specific activity and the slow fibers contained myosin ATPase of a relatively low specific activity.

In the present study similar indirect evidence of relative fiber type speeds is obtained. The SOL which contains a component of Type I fibers is usually considered to be a slow muscle in the mouse. It does however have a high complement of II_{ox} fibers which may also prove to be a fiber of relatively slow or intermediate speed (Refer to discussion section). However, in the fast EDL (Type II_{glyc} and II_{ox} only) the contraction is very fast suggesting both these fibers are fast contracting.

It is of interest that Briscoe and Taylor (1967) hypothesize that it takes a larger admixture of fast fibers in a muscle than it

does slow fibers to cause an inflection in the isometric twitch tension-time record. This points to the possibility that each fiber type does not contribute equally to the physiological characteristics of the muscle and further complicates the issue.

Close (1967) found that the rat soleus had 3 out of 30 motor units which were of intermediate speed and 27 which were slow which correlates well with the histochemical stains if one assumes that II_{ox} is of intermediate speed. However, in the rat EDL which contains II_{ox} and II_{glyc} fibers the motor units had very small variations of contraction times.

Edstrom and Kugelberg (1968) also found that II_{ox} and II_{glyc} motor units were fast. They found the II_{glyc} motor units to fatigue rapidly, the II_{ox} units slower and the Type I units not at all.

Burke et al (1971) in a motor unit study of cat gastrocnemius found 3 types of motor units classified as fast fatiguing (FF), fatigue resistant (FR) and slow (S). Of these he found the FF and FR units to be fast contracting whereas the S units contracted slowly. When the units were examined as a whole there was a considerable spread of contraction speeds.

Close (1972) said that the available evidence shows that the fibers of some heterogenous muscles can be classified into 3 different groups on the basis of their morphological and histochemical properties but into only two groups on the basis of the time course of their isometric twitch and kinetic properties of myosin ATPase at neutral pH. I believe however that it is not wise to apply information from

one muscle to another until direct proof becomes available.

There is considerable debate as to whether the individual histochemical stains have as their basis a physiological correlate.

One correlation which does seem to fit rather well is the oxidative enzyme staining intensity and the resistance to fatigue of the fiber. Burke and Tsairis (1974) and Burke et al (1974) suggest that this is further related to the richness of capillary supply. In addition fibers with a large oxidative capacity and which use the Krebs cycle fatigue very slowly whereas fibers which rely on glycolysis for energy are capable of rapid phasic activity but fatigue rapidly. This latter occurrence is possibly due to the depletion of intracellular glycogen. (Burke and Tsairis 1974).

In particular Burke and Tsairis found 3 types of motor units in the cat which were classified according to fatigue resistance. Single units were tetanically stimulated at 40 Hz for 330msec and this was repeated every second for two minutes. They devised a "fatigue index" to define the ratio of tension decline from the start to the end of the two minute interval. They found "fast fatiguing" units which have little oxidative enzyme activity and depend on anaerobic enzymes for ATP. They found also two types of "fatigue resistant" units which fatigue slowly and the "slow" units which showed no fatigue even after an hour of such stimulation.

Likewise Edstrom and Kugelberg (1968) and Kugelberg (1973, 1976) presented strong arguments that the oxidative enzyme staining intensity

is related to fatigue resistance in their motor unit studies.

A more interesting situation appears when attempts are made to correlate myofibrillar ATPase with speed of contraction. From a superficial viewpoint the relationship does appear to exist. Engel (1970) showed that in the slow contracting guinea pig soleus and cat soleus the histochemical myofibrillar ATPase is of uniform low intensity. Conversely, in the fast gastrocnemius and EDL intense staining type II fibers predominate. A similar relationship was noted by Edgerton and Simpson (1969) and Buller and Mommaerts (1969) stated that ATPase was a significant determinant of CT. Kugelberg (1973) reported that the CT could be predicted from the intensity of the ATPase reaction and in 1976 suggested that fiber types are continuously in a state of transformation due to altered functional demands and that these changes are mediated by the motoneurons.

In 1967 Barany obtained evidence for a direct relation between the speed of shortening of a muscle and its actomyosin ATPase activity using quantitative methods. He suggested that this difference in dynamic properties is correlated with and probably the result of differences in the kinetics of myosin ATPase.

In 1971 Barany and Close confirmed and extended these findings in their work on cross-innervated rat muscles in which they report reciprocal changes in the CT of cross-innervated fast and slow muscles. They showed that the ATPase activity which is normally greater in fast muscles, changes reciprocally as do the contractile properties and the myosin structures of the cross-innervated muscles.

Furthermore, they demonstrated that of the 5 activators of myosin ATPase used the physiological one, actin, gives the best correlation. Thus, this evidence for the first time showed a direct proportionality between the intrinsic speed of shortening of sarcomeres and actin-activated ATPase activity. Moreover, this provided excellent evidence that the neural influences determine the fundamental dynamic properties of the contractile material by an effect at the ATPase site of myosin.

Therefore, at first appearance the direct correlation of ATPase activity to speed of contraction appears intact. In practice however, there are several instances which raise serious objections to this relationship. For example, Guth and Samaha (1972) have noted that newborn animals show an intense ATPase reaction when examined histochemically but quantitatively the ATPase activity is very low and the speed of shortening is slow (Bulter et al 1960, Close 1964). They explained this as possibly reflecting properties of the pH stability of the ATPase of young muscle. Therefore one can't assume that an intense ATPase reaction is tantamount to high actomyosin ATPase activity at physiological pH.

Burke, Levine and Zajac, Tsairis and Engel (1971) have found 3 non-overlapping populations of motor unit types in the cat gastrocnemius which were separated by several physiological parameters. The 3 basic histochemical fiber types exactly matched the physiological groups but on the basis of contraction time alone they were unable to discern the dividing point between motor units.

Burke, Levine, Saligman, Tsairis (1974) in looking at cat soleus motor units saw some correlations from the "coarse view". But, from the "fine view" they noted a two fold range in CT for soleus motor units despite the fact that all fibers stained the same intensity for myofibrillar ATPase. Further, the ATPase reaction is more intense in the cat soleus than in presumably similar such units of the gastrocnemius even though they contract more slowly in soleus. This last point not only emphasizes the problem of histochemical-physiological correlation but also nicely illustrates the lack of identity between motor units of apparently similar types in different muscles of the same animal.

Burke and Tsairis (1974) similarly observed a lack of consistency in the ATPase staining and CT with both the fast twitch and slow twitch units of the gastrocnemius. There was considerable overlap of motor unit contraction time despite ATPase staining similarities. They concluded that it is probable that the histochemical appearance of fibers may give an imperfect indication of the speed of contraction of the same fibers at least as viewed from "fine grain".

Robbins et al (1969) studied cross-innervated guinea pig soleus which I feel helps clarify the situation. From the above information it is clear that the ATPase staining intensity-contraction speed controversy requires a compromise until additional information fully resolves the issue.

They found that when soleus was cross-innervated with a nerve of a mixed muscle the CT of the muscle as a whole decreased and the

number of ATPase dark (Type II) fibers increased as expected. But, closer examination led to the observation that the CT of the light staining fibers decreased also. This indicated that factors other than the increase in the ATPase dark fibers were responsible for the decrease in contraction time. They proposed that a correlation between histochemical and physiological results doesn't necessarily imply that in the cross-innervated muscle every Type I or II fiber is slow or fast respectively. Rather, these results suggested to Robbins et al that the percent of Type II fibers can only act as a gross indicator of the degree of speeding of the whole muscle. They hypothesized that a fiber could perhaps change its ATPase staining reaction only when speeding or slowing had proceeded to some critical "turnover point".

Thus the principal point made is that if the ATPase reaction only marks the fibers which have reached this point then this could explain the incomplete correlation of contraction speed and ATPase staining intensity.

Finally, in defence of muscle histochemistry it is imperative that these techniques not be over-looked despite the above mentioned shortcomings. While histochemistry (like most disciplines) does have finite limitations, if these are accounted for and are not exceeded, the stains can be very useful indeed. Few techniques have been able to expand an area of knowledge as rapidly as has the, histochemistry of muscle. It has enabled the study of muscle to proceed from a new vantage point whereby many important discoveries

have already been made, and the potential is great. While a particular stain may not necessarily represent the actual biochemical relationships of the fibers at least the possibility is presented and this is a challenge to physiologists.

Perhaps one of the major benefits of histochemistry is its applicability in conjunction with physiology as is done in the present work. By interpretation of data from both points of view a much more concrete appraisal of the situation can be obtained. Moreover, there is no reason to believe that histochemistry would not provide consistent results when examining a particular muscle such as the SOL of animals of the same age and strain. In this respect much can be gained from such a study especially when applied to disease. Brooke and Kaiser (1974) and Engel (1974) have suggested that perhaps the major use of histochemistry is as a muscle marker for fiber types and in this sense has provided great strides in the study of muscle function and disease.

6. Experimental Rationale

The findings of Brust (1966) sparked a number of investigators to address the question of preferential involvement of one muscle type, and more specifically one fiber type in murine muscular dystrophy. In particular the suggestion was firstly that fast muscles are slowed and secondly that slow muscles are spared by the disease. (It is known that all fibers are eventually affected by the disease). A great deal of evidence has accumulated consistent with this hypothesis and if proven correct may give some support to the neurogenic hypothesis of McComas et al (1971).

After preliminary observation of the contraction times of dystrophic muscle indicated that the hypothesis of Brust may hold in dy^{2j} muscle a time course study was devised to further investigate the hypothesis. Several fundamental physiological characteristics of fast and slow muscles were examined for signs of conversion to determine whether these muscles had undergone intrinsic or extrinsic fiber property changes. The properties monitored were CP/CD, PTP/PTD and DASP.

If indeed the cause was found to be alterations of intrinsic muscle fiber properties, a histochemical study would be undertaken to more specifically characterize the nature of the property conversions. This phase of the work was designed to determine whether there was a selective involvement of a single fiber type or whether there was a more general slowing of all fiber types. In this study mice of 3-4 weeks of age were examined because older dystrophics could not be examined accurately with the histochemical methods available.

CHAPTER II

MATERIALS AND METHODS

I. Animal Models

A. Mice

Both normal (C57 BL/6J) and dystrophic (dy^{2j}/dy^{2j}) mice were originally obtained from the Jackson Laboratories in Bar Harbour, Maine and have since been successfully bred in our own animal quarters. Because this form of muscular dystrophy is caused by an autosomal recessive gene the F_1 offspring of a heterozygous-heterozygous mating will, on the average produce $\frac{1}{4}$ normal animals, $\frac{1}{2}$ heterozygous and $\frac{1}{4}$ dystrophics. In fact we normally get fewer dystrophics, presumably because some die at birth. Dystrophic-dystrophic matings are very uncommon. It is of interest that there are no obvious phenotypic differences between the normal and heterozygous animals so the experiments involving "control" animals likely also contained heterozygous animals included in the pool. There are also no apparent functional differences between the muscles of $+/+$ and $dy^{2j}/+$ mice.

The dystrophics begin to show clinical signs of the disease at about 21 days of age but this is variable over a few days. It was of importance that these animals be detected at the earliest possible stage of dystrophy and two signs helped in the early diagnosis. Firstly, if a litter was agitated the dystrophics would often limp slightly and hyperextend their hindlimbs while walking

as in voluntary stretching. The second and most reliable sign was to suspend an animal by the tail. The dystrophics clasped their hindlimbs close to the body unlike their normal counterparts which would extend their hindlimbs outwardly.

Since a time-course study was undertaken throughout my experiments the controls and dystrophics were grouped according to their ages. In the contractility experiments the "young" group of animals were 6-12 weeks of age and the "old" group was pooled from animals greater than 6 months of age.

In the histochemistry experiments the mice were from 21-29 days of age. Histochemically this age period was critical since it was found that with dystrophics the stains employed lose their specificity soon after this period. Therefore in staining this young tissue I was able to study dystrophic tissue which still retained its fiber types and thereby make judgments as to possible preferential fiber type involvement.

B. The Guinea Pig

Male albino guinea pigs of the Hartley strain were purchased from the Canadian Breeding Farm and Laboratories in Montreal.

These animals were from 6-8 weeks of age at the time of experimentation. The solei of these animals are composed entirely of Type 1 fibers. Karpati and Engel (1968) report that it is not until the 6th week of age that the guinea pig soleus matures from a mixed fiber population to an entirely Type 1 population. I have confirmed this histologically

with 6 week soleus tissue but I have also found the soleus of one 3 week animal to be homogenously Type 1.

The animals were fed ordinary guinea pig chow but this was supplemented with ascorbic acid in their drinking water (guinea pigs cannot synthesize Vitamin C) and fresh lettuce to improve their blood clotting ability and general health. This was implemented since they are prone to more copious bleeding under surgery during the hot summer months and since it was thought that some animals had scurvy on arrival.

2. Experiments Involving Contractility

A. Preparation of the Mice

All contractility experiments were done in vivo with the aim of maintaining an intact nerve and blood supply to the muscle. The mice were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal) at an initial dosage of 85mg/Kg (Taber and Irwin 1969). Supplementary doses were administered as required. The animals were prepared for recording with the aid of a Nikon stereomicroscope.

In the case of the EDL muscles the cutaneous tissue of the anterior aspect of the hindlimb was removed and a length of 6-0 silk was tied around the distal tendon of the anterior tibialis muscle, which overlies the EDL. This tendon was cut and the anterior tibialis was reflected proximally, care being taken to avoid the local vasculature. This exposed the EDL and in like fashion a length of 6-0 silk was secured to its distal tendon near its insertion. In an effort to maintain strict isometric conditions a stainless steel wire was tied to the EDL tendon which was then coupled to a force transducer.

If the mouse was dystrophic the sciatic nerve was transected bilaterally at mid thigh level to reduce extraneous contractures caused by the ever-present spontaneous muscle twitching. The leg was further immobilized at the ankle with a clamp and at the knee with a pin inserted through the patella and into the matrix of the base of the bath (Sylgard encapsulating resin: Dow Corning).

The soleus was more difficult to prepare since it is a deep muscle in the posterior compartment of the hindlimb and utilizes a common distal tendon, the Achilles tendon along with the plantaris muscle and the gastrocnemius muscle. I cauterized and tied off the major vessels which must be severed in order to properly expose the soleus. The distal $\frac{1}{2}$ of the gastrocnemius and a portion of the plantaris was removed so that the only muscle left attached to the Achilles tendon was the soleus. A steel wire was connected as above and the Achilles tendon was severed near the calcaneum for recording. The motor nerves were transected as above if the mouse was dystrophic and in most cases if it was normal. The muscles were stimulated either indirectly by means of the sciatic nerve or directly by field electrodes. Indirect stimulation was done as close to the experimental muscle as possible to avoid stimulating other muscles subserved by the nerve trunk.

B. Preparation of the Guinea Pig

The guinea pig posed some special problems of its own not already described in the mouse section. The guinea pigs were anesthetized by I.P. injection of Nembutal at 40mg/kg initial dose (Hoar 1969):

Bleeding was found to be a major problem and the following measures were taken. The animal was kept in a cool air-conditioned room 12 hours prior to surgery and after anesthesia the leg was packed with ice to further reduce peripheral blood flow. The smaller vessels were blocked by cauterizing and oozing was controlled with

Gelfoam sponges of absorbable gelatin (UpJohn). A neuro-coagulator was used to seal some vessels and the largest ones were tied off with Ethicon 3-0 surgical silk.

A tracheostomy was used since it was found that occasionally mucus accumulation would occlude the airway. Since it is difficult to control the plane of anesthesia by I.P. injection and since guinea pigs can react unpredictably to the drug, (Hoar 1969) a Harvard 680 respirator was used in case of accidental barbiturate overdose. The respirator was implemented when the pupillary and pinch reflexes became depressed and resuscitation was usually effective.

C. The Animal Baths, Solutions and Temperature Control

The prepared animals were transferred to a plexiglass bath and fixed in position. The bath was filled obliquely with Sylgard encapsulating resin (Dow Corning Corpⁿ) which remained gelatinous enough to allow penetration of the immobilizing pins. The animal was placed so that its head was at the elevated end of the bath and its leg was at the lower end. Thus, Ringer could be introduced into the bath completely immersing the animal to its upper thigh while the head remained out of the fluid. The Kreb's Ringer solution was composed of the following constituents:

NaCl = 121.00mM	NaHCO ₃ = 25.00mM	glucose = 11.1mM
KCl = 4.75mM	KH ₂ PO ₄ = 0.50mM	
CaCl ₂ = 1.50mM	MgCl ₂ -6H ₂ O = 0.23mM	

The Ringer was constantly gassed with 95% O₂ and 5% CO₂ and

was gravity fed to the animal bath either via a hot water bath (37°C) or directly at Room temperature ($20-26^{\circ}\text{C}$). A yellow Springs Instruments Telethermometer thermistor probe was submerged in the bath near the muscle so the bath temperature could be continuously monitored. A suction apparatus removed the surplus Ringer during the temperature changes and kept the Ringer level constant.

D. The Experimental Protocol for Recording

The optimum point on the length-tension curve was obtained as the muscle was stimulated at a rate of 1 per 5 seconds at slightly above the voltage required to elicit a maximal isometric contraction. The duration of this stimulus was 1 msec. The required voltage was then usually determined for indirect stimulation at a duration of 0.1 msec. in a similar fashion as above.

The muscle was allowed to equilibrate at each new bath temperature for a period of 10 minutes and over this period photographs were taken of the contractions at the 0, 3, 7 and 10 minute marks to insure the muscle was stabilizing. The muscle was then tetanized at a rate of 50/sec for 2 seconds and approximately 5 seconds after this, stimulation was resumed at the previous rate of 1/5 seconds. Photographs of each post-tetanic twitch were recorded for a period of two minutes then at every minute for 5 minutes and finally on the 10th minute post tetanus.

The parameters chosen for the present experiments of 50/sec for 2 seconds are within the guidelines of Close & Hoh (1968) such that PTP should be seen in the fast muscle without alteration of the CT or $\frac{1}{2}\text{RT}$.

If indirect records were desired this procedure would usually be repeated with indirect stimulation at this temperature. Otherwise, the bath temperature was raised to 37° and the above sequence of events repeated. The order of direct vs indirect recording was varied in several experiments to act as an internal control. Likewise experiments were discarded when the post-tetanic twitches did not return to 75% of the pre-tetanic values after 10 minutes.

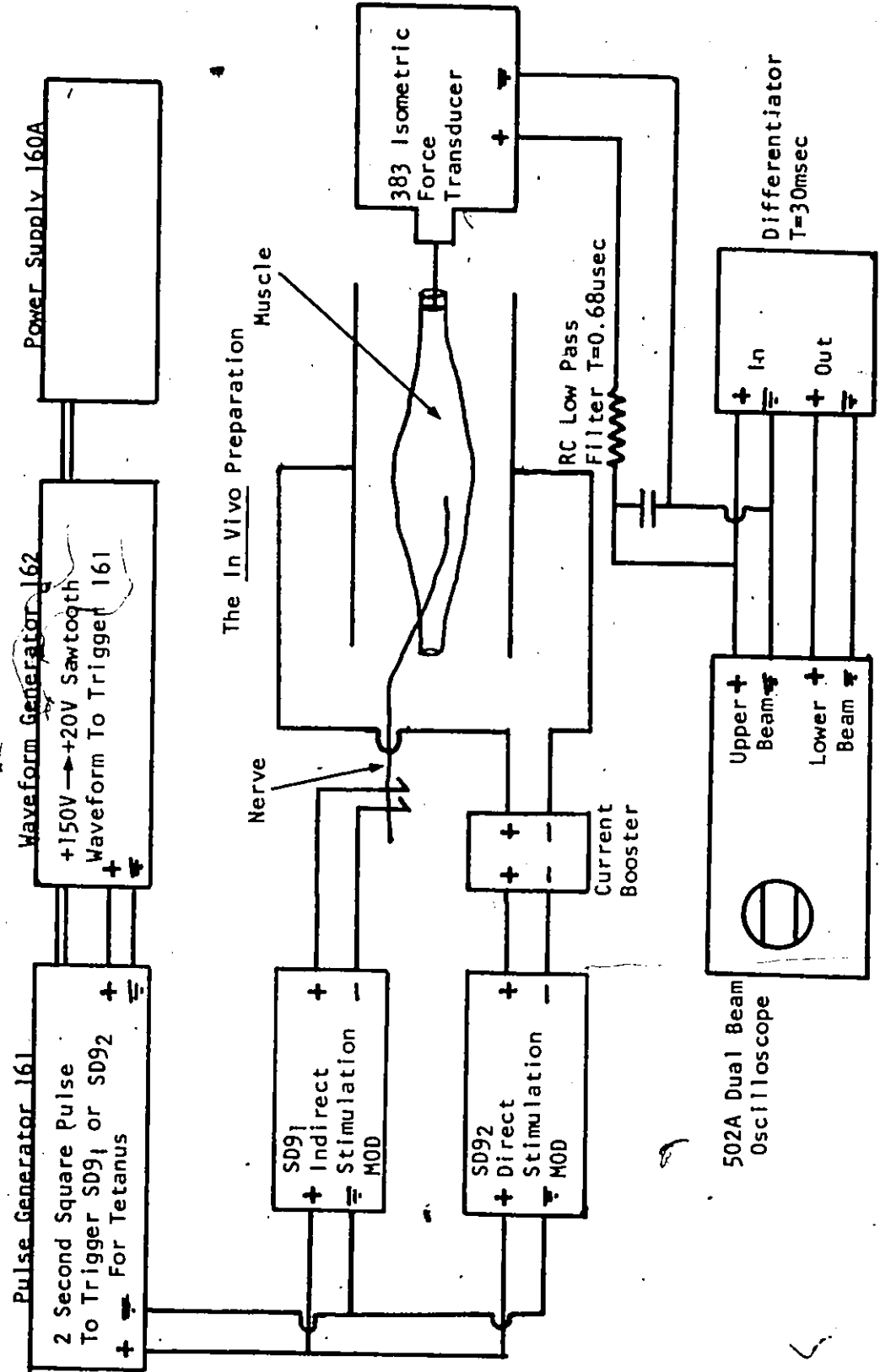
If duration of active state plateau measurements were required photographs were taken at room temperature either before or after the tetanus experiments using the Macpherson and Wilkie (1954) technique.

In these experiments no attempt was made to test for the presence of the "back-response" as defined by Brown & Matthews (1960) and which was further examined by Buller & Proske (1978). Since the muscles were examined at optimum length, little back-response would be expected. Essentially they have found that it is possible to restimulate some fibers by means of the "back-response" caused by a single maximal stimulus applied to the motor nerve. The resultant potentiation is presumably caused by electrical activity associated with the first contraction and they suggested that it is more likely to occur in the low threshold axons.

E. Characteristics of the Stimulating and Recording Apparatus

A schematic diagram of the apparatus is shown on p.49. The basic stimulus for contraction was provided by either two Grass SD9 square wave stimulators, one for direct stimulation and one for

Figure 1. DIAGRAM OF STIMULATION AND RECORDING APPARATUS



indirect stimulation, or by a single Grass S88 multi-function square wave stimulator. The SD9 used for direct stimulation was assisted by a current booster but this was usually not necessary for the S88. When a tetanic train was desired the SD9's were driven by 2 second external triggering pulses fed into their MOD input. This was achieved by a sequential arrangement as follows. A Tektronix Type 160A Power Supply supplied the operating voltages for the Type 162 and 161 units. The 162 Waveform generator when required initiated a negative going sawtooth wave form which triggered the 161 Pulse Generator. This, in turn triggered the SD9 for 2 seconds with a 10V rectangular pulse. In the case of the S88, these functions can be generated internally without external modification.

Direct stimulation was achieved by two large platinum field electrodes which surrounded the muscle and indirect stimulation by two platinum wire leads which were hooked around the distal sciatic nerve stump.

The equipment was calibrated and to maintain consistency throughout, the various settings were marked with ink.

The contractions were transmitted by a fine steel wire coupled to either a Harvard Apparatus 373 (0-100gm) or 383 (0-1000gm) isometric force transducer for mouse and guinea pig contractions respectively. These contractions were displayed on a Tektronix Type 502A dual beam oscilloscope after filtering some very high frequency noise generated by the transducers. An RC lowpass filter was designed for this purpose with a time constant of 0.68usec.

The photographs of the oscilloscope traces were taken with a Nikon continuous recorder PC-2A camera which was manually operated. Kodak RAR black and white 35mm film (2495) was used from 250 foot reels and I developed this with Kodak chemicals. The subsequent footage was edited and analysed on a back-illuminated film viewer.

Estimations of the performance of the transduction systems were made since this is important when attempting to make accurate measurements of the isometric contraction times.

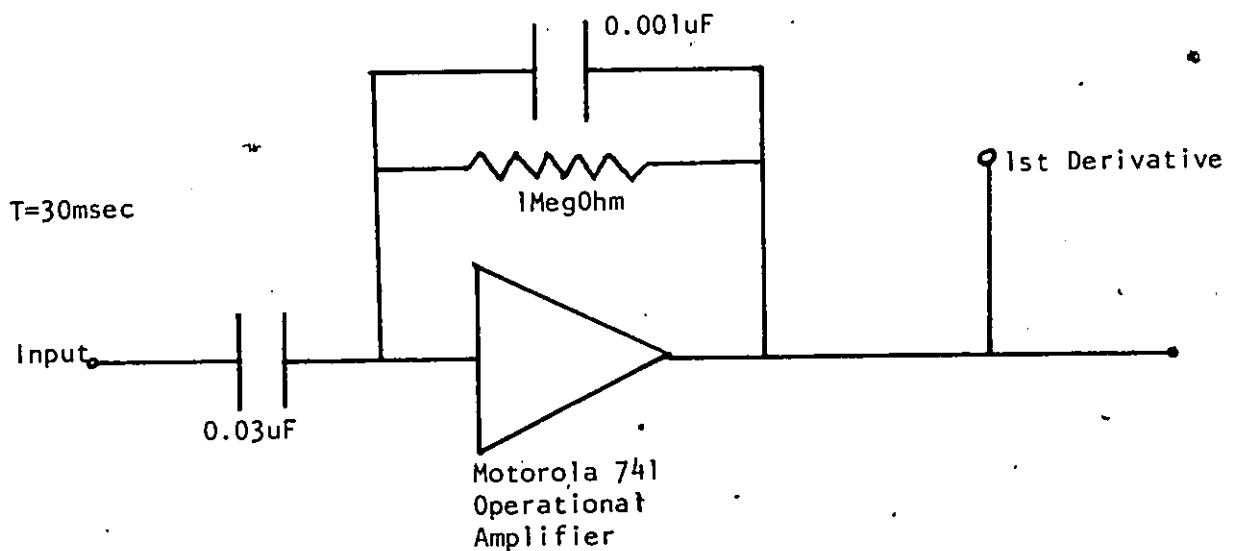
With the 373 transducer a natural resonant frequency of 450Hz was calculated by photographs taken as the transducer beam was lightly tapped. An estimate of the compliance of the system was found by connecting the steel wire to the transducer as in an actual experiment and applying various amounts of pulling force to the other end by retracting a micro-manipulator. The deflection of the transducer beam was recorded with a Mercer micrometer and the average calculated compliance was 1.58 u/gm tension.

The frequency response of the system was estimated by stretching a length of 5-0 silk from the transducer to a micromanipulator under about 10 gms of force. The thread was then rapidly burnt through with a propane torch and the resultant rate of tension decline was recorded. The average time to drop 95% of the original tension was in the order of 2msec. The assumption here was that a system having an ideal frequency response should display an instantaneous change of tension. As pointed out this time period is only an estimate and may be excessively long since the time taken for the thread to burn through was probably not instantaneous.

The corresponding values obtained for the Model 383 were: natural resonant frequency 1000Hz, compliance 1.0 u/gm and response time in the order of 2msec.

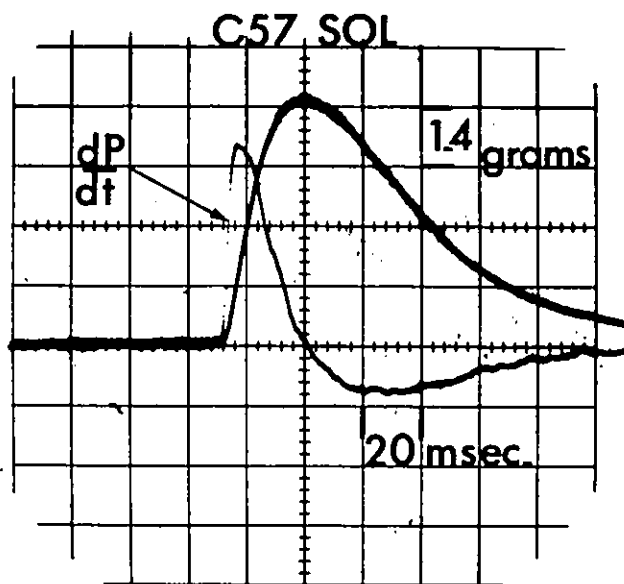
The second beam of the oscilloscope was used to obtain the first derivatives of twitches for use in active state determinations. The differentiator was designed with a 30msec time constant. (See circuit diagram below). The shape of the first derivatives were checked with phase plane plots (See figure two). A Motorola 741 operational amplifier was used here.

CIRCUIT DIAGRAM OF DIFFERENTIATOR

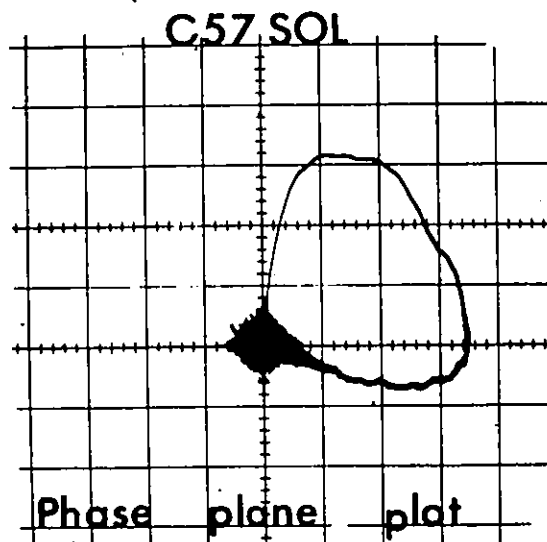


- Figures 2a & b
- Typical isometric contraction with first derivative of mouse C57 SOL.
 - Phase plane plot of this contraction showing that the differentiator has not altered the contraction time relationships.

2a



2b



3. Histochemistry Experiments.

A. Tissue Preparation

The mouse and guinea pig tissue was removed from anesthetized animals and was rapidly frozen by immersion for about 15sec. in isopentane pre-cooled to -170°C in liquid nitrogen (-196°C). If this tissue was not immediately used it was stored at (-45°C) for variable periods of time. Old tissue becomes more dehydrated.

The frozen tissue was transferred on dry ice to a Damon IEC cryostat and allowed to warm to -20°C at which time serial cross sections were cut at about 10 μ thickness. The serial sections were placed on microscope coverslips which were warmed to room temperature by placing a finger on its lower surface.

The sections were usually stained immediately but if not it was found that enzymatic preservation was better if the sections were stored in the freezer.

B. Stains

Three stains were used on the serial sections. One section was stained with Hematoxylin and Eosin (H&E) and this was used as a morphological stain (Lillie 1974).

A second serial section was stained for oxidative enzymes with the NADH tetrazolium reductase (NADH-TR) stain using modifications of the methods of Nachlas et al (1957), Scarpelli et al (1958) and Novikoff et al (1961).

A third serial section was stained for alkali-stable myofibrillar ATPase (ATPase) using the methods of Padykula and Herman (1955) as modified by Guth and Samaha (1969).

Since the procedure for the H&E stain is well known a description is not provided here. However, complete descriptions of the procedures for the ATPase and NADH-TR stains are included below. It is assumed that the tissue has dried on the coverslips for a period of at least 30 minutes.

i. Demonstration of NADH-TR

1. Incubate for 30 minutes at 37°C in the following well mixed solution which has pH adjusted to 7.4 (HCl and NaOH):

0.2M Tris buffer (pH 7.4)	10mls
NBT	10mgm
NADH	8mgm

2. Rinse in cool distilled water 3 x 1 minute
3. Mount on microscope slide with water soluble mounting medium.

ii. Solutions

1. 0.2M Tris buffer (pH 7.4):

Tris hydroxymethylaminomethane	0.606gms
distilled water	58.0 mls
0.1M hydrochloric acid	42.0 mls

adjust pH to 7.4

2. Nitro Blue tetrazolium (NBT) salt 816.0 mw
3. Reduced diphosphopyridine nucleotide (NADH) 709.4mw

NB It was through the preliminary work of Mr. Jacques Dallaire and Dr. A. Reed on the fiber type formaldehyde sensitivities that the ATPase stain was adaptable for mouse studies as employed below.

iii. Demonstration of ATPase

1. Fixative - 10 min. in cold (4°C) fixative medium
2. Fixative rinse - 4x3 min. in cold distilled water rinse
3. Pre-incubation - 15 min in cold pre inc. medium
4. Incubation - 45 min at 37°C in incubation medium
5. 1% CaCl_2 - 3x30 sec rinse
6. Drain and blot coverslips
7. 2% CoCl_2 - 1x3 min. wash
8. 0.1M buffer rinse 4x30 sec rinse (re establish pH)
9. 1% $(\text{NH}_4)_2\text{S}$ - 1x3 min.
10. tap water rinse (cold) - 1x5 min.
11. Ascending alcohols : 95% ethanol - 1x1 min.
99% ethanol - 2x1 min.
12. Xylene - 1x2 min.
13. Mount - mounting medium

iv. Solutions

1. Fixative: (To be made up freshly each time).
 - a. formaldehyde (5%) 1ml
 - b. sucrose mw (342.3) 2.3276gm
 - c. calcium chloride mw (110.99) 0.1509gm
 - d. sodium cacodylate mw (214.02) 0.856 gm

-make up to 20mls with distilled water

-adjust pH to 7.6 in cold and use in cold (4°C)
2. Fixative rinse: cold (4°C) distilled water
3. 0.2M stock buffer: (make up to 75ml with distilled water)
 - a. 2 amino 2 methyl 1 propanol mw(89.14) 1.3371gm

4. Pre-incubation medium: (fresh each use)

a. calcium chloride 0.1gm

b. stock buffer 12.5ml

-make up to 25ml with distilled water

-pH adjusted to 10.4 with NaOH at 4°C

5. Incubation medium: (fresh each use)

a. stock buffer 12.5ml

b. calcium chloride 0.0499gm

c. disodium ATP mw (551.2) 0.062 gm

-make up to 25mls with distilled water

-adjust pH to 9.4 with HCl at room temperature

6. 0.1M Buffer, rinse: (fresh each use)

a. stock buffer 50mls

-make up to 100mls with distilled water

-pH adjusted to 9.4 with HCl at room temperature

7. 1% Calcium chloride:

a. calcium chloride 1gm

-add 100mls of distilled water

8. 2% Cobaltous Chloride, mw(237.95):

a. CoCl_2 2gm

-add 100ml of distilled water

9. 1% Ammonium Sulphide (assay 23.4%):

a. $(\text{NH}_4)_2\text{S}$ 1ml

-add 99mls of distilled water

NB: above solutions 7, 8, 9 must be stored in brown glass bottles at 4°C if made up as stock.

The photomicrography was taken with a Carl Zeiss Ergaval microscope. Black and white prints were made from Kodak Panatomic X ASA 32 film and when colour slides were required they were taken with Kodak Ektachrome tungsten ASA 50 film and were sent out for commercial developing.

The histochemical portion of this study required that muscle fiber types and certain morphological structures of fibers be counted with accuracy. In order to do this, black and white prints were taken of the muscles. Since the entire cross-section of the muscle was to be counted, 4 to 7 photos had to be taken of each muscle and these photos were reconstructed so as to include the complete muscle cross-section. The photos were taken at 100x magnification to provide sufficient detail. Acetate sheets were then placed over the reconstructed cross-sections and the various structures of interest were identified with a dot from different coloured marker pens.

The protocol for this was as follows. Only the ATPase and H&E stains were used in this study. First the ATPase stained cross section was analysed. All fibers were identified with one of four different coloured dots for four of the criteria which are defined in the histochemistry portion of the results chapter. These criteria were 1. Total Type II_{ox} 2. Total Type I 3. Abnormal staining and 4. Abnormal structure.

Next, each fiber identified above had to be re-located on the H&E stained serial cross section of the same muscle. Here the individual

fibers were further categorized with a coloured dot. From the H&E stains judgements were made regarding whether the fibers were centrally nucleated, again according to defined criteria found in the results chapter. (Central nucleation cannot be determined from the ATPase stain).

In this fashion 10 whole normal and 10 dystrophic SOL cross sections were completely counted and this data is contained in the results chapter.

4. Statistical Analysis

A. Contractility Experiments

For the most part standard statistical tests were used to determine significance. Means, standard deviations, standard error of the mean and student t tests were performed with the aid of a Wang 600 Computer. Significance was set at the 95% level. A 2 by 7 analysis of variance was also used to obtain information from the summarized physiological data. Parametric and non-parametric methods were used.

B. Histochemical Experiments

Similar tests were employed here as above. A Chi square test was used to determine significance for the fiber type population distributions.

CHAPTER III

RESULTS

1. Physiological Study

A. Mouse

a. Muscle Weights and Tensions (Absolute and Normalized)

i. Introduction In this section and in the sections to follow dealing with the physiological aspects I have examined the data from five points of view. The first variable deals with muscle types ie fast and slow. Thus the extensor digitorum longus (EDL) was chosen as a representative fast muscle and the soleus (SOL) was chosen as a representative slow muscle. The second variable was the state of health of the muscle, healthy (C57) and dystrophic (dy^{2j}). The healthy muscles would provide the baseline control data. Thirdly, since the study was essentially one of time courses the age of the mouse was examined as well, 6-12 weeks or greater than six months, from which the disease progression could be monitored. Fourthly, although these muscles were examined at the baseline temperature of 37°C, they were also carried out in duplicate at room temperature since some fast and slow muscle responses are best observed at this lower temperature. Finally, the muscles were either stimulated directly by massive "field" electrodes or indirectly via the motor nerve. The purpose of the indirect stimulation was originally intended to act simply as a control for the guinea pig stimulation experiments.

Since one cannot be sure that all of the innermost muscle fibers are stimulated when direct shocks are applied to the guinea pig soleus it was necessary to elicit contractions via the motor nerve. However, apart from this core information, additional "spin-off" information could be extracted. For instance, the indirect stimulation experiments of mouse not only provided controls for the guinea pig muscles but also could be used to measure differences between direct and indirect stimulation. This type of information which was not considered critical in terms of the original intentions but which was nevertheless of interest and value is deferred to the appendices.

ii. Muscle Weight and Tension Data in Mouse Data are given in Table 1 for the absolute twitch tensions of the EDL and SOL muscles at 37°C. In addition the weight of the whole excised muscles were recorded after each experiment and these were used to calculate the normalized twitch tensions (twitch tension (gms)/gm of wet muscle weight).

From these data it is apparent that one of the most obvious effects of the disease is to reduce the muscle weights and absolute tensions. According to the hypothesis, if SOL is spared and EDL is slowed with time one would expect to see EDL progressively taking on characteristics of the normal slow SOL and relatively unchanged effects upon SOL.

In order to interpret the information contained on Table 1 student's t tests were performed and only the core information is presented here. For a full analysis refer to the Appendix I which contains data obtained at room temperature.

TABLE I

The Effect of Muscular Dystrophy on the Weights and on
Absolute and Normalized Tensions of Fast and Slow Muscles
in Mice of Different Ages at 37°C (a) (b) (c)

Muscle Description	Muscle Weight (mgms)	37°C	
		Absolute Tension(gms)	Normalized Tension(gms/gm)
1. Direct Old C57 EDL (15)	11.0±0.3	6.4±0.4	595.5±34.5
2. Direct Old dy ^{2j} EDL (14)	6.6±0.4	4.7±0.4	725.4±54.7
3. Direct Young C57 EDL (14)	8.1±0.4	4.0±0.3	489.0±23.2
4. Direct Young dy ^{2j} EDL (13)	7.8±0.4	2.1±0.2	272.3±22.0
5. Direct Old C57 SOL (15)	12.3±0.4	6.1±0.4	488.8±26.3
6. Direct Old dy ^{2j} SOL (14)	6.4±0.6	3.4±0.3	564.1±42.4
7. Direct Young C57 SOL (11)	8.8±0.5	3.3±0.4	373.1±24.2
8. Direct Young dy ^{2j} SOL (12)	7.3±0.5	1.8±0.2	244.4±16.2

(a) Values are Mean ± SEM, values in parenthesis are N=Number of Mice

(b) Normalized Tension = gms tension/gm wet muscle weight

(c) Crossed Vertical bars indicate significance at the 5% level
with student "t" test

Dealing firstly with the muscle weights there is a clear trend that in all age groups studied the dystrophic muscles are lighter than control. It is further apparent that the young dystrophic mice are less impaired in this respect than are the older dystrophics presumably because in the young animals the disease progression has not yet advanced as far.

It is important to establish the situation in the normal SOL and EDL muscles before examining the dystrophics.

Looking at the young and old C57 EDL there is a weight gain with age and this is also reflected in an increased absolute tension (rows 1 and 3). The normalized tension increases as well which may indicate that the contractile mechanism itself develops to a greater extent with age. In the C57 SOL similar changes are observed showing that these muscles gain weight and develop greater absolute and normalized tensions with age (rows 5 and 7).

Thus having established the baseline situation for C57 muscle the dystrophic muscle can be examined in reference to this. Directing attention to the young animals it appears that while the young dy^{2j} EDL has not lost weight relative to its young control, it has lost both absolute and normalized tension (rows 3 and 4). This is interpreted as evidence that the contractile mechanism is being adversely affected at this early age. The young dystrophic SOL has lost weight as well as absolute and normalized tension (rows 7 and 8).

The older animals exhibit marked changes as well. In the old dy^{2j} EDL almost a 50% weight loss is incurred relative to control,

twitch tension drops about 1/3 but the normalized tension actually increases (rows 1 and 2). In the old dy^{2j} SOL similar losses occur in weight but the twitch tension drops by about 1/2 and the normalized tension doesn't change relative to control (rows 5 and 6). That the normalized tension should increase in the dy^{2j} EDL is rather curious and may reflect an enhanced activation of the contractile mechanism of the surviving fibers.

Finally to depict the changes occurring within the dystrophic categories themselves further t tests were used. The young dy^{2j} EDL weighs more than the old dy^{2j} EDL due to the severe wasting of dystrophy in the older muscle. The older dy^{2j} EDL however produces more absolute and normalized tension than the young dy^{2j} EDL (rows 2 and 4). Similar observations are made for the dystrophic soleus (rows 6 and 8). Why this should be so is not clear but it would appear on this basis that the younger dystrophic fast and slow muscles are more severely debilitated than the older muscles in terms of tension development.

To summarize the above information, it is clear that muscle mass and absolute muscle tension are lost in both fast and slow muscles in dystrophy as is commonly observed. It would appear that the slow muscle is affected at least as severely as the fast muscle in this respect. However, normalized tension is depressed in the young dy^{2j} fast and slow muscles relative to their age matched controls but is augmented in the old dystrophic relative to control. These basic data represent preliminary information required before a more detailed

study of the contractile properties of these fast and slow muscles can be undertaken, the most important of which is their speed of contraction.

b. Study of the Time Parameters of the Contraction

i. Introduction The most fundamental properties which define fast and slow muscles are their contraction times (CT) and one half relaxation times ($\frac{1}{2}$ RT). These parameters were examined in the dystrophic mouse for evidence of slowing in fast muscle and sparing (or small change) in the slow muscle as was recorded by Brust (1966) in the ReJ 129 mouse. If the above findings are also observed in the dy^{2j} strain of mouse a more detailed study of the nature of this occurrence would be undertaken.

ii. Mouse Contraction Times and Half Relaxation Times

The CT and $\frac{1}{2}$ RT data obtained for mouse SOL and EDL at 37° are given in Table 2 and as before the room temperature data are given in the Appendix number 2. The student's t test was used to determine significance and some of the more important comparisons are shown on the table. It was found as expected that the young and old C57 EDL muscles are significantly different from their age matched C57 SOL muscles although this comparison is not drawn out in Table 2.

The effect of maturation on the C57 EDL can be seen by examining rows 1 and 3. Since there is no significant difference in the young and old muscle times it would appear that these parameters were established and maintained at the same speeds over the time period examined in this study.

It is of interest that the C57 SOL (rows 5 and 7) develops a significantly slower CT as it ages. The $\frac{1}{2}$ RT however does not change.

TABLE 2

ISOMETRIC TWITCH

Contraction Times and Half Relaxation Times in Normal and
Dystrophic Mouse Fast and Slow Muscles at 37°C (a) (b)

Muscle Description	Contraction Time (msec)	Half Relaxation Time (msec)
1. Old C57 EDL (14)	9.0±0.4	8.6±0.5
2. Old dy ^{2j} EDL (14)	16.6±1.1	19.1±1.6
3. Young C57 EDL (14)	9.1±0.2	9.2±0.6
4. Young dy ^{2j} EDL (13)	11.7±0.5	11.8±1.6
5. Old C57 SOL (11)	20.6±0.9	23.1±1.1
6. Old dy ^{2j} SOL (14)	20.6±0.9	27.6±1.8
7. Young C57 SOL (11)	17.4±1.0	21.5±1.8
8. Young dy ^{2j} SOL (12)	14.9±0.4	16.7±0.8

(a) Values are mean ± SEM, figures in parenthesis are N=Number of muscles.

(b) Crossed vertical bars indicate significance at 5% level using student's t test.

It is likely that this baseline slowing of SOL with age is due to a shift with maturation toward a proportionately larger population of slow fiber types as seen in the rat (Kugelberg 1976).

From rows 3 and 4 it appears that dystrophy does indeed slow the CT of young dy^{2j} EDL which is in agreement with the findings of Brust (1966). The $\frac{1}{2}$ RT is not prolonged. The effect of the disease is seen even more clearly by examining rows 2 and 4. Here the old dy^{2j} EDL shows considerably more slowing of both CT and $\frac{1}{2}$ RT than does the young dy^{2j} EDL. The course of the disease would thus appear to bring out the slowing effect to an even greater degree.

The young dy^{2j} SOL on the other hand actually speeds relative to control (rows 7 and 8). This finding might not be anticipated according to the working hypothesis. The old dy^{2j} SOL does however conform to the hypothesis in that it appears reasonably unchanged relative to control. The $\frac{1}{2}$ RT is prolonged in this muscle.

In summary, it has been found from this preliminary study that for the most part the properties of dy^{2j} fast muscles do appear to shift towards those of slow muscles as previously suggested by Brust. The young dy^{2j} SOL speeded while the old dy^{2j} SOL slowed relative to control. Consequently a more detailed examination of the cause of these observations was considered worthwhile. In order to determine whether these observations were due to intrinsic or extrinsic fiber property changes the following exemplary fast and slow muscle properties were examined: cooling potentiation/depression, post tetanic potentiation/depression and the duration of active state plateau.

c. Cooling Potentiation/Cooling Depression

i. Introduction The first intrinsic muscle property to be presented is the cooling potentiation (CP) and cooling depression (CD) phenomenon. Fast and slow muscle responses are polarized with respect to this property. When cooled fast muscle normally develops a greater amount of tension while slow muscles develop the same or a lesser amount of tension. Fig. 3 illustrates the effect of lowering the bath temperature from 37° to room temperature in C57 and dy^{2j} fast and slow muscles.

ii. Mouse Cooling Potentiation/Depression Table 3 shows the data obtained for mouse fast and slow muscle. To first confirm that these muscles really do differ in their responses, it was found by t testing that the young and old control EDL responses were significantly different from the respective control SOL responses ie the EDL developed a CP response while the SOL developed either a CD response or showed no change. Secondly, there was a difference between the young and old C57 EDL (rows 1 and 3) and the young and old C57 SOL (rows 5 and 7). The young C57 EDL showed the fastest response, (largest CP) and the old C57 SOL the slowest response (largest CD). These differences are probably due to muscle changes associated with maturation. It is however curious that the directions of the shift of these properties with age should be in opposite directions for the two types of muscle.

Figure 3 Isometric contractions of mouse fast and slow muscles at room temperature and at 37°C (showing cooling potentiation/cooling depression).

Insets: Vertical bars = 1 gram tension.
Horizontal bars = 20 msec.

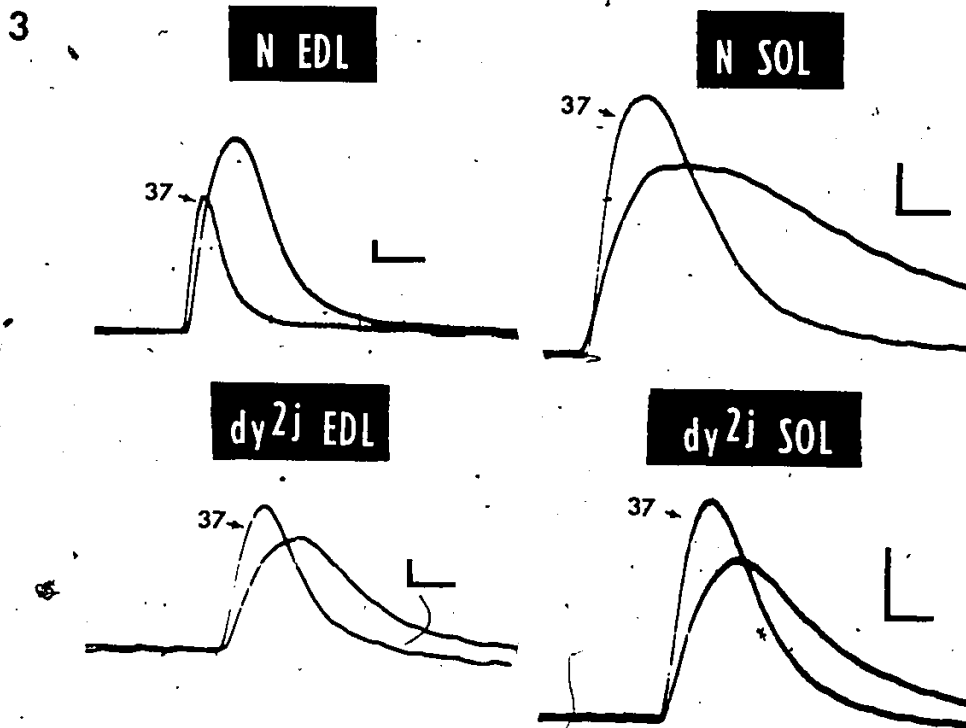


TABLE 3

Cooling Potentiation and Cooling Depression in Normal and
Dystrophic Mouse SOL and EDL Muscle (a) (b)

Muscle Description			Tension at RmT. (gms)	Tension at 37°C (gms)	Twitch Tension at RmT. as a Percent of 37°	
1.	Old C57 EDL	(15)	9.0±0.5	6.4±0.4	143.5±4.9	] X]
2.	Old dy ^{2j} EDL	(14)	4.6±0.4	4.7±0.4	98.6±4.5	
3.	Young C57 EDL	(14)	6.6±0.4	4.0±0.3	169.9±7.2	] X]
4.	Young dy ^{2j} EDL	(13)	3.1±0.3	2.1±0.2	149.0±6.0	
5.	Old C57 SOL	(15)	5.2±0.3	6.1±0.4	88.6±2.6	] X]
6.	Old dy ^{2j} SOL	(14)	3.0±0.2	3.4±0.3	88.0±2.5	
7.	Young C57 SOL	(11)	3.3±0.3	3.3±0.4	102.8±5.4	] X]
8.	Young dy ^{2j} SOL	(12)	2.2±0.1	1.8±0.2	125.0±6.2	

(a) Values are mean ± SEM, numbers in brackets are number of animals.

(b) Significance at 5% level using student's t test is indicated by
crossed vertical bars. RmT = room temperature.

Thus having described the situation in the normal animals a comparison can now be made with the dystrophic muscle. While the young dy^{2j} EDL demonstrates significantly less CP than does its control, it nevertheless still shows some CP, (row 4). Probably the slowing effect of dystrophy has not yet had sufficient time to exert its full influence. The old dy^{2j} EDL which has been subjected to the disease for a longer period of time shows no change as the temperature is varied which is a response much like a slow muscle's. The old dy^{2j} EDL shows much less CP than does the young dy^{2j} EDL (rows 2 and 4) which would also indicate that further slowing occurs as the disease progresses.

Interestingly, in the young dy^{2j} SOL an enhanced CP is observed compared to the controls (rows 7 and 8). This is in agreement with the speeding of contraction time data which was previously presented. It should be reemphasized however that the young C57 SOL shows relatively fast characteristics to start with.

The old dy^{2j} SOL is not significantly different from the control, possibly indicating that a small effect if any has occurred to the slow muscle response (rows 5 and 6). The old dy^{2j} SOL shows significantly more cooling depression than does the young SOL since the young SOL speeded.

In summary, it would appear that the CT slowing of the dy^{2j} EDL of both age groups has been substantiated by the CP/CD data. The old SOL shows small change with dystrophy, however the young dy^{2j} SOL showed speeding as it did for the contraction parameters.

d. Post-Tetanic Potentiation/Post-Tetanic Depression

i. Introduction Since post-tetanic potentiation (PTP)

is a characteristic of fast muscle and post tetanic depression (PTD) is a characteristic of slow muscle these properties were also investigated to help elucidate the nature of the changes occurring in the dystrophic muscle. If for instance the fast muscle contraction speed shift toward slow muscle is accompanied by a shift of post-tetanic response toward slow muscle properties this could be interpreted as evidence that the fibers themselves have undergone intrinsic property changes.

ii. The First Post Tetanic Twitch in Mouse

PTP is most evident at 37°C in fast muscle because the more complete activation of this muscle at room temperature precludes its presence. Table 4 shows data obtained at 37°C for the first post-tetanic twitch only in the different muscle categories. Since PTP and PTD are maximal shortly after the tetanus and then decay, these data obtained approximately five seconds post-tetanus are as close to the maximal responses as was possible using the equipment available. The data obtained at room temperature are examined in Appendix 3.

From Table 4 it is of interest that both the young and the old control EDL values display significantly more PTP than their SOL counterparts (Statistical tests not shown on Table 4). Thus the C57 EDL has a rather strong PTP response (rows 1 and 3) while the

TABLE 4

First Post-Tetanic Twitch as a Percent of Pretetanus in
Normal and Dystrophic Mouse, Fast and Slow Muscle at 37°C

(a) (b)

Muscle Description	First Post Tetanic Twitch as a Percent of Pretetanus at 37°C
1. Old C57 EDL (11)	131.7±5.1
2. Old dy ^{2j} EDL (13)	107.4±2.1
3. Young C57 EDL (12)	142.7±4.4
4. Young dy ^{2j} EDL (10)	124.4±2.7
5. Old C57 SOL (10)	102.7±1.8
6. Old dy ^{2j} SOL (8)	105.0±3.3
7. Young C57 SOL (10)	110.3±2.1
8. Young dy ^{2j} SOL (11)	111.1±1.8

(a) Values are mean ± SEM, values in parenthesis are N=Number of muscles.

(b) Crossed vertical bars indicate significance at 5% level using student's t test.

C57 SOL exhibits a lesser response (rows 5 and 7). It is probably not possible to assign a true PTD response to the C57 SOL values since if anything they show a slight tendency toward PTP. This outcome can be explained if one recalls that the SOL does have a rather large fast muscle component. It is likely that the two types of components work in opposite directions so that the net response would really reflect to some degree the ratios of these components in the muscle. Since the SOL has a larger population of fast fibers than slow fibers (see histochemistry section) one would perhaps anticipate the small net amount of PTP observed. Likewise as previously mentioned if the old C57 SOL has a roughly equal proportion of fast and slow fibers (see histochemistry section) then one could expect the observation of no change post-tetanus as was found here.

Now, considering the muscle types separately there was no difference in response between the young and old C57 EDL muscles (rows 1 and 3) as was the case with the C57 SOL muscles (rows 5 and 7). This might be interpreted as evidence that these properties mature early in these mice and remain stable over the age groups examined.

The young dy^{2j} EDL shows significantly less PTP than control (rows 3 and 4) as does the old dy^{2j} EDL (rows 1 and 2). Also, since the old dy^{2j} EDL shows less PTP than does the young dy^{2j} EDL (rows 2 and 4) it would appear that as the disease advances there is a progressive shift of fast to slow muscle properties in the EDL.

In the old dy^{2j} SOL there is no significant difference from the control which could indicate a sparing of slow properties (rows 5 and 6).

There is no difference between the control and diseased young SOL muscles but, as previously noted both of these demonstrate rather "fast" characteristics.

Finally, there is no difference between the young and old SOL values indicating that the effect of the disease has been relatively small regarding this property.

In summary, data gathered on the first twitch post tetanus have suggested that the disease does transform the intrinsic fiber type properties of the fast muscle and leaves those of slow muscle relatively intact. One further bit of information was also derived from the post tetanic phase of these experiments. It was found that the CT and $\frac{1}{2}$ RT of the post-tetanic twitches were not statistically altered by the tetanus for any category of muscle examined. For this reason a detailed chart was not included. In the EDL which demonstrates an increase in twitch tension post-tetanus (PTP) but no change in CT or $\frac{1}{2}$ RT, this must mean that the average rate of rise has increased and this may be interpreted as an increase in activation of the muscle. In addition, this may be an indication that the rate of rise of the active state curve has changed. In the section following the PTP/PTD study a portion of the active state curve was investigated, this was the Duration of the Active State Plateau or DASP.

iii. Further Characterization of the Post-Tetanic Potentiation/Post-Tetanic Depression Response

As previously noted, since the PTP/PTD response is maximal very soon after a tetanus it is desirable to use the first post-tetanic twitch as the reference point from which fast and slow muscle differences are measured. In addition, it is a fundamental property of fast and slow muscle to decay at a characteristic rate. It is important also to insure that the decay of these curves is stable and reproducible and that there is an acceptable return to pre-tetanic levels. Any experiments which did not return to 75% of pre-tetanic tension within 10 minutes post-tetanus were discarded.

Therefore, in order to further characterize and insure the reproducibility of these phenomena the time course of decay was monitored for a period of 10 minutes post-tetanus.

The following figures depict the time course of PTP decay in the muscle categories studied at 37°C.

Fig. 4 shows the interrelationships between the young C57 EDL, dy2j EDL, C57 SOL and dy2j SOL muscles and the time course of decay of their post-tetanic responses. The young C57 EDL clearly demonstrates the greatest amount of PTP while the C57 SOL shows the least. The dy2j EDL however has made a noticeable shift toward the C57 SOL ie toward the normal response of the mouse slow muscle. It would thus appear that there is a conversion of intrinsic muscle properties taking place.

It is of interest that the young dy^{2j} SOL demonstrates once more its tendency to behave like a fast muscle. The decay curve for this muscle shows considerable PTP and takes a long time to lose its potentiation. The young C57 SOL also shows some fast muscle inclinations.

Fig. 5 depicts the situation in similar fashion to Fig. 4 except that old muscles are considered. The most noteworthy aspect of this graph is that the old dy^{2j} EDL shifts toward the C57 SOL to an even greater extent than was seen in the young dy^{2j} EDL. This suggests that the slowing effect is time related and intensifies as the disease progresses. Moreover, the time course of the old dy^{2j} SOL is unchanged relative to its control.

The curves obtained for the room temperature data are also included in Figures 6 and 7. An important characteristic illustrated by these graphs is the general compression of response around the 100% level. At 37°C the post-tetanic responses are usually larger.

In Figures 6 and 7 the dy^{2j} EDL swings from control toward the C57 SOL values, again illustrating their tendencies toward slowness. The young dy^{2j} SOL swings toward the C57 EDL.

Figure 7 is also a compressed version of Figure 5. Here the old dy^{2j} EDL has made a larger shift toward the C57 SOL than had the young dy^{2j} EDL. Also here the old dy^{2j} SOL shows a small tendency to speed up by approaching the C57 EDL values.

In summary the graphs have shown in a detailed manner some of the tendencies of the different muscles and have verified that the post tetanic decay of the muscles proceeds with reproducibility.

Figure 4
Time course of PTP decay in Young mouse, C57 and dy^{2j} EDL and in C57 and dy^{2j} SOL stimulated directly at 37°C.

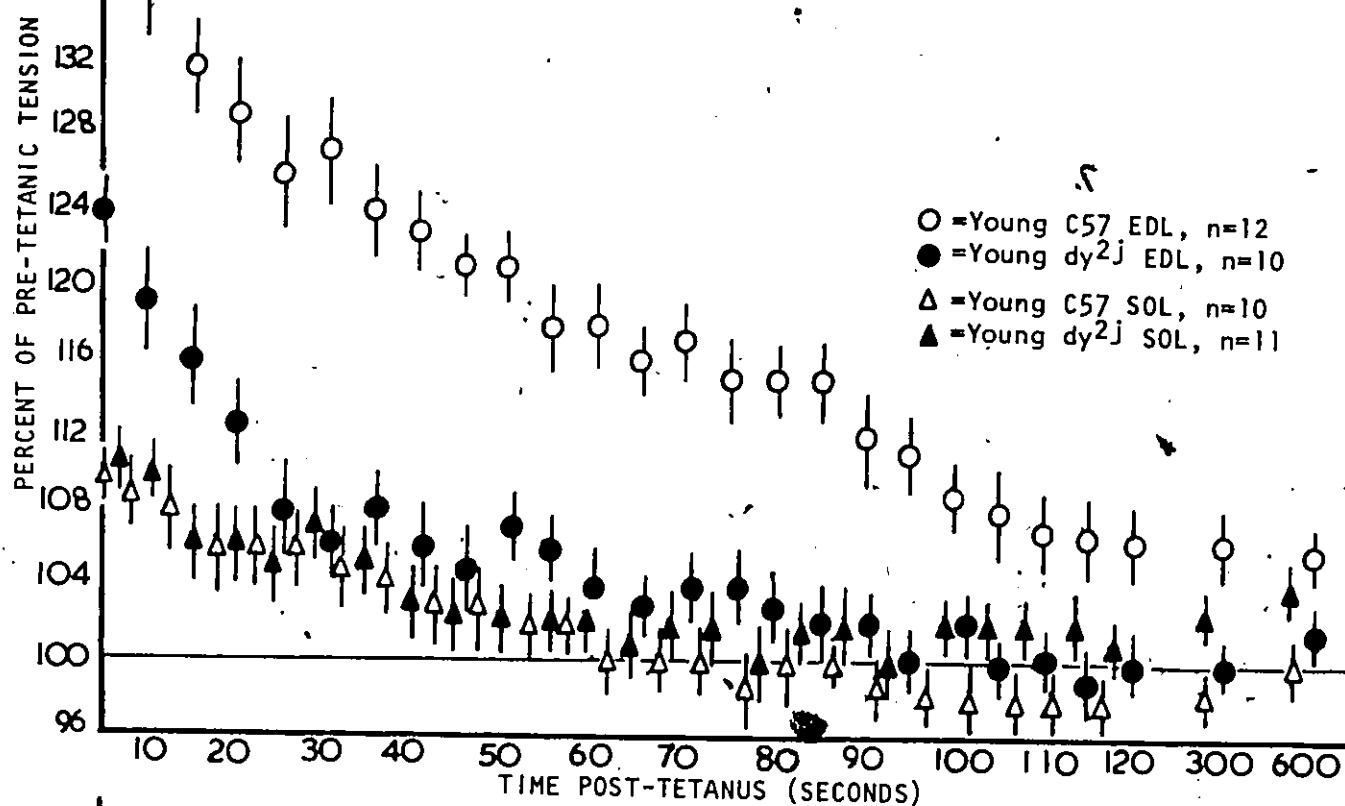
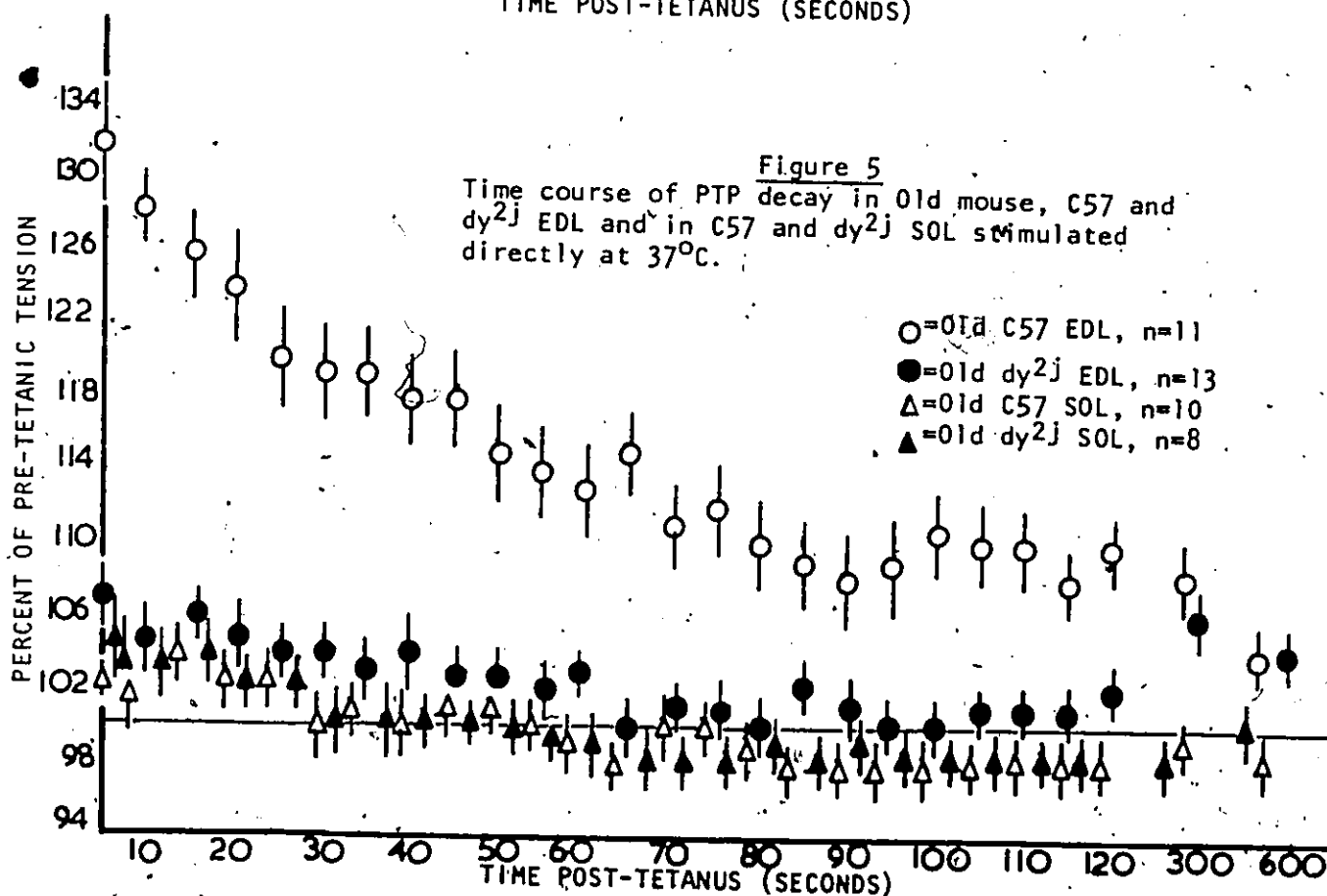
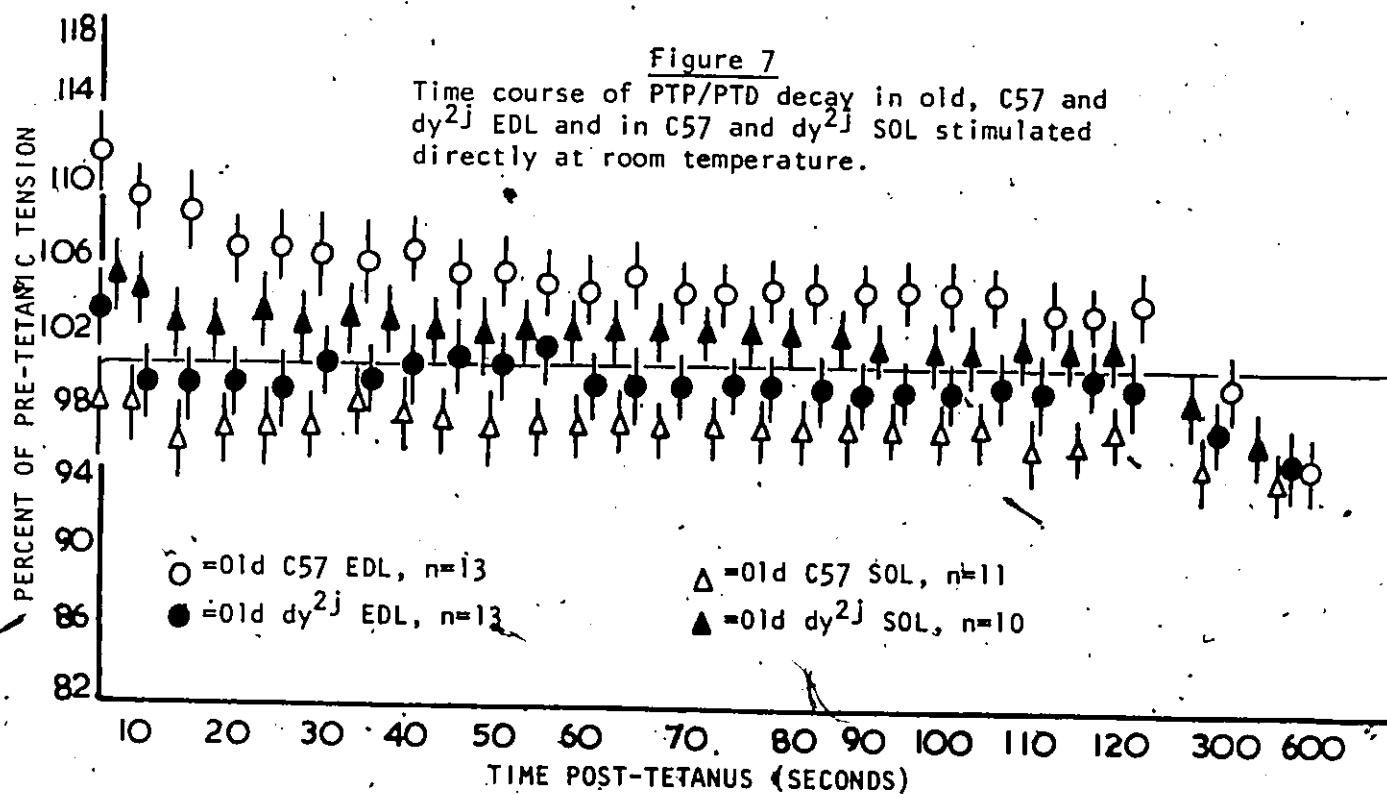
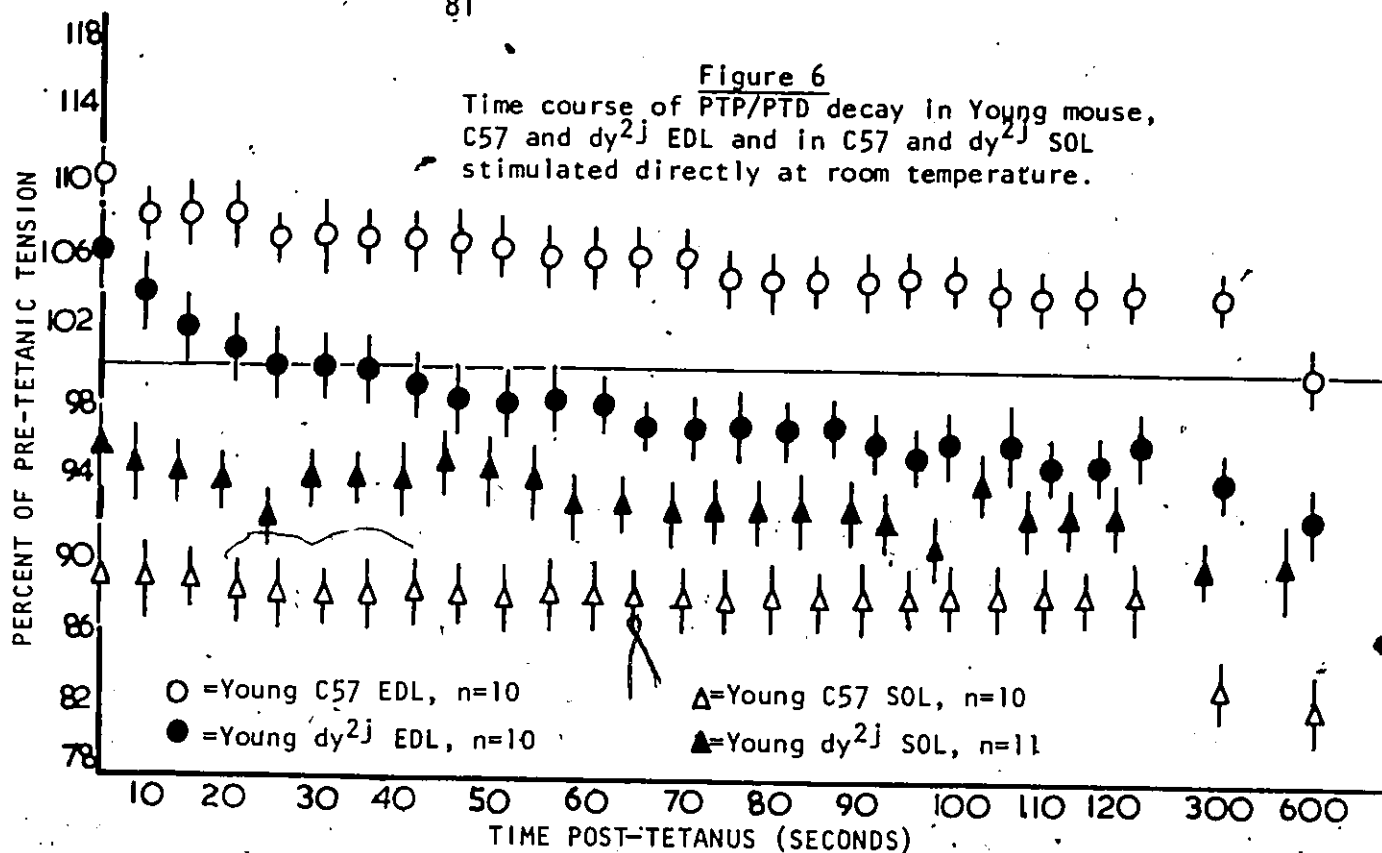


Figure 5
Time course of PTP decay in Old mouse, C57 and dy^{2j} EDL and in C57 and dy^{2j} SOL stimulated directly at 37°C.





e. Duration of the Active State Plateau

i. Introduction Fast and slow muscles normally have characteristic differences in their duration of active state plateaus (DASP), the fast muscle having a shorter DASP than the slow muscle. It was thought that a conversion of this property could be possible in dystrophy. The observed slowing of fast muscle could be in part due to a prolongation of a portion of the active state curve, possibly the DASP, and this could reflect changes in the degree of activation of this muscle.

ii. DASP in the Mouse Figure 8a,b contains two photographic records of a typical DASP measurement in the mouse. The upper photo shows at a slow sweep speed the muscle contraction and its first derivative. Superimposed upon this (and of greater amplitude) is the muscle contraction and its derivative resulting from a pair of closely timed stimuli.

The lower photo shows at faster sweep speed the point of separation of the two superimposed derivatives. The time from first deviation of the baseline to the point of separation is considered to be the DASP, and this is indicated on the photo.

Table 5 gives the values obtained for the DASP of the old C57 and dy^{2j} fast and slow muscles at room temperature and compares these values for significance using the student's t test. The DASP values were measured at room temperature so that the contractions would be slower and thereby improve the accuracy of the technique.

Figures 8a & b

- a. Typical photograph of isometric contraction and first derivative upon which is superimposed the contraction and derivative resulting from two closely timed stimuli.
- b. The lower photo taken at the faster (1 msec/division) sweep speed shows a typical measurement of the DASP in C57 EDL.

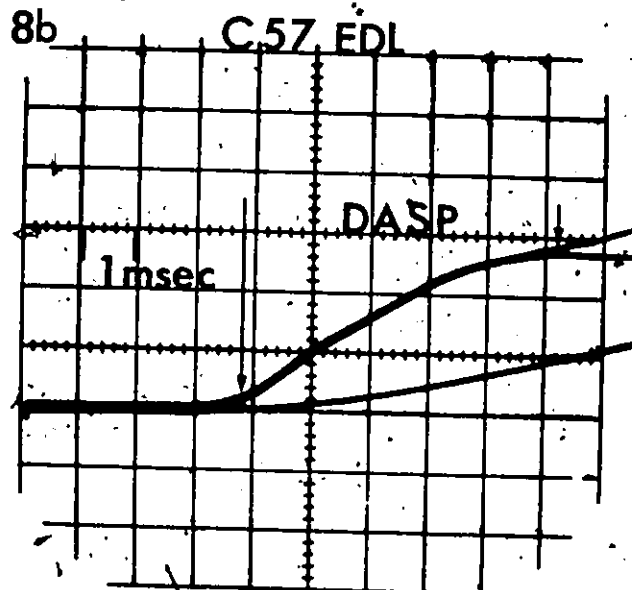
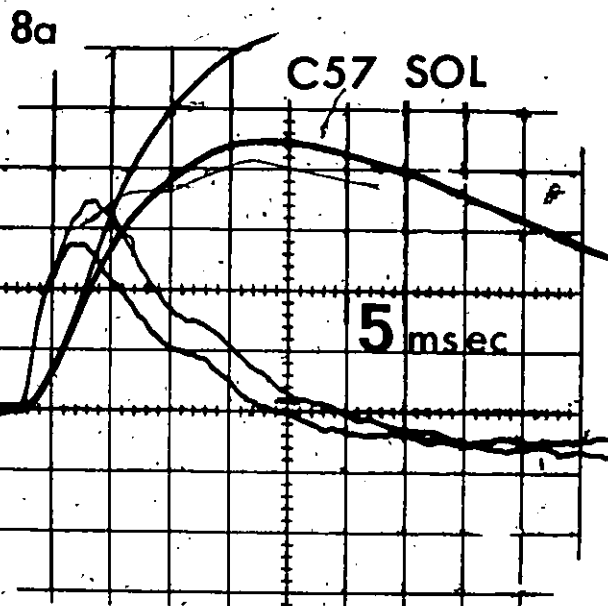


TABLE 5

Duration of Active State Plateau at Room Temperature in
Normal and Dystrophic Mouse Fast and Slow Muscle (a)

Muscle Description	DASP	Significance Level
1. Old C57 EDL (6)	7.2±0.3	
2. Old dy ^{2j} EDL (10)	7.4±0.3	
3. Old C57 SOL (7)	8.3±0.3	
4. Old dy ^{2j} SOL (10)	8.2±0.3	

(a) Values are mean ± SEM. Crossed vertical bars indicate significance at the 5% level using the student's t test. Values in parenthesis are N=Number of animals.

Similarly old animals were used so the changes, if present would be fully developed.

Table 5 shows that the normal EDL and SOL muscles are significantly different, confirming previous reports that fast muscle has a shorter DASP than does the slow muscle (rows 1 and 3).

The table also indicates that the dy^{2j} EDL is not different from control (rows 1 and 2) and the dy^{2j} SOL is not different from control (rows 3 and 4). This outcome is somewhat surprising in the case of the EDL because the previous data has shown a conversion of intrinsic muscle properties. In the SOL on the other hand, one might not anticipate any change if indeed the hypothesis is correct. The dy^{2j} EDL and the C57 SOL are dissimilar (rows 2 and 3) as are the dy^{2j} EDL and the dy^{2j} SOL (rows 2 and 4), the dy^{2j} EDL values being smaller. This is not unexpected in view of the finding that the control values were different from each other and that the respective dystrophic values were not different from their controls.

To summarize the DASP findings, no indication of a change in this property is seen in dystrophy. However, the bulk of the data presented thus far has indicated that there is a property shift in fast muscle and basically no change in the slow muscle.

One problem alluded to previously is that the mouse SOL is not a pure slow muscle because of its large fast fiber content. Indeed, on the basis of fiber type content it would probably be more accurate to call the SOL a fast muscle (see histochemistry section). Therefore it would be necessary to examine a muscle which is composed of entirely

slow fibers to illustrate how an ideal slow muscle should respond.

The guinea pig SOL is such a muscle and this muscle was chosen for this purpose. However, before it is possible to use the guinea pig data as a reference for slow muscle several considerations are necessary. Of primary importance is the problem that the guinea pig SOL is too large to stimulate directly as can be done in the case of the mouse SOL, and stimulation must be carried out via its motor nerve. Therefore, a parallel set of mouse experiments were carried out using indirect stimulation to investigate whether this form of stimulation causes any significant changes in the muscle responses. In this manner an attempt is made to reduce the likelihood of error in such an intraspecies comparison and these results are presented next.

B. Guinea Pig

a. Preliminary Study of Indirect Stimulation in Mouse Soleus

i. Introduction Old mouse SOL muscles from C57 and dy^{2j} animals were stimulated indirectly. The following parameters were examined for evidence of deviation from their directly stimulated counterparts: 1. absolute tension 2. normalized tension 3. CT 4. $\frac{1}{2}$ RT 5. PTP/PTD and 6. CP/CD. The muscles were stimulated at 37°C and at room temperature, the latter data are once again deferred to the Appendix 4 with the exception of the PTP/PTD time course of decay curves. Data are presented here in Table 6.

ii. Indirect Stimulation of Mouse Muscle The absolute tensions were not altered for C57 and dy^{2j} SOL muscles when indirectly stimulated, nor were the normalized tensions. The CT and $\frac{1}{2}$ RT values were faster in the indirectly stimulated C57 SOL but the dy^{2j} SOL showed no change in these parameters. The cause of this is not readily apparent. There was no change in the PTP/PTD response for the first twitch post-tetanus. Figures 9 and 10 depict the time course of decay of PTP/PTD in C57 and dy^{2j} SOL muscles using both methods of stimulation at both 37°C and at room temperature. From these figures it is seen that indirect stimulation causes very little change in the pattern of response at 37°C and has extremely limited effect at room temperature.

Finally, the CP/CD data show considerable change when indirectly stimulated. While the directly stimulated muscles display substantial

TABLE 6

Effect of Indirect Stimulation on Normal and Dystrophic
Mouse SOL Muscle at 37°C (a)

Physiological Parameter	Indirect C57 SOL/ Direct C57 SOL	Indirect dy ^{2j} SOL/ Direct dy ^{2j} SOL
Absolute Tension (gms)	5.1± 0.3 (7) 6.1± 0.4 (15)	3.2± 0.4 (7) 3.4± 0.3 (14)
Normalized Tension (gms/gm)	416.5±29.8 (7) 488.8±26.3 (15)	617.6±63.3 (7) 564.1±42.4 (14)
CT (msec)	17.6± 1.0 (7) 20.6± 0.9 (11) *	20.7± 0.9 (7) 20.6± 0.9 (14)
½RT (msec)	18.6± 0.5 (7) 23.1± 1.1 (11) *	25.6± 2.2 (7) 27.6± 1.8 (14)
PTP/PTD (%)	104.0± 1.6 (7) 102.7± 1.8 (10)	105.7± 3.0 (7) 105.0± 3.3 (8)
RmT. as % 37°C	102.0± 2.5 (7) 88.6± 2.6 (15) *	97.5± 2.7 (7) 88.0± 2.5 (14) *

(a) Values are mean ± SEM, numbers in parenthesis are numbers of muscles. Asterisk denotes significance at 5% level using student's t test. RmT = room temperature

Figure 9
Time course of PTP decay in directly and indirectly stimulated, old, C57 and dy^{2j} mouse SOL at 37°C.

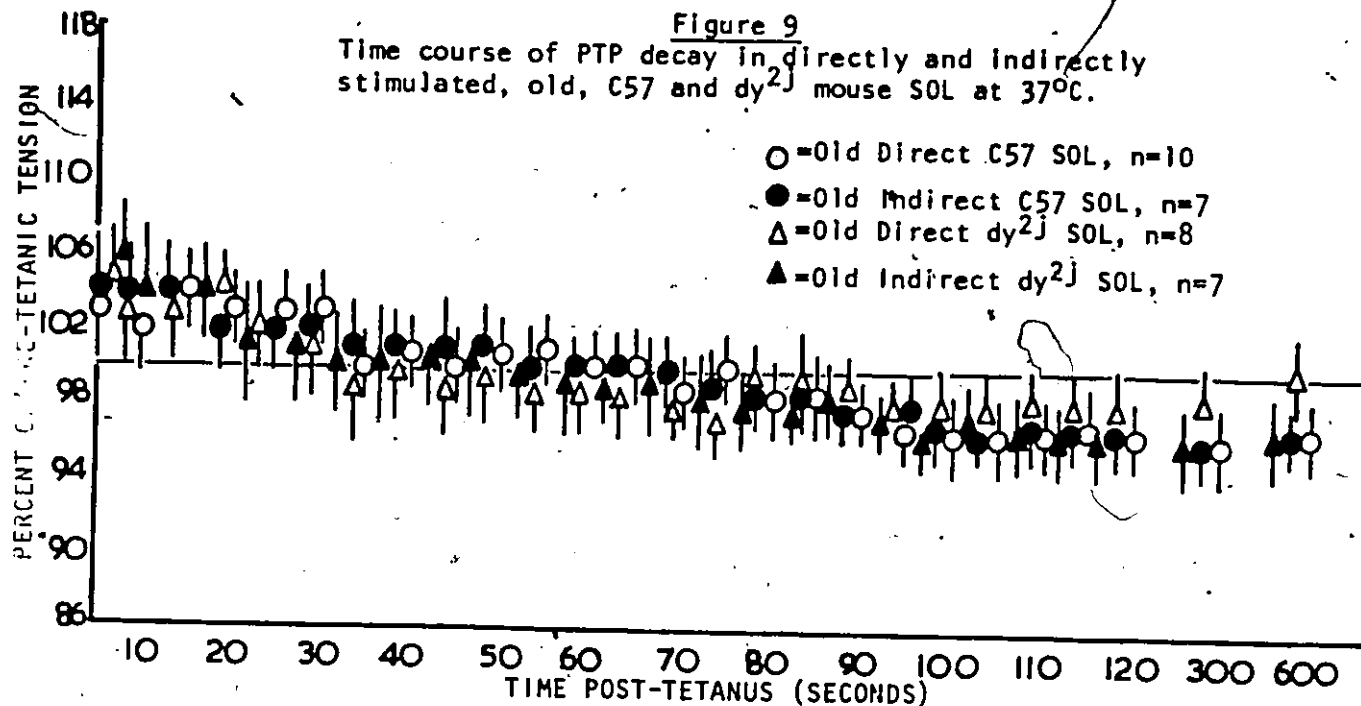
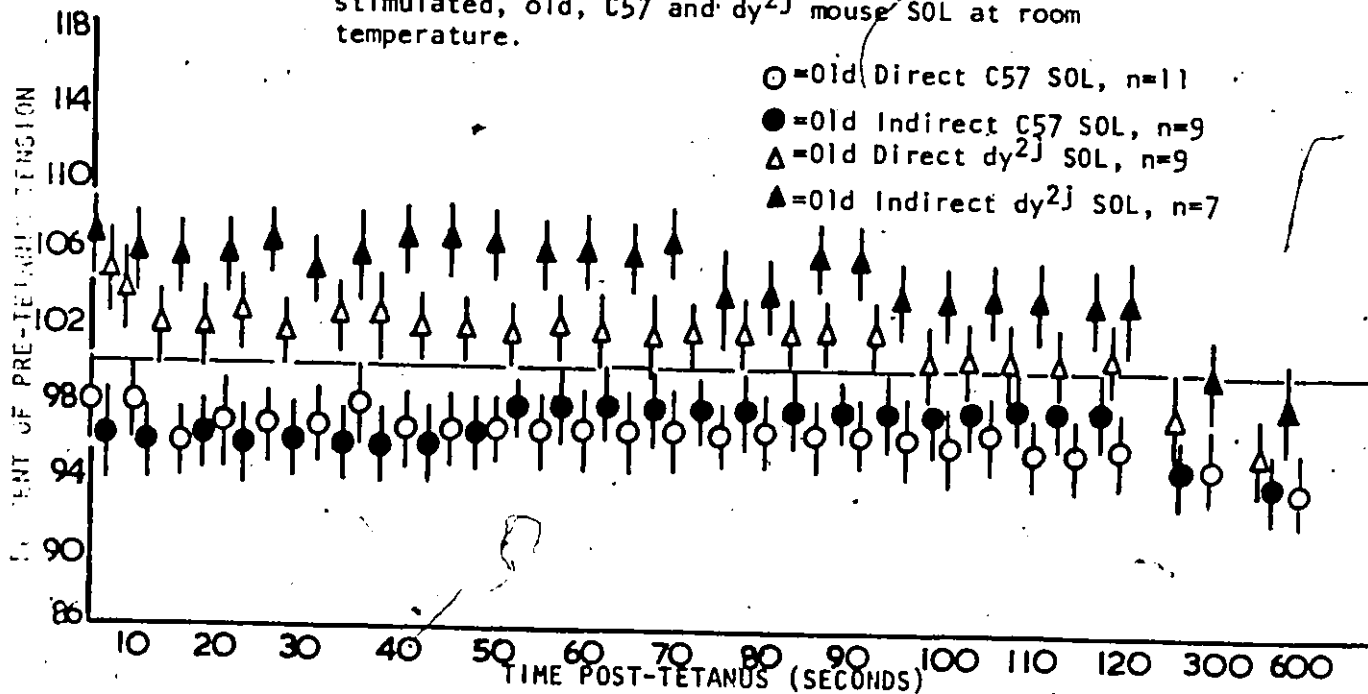


Figure 10
Time course of PTP/PTD decay in directly and indirectly stimulated, old, C57 and dy^{2j} mouse SOL at room temperature.



CD the indirectly stimulated muscles show virtually no change due to temperature. This may be interpreted as a swing toward faster characteristics in indirect C57 SOL at least, is consistent with the observed faster CT and $\frac{1}{2}$ RT. The dy^{2j} SOL does not however show a CT and $\frac{1}{2}$ RT speeding concomitant to the faster appearing CP/CD response. The cause of this is unclear.

b. Physiological Study of the Guinea Pig Soleus

i. Introduction Since the guinea pig soleus is a true slow muscle it was thought that it could provide a useful reference from which one could base comparisons about the degree of slowness of the mouse muscles studied. If the physiological and histochemical data acquired from this muscle are taken as the extreme of slowness then the closer the other muscles approach this data, the slower are their properties. This type of comparison is necessary because of the apparent lack of any true slow muscles in the mouse from which to gauge slowness.

ii. Guinea Pig Soleus Properties Having considered some of the factors necessary for the intraspecies comparison, Table 7 gives the physiological data obtained at 37° from eight guinea pig muscles. The room temperature data are given in Table 8.

Using the student's t test there was no difference between the pre and post-tetanic CT and the pre and post-tetanic $\frac{1}{2}$ RT values. As well the CT and $\frac{1}{2}$ RT values were significantly faster at 37° than at room temperature so that tables were not included to compare this information. Table 7 shows that the guinea pig SOL weighs about 200mg at 6 weeks of age, develops about 28 grams of absolute tension and 150gms/gm of normalized tension. There is a very strong cooling depression and this was not seen to such a great extent in the mouse. There is about 10% PTD which is considered to represent the normal slow muscle response under the given conditions (see discussion).

TABLE 7

Physiological Characteristics of Normal 6 Week Old Guinea
Pig Soleus Stimulated Indirectly at 37°C (a)

Physiological Property	Numerical Value
1. Muscle Weight (mgms)	206.2±20.4
2. Absolute Tension (gms)	27.8± 2.1
3. Normalized Tension (gms/gm)	143.7±25.0
4. Tension at RmT as % 37°C	56.8± 0.9
5. PTD (%)	91.4± 2.5
6. CT (msec)	54.0± 1.4
7. ½RT (msec)	40.9± 2.0

(a) Values are mean ± SEM, N=8=Number of animals. The post-tetanic CT and ½RT values are not significantly different from pre-tetanic using 5% level student's t test. RmT = room temperature.

TABLE 8

Physiological Characteristics of Normal 6 Week Old Guinea
Pig Soleus Stimulated Indirectly at RmT. (a)

Physiological Property	Numerical Value
1. Muscle Weight (mgms)	206.2±20.4
2. Absolute Tension (gms)	16.3± 1.1
3. Normalized Tension (gms)	80.8±13.9
4. PTD (%)	99.5± 1.0
5. CT (msec)	103.6± 3.5
6. ½RT (msec)	85.0± 3.9

(a) Values are mean ± SEM, number of animals = 8.

The CT and $\frac{1}{2}$ RT are greatly prolonged in relation to the mouse SOL and this is partially due to the larger linear dimensions (see discussion section).

Figures 11 & 12 compare the time courses of PTD decay of the guinea pig SOL with the decay of PTP response of the C57 mouse SOL at 37°C and at room temperature. The two plots initially start at different levels but merge with each other as the curves decay.

Table 8 contains the physiological data for guinea pig SOL obtained at room temperature. In comparison to the 37°C data there is a striking increase in CT and $\frac{1}{2}$ RT values. There is less PTD to the extent that the post-tetanic tension is not different from the pre-tetanic tension.

In summary, the guinea pig SOL characteristics as shown here represent properties of a true slow muscle. These properties will be used as a bench mark to rank other muscles for slowness in the following section.

Figure 11

Time course of PTP/PTD decay in old indirectly stimulated mouse C57 SOL and in normal 6-8 week old guinea pig SOL at 37°C.

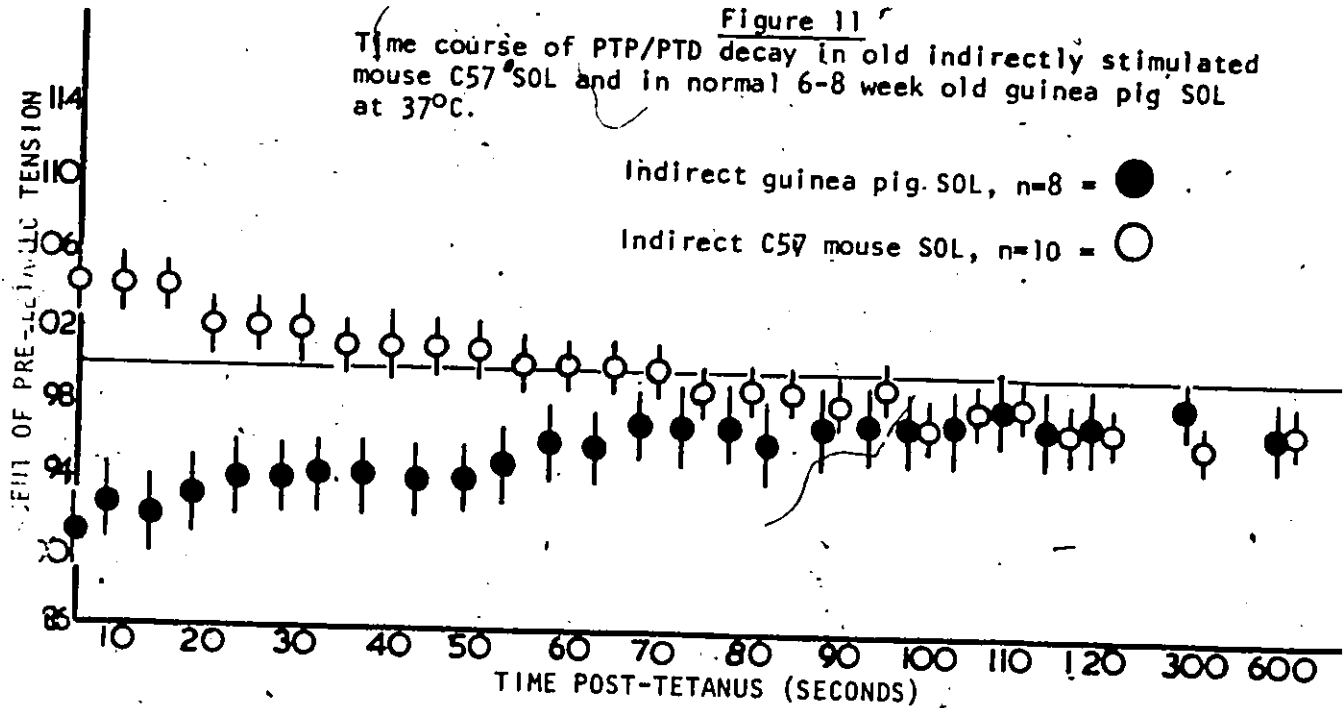
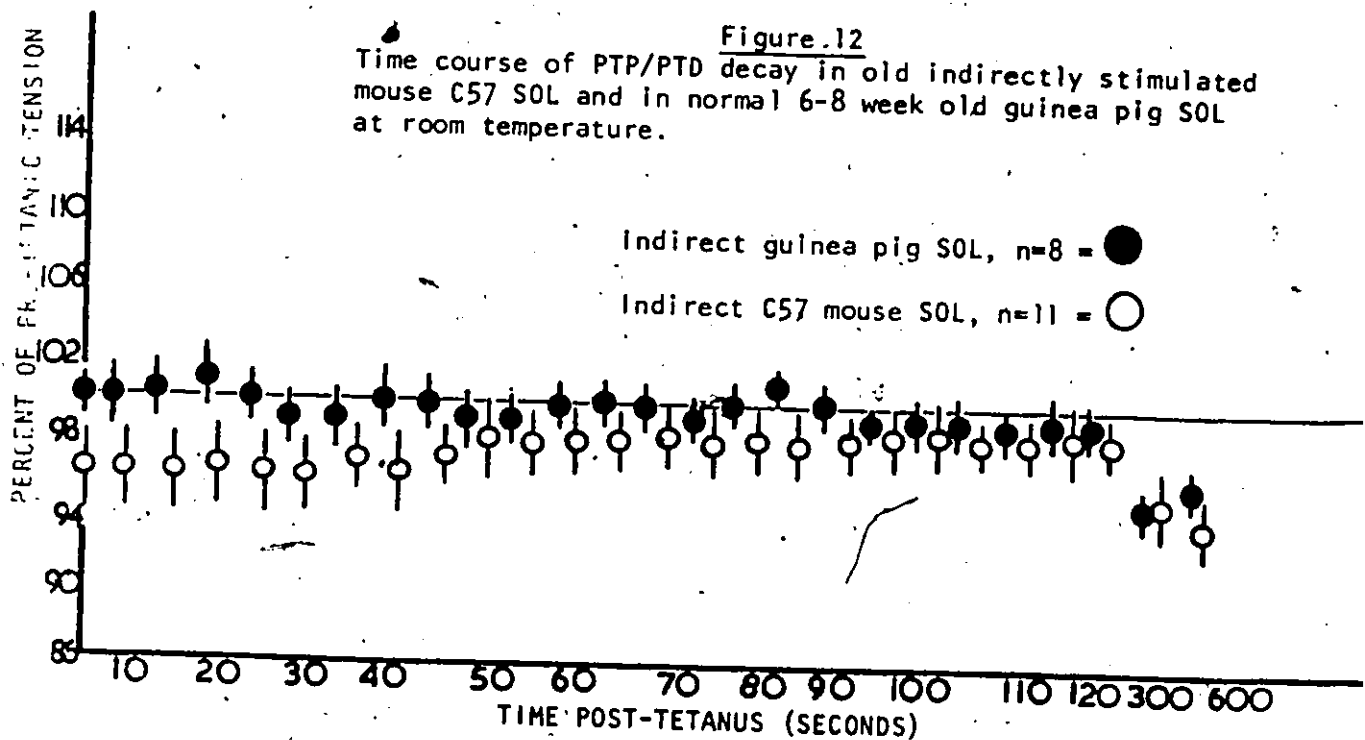


Figure 12

Time course of PTP/PTD decay in old indirectly stimulated mouse C57 SOL and in normal 6-8 week old guinea pig SOL at room temperature.



c. Physiological Ranking of Muscles for Degree of Slowness

i. Introduction Since it does appear that slowing occurs in the dy^{2j} EDL and the old dy^{2j} SOL is slowed slightly, an attempt is made here to quantify these changes. The muscles examined have a spectrum of physiological properties associated with muscle "speed". At the extremes, the C57 EDL is a fast muscle while the guinea pig SOL is a slow muscle. But, the C57 SOL, dy^{2j} SOL, and dy^{2j} EDL show intermediate tendencies. Using the C57 EDL and guinea pig SOL as end points the muscles were rated on the basis of several parametric and non-parametric variables for degree of slowness. In this manner it becomes clear that the C57 SOL is physiologically only intermediate in terms of slowness (further corroborated by histochemistry). Additionally, the dy^{2j} muscles have a slower nature than their control counterparts.

It should be emphasized that the properties chosen to judge muscle speed are not necessarily all of the same merit. Obviously the CT and $\frac{1}{2}$ RT are the best and most basic properties examined while some of the others may reflect more subtle aspects of the nature of these muscles. The problem is complicated also by the different sizes of fiber type populations in each muscle type.

ii. Ranking the Muscles The muscles have been analysed in four ways to give an idea of how they rank in terms of degree of slowness. These are: 1. table of means $\pm$ 95% confidence intervals, 2. ranking (1-5) by means, 3. t testing, 4. analysis of variance.

Table 9 lists the properties examined in this study which are

TABLE 9

Physiological Properties Associated With Muscle Speed in
Various Categories of Muscle (a)

Physiological Property	Old C57 EDL	Old dy ^{2j} EDL	Old C57 SOL	Old dy ^{2j} SOL	4-6 week G.Pig SOL
CT at RmT	20.3±3.0	34.6± 6.7	33.4±3.2	38.4± 4.0	103.6±8.3
½RT and RmT	15.0±2.8	50.8±21.7	45.6±8.4	64.1±13.3	85.0±9.2
CT at 37°C	8.1±0.9	19.3± 4.3	17.6±2.4	20.7± 2.1	54.0±3.3
½RT at 37°C	7.8±0.9	24.4± 8.3	18.6±0.9	25.6± 5.2	40.9±4.7
Tension at RmT as % 37°C	143.5±4.9	98.6± 4.5	88.6±2.6	88.0± 2.5	56.8±0.9
First Twitch PTP/ PTD at RmT	113.6±5.4	93.1± 9.7	96.2±3.8	106.6± 6.4	99.5±2.4
First Twitch PTD at 37°C	151.1±6.9	105.2±16.2	104.0±3.8	105.7± 7.1	91.4±5.9
DASP at RmT	7.2±0.7	7.4± 0.7	8.3±0.7	8.2± 0.7	Not Avail.

(a) Values are mean ± 95% confidence interval. RmT = room temperature.

considered to be representative of muscle speed and displays the data obtained for each of the muscles studied. Here the 95% confidence interval was used to provide a particularly stringent criterion for visual evaluation of significant differences. It is assumed that the degree to which the intermediate speed muscles approach either the ideal fast or slow muscle data is an indication of their relative tendencies. Table 9 shows the fast muscle properties of C57 EDL to consist of a fast CT and $\frac{1}{2}$ RT, a strong CP, a strong PTP and a short DASP. The slow properties of the guinea pig SOL are the opposite of those just described.

Table 10 ranks the muscles by their means in order of slowness by assigning a value from 1 to 5, 5 being the slowest score. The respective standard errors are not considered here and the table is presented merely to give a non-parametric perspective of the tendencies. The C57 SOL has several values which rate at the fast end of the scale. The dy^{2j} EDL ranks 3rd slowest on several of the properties.

Table 11 uses student's t test to examine pertinent muscle comparisons. Several interesting points arise here, in particular the finding that for most properties the dy^{2j} EDL has slowed to the point that it is indistinguishable from the C57 SOL.

The C57 EDL is faster than the dy^{2j} EDL on all but one criterion. The C57 SOL was faster than the dy^{2j} SOL in the CT and $\frac{1}{2}$ RT parameters only. Finally, the dy^{2j} EDL was not significantly faster than the dy^{2j} SOL in any property examined.

TABLE 10

Ranking by Means For Degree of Slowness in Certain Muscle
Types (a)

Physiological Property	C57 EDL	dy ^{2j} EDL	C57 SOL	dy ^{2j} SOL	G. Pig SOL
CT at RmT	1	3	2	4	5
½RT at RmT	1	3	2	4	5
CT at 37°C	1	3	2	4	5
½RT at 37°C	1	3	2	4	5
Tension at RmT as % 37°C	1	4	2	3	5
First Twitch PTD at RmT	1	3	4	2	5
First Twitch PTD at 37°C	1	3	4	2	5
mean (x)	1	3.1	2.6	3.3	5
Std. Dev. (s)	0	0.4	1.0	1.0	0
Std. Error Mean SEM	0	0.2	0.4	0.4	0

(a) Rankings are from 1-5 where 5 is the slowest rating and is always assumed to be present in the guinea pig soleus.

RmT = room temperature.

TABLE 11

Comparison of Physiological Properties Associated With
Muscle Speed by Student's t Test in Various Mouse Muscle
Categories (a) (b)

Physiological Property	(b) C57 EDL X dy ^{2j} EDL	(b) dy ^{2j} EDL X C57 SOL	(b) C57 SOL X dy ^{2j} SOL	(b) dy ^{2j} EDL X dy ^{2j} SOL
CT at RmT	< .001	NS	< .05	NS
½RT at RmT	< .001	NS	< .01	NS
CT at 37°C	< .001	NS	< .05	NS
½RT at 37°C	< .001	NS	< .001	NS
Tension at RmT as % 37°C	< .001	NS	NS	NS
First Twitch PTD at RmT	< .02	NS	NS	NS
First Twitch PTD at 37°C	< .001	NS	NS	NS
DASP at RmT	NS	< .05	NS	NS

(a) Significance for student's t test set at 5% level

(b) X means: Faster than

RmT = room temperature.

Table 12 is a final attempt to show how these muscles rank with respect to slowness. It involves the use of a series of four 2×7 analyses of variance in which all the test criteria from each muscle type are evaluated in terms of their similarity to the data from true slow muscle, the guinea pig SOL. An "F" value is derived for each muscle which is a measure of how closely all of the properties of slowness for that particular muscle approach those for the guinea pig SOL. In column 1 since the "F" value is large the C57 EDL is quite different from the guinea pig SOL while the C57 SOL (column 3) and the dy^{2j} SOL (column 4) show progressively more similarities.

The dy^{2j} EDL gives the smallest "F" value and would thus appear to resemble the guinea pig SOL the most closely. This however is an artefact caused by the large standard error of the various tests in this muscle. Since there was a large spread of data for the dy^{2j} EDL this has artificially caused it to appear similar to the guinea pig SOL.

From the preceeding information it is clear that 1. the C57 EDL is the fastest muscle, guinea pig SOL the slowest, 2. C57 SOL is only intermediate between these, 3. the dy^{2j} EDL has slowed until indistinguishable from C57 SOL, 4. the dy^{2j} SOL slows slightly, and 5. the C57 SOL, dy^{2j} SOL and dy^{2j} EDL properties show a considerable overlap.

It is important to note that the old dy^{2j} SOL might be seen as being spared from the viewpoint that its slow properties are preserved to some extent. However this is peculiar if one considers that the histological damage is more severe than in that of the young dy^{2j} SOL (personal observations).

TABLE 12

Four, 2 by 7 Analyses of Variance For Properties Associated
With Muscle Speed in Mouse Muscle and Guinea Pig Muscle

Statistical Parameter		1 G. Pig SOL C57 EDL	2 G. Pig SOL dy ^{2j} EDL	3 G. Pig SOL C57 SOL	4 G. Pig SOL dy ^{2j} SOL
Sum of Squares Degrees of Freedom Mean Square F	C	255758.3	50934.2	65549.2	66675.1
	G	1	1	1	1
	M	255758.3	50934.2	65549.2	66675.1
	F	1640.1	94.7	329.9	225.4
C G M F	B	0.340	25580.3	32813.8	9008.2
		6	6	6	6
		0.056	4263.4	5468.9	1501.4
		0.0	7.9	27.5	5.1
C G M F	AB	0.06	26094.1	32851.0	9002.0
		6	6	6	6
		0.1	4349.0	5475.2	1500.3
		0.0	8.1	27.6	5.1
C G M	Within Cells	13878.4	46262.9	17881.8	24851.9
		89	86	90	84
		155.9	537.9	198.7	295.9

(The "F" values in "A" indicate the significance in terms of differences with respect to the guinea pig SOL. The values are ordered (degree of slowness) except for the dy^{2j} EDL. This occurred because the numerical values entered in this test, for dy^{2j} EDL, were quite variable). ie 1640.1 for C57 EDL column indicates C57 EDL is fastest muscle tested with respect to guinea pig SOL etc.

The young dy^{2j} SOL has actually speeded at 37°C (but not at room temperature). On this basis it is also difficult to say that the slow components have been spared. From the physiological evidence it would appear that the SOL does not strictly adhere to the hypothesis of Brust. The EDL behaves in a manner which is consistent with the hypothesis in that it is progressively slowed with age.

To further investigate the problem a histochemical study was carried out and this is presented in the following section.

2. Histochemical Study

Mouse and Guinea Pig

A. Introduction

The physiological data have shown that several characteristic fast muscle properties are shifted in the dystrophic mouse toward some of the properties of slow muscle. Furthermore, the old mouse SOL which is not a pure slow muscle becomes even slower. These occurrences can mean that either there is a generalized slowing of all muscle fibers in dystrophy or there is a selective loss of the fast fibers leaving the slow fibers to determine the contraction characteristics.

In order to arrive at a more specific conclusion as to the actual cause of these physiological observations a histochemical study was undertaken on the fiber populations of the 4 week old C57 and dy^{2j} SOL of the mouse and the 6 week old normal guinea pig SOL.

B. Histochemical Fiber Typing in Normal Mouse

The histochemical reactions had to first be established in the normal animals. Three stains were used for this purpose, a morphological stain (H&E) and two fiber typing stains (NADH-TR and the alkali-stable myofibrillar ATPase stain). By staining adjacent serial cross-sections 3 fiber types were found in the four week mouse. These fibers have been termed Type I, Type II_{ox} and Type II_{glyc} and their staining properties are listed in Table 13. These fiber types are pictorially presented in Plates 1 to 3 and the typing system is described below.

TABLE 13

Histochemical Staining Reactions of the Various Fiber Types
in C57 Mouse

Histochemical Stain	Staining Intensity		
	Type I	Type II _{oxidative}	Type II _{glycolytic}
Hematoxylin and ϵ	Blue-Nuclear	Blue-Nuclear	Blue-Nuclear
Eosin	Red-Myoplasm	Red-Myoplasm	Red-Myoplasm
NADH-TR*	Intermediate	Positive ^o	Negative
Alkali-Stable* Myofibrillar ATPase	Negative	Positive	Intermediate

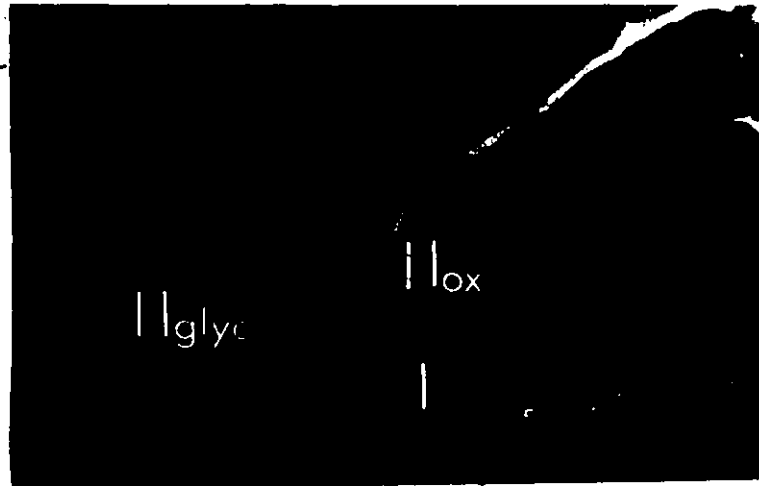
ϵ This stain is used as a morphological stain.

* These stains are used for fiber typing.

Plates 1, 2 and 3

Fiber Types in C57 Mouse

Plate 1



H&E stain
100x

Plate 2



alkali-stable
myofibrillar
ATPase stain
100x

Plate 3



NADH-TR stain
100x

Since it is known that the H&E stain colours the sarcoplasm red and the nuclei blue this stain was useful in detecting abnormal morphological features such as central nucleation. Occasionally the H&E stain will crudely outline different fiber types since the Type I and Type II_{ox} fibers can stain darker than the Type II_{glyc} fibers.

The blue NADH-TR diformazan salt stains in a more granular fashion and this leads to a generally less homogenous particle distribution. The Type II_{ox} fibers stain the darkest blue and normally have a very intense blue rim due to heavy staining of the subsarcolemmal region.

The Type I fibers stain less intensely and have a more evenly distributed salt deposition. The Type II_{glyc} fibers stain with the least intense blue and are relatively homogeneously stained.

The alkali-stable myofibrillar ATPase stain differentiates 3 fiber types as well. The stain itself results in a brown colour of various intensities. As with the NADH-TR stain the Type II_{ox} fibers stain the most intensely yielding a homogeneously distributed strongly positive brown reaction product. The Type II_{glyc} fibers stain less intensely, but equally homogeneously as an intermediate brown colour. Finally the Type I fibers stain negatively and this is homogeneously distributed.

While W.K. Engel prefers the ATPase stain alone for fiber typing the system used here employs the combined NADH-TR and ATPase stains for definitive typing. I would say however that the ATPase stain is by far the more precise of the two stains due to the relatively

uneven staining pattern seen with NADH-TR. This introduces a greater degree of subjectivity into the typing procedure. Engel also suggests that the ATPase stain is able to retain its typing specificity best under pathological conditions. I have come to the same conclusion and have found that the NADH-TR stain afforded no advantages in typing young dystrophic tissue. Consequently the fiber typing is based on the ATPase reaction alone. In fact, only young mice (3 weeks 3 days to 4 weeks 1 day) were used in this typing study because even the hardy ATPase stain loses its specificity as dystrophy continues past this age. For instance, there is no guarantee that one could confidently type fibers in a 5 week old dystrophic. The reaction product generally blends so that all fibers stain similarly in the advanced stages, and there are many gradations of staining intensities in the middle stages of disease.

Consequently, typing had to be done at the instant in time when the muscle was just beginning to show histochemical signs of disease but not to the degree that it rendered the fibers untypable. Part of this study involved finding this transition point.

The complete intact SOLs were examined in 10 C57 and 10 dy^{2J} mice of approximately 4 weeks of age. It was found that the C57 SOL normally contains only Type II_{ox} and Type I fibers however occasionally a handful of untypable fibers were present as well.

C. Criteria for Comparison of Fiber Type Populations in C57 and in
dy^{2j} Mice

Since in the dystrophic animals a variety of abnormalities occur which are not normally seen in the controls, special categories had to be devised so as to insure the inclusion of all normal and abnormal muscle features. It must be emphasized however that there are some abnormalities at a basal level even in the normal animals and it is reasonable to expect a small percentage turnover of fibers as a normal condition. Also, as I pointed out earlier, some of the "normal" animals may actually have been heterozygotes and this could potentially have increased the basal levels of abnormal fibers to a small degree.

The category definitions decided upon for the purpose of fiber type classification consist of 4 major groups each with a subgroup as shown below. Each category is represented in the H&E and ATPase photos shown in Plates 4 and 5.

1. Total Type II_{ox} - Those fibers staining dark with the ATPase stain. Can be any size as long as they are recognizable as a fiber and not as debris, connective tissue etc.
2. Type II_{ox} (C) - Those fibers of group 1 with central nuclei as judged by the H&E stain.
3. Total Type I - Those fibers staining negatively with the ATPase stain. Can be any size as long as they are recognizable as a fiber.

Plates 4 and 5

Fiber Type Categories in 4 Week dy^{2j} SOL of Mouse

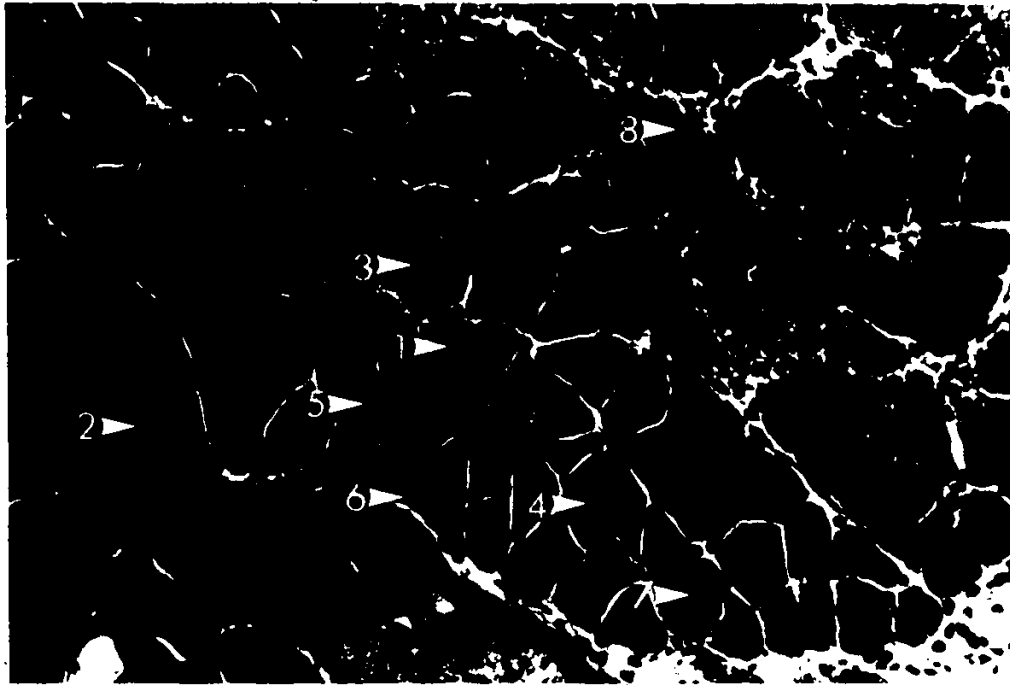


Plate 4

H&E stain
100x

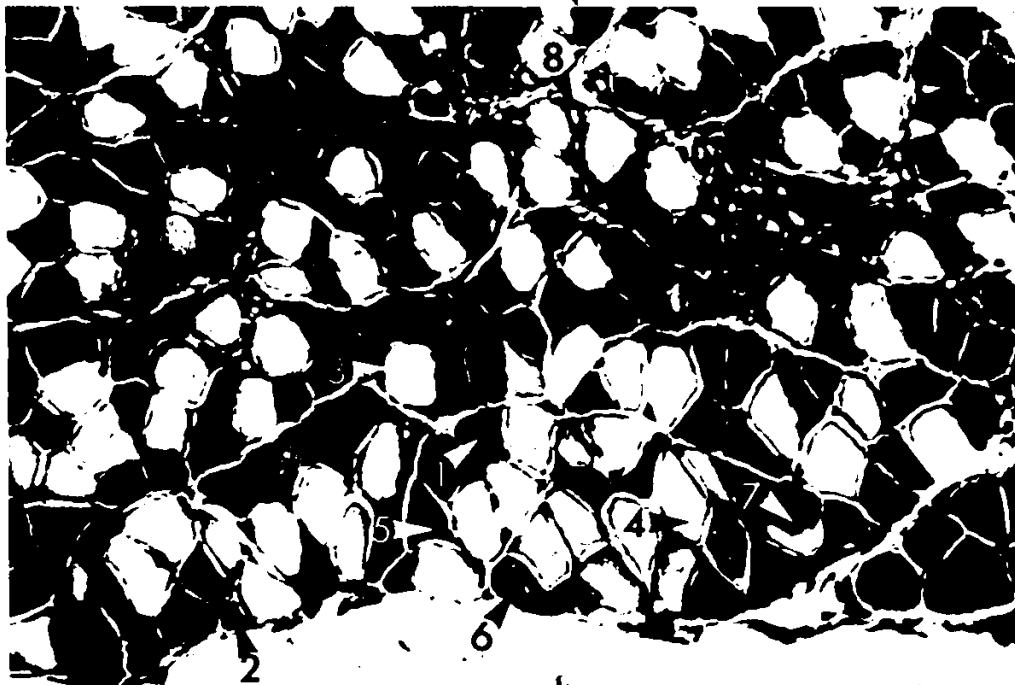


Plate 5

alkali-stabl
myofibrillar
ATPase stain
100x

4. Type I (C) - Those fibers of group 3 with central nuclei as judged by the H&E stain.
5. Abnormal Staining - Those fibers which stain intermediate between those of groups 1 and 3 and those fibers which stain unevenly as judged by the ATPase stain.
6. Abnormal Staining (C) - Those fibers of group 5 with central nuclei as judged by the H&E stain.
7. Abnormal Structure - Those structures of unclear identity due to very small size, peculiar shape, texture etc as judged by the ATPase stain.
8. Abnormal Structure (C) - Those structures of group 4 which appear to have a central nucleus as judged by the H&E stain.

It should be noted that each subgroup category (followed by the subscript C) as in Type II_{ox} (C) refers to a group of fibers already counted in the major group heading (Total Type II_{ox}). Since centrally nucleated fibers are considered diseased, in order to calculate the number of healthy fibers, one would subtract as follows: (Total Type II_{ox} - Type II_{ox} (C)). Likewise to calculate the number of healthy Type I fibers one would subtract as follows: (Total Type I - Type I (C)).

The primary point of interest in this study is whether the fibers can be classified as healthy or unhealthy. A fiber is considered unhealthy if it has a. a central nucleus, b. abnormal staining

properties, c. is of abnormal structure of, d. any combinations of the above.

The values obtained for C57 and dy^{2j} mouse and guinea pig by direct counting and by calculations are given in Tables 14 and 15. Table 15 shows summarized, calculated information derived from the actual counts shown in Table 14. Table 14 contains rather detailed information directly from the fiber counts. The purpose of this detail was to hopefully better elucidate the sequence of events involved in the destruction of the individual fibers.

D. General Comparison of C57 SOL and Guinea Pig SOL

A perusal of Tables 14 and 15 reveals that the C57 SOL contains only 26.2% (240) healthy Type I fibers and 71.5% (654) healthy Type II_{ox} fibers. This gives a II_{ox}/I ratio of 2.8. If as is assumed, the Type I fiber is the slow fiber and II_{ox} is the fast fiber, then we readily see that the C57 SOL (4 weeks) is by no means a pure slow muscle but it does contain sufficient slow fibers to provide it with some slow muscle properties. This would account for the observations of physiological properties as examined above.

It is of interest to consider the findings of other authors. Sushella et al (1968) examined a single cross section of normal 3-4 month old SOL in which they counted a total of 87 fibers. They found 30 were "Type I" (probably similar to the Type I fibers here) and 57 were "Intermediate" (probably the II_{ox} fibers here). This is to say their sample contained 34.5% Type I and 65.5% Type II_{ox} .

TABLE 14

Detailed Fiber Type Counts For 4 Week Old C57 and dy^{2j}
 Mouse Soleus and 6 Week Old Guinea Pig Soleus (a)

Category	Histochemical Category	C57 (10)	dy ^{2j} (10)	G. Pig (4)
1.	Total Type I	240.5± 7.4	176.0±10.2	(100)
2.	Type I _c	1.2± 0.4	3.4± 1.1	-
3.	Total Type II _{ox}	659.0±17.1	445.0±18.7	(0)
4.	Type II _{ox c}	5.1± 0.8	64.6±10.3	-
5.	Total Abnormal Stain	9.8± 0.9	82.4±10.6	-
6.	Abnormal Stain _c	0.3± 0.2	38.0± 6.9	-
7.	Total Abnormal Structure	5.3± 0.9	63.7± 8.8	-
8.	Abnormal Structure _c	1.3± 0.4	24.0± 5.2	-

(a) Mouse values are mean ± SEM, (n=10). Guinea pig values in parenthesis are percents, (n=4).

TABLE 15

Summary of Calculated Histochemical Population Values For
4 Week Old C57 and dy^{2j} Mouse Soleus (a) (b)

Calculated (a) Information	C57		dy ^{2j}		Population (b) Change (%)
	Mean ± SEM	% of Total	Mean ± SEM	% of Total	
Total Fiber Population	914.5±20.6	100.0	767.1±22.4	100.0	16.1(147)
Total Unhealthy Fiber Popl	21.4± 1.4	2.3	214.1±16.0	27.9	1000.0(193)
Total Healthy Type II _{ox}	653.8±17.3	71.5	380.5±12.8	49.6	41.8(273)
Total Healthy Type I	239.3± 7.4	26.2	172.5± 9.9	22.5	27.9(67)
Healthy II _{ox} /I	2.8± 0.1	-	2.3± 0.2	-	-

(a) The C57 and dy^{2j} healthy Type II_{ox}/Type I ratios are significantly different at the 0.025% level using a student's t test.

(b) Values are percents, values in parenthesis are numbers of fibers.

Number of animals = 10 C57, 10 dy^{2j}.

Likewise, Dribin and Simpson (1977) counted about 100 fibers/muscle in 10 muscles at 2.5 to 5.5 months of age and averaged 60.2% "red" (Type II_{ox}) and 38.6% "intermediate" (Type I) per SOL. The above findings both show a greater number of Type I fibers than I have found and there are at least two explanations for this. First of all, as previously mentioned it is possible that there is an age related shift in fiber type populations since an increase in Type I fibers with age has previously been reported in other species (Kugelberg 1976). Since these experiments were performed on older mice this fits well. In addition, these authors sampled at most only 1/10 of the whole muscle fiber population, whereas I have counted all fibers. I have noted in my counts that the fiber type distribution is not a uniform checkerboard mosaic but rather contains areas of high density for a single fiber type so that if a single small sample were counted this could lead to inaccuracies.

I have since attempted to resolve this issue by quickly examining two, 25 week old C57 mouse solei: I found in these muscles which contained an average of 891 fibers:

57.8%	Type I
39.9%	Type II _{ox}
2.3%	Unhealthy

This finding appears to fit nicely with Kugelberg's observations. Sushella and Dribin and Simpson found somewhat fewer Type I fibers in their mice than I have. Since my 25 week old mice were at the top end of the age range that they used it would appear that the fiber type conversion has proceeded to a greater extent, and this may account for the greater number of Type I fibers found here.

Table 14 shows that the guinea pig SOL which is a pure slow muscle is composed of 100% Type I fibers. These data are derived from the

visual inspection of 4, 6 week old guinea pig solei in which it was clear that the fibers were uniformly Type I. Plates 6, 7 and 8 show photographs of a representative field in one of the SOL muscles examined. Thus, it would appear that the percentage of slow fibers may determine the degree of slowness of a muscle since this muscle also contracts very slowly, and the old C57 SOL contracts slower than the young C57 SOL.

It was found in the C57 SOL that 2.3% of the fibers could be classified as unhealthy according to the given criteria.

E. Comparison of C57 SOL and dy^{2j} SOL

When the C57 and dy^{2j} SOL muscles are compared, Table 15 one sees firstly a smaller overall fiber population by 16.1% (147) in the dy^{2j} SOL. Moreover the unhealthy fiber population has jumped markedly by 193 fibers.

The most important comparison however, has to do with the shift in proportions of healthy Type I and II_{ox} fibers. Whereas in the dy^{2j} the Type II_{ox} fibers have dropped by 42% (274), the Type I fibers have dropped by only 28% (67). When the healthy II_{ox}/I ratios are compared in the C57 and dy^{2j} (2.8 and 2.3 respectively) it is found that the II_{ox} fibers have decreased significantly more than the Type I fibers using the student's t test. Significance was also confirmed by the use of a Chi square test.

This then is evidence that while at the four week age, there is definitely a loss of fibers from both healthy II_{ox} and I pools, there is a greater detrimental effect upon the Type II_{ox} fibers than upon the Type I fibers.

Plates 6, 7 and 8

Fiber Type Composition of 6 Week Old

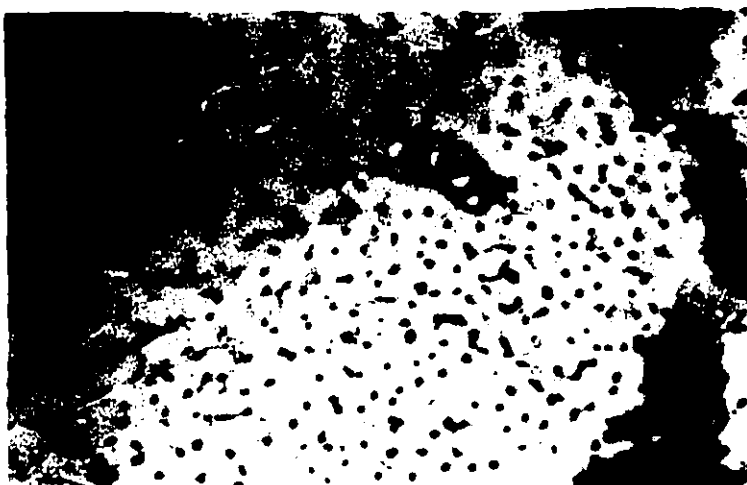
Guinea Pig Soleus (100% Type I)

Plate 6



H&E stain
100x

Plate 7



alkali-stable
myofibrillar
ATPase stain
100x

Plate 8



NADH-TR stain
100x

This information correlates with the physiological findings for the old dy^{2j} SOL but not necessarily for the young dy^{2j} SOL since we do see a slowing in the old dy^{2j} SOL but a speeding in the young dy^{2j} SOL. Since the Type I fiber is currently thought to be a slow fiber, and if the only effect of dystrophy is to increase the proportion of healthy Type I fibers relative to Type II fibers, then one might expect a slower whole muscle contraction. In other words the contraction would be governed to a greater extent by the slow fibers and therefore the contraction would appear more like that of a pure slow muscle such as the guinea pig SOL.

Since the Type I fiber is spared in relation to the Type II_{ox} fiber in the 4 week mouse but the contraction speeds up, it would appear that another superimposed factor must be involved to cause these changes apart from a simple fiber type ratio change.

CHAPTER. IV

DISCUSSION

In this section I will address the data in approximately the same order as they were presented in the results section. Here attention will be drawn to the findings of other workers, possible sources of error and to potential future directions. As well, an effort is made to integrate the physiological and histochemical data and to draw conclusions about how dystrophy has affected the parameters which I have studied.

The results have shown firstly that the dystrophic mice exhibit a decrease in developed absolute tension and in muscle weight. In the ReJ 129 strain of mouse this is probably the defect of dystrophic muscle most commonly reported in the literature (Sandow and Brust 1958, 1962, Brust 1966, Douglas and Baskin 1971, Taylor et al 1971, 1974, Hofmann and Ruprecht 1973 and Sabbadini and Baskin 1976).

There is a relative paucity of data regarding this in the dy^{2j} strain of mouse but several authors have come to similar conclusions (Hironaka and Miyata 1975, Harris and Montgomery 1975 and Parry 1977). It is important to note from the onset however that excessive extrapolations between these two strains may be dangerous since the etiologies of the diseases may be different (Parsons 1974).

In this study the relative weight loss in both the young and the old groups of dystrophic soleus was greater than in the EDL groups.

This would seem to detract from the general hypothesis that the soleus is less detrimentally affected than the EDL.

One other peculiar finding was that the normalized tensions are actually greater in the old dystrophic fast and slow muscles than in their control counterparts. The young dystrophics on the other hand show a decreased normalized tension in relation to their controls. This would suggest that the dystrophic muscles actually became stronger on a grams tension/grams wet weight basis as the disease progressed. This observation has not to the best of my knowledge been previously reported in the literature on the ReJ 129 strain of mouse and generally there is a reduction of normalized tension concomitant with the weight loss. This latter situation would also intuitively seem more likely to occur.

In the dy^{2j} strain the literature has been more difficult to summarize due to inconsistencies in data presentation and muscles studied. However, Harris and Montgomery (1975) found a decrease in gm/gm tension in anterior tibialis, a fast muscle, from mice of 2-3 months of age. Another study (Parry 1977) examined the SOL and EDL muscles of mice of the same age groups as in this study. It was found that the dystrophic EDL showed progressively greater decrements in normalized tension with age whereas the young soleus showed no change. However, the old soleus did show a significant decrement.

Goldspink and Rowe (1969) pointed out that in addition to a decrease in sarcomeres per fiber there are also fewer fibers in the ReJ 129 mouse and noted that the dystrophic muscle has a much greater

fiber diameter variation than does normal muscle. They suggest that this may possibly be due to the fact that the reduced fiber number may mean less competition for metabolic supplies for the remaining fibers and therefore may explain the fiber hypertrophy since normal size limitations may no longer apply. In view of these major alterations in fiber structure and integrity it is possible that this will be reflected in some form in the normalized tensions of the muscles.

In addition, the normalized tension data does not preclude the possibility that the dystrophic muscles are activated to a greater degree during a single twitch. This would mean for instance an increased twitch tetanus ratio. However, since the tetanic tensions were not recorded this could not be verified.

Another possible but unlikely explanation for the normalized tension data is that an error was systematically incorporated into the study dealing with the dystrophics and rendered them falsely high. For instance since the old dystrophic muscles weighed the least of all muscles studied they could possibly be the most susceptible to dehydration. Thus even if a similar time was taken to blot and weigh these muscles as was done for the controls they may have lost proportionately more water. This could have the effect of increasing their normalized tensions.

Thus, while the soleus would not appear spared from the point of view of weight loss it does do relatively better than the EDL from the point of view of absolute tension loss. As well it was

seen that the normalized tensions of dystrophic muscles increase with age relative to the controls.

The data obtained on the speeds of contraction have clearly shown a slowing of dystrophic EDL and this is more prominent in the older muscles than the younger ones. This suggests that as the disease advances the effect of "slowing" becomes progressively more pronounced. Both the CT and the $\frac{1}{2}$ RT are prolonged, the $\frac{1}{2}$ RT to the greatest extent.

Evidence has been accumulating that alterations occur in the SR and it has been suggested that the observed reduced capacity for Ca^{+2} uptake is attributed to the proliferation of membranes in the junctional and tubular sarcomere regions of the dystrophic muscle (Scales et al 1977). This may explain the observed slowing. They further suggest that this accounts for the altered excitation-contraction coupling. Likewise, it has been suggested that the observed abnormal membrane lipid composition of the SR may account for the mechanical alterations of dystrophic muscle (Mrak and Baskin 1978).

In the old dystrophic soleus the CT is not changed but the $\frac{1}{2}$ RT is prolonged. This may be interpreted as a relative sparing of the slow components of the muscle. However, in the young dystrophic soleus both the CT and $\frac{1}{2}$ RT are actually speeded which is contrary to the argument that slow properties are spared. Presumably a speeding of the young dy^{2j} soleus can mean that either the slow components selectively drop out (or become faster) or that there

could be a generalized speeding of all components. This involves the assumption of the current view that the contraction time parameters are controlled by the fiber types present.

One other possibility not yet discussed is that the time course of maturation of the dy^{2j} SOL may be altered. We have seen that C57 SOL normally slows with age. Perhaps in the dy^{2j} SOL there is a maturation lag at first which is followed by a small overshoot as the muscle ages.

Other workers examining fast and slow muscles have considered the SOL. Brust (1966) working on in vitro ReJ 129 muscles found no change in the $\frac{1}{2}$ RT of 3 month old SOL. Likewise Douglas and Baskin (1971) working on 1-12 week old SOL found only slight changes in its time parameters. On the other hand Brust (1966) found the fast gastrocnemius $\frac{1}{2}$ RT doubled in dystrophy, Douglas and Baskin (1971) noted an increase in the $\frac{1}{2}$ RT of EDL but no change in CT and Sabbadini and Baskin (1976) found an increase in $\frac{1}{2}$ RT but a decrease in CT of 6-7 week old EDL.

Hironaka and Miyata (1975) worked on the dy^{2j} EDL and found an increase in CT and in $\frac{1}{2}$ RT which was magnified with age as were the results of this study.

Thus the presently observed slowing of dystrophic EDL is consistent with the findings of other workers and with the hypothesis that fast muscle is slowed.

The fact that the young dy^{2j} soleus is speeded is more difficult to interpret. Whereas Douglas and Baskin (1971) have found only a

slight slowing of the 4 week old ReJ 129 soleus, in this study a definite speeding was obtained. Clearly, the strain differences again may have some bearing on the observation inconsistencies.

Likewise, their results were obtained from in vitro work so that they were probably dealing with a greater number of unphysiological influences.

However, the speeding seen here in young soleus can be interpreted as evidence against the sweeping general hypothesis that all slow muscle is spared, and, in fact it would appear that a modification of this hypothesis may be in order. If several more characteristics of muscle contraction speed were altered, even better evidence against this general hypothesis would be obtained. Indeed, as will be shown below the young dy^{2j} soleus appears to be "speeded" also with respect to the cooling and the post-tetanic effects. It is of interest that even the normal young soleus demonstrates rather "fast" characteristics and this also will be addressed below.

Thus; while some speeding does occur in the young dy^{2j} soleus, it is evident from the results that it is an exception since slowing occurs in the dy^{2j} EDL of both age groups as well as in the old dy^{2j} soleus.

Consequently, it was considered worthwhile to examine whether these transformations were due to intrinsic property changes of the fibers or whether they were due to extrinsic alterations such as the damping effect which could result from connective tissue infiltration. The properties of CP/CD, PTP/PTD and DASP were examined for this purpose.

An analysis of the CP/CD data reveals that the normal EDL undergoes a CP while the normal soleus shows a CD, or remains the same as the temperature drops from 37° to room temperature. These responses therefore represent the baseline characteristics of healthy mouse muscles. In the dystrophic condition the young and old EDL both show CD. Since this is the characteristic mouse slow muscle response it would appear that there has been a conversion to slow muscle properties of this muscle. Likewise, since the old dy^{2j} SOL shows even greater CD than does the control, this would suggest that the old dy^{2j} soleus has become even slower. The EDL and the old SOL therefore respond as would be expected according to the hypothesis of slow muscle sparing (at least the slow components of this muscle) and fast muscle slowing in shifting their basic properties towards those of slow muscle.

The young dy^{2j} soleus as mentioned above does not however show enhanced CD. Since it responds with CP which is a fast muscle property it has once again deviated from the general hypothesis of slow muscle sparing.

The PTP/PTD data illustrate yet another phenomenon which is known to differentiate fast and slow muscle. Since fast muscles are transiently potentiated post-tetanicly and slow muscles are depressed or show no change, one would expect the dy^{2j} EDL to take on the slow muscle characteristic of depression. Indeed this occurs in the young and old dy^{2j} EDL.

On the other hand, it is reasonable to expect that the SOL would not show much of a change or would retain its original characteristics if spared. In both the young and the old dy^{2j} SOL there is no significant change from the control values. It should be noted however, that unlike the old control SOL, the young control SOL values are quite "fast" with respect to this parameter. They display a considerable amount of PTP, a fast muscle property. Thus while the young dy^{2j} values are slightly but not significantly faster than the control values, both values are significantly faster than those of the old control SOL.

There has been a consistent pattern throughout this study whereby the young control soleus displays physiological characteristics which have a strong fast muscle resemblance. On the contrary, the old control soleus has generally responded much more like a slow muscle. The cause of the disparity between these control muscles of different ages can probably be explained on the basis of fiber type populations. It has been observed that neonatal rat and cat soleus muscles contract slowly but subsequently speed up until at about 4-6 weeks of age they obtain their fastest contraction speeds. They then promptly slow down to contract at their final slow speeds (Buller et al 1960, Close 1964). The work of Douglas and Baskin (1971) suggest that this is also true of the mouse soleus. A physiological explanation for this type of phenomenon has been elucidated by Kugelberg (1976) who found that the contraction times of the rat SOL at a particular age were related to the

percentages of the fiber type populations in the muscle. In other words as the SOL ages there is a conversion of Type II (fast) fibers toward Type I (slow) fibers and the predominant type would appear to have the greatest influence on contraction speed.

Since it was shown here histochemically that the young SOL has a high percentage of Type II_{ox} fibers (72%) it is clear that the contraction will be strongly dominated by these fibers. Therefore one would expect the fast characteristics which were observed in the young C57 SOL. The results of this work have also shown that the old C57 SOL shows considerably slower characteristics. This shift is easily explained if one considers that a fiber type population shift has occurred as described above. The results of this study provide histochemical evidence for this in 25 week old muscles and are consistent with the smaller Type II_{ox} populations of (39%) in 2.5 to 5.5 month old mice (Dribin and Simpson 1977) and 66% in 3-4 month old mice by Sushella et al (1968).

The causes of PTP and PTD as reviewed in the introduction have been considered in terms of degree of activation and the effects of Ca^{+2} as an activator. It is possible the PTP is seen in fast muscles because its abundant and efficient SR is able to rapidly recapture Ca^{+2} in the process of a single twitch. This uptake is thought to be so rapid that it denies the full expression of the potential of the muscle and thus explains why tetanic tension is not reached in a single twitch. However, after a tetanus it is thought that the Ca^{+2} slug released into the sarcoplasm is more than

sufficient, to fully saturate the reuptake mechanism. Consequently, a greater time is required to remove this Ca^{+2} and the twitch is potentiated. However, recent work using aequorin to mark Ca^{+2} transients in frog muscle has shown the Ca^{+2} is decreased during the PTP period (Rudel and Taylor 1973). This finding may argue against the proposal that PTP is due to an extra amount of Ca^{+2} which more fully saturates the contractile apparatus and increases the activation of the fibers. S.R. Taylor, stated (Hammond and Ridge 1978) that it may not be so much the amount of Ca^{+2} which is present but rather the length of time that it is present which is most important in determining twitch amplitude. This tends to add credibility to the Ca^{+2} movement theory described above.

I have not found any references in the literature which have studied the DASP of dy^{2j} animals and there are only a few such measurements in the ReJ 129 mice. A decrease in the DASP of ReJ 129 SOL and gastrocnemius (fast muscle) was reported by Brust (1966) and a decrease in the DASP of ReJ 129 EDL was also reported (Sabbadini and Baskin 1976). No change in the DASP of tetanic SOL contractions was reported by Taylor et al (1971, 1974). Since the DASP depends heavily on the temporal and the quantitative interrelationships of Ca^{+2} , the presumed activator, it is of interest to examine the DASP in that light. It is possible that slow muscle which has a less efficient SR than fast muscle may have a longer DASP because the Ca^{+2} is taken up at a slower rate than in fast muscle. This would have the effect of increasing the duration of the

contraction as well. Since this study shows a prolongation of the contraction time parameters in dystrophy it was considered possible that a cause was an increased DASP.

The DASP study was intended to examine very basic properties of the fast and slow muscle contractile mechanisms. Since fast muscles are known to have a shorter DASP than slow muscles this property was examined for evidence of prolongation in the dy^{2j} EDL and preservation in the dy^{2j} SOL. Since the data showed no change for both types of muscle, this information is difficult to interpret. It is certainly possible that dystrophy had no effect on the DASP. However, it could also have been that the test was not sensitive enough to pick up the changes. The latter possibility is considered unlikely for two reasons. Firstly the DASPs were measured in old animals, so that any changes due to dystrophy would be well established. Secondly, the DASPs were measured at room temperature so as to maximize the individual durations.

The above quoted results for ReJ 129 mice are difficult to interpret on the basis of a simple Ca^{+2} uptake mechanism. Under the above conditions one would expect a prolongation rather than a decrease of the DASP.

In view of the fact that the results of this study are at variance with the studies on the ReJ 129 mouse it is possible that alternative means may be necessary to account for the discrepancy.

It is clear that the Ca^{+2} transients are but one link in the chain of events responsible for the onset, maintenance and decay of

active state. Many other intermediates are involved each of which could potentially be the rate-limiting step. For instance, ATP is a possible but unlikely candidate since it has been found that steady state levels of adenosine triphosphate, diphosphate, monophosphate and creatine phosphate are unchanged compared to normal muscle on a non-collagen, soluble muscle protein basis (Farrell and Olson, 1973). In addition, as previously noted, measurements of the DASP supply information on only one portion of the active state curve. It is possible for instance that it is the rate of decay of active state which is prolonged as suggested by Sabbadini and Baskin (1976). If this were true, one would expect that the Ca^{+2} activator would be present for a slightly longer time though not at full saturation during this period. This could prolong the contraction and account for the longer twitch duration observed here in dy^{2j} EDL.

Table 16 summarizes the physiological data obtained for mouse muscles. It is evident from this table that the EDL of both ages has slowed in all properties studied except the DASP. The young SOL has speeded to some extent while the old SOL has slowed in $\frac{1}{2}\text{RT}$ only.

This concludes the information from the physiological study on the mouse. As previously mentioned there is a dearth of slow muscles in the mouse and the soleus was chosen as a representative slow muscle despite the fact that it has a large complement of fast fibers.

It was considered necessary to examine the properties of the

TABLE 16

Summary of the Effect of Dystrophy in the Alteration and
Conversion of Some Fast and Slow Muscle Properties in
Mouse SOL and EDL Muscle (a) (b)

Physiological Property			SOL			EDL		
			C57	dy2J	*	C57	dy2J	*
CT	(msec)	Y	17.4±1.0	14.9±0.4	↑	9.1±0.2	11.7±0.5	
		0	20.6±0.9	20.6±0.9	↔	9.0±0.4	16.6±1.1	
½RT	(msec)	Y	21.5±1.8	16.7±0.8	↑	9.2±0.6	11.8±1.6	
		0	23.1±1.1	27.6±1.8	↓	8.6±0.5	19.1±1.6	
PTP/PTD	(%)	Y	110.3±2.1	111.1±1.8	↔	142.7±4.4	124.4±2.7	
		0	102.7±1.8	105.0±3.3	↔	131.7±5.1	107.4±2.1	
CP/CD	(%)	Y	102.8±5.4	125.0±6.2	↑	169.9±7.2	149.0±6.0	
		0	88.6±2.6	88.0±2.5	↔	143.5±4.9	98.6±4.5	
DASP	(msec)	0	8.3±0.3	8.2±0.3	↔	7.2±0.3	7.4±0.3	

(a) Values are mean ± SEM Y=Young, 0=Old

↑ =Conversion to a Faster Property

↓ =Conversion to a Slower Property

↔ =No Change

(b) All data were obtained at 37° except for DASP property
(Room Temperature).

guinea pig SOL which is a histochemically pure slow muscle uncomplicated by extraneous populations of Type II fibers. This would establish a baseline reference point from which to evaluate other muscle types.

The data for the guinea pig soleus show that indeed the CT and $\frac{1}{2}$ RT are longer. It is of interest that the linear dimensions of animals have some bearing on the speed of contraction. It has been pointed out that in homologous muscles of different species the speed of shortening is inversely proportional to body size (Hill 1950). Thus, a small animal such as a mouse would have much more rapid limb movements than would the guinea pig. However the habitat and life style is also important. Since Barany (1967) showed that the intrinsic speed of shortening is proportional to the specific activity of ATPase of myosin there should also be a correlation between animal size and fiber types present. The mouse SOL has few slow Type I fibers whereas the rat has more and the cat and guinea pig solei are pure slow muscles.

In addition to the slow speed of contraction of the guinea pig SOL, a definite but small PTD was observed as well as a strong CD. These are considered as slow muscle properties.

It is of interest that only a weak PTD was recorded but a strong CD appeared. A likely possibility for this outcome was provided by a personal communication with Dr. D.M. Lewis and Dr. K.W. Ranatunga. PTD is apparently maximal within about one half second post-tetanus but rapidly returns towards pre-tetanic levels. At about two seconds post-tetanus it plateaus at a tension about 10% below pre-tetanus.

This plateau which is quite flat persists until about 10 seconds post-tetanus and then returns to pre-tetanic levels. Since in the experimental protocol used here the post-tetanic twitches were recorded approximately 5 seconds post-tetanus only a limited amount of PTD would remain at this point.

A similar explanation is presumably valid with respect to the mouse SOL in which no PTD was observed. Since this muscle contains a mixture of fast and slow fibers, the post-tetanic twitch tension would be in part the result of potentiation of fast fibers and in part a small degree of PTD of the slow fibers.

The CD is a phenomenon however, which was recorded in its fully developed state due to the experimental design. This occurred since sufficient time was allowed for thermoequilibration after changes in the bath temperature.

The physiological data discussed above were summarized by parametric and non-parametric tables. These tables were intended to give some idea of the ranking of each old muscle type studied in relation to the physiological properties associated with contraction speed. It is evident from these tables that at the extremes the mouse EDL is the fastest muscle and the guinea pig soleus is the slowest muscle. The intermediate categories (C57 SOL, dy^{2j} SOL and dy^{2j} EDL) are blurred due to the overlap of the error bars. Nonetheless, it is clear that the old dy^{2j} EDL shifts considerably towards slow muscle properties. The old dy^{2j} SOL makes only limited changes from its control properties.

Since the conversion of fast to slow properties does occur in the EDL it is concluded that the cause of this slowing is a transformation of fast muscle properties to slow muscle properties.

Since the old dy^{2j} SOL has shown only little change, this is interpreted as a relative sparing of slow muscle properties in this muscle.

There are several important considerations relating to the above physiological study.

Firstly, the hypotheses of fast muscle slowing and slow muscle sparing are not necessarily causally related. Indeed the results presented above would argue against the idea that similar mechanisms are involved on the different muscle types.

The young dy^{2j} soleus speeds to act more like a fast muscle which is in direct opposition to what one would expect from a dystrophic condition. Consequently this effect is possibly not due to the dystrophy per se but may be due to other factors. It appears that an age dependent factor is involved. Moreover, the dy^{2j} EDL which behaves consistently like a slow muscle in both age groups cannot be the result of slow fiber type sparing since there are no Type I fibers in this muscle to begin with (personal observations). Therefore, the effect of dystrophy on this muscle could be due to one of the following:

- (a.) a generalized slowing of all fibers or
- (b.) those fibers which succumb contract with a slow contraction speed.

It is impossible to determine from these experiments which of the above possibilities is correct. On the basis of these considerations it

now appears naive to assume that the hypothesis as suggested by Brust in 1966 should be borne out 100% but rather that agreement would be found in some respects only.

In an attempt to demonstrate influences which may have been responsible for the data the following factors are considered.

Firstly, the fact that in some cases heterozygous animals were undoubtedly included in the "control" pool may have had some effect on the results. The degree of such an effect is difficult to determine except possibly by observing whether in any control category certain muscles responded in a "dystrophic-like" fashion.

Secondly, the "old" category of mouse contained mice of widely varying ages from 6 months to more than 1 year. This could also contribute to the variance.

In comparison with the work of other investigators, few comparable studies have been completed on the dy^{2J} strain of mouse and care must be taken when comparing these results to the ReJ 129 ones. Likewise, the present experiments were carried out in vivo to preserve physiological conditions as well as possible. One must be careful when comparing these results to the in vitro studies, especially those involving muscle strips.

In addition, a criticism which could be levelled against this study was suggested to me by Dr. K.W. Ranatunga who pointed out that a staircase phenomenon could possibly have occurred in the mouse muscle at the rates of stimulation employed ($\frac{1}{5}$ sec). If indeed this were so it may obscure some of the real twitch tension effects and

the degree of obscuring could be different for fast and slow muscles as well as dystrophic muscles. The degree to which this occurred, if any, is not known.

The histochemical data obtained from the 3-4 week old mice have shown that the Type II_{ox} healthy fiber population drops by 42% relative to the control whereas the healthy Type I fibers drop by only 28%. This difference was found to be significant using a Chi-square test. These data may be interpreted as being indicative of a preferential involvement of the Type II_{ox} fibers. It is however difficult to determine how much affect this will have physiologically. It would appear though that it is really the rate of progress of the disease which differs in the two fiber type populations and it is not a question of complete differential involvement. It is further apparent that all fibers eventually become affected (personal observations).

Sushella et al (1968) studied the histochemistry of the ReJ 129 mouse in which they found that dystrophy affects the "Type I fiber area" of the mouse gastrocnemius and affects the mouse SOL uniformly. This of course would run counter to the evidence of Brust and his supporters. I think several explanations are available for this type of result. Perhaps most importantly is that ReJ 129 mice were used which were severely affected at 3-4 months of age. Histochemically as indicated in their paper this causes great difficulty in fiber typing and is subject to a large margin of error. Additionally the criteria used for typing were not methods now accepted for definitive

typing. However, one observation made which is consistent with the present findings is that the muscle is not affected uniformly with some areas of the same muscle being differentially affected.

There have been only 2 papers dealing with the newer dy^{2j} strain of mouse.

Dribin and Simpson (1977) studied the SOL and gastrocnemius of dy^{2j} mice using perhaps the best available fiber typing techniques ie the acid and ~~alkali~~ stable myofibrillar ATPase stains and an oxidative enzyme stain. However here also they encountered serious problems in typing because the mice were very severely affected at 2.5 - 5.5 months of age. In addition they were totally unable to rely on the oxidative enzyme stain for the dystrophics since it no longer differentiated fiber types. They observed that even the myofibrillar ATPase stains showed subtle gradations of staining. As a result, there was considerable variation in their results. In the present work mice of 3-4 weeks of age were examined for the very reason that it was judged impossible to make accurate assessments of the fiber type populations in older dystrophics.

Dribin and Simpson observed in the gastrocnemius the "red" region was more severely affected. Presumably this is the region referred to by Sushella et al above which is composed of red (Type II_{ox}) and intermediate (Type I) fibers. In the SOL they concluded it was the intermediate fibers which were preferentially decreased in number compared to the red fibers.

One paper which would appear to substantiate the present findings was by Butler and Cosmos (1977) in which they compared the ReJ 129 and dy^{2j} strains histochemically. They studied mice from 3 days to 15 months of age including mice of 4 weeks of age as done in the present work. They have concluded that despite the fact that the two dystrophic genetic expressions originate at a common locus, the phenotypic expression and the time courses of the diseases are different. They reported the ReJ 129 to have an early preferential involvement in their "white, fast-twitch glycolytic fibers" and the dy^{2j} in their "red, slow-twitch oxidative fibers". They have based these findings on their observations that in the ReJ 129 mouse the crown portion of the fast anterior tibialis muscle is largely wasted. They reported that this area normally contains mostly "white, fast-twitch glycolytic fibers" and that the SOL and the core of the anterior tibialis which contains mostly "red, slow-twitch oxidative fibers" was spared. Since the reverse was found in the dy^{2j} mouse they have concluded that there is a differential temporal involvement of different fiber types in these strains.

In their paper it remained unclear exactly which fibers the above mentioned nomenclature referred to. There was no mention made of the 3 fiber types which are now generally accepted (Close 1972) and which has been specifically demonstrated in the mouse (Sher and Cardasis 1976). In addition their fiber typing was based on stains for phosphorylase and succinic dehydrogenase without the aid of the more generally accepted myofibrillar ATPase stain. Karpati and Engel

(1968) have suggested that the ATPase stain is the single best stain and Engel (1974) has criticized the phosphorylase and the oxidative stains. Likewise, Ashmore and Doerr (1971) have cautioned against interpretations made by the use of the phosphorylase stain and both Dribin and Simpson (1977) and myself have observed limited usefulness of the oxidative stains when applied to dystrophic tissue.

I have noticed that some mouse muscles such as gastrocnemius seem to have a geographical distribution of regions which contain different densities of a particular fiber type and this has been reported by Sushella et al (1968), Ashmore and Doerr (1971), Dribin and Simpson (1976) and Sher and Cardasis (1976). The superficial portions of these muscles are composed of Type II_{glyc} fibers. As one nears the bone there is a gradual transition through an area of relatively high density II_{ox} fibers mixed with an area quite high in Type I fibers adjacent to the bone.

It would appear likely that the system used by Butler and Cosmos did not differentiate the 3 fiber types at all and in particular did not identify the II_{ox} fiber. The SOL of course has a large number of these fibers as does the gastrocnemius "Type I area" of Sushella 1968, the "red region" of Dribin and Simpson, the "inner zone" of Sher and Cardasis, the area observed by Ashmore and Doerr and the "crown portion" of the anterior tibialis of Butler and Cosmos.

Consequently, if as it appears the II_{ox} fiber was lumped together with the Type I fiber by Butler and Cosmos it is possible that it is actually the II_{ox} fiber which is preferentially involved.

According to Close (1972) the available evidence is such that the fiber types can be grouped into 3 categories (1) fast twitch white (2) fast twitch red and (3) slow twitch intermediate. Thus, the "slow twitch red" fibers referred to by Butler and Cosmos may actually include both the II_{ox} and Type I fibers in the anterior tibialis muscle of the mouse so that the fibers which they are calling "slow oxidative" are almost certainly the Type II_{ox} fibers (personal observations).

The implications of the above information are interesting when attempting to correlate the physiological findings with those of the histochemistry. Since physiologically it was found that there was a distinct tendency of the young dy^{2j} SOL muscles to shift towards faster muscle characteristics the simplest explanation for this would be that it was due to a greater detrimental effect upon the slow fibers (Type I) than on the fast fibers (Type II_{ox}), the reverse of what was actually found. This discrepancy is difficult to explain using conventional approaches.

In order to explain the above inconsistency it is likely that one of the following is responsible: (a) The histochemical-physiological correlation is not as predicted for this age group or (b) some aspect of the experimental technique gave rise to false observations.

Regarding the former possibility, evidence has been accumulating which suggests that a rigid view of fiber type physiological properties is not entirely accurate. For example the preliminary findings of Lewis & Parry (1979) have given some evidence for a continuum of motor

unit contraction speeds in mouse SOL. Motor unit contraction times ranged from 7 to 37 msec with no sign of bimodal distribution despite the clearly bimodal distribution of myofibrillar ATPase activity of fibers.

Thus, once again, a rigid classification of Type I and Type II into slow and fast fibers respectively may not be entirely accurate at least in the mouse SOL. Likewise, according to the work of Burke and Tsairis (1974) a particular fiber type may have different properties depending on the muscle.

In using a similar approach to that of Robbins et al (1969), which pointed to the possibility that histochemical changes may lag behind the physiological changes, it is possible that the diseased muscle has undergone a physiological speeding prior to any demonstrable histochemical alteration.

Another possible explanation for these results would be to consider that the diseased fibers placed in the "abnormal" histochemical categories actually contract more rapidly than when they were healthy. This is not considered likely however because it would require that these fibers reverse their contraction speeds when they become older. This would be necessary to explain the slow contracting old SOL which has many more diseased fibers than the young SOL.

A further aspect of the histochemistry which has not yet been dealt with involves the role played by the Type II glycolytic fibers. It is interesting that the normal soleus contains only Type I and II_{ox} fibers while the EDL contains only Type II_{ox} and II_{glyc} fibers. The

Type II_{ox} fiber is thus common to both muscles. The II_{glyc} fibers are thought to contract rapidly and generally, these fibers appeared very well preserved in most of the tissue examined which contained this fiber type. On this basis it would appear that it is primarily the II_{ox} and I fibers which are first affected at this early age. Otherwise, not much can be said regarding the II_{glyc} fibers.

The fiber count data have shown that at 3-4 weeks of age there are already about 150 fewer fibers in the dystrophic soleus than in the control. There is good evidence that these fibers actually were lost and were not simply missing to start with because Montgomery and Swenarchuk (1977) obtained total fiber counts in preclinical mice which were very similar to the totals I obtained for the control mice.

The finding that there were many different stages of fiber damage in any histological section led me to consider the 3-4 week tissue as representing a "snap shot" in time wherein all the stages of the disease were under way simultaneously. In other words, assuming a consistent pattern and course of degradation, all of the stages from healthy fibers through to fibers which are completely destroyed should be present in the histological sections. Thus, the classification of the unhealthy fibers was carried out in some detail in this study. It was hoped firstly that by tallying the fibers in each unhealthy category it may be possible to determine which healthy category contributed to the particular unhealthy categories and thereby account for the fiber population redistribution. However, it became clear

that there were too many abnormal groups to enable this sort of inventory procedure. The second purpose for the detailed examination was to gain some insight into the fate and structural change of a fiber as it progresses from the healthy condition through its stages of necrosis until it is finally destroyed. Again, however the sheer number of abnormal stages rendered such a procedure at best speculative: but would be an interesting study for the future.

A further aspect of mouse histochemistry which has not yet been considered is the effect that altered functional demands may have on the ratios of the fiber types. The hindlimb and gluteal regions of the dystrophic mice can be seen to constantly jerk spasmodically. This activity in itself may cause a superimposed effect upon the basic changes which would occur due to the disease by itself. A rather large volume of literature has accumulated on the effects of altered activity levels and its effects on the histochemical and contractile responses. (Barnard et al 1970, Olson and Swett 1969, Fitts and Holloszy 1977, Gutmann et al 1971). In addition, the young animals drag their hindlimbs (the spastic activity seems to develop a little later) and in the older animals the knee joints become fused. These abnormal conditions may well contribute to the final histochemical and physiological pattern. The abnormal fiber size variation in ReJ 129 mice noted by Goldspink and Rowe (1969) was investigated in dy^{2j} animals by Dribin and Simpson (1976). The latter group found that the Type I fibers were hypertrophied in their older mice (2.5 - 5.5 months) and were

reduced in number while the Type II_{ox} fibers were variable in number but of the same size. Their abnormal group consisted of atrophied fibers.

Since morphometric techniques were not used in this study the fibers were not checked for size variations however the Type I fibers did look hypertrophied in several sections (personal observations).

In summary it would appear that the physiological study has confirmed that there is indeed a real transition of fast EDL to slow muscle properties and that there is relatively little change in the slow soleus muscle with the exception of the young soleus. The histochemical study which was designed as a means of determining in a more refined manner the changes occurring at the fiber type level could be interpreted as indicating that there is a preferential sparing of the Type I, supposedly slow muscle fiber. However this histochemical data does not necessarily fit with the physiological speeding found in this muscle. One would expect a slowing of the young dystrophic soleus if indeed the Type I fibers are slow and if they are spared.

Since this is apparently not the case it is possible that something even more basic than the fiber type level is required to explain the situation. Perhaps the effect is a general one but for some reason the Type I fiber type staining reaction remains slightly more resilient. It is clear however that the best way to resolve this problem is to examine individual motor units in both control

and dystrophic animals and determine the characteristics of each fiber type and the nature of alterations which occur to each fiber type.

CHAPTER V

APPENDICES

Appendix 1

Muscle Weights and Tensions (Absolute and Normalized)

In addition to the data presented in Table 1 concerning absolute and normalized twitch tensions at 37°C a duplicate set of data was obtained at room temperature and these data will be analysed here in Table 1A. At this lower temperature the fast muscle tensions are larger than at 37°C whereas the slow muscle develops slightly less tension.

Table 1A shows that there is an increase in absolute tension as the normal EDL ages (rows 1 and 3) similar to the 37°C data but there is now no difference in normalized tensions which is unlike the 37°C data. It would thus appear that reducing the temperature has a greater enhancing effect on the contractile mechanism of the young C57 EDL than on the old C57 EDL.

The young and old C57 SOL (rows 5 and 7) data are consistent with the 37°C data in that both absolute and normalized tensions are significantly increased in the older muscle. This increase in normalized tension probably reflects developmental change of the SOL contractile mechanism.

Now, examining the dystrophic muscle, the young dy^{2j} EDL shows a substantial reduction in absolute and normalized tension (rows 3 and 4)

TABLE 1A

The Effect of Muscular Dystrophy on the Absolute and Normalized Tensions of Fast and Slow Muscles of Mice of Different Ages at Room Temperature (a) (b) (c)

Muscle Description	Room Temperature	
	Twitch Tension (gms)	Normalized Tension (gms/gms)
Old C57 EDL (15)	9.0±0.5	816.2±35.8
↓ Old dy ^{2j} EDL (14)	4.6±0.4	704.0±54.0
Young C57 EDL (14)	6.6±0.4	815.9±30.9
Young dy ^{2j} EDL (13)	3.1±0.3	399.0±30.4
Old C57 SOL (15)	5.2±0.3	428.0±19.6
Old dy ^{2j} SOL (14)	3.0±0.2	496.4±40.0
Young C57 SOL (11)	3.3±0.3	374.0±15.6
Young dy ^{2j} SOL (12)	2.2±0.1	301.3±18.7

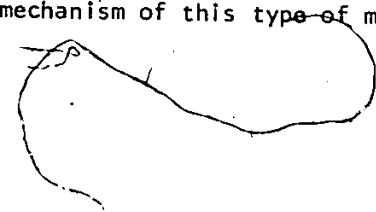
- (a) Values are mean ± SEM, values in parenthesis are N=Number of mice.
- (b) Normalized tension = grams tension/gram wet muscle weight.
- (c) Crossed vertical bars indicate significance at 5% level using student's t test.

as was the case at 37°C. The SOL (rows 7 and 8) also shows some decrement here but to a lesser extent than does the EDL.

The older dystrophic EDL is reduced in both tensions (rows 1 and 2) in contrast to the findings at 37°C where the normalized tension was found to have actually increased relative to the control value. The old dystrophic SOL (rows 5 and 6) was consistent with the 37°C data in that the absolute tension was reduced and the normalized tension showed no change from control.

Finally, the young dy^{2j} EDL exerts less tension (absolute and normalized) when compared with the older dy^{2j} EDL (rows 2 and 4) which is similar to the 37°C data. This is also true of the dy^{2j} SOL muscle (rows 6 and 8).

In summary, the room temperature data is for the most part in agreement with the 37°C data for absolute and normalized tensions. There were some interesting inconsistencies which are difficult to interpret. For instance the observation that normalized tension of the old dy^{2j} EDL was significantly lower than the control at room temperature but at 37°C it was greater. It would appear that the change in temperature causes a marked alteration of some sort within the contractile mechanism of this type of muscle.



Appendix 2

Study of the Time Parameters of the Contraction

In addition to the data obtained at 37°C observations were made at room temperature. While the lower temperature has the effect of slowing both the CT and the $\frac{1}{2}$ RT of fast and slow muscle, the fast muscle remains faster than the slow muscle response. Table 2A contains these data and indicates which of the muscles differed significantly. The data are analysed below and are considered in relation to the results at 37°C.

Firstly, the young and old C57 EDL muscles were found to be significantly faster than their respective C57 SOL counterparts so this information is not included on Table 2A.

The time parameters are not significantly different in the young and old C57 EDL as was found at 37°C (rows 1 and 3) and the old C57 SOL is significantly slower than the young C57 SOL which is also similar to the 37°C findings (rows 5 and 7).

The young dy^{2j} EDL is slower than control in both time parameters whereas it was only significantly slower in CT at 37°C (rows 3 and 4).

Interestingly, at room temperature the young dy^{2j} SOL is not significantly different from control whereas it had been speeded at 37°C (rows 7 and 8).

The old dy^{2j} EDL is much slower than control at room temperature (rows 1 and 2) and indeed it has become so slow that it is not significantly different in CT from the old C57 SOL. The $\frac{1}{2}$ RT of the old C57 SOL is slower however (not shown on table).

TABLE 2A

Isometric Twitch Contraction Times and Half Relaxation Times
in Normal and Dystrophic Mouse Fast and Slow Muscle at Room
Temperature (a) (b) (c)

Muscle Description			Contraction Time (msec)		Half Relaxation Time (msec)		
1.	Old C57 EDL	(22)	22.5±0.9	] X]	18.1±0.7	] X]	] X]
2.	Old dy ^{2j} EDL	(24)	33.6±1.3		38.9±2.8		
3.	Young C57 EDL	(14)	22.2±0.8	] X]	19.9±1.0	] X]	] X]
4.	Young dy ^{2j} EDL	(13)	27.1±0.9		27.2±3.5		
5.	Old C57 SOL	(18)	36.2±1.6	] X]	50.1±2.7	] X]	] X]
6.	Old dy ^{2j} SOL	(14)	41.9±2.2		63.9±4.5		
7.	Young C57 SOL	(11)	28.8±1.4	] X]	36.3±3.7	] X]	] X]
8.	Young dy ^{2j} SOL	(12)	31.7±0.9		33.9±2.8		

- (a) Values are mean ± SEM, figures in parenthesis are N=Number of muscles.
- (b) Crossed vertical bars indicate significance at 5% level using student's t test.
- (c) Each value shown here is significantly greater than its counterpart at 37°C.

The old dy^{2j} SOL has slowed relative to control in both CT and $\frac{1}{2}$ RT (rows 5 and 6) while at 37°C only the $\frac{1}{2}$ RT was significantly slower.

In addition the old dy^{2j} EDL was significantly slower than control (rows 2 and 4) as was the old dy^{2j} SOL (rows 5 and 8) which is consistent with the 37°C data.

In summary there is quite good agreement of the room temperature data with the 37°C data. However, the young dy^{2j} SOL which showed speeding at 37°C was not significantly different from control at room temperature. This is potentially of importance and an explanation is not readily available.

Appendix 3

Post Tetanic Potentiation/Depression

Supplementary to Table 4 which contains data pertaining to PTP/PTD at 37°C is the following Table 3A which contains similar data but at room temperature. These data will be compared as before but with reference also to the effect of lowering the temperature. As will be shown below the lower temperature resulted in a changed pattern of statistical significance.

However, as seen before the young C57 EDL demonstrated more PTP than did the young C57 SOL and this was also true of the old control muscles (not shown on Table 3A). Also as was previously found for the 37°C data, there was no significant difference between the pre and post-tetanic CT and ½RT values for any category of muscle so that a separate chart was not included here.

As at 37°C the young C57 EDL was statistically similar to the old C57 EDL (rows 1 and 3). The young and old C57 SOL values were not significantly different at 37°C but the old C57 SOL showed a lesser PTD than the young C57 SOL at RmT (rows 5 and 7). It seems paradoxical that the old C57 SOL should show a lesser PTD (a fast muscle tendency) than the young C57 SOL since I have previously suggested that the older C57 SOL appears to become more like a slow muscle with age. This puzzle can be simply explained if one considers the data more fully. While the old C57 SOL gives similar responses at both temperatures, the young C57 SOL is strongly affected by the temperature change. It displays a distinct PTP at 37°C but a distinct

TABLE 3A

First Post Tetanic Twitch as a Percent of Pre-Tetanus In
Normal and Dystrophic Mouse Fast and Slow Muscles at Room
Temperature (a) (b)

Muscle Description	First Post-Tetanic Twitch as a Percent of Pre-Tetanus at Room Temperature	RmT vs 37°C (b) Student t test
1. Old C57 EDL (13)	110.8±2.5	<
2. Old dy ^{2j} EDL (13)	103.3±2.4	
3. Young C57 EDL (10)	110.0±1.4	<
4. Young dy ^{2j} EDL (10)	105.7±2.1	
5. Old C57 SOL (11)	98.3±2.2	<
6. Old dy ^{2j} SOL (10)	105.2±2.1	
7. Young C57 SOL (10)	88.8±2.6	<
8. Young dy ^{2j} SOL (11)	96.4±2.2	

(a) Values are mean ± SEM. Values in parenthesis are N=Number of muscles. Crossed vertical bars = significantly different at 5% level using student's t test.

(b) < = RmT significantly less than 37°C at 5% level using student's t test.

PTD at room temperature. The cause of this erratic behavior is unclear but the strong PTD at room temperature, if taken by itself could mislead one to think that the old C57 SOL has the faster tendencies of the two muscles.

The young C57 EDL shows greater PTP than the young dy^{2j} EDL at 37 degrees (rows 3 and 4) but there was no difference between them at room temperature.

The old C57 EDL values are greater than the dy^{2j} values at both temperatures (rows 1 and 2). Interestingly, whereas the young dy^{2j} EDL showed more PTP than the young C57 SOL the old dy^{2j} EDL was not different from the old C57 SOL (not shown on Table 4A). This could indicate that as the disease advances the EDL is gradually transformed toward slow muscle properties as has previously been seen.

The young dy^{2j} EDL showed more PTP than did the old dy^{2j} EDL at 37°C but there was no difference at room temperature (rows 2 and 4). This is reasonable since fast muscles show less PTP at room temperature and this would tend to bring the two values closer together.

The young and old dy^{2j} SOL show more PTP than their respective controls at room temperature only (rows 5 and 6), and (7 and 8), and the young dy^{2j} SOL gives significantly more PTD than the old dy^{2j} SOL at room temperature only. It thus appears that the lower temperature has resulted in considerably different responses than at 37°C. Moreover, at the lower temperature the young and old dy^{2j} SOL responses show a shift toward PTP, relative to control. The cause of this is unclear.

An additional set of comparisons were carried out to complete the data on the first post-tetanic twitch. In order to determine the effect of temperature on each muscle category, t tests were done to find out whether significant post-tetanic changes occurred as a result of the temperature change. The last column of Table 3A contains this information. It shows that the post-tetanic response of the old dy^{2j} EDL, old C57 SOL and old dy^{2j} SOL are not altered by the change in temperature while the remaining muscle categories were significantly changed by the temperature. Since neither the old C57 SOL nor the old dy^{2j} EDL values changed with temperature, this is yet another bit of evidence illustrating that the dy^{2j} EDL becomes similar to a slow muscle.

In summary, the room temperature data differed considerably from the 37°C data in the PTP/PTD response. It would therefore appear that the post-tetanic effects are strongly influenced by the temperature of the experiment. In general the EDL muscle comparisons became more significant at 37°C whereas the SOL comparisons were more significant at room temperature.

Appendix 4

Indirect Stimulation of Mouse SOL at Room Temperature

Whereas the data obtained for SOL at 37°C showed several differences between direct and indirect stimulation there were no such differences at room temperature.

Table 4A shows the room temperature data for both methods of stimulation. While at 37°C the indirectly stimulated SOL was significantly faster this was not true at room temperature. In addition the PTP/PTD response became more similar to that of a fast muscle when indirectly stimulated at 37°C but here no such conversion was observed. The time course of the PTP/PTD response of SOL was recorded at room temperature for indirectly stimulated muscle has already been given in Fig. 10. There is very little difference between the curves of these pairs.

It would thus appear that the speeding of some SOL responses seen only at 37°C is due to a temperature dependent factor and is difficult to explain in more specific terms.

TABLE 4A

Effect of Indirect Stimulation on Normal and Dystrophic Mouse
SQL Muscle at Room Temperature (a)

Physiological Parameter	Indirect C57 SOL/ Direct C57 SOL	Indirect dy ^{2j} SOL/ Direct dy ^{2j} SOL
✓ Absolute Tension (gms)	5.2± 0.4 (7) 5.2± 0.3 (15)	3.1± 0.3 (7) 3.0± 0.2 (14)
Normalized Tension (gms/gm)	422.0±33.0 (7) 428.0±19.6 (15)	500.0±61.0 (7) 496.4±40.0 (14)
CT (msec)	33.4± 1.4 (9) 36.2± 1.6 (18)	38.4± 1.7 (7) 41.9± 2.2 (14)
½RT, (msec)	45.6± 3.7 (9) 50.1± 2.7 (18)	64.1± 5.6 (7) 63.9± 4.5 (14)
PTP/PTD (%)	96.2± 1.7 (9) 98.3± 2.2 (11)	105.2± 2.1 (10) 106.6± 2.7 (7)

(a) Values are mean ± SEM, numbers in parenthesis are numbers of muscles. None of the paired values above were significant at the 5% level using the student's t test.

REFERENCES

- Ashmore, C. and L. Doerr. 1971. Comparative aspects of muscle fiber types in different species.
Exp. Neurol. 31:408-418
- Asmundson, V. and L. Julian. 1956. Inherited muscle abnormality in the domestic fowl.
J. Hered. 47 p.248-252
- Bahler, A., J. Fales and K. Zierler. 1967. The active state of mammalian skeletal muscle.
J. Gen. Physiol. 50:2239-2253
- Ballantyne, J. and S. Hansen. 1974. New method for the estimation of the number of motor units in muscle.
J. Neurol. Neurosug. and Psych. 37:1195-1201
- Barany, M. 1967. ATPase activity of myosin correlated with speed of muscle shortening.
J. Gen. Physiol. 50 (suppl. part 2) 197-218
- Barany, M. and R.T. Close. 1971. The transformation of myosin in cross-innervated rat muscles.
J. Physiol. 213:455-474
- Barnard, R., V. Edgerton and J. Peter. 1970. Effect of exercise on skeletal muscle. I. biochemical and histochemical properties.
J. Applied Physiol. 28:762-766
- Barnard, R., V. Edgerton, T. Furukawa and J. Peter. 1971. Histochemical, biochemical and contractile properties of red, white and intermediate fibers.
Am. J. Physiol. 220:410-414
- Biscoe, T. and R. Taylor. 1967. The effect of admixture of fast and slow muscle in determining the form of the muscle twitch.
Med. Biol. Eng. 5:473-479
- Bowman, W., A.A. Goldberg and C. Raper. 1962. A comparison between the effects of a tetanus and the effects of sympathomimetic amines on fast and slow contracting mammalian muscles.
Brit. J. Pharm. 19:464-484

- Brady, A. 1968. Active state in cardiac muscle.
Physiol. Rev. 48:570-600
- Brooke, M. and K. Kaiser. 1970. Muscle fiber types: How many and what kind?
Arch. Neurol. 23:369-379
- Brooke, M. and K. Kaiser. 1974. The use and abuse of muscle histochemistry.
Annals N.Y. Acad. Sc. 121-144
- Brown, G.L. and U. von Euler. 1938. The after-effects of a tetanus on mammalian muscle
J. Physiol. 93:39-60
- Brown, M. and P. Matthews. 1960. The effect on a muscle twitch of the back-response of its motor nerve fibers.
J. Physiol. 150:332-346
- Brust, M. 1965. Combined effects of nitrate and caffeine on contractions of skel. muscles
Am. J. Physiol. 208:431-435
- Brust, M. 1966. Relative resistance to dystrophy of slow skeletal muscle of the mouse.
Am. J. Physiol. 445-451
- Buchthal, F., H. Schmalbruch and Z. Kamieniecka. 1971. Contraction times and fiber types in patients with progressive muscular dystrophy.
Neurology (Minn.) 21:131-139
- Buller, A.J., J.C. Eccles and R.M. Eccles. 1960. Differentiation of fast and slow muscle in the cat hindlimb.
J. Physiol. 150:399-416
- Buller, A.J. and D.M. Lewis. 1965. The rate of tension development in isometric tetanic tension development in tetanic contractions of mammalian fast and slow skeletal muscle.
J. Physiol. 176:337-354
- Buller, A.J., K.W. Ranatunga and J. Smith. 1968. Influence of temperature on the isometric myograms of cross-innervated mammalian fast twitch and slow twitch skeletal muscles.
Nature 218:877-878
- Buller, A.J., K.W. Ranatunga and J. Smith. 1968. The influence of temperature on the contractile characteristics of mammalian fast and slow twitch skeletal muscles.
J. Physiol. 196:82P

- Buller, A.J. and W. Mommaerts. 1969. Myofibrillar ATPase as a determining factor for contraction velocity, its changes upon experimental cross-innervation. *J. Physiol.* 201:46P
- Buller, A.J., C. Kean, K. Ranatunga and J. Smith. 1971. Some characteristics of the PTD of the isometric twitch response in cat slow-twitch muscle. *J. Physiol.* 217:62P-63P
- Buller, A.J. and U. Proske. 1978. Further observations on back-firing in the motor nerve fibers of a muscle during twitch contractions. *J. Physiol.* 285:59-70
- Burke, R., D. Levine, F. Zajac, P. Tsairis and W.K. Engel. 1971. Mammalian motor units: physiological-histochemical correlation in 3 types in cat gastrocnemius. *Sci.* 174:709-712
- Burke, R., D. Levine, M. Salzman and P. Tsairis. 1974. Motor units in cat soleus muscle: Physiological, histochemical and morphological characteristics. *J. Physiol.* 238:503-514
- Burke, R. and P. Tsairis. 1974. The correlation of physiological properties with histochemical characteristics in single muscle units. *Annals N.Y. Acad. Sc.* 145-158
- Butler, J. and E. Cosmos. 1977. Histochemical and structural analyses of the phenotypic expression of the dystrophic gene in the 129/ReJ dy/dy and the C57BL/6J dy^{2j} dy^{2j} mice. *Exp. Neurol.* 57:666-681
- Close, R. 1964. The relation between intrinsic speed of shortening and DAS of muscle. *J. Physiol.* 180:542-559
- Close, R. 1964. Dynamic properties of fast and slow skeletal muscles of the rat during development. *J. Physiol.* 173:74-95
- Close, R. 1967. Properties of motor units in fast and slow skeletal muscles of the rat. *J. Physiol.* 193:45-55
- Close, R. and J. Hoh. 1968. Influence of temperature on isometric contractions of rat skeletal muscles. *Nature* 217:1179-1180

- Close, R. and J. Hoh. 1968. The after-effects of repetitive stimulation on the isometric twitch contraction of rat fast skeletal muscle.
J. Physiol. 197:461-477
- Close, R. and J. Hoh. 1969. PTP of twitch contractions of cross-innervated rat fast and slow muscles.
Nature 221:179-181
- Close, R. 1972. Dynamic properties of mammalian skeletal muscles.
Physiol. Rev. 52:129-197
- Curran, M. and D.J. Parry. 1975. Neuromuscular function in fast and slow muscles of genetically dystrophic mice.
Exp. Neurol. 47:150-161
- Denny-Brown, D. 1929. The histological features of striped muscle in relation to its functional activity.
Proc. Roy. Soc. Ser. B. 104:371-411
- Douglas, W. and R. Baskin. 1971. Contractile properties of developing mouse dystrophic muscle.
Am. J. Physiol. 220:1344-1354
- Drachman, D., S. Murphy, M. Nigam and J. Hills. 1967. Myopathic changes in chronically denervated muscle.
Arch. Neurol. 16 p.14-24
- Dribin, L. and S. Simpson. 1977. Histochemical and morphological study of dystrophic (C57BL/6J dy^{2j}/dy^{2j}) and normal muscle
Exp. Neurol. 56:480-497
- Dubowitz, V. 1969. Chemical and structural changes in muscles: the importance of the nervous system in some inherited disorders of brain and muscle.
(Ed. J. Allen and D. Raine) (Livingstone, Eng.)
- Dubowitz, V. and A. Pearse. 1970. Reciprocal relationships of phosphorylase and oxidative enzymes in skeletal muscle.
Nature 185:701-702
- Duchenne, G. 1868. Recherches sur la paralysie musculaire pseudo-hypertrophique ou paralysie myo-sclerosique.
Arch. Gen. Med. Series 11;5, 179, 305, 421, 552.
- Duchenne, G. 1872. De l'électrisation localisée et son application a la pathologie et la therapeutique.
3rd Ed. Paris: Bailliere et Fils.

- Ebashi, S. and M. Endo. 1968. Calcium ion and muscle contraction. *Quart. Rev. Biophys.* 2:351-384
- Eccles, J.C., R.M. Eccles and A. Lundberg. 1958. The action potentials of the alpha motoneurons supplying fast and slow muscles. *J. Physiol.* 142:275-291
- Edgerton, V. and D. Simpson. 1969. The intermediate muscle fibers of rats and guinea pigs. *J. Histochem. Cytochem.* 17:828-838
- Edman, K. 1970. The rising phase of the active state in single skeletal muscle fibers of the frog. *Acta. Physiol. Scand.* 167-173 V.79
- Edstrom, L. and E. Kugelberg. 1968. Histochemical composition, distribution of fibers and fatiguability of single motor units. *J. Neurol. Neursurg. Psych.* 31:424-433
- Engel, W.K. 1962. The essentiality of histo and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. *Neurology.* 12:778-784
- Engel, W.K. 1970. Selective and non-selective susceptibility of muscle fiber types. *Arch. Neurol.* 22:97-117
- Engel, W.K. 1974. Fiber type nomenclature of human skeletal muscle for histochemical purposes. *Neurology* 24:344-348
- Erb, W.H. 1883. Ueber die "juvenile form" der progressiven. Muskelatrophie ihre beziehungen zur sogenannten pseudohypertrophie der muskeln. *Dtsch. Arch. Klin. Med.* 34:467-480
- Fahimi, H. and P. Roy. 1966. Cytochemical localization of LDH in muscular dystrophy of the mouse. *Aci.* 152:1761-1763
- Farréll, P. and R. Olson. 1973. Creative phosphate and adenine nucleotides in muscle of animals with muscular dystrophy. *Am. J. Physiol.* 225:1102-1106
- Fiehn, W. and J. Peter. 1971. Properties of the fragmented SR from fast-twitch and slow twitch muscles. *J. Clin. Invest.* 50:570-573

- Fischer, H. 1908. Zur physiologie der quergestriefften Musklen der Säugetiere.
Arch. Ges. Physiol. 125:541-583
- Fitts, R. and J. Holloszy. 1977. Contractile properties of rat soleus muscle effects of training and fatigue.
Am. J. Physiol. 233(3) C86-C91
- Gabel, L., C. Carson and E. Vance. 1970. Active state of muscle and the second and third derivatives of twitch tension.
Am. J. Physiol. 214 p.1025-1030
- Gardner, D. and D. Medwin. 1970a. Mutation rate in Duchenne M.D.
F. Med. Genetics 7 p.334
- Goffart, M. and J.M. Ritchie. 1952. The effect of adrenaline on the contraction of mammalian skeletal muscles.
J. Physiol. 116:357-371
- Goldspink, G. and R. Rowe. 1968. The growth and development of muscle fibers in normal and dystrophic mice. In: Research in muscular dystrophy (Proc. 4th Symp.) Ed. by members of the Research Committee of the M.D. group, Pitman Medical, London p.116-128
- Gowers, W.R. 1879. Pseudohypertrophic muscle paralysis
Churchill, London
- Grützner, P. 1884. Zur anatomie und physiologie der quergestreiften muskeln.
Rec. Zool. Suisse 1:665-684
- Guth, L. 1968. "Trophic" influences of nerve on muscle.
Physiol. Rev. 48:645-687
- Guth, L. and F. Samaha. 1969. Qual. differences between actomyosin ATPase of fast and slow muscle.
Exp. Neurol. 25 p. 138
- Guth, L. and H. Yellin. 1971. The dynamic nature of the so-called "Fiber types" of mammalian skeletal muscle.
Exp. Neurol. 31:277-300
- Guth, L. and F. Samaha. 1972. Erroneous interpretations which may result from applications of the myofibrillar ATPase histochemical procedure to developing muscle.
Exp. Neurol. 34:465-475

- Guth, L. 1973. Fact and artifact in the histochemical procedure for myofibrillar ATPase.
Exp. Neurol. 41:440-450
- Gutmann, E., S. Schiaffino and V. Hanzlihova. 1971. Mechanism of compensatory hypertrophy in skeletal muscle of the rat.
Exp. Neurol. 31:451-464
- Hadlow, D.J. 1973. Myopathies of animals in: "The striated muscle".
Ed. Pearson (Williams and Wilkins, Baltimore) p. 364-409
- Hall-Craggs, E. 1968. The contraction time and enzyme activity of 2 rabbit laryngeal muscles.
J. Anat. 102:241-255
- Hammond, G. and R. Ridge. 1978. Post-tetanic potentiation of twitch motor units in snake costocutaneous muscle.
J. Physiol. 276:535-554
- Harris, J.B. 1971. The resting membrane potential of fibers of fast and slow twitch muscles of normal and dystrophic mice.
J. Neurol. Sci. 12:45-52
- Harris, J. and A. Montgomery. 1975. Some mechanical and electrical properties of distal hindlimb muscles of genetically dystrophic mice (C57 BL/6J dy^{2j}/dy^{2j}).
Exp. Neurol. 48:569-585
- Hill, A.V. 1949. The abrupt transition from rest to activity in muscle.
Proc. Roy. Soc. B136 p. 399-421
- Hill, A.V. 1950. The development of the active state of muscle during the latent period.
Proc. Roy. Soc. B137 p. 320-329
- Hill, A.V. 1950. The dimensions of animals and their muscular dynamics.
Sci. Prog. 38:209-230
- Hill, A.V. 1951. The transition from rest to full activity in muscle: the velocity of shortening.
Proc. Roy. Soc. Sec B. 138:329-338
- Hironaka, T. and Y. Miyata. 1975. Transplantation of skeletal muscle in normal and dystrophic mice.
Exp. Neurol. 47:1-15

- Hoar, R. 1969. Anesthesia in the guinea pig.
Fed. Proc. 28, #4 p. 1517
- Hofmann, W. and E. Ruprecht. 1973. Observations on the efficiency of dystrophic muscle in vitro.
J. Neurol, Neurosurg. and Psych. 36:565-573
- Hoh, J. 1974. Neural regulation of muscle activation.
Exp. Neurol. 45:241-256
- Hoh, J. and M. Radulovacki. 1973. Electrolyte composition of fast-twitch and slow-twitch muscles in normal and dystrophic mice.
p. 331-336 In "Clinical Studies in Myology" B. Kukules(Ed)
Excerpta Medica, Amsterdam.
- Homburger, F., J. Baker, C. Nixon and G. Wilgram. 1962. New hereditary disease in Syrian hamsters. Primary generalized polymyopathy and cardiac necrosis.
Arch. Intern. Med. 110:660-662
- Iec. Cryotomy Handbook. 1975. 1st ed.
Damon Corp.
- Isaacson, A., M. Hinkes and S. Taylor. 1970. Contracture and twitch potentiation of fast and slow muscles of the rat at 20 and 37 C.
Am. J. Physiol. 218(1):33-41
- Julian, F. and R. Moss. 1976. The concept of active state in striated muscle.
Circ. Res. 1976 38:53-59
- Karpati, G. and W. Engel. 1968. Histochemical investigation of fiber type ratios with myofib. ATPase Rn. in normal and denervated skeletal muscle of guinea pig.
Am. J. Anat. 122, p. 145
- Karpati, G. and W. Engel. 1968. Correlative histochemical study of skeletal muscle after suprasegmental denervation peripheral nerve section and skeletal fixation.
Neurol. 681-692
- Knoll, P. 1891. Über protoplasmaarme und protoplasmareiche Muskulatur Denschr. Akad. Wiss. Wien.
Math-Nat. Kl 58:633-700
- Kruger, P. 1952. Tetanus und tonus der quergestreiften skelettmuskeln der wirbeltiere und des menschen.
Leipzig Acad. Verlag

- Kugelberg, E. and L. Edstrom. 1968. Differential histochemical effects of muscle contractions on phosphorylase and glycogen in various types of fibers: relation to fatigue. *J. Neurol. Neurosurg. Psych.* 31:415-423
- Kugelberg, E. 1973. Histochemical composition, contraction speed and fatiguability of rat soleus motor units. *J. Neurol. Sc.* 20:177-198
- Kugelberg, E. 1976. Adaptive transformation of rat soleus motor units during growth. *J. Neurol. Sc.* 27:269-289
- Lammergren, J. and R.S. Smith. 1966. Types of muscle fibers in toad skeletal muscle. *Acta Physiol. Scand.* 68:263-274
- Lewis, D.M. and D.J. Parry. 1979. *J. Physiol.* (in press).
- Lillie, R. 1964. Histopathologic technic and practical histochemistry. 3rd ed. N.Y., Blakiston Cpy. p. 174
- MacPherson, L. and D.R. Wilkie. 1954. The duration of the active state in a muscle twitch. *J. Physiol.* 124:292-299
- McComas, A. and A. Thomas. 1968. A study of the muscle twitch in the Duchenne type muscular dystrophy. *J. Neurol. Sci.* 7:309-312
- McComas, A., R. Sica and S. Currie. 1970. Muscular dystrophy: Evidence for a neural factor. *Nature (L)* 226:1263
- McComas, A., R. Sica and M. Campbell. 1971. "Sick" motoneurons, a unifying concept of muscle disease. *Lancet* 1, 321
- McComas, A., M. Campbell and R. Sica. 1971. Electrophysiological study of dystrophina myotonica. *J. Neurol. Neurosurg. Psych.* 34:132-139
- McComas, A.J. 1977. Muscular dystrophy in animals in neuromuscular function and disorders. A.J. McComas (Butterworths, London-Boston) p. 170-178
- Meier, H. and J. Southard. 1970. Muscular dystrophy in the mouse caused by an allele at the dy-locus. *Life Sce.* 9:137-144

- Meryon, E. 1852. On granular and fatty degeneration of the voluntary muscle.
Med. Chir. Trans. 35:73-84
- Michelson, A., E. Russell and J. Harman. 1955. Dystrophia muscularis: A hereditary primary myopathy in the house mouse.
Proc. Nat. Acad. Sci. U.S.A. 41:p. 1079-1084
- Montgomery, A. and L. Swenarchuk. 1977. Dystrophic mice show age related muscle fibre and myelinated axon losses.
Nature 267:167-169
- Mrak, R. and R. Baskin. 1978. Calcium transport and phosphoenzyme formation in dystrophic mouse sarcoplasmic reticulum.
Biochem Med. 19:47-70
- Nachlas, M., D. Walker and A. Seligman. 1957. A histochemical method for demonstration of DPNH diaphorase.
J. Biophys. and Biochem. Cytol. 4
- Novikoff, A., W. Shin and J. Drucker. 1961. Mitochondrial localization of oxidative enzymes. Staining results with 2 tetrazolium salts.
J. Biophys. and Biochem. Cytol. 9
- Olson, C. and C. Swett. 1969. Speed of contraction of skeletal muscle. The effect of hypoactivity and hyperactivity.
Arch. Neurol. 20:263-270
- Olson, C. and P. Swett Jr. 1969. Effect of prior activity on properties of different types of motor units.
Neurology 19:1-16
- Padykula, H. and E. Herman. 1955. The specificity of the histochemical method for ATPase.
J. Histochem. Cytochem. 3 p. 170
- Padykula, H. and G. Gauthier. 1967. Ultrastructural features of 3 fiber types in the rat diaphragm.
Anat. Rec. 157:157-296
- Parry, D.J. 1977. A study of functional denervation in fast and slow muscles of dystrophic mice of various ages.
Exp. Neurol. 55:556-566
- Parsons, R. 1974. Expression of the dystrophia muscularis (dy) recessive gene in mice.
Nature 251:621-622

- Paukul, E. 1904. Die zuckungsformen von koninchenmuskeln verschiedenen farbe und struktur.
Arch. Anat. Physiol. 100-120
- Pearce, G. and J. Walton. 1963. A histological study of muscle from the Bar Harbor strain of dystrophic mice.
J. Path. Bact. 86:25-33
- Peter, J., R. Barnard, V. Edgerton, C. Gillespie and K. Stempel. 1972. Metabolic profiles in 3 fiber types of skeletal muscle in guinea pig and rabbits.
Biochem. 11:2627-2633
- Ranatunga, K.W. 1977. Influence of temperature on the characteristics of summation of isometric mechanical responses of mammalian skeletal muscle.
Exp. Neurol. 54:513-532
- Ranvier, L. 1874. De quelques faits relatifs a l'histologie et a la physiologie des muscles striaes.
Arch. Physiol. Norm. Path. (Paris) 1:5-15
- Ritchie, J.M. and D.R. Wilkie. 1955. The effect of previous stimulation on the active state of muscle.
J. Physiol. 130:488-496
- Robbins, N., G. Karpati and W.K. Engel. 1969. Histochemical and contractile properties in the cross-innervated guinea pig soleus muscle.
Arch. Neurol. 201:318-329
- Romanul, F. 1964. Enzymes in muscle I. Histochemical studies of enzymes in individual muscle fibers.
Arch. Neurol. 11:355-368
- Romanul, F. and J. Van Der Meuler. 1967. Slow and fast muscles after cross-innervation. Enzymatic and physiological changes.
Arch. Neurol. 17:387-402
- Rowe, R. and G. Goldspink. 1969. Muscle fiber growth in five different muscles in both sexes of mice. II. Dystrophic mice.
J. Anat. 104:531-538
- Rowland, L. 1974. Part IV Clinical disorders of trophic function: Muscular dystrophy are the muscular dystrophies neurogenic?
Ann. N.Y. Acad. Sci. 244-260
- Rudel, R. and S. Taylor. 1973. Aequorin luminescence during contraction of amphibian skeletal muscles.
J. Physiol. 233 5-6R

- Sabbadini, R. and R. Baskin. 1976. Active state of normal and dystrophic mouse muscle.
Am. J. Physiol. 230(4):1138-1147
- Salafsky, B. 1971. Functional studies of regenerated muscle from normal and dystrophic mice.
Nature (L) 229:270-272
- Sandow, A. and M. Brust. 1958. Contractility of dystrophic mouse muscle.
Am. J. Physiol. 194 (3):557-568
- Sandow, A. and M. Brust. 1962. Effects of activity on contractions of normal and dystrophic mouse muscles.
Am. J. Physiol. 202 (5):815-820
- Scales, D., R. Sabbadini and G. Inesi. 1977. The involvement of sarcotubular membranes in genetic muscular dystrophy.
BBA 465 (3):535-549
- Scarpelli, D., R. Hess and A. Pearse. 1958. The cytochemical localization of oxidative enzymes: DPNH diaphorase and TPNH diaphorase.
J. Biophys. and Biochem. Cytol. 4 p. 747-760
- Schiaffino, S., V. Hanzlikova and S. Pierobon. 1970. Relations between structure and function in rat skeletal muscle fibers.
J. Cell Biol. 47:107-119
- Shafiq, S., M. Gorychi and A. Milhorat. 1969. An electron microscope study of fiber types in normal and dystrophic muscles of the mouse.
J. Anat. 104 p. 281-293
- Sher, J. and C. Cardasis. 1976. Skeletal muscle fiber types in the adult mouse.
Acta. Neurol. Scand. 54:45-56
- Sherrington, C. 1929. Some functional problems attaching to convergence.
Proc. Roy. Soc. B 105:332-362
- Sica, R. and A. McComas. 1971. An electrophysiological investigation of limb-girdle and facioscapulohumeral dystrophy.
J. Neurol. Neurosurg. and Psych. 34:469-474
- Smith, J. 1974. A comparison of the mechanical responses of cat fast and slow twitch skeletal muscle following repetitive stimulation.
Phd. thesis University of Bristol

- Standaert, F. 1963. Post-tetanic repetitive activity in the cat soleus nerve. Its origin, course and mechanism of generation. *J. Gen. Physiol.* 47:53-70
- Standaert, F. 1964. The mechanisms of PTP in cat soleus and gastrocnemius muscles. *J. Gen. Physiol.* 47:987-1001
- Stein, J. and H. Padykula. 1962. Histochemical classification of individual skeletal muscle fibers of rat. *Am. J. Anat.* 110:103-123
- Sushella, A., P. Hodgson and J. Walton. 1968. Murine muscular dystrophy. Some histochemical and biochemical observations. *J. Neurol. Sci.* 7:437-463
- Taber, R. and S. Irwin. 1969. Anesthesia in the mouse. *Fed. Proc.* 28, #4 p. 1528
- Taylor, R., W. Fowler, D. Mason and J. Spann. 1971. Contractile properties of skeletal muscle in dystrophic mice. *Arch. Phys. Med. Rehab.* 52:511-514
- Taylor, R., W. Fowler and D. Mason. 1974. Contractile properties of soleus muscle during development in normal and dystrophic mice. *Arch. Phys. Med. Rehab.* 55:531-539
- Truong, X., B. Wall and S. Walker. 1964. Effects of temperature on isometric contraction of rat muscle. *Am. J. Physiol.* 207 (2) 393-396
- Walton, J. and F. Nattrass. 1954. On the classification, natural history and treatment of the myopathies. *Brain* 77:169-231
- Walton, J. 1962. Clinical aspects of human muscular dystrophy. In muscular dystrophy in man and animals. (Ed. G. Bourne and N. Golaz) (Karger, Basle & N.Y.)
- Walton, J. 1973. Progressive muscular dystrophy: Structural alterations in various stages and in carriers of duchenne dystrophy. In the striated muscle. Ed. Pearson, C. p. 263-291
- Walton, J. and D. Gardner Medwin. 1974. Progressive muscular dystrophy and myotonic disorders in: Disorders of voluntary muscle. Ed. J. Walton p. 561-613

- Wells, J. 1964. Comparison of mechanical properties between slow and fast mammalian muscles.
J. Physiol. 178:252-269
- Vrbova, G. 1963b. The effect of motoneurone activity on the speed of contraction of striated muscle.
J. Physiol. 169:513-526
- Yellin, H. and L. Guth. 1970. The histochemical classification of muscle fibers.
Exp. Neurol. 26:424-432