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THE MECHANICAL PROPERTIES OF MOTOR UNITS OF EXTENSOR DIGITORUM LONGUS FROM NORMAL AND DYSTROPHIC MICE

by

DOUGLAS STUART BATESON

A thesis submited 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

The extensor digitorum longus muscle (EDL) of the mouse consists almost entirely of Type II fibres (fast-twitch). The motor unit composition of this muscle in both normal (C57BL) and dystrophic (dy2J/dy2J) mice has been examined. 64 motor units have been investigated in 15 normal mice of average age 84 days and weight 29 grams. 60 motor units have been studied in 15 dystrophic mice of average age 81 days and weight 22 grams.

Anaesthesia was by Na pentibarbitone 75 mg/Kg I.P. initially, supplemented via an indwelling peritoneal cannula as required. Whole blood from a healthy donor mouse was citrated and infused (0.7 to 1.0 ml) via a thin polyethelene cannula, drawn to a fine taper, and inserted into the femoral vein of the contralateral leg. Complete denervation of all muscles except EDL, was followed by laminectomy from L1 to L5. Motor units were isolated functionally by ventral root splitting of the L3 and L4 roots. Units were determined to be single by all or none contraction, EMG and antidromic ventral root action potential.

Motor unit tetanic tension, expressed as a percentage of whole muscle tetanic tension for both normal and dystrophic mice showed little variation, 1.0 to 11.7% (mean value $\pm$ SD = 4.6 $\pm$ 2.2%) and 1.0 to 18.4% (mean value $\pm$ SD = 6.7 $\pm$ 4.0%). This suggests that the C57 EDL contains about 22 motor units while

dystrophic EDL is composed of about 15.

Normal mice demonstrated a distribution of motor unit times to peak twitch tension that was unimodal. The mean value was 8.7 ± 1.2 msec (standard deviation). In EDL of dystrophic mice the mean time to peak twitch tension was 11.9 ± 2.7 msec (SD). These times to peak showed a tendency to fall into a bimodal distribution with mean values of 7.0 msec and 12.6 msec. Statistically, however, cumulative plotting of unit CT on probability paper failed to indicate anything but a normal distribution for dystrophic motor units.

The mean conduction velocity for normal mice was 70 m/sec (± 11.8 SD) and 46.3 m/sec (± 5.7 SD) for dystrophic mice. No simple relationships between unit tension or CT and conduction velocity were observed. No interrelationship between motor unit CT, Po or Pt/Po was observed.

The results presented are interpreted as supporting a neurogenic etiology for murine muscular dystrophy.

I THE MUSCULAR DYSTROPHIES: A BRIEF HISTORICAL REVIEW

The muscular dystrophies have long held a fascination for scientists since they were first clinically described (for historical review see Rowland 1974). Progress in the understanding of this disease was probably first approached systematically in the mid 19th century by the French school of neurologists. Of particular note was the pioneering work of Duchenne. It was he who described clearly the type of dystrophy that now bears his name.

Around this same time an English physician Edward Meryon described his clinical observations of four brothers afflicted with dystrophy (1852). He observed the disease to be hereditary, affecting males, and on post-mortem saw no abnormality in the nervous system. He postulated the disease reflected a primary defect in the muscle fibres, a conclusion Duchenne subsequently arrived at in 1872. This has remained relatively unchallenged until the mid-20th century.

In the next decades the emergence of several other forms of dystrophy led to a general confusion as to the classification of this disorder (reviewed by Walton and Nattrass 1954). It was Erb (1844) who did the most to define the histopathologic criteria common to the dystrophies (for review see Walton 1973a). So accurate was his work that today it is still valid for use in

diagnosis.

These consisted of:

1) rounding of muscle fibres shown in transverse sections with hypertrophy of some fibres and atrophy of others;
2) random variation in muscle fibre size and shape;
3) central migration of sarcolemmal nuclei in many fibres;
4) fibre splitting; and
5) extensive infiltration of fat and connective tissue.

Even with clear histopathologic criteria the classification of the muscular dystrophies is not easy. Its mode of inheritance, age of onset, rate of progression, distribution of affected muscle, etc. have made classification ambiguous at best. Walton and Gardner-Medwin (1974) have attempted to use an analysis of genetic and clinical data to arrive at a classification which divides progressive dystrophy into its "pure" form and that associated with myotonia (see Table 1).

The fact that Erbs criteria for dystrophy are also the histopathological description for a chronically denervated muscle (Walton 1973a) and data to be discussed in the following chapters have led investigators to a critical time in dystrophy research; elucidation of the etiology of the disease.

While it has now become possible to diagnose muscular dystrophy and some degree of stability has been achieved in its

TABLE I

CLASSIFICATION OF THE MUSCULAR DYSTROPHIES

I. "Pure" Muscular Dystrophies

a. Sex-linked muscular dystrophy

severe: Duchenne type

benign: Becker type

b. Autosomal recessive muscular dystrophy

limb-girdle type

c. Autosomal dominant muscular dystrophy

fascioscapulohumeral type (FSH type)

d. Distal muscular dystrophy

e. Ocular muscular dystrophy

f. Oculopharyngeal muscular dystrophy

II. Autosomal Dominant Myotonic Dystrophy

classification the direction of future research hinges on the defining of the primary site of action of the responsible genetic lesion. By our modern concepts, a genetic lesion would imply a protein (enzymatic) abnormality(s). But whether this abnormality occurs at a neurogenic or myogenic site still remains highly contested.

II THE ETIOLOGY OF HUMAN DYSTROPHY : MYOGENIC VS NEUROGENIC

Muscular dystrophy has been primarily considered as a classical myopathy i.e. a disease that originates within the muscle fibre itself. This has been the situation historically (Meryon 1852, Duchenne 1872). Recently, however it has been shown that at a stage when the muscle fibres show signs of severe degeneration their axons and spinal cord appear normal (Meier and Southard 1970).

But appearance of normality and normality of function do not necessarily go hand in hand. This has been the basis for a recent challenge to the myopathic hypothesis in the form of a neural hypothesis for dystrophy (McComas et al 1971).

For many years the influence of nerve on muscle in terms of its electrical impulse patterns (Buller, Eccles and Eccles 1960, Vbrova 1963, Salmons and Vbrova 1969) and the less precisely defined trophic biochemical influence (for review see Guth 1968, Lewis 1972 and Gutmann 1976) have been known.

Guth (1968) defined trophic influences of nerve on muscle as "those functions of the nerve that affect or regulate the metabolism of the muscle cell". Because of the complex nature of this relationship one could envision a metabolic defect of a motorneurone which produces no morphologic alteration in itself, but, which results in disorder for the muscle fibre (presumably

via an altered trophic effect). Recently, experiments have shown the existence of a transport of various proteins from muscle to nerve (H^3-lysine labelled proteins, Watson 1968, and horseradish peroxidase (HRP), Kristenssen and Olsson 1971). These data provide the possible mechanism for a trophic influence of muscle on nerve. We must therefore concede a primary genetic defect in the muscle fibres could give rise to its degeneration and induce a major abberation(s) in the motorneurone. This could subsequently result in further myopathic degeneration.

Some experimenters have begun to question the myopathic origin of dystrophy. Even early workers in the 1900's on the gross observation of the selectivity of muscles affected by the various dystrophies suggested the involvement of a neural component.

The chief proponent of a neural hypothesis was McComas who formulated a "sick motorneurone" hypothesis (McComas et al 1971). Their work hinged on an indirect method in which they were able to estimate the numbers and sizes of functioning motor units in a muscle.

McComas reasoned that if dystrophy were a myopathic process and muscle fibres were affected in a random fashion (this may in fact be untrue see Chapter V) one would expect to see all motor units being affected. However, in the early stages of the

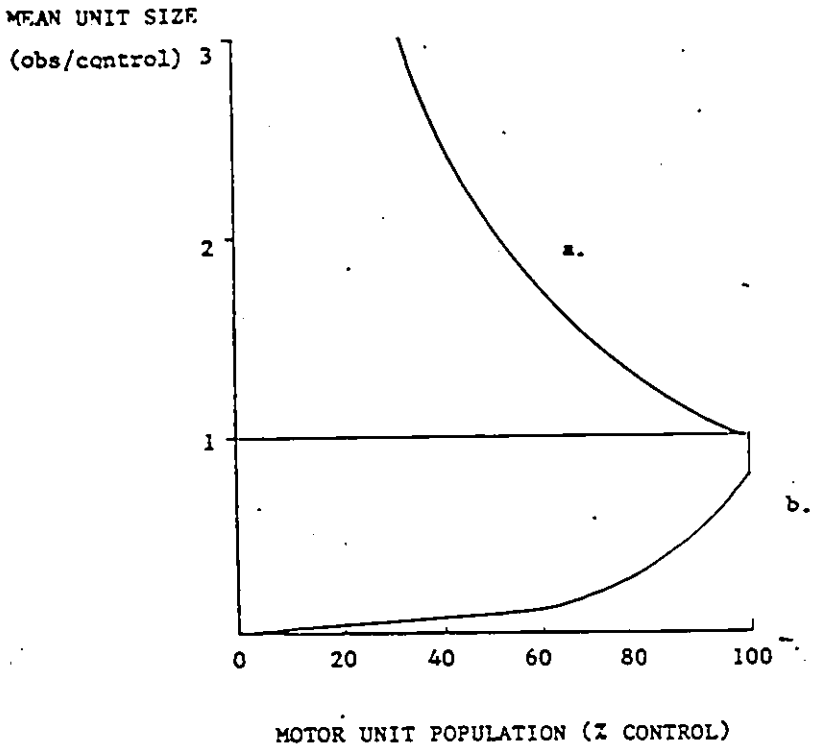
disease there should still be a full complement of motor units, though their mean size would be reduced. With progression of the disease, more muscle fibres would degenerate and eventually some motor units would lose all of their muscle fibres or become so small they could no longer be detected. The number of functioning motor units would decrease but concomitantly the mean sizes of motor units remaining would become significantly less. With further progression additional motor units are lost while the survivors mean amplitude would be even smaller. This can be expressed graphically in Figure 1b (after McComas et al 1971)

If however one considers the case of a chronic denervating disorder (indicative of a neural abnormality) a different picture evolves for dystrophy. While the number of motor units would decrease as the disease progresses, one would expect the average unit size to increase due to collateral innervation by surviving motorneurones. In this way surviving motor unit territories would be expected to increase by the capturing of still functional but now denervated muscle fibres. Ideally this is represented in Figure 1a.

Using the motor unit counting (estimating) technique in patients with various dystrophies, McComas reported losses of functioning motor units with the remaining ones having normal or only slightly reduced numbers of indirectly estimated muscle

FIGURE 1

Figure 1 shows the predicted changes in motor unit size as a result of a) chronic denervation and b) a random myopathic disorder. (After McComas et al 1971).


MEAN UNIT SIZE
(obs/control)
3
2
1
a.
b.
0
20
40
60
80
100
MOTOR UNIT POPULATION (% CONTROL)

fibres. On the basis of this observation and the previous lines of thought he proposed the "sick motorneurone" hypothesis which expounded a neural defect for myotonic dystrophy, limb-girdle and FSH types and Duchenne dystrophy (for review see McComas et al 1974).

This hypothesis suggests that motorneurones may exist in three states: healthy, dead and a previously overlooked state between these two extremes in which the neurone is dysfunctional or, in other words, sick. Healthy motorneurones he defined as being able to maintain all the muscle fibres of the motorunit in terms of conduction of impulses, trophic influences, etc. As well, they have the ability to successfully establish collaterals with any denervated fibres and can therefore enlarge their motor unit territory. This can clearly be seen in partial denervation of muscle.

Sick motorneurones have difficulty in satisfactorily maintaining the viability of their muscle fibres. In addition, although each motorneurone may command a normal or slightly reduced number of muscle fibres they are unable to successfully send out collaterals to reinnervate denervated muscle fibres.

Dead motorneurones exert no excitatory or trophic influence on muscle fibres.

McComas' observation of motor unit numbers and sizes

does not reflect a myopathic progression but resembles a modified denervation phenomenon in which remaining motorneurones are sick and therefore unable to incorporate denervated muscle fibres into their motor unit territory via axonal sprouting.

This hypothesis therefore suggests a neurogenic defect in muscular dystrophy. This subsequently manifests itself in muscle and the resultant myopathic observations are a secondary expression of a primary neural lesion.

The principle of the estimating method is to compare the sizes of potentials generated by individual motor unit discharges, through graded stimulation of the nerve, with that of the whole muscle. The mean motor unit size is estimated by averaging the first 10-12 putative motor unit responses. This value is then divided into the maximal muscle response thereby giving an estimate of the number of functioning motor units present.

McComas makes four assumptions that are necessary for this technique to be valid. They are:

1. electrical activity is derived from a single muscle;
2. the incremental responses evoked by graded stimulation correspond to the activation of successive individual units;
3. the evoked motor unit potentials summate algebraically; and
4. the motor unit potentials used in the calculation of the mean potential amplitude are representative of those generated by

the total population of units.

The validity of this technique has been strongly criticized.

Desmedt and Borenstein (1973) have questioned the use of the EDB muscle on the basis that it exhibits neurogenic changes even in normal young subjects with no history of neurological disease.

Panayiotopoulos et al (1974, 1975) suggest that the noise level of the recording system of McComas was sufficiently high as to obscure the small amplitude MUAP's (muscle unit action potentials) that electromyographic data suggest exist. They question whether the incremental responses to graded stimuli correspond to activation of single units by pointing out the possibility of two motor units of the same threshold being mapped as one. This raises the question of whether the first 10-12 putative motor unit potentials used to determine the mean MUAP value are representative of the total unit population. This point has also been questioned by Feasby and Brown (1974).

Using a method of superimposing enlarged photographs of motor unit responses and analysing these by computer Panayiotopoulos and co-workers claim to be able to distinguish and count the very small units. Otherwise, they have used essentially the same technique to estimate motor unit numbers but have arrived

at an opposing conclusion to McComas. They demonstrated no significant difference between the numbers of motor units in patients with Duchenne dystrophy and controls but showed both the maximal muscle response and the average MUAP to be significantly reduced with dystrophy. In patients suffering limb-girdle muscular dystrophy (1975) they could show no evidence of a functional loss of motor units and concluded their results were in accordance with the traditional concept of a myogenic origin for dystrophy.

Their technique still requires the same assumptions as McComas. They claim to overcome the most significant problem (noise obscuring small amplitude MUAP) by photographing many responses, superimposing the enlarged photographs and analyzing by a computer of average transients. This enables responses of similar amplitude to be more easily discriminated and thereby minimizes missing small amplitude MUAP. However, Ballantyne and Hansen (1974) point out that whether analysed by eye or by on-line computer criticism of this method is still valid.

Parry, Mainwood and Chan (1977) have demonstrated on theoretical grounds that the necessary conditions for algebraic additivity of MUAP's are not met. In the hypothenar group an underestimate of 26% of the number of motor units was found. Since McComas (1974) observed a decrease in motor unit numbers of

36% in the hypothenar group of dystrophic patients this may be significant. This criticism applies equally to Panayiotopoulos's work.

Therefore while the method of motor unit estimation is a valuable tool, its results are variable and can only be taken as indicative and not conclusive.

Considerable debate has centred around this neurogenic approach to dystrophy and several diverse and suggestive lines of investigation have arisen due to this hypothesis.

Because most experiments are not able to be carried out using human dystrophics, it becomes necessary to have experimental models that represent the human situation from which hopefully valuable information can be attained.

III THE MOUSE MODEL

One of the greatest difficulties man faces in his quest to understand himself and his afflictions is his ability to obtain useful experimental models. Without such models, man's research is limited to observation and theoretical conjecture. This has been the case with the muscular dystrophies. Relying on superficial observation, autopsy results and surmize, the advancement of knowledge in this disease has been slow and yet still quite remarkable; a tribute to the integrity and astuteness of early scientists in this field. However, with the emergence of several animal models research has progressed at a tremendous rate.

Several animal species with inherited muscular dystrophy are currently being investigated. These include the domestic chicken (Asmundson and Julian 1956), in which dystrophy exhibits a preferred involvement of the pectoral muscles, the Syrian hamster and related lines (Homburger et al 1962) and more exotic creatures such as the turkey, mink and Peking Duck (Hadlow 1973).

However, the most widely studied animal model is the common house mouse. This model may be the most useful since its clinical features of dystrophy most closely resemble human muscular dystrophy. Still, debate continues regarding the relevance of these models for human forms of dystrophy. Mendell

et al (1979) reviewed the relevance of genetic animal models to human muscular dystrophy. They looked at characteristics of early muscle fibre breakdown i.e. Horseradish peroxidase (HRP) leakage into cells, Ca+2 accumulation, and the similarities and differences in the overall "dystrophic" process between various animal models and the human muscular dystrophies. They concluded that while no animal model totally mimics the human disorder each offers significant contributions to our understanding of dystrophy and this is particularly true of the mouse model.

In 1951 at the R.B. Jackson Memorial Laboratory (Bar Harbor, Maine) an autosomal recessive mutation appeared within the colony of inbred strain 129 mice. It was characterized by a progressive ataxia, atrophy and hindlimb paralysis. No demonstrable pathology could be found in any examined neural tissue. However, muscle tissue showed loss of striations, necrosis, variation in fibre size, fibre rounding and central nucleation, all compatible with Erb's classical definition of muscular dystrophy.

Because this so closely resembled human muscular dystrophy, the most notable difference being the lack of fat infiltration and the non-sex linked genetic background, this hereditary myopathy was termed "dystrophia muscularis". The genetic symbol "dy" was assigned and description of the afflicted

mice was therefore 129 ReJ dy/dy (Michelson et al 1955).

Due to the rapid onset, progression and severity of this disease successful breeding of homozygotes is virtually impossible unless special precautions are taken (Butler and Cosmos 1977). One must therefore breed heterozygotes (dy/+) and use the 1/4 dy/dy progeny. Thus, because it is not possible to recognise the mice of dy/dy genotypes before the age of 3 weeks one cannot study the very early ontogenic stages. At the time when afflicted individuals can be identified the disease state is quite advanced. This makes this particular strain very difficult to work with.

More recently a newly discovered hereditary myopathy in inbred WK/ReJ mice, resembling the early dy mutant, has been reported (Meier and Southard 1970). Since the two mutations represent separate alleles at the same gene locus, by genetic nomenclature this new allele was designated "dy2J". This genetic trait, also autosomal recessive, was subsequently transferred to the strain C57BL/6J and therefore afflicted individuals have the title C57BL/6J dy2J/dy2J. It is this strain of mice that were used throughout the experiments reported in this thesis. Although the two mutant genes dy and dy2J, are allelic, the progression and degree of severity of the disease differs drastically. With a much later onset and progression of clinical symptoms in the dy2J

mice, both affected males and females reach sexual maturity and may successfully breed. This provides an enormous advantage over 129 ReJ mice.

The clinical signs of dystrophy can be detected at approximately 21 days of age. The hindlimb musculature seems to be principally affected and progressive muscle weakening soon leaves the hindlimbs useless and in a paralysis-like state. Accompanying this is a spontaneous spastic twitching of the hindlimb and pelvic musculature. This phenomenon may take on importance in the expression of the dystrophy in these muscles. The wasting process is paralleled by an arching of the spine as abdominal and spinal muscles weaken. The afflicted mouse drags itself by its forelimbs, which though clearly histologically dystrophic do not exhibit the spasticity of the hindlimbs (personal observation). As the disease progresses the fur thins and obvious wasting occurs. Still, these mice are capable of living in excess of 1 year with proper conditions.

With the availability of such a model a much more comprehensive understanding of muscular dystrophy has become possible. However, it must be noted here that while many similarities exist (clinically, histologically, biochemically and physiologically, see Ann. NY Acad. Sci. Vol 317 1979) between murine dystrophy and human muscular dystrophies the two may not

necessarily be related in terms of their primary genetic defect and expression. Still no substantial evidence has been presented to indicate this model as unacceptable and therefore a more complete understanding of murine dystrophy can only lead to a better understanding of the human dystrophies.

IV THE ETIOLOGY OF MURINE DYSTROPHY: MYOGENIC VS NEUROGENIC

Together with McComas' neurogenic hypothesis, recently documented abnormalities of the peripheral nerves and neuromuscular junction in dystrophic mice has strengthened the inclusion of a neural lesion in the etiology of murine muscular dystrophy. For example the proximal amyelination of the sciatic nerve and its spinal rootlets (Bradley and Jenkison 1973, Stirling 1975a, Bray and Aguayo 1975) may be part of the phenotypic expression of the dy gene manifested through a Schwann cell abnormality. As well, altered axoplasmic transport of neural metabolites and proteins (Komiya and Austin 1971, Tang, Komiya and Austin 1974) in the sciatic nerve of dystrophic mice have strongly suggested abnormal motorneurone activity. This may have a direct effect on the post junctional changes seen in dystrophic muscle. Both Ragab (1971) and Gilbert et al (1973) observed an altered ultrastructure of the end plate region in dystrophic mice. They noted significant decreases in the number of synaptic vesicles and a reduction in number and complexity of post junctional folds.

In order to determine if such neural abberations cause or are major contributors to the dystrophic process, experiments to directly test the etiology of the disease have been devised. The first attempts to elucidate the genetic lesion of murine dystrophy came in the form of muscle transplantation experiments.

Salafsky (1971) using minced muscle transplants between normal and dystrophic mice claimed that dystrophic muscle took on a normal appearance (in terms of measured contractile properties) when transplanted and allowed to regenerate in normal hosts. The opposite occured to normal muscle minces regenerated in dystrophic hosts. He concluded that dystrophic muscle was rendered defective by the environment of the dystrophic mouse and therefore murine muscular dystrophy is not a primary myopathy. This suggested a neural lesion for dystrophy that probably resides within the motorneurone and results in the synthesis and/or transport of defective trophic substances which produce in muscle the characteristic changes associated with muscular dystrophy.

In a similar experiment Hironaka and Miyata (1973b, 1975) transplanted whole extensor digitorum longus (EDL) muscles rather than minces. Their results supported Salafsky's observations and conclusion that dystrophy is not a primary myopathy but most likely a failure within the nervous system. However, in both these cases a major criticism arises in the fact that regenerated muscle tissue may originate from cells remaining from the host muscle which had been removed or from host neighbouring muscle rather than from precursor cells derived from within the transplant.

Neerunjun and Dubowitz (1975) investigated the source of

regenerating cells by transplanting minced dystrophic and normal anterior tibialis muscle labelled with tritiated thymidine into normal unlabelled hosts. Both autoradiography and radioactivity counts showed that the transplanted muscles were regenerated from muscle cells derived from within the donor tissue. It would therefore seem likely that normal motor innervation determines the state of the regenerated transplanted dystrophic muscle as proposed by Salafsky. This is further supported by the fact that the dystrophic environment does not support the regeneration of either normal or dystrophic transplants (Neerunjun and Dubowitz 1974 (in hamster) and 1975 (in 129 ReJ mice)).

But the transplantation picture is by no means definitive. Bradley, Jenkison and Montgomery (1975) point out that the extent of the abnormalities in muscle regenerates makes interpretation of trends towards normality in previously dystrophic muscle and vice versa virtually impossible.

On the other hand work emanating principally from Cosmos' laboratory has provided evidence against a neural involvement. Her transplantation studies have involved not only dystrophic mice but dystrophic chickens. In the latter model they concluded that the dystrophic abnormality was inherent in the muscle cell (Cosmos and Butler 1971a, 1972). In an elegant experiment to eliminate the possibility that a neuronal influence

may be pre-established during embryogenesis they transplanted pectoral muscles from 12 day embryos to newly hatched chicks. Here the host could not alter the characteristics of the regenerates.

In transplation studies of the minced gastrocnemius and anterior tibialis of 129 ReJ littermates Cosmos (1973) and Cosmos, Butler and Milhorat (1973) were again unable to confirm Salafsky's conclusion. They followed the structural changes of minced transplants over a period of 9 - 36 weeks. Dystrophic muscle in normal hosts showed no regeneration and trahsplants were eventually infiltrated with fat and connective tissue. The environment of the normal host could not prevent the degeneration of dystrophic transplants. Normal transplants in dystrophic hosts were maintained in a state closely resembing normality. Since the dystrophic environment did not totally inhibit normal transplant regeneration and in view of the embryonic chick studies, dystrophy appeared to be a myogenic disorder independent of neural considerations. Physiological measurements of similar transplants confirmed the morphologic and histochemical findings (Hsieh et al 1973).

Because of conflicting results and questions as to the validity of using transplants of whole and particularly minced muscle more clear cut and definitive techniques have been sought.

One such promising line has been the use of tissue culture techniques to provide an *in vitro* system to examine the neurotrophic effects, if any, of spinal cord on developing dystrophic muscle. Paul and Powell (1974) have examined the morphologic development of coupled cultures of normal and dystrophic embryonic cord with normal and dystrophic adult mouse muscle (strain dy) in an attempt to analyze their regenerative capabilities.

Regeneration of teased muscle strips (normal or dystrophic) was virtually non-existent in cultures devoid of fetal spinal cord. Initial cellular outgrowth from spinal cord sections of both normal and dystrophic explants were similar. Dystrophic muscle initiated myotube formation (regeneration) statistically sooner than normal muscle fragments in the presence of either normal or dystrophic spinal cord. This may however merely be a reflection of more myogenic cells being available in the dystrophic muscle. Dystrophic myotubes were however incapable of maturation unlike normal myotubes, irrespective of the type of cord present in the culture. They suggest this would indicate normal fetal cord does not exert a "curing" effect on dystrophic muscle regenerates nor does the dystrophic cord interfere with normal regeneration. Hamburgh *et al* (1975), using the same type of culture system, came to the same conclusions using the dy2J

strain of mouse. They did however demonstrate maturation of dystrophic myotubes in the presence of both normal and dystrophic cord cultures. Degenerative changes did not appear in dystrophic cells until approximately 50 days. This may be related to the differences in the degree of severity and rate of onset of the two strains of dystrophy (see Chapter II). It therefore seems that neural influence is necessary for regeneration, but the type of influence has no subsequent effect on the phenotypic expression of the regenerate.

In a similar study involving chick cell cultures Peacock and Nelson (1973) were able to prepare cultures of dystrophic myotubes from pectoral muscle to which they added neurones from either dystrophic or normal embryos. Under both conditions they showed a high degree of synapse formation and electrical coupling between nerve and muscle cells. However, no obvious improvements of dystrophic myotubes when innervated by normal cord as compared to dystrophic cord innervation could be shown. These culture experiments, though involving different strains of mouse and species (chicken), still all point to a myogenic and not neurogenic etiology for the disease.

There is however opposing evidence. Gallup and Dubowitz (1973) demonstrated that regeneration of normal and dystrophic mouse muscle in culture is influenced by the genotype of fetal

mouse cord present. It must be pointed out that no explanation for the differences in results reported between these and other workers has been made and both experiments appear equally valid. Paul and Powell (1974) themselves point out the difficulty of reconciling such conflicting results due to the large numbers of variables between experiments (see their discussion).

In the presence of normal spinal cord Hamburgh (1972) demonstrated both normal and dystrophic muscle fused to form new muscle fibres with well developed cross-striations and peripheral nuclei. Fibre groups demonstrated spontaneous synchronous contractions with activity maintained throughout the culture time (7 weeks). No difference could be seen between normal and dystrophic muscle in this culture except, as previously seen, a slightly quicker and greater regeneration occured with dystrophic muscle. In contrast, muscle coupled with dystrophic spinal cord showed an initial cellular regeneration response (in the first week) but was quickly followed by degeneration and loss of muscle elements leaving only strands of connective tissue. Both significant quantitative and qualitative differences could be shown between myotubes and muscle fibres coupled with normal and dystrophic cord. These workers concluded "that murine dystrophic muscle behaves morphologically quite normally when regenerating in the presence of normal spinal cord; and that regeneration of both

normal and dystrophic muscle is severely affected when coupled with spinal cord from dystrophic mice. The state of muscle is therefore dominated by the spinal cord."

Tissue culture too then has yielded inconsistent results. The artificial culture conditions and the vast difference in the stage of progression of dystrophy between cord and muscle explants may account for, or contribute to, this inconsistency. It does however, seem clear that a humoral or vascular etiology may be discounted.

A more "natural" experimental approach to study nerve-muscle interaction is the technique of parabiotic cross-reinnervation. This avoids the criticism of muscle transplant experiments. It allows the motorneurone cell bodies to remain undisturbed in the donor spinal cord (a possible site of damage when these are removed for tissue culture) and enables the recipient muscle to be neither surgically injured nor deprived of circulation.

Douglas (1975) and Montgomery (1975a) both independently studied in detail the physiological parameters of self-innervated and cross-reinnervated N- N, dy- dy, N- dy & dy- N (donor motorneurone- recipient muscle) parabionts. Reinnervation was assessed to have occured by sensory and motor reflex testing and was first detected in all parabionts by approximately 30 days post

surgery. This time for reinnervation to occur is in agreement with that observed by others (Guth 1956, Parry 1979). After 180 - 190 days post surgery, animals were examined for experimental parameters and histochemistry. To be sure muscles were fully functionally reinnervated two criteria were utilized. First muscles were tested for response to indirect stimulation of the regrown nerve. Secondly, in both groups morphologic and histochemical analyses were made (Douglas and Cosmos 1973, Johnson and Montgomery 1975b). Reinnervation was indicated since fibre type grouping did occur. This is taken as evidence of successful reinnervation (Kugelberg et al 1970). In addition end-plate formation typical of normally innervated muscle was demonstrated by acetylcholinesterase staining.

The time period prior to physiological measurements appears to have been sufficient for donor nerves to impress their effects on recipient muscle since differences between twitch and tetanus responses of group N- N, self-innervated normals, unoperated normals and group dy- dy, self-innervated dystrophics and unoperated dystrophics were statistically insignificant within each group.

Dystrophic nerves showed no problems in their ability to reinnervate despite regions of root and nerve amyelination and disturbed axoplasmic flow. It should be noted that the site of

nerve transection in the peripheral portion of the sciatic has been shown to be morphologically normal (Bradley and Jenkison 1973).

Most important, dystrophic muscle cross-reinnervated with normal nerves having presumptive normal neuotrophic and electric activity showed no differences from dy- dy parabionts. As well dy-N cross-reinnervates developed to normal unoperated twitch and tetanus levels.

The results of these experiments agree with earlier transplantation experiments by Cosmos' group and again fail to support a neurogenic expression of the dy gene. A primary myopathy is indicated.

Montgomery and Johnson (1975a & b), from the morphologic point of view, concluded that the introduction of a dystrophic nerve into a normal muscle does not result in induction of dystrophic changes in the recipient muscle. At the same time, N-dy cross- reinnervation does not induce any "curing" effect on recipient dystrophic muscle. Like Douglas and Cosmos (1973) they saw no difference in muscle weights within groups N- N, dy- N and dy- dy, N- dy. This is a parameter that is acknowledged to be under neural control (Gutmann 1962). In terms of isometric twitch characteristics (twitch tension, Pt; time to peak tension, CT; half-relaxation, $\frac{1}{2}$RT; and tetanic tension, Po) no statistical

difference within the following groups could be shown;

a). unoperated N, self-innervated N, N- N, dy- N, and

b) unoperated dy, self-innervated dy, dy- dy, N- dy.

In combination with their histochemical findings a neurogenic etiology for muscular dystrophy could not be upheld.

In an elegant derivation of this experiment Law et al (1976) used a double crossed preparation in which the slow solei of parabionts were cross-reinnervated with the "fast" tibial nerve of the partner.

This method allows one to more directly assess the neurotrophic effects of a "fast" nerve on a slow muscle and observe specifically the influence of this nerve on dystrophy.

Normally slow muscles like soleus do not exhibit any post tetanic potentiation (PTP) and have contractile parameters i.e. CT, ½RT that are markedly slower than fast muscles such as EDL. On cross-union of nerve with muscle it has been observed that the muscle characteristics (contractile and histochemical) convert to those of the type of innervation it receives i.e. slow soleus converts to fast properties when innervated by a "fast" nerve (Buller et al 1960, Close 1969). In this study the slow soleus of the normal parabiont cross-reinnervated by the "fast" tibial nerve of the dystrophic partner, as well as the opposite cross (N- dy), showed a PTP and decrease in CT and ½RT.

Histochemically, the cross-reinnervated muscles showed a conversion of fibre type to one resembling fast muscle (i.e. appearance of pale SDH-staining fibres). It therefore seems clear that dystrophic nerve can supply the necessary neurotrophic and/or nerve impulse-related activity to support muscle and the cross-reinnervated muscles are capable of responding to this re-innervation.

From their histochemical studies however, it was clearly shown that normal nerves could not arrest the dystrophic process nor can dystrophic nerves impose dystrophic characteristics on cross-reinnervated normal solei.

The elegance of these experiments lies in the demonstration that the dystrophic nerves are capable of exerting normal neurotrophic influences but these nerves are incapable of inducing dystrophic characteristics in normal muscles. A neurogenic etiology is not supported.

Criticism of these experiments with regards to the stage of progression of the disease in relation to its first detection for experimentation and the need for surgical preparation makes a more ideal situation desirable. One such ideal would be to study genetically dystrophic muscle innervated by a normal neural input or genetically normal muscle innervated by a dystrophic nervous system in a naturally occuring system. Though this might at first

seem more like science fiction, Peterson (1974) has been able to do so with the mouse chimaera model.

Chimaeras are derived from aggregating embryonic cells of different genotypic backgrounds. In these experiments normal C57Bl/6J and dystrophic C57Bl/6J dy2J mice were used. The mosaic morula produced by aggregation is cultured until the single blastocyst stage (24 hrs.) and then transferred to pseudopregnant females for complete development.

If muscles of identified dystrophic genotype can be found that have normal characteristics it would suggest a non-myopathic origin for the dystrophy. If genetically normal muscle shows dystrophic characteristics this too would then support an extramuscular origin for the disease.

The primary requirement for these experiments is an unambiguous genetic marker with which to determine muscle genotype. Several such markers do exist. Peterson chose to use the electrophoretic isoenzyme pattern of the malic enzyme. He observed, in some chimaeras, muscle whose genotype was 83% dystrophic but showed no detectable abnormality (only occasional central nuclei). Conversely, muscle from a chimaera of no detectable dystrophic genotype showed extensive fibre degeneration and central nucleation. These results are not compatible with a myogenic etiology. Still, criticism that

sufficient normal nuclei may be retained within mostly dystrophic fibres and therefore are somehow capable of preventing dystrophy make these results only suggestive and not conclusive.

We arrive then at a time where it appears naive to ask the question "Is muscular dystrophy myogenic or neurogenic?" and expect a simple answer. This complexity may be attributed to a possible pleiotropic expression of the primary genetic lesion or it may be a result of differences in the animal model as a model for human muscular dystrophy. However, until a complete understanding of the animal model is achieved, our understanding of its application to human dystrophy must remain limited.

V DYSTROPHIC MUSCLE

i) PHYSIOLOGICAL EFFECTS OF DYSTROPHY

The principal effect of the gene defect in muscular dystrophy has been felt to be skeletal muscle wasting. As discussed the myogenic etiology has been challenged by a neuronal hypothesis. However, regardless of the etiology, skeletal muscle is devastated by this disease. To better understand muscular dystrophy, its mechano-chemical effects, etiology and progression, it becomes important to understand how its apparent main target, skeletal muscle, is affected. Questions arise regarding whether extrinsic factors (e.g. increased connective tissue) or intrinsic factors (e.g. active state) are the target resulting in the altered mechanics of afflicted muscle. How do the mechanical properties of muscles alter with the progression of dystrophy? Are these alterations the same for both the 129 ReJ dy and C57 Bl/6J dy2J strains? These are the types of questions that have led workers to investigate the physiological effects of dystrophy on skeletal muscle.

With the early discovery of the dy form of dystrophy, considerable work has evolved regarding the effects of muscular dystrophy on muscles of the 129 ReJ mouse. It will be seen in this discussion that relatively little work has been reported for mice of the dy2J strain (the strain used in this study). Still,

sufficient data do exist to enable a good basic understanding of what dystrophy is doing to skeletal muscle in these two strains and what differences exist between the two in terms of the effects of dystrophy.

In attempting to summarize the data available and determine its significance it may be easiest to approach this topic in a chronological manner.

During the late 1950's and the 1960's Sandow and Brust (1958, 1962) studied various aspects of the muscle mechanics of the 129 ReJ mouse. They examined excised, whole field-stimulated gastrocnemii and teased gastrocnemius muscle strips. From their data Sandow and Brust (1958) concluded that in comparison to normal muscle, dystrophic muscle fibres are inherently altered so that their rates of development of tension and fall of tension are significantly reduced and their active state plateau is of shorter duration. It is this reduced duration of active state that may account totally, or at least in part for the reduced tension and rate of development they saw in dystrophic gastrocnemius muscle. The extended $\frac{1}{2}$RT they felt was due to an alteration of that part of the mechanism of the contractile system responsible for relaxation (Ca^{+2} reuptake, which has subsequently been shown to be reduced in dystrophic muscle, Sreter et al 1964, Sabbadini and Baskin 1976, Mrak and Baskin 1978 a & b). In a follow-up paper

(Sandow and Brust 1962) the changes in gastrocnemius with the onset of fatigue in both the twitch and tetanus were examined. Quite unexpectedly their data showed that dystrophic muscle was much less fatiguable than normal muscle. In order to be sure this was not an artefact of poor diffusion between the muscle and the bath, in some experiments they used teased strips of normal gastrocnemius that approximated the size of whole dystrophic muscle. Experiments on normal whole and teased strips revealed no differences between the two.

Examining a twitch series for fatigue (stimulus 1/sec for 1200 twitches) they saw that the peak tension output for dystrophic muscle was rather constant, dropping steadily by only 20% over the test fatigue, while normal muscle tension fell off rapidly by 90% after 600 twitches. This strongly indicated dystrophic muscle was much less fatiguable than normal muscle. Looking at the fatigue during tetanus they saw that the tetanic tension output of dystrophic muscle was less rapidly decreased by activity than normal muscle. These results, for gastrocnemius subjected to a long series of twitches and tetani, show the fatigue pattern of dystrophic muscle is markedly different from normal muscle. Dystrophic muscle is less fatiguable to both a series of twitches and tetani.

From these observations they noted that the slow rate of

decline of twitch tension that prevailed over the test series in dystrophic muscle was similar in appearance to the very late portion of fatigued normal muscle, suggesting that rested dystrophic muscle is generally already in a state similar to the normal fatigued muscle and that this may mean that the transformation of normal to dystrophic muscle in mouse results from the conversion to new properties resembling those of slow muscle. This concept of a conversion of muscle properties prompted a comparative investigation between the fast gastrocnemius and slow soleus muscle (Brust 1966), and was the first investigation of a slow dystrophic muscle type. In all measured parameters (Pt, Po, $\frac{1}{2}$RT, muscle weight, fatigue and duration of active state plateau) soleus was either unaffected or significantly less affected than gastrocnemius.

Brust (1966) made several conclusions from this and prior studies. Firstly, the physiological effect of dystrophy on slow muscle was more limited and less severe than that in fast muscle. This suggested to him a sparing of slow muscle from dystrophy. Secondly, the primary effect of dystrophy was to shift the characteristics of fast muscle towards those of slow muscle.

Other evidence to support this hypothesis has come from biochemical studies of staining of lactate dehydrogenase (Fahimi and Roy 1966). Their results suggested that the "white" fibres

(fast fibres) in the 129 ReJ mouse were primarily affected by dystrophy.

In a morphologic study on muscle fibre growth, Rowe and Goldspink (1969) showed that although soleus was affected to the same degree as anterior tibalis and EDL (both fast muscles) in terms of muscle weight loss, it was spared in terms of total fibre number loss, mean fibre diameter reduction and total fibre cross-sectional area change. Their results demonstrated a clear derangement of orderly relationships found in normal muscle but indicated the slow soleus to be significantly spared.

Shafiq et al (1969) presented a detailed study of muscle fibres of both fast and slow type in normal and dystrophic 129 ReJ mice. Degenerative effects could be seen in both the slow soleus and fast EDL and gastrocnemius though they were very much more pronounced in the fast muscle types. By localizing calcium oxalate deposits as a measure of Ca+2 uptake, they noted a greater accumulation of Ca+2 in the EDL fibres than in the soleus of normal mice. In dystrophic EDL fibres the uptake of Ca+2 was judged to be significantly less while no alteration occured in the dystrophic soleus muscle fibres. Concomitantly dystrophic EDL exhibited an increase in the number of its mitochondria while dystrophic soleus showed no alteration. Again slow muscle fibres seemed to be spared from dystrophy while there appeared to be a

conversion of features in fast muscle that caused them to resemble slow ones.

Taylor et al (1971, 1974) examined the contractile properties of the soleus muscle of normal and dystrophic 129 ReJ mice with the progression of the disease. They saw a significant reduction in muscle weight, muscle length and cross-sectional area between dystrophic and normal muscle. As others had noted tension and dP/dt were significantly reduced. In contrast to Brust (1966) however, Taylor and co-workers saw either no change or an increase in the duration of active state coupled with an increased ½RT. It may be that the less efficient sarcoplasmic reticulum of dystrophic muscle (Sreter et al 1967) may result in the longer active state plateau and increased ½RT seen. They concluded that the mechanical alterations of dystrophy in soleus are not due to an abnormal shortening of the active state (since this was not seen) or an incomplete fusion at stimulus frequencies equivalent to natural motor unit firing rates as others previously claimed. They suggested an altered state of intrinsic contractile properties occurs in muscular dystrophy.

In a later paper, Taylor and Fowler (1976) reported on the contractile properties of 129 ReJ fast (EDL) and slow (soleus) muscles. Their results confirmed an earlier paper by Douglas and Baskin (1971) on these same muscles. EDL was impaired much

earlier than soleus and much more severely in terms of developed tension, dP/dt, passive length-tension properties and an increased ½RT. With age, dystrophic EDL became weaker but more resistant to fatigue. This may suggest a loss of fast anaerobic (fast fatigue) fibres and a conversion to slow (fatigue resistant) aerobic fibres (for fibre type classifications see Chapter V. (ii) and Chapter VI.). These results are in good agreement with prior conclusions regarding slow muscle sparing and fast muscle slowing in the 129 ReJ dystrophic mouse.

Hofmann and Ruprecht (1973) however were unable to confirm all the observations made on the 129 ReJ EDL and soleus. In particular they saw no increased fatigue resistance of dystrophic fast muscle or any indication that fast muscle was preferentially affected. They point out that in Brust's original work using gastrocnemius, most experiments made use of teased muscle strips. These, they felt, were subject to a more rapid in vitro deterioration and they suggested that his results were due to experimental artefact. This however does not apply to the work of Taylor or Douglas and Baskin, whose use of whole muscles gave conclusions that supported Brust's. Hofmann and Ruprecht did however show the dystrophic EDL to have much longer twitch times than normal and a reduced contractile strength, both in agreement with Brust.

This then seems to leave a general concensus that dystrophy in the 129 ReJ dystrophic mouse causes a shift of fast muscle properties towards those of slow muscle, while slow muscles themselves remain relatively spared.

Can this conclusion however be applied to dy2J allelic mice? Though the disease caused by the dy2J allele appears similar but less severe than the original dy mutation, does this reflect only a quantitative difference or is there a qualitative difference in the expression of these two alleles?

Relatively little work has been done on the C57BL/6J dy2J/dy2J strain of mouse to answer these questions. Harris and Montgomery (1975) produced the first detailed examination of mechanical and electrical properties of muscle (anterior tibialis, EDL and soleus) of this dy2J strain. They observed many similarities to dy affected whole muscles from 129 ReJ dystrophic mice in terms of muscle weight, twitch tension, tetanic tension, contraction time and dP/dt. Half-relaxation time, however, was not significantly different from control values. Both dy2J soleus and EDL showed a lower than normal resting potential with EDL significantly less than soleus. This agrees with an earlier study by Harris (1971) on 129 ReJ mice in which he showed the resting membrane potential was reduced for both soleus and EDL but relatively more reduced for EDL.

In terms of the action potential, normal EDL fibres had a greater rate of rise and overshoot amplitude than SOL. In dystrophics however, action potentials in EDL had a lesser rate of rise and lesser overshoot while soleus parameters were unaffected. This is in line with the general but not unanimous belief of fast slowing and slow sparing seen in 129 ReJ mice. Similar action potential properties were seen by Harris and Marshall (1973) in 129 ReJ mice.

Harris and Montgomery also demonstrated miniature endplate potentials were present in all dystrophic muscle fibres examined. Many fibres in both EDL and soleus were sensitive to ACh outside the endplate region and were able to generate action potentials in the presence of 10^{-6}M TTX. These are considered features of immature and developing or functionally denervated muscle. Because they could not find any fibres that did not produce an action potential when stimulated indirectly they concluded their data did not support the idea of these fibres being denervated. They suggest that they represent muscle fibres in the later stages of regeneration. They concluded that this capacity to regenerate may represent a major qualitative difference from dy muscle, in which attempts at regeneration are generally abortive (Sushella et al 1969).

Curran and Parry (1975) examined the miniature end plate

potential frequency in both developing EDL and soleus muscles of the C57BL/6J dy2J strain. Normal EDL reached a higher discharge frequency than normal soleus. Dystrophy did not affect the discharge frequency of soleus but the adult EDL frequency was significantly lowered.

Parry (1977) addressed the question of functional denervation by examining the functional innervation ratio for EDL and soleus in vivo. This ratio:

$$\frac{\text{max. twitch tension from nerve stimulation}}{\text{max. twitch tension from direct muscle stimulation}} \times 100$$

will reflect any functional denervation phenomena. A decrease in the functional innervation ratio was seen in both muscles but it occurred some time after the symptoms of dystrophy were clearly apparent. It therefore appeared that there was not a significant degree of functional denervation in dystrophic mice at an age up to 10 weeks. Parry noted that it is possible denervation could be occurring but that the denervated fibres are captured by collaterals from healthy motor units. This however would not be consistent with McComas' neurogenic hypothesis. It was concluded that a primary neurogenic etiology could not be supported. Parry acknowledged that clearly there is evidence favoring a neurogenic abnormality but this may be only a secondary result of a primary

myopathy.

Parslow and Parry (1981) examined both intrinsic contractile properties (CT, ½RT, duration of active state plateau, post tetanic potentiation (PTP) and cooling potentiation (CP)) as well as histochemistry of young (4-6 weeks) and old (6 months) fast (EDL) and slow (soleus) muscles of dy^{2J} mice.

They clearly demonstrated several points:

1) fast-twitch muscles of dystrophic EDL contracted more slowly than normals. No difference was seen with soleus.
2) the slowing they observed was accompanied by a conversion to slow properties in cooling potentiation and post tetanic potentiation (CP to CD and PTP to PTD). (Note: Fast muscles normally exhibit a twitch cooling potentiation (CP) when temperature is decreased while slow muscles exhibit a cooling depression (CD) of thier twitch. Similarily after tetanus, fast muscles will normally exhibit a post tetanic twitch potentiation (PTP) whereas slow muscle exhibits a depression (PTD).)
3) no change was seen in the DASP.

They concluded that the fast twitch EDL clearly shifts its contractile characteristics towards those of a slow twitch muscle in dy2J mice. Old dystrophic soleus on the other hand was not significantly different from control mice whereas young

dystrophic soleus actually demonstrated faster characteristics than age matched controls. They felt this reflected the changing fibre-type composition of the soleus with development and it is the increase in presumed slow twitch Type I fibres (26% to 60%) that accounts for the slowing of the normal soleus with age. In EDL however, very few Type I fibres (if any) exist and therefore its slowing with the progression of dystrophy cannot be attributed to a selective involvement of fast twitch Type II fibres and a Type I sparing. It may be that continuous spontaneous activity bombarding the hindlimb musculature is in fact responsible for its changed contractile parameters.

It therefore seems apparent that in both strains of dystrophic mice the disease produces similar effects. However, the rate of onset differs. The general tendency is for a conversion of fast muscle properties to those resembling slow muscle. Slow muscle itself appears relatively spared, although Parslow and Parry (1981) did note that young dystrophic soleus was faster than young control soleus and slowed with maturation, though old dystrophic soleus was not significantly different from old control soleus.

The alterations in intrinsic mechanical parameters may be the result of either fibre type conversion or the selective loss of specific fibre types. It is important to note that

selective attack of specific fibre types is not foreign to man and therefore lends credibility to this concept. For example, in nemaline myopathy and central core disease there is a selective loss of Type I fibres. In muscle wasting due to disuse or cachexia selective Type II atrophy occurs.

Sica and McComas (1971) noted that patients with limbgirdle and FSH dystrophies exhibited a slowing in both CT and ½RT in examined muscles. This was also seen in patients suffering Duchenne dystrophy (McComas and Thomas, 1968). This suggests a selective loss of Type II fibres or their possible conversion to slow Type I properties. Buchthal et al (1971) correlated increased slowing of muscle bundles in human dystrophies with increased Type I fibres, again supporting specific fibre type involvement.

In an attempt to "visually" define the effects of dystrophy on the muscle fibre population considerable work has emerged making use of various histochemical stains. By comparative studies of normal and dystrophic muscle it has been hoped that the precise effects of dystrophy on each fibre type can be determined and related to the altered physiological parameters previously described.

ii) HISTOCHEMICAL EFFECTS OF DYSTROPHY

Since the early work of Grutzner (1884) and Knoll (1891) distinct and striking differences in morphology and cytochemistry have been noted in skeletal muscle. Most muscles had been grossly divided into either "red" or "white" fibres. Red fibres are usually thin, dark in tone and contain many mitochondria and fat globules. White fibres on the other hand, are thicker, opaque-like in tone and contain few mitochondria and little fat deposition. These early workers also noted that muscles examined were not totally homogeneous for one particular fibre type.

Later, more dissimilarities between muscles were pointed out on a comparative basis with their contractile properties. Sreter et al (1966) demonstrated that fast and slow muscles, defined so by their contraction times, have distinct myosins which possess several distinct structural differences. Related to this was the demonstration of differences in the specific activity of myosin ATPase in fast and slow muscle (Barany 1967).

Schiaffino et al (1970) studying the relationships between structure and function in a comparison of fast EDL and slow soleus in rat reported several key differences. They noted most fibres of rat EDL were large, had few mitochondria, a lesser myoglogin concentration and thicker Z lines than the slow soleus. In soleus they found significantly less sarcoplasmic reticulum

(SR) which was discontinuous at points between itself and the t-system. As well, they noted the presence of many dyads as opposed to typical triads. Based on the known contractile ditterences between fast and slow muscle they then suggested their observations were related to the physiological parameters of contraction time and resistance to fatigue. This gained support rather quickly when Fiehn and Peter (1971) reported on the properties of fragmented SR from fast-twitch and slow-twitch muscles. They concluded that fast SR takes up Ca+2 4 - 11 times faster than slow muscle SR. Much speculation has been made with regards to correlations of histochemical reactions with physiological parameters. The question of this correlation will be dealt with later in Chapter VI.

Though earlier workers noted that whole muscle color was not solely a function of vascularity it was not until Dubowitz and Pearse (1960) described a reciprocal relationship between phosphorylase and oxidative enzyme contents in human normal and dystrophic muscle fibres as well as for various other animal skeletal muscles that systematic histochemical classification of muscle fibres began. In 1962, Stein and Padykula examined glycogen, succinic dehydrogenase, ATPase and non-specific esterase in serial sections of rat gastrocnemius and soleus muscle fibres and developed a tripartite classification scheme based on the

histochemical staining patterns. Peter et al (1972) developed a fibre type classification based on quantitative biochemical, histochemical and physiological measurements. Using guinea pigs and rabbits they were able to examine muscles that were virtually homogeneous for a particular fibre type. These they compared to other muscles within the animal on the basis of contraction time, glycolytic and oxidative capacity. Using rabbit semimembranosus accessorius and the white portion of the guinea pig vastus lateralis they showed these fast-twitch white fibres to be fast-twitch glycolyic fibres. The red portion of the guinea pig vastus lateralis could be identified as fast-twitch oxidative-glycolytic in nature while the guinea pig soleus as slow-twitch oxidative fibres.

While this work clearly related histochemistry and physiology previous work on guinea pig muscles had provided good evidence for Type I fibres being slow fibres and Type II fibres as being composed of two groups, fast-twitch red and fast-twitch white. (Karpati and Engel 1968, Edgerton and Simpson 1969, Barnard et al 1971).

However, these studies involved whole muscle histochemistry of muscle fibres and shed no light on the relationship between the functional unit of muscle (the motor unit), its physiology and the observed histochemistry.

Currently, the literature is confusing in terms of fibre typing with no common, agreed nomenclature. This has led to fibre type classifications ranging from two to an unlimited number (Dubowitz 1960, Guth and Yellin 1971). The difficulty arises from a lack of agreed parameters with which to define muscle fibre types. Fibres have been classified by any number and combination of differences in contractile properties, morphology, color, ultrastructure, myosin types, myosin ATPase activity, pH lability, metabolism and oxidative and glycolytic enzyme content. Generally speaking a compilation system based in Dubowitz (1960) and Engel (1962) has found good acceptance and is used within this thesis. This is based upon the Type I and Type II system. It avoids the use of physiological terms, such as fast and slow, to describe a histological observation and the erroneous implications this might have.

The method of fibre typing referred to in this thesis (see Parry and Montpetit 1978) is based on myofibrillar ATPase at pH 10.4 and 4.5 (Guth and Samaha 1969) and NADH diaphorase for oxidative capacity (Novikoff et al 1961). Type I fibres stain pale with alkali-stable ATPase and intermediate with NADH diaphorase. Type II oxidative fibres stain darkly with both alkali-stable ATPase and NADH diaphorase. Type II glycolytic show an intermediate staining with alkali-stable ATPase and stain pale

with NADH diaphorase. Parry points out (Parry and Parslow 1981) that while these fibre types can most probably be related to contractile parameters, without motor unit data to confirm any correlation its assumed validity should be avoided. Table II is a tabulation of several of the current fibre type classifications and how they relate to each other (compiled after Dubowitz 1966, Close 1972, Syret and Munson 1981).

These three distinct fibre types can be functionally described as follows:

Type I (I, SO, S, intermediate) fibres are small with an extensive blood supply, a high number of mitochondria, and a high myoglobin content. Ultrastructure studies reveal a moderate number of end plate presynaptic vesicles; a few short junctional folds; and a wide Z band. Chemically, these fibres are amply supplied with oxidative enzymes and show little capacity for glycolysis or anaerobic metabolism. The muscle has a slow twitch that is small in amplitude, and is almost infatiguable with repetitive stimulation (tetanus).

Type II glycolytic (IIb, FG, FF, white) fibres are large and have a relatively sparse capillary supply, few mitochondria, and a low myoglobin content. Ultrastructurally there are abundant motor end plate presynaptic vesicles; numerous long junctional folds; and a narrow Z band. Biochemically, the fibres are well

TABLE II

CURRENT FIBRE TYPE CLASSIFICATIONS AND THEIR RELATIONSHIP TO THE SYSTEM USED IN THIS THESIS.

		Fibre Types		
Present System		I	II_{ox}	II_{glyc}
Dubowitz and Pearse	(1960)	I	II	II
Engel	(1962)	I	II	II
Stein and Padykula	(1962)	B	C	A
Romanul	(1964)	III	II	I
Padykula and Gauthier	(1966)	Intermed.	Red	White
Kugelberg and Edstrom	(1968)	C	B	A
Yellin and Guth	(1970)	b	a	ab
Brooke and Kaiser	(1970)	I	IIa	IIb
Burke et al	(1971)	S	FR	FF
Peter et al	(1972)	SO	FOG	FG

supplied with glycogen and phosphorylase, but have few oxidative enzymes, indicating a high capacity for anaerobic metabolism. Mechanically, the muscle twitch is fast and large amplitude but fatigues rapidly with tetanic stimulation.

Type II oxidative (IIa, FOG, FR, red) fibres fall between the Type I and Type II glycolytic fibres in cytological characteristics. Biochemicaly, they have moderate amounts of both oxidative and glycolytic enzymes. Mechanically, they produce fast twitches of low tension and are relatively resistant to fatigue when compared to Type I and Type II glycolytic fibres.

The results of histochemical analysis of dystrophic muscles have not been totally conclusive or in total agreement amongst various laboratories. The greater amount of information has arisen from studies of the dy2J strain of dystrophy.

Dribin and Simpson (1977) were the first to exploit histochemical analysis with regards to dystrophic (dy2J) muscle. They compared normal and dystrophic soleus and gastrocnemius muscle using the acid and alkali-stable ATPase (pH 4.35 and 10.4 respectively) and succinic dehydrogenase (SDH), for oxidative capacity. For ease of comparison with following papers the Type I & II system will be used. In their original work Dribin and Simpson use a red (Type II oxidative), intermediate (Type I) and

white (Type II glycolytic) nomenclature.

In terms of morphologic abnormalities in dystrophy both soleus and gastrocnemius exhibited fibre splitting, fibre necrosis, central nucleation, atrophic fibres, connective tissue infiltration and fibre diameter variations. In soleus these abnormalities were randomly distributed throughout the muscle. In gastrocnemius, however, morphologic alterations were not randomly distributed. They noted that overall the outer region of dystrophic gastrocnemius exhibited fewer gross abnormalities than the deeper region near the bone.

Histochemically dy2J soleus fibres were classified solely on the basis of the ATPase reaction as the SDH staining at the age of study (3 - 6 months) was not useful in differentiating between fibre types. Fibres were classified as Type I, Type II oxidative or abnormal. The dystrophic soleus exhibited a substantial number of abnormal atrophied fibres; a significant decrease in the numbers of Type I fibres; and variable numbers of Type II oxidative fibres, with a general overall decrease seen.

In gastrocnemius both ATPase and SDH staining were used to classify fibre types. The normal gastrocnemius exhibited a distinct grouping of fibre types. The region near the bone surface is composed primarily of Type II oxidative and Type I fibres with few interspersed Type II glycolytic fibres. This is

sometimes referred to as the red region. The outer region of gastrocnemius is composed almost entirely of Type II glycolytic fibres and is sometimes referred to as the white region.

Dribin and Simpson concluded that for gastrocnemius the white region (Type II glycolytic) is both morphologically and histochemically more affected than the red region. In soleus the Type I fibres appear preferentially decreased in number compared to Type II oxidative fibres and as well, existing dystrophic Type I's were selectively hypertrophied.

In the preceding section, we saw the hypothesis of whole fast muscle properties becoming slower compared to slow muscle. Part of this hypothesis of fast muscle slowing is the suggestion of a conversion of biochemical and physiological properties. Dribin and Simpson point out that their results suggest that Type II glycolytics became "redder" in terms of ATPase staining. Nevertheless even if fast fibres become more like slow fibres the question remains whether or not this reflects i) a preferential destruction of Type II glycolytics; ii) a defective maturation of Type II glycolytics; or iii) a conversion of fibre types. Though their study can not answer these questions they point out that since the observed histochemical alterations preceded gross morphlogic abnormalities a preferential destruction of Type II glycolytic fibres seems improbable. They suggest that the

alterations, seen also in previous studies reflect not only effects of dystrophy but may be the indirect result of altered functional demands on the muscle causing a conversion in fibre typing. Clearly the rigid paralysis of the hindlimbs and the continual phasic twitching must somehow alter the expression of dystrophy within the muscle. Silverman and Atwood (1980) have shown a correlation between this activity and the conversion of the level of oxidative enzymes in dystrophic muscle.

Two major criticisms of the Dribin and Simpson paper do exist. Firstly, the age of mice used (3 - 6 months) are such that the disease is highly advanced, muscle wasting is extensive and the ability to clearly distinguish different staining patterns is rather poor (Parry personal communication). Secondly, their comparison to early physiological studies (i.e. Brust 1966) involves comparison of dy and dy2J strains in an attempt to correlate physiological and histochemical observations. As has been pointed out these two strains should be considered as two separate disease proceses and as will be shown later (Chapter VI.) unless motor unit data is available the question of correlating histochemical and physiological observations must be treated with great care. Butler and Cosmos (1977) recognizing the distinction between the two alleles of dystrophy and the age related criticism have approached a similar histochemical study in a different

manner. They examined both dy/dy and dy2J/dy2J mice (soleus and anterior tibialis) from 2 - 3 weeks postpartum to 6 months. They noted that at older age both fast and slow-twitch fibres are affected by dystrophy in both strains of mice. However, they demonstrated a "different temporal involvement of different fibre types in the two strains of mice" at younger ages. Their conclusions were that in 129 ReJ dy/dy mice the early expression of the disease is in the white, fast-twitch glycolytic fibres while red, slow twitch fibres are spared until later. In the C57BL/6J dy2J/dy2J however, the primary expression is in the "red, slow-twitch oxidative fibres". Although these results somewhat agree with Dribin and Simpson (for C57 mice) they have been justifiably criticized. Parry and Parslow (1981) point out that Butler and Cosmos erroneously classified their fibre types. Their classification was based on SDH and phosphorylase activity. This has lead them to categorize the core region of anterior tibialis as "red, slow-twitch oxidative fibres." Parry and Parslow point out the principally affected fibres here are in fact Type II oxidative fibres and are therefore probably not slow-twitch by nature. Similarly, Cosmos and Butler describe the soleus as being composed of "slow red" fibres, when in fact its constitution is a mixture of Type I and Type II oxidative fibres. Both these fibre types exhibit high oxidative capacity and by

absence of a corroborating stain (i.e. ATPase) they erroneously claim in soleus that "slow red" fibres are primarily affected.

Parry and Parslow (1981) demonstrated in young soleus (4 weeks old) that the proportion of both Type I and Type II oxidative fibre loss was approximately equal, suggesting no preferential involvement of either fibre type. This trend was seen by Dribin and Simpson (1977) but due to the age of their mice the majority of fibres examined could not be unambiguously classified.

In EDL (6 - 7 weeks) Parry and Parslow showed a marked decrease in the proportion of Type II glycolytic fibres in dystrophic muscle. This agrees with Dribin and Simpson who concluded in gastrocnemius that the primarily white (Type II glycolytic) region was affected by the disease. It is interesting to note that Parry and Parslow saw that the total reduction of fibre numbers in dystrophic EDL coincided almost exactly with the loss of Type II glycolytic fibres. However, this cannot be interpreted to mean only Type II glycolytics are affected. Parry notes, and as Dribin and Simpson suggested, this observation may represent a shifting from II glycolytic - intermediate - II oxidative with a concomitant loss of Type II oxidative fibres, as a result of the spontaneous hindlimb activity (i.e. altered functional overload).

EDL fibres identified as dystrophic had staining characteristics of Type II oxidative's. In soleus, identified dystrophic fibres were also Type II oxidative's. Virtually no dystrophic Type I fibres were seen. Parry indicates this too may reflect a transformation; Type I - intermediate - central nucleation, etc.

These results and corresponding physiological measurements (Parslow and Parry 1981, see previous section) lead to the conclusion that although there may be a preferential (though not exclusive) involvement of a particular fibre type, there was no conclusive evidence that the same fibre type is involved for all muscles.

VI THE MOTORUNIT

i) HISTORICAL REVIEW

In the 1900's Sherrington (1906) evolved our current working concept of the "motor unit". This term was used to describe a single alpha motorneurone (the final common path of descending CNS influence on motor behavior) and the group of muscle fibres it innervated, also called the muscle unit. Skeletal muscle is essentially a collection of muscle units whose fibres run parallel to each other over the length of the muscle.

Most mammalian skeletal muscles contain motor units which exhibit a wide range of mechanical, morphological and histochemical properties making them heterogeneous muscles. The contractile properties of a muscle are the result of this mixture of muscle fibre types.

The earliest studies of motor unit properties was the work of Wuerker, McPhedran and Henneman (1965 a & b) on cat soleus and medial gastrocnemius muscle. After hindlimb denervation, the L7 and S1 ventral roots were exposed by lumbar laminectomy and cut proximally. Ventral roots were teased down to progressively finer filaments until single stimuli to the muscle nerve elicited an all or none antidromic action potential signifying isolation of one functional motor unit.

They showed an indirect correlation (not statistically

significant) between twitch contraction time and tetanic tension i.e. motor units producing large tetanic tensions had fast twitches and units producing small tetanic tensions had either fast or slow twitch contraction times. Motor units with slow twitch contraction times always produced small tetanic tensions.

Also demonstrated was "an approximately" linear relationship between axonal conduction velocity and tetanic tension for units that produced less than 20 grams. However, this conclusion was based on a relatively small number of motor units in only 6 experiments (3 experiments with soleus and 3 with medial gastrocnemius) and could only be applied to the individual experiments since grouped data showed no relationship existed. In medial gastrocnemius the linearity for units producing less than 20 grams was considerably less evident than in soleus and units with greater than 20 grams tension (more than 60%) showed no simple relationship between tension and conduction velocity.

Based on these observations and a subsequent study of motorneurone cell size in relation to motor unit function and recruitment, Henneman proposed the "size principle" which related motor unit recruitment and motor unit properties to axonal size and hence alpha-motorneurone size. Henneman felt the motorneurone pool was continuously graded according to size and a relationship existed between motorneurone cell size, axon

diameter, conduction velocity, motor unit contraction time and tetanic tension. These properties were all related to the order of recruitment of motor units. Though supported by some workers (Emonet-Dénand et al 1971, Bagust 1974, Proske and Waite 1974) several of the assumptions and implicit conclusions of this principle have been criticized and not borne out in other work (Mosher et al 1972, Stephens and Stuart 1975 a & b). This will be further examined in Chapter X.

Close (1967) using a graded threshold stimulus of the sciatic nerve to evoke motor units sequentially in rat soleus and EDL was able to divide motor units into three classes based on twitch times to peak. He was then able to draw some inferences between these unit groups and the whole muscle histochemistry. He found rat soleus contained 3 out of 30 motor units which were intermediate speed while the remainder were slow. Assuming that the Type II oxidative fibre is of intermediate speed a good correlation with whole muscle histochemistry existed. In EDL, which is composed primarily of Type II oxidative and Type II glycolytic fibres, though some Type I fibres can be found on occasion, motor units exhibited a very small variation in contraction times. This work was however only indirect and criticism of this technique follows in section iii.

This work was followed by various indirect attempts to

correlate histochemical and morphological characteristics with physiological parameters. Until Edstrom and Kugelberg (1968, 1973), studying motor units of the rat anterior tibialis muscle, utilized the method of glycogen depletion the direct study of the interrelationship between physiology and histochemistry was impossible.

After functionally isolating a single motor unit by conventional techniques, glycogen in the muscle fibres was depleted by stimulation during ischaemia produced by clamping the popliteal artery. Using short current pulses (40/sec for .5 seconds repeated every second), applied to the ventral root filament, this regime was maintained until tetanic tension had dropped to zero. The muscle was then frozen and sectioned for histochemical staining. PAS staining for glycogen enabled the identification and mapping of this motor unit's fibres within the muscle cross-section. Depleted fibres (exhibiting a negative staining) showed white against the dark background of glycogen - containing fibres. With serial section staining (for SDH, esterase and ATPase) it was then possible to directly relate histochemical and physiological data.

They found Type I units to be slow and virtually unfatiguable, Type II oxidative units to be fast and intermediate in fatigue and Type II glycolytic units to be fast and rapidly

fatigued.

Burke and co-workers (1967, 1971, 1973, 1974 a & b) exploited this technique and combined with the physiological parameters of twitch, tetanus and fatigue (as described by the fatigue index which defined the ratio of tension decline from the start of a given tetanic regime to the end of a two minute interval) described a tripartite classification of cat motor unit types based on studies on triceps surae, soleus, lateral and medial gastrocnemius muscles. Their classification system has proven both viable, as more data has been accumulated and useful in predicting patterns for new data.

Burke divided motor unit populations into three categories:

1. fast twitch, fast-fatiguing (FF),
2. fast twitch, fatigue resistant (FR), and
3. slow-twitch, unfatiguable (S).

(Note: In later work Burke has included a fourth type of motor unit; this is fast-twitch and intermediate in fatigue resistance (FI).)

They demonstrated excellent correlations between oxidative enzyme staining and resistance to fatigue and suggested that this may be further related to the richness of capillary blood supply (Burke and Tsairis 1974, Burke et al 1974). Those

fibres with a large oxidative capacity utilizing Krebs cycle fatigued slowly (FR and S units) while those (FR) utilizing glycolysis for energy fatigued rapidly, possibly due to a total depletion of intracellular glycogen (Burke and Tsairis 1974).

In terms of unit discrimination based on CT a totally unambiguous separation was not possible (Burke et al 1971, Burke and Tsairis 1974) nor was ATPase staining directly correlated to unit CT.

However, from these studies the motor unit classification of Burke could be clearly related as follows:

FF units = Type II glycolytic fibres

FR units = Type II oxidative fibres

S units = Type I fibres

From Kugelberg's and Burke's work several general principles have been evolved. All the muscle fibres of a single motor unit have essentially identical histochemical profiles and are assumed to produce identical mechanical responses. The fibres of each individual motor unit are not as a rule arranged as a homogeneous group. Rather, they are scattered throughout the muscle with none or only a few of any unit's fibres being contiguous (Kugleberg and Edstrom 1968, Edstrom and Kugelberg 1968, Burke and Tsairis 1974). This dispersion of muscle unit fibres is proposed to permit mechanical interaction between them.

to "smooth" the summation of their active tensions during asynchronous low frequency motorneurone firing (Rack and Westbury 1969, Westbury 1972).

These general principles can be assumed to hold for all species (mice included) though actual numerical relationships or distributions may not hold.

ii) MOTOR UNITS IN NORMAL AND DYSTROPHIC MICE

Though considerable motor unit data have been reported for rat and cat very few direct motor unit studies have been reported using mice and with the exception of this thesis no direct motor unit investigation involving dystrophic mice has been reported. Two studies have been published that make use of estimating techniques for motor unit numbers (Harris and Wilson 1971, Law and Caccia 1975) and while subject to criticism must form the sole background for data regarding dystrophic motor units in mouse.

Direct unit data for normal mice of the C57BL/6J strain have been reported for the soleus muscle (Lewis and Parry 1979, Lewis, Parry and Rowlerson 1982). The range of tetanic tensions of motor units was small compared with that seen in larger mammals. As a percentage of the whole muscle tension, soleus motor unit tetanic tensions showed an eight fold range (1.20 to 9.60%) that was unimodal and symmetrically distributed. The reciprocal of the mean motor unit tetanic tension as a fraction of the whole muscle tension gave an estimate of 21 motor units for soleus.

The distribution of motor unit times to peak was unimodal. Motor unit CT was inversely related to axonal conduction velocity. However this correlation was not seen for

the data as a whole but only in individual experiments in which several motor units from the same mouse were investigated.

The unimodal distribution of unit CT which contrasts with the two clearly defined fibre types of soleus from histochemistry and antibody response was explained in two ways. Firstly, assuming motor unit fibres are homogeneous as in other species, there may be no differences between Type I and Type II oxidative fibres. Or, and their favored explanation, the two populations of motor units exhibit so wide a variation that an apparent unimodal distribution is present.

No direct motor unit studies have been carried out with dystrophic mice. Harris and Wilson (1971) made use of an indirect estimation method to determine the effects of dystrophy on the fast anterior tibialis of 129 ReJ mice. Here, after denervation and ventral root section (after Close 1967), they recruited motor units by graded threshold stimulation of the sciatic nerve. The mechanical tension of the first four to six putative motor units were recorded. After recording the maximal muscle Pt motor unit estimates were made by two methods. Firstly the maximal Pt was divided by the amplitude of the most excitable unit and secondly the maximal whole muscle twitch tension was divided by the mean unit tension of the series. By both methods of estimating motor unit numbers, dystrophic mice showed a significant reduction in

the number of motor units by 70% ($p < 0.01$).

Since most dystrophic motor unit twitch tensions were within the normal range (though their mean value was significantly less than the normal mean) they felt the loss of motor units was not due to random loss of muscle fibres eventually resulting in unit loss. If the primary event were to occur in muscle one would expect normal numbers of very weak units. Their results support a denervation like phenomenon as the primary effect of dystrophy. The observation of many weak motor units is not inconsistent but reflects "sick" motorneurones that may be expected to become weaker before dropping out.

Law and Caccia (1975) applied McComas' technique of estimating motor unit size based on extracellularly recorded muscle compound potentials and tension output for comparison. They used 129 ReJ dy mice to study the soleus muscle. Mechanical tensions and electrical responses at the endplate region (detected by a cotton-agar electrode soaked in Krebs solution) were recorded. Each was used to estimate the motor unit population number by dividing the motor unit mean into the maximal muscle response.

Dystrophic soleus showed a reduction of 27% in the number of motor units estimated. This suggests the soleus is less affected by dystrophy than the relatively fast anterior tibialis.

Their estimate of 22 motor units for normal soleus is in agreement with that of Lewis and Parry (1979). Law and Caccia claimed that the significantly reduced mean motor unit twitch tension (0.20 gm to 0.12 g.) reflects the presence of "functional denervation" within surviving dystrophic motor units and they suggest that 40% of the fibres in dystrophic soleus muscle are "functionally denervated". While the maximal muscle action potential was less than normal, as would be expected from the reduced number of motor units in dystrophic soleus, the mean evoked potential of surviving motor units was not significantly different from normals. This is similar to the case of human dystrophy (McComas et al 1970) and is compatible with the concept of "sick" motor units.

However, Parry (1977) notes that while a 40% reduction in tension has occured the presence of normal size motor unit potentials (presumably indicative of the number of fibres producing action potentials) would most easily be explained by no loss in numbers of fibres but an overall reduced tension output per fibre (indicative of a myopathy). Further criticism of this work follows in Chapter X.

iii) DIFFICULTIES WITH DYSTROPHIC MOTOR UNITS

Though various difficulties unique to the dystrophic animal are present they can be successfully overcome to enable accurate data collection.

Dystrophic hindlimb muscles are subject to a continual spontaneous bombardment. This phenomenon has been the subject of some investigation, see Appendix 1. This spastic twitching requires a surgical preparation technique and mounting apparatus that eliminates its effect on transducer recordings. Such a technique (denervation followed by dorsal and ventral root section) removes this nuisance twitching from the experimental leg. The mounting apparatus and leg pool serve to isolate the experimental leg from any influence of the still innervated and twitching opposite limb.

An earlier criticism of the McComas and Panayiotopoulos work was that of noise. As with most recordings of small voltage deflections using high amplifier gains, inherent machine noise can present problems. Noise problems became significant when considering dystrophic motor units whose twitch tensions are considerably less than in normal mice. However, with proper component matching, effective grounding and the use of signal averaging (as in this study) enhanced signal to noise ratios can be achieved.

Transducer characteristics must be designed to enable an accurate reflection of the muscle and motor unit properties. Both compliance and unloaded natural resonant frequency must be caretully chosen.

It is sufficient to say at this point that both the work of Harris and Wilson (1971) and Law and Caccia (1975) fall under the same criticisms as given in Chapter III for motorunit estimates in human dystrophies. The results must be regarded with some caution and cannot be considered definitive.

VII RATIONALE AND STATEMENT OF THE PROBLEM

This thesis has been designed to examine the mechanical properties of motor units in normal and dystrophic mice of the C57Bl/6J strain. The motor units studied were obtained from the extensor digitorum longus (EDL).

Considerable efforts have been directed towards determining the etiology of murine muscular dystrophy in the hopes it will lead to a better understanding of the human muscular dystrophies. However, the results have been inconclusive. The primary suggestion for a neuronal involvement in dystrophy has come from indirect estimates of motor unit sizes and numbers in both man and mouse. Yet significant criticism of these techniques and their results leaves them suggestive at best.

Ideally, a method in which the component muscle fibres of a single motor unit could be identified (i.e. glycogen depletion technique) would enable one to directly test the etiology of dystrophy and its method of action at this level. However, in the absence of such a technique it is hoped that by a comparative study of the mechanical properties of motor units isolated from normal and dystrophic mice that not only can an understanding of how dystrophy is affecting the motor unit be made, but also whether it acts through a myogenic or neurogenic process be determined.

To better understand how the data to be presented can be used to elucidate these problems it will be advantageous to understand theoretically what would be expected of the distribution of mechanical parameters (absolute tension, relative unit size and time to peak tension).

The cases to be considered:

1) a random myopathy in which muscle fibres are randomly affected, and,

2) a pure (acute) neuropathy in which units become affected and drop out. (Units remaining are not affected and therefore normal).

We will make two assumptions in considering these distributions. First, the sample size of each group (normal and dy2J) is the same. Secondly, the distributions described are unimodal normal distributions (as will be shown in Chapters VIII and IX).

In the case of absolute tension (Pt or Po) the sequence in Figure 2a shows what would be predicted for a random myopathy and a pure (acute) neuropathy.

In a random myopathy muscle fibres from all units are

FIGURE 2

Theoretical frequency distributions for:

a) absolute tension,

b) relative unit size, and

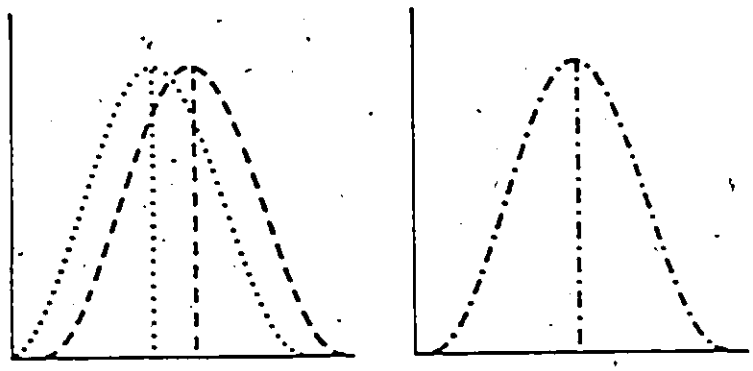
c) time to peak twitch tension

for units from normal and dystrophic mice (see text for details).

– – – – motor units from normal mice

. . . . . motor units from dystrophic mice

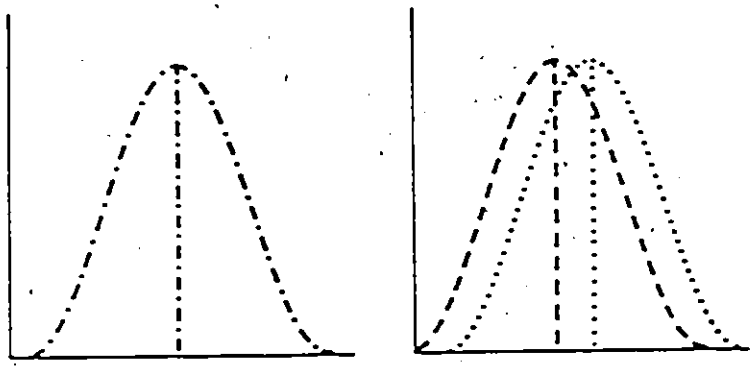
a) Absolute Tension (Pt or Po)



random myopathy

pure (acute) neuropathy

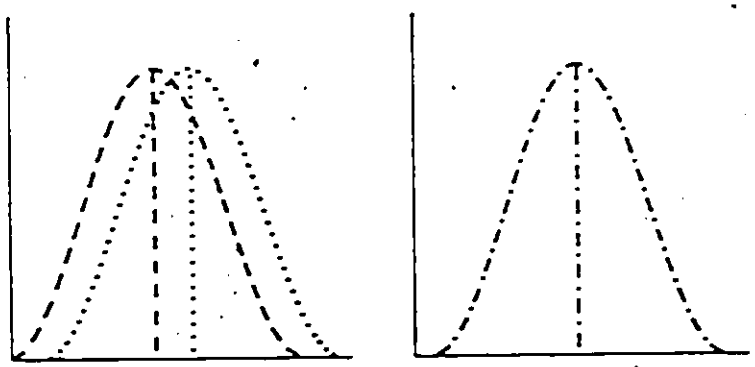
b) Relative Size ($\simeq 1/\#$ of units)



random myopathy

pure (acute) neuropathy

c) Time to peak twitch tension



random myopathy

pure (acute) neuropathy

randomly affected. Therefore the mean unit tension will decline. The number of motor units remains constant. We would predict a normal distribution for absolute tension shifted towards a smaller mean value.

If dystrophy were a pure neuropathy where whole units drop out, those units that remain are unaffected. Thus, the distribution and mean absolute tension of remaining motor units would be unaltered.

For the relative unit size distribution, sequence Figure 2b, in a random myopathy the distribution and mean unit size would be unaltered, since each unit's contribution to the muscle tension (expressed on a relative percent basis) remains the same. For example, if a muscle produced 10 gm tension and had 10 units each contributing 1 gm tension their relative size would be 10%. In a myopathy fibres are randomly affected; suppose that at some stage approximately 50% of the fibres of each unit are affected. The units now each produce only .5 gm tension but, the total muscle

tension has also fallen by 50% to 5 gm. Therefore, the mean relative size is still 10%. Thus in a myopathy, provided it has not progressed to the stage where some units have become vanishingly small and lost in noise, the number of units (1/mean relative size) should remain the same.

For a pure neuropathy a normal distribution with increased mean relative size is predicted (Figure 2b). As whole units drop out, those units that remain have a greater relative per cent contribution towards muscle tension and therefore the mean relative size is greater. A decreased number of units would be calculated. For example, consider 10 units each producing 1 gm tension for a whole muscle tension of 10 gm. Each units relative size is 10%. Suddenly 5 units are lost (pure neuropathy). Whole muscle tension is now 5 gm produced by 5 remaining units of 1 gm each. The mean relative size of each is now 20%.

For CT, if we assume that in the C57 mouse the unimodal distribution seen arises from two overlapping normal distributions (eg. II oxidative and II glycolytic) sequence Figure 2c shows what we would predict. A further assumption is that when a muscle fibre becomes dystrophic its CT is prolonged.

In a myopathy all units would be slowed. The distribution is shifted towards a slower mean CT.

In a pure neuropathy units are lost while remaining units are unaffected. Therefore the distribution and mean of remaining units is unaltered.

We can therefore see that distinct predictions can be made for the frequency distributions of motor unit mechanical parameters from normal and dystrophic mice. Thus we have a means to examine the results of this thesis in terms of these predictions for myogenic and neurogenic etiologies.

VIII METHODS AND MATERIALS

i) ANIMALS

Mice used were of the C57BL strain originally obtained from the Jackson Laboratories, Bar Harbor, Maine. Both normal mice (C57BL/6J) and dystrophic mice (C57BL/6J dy2J/dy2J) have been successfully bred in our own animal quarters. Mice were quartered in standard cages with food and water supplied ad lib. Constant temperature and relative humidity were maintained for optimal health conditions. There seems at present no problems with regards to post natal care by new mothers and consequently our colony has flourished.

For an autosomal recessive gene the F1 ratios are:

dy2J/+ x dy2J/+ :	25% +/+;	50% dy2J/+; 25% dy2J/dy2J
dy2J/+ x dy2J/dy2J :		50% dy2J/+; 50% dy2J/dy2J
dy2J/dy2J x dy2J/dy2J :		100% dy2J/dy2J

Since murine muscular dystrophy is caused by an autosomal recessive gene, motor units collected from phenotypically "normal" mice have undoubtedly included a mixture of +/+ and dy2J/+ genotypes. However, no known phenotypic differences exist between homozygotes and heterozygotes. Phenotype rather than genotype was the criterion for normality in this study.

Detection of dystrophic mice can be made as early as 21 days on average, while unambiguous identification is possible by

28 - 35 days. This can be achieved by several characteristic observations. A limping or partial paralysis-like extension of the hindlimbs during voluntary movement can be seen at an early age. By then suspending the mouse by its tail and gently blowing on its hindlimbs, dystrophic mice will draw their legs towards the body instead of the normal extension retlex.

Since mice used in this study were of an age 70 - 91 days, progression of the dystrophy was quite complete. At this advanced state of the disease selection of dystrophics becomes obvious with the hindlimbs being permanently extended and rigid while exhibiting a spastic-like twitching.

ii) EXPERIMENTAL SOLUTIONS

For transfusion, whole blood was citrated with an anticoagulant solution of:

citric acid	1.10 mM
Na citrate	5.60 mM
Na_2HPO_4	0.50 mM
dextrose	8.90 mM

and used in a 1:7 dilution with whole blood.

During surgery and throughout the experiment, tissues were bathed or immersed in a Krebs Ringer whose composition was:

NaCl	121.00 mM
$CaCl_2.2H_2O$	1.13 mM
$MgCl_2.6H_2O$	2.30 mM
KH_2PO_4	6.00 mM
KCl	4.75 mM
$NaHCO_3$	25.00 mM
dextrose	11.00 mM

The Ringer was constantly gassed with a mixture of 95% O_2 and 5% CO_2 to maintain pH at 7.4.

iii) SURGICAL AND EXPERIMENTAL PREPARATION OF MICE

Mice were initially anaesthetized by intraperitoneal injection of Na pentabarbitone (Nembutal) 70 mg/Kg. A length of Intramedic Polyethylene Tubing PE-50 was sealed into the peritoneal cavity with cyanoacrylate adhesive, through which supplementary anaesthesia was administered as required.

Surgery was aided by a Carl Zeiss - Jena stereomicroscope of variable magnification. After shaving the leg and back; a cutaneous incision was made on the lateral surface of the right hind leg from the ankle to the sciatic notch. This cutaneous layer was cut away exposing the hindlimb musculature. Biceps femoris was cut away so as to expose the sciatic nerve.

All branches except the deep peroneal nerve were transected. (Figure 3)

The EDL was exposed by reflecting medially the anterior tibialis after sectioning its distal tendon. By rolling the EDL laterally its dual innervation could be clearly distinguished from that serving the anterior tibialis and lower foot (Figure 4) This enabled denervation of all muscles except the EDL. By then gently teasing back and separating the cut nerve stumps the EDL inervation could be clearly exposed.

A length of silk thread was looped around the EDL tendon of origin to facilitate its manipulation for clamping by stainless steel forceps in the muscle bath. To complete isometric conditions a stainless steel wire, looped at both ends, was tied to the distal EDL tendon which was then cut free. The wire loop was subsequently attached to a force transducer.

A cannula pulled from Intramedic PE-50 tubing was used to administer approximately 1 cc of citrated whole blood (see ii) via the femoral vein. Blood for transfusion was removed via the left ventricle of a healthy donor mouse. This was administered throughout the surgery and experiment in about 0.1 cc doses given over 1 - 2 minutes approximately every 10 - 15 minutes.

Laminectomy of L1 to L5 was carried out. This procedure involved first cleaning the vertebrae free of muscle.

FIGURE 3

Lower leg innervation of the mouse. All branches of the sciatic except the deep peroneal nerve to EDL and anterior tibialis were transected.

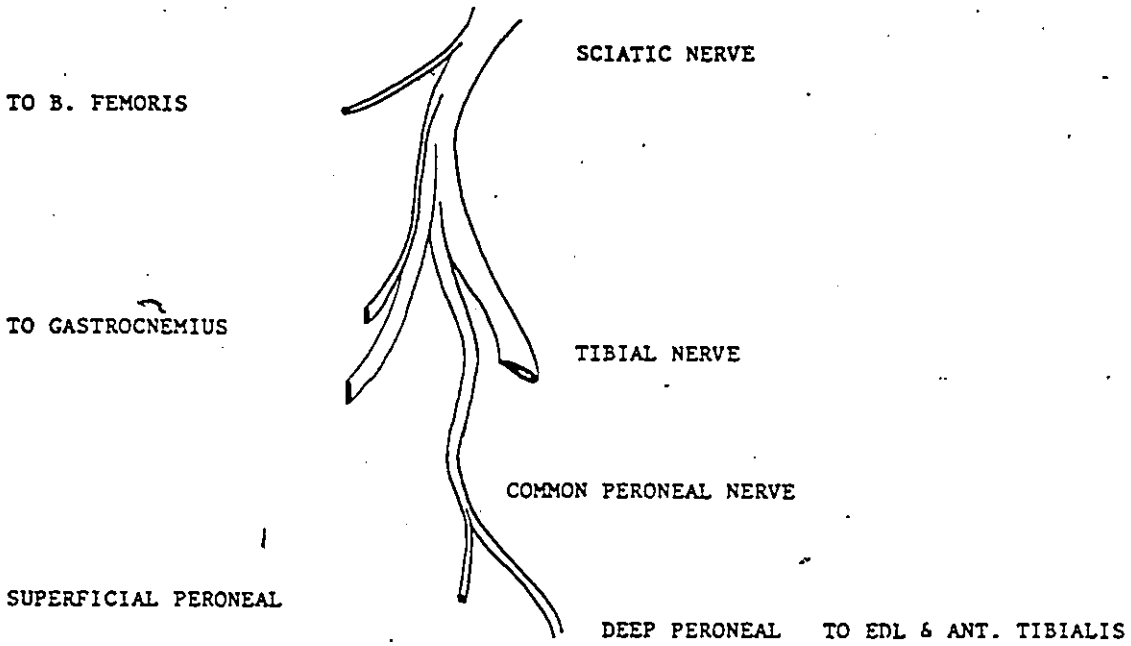
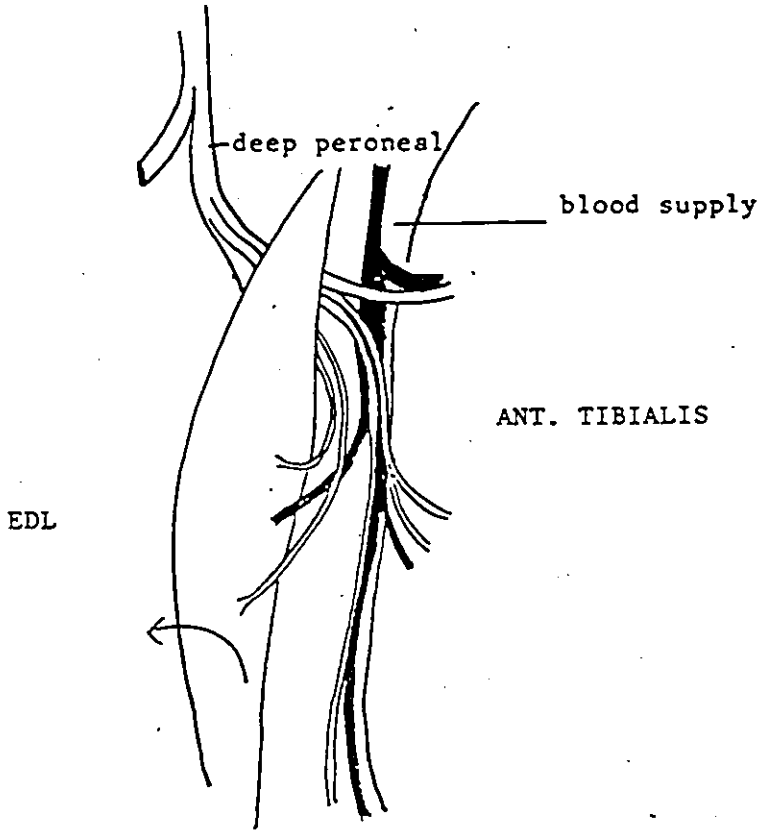

SCIATIC NERVE
TO B. FEMORIS
TO GASTROCNEMIUS
TIBIAL NERVE
COMMON PERONEAL NERVE
SUPERFICIAL PERONEAL
DEEP PERONEAL
TO EDL & ANT. TIBIALIS

FIGURE 4

The dual innervation of the EDL is shown as well as its more prominent blood supply. By gently rolling back EDL the anterior tibialis could be denervated.


deep peroneal
blood supply
ANT. TIBIALIS
EDL

Using DUMONT 15A TAB NIPPERS the lateral dorsal processes of the vertebrae were then cut away. Steadying the vertebrae with forceps, by the dorsal spinal process, spinal segments were removed sequentially by cutting through each vertebra at approximately its mid point. The upper half of the vertebrae could then be removed and the spinal cord and spinal roots exposed. Great care was taken not to exert any excess mechanical pressure on the spinal roots while the spinal processes were clipped and the vertebrae cut and removed. After clearing the muscle and exposing the sacrum region, the preparation was then ready for mounting in the experimental apparatus.

Mounting was achieved by supporting the mouse with adjustable opposing pin-like calipers. These were arranged so as to support the animal by the sacrum and thoracic vertebrae. Excess skin flaps were tied to the holders so as to form a pool surrounding the spinal column. Body temperature was maintained by a heating blanket resting underneath the supported mouse. Temperature was maintained at 36° - 36.5° through a thermistor probe and feedback circuit (see Appendix 2).

The experimental leg was placed into the bath chamber and clamped at the ankle by stainless steel forceps. A 2.8% by weight solution of agar (Fisher Bacteriological grade Agar/Agar) was made. Before hardening this was used to first seal the body

and skin flaps of the spinal pool. The experimental leg was then sealed into the leg bath by agar dipped cotton wool. On hardening the spinal pool was filled with parafin oil and the leg bath with Ringer.

A thermistor probe was used to monitor and maintain bath temperature at 36 - 36.5°C via a heating coil. 95% 02/5% C02 gas was bubbled throughout the bath continuously. To establish isometric conditions the upper tendon of the EDL was clamped by a second set of forceps with the lower tendon attached to the force transducer. The same procedure was used for both normal and dystrophic mice.

iv) EXPERIMENTAL PROTOCOL FOR DATA RECORDING

After mounting the preparation bipolar silver wire electrodes were placed under the EDL nerve and a flat surface bipolar silver wire EMG electrode was positioned over the belly of the muscle. Stimulating electrodes (made of Ag or stainless steel) were made by bonding two lengths of wire approximately 1 mm apart with epoxy resin which also acted as insulation. EMG electrodes were made in a similar fashion but wires were totally embedded in resin. Upon hardening the tip was ground flat so as to expose the wire tips. Electrodes were shielded in ground cable and were held in micro manipulators.

The point of optimum muscle tension was obtained by

stimulating the whole nerve at a rate of 1 per 5 seconds, 50 microseconds duration, at 50% above the voltage that elicited a maximal contraction. The muscle length was adjusted by a micrometer to which the transducer was attached until a maximum twitch tension was obtained.

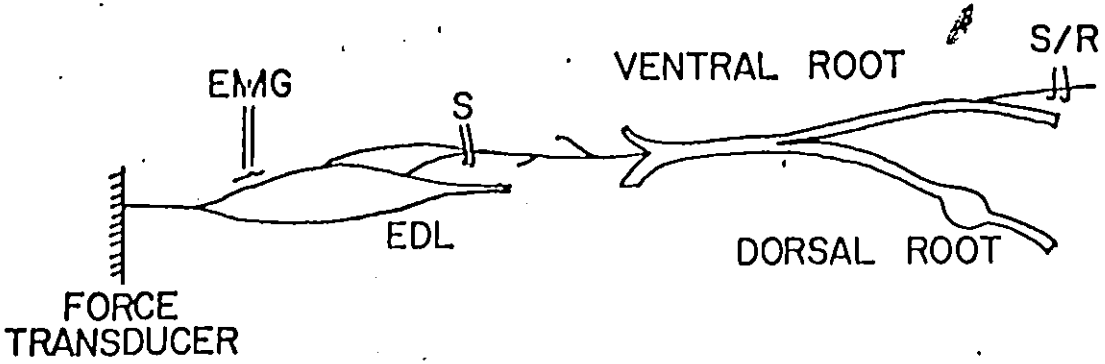
The muscle was allowed to equilibrate while the dura was removed from the exposed spinal cord. Dorsal roots were transected near their entrance to the spinal canal. The cord was then gently rolled back with fine glass needles to expose the ventral roots. These were sectioned approximately midway between their exit from the spinal cord to their exit from the spinal canal. This left approximately 0.5 cm of ventral root to work with. Motor units were isolated from the L3 and L4 ventral roots.

Ventral root filaments were separated on a stainless steel plate immersed in the preparation pool. A stimulating/recording electrode of stainless steel wire was used for ventral root stimulation and antidromic ventral root action potential recording. This electrode was arranged so as to be monopolar with a length of dissected root of approximately the same diameter as the filament acting as ground to the animal itself. A diagrammatic arrangement of the experimental set up is shown in Figure 5.

Motor units were determined to be single by three

FIGURE 5

A diagrammatic representation of the experimental set up is shown. For details see Chapter VIII. (iv). Motor units were determined to be single by the three criteria listed.



1. ALL OR NONE CONTRACTION
2. ALL OR NONE EMG
3. ALL OR NONE ANTIDROMIC VENTRAL ROOT ACTION POTENTIAL

criteria:

1) all or none contraction;

2) all or none emg and;

3) all or none antidromic ventral root action potential.

With a teased ventral root filament across the stimulating/recording electrode a stimulus of 50 microseconds duration every 10 seconds was administered until threshold for contraction and emg was achieved. The stimulus was set at threshold for several pulses to confirm the all or none phenomenon, i.e. at threshold, contraction and emg should occur about 50% of the time. If this was the case, the stimulus voltage was then increased by 50% to observe that no alteration occured in either the contraction or emg. The unit's antidromic ventral root action potential was elicited by stimulation of the whole EDL nerve. Threshold was found to see that this potential was all or none in the same fashion as for the contraction and emg. If all three criteria were met then the unit being examined was considered single.

Experimental data were then collected. Unit contractions were fed to a TRACOR NORTHERN NS-575 DIGITAL SIGNAL ANALYZER. For normal mice the average of 4 or 8 twitches (1 per 10 seconds) was taken, stored and transferred to a TEKTRONIX 502 A

oscilloscope as a standing wave pattern. This was photographed by a NIHON KOHDEN PC-2A continuous recorder on KODAK RAR FILM 2495 (35 mm). For dystrophic mice averages of 16 or 32 twitches were used to obtain measurements. In a similar manner the unit tetanus was obtained (TRACOR average of 1). A pulse of 150 stimuli/second, duration 200 milliseconds, was used to elicit tetani in both units and whole muscle of normal and dystrophic mice. After tetanus, the antidromic ventral root action potential was then recorded and stored on a TEKTRONIX 5111 storage oscilloscope and the conduction time measured. The twitch and tetanus of the whole muscle were than recorded and photographed. This constituted the necessary data for analysis of a single unit. This same protocol was followed for each unit isolated. The termination of an experiment was determined by a fall in whole muscle tetanic tension of greater than 15% of the initial tetanic tension. This was taken to be an indication of a deteriorating preparation. After termination of an experiment, the conduction distance between the electrodes on EDL nerve and the ventral root was measured by dissecting out the nerve and laying a thread along side. Conduction velocities could then be calculated. Muscle weight was determined by quickly excising the tissue and weighing on a torsion balance.

v) CHARACTERISTICS OF THE APPARATUS

A schematic diagram of the experimental apparatus is shown in Figure 6.

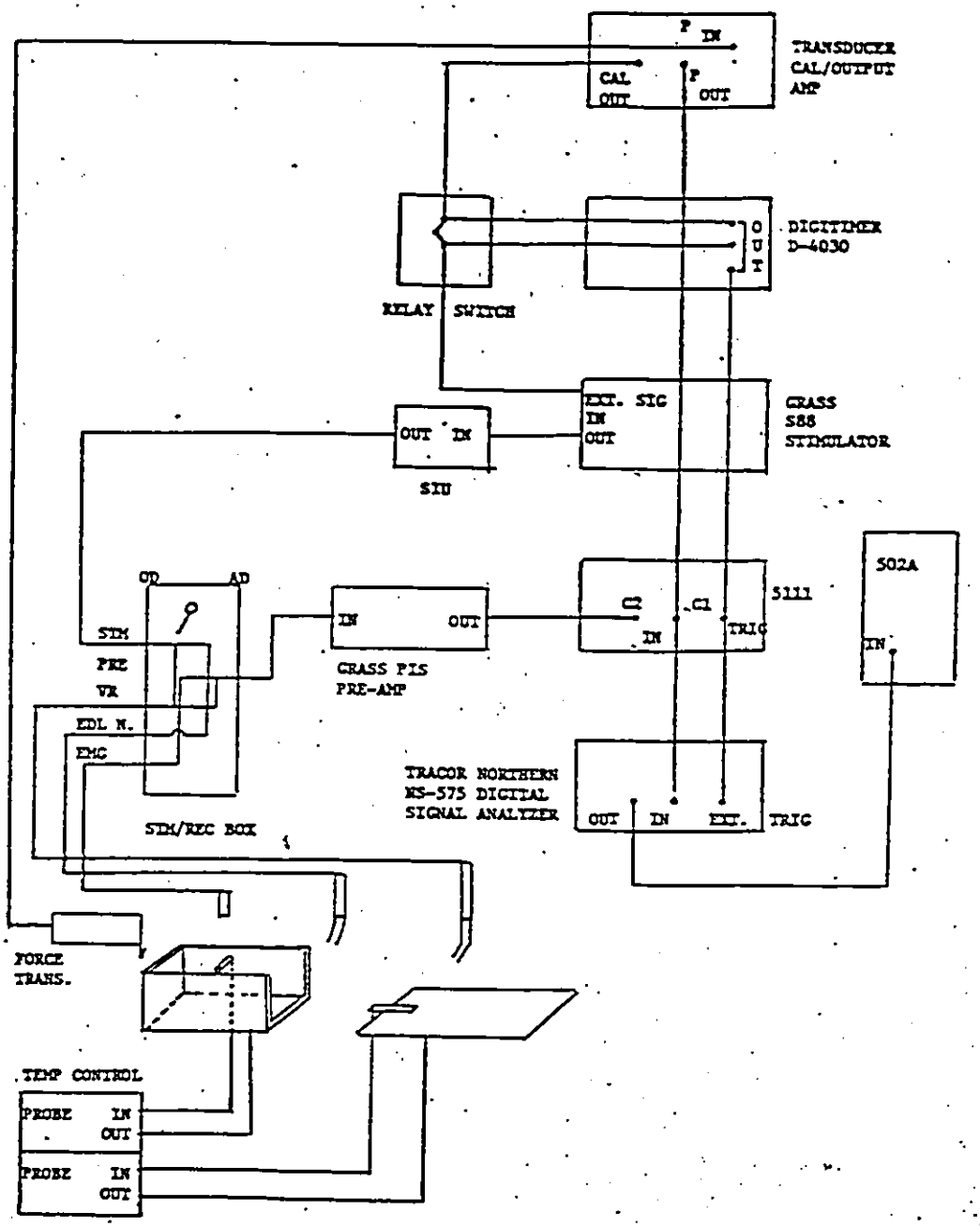
As previously discussed, body temperature was monitored by a thermistor probe and a constant temperature maintained through a heating coil embedded in a supporting blanket. For the leg pool a separate thermistor probe and heating coil maintained a constant 36 - 36.5°C temperature.

Tension was recorded by a transducer arranged as a stainless steel cantilever beam formed into a hook at one end. Four semi-conductor strain gauges (Kulite UDP - 360-150) were bonded to the beam; the whole of which was encased in a stainless steel housing. The gauges were connected as a full bridge at the transducer amplifier (see Appendix 3 for complete circutry). The compliance of the transducer was approximately 1 uM/gm with an unloaded natural resonant frequency of 1660 HZ. Some data were obtained using a transducer of similar design but whose compliance was approximately 1.5 uM/gm with an unloaded natural resonant frequency of 1250 HZ. No difference could be detected between the two.

The stimulus for contraction was provided by a GRASS S88 stimulator. The site of application of this stimulus, was determined by the stimulus/recording box setting. With an

FIGURE 6

A schematic diagram of the experimental apparatus is shown. For details see text (Chapter VIII. (v)).


P IN
TRANSDUCER
CAL/OUTPUT
AMP
CAL
OUT
P
OUT
DIGITIMER
D-4030
O
U
T
RELAY
SWITCH
EXT. SIG
IN
OUT
GRASS
S88
STIMULATOR
OUT
IN
SIU
OD
AD
STIM
PRE
VR
EDL N.
EMG
STM/REC BOX
IN
OUT
GRASS P15
PRE-AMP
C2
C1
IN
TRIG
5111
502A
IN
TRACOR NORTHERN
NS-575 DIGITAL
SIGNAL ANALYZER
OUT
IN
EXT.
TRIG
FORCE
TRANS.
TEMP CONTROL
PROBE
IN
OUT
PROBE
IN
OUT

orthodromic setting the stimulus pulse was applied to the ventral root electrodes enabling stimulation of motor units. Force of contraction was recorded as was the unit emg response. On an antidromic setting the S88 stimulus impulse was delivered to the EDL nerve enabling whole muscle stimulation. The evoked antidromic action potential was recorded via the ventral root electrode. This signal, as well as the emg, when set orthodromically, was amplified by a GRASS P15 preamplifier and recorded by the TEKTRONIX 5111 oscilloscope.

Twitch tensions were processed as follows. The force transducer signal was fed from the transducer amplifier to channel 1 of the TEKTRONIX 5111 storage osciloscope. This same signal was also passed to the input of a TRACOR NORTHERN NS-575 DIGITAL SIGNAL ANALYZER. Here the predetermined number of inputs were summated and averaged. The averaged output was then fed to a TEKTRONIX 502A oscilloscope and displayed as a standing wave pattern for photographing.

Immediately before the S88 was triggered a calibration pulse was obtained by operating a relay which presented a known resistance in parallel with one of the arms of the bridge. A DIGITIMER D-4030 was used to trigger the oscilloscope (and signal averager) sweep, the calibration pulse and the stimulator output. The calibration pulse was of variable amplitude (controlled at the

transducer amplifier by switching in resistors of different values) and duration (controlled by the DIGITIMER D-4030). In this way each sweep consisted of the calibration pulse followed by the evoked twitch response, with the corresponding emg (if set OD) or the antidromic ventral root action potential (if set AD). Only the calibration and twitch response were averaged by the TRACOR unit and subsequently photographed. For conduction velocity calculations, the antidromic ventral root action potential conduction time from the stimulus artifact was directly measured from the TEKTRONIX 5111 scope.

vi) METHODS OF DATA ANALYSIS

The criteria for acceptance of a set of motor unit data was chosen based on the whole muscle tetanic tension. Any whole muscle tetanic tension measured that had fallen by 15% or more of the initial whole muscle tetanic tension was considered as indicative of a failing preparation and the unit's data associated with that tetanic tension were rejected and the experiment terminated.

Within experiments tetanic tension for whole muscles showed no consistent trend i.e. continual decline in tension. However, in some cases whole muscle twitch tension did decline by as much as 30%, though the corresponding tetanic tension exhibited less than 10% decline. This is similar to results reported for

cat by Buller et al (1981) who noted on an average twitch tension (at the end of a 2 to 6 hour experiment) was only 77% of the initial tension while the corresponding tetanic tensions were within 5% of the first. For this reason estimates of motor unit numbers are based on whole muscle and motor unit tetanic tensions.

The tabulated data of results is composed of mean values of measured parameters. For this reason data were expressed two ways. First the mean value $\pm$ standard deviation (SD), to yield a description of the dispersion or spread of data about that mean, and this followed by $\pm$ standard error of the mean (SEM) to give a measure of the precision or reliability of the sample mean. That is to say, the SEM indicates how close our mean is to the true population mean.

Samples were determined to be independent and random by using the Run Test for Randomness (Choi 1978) significant to the $P < 0.0005$ level.

All data parameters were first examined as the cumulative probability of an event ranked and plotted on a normal probability ordinate. In all cases (for example see Figure 12 and 14) both normal and dystrophic data did not deviate significantly from the straight line which corresponds to a normal distribution.

Linear regression coefficients were calculated as an indication of nearness to linearity. Their significance was set

at the 1% level.

By dealing with independent random samples from normal populations having unknown means and unknown variance, testing of the null hypothesis ul = u2 can be achieved using the Student's t test for unpaired data (Brownlee 1965). This statistic enables us to accept or reject the null hypothesis for unpaired data of equal or unequal populations.

Mean, standard deviation, standard error of the mean, regression coefficient, and the Student's t, were calculated with the use of a Hewlett Packard HP-65 Programmed Calculator. Significance in the t was set at the most powerful level.

For comparison of the interrelationships between axonal CV and muscle parameters in individual experiments slope, intercept and linear regression coefficients were calculated.

IX. RESULTS

1. WHOLE MUSCLE DATA

i) Mice

The average age of animals was 84 and 81 days for normal and dystrophic mice, respectively. In terms of body weight normal mice were significantly heavier than dystrophics; 29.1 $\pm$ 3.2 gm (SD) and 22.0 $\pm$ 2.5 gm (SD) (P 0.005).

ii) Muscle Weight and Tension Data

Data for the absolute tensions (Pt and Po) of normal and dystrophic EDL are shown in Table III. Following each experiment the weight of the whole excised muscle was recorded and this was used to calculate the normalized tensions (expressed as gm tension/gm of wet muscle weight).

It is obvious that the total wet muscle weight is significantly less in the dystrophic EDL. Absolute tension was significantly reduced with dystrophy in both twitch and tetanus by approximately 50%. This reduction in tension was also borne out in terms of normalized tensions for the whole muscle. Normalized twitch tension was reduced by 39% and normalized tetanic tension by 45% when compared with normal muscles.

Figure 7 shows representative whole muscle isometric twitch and tetanus responses for both normal and dystrophic EDL. In addition to the obviously reduced tensions, the characteristic

TABLE III

THE EFFECT OF MUSCULAR DYSTROPHY ON THE WEIGHTS AND ON ABSOLUTE AND NORMALIZED TWITCH AND TETANIC TENSIONS OF EDL MUSCLE AT 36.5° C

MUSCLE DESCRIPTION	MUSCLE WEIGHT (mgm)	ABSOLUTE TENSION Pt (gm)	NORMALIZED TENSION (gm/gm)	ABSOLUTE TENSION Po (gm)	NORMALIZED TENSION (gm/gm)	Pt/Po
C57 EDL (15)	12.1 ± 1.0 ± 0.3	4.5 ± 0.8 ± 0.2	392.7 ± 108.6 ± 25.0	29.7 ± 5.7 ± 1.4	2558.5 ± 661.8 ± 170.8	0.16 ± 0.03 ± 0.01
dy2J EDL (15)	9.8 ± 0.9 ± 0.2	2.3 ± 0.7 ± 0.2	240.7 ± 82.3 ± 21.3	13.8 ± 3.1 ± 0.8	1434.5 ± 405.1 ± 104.6	0.17 ± 0.03 ± 0.01
P	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	NS

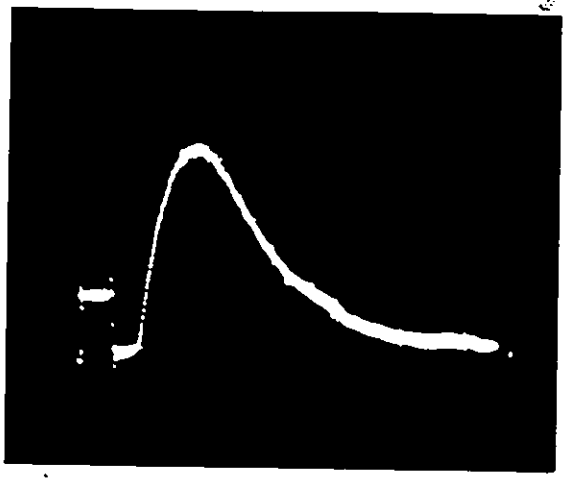
a) Number in parenthesis is N = number of mice studied.

b) Values are Mean ± standard deviation (SD) followed by ± standard error of the mean (SEM).

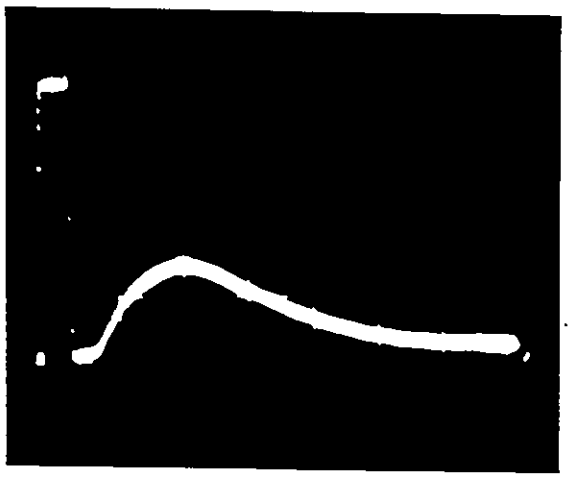
c) P indicates levels of significance using the Student's t test for unpaired data.

FIGURE 7

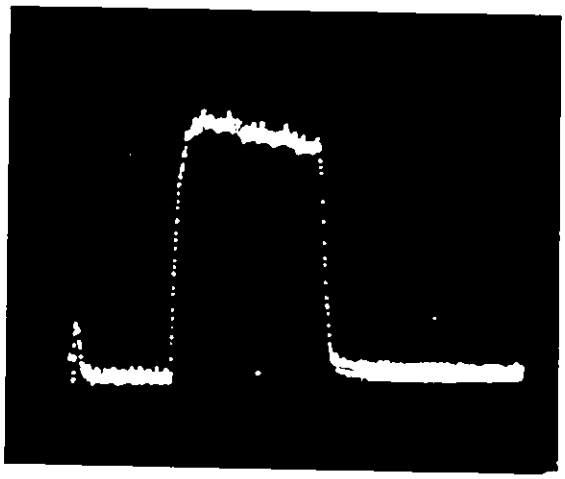
Representative whole muscle twitch and tetanus. a) and c) correspond to C57 and b) and d) to dy2J twitch and tetanus respectively. Calibrations are shown.



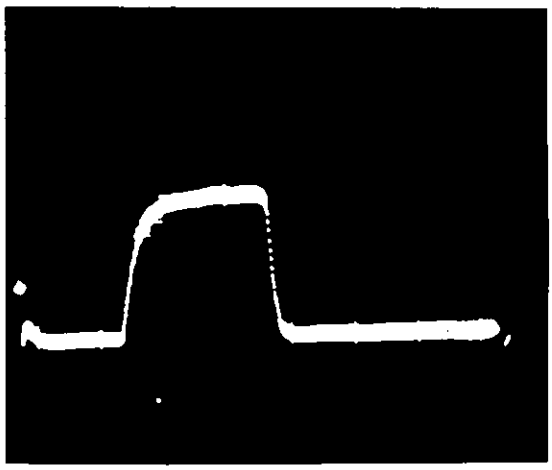
a. C57 EDL twitch
CAL 3 msec, 1.10 gm
Pt 3.36 gm



b. dy2J EDL twitch
CAL 3 msec, 3.36 gm
Pt 1.29 gm



c. C57 EDL tetanus
Po 24.70 gm



d. dy2J EDL tetanus
Po 7.01 gm

slower time parameters (CT and ½RT) of the dystrophic EDL are apparent.

The frequency distributions of both Pt and Po were normally distributed in control and dystrophic EDL. In normal mice the range of absolute twitch tension was 2.6 gm to 6.0 gm (mean 4.5 ± 0.8 gm (SD)) while dystrophic mice exhibited a range of 1.3 gm to 3.6 gm (mean 2.3 ± 0.7 gm (SD)).

Tetanic tension reflected this range of twitch tension; 16.5 gm to 37.7 gm compared to 7.6 gm to 19.7 gm for dystrophic muscle.

No significant difference was seen between twitch/tetanus ratios of normal and dystrophic muscle.

iii) Time Parameters of Contraction

The most fundamental parameters used in categorizing whole muscles are those of time to peak twitch tension (CT) and half relaxation time (½RT). The CT and ½RT data obtained for mouse EDL are given in Table IV.

It can be seen there is a significant slowing in the dystrophic EDL when compared with controls. Both CT and ½RT are affected. This is in good agreement with previously reported slowing associated with dystrophy (Parry and Parslow 1981). This slowing is apparent in the representative twitch contractions shown in Figure 7.

TABLE IV

ISOMETRIC TWITCH TIMES TO PEAK TWITCH TENSION AND ONE HALF RELAXATION TIMES IN NORMAL AND DYSTROPHIC EDL MUSCLE AT 36.5°C

MUSCLE DESCRIPTION[a]	TIME TO PEAK TWITCH TENSION (msec)	ONE HALF RELAXATION TIME (msec)
C57 EDL (15)	8.7 ± 0.9 ± 0.2	7.6 ± 1.5 ± 0.4
dy2J EDL (15)	12.4 ± 2.3 ± 0.6	13.2 ± 2.9 ± 0.8
P	< 0.0005	< 0.0005

a) Number in parenthesis is N = number of mice studied.

b) Values are Mean ± SD, ± SEM.

c) P indicates level of significance using the Student's t test for unpaired data.

2. MOTOR UNIT DATA

i) Motor Units

A total of 64 motor units were isolated from 15 normal EDL muscles and 60 motor units from 15 dystrophic EDL muscles. Between two and seven motor units were examined in individual muscles. Representative twitch and tetani of both normal and dystrophic motor units are shown in Figure 8. Both a reduced tension output and slower time parameters in dystrophic motor units can be seen. This is reflected in the numerical analysis.

ii) Motor Unit Tension Data

Tension data for the motor units studied are shown in Table V. The frequency distributions of motor unit Pt, Po and motor unit size (expressed as unit tetanic tension as a percent of whole muscle tetanic tension) are shown in figures 9, 10 and 11.

The mean motor unit twitch tension is seen to be significantly less for dystrophic motor units (170.3 + 68.8 mgm (SD)) than for normal motor units (302.2 + 145.0 mgm (SD)). From the frequency distribution, Figure 9, it appears that the distribution of unit twitch tensions of dystrophic mice is narrower in range than those seen in normal mice. However, dystrophic twitch tensions (range 45.0 to 349.0 mgm) still show considerable overlap with normal tensions (range 62.9 to 744.9 mgm).

The frequency distribution of maximum tetanic tensions

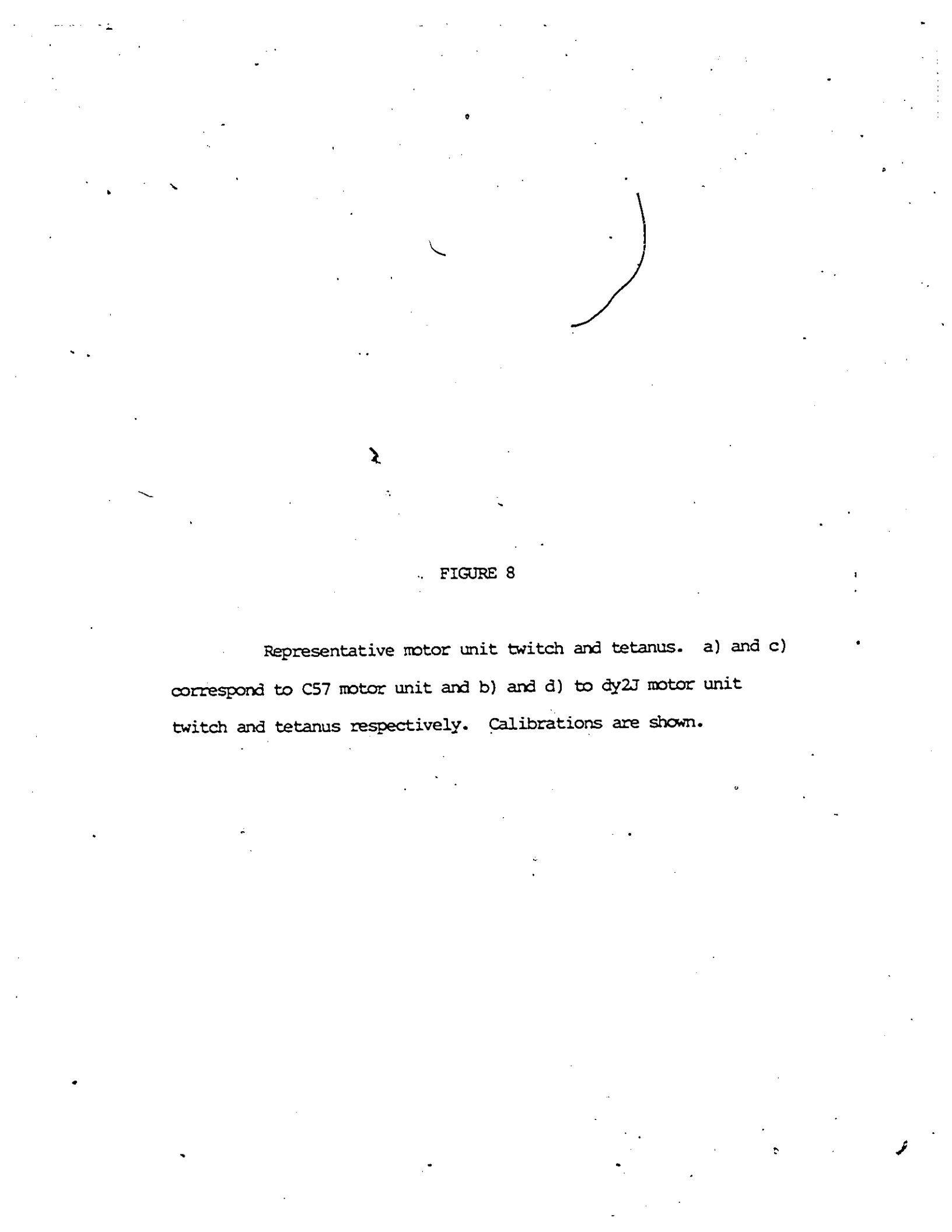
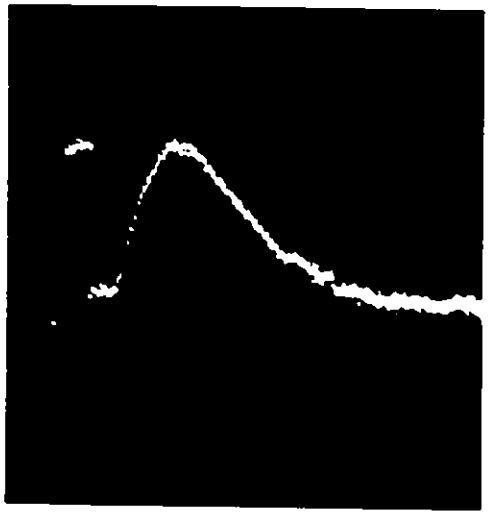
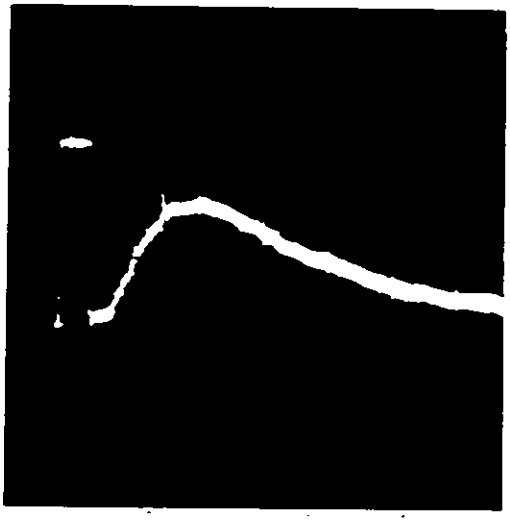


FIGURE 8

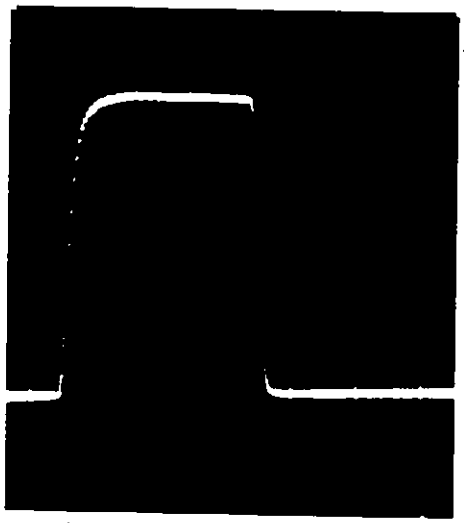
Representative motor unit twitch and tetanus. a) and c) correspond to C57 motor unit and b) and d) to dy2J motor unit twitch and tetanus respectively. Calibrations are shown.



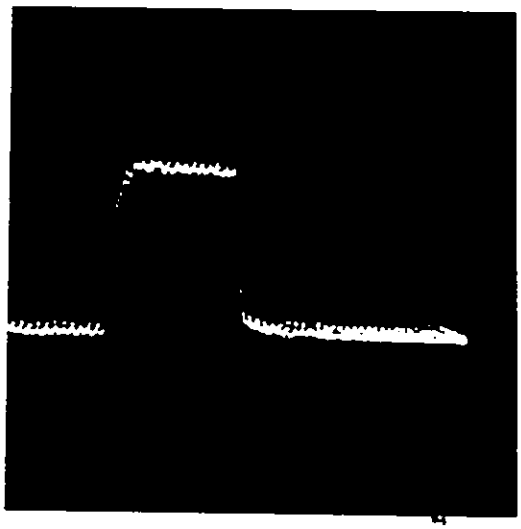
a. C57 motor unit twitch
CAL 4 msec, 385 mgm
Pt 405 mgm



b. dy2J motor unit twitch
CAL 4 msec, 220 mgm
Pt 153 mgm



c. C57 motor unit tetanus
Po 1450 mgm



d. dy2J motor unit tetanus
Po 960 mgm

TABLE V

MOTOR UNIT TWITCH, TETANUS AND SIZE DATA FOR EDL MOTOR UNITS ISOLATED FROM NORMAL AND DYSTROPHIC MICE AT 36.5°C

MOTOR UNIT DESCRIPTION	Pt (mg)	Po (mg)	Pt/Po	UNIT x 100/EDL Pt	% Po
C57 (n = 64)	302.2 ± 145.0 ± 18.2	1438.7 ± 705.8 ± 88.2	0.22 ± 0.06 ± 0.01	6.8 ± 3.6 ± 0.5	4.6 ± 2.5 ± 0.3
dy2J (n = 60)	170.3 ± 68.8 ± 8.8	840.8 ± 355.5 ± 45.9	0.22 ± 0.07 ± 0.01	8.2 ± 4.8 ± 0.6	6.7 ± 4.1 ± 0.5
P	< 0.0005	< 0.0005	NS	< 0.0005	< 0.0005

a) Number in parenthesis is N = number of units studied.

b) Values are Mean ± SD, ± SEM.

c) P indicates levels of significance using the Student's t test for unpaired data.

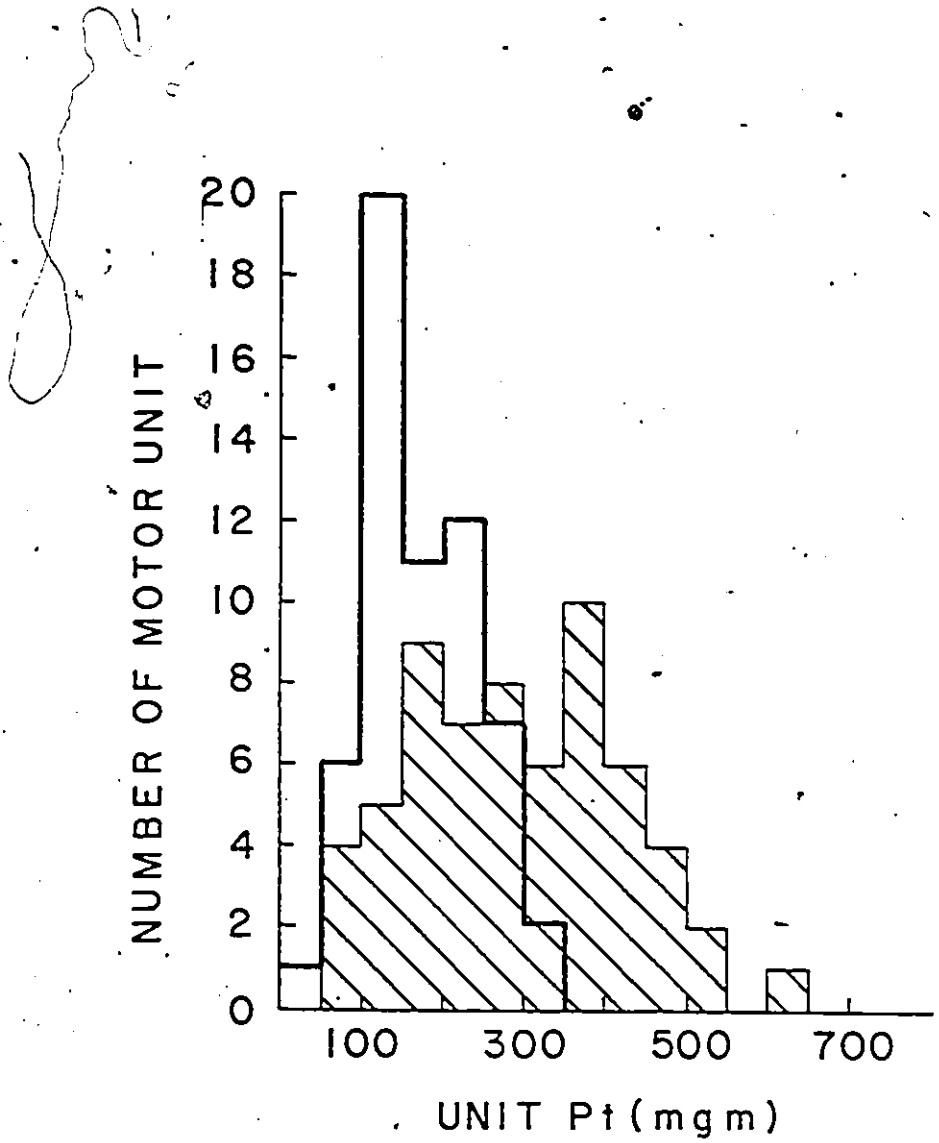
FIGURE 9

Frequency distribution of twitch tension, for motor units from normal and dystrophic EDL.

motor units from normal EDL (64)

motor units from dystrophic EDL (60)

Lower limit or resolution was 40 mgm.


NUMBER OF MOTOR UNIT
0
2
4
6
8
10
12
14
16
18
20
100
300
500
700
UNIT Pt (mgm)

of all motor units is shown in Figure 10. Tensions are arranged in 300 mgm bins. Both sets of data indicate normal distributions with a 10 fold range of data. For dystrophic animals the smallest unit developed a maximal tetanic tension of 150.0 mgm and the largest 1570.0 mgm. For normal mice the smallest tetanic tension was 300.0 mgm while the largest was 3420.1 mgm. The mean tetanic tensions produced by motor units of normal and dystrophic mice was significantly different, Table V.

The frequency distribution of Figure 10 shows the majority of motor units from dystrophic EDL as being located at the lower end of the distribution of normal tetanic tension. Unlike motor units of normal mice, units from dystrophic mice exhibited a much narrower dispersion of tetanic tensions. Although the average Po was reduced the majority of units from dystrophic EDL were within normal confines.

Table V shows that the twitch/tetanus ratio was not significantly different suggesting no difference in the degree of activation of motor unit twitches.

The mean unit size is given in Table V derived from both the unit twitch tension as a percent of the whole muscle twitch and the unit tetanic tension as a percent of the whole muscle tetanus. As indicated in Chapter VIII (vi) the value of unit Po x 100/EDL is used to calclate numbers of motor units.

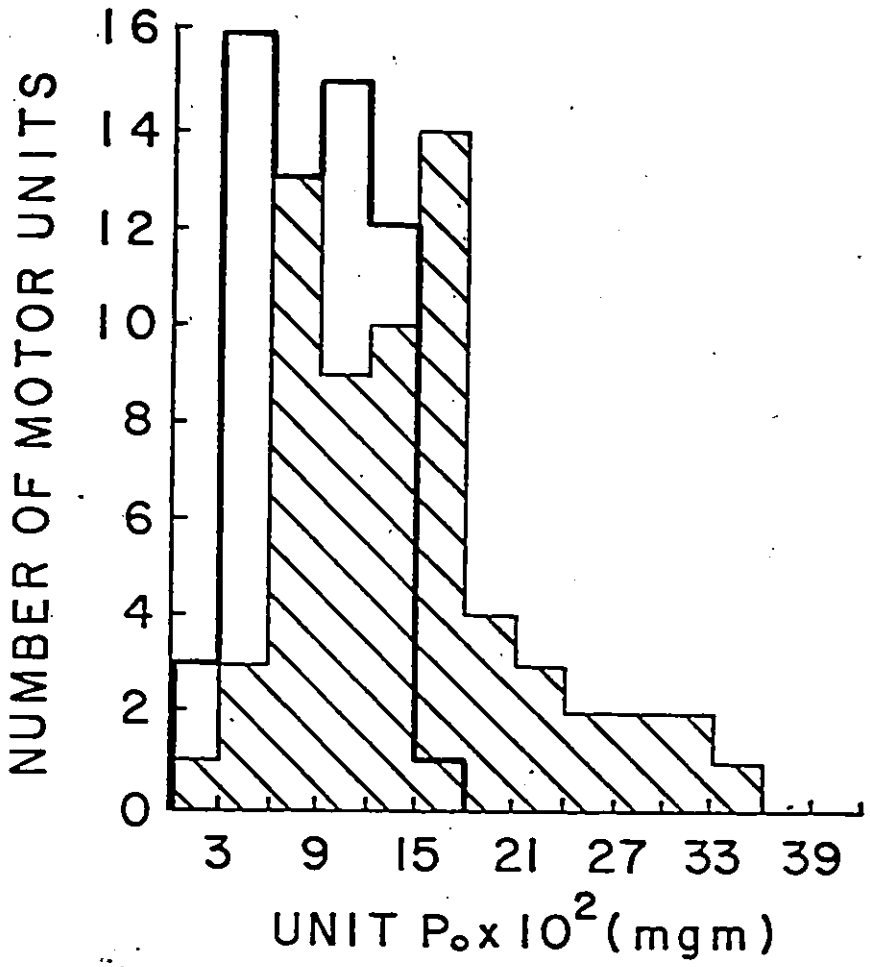
FIGURE 10

Frequency distribution of tetanic tension for motor units from normal and dystrophic EDL.

motor units from normal EDL (64)

motor units from dystrophic EDL (60)

Lower limit or resolution was 40 mgm.


NUMBER OF MOTOR UNITS
16
14
12
10
8
6
4
2
0
3
9
15
21
27
33
39
UNIT $P_o \times 10^2$ (mgm)

For normal mice the value of unit tetanic tension as a percentage of whole muscle tetanic tension indicates the average motor unit contributed 4.6% of the whole muscle tetanic tension, little variation was seen in normal mice (1.0% to 11.7%.). For dystrophic mice this value indicates the average motor unit contributed 6.7% of the whole muscle tetanic tension (range 1.0% to 18.4%.). By taking the reciprocal of these values we can estimate the number of motor units in normal EDL to be approximately 22 while for dystrophic EDL there are approximately 15 motor units.

Figure 11 shows the frequency distribution of motor unit sizes. Both are normally distributed as indicated by their cumulative probability plots, Figure 12. However, dystrophic animals exhibited several units whose mean relative size was considerably larger than any normal motor unit size.

iii) Time Parameters of Contraction

The twitch time to peak tension and half relaxation data obtained for the EDL motor units studied are presented in Table VI. Motor units from dystrophic EDL were significantly slower in both CT and ½RT. Figure 8 also reflects this. In both parameters the data dispersion was considerably greater for motor units of dystrophic mice than for units from normal animals. Figure 13, the frequency distribution of unit CT in 1 msec bins, clearly

FIGURE 11

Frequency distribution of tetanic tension as a percentage of the whole muscle tetanic tension for motor units from normal and dystrophic EDL.

motor units from normal EDL

motor units from dystrophic EDL

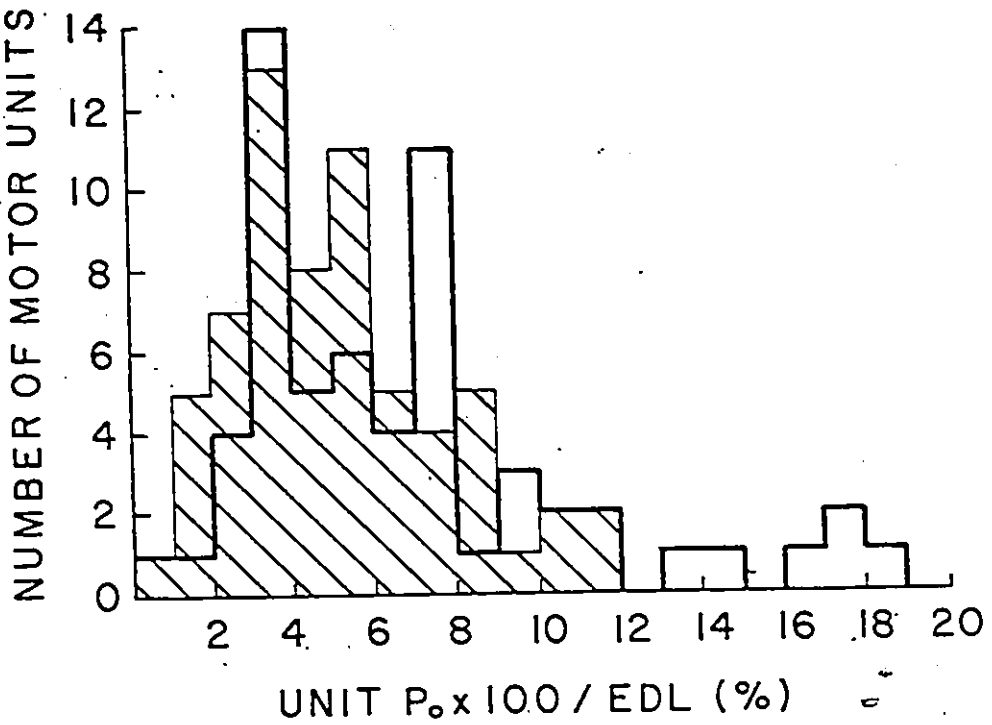

NUMBER OF MOTOR UNITS
0
2
4
6
8
10
12
14
2
4
6
8
10
12
14
16
18
20
UNIT P_o x 100 / EDL (%)

FIGURE 12

Cumulative probability plot of motor units ranked in order of UNIT Po x 100/EDL plotted on a normal probability ordinate. Both normal and dystrophic data do not deviate significantly from the straight line which corresponds to a normal distribution.

(normal ● $r = .97$ $p < .01$

dystrophic O $r = .95$ $p < .01$)

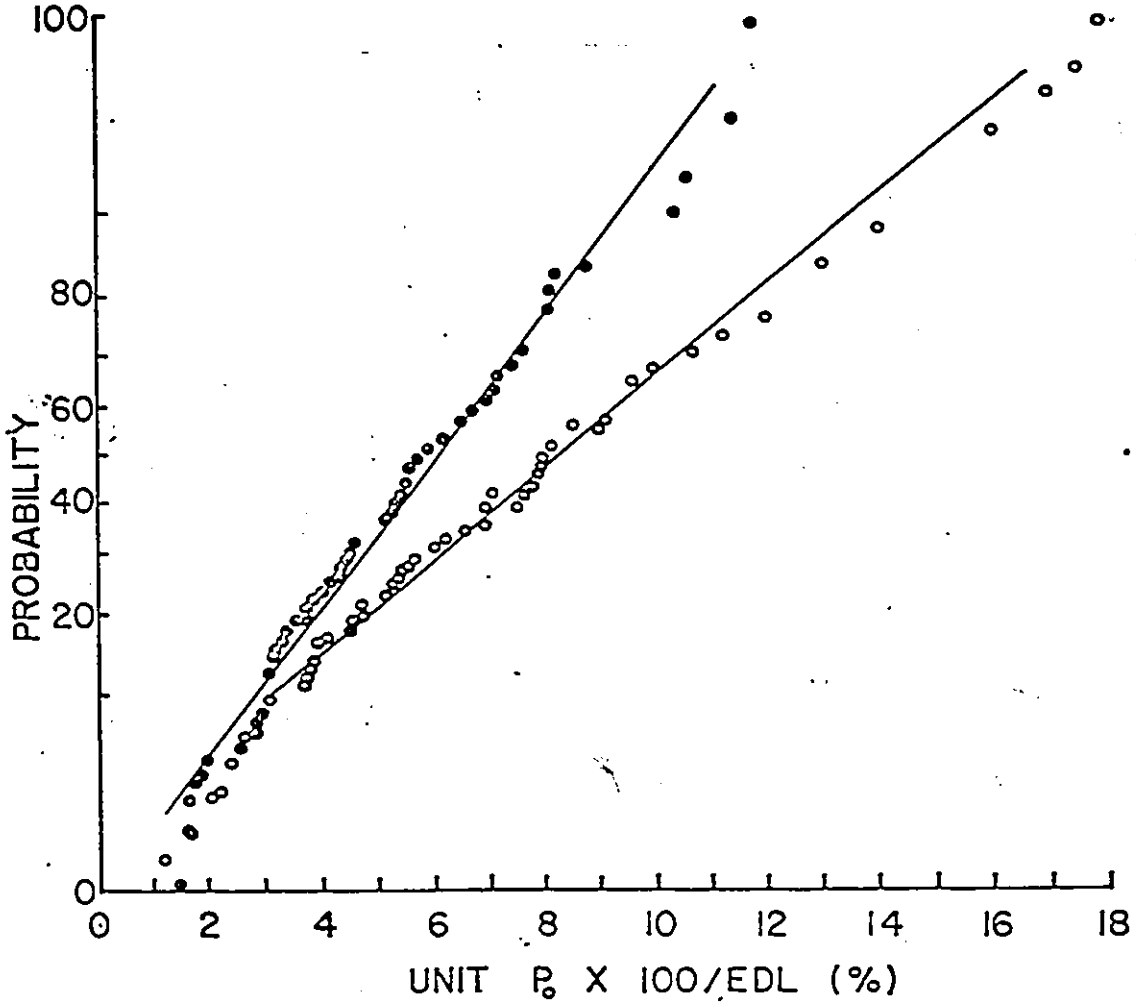

100
80
60
40
20
0
PROBABILITY %
0
2
4
6
8
10
12
14
16
18
UNIT P_o X 100/EDL (%)

TABLE VI

ISOMETRIC TWITCH TIMES TO PEAK TENSION AND ONE HALF RELAXATION FOR MOTOR UNITS OF EDL FROM NORMAL AND DYSTROPHIC MICE AT 36.5° C

MUSCLE DESCRIPTION	TIME TO PEAK CONTRACTION (msec)	ONE HALF RELAXATION TIME (msec)
C57 (64)	8.7 ± 1.2 ± 0.2	8.6 ± 2.1 ± 0.3
dy2J (60)	11.9 ± 2.7 ± 0.4	14.0 ± 4.0 ± 0.5
P	< 0.0005	< 0.0005

a) Number in parenthesis is N = number of motor units studied.

b) Values are Mean ± SD, ± SEM.

c) P indicates level of significance using the Student's t test for unpaired data.

demonstrates this. Both groups of data are normal distributions about their respective means. Figure 14, the cumulative probability plot of CT, demonstrates no significant deviation from the straight line indicative of a normal distribution.

In normal muscle, motor unit CT are closely grouped about their mean 8.7 + 1.2 msec (SD), range 5.4 to 11.9 msec. Dystrophic motor units exhibit a somewhat wider range of values 6.0 to 17.6 msec, mean 11.9 + 2.7 msec (SD). For dystrophic EDL motor unit CT a bimodal distribution suggested itself (with means at 7.0 and 12.6 msec). However, as noted, cumulative plotting on probability paper (Figure 14) was unable to indicate this as a separate population and gave results suggesting a normal distribution. Approximately 75% of the motor units of dystrophic EDL examined fell outside the range found in normal mice. Some units did exist in dystrophic mice however, whose CT was equal to the fastest normal units, Figure 12.

One half relaxation time was significantly prolonged in motor units from dystrophic EDL, Table VI. The data dispersion was considerably greater for dystrophic mice than the normals.

iv) Interrelationships between axonal conduction velocity and motor unit mechanical parameters

Table VII shows the axonal conduction velocity and conduction time for normal and dystrophic motor units. The mean

FIGURE 13

Frequency distribution of motor unit times to peak tension (CT) for normal and dystrophic EDL.

motor units from normal EDL

motor units from dystrophic EDL

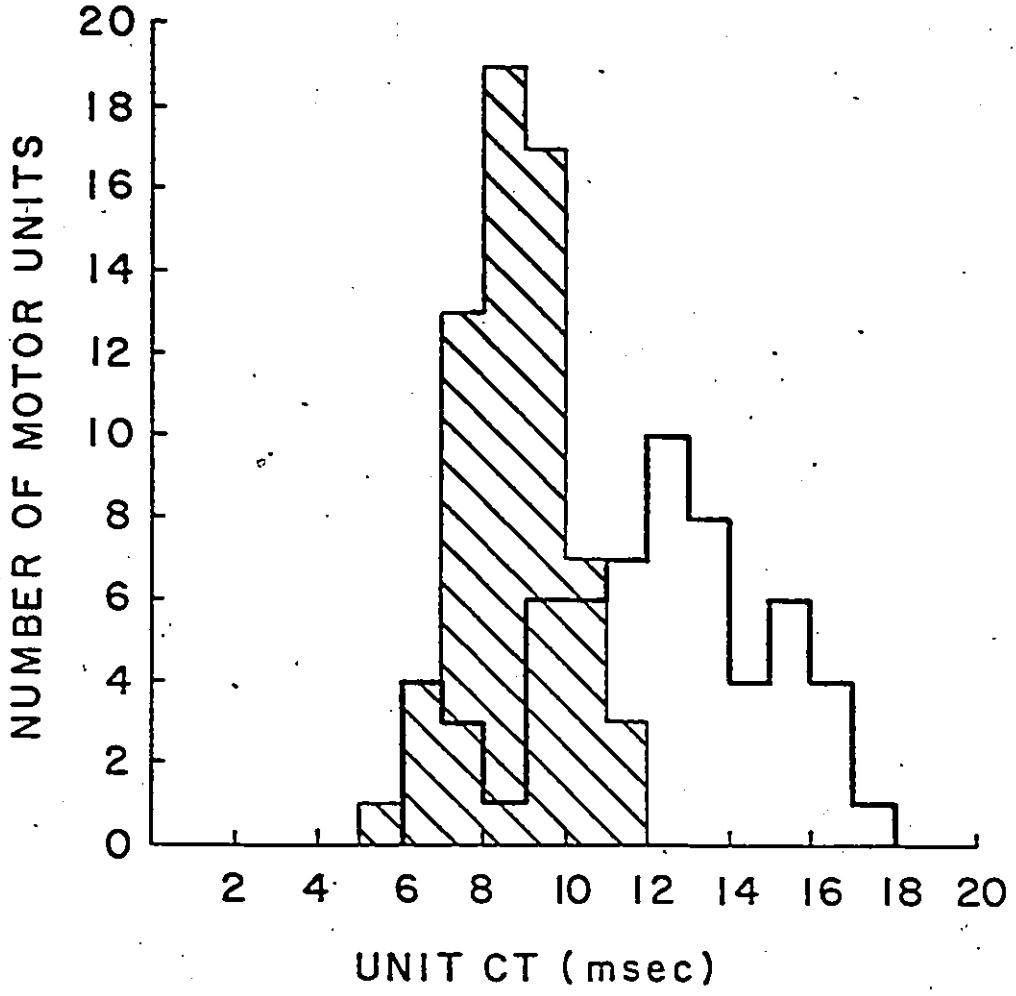
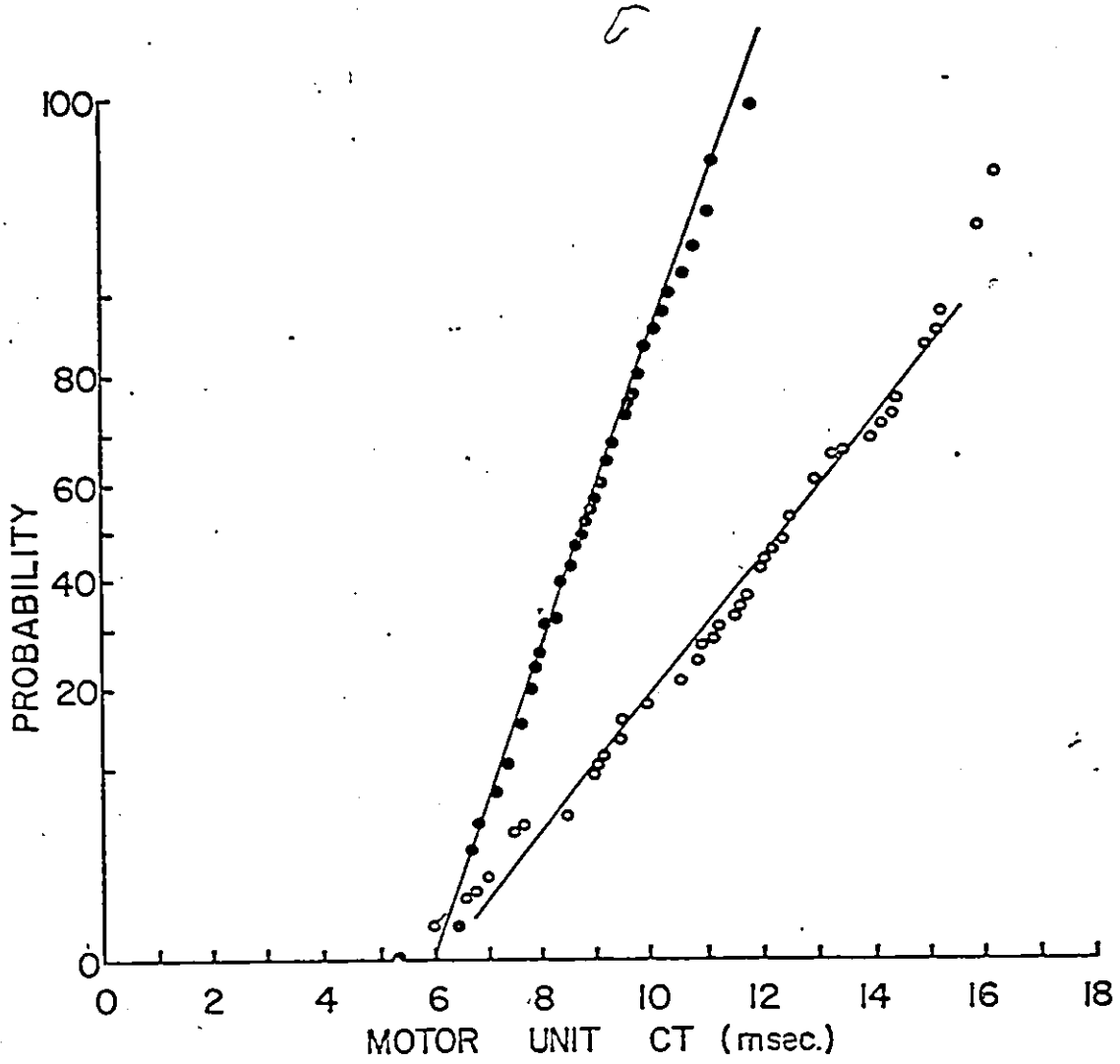

NUMBER OF MOTOR UNITS
0
2
4
6
8
10
12
14
16
18
20
2
4
6
8
10
12
14
16
18
20
UNIT CT (msec)

FIGURE 14

Cumulative probability plot of motor units ranked in order of CT plotted on a normal probability ordinate. Both normal and dystrophic data do not deviate significantly from the straight line which corresponds to a normal distribution.

(normal ● $r = .98$ $p < .01$

dystrophic ○ $r = .97$ $p < .01$)


100
80
60
40
20
0
PROBABILITY
0
2
4
6
8
10
12
14
16
18
MOTOR UNIT CT (msec.)

axonal conduction velocity was significantly faster for normal mice than for dystrophics. The mean conduction time was significantly shorter for normal mice than for dystrophics.

Units from normal mice exhibited a wider range of conduction velocity than dystrophics. Save for one unit whose conduction velocity was 110 M/sec normal units ranged from 52 to 88 M/sec. Units from dystrophic mice ranged from 33 to 55 M/sec. A similar trend was seen with unit conduction time. Normal mice ranged from 0.50 to 0.88 msec while dystrophic mice exhibited a range from 0.80 to 1.40 msec. In both cases little overlap of distributions occured betweeen normal and dystrophic data.

It has been suggested that various relationships exist between the axonal conduction velocity and the mechanical parameters of motor units. Various relationships among axonal conduction velocity, conduction time, CT, Po, Pt/Po and motor unit size were examined. Only animals from which five or more units were isolated were considered. The data are presented graphically in Figures 15, 16 and 17.

Figure 15 a,b shows the relationship between axonal CV and CT for units from 5 normal mice and 4 dystrophic mice respectively. The idea that axons with fast conduction velocities have fast times to peak is examined. Lines represent a best fit from linear regression analysis. No obvious relationship can be

TABLE VII

AXONAL CONDUCTION VELOCITY AND CONDUCTION TIMES FOR MOTOR UNITS FROM NORMAL AND DYSTROPHIC EDL AT 36.5° C

MOTOR UNIT DESCRIPTION	CONDUCTION VELOCITY (M/sec)	CONDUCTION TIME (msec)
C57 (49)	70.1 ± 11.8 ± 1.7	0.67 ± 0.12 ± 0.02
dy2J (46)	46.3 ± 5.7 ± 0.8	0.99 ± 0.13 ± 0.02
P	< 0.0005	< 0.0005

a) Number in parenthesis is N = number of motor units studied.

b) Values are Mean ± SD, ± SEM.

c) P indicates level of significance using the Student's t test for unpaired data.

seen for the data.

As large displacements along the conduction velocity axis might obscure a relationship, the reciprocal of conduction time was used to eliminate this horizontal dispersion in Figure 15 c,d (Ridge 1967, also see Chapter IX). Here too no significance could be shown within experiments or between experiments.

If one accepts the belief that large diameter axons have large conduction velocities and because of their size are able to innervate greater numbers of muscle fibres, then a relationship between axonal conduction velocity and motor unit Po should exist. Such a relationship is studied in Figure 16 a,b. From the regression coefficients of experiments no linear relationship exists for individuals and examination of the best fit lines shows no obvious relationship.

Here too Po has been plotted against reciprocal conduction time but again no relationship is evident.

Figure 17 a,b and c,d examine the relationships of motor unit Po, Pt/Po and CT. Again no relationships could be detected in either normal or dystrophic motor units.

FIGURE 15

15 a, b Motor unit twitch time to peak tension plotted against axonal conduction velocity. a) corresponds to units from normal EDL, b) to units from dystrophic EDL. (r is the linear regression coefficient).

a) normal ▲ r1 = .40 (NS), + r2 = .17 (NS), ■ r3 = .10 (NS), × r4 = .97 ($p<.05$), ● r5 = .10 (NS);

b) dystrophic ▲ r1 = .79 (NS), + r2 = .28 (NS), ■ r3 = .44 (NS), × r4 = .85 ($p<.05$)

15 c, d Data of a) and c) replotted using the reciprocal of axonal conduction time on the abscissa. c) corresponds to units from normal EDL, d) to units from dystrophic EDL.

c) normal ▲ r1 = .35 (NS), + r2 = .44 (NS), ■ r3 = .14 (NS), × r4 = .56 (NS), ● r5 = .75 (NS);

d) dystrophic ▲ r1 = .68 (NS), + r2 = .20 (NS), ■ r3 = .41 (NS), × r4 = .70 (NS).

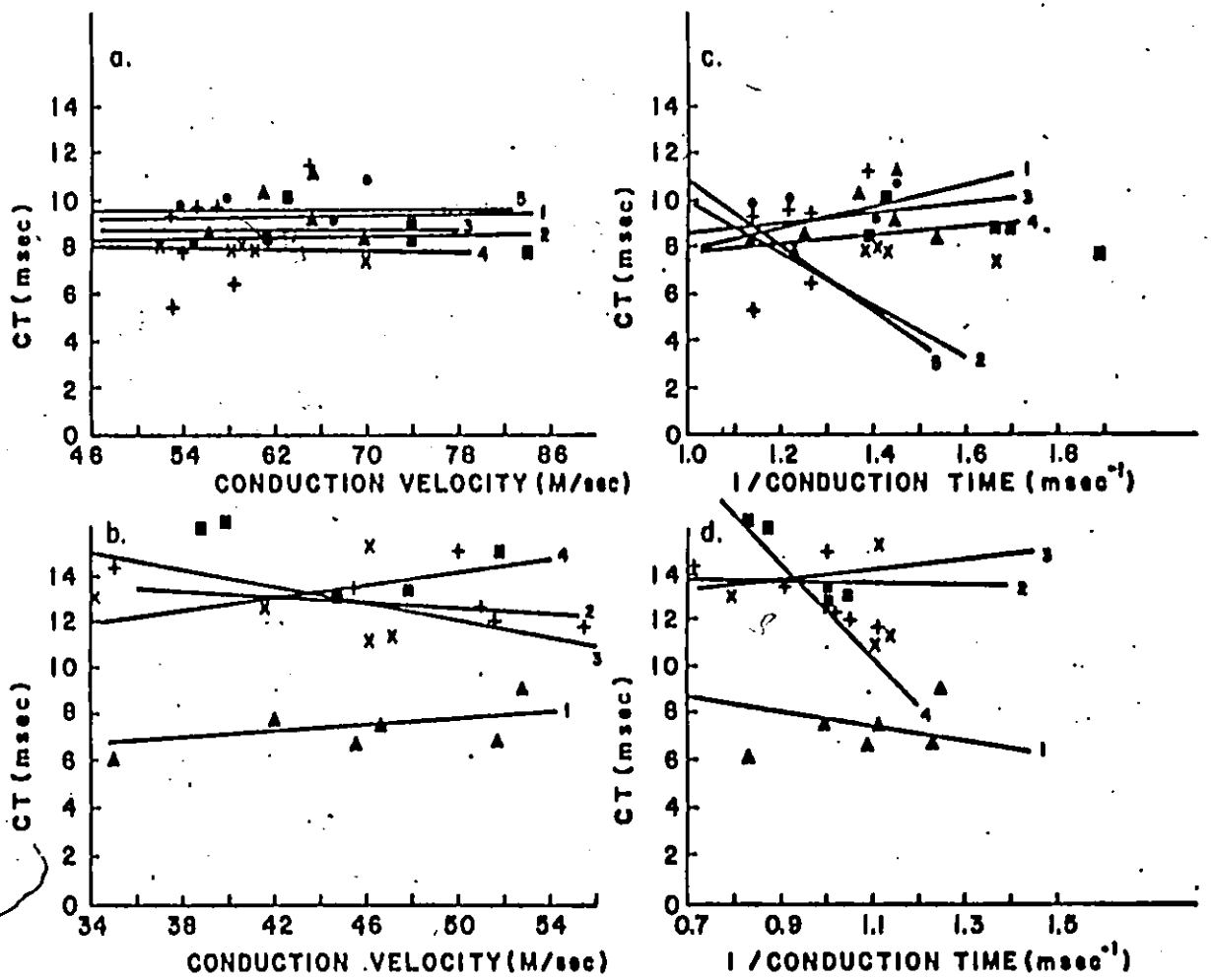

a.
CT (msec)
CONDUCTION VELOCITY (M/sec)
c.
CT (msec)
1/CONDUCTION TIME (msec⁻¹)
b.
CT (msec)
CONDUCTION VELOCITY (M/sec)
d.
CT (msec)
1/CONDUCTION TIME (msec⁻¹)

FIGURE 16

16 a, b Motor unit tetanic tension plotted against axonal conduction velocity. a) corresponds to units from normal EDL, b) to units from dystrophic EDL. (r is the linear regression coefficient).

a) normal ▲ r1 = .57 (NS), + r2 = .10 (NS), ■ r3 = .41 (NS), × r4 = 0 (NS), ● r5 = .39 (NS);

b) dystrophic ▲ r1 = 0 (NS), + r2 = .44 (NS), ■ r3 = .49 (NS), × r4 = .28 (NS)

16 c, d Data of a) and b) replotted using the reciprocal of axonal conduction time on the abscissa. c) corresponds to units from normal EDL, d) to units from dystrophic EDL.

c) normal ▲ r1 = .32 (NS), + r2 = .10 (NS), ■ r3 = .10 (NS), × r4 = 0 (NS), ● r5 = .17 (NS);

d) dystrophic ▲ r1 = .20 (NS), + r2 = .26 (NS), ■ r3 = .45 (NS), × r4 = .41 (NS).

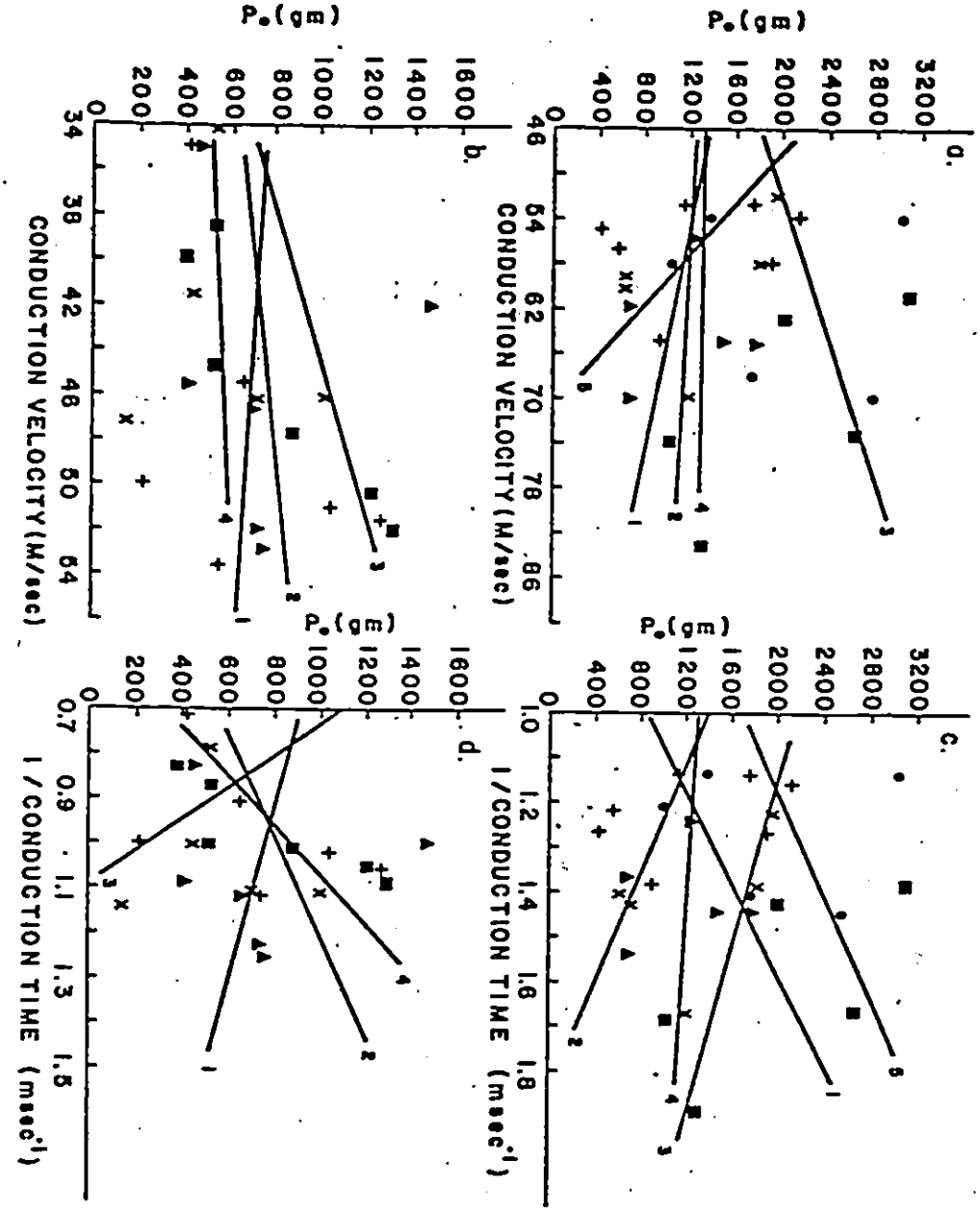

a.
b.
c.
d.
P₀(gm)
CONDUCTION VELOCITY (M/sec)
1 / CONDUCTION TIME (msec⁻¹)

FIGURE 17'

17 a, b Motor unit tetanic tension plotted against time to peak tension. a) corresponds to units from normal EDL, b) to units from dystrophic EDL. (r is the linear regression coefficient).

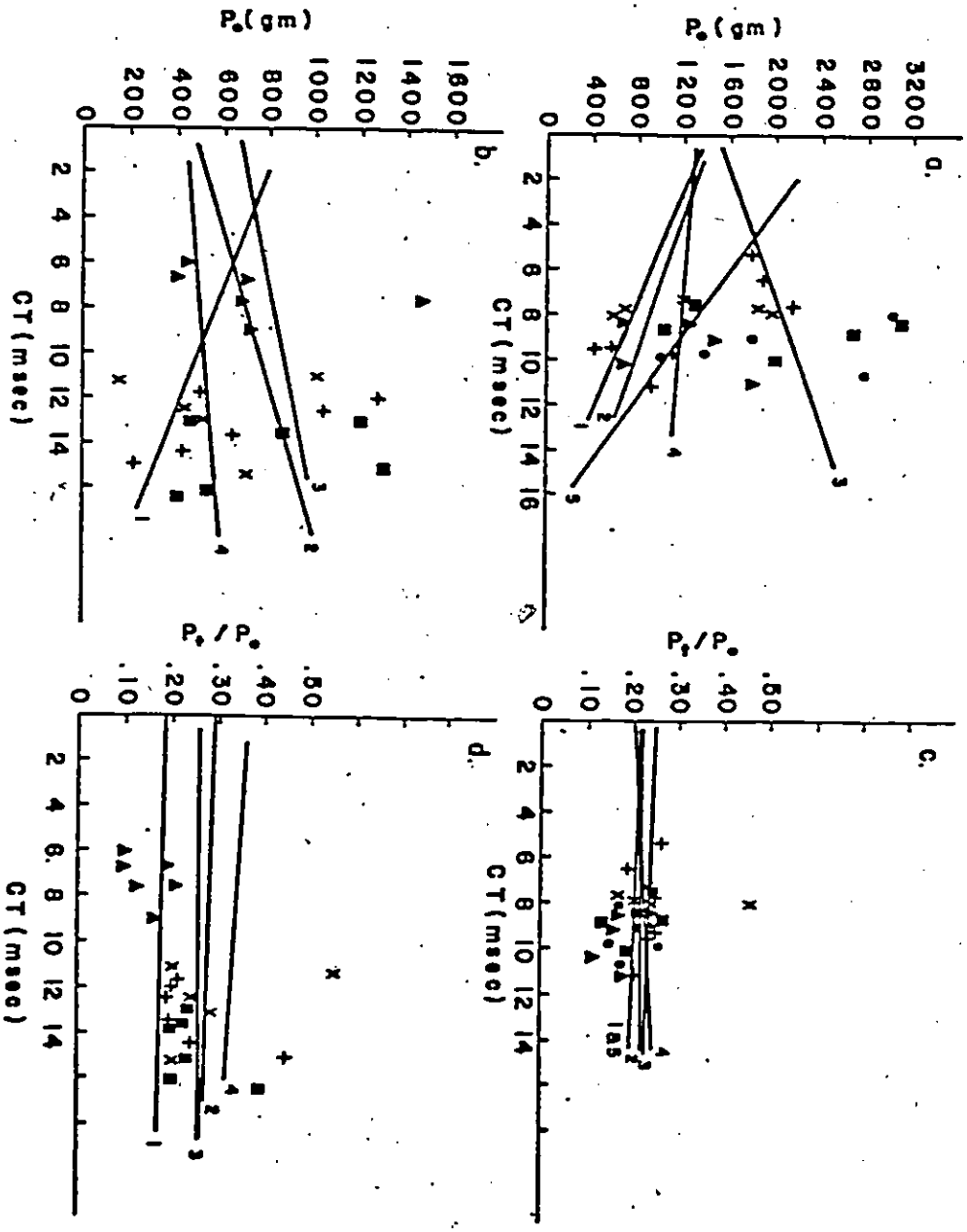
a) normal ▲ r1 = .52 (NS), + r2 = .17 (NS), ■ r3 = .39 (NS), × r4 = 0 (NS), ● r5 = .36 (NS);

b) dystrophic ▲ r1 = .20 (NS), + r2 = .57 (NS), ■ r3 = .45 (NS) × r4 = .41 (NS)

17 c, d Motor unit twitch/tetanus ratio plotted agains time to peak tension. c) corresponds to units from normal EDL, d) to units from dystropic EDL.

c) normal ▲ r1 = .71 (NS), + r2 = .28 (NS), ■ r3 = .70 (NS), × r4 = .30 (NS), ● r5 = .48 (NS);

d) dystrophic ▲ r1 = .36 (NS), + r2 = .36 (NS), ■ r3 = .17 (NS), × r4 = .45 (NS).


a.
b.
c.
d.
P_s (gm)
P_s / P_o
CT (msec)

X DISCUSSION

As a first report of the motor unit properties of a mouse fast muscle the findings for normal EDL motor units are of significant interest in themselves. Having also been able to successfully functionally isolate EDL motor units from dystrophic dy2J mice, comparison of the two will represent the first report of its kind. As only indirect studies of dystrophic motor units have been reported (Harris and Wilson 1971, Law and Caccia 1975) some of the conclusions to be drawn must stand on their own. However, whenever possible comparison with the literature will be made.

As has been indicated certain distinct predictions can be made for motor unit properties if dystrophy is considered as either myogenic or neurogenic. In addition to examining the effects of dystrophy on EDL motor unit properties these predictions will be examined in an attempt to indicate the etiology of murine muscular dystrophy.

Some of the relevant literature discussed is derived from studies using the 129 ReJ dy/dy strain of mouse and it must be pointed out again that while this genetic mutation and that of the dy2J/dy2J mouse (used in this study) are allelic, their etiologies and phenotypic expressions are not necessarily

identical (Parsons 1974, Harris and Montgomery 1975).

Significant decrease in whole EDL muscle weight occured with dystrophy. This weight loss can be attributed to muscle fibre loss (Rowe and Goldspink 1969, Parry and Parslow 1981). Associated with this loss of whole muscle weight was a decrease in absolute twitch tension, tetanic tension and a decreased normalized Pt and Po. These observations are probably the most commonly reported effect of dystrophy on whole muscle.

Others have reported on significant decreases in tension for the dy strain of mouse with soleus, gastrocnemius and EDL (Sandow and Brust 1958, Brust 1966, Douglas and Baskin 1971, Taylor et al 1971, 1974 and Hofmann and Ruprecht 1973).

Considerably fewer studies using the dy2J strain have been reported. Harris and Montgomery (1975) examined the anterior tibialis. They noted decreased muscle weight, decreased Pt and Po and decreased normalized tensions with dystrophy. As in this study they also saw no significant difference between normal and dystrophic Pt/Po ratios. It therefore seems that despite the morphological alterations due to the disease the degree of activation of the muscle is not altered between normal and dystrophic animals. Parry (1977) examined the EDL (dy2J) of mice of approximately the same age as those used in this study and showed the same trend with regards to muscle weight and maximal

twitch tension.

Oba et al (1978) examined the electrophysiological characteristics and ultrastructural features of single muscle fibres from normal (C57) and dystrophic (dy2J) EDL muscle. They showed single muscle fibres exhibited an age-dependent reduction in tension generation despite a myofibrillar structure that was relatively well preserved, even at advanced stages of the disease. They did show dystrophic fibres had a poorly developed T-system with disrupted tubules and suggest that the decreased tension in dystrophic muscle is primarily caused as a result of the immature disrupted T-system which is essential in excitation/contraction coupling process.

The data for speed of contraction and half relaxation clearly demonstrate a slowing of the dystrophic EDL. The $\frac{1}{2}$RT was more affected, being prolonged by 43% while the CT was increased by 30%. These observations are consistent with previous studies (Parslow and Parry 1981) in which a significant slowing in old dystrophic EDL was reported. The actual values reported for dystrophic EDL were slower than reported here but this may be related to the age difference of mice, greater than 6 months versus 3 months, used in this study. They demonstrated a progressive slowing from young (4-6 weeks) animals to old (6 months) animals. One might reasonably expect 3 month animals to

fall somewhere between the values they report for young and old mice; and in fact this is the case.

Comparison with other literature using dy mice is difficult due to strain differences. Brust (1966) studying dy gastrocnemius saw a slowing of both the contraction and relaxation phases of the twitch with dystrophy while Douglas and Baskin (1971) saw only an increase in ½RT and no significant change in CT with dy EDL compared to controls. Harris and Wilson (1971) reported an increase in CT while Montgomery (1975) reported no change in this parameter. Sabbadini and Baskin (1976) reported a decrease in CT which they attributed to a decreased intensity, duration, and rate of decay of active state.

In dy2J mice Harris and Montgomery (1975) showed an increase in CT for anterior tibialis while Hironaka and Miyata (1975) using EDL found an increase in both time to peak tension and half relaxation time.

Parry and Parslow (1981) suggested that the slowing observed in EDL represents an intrinsic change in the properties of dystrophic muscle based on their observed conversion to "slow" properties with regard to both cooling and post-tetanic potentiation. This intrinsic conversion resulting in an increased CT and extended ½RT may be due to several interrelated parameters. Both Dribin and Simpson (1977) and Parry and Parslow

(1981) have implicated the spontaneous hindlimb activity as a key factor in the histological and physiological observations of dystrophic muscle. Silverman and Atwood (1980) suggested that this activity results in a shift towards a more oxidative profile. In addition it has been shown (Pette et al 1976) that continuous stimulation of fast-twitch muscle at 10 Hz results in a conversion towards a more oxidative profile and an associated increase in time parameters. This is accompanied by a shifting of the myofibrillar ATPase activity towards that of Type I fibres. Therefore it seems clear that the altered hindlimb activity pattern seen in dystrophic animals contributes to the overall slowing seen in fast muscle.

Both Dribin and Simpson (1977) and Parry and Parslow (1981) demonstrated a significant loss of Type II glycolytic fibres in gastrocnemius and EDL respectively with dystrophy. This leaves a predominance of Type II oxidative fibres in these dystrophic muscles. This result may be achieved by a selective loss of Type II glycolytic fibres, due to dystrophy, or it may result from a conversion to intermediate then Type II oxidative fibres caused by spontaneous hindlimb activity and a subsequent loss of Type II oxidatives due to dystrophy.

Burke et al (1973) in cat have shown motor units of the Type II oxidative fibre type to be slower in CT and more prolonged

in ½RT when compared to Type II glycolytic units. It therefore seems plausible to propose that the increased contribution of Type II oxidative fibres serves to slow the time parameters of a muscle in which it predominates.

In addition, prolongation of the ½RT in whole dystrophic EDL may be contributed to by the decreased Ca+2 reuptake mechanism. This observation has been clearly defined in isolated sarcoplasmic reticular fractions from dystrophic muscle (Martonosi 1968, Mrak and Baskin 1978) and is supported by an altered ultra structure in dystrophic sarcoplasmic reticulum (Shafiq et al 1969, Mrak and Baskin 1978).

These muscle parameters reflect the cumulative contribution of the motor units that make them up. Therefore to better understand muscle in normal and diseased states an understanding of the constituent motor unit properties is invaluable. The results presented in this thesis for motor units represent the first such report of functionally isolated units from a fast muscle of mouse. More importantly, it is the first report of functionally isolated motor units from dystrophic animals.

The relative mean tetanic tension of motor units from normal mice was 4.6 % of the whole muscle tension. The 11 fold range seen in normal EDL unit size was not significantly greater

than that reported for the slow soleus motor units (8 fold) of normal C57 mice (Lewis et al 1982). The range of motor unit size for dystrophic units was similar to that of normal units (Figure 11). The mean value of unit size was however significantly greater, 6.7%.

As has been demonstrated for the theoretical distribution of relative motor unit size (Figure 2b) a shift towards a greater mean unit size is not consistent with a random myopathy in which mean relative size would remain unchanged. However, such an observation (Table V and Figure 11) is consistent with an acute neuropathy.

The reciprocal of the mean motor unit size gives an estimate of the number of motor units in the muscle. For normal EDL this corresponds to 22 while for dystrophic EDL there are approximately 15, a reduction of 35% in the motor unit population.

Both Harris and Wilson (1971) and Law and Caccia (1975) claimed a decreased number of motor units in dystrophic dy anterior tibialis and dy soleus, respectively. However, as has been pointed out in Chapter III and VI the method of incremental stimulation of the whole nerve to evoke motor units requires assumptions that have been strongly criticised. In terms of mechanical measurements (i.e. Po) non-random recruitment of motor units, that may result from this technique, can cause erroneous estimates of motor unit numbers. With regards to the evoked action potential measurements made by Law and Caccia (1975), the assumption of linear summation of the evoked motor unit responses has been criticised and may lead to underestimates of motor unit numbers (Parry, Mainwood and Chan 1977).

This thesis represents the first report of a statistically significant decrease in motor unit numbers in the EDL of dy2J mice. The technique of functionally isolating motor units avoids the criticisms of previous work in enabling the determination of motor unit numbers.

If muscular dystrophy were of myogenic origin then one would expect a random loss of muscle fibres resulting, with the progression of the disease, in a normal or only slightly reduced number of very weak motor units. The results reported here indicate a significantly reduced number of motor units whose

tensions are within the range of normal motor unit tensions. This may be construed as support for a neurogenic etiology to muscular dystrophy.

In contrast to McComas' sick motorneurone hypothesis Desmedt and Borenstein (1973) have suggested that in man dystrophic motor axons are capable of reinnervating muscle fibres. If we assume for the moment that this might be true also in murine dystrophy then we would predict dystrophic muscle would exhibit large size units (as a percent of the whole muscle tension) that also produced significantly larger tensions when compared to controls. This is not the observed case. Though Figure 11 shows some dystrophic units contribute as much as 14% - 18% of the whole muscle tension, their mean absolute tetanic tension is still less than the mean normal unit tetanic tension (1217 gm versus 1429 gm respectively, though these values are not statistically significant). It would therefore seem that such a unit in dystrophic muscle appears relatively large only due to the loss of other motor units within that muscle, but is not larger in an absolute sense.

The absence of units from dystrophic EDL whose absolute tensions are greater than units from normal EDL is in agreement with the "sick" motorneurone hypothesis. The remaining motorneurones in dystrophic muscle may be sick and incapable of

successfully reinnervating by collaterals those fibres that have lost their neuronal influence. Parry and Melenchuk (1981) have shown that, following nerve section, reinnervation of EDL is less complete in dy2J mice than in normal animals. This may be considered also as evidence in favour of the presence of "sick" motorneurones. McComas et al (1974) have shown an absence of larger than normal motor units in dystrophic patients which supports the idea of an inability of motor axons in dystrophic mice to send out successful collaterals.

The actual estimate of the number of motor units for normal and dystrophic EDL presented here cannot be verified directly through the literature. However, the estimate of 22 motor units for normal EDL is similar to that reported by Lewis, Parry and Rowlerson (1982) for normal C57 soleus, using a similar technique. Soleus is a muscle of approximately the same size and weight and therefore it would seem reasonable to assume its motor unit pool would approximate that of EDL.

Similarly Parry, McHanwell and Haas (1982) have reported on the motorneurone pool of normal and dystrophic C57 soleus. They used the retrograde transport of Horseradish peroxidase to label the motorneurones in these muscles and estimated the number of alpha motorneurones stained was 25 for normal soleus and 20 for dystrophic soleus. This again is suggestive that the numbers of

motor units that have been reported in this thesis for EDL are reasonable.

The value reported by Parry et al (1982) for normal soleus motorneurones was similar to that obtained in ReJ 129 mice by Law and Caccia (1975) using an electrophysiological estimating method. It therefore seems that despite reported abnormalities in axoplasmic flow in the dystrophic mouse (Bradley and Jaros 1973, Komiya and Austin 1974, Tang, Komiya and Austin 1974, Kuffer, Komiya and Austin 1977) this method of labelling is quite reasonable and retrograde transport of HRP is not impaired in alpha motorneurones of dystrophic mice.

Other indirect evidence exists to support a neurogenic concept of dystrophy suggestive of a dropping out of whole motor units as opposed to a random myopathic degeneration. This comes in a number of reports dealing with nerve fibre counts.

Harris, Wallace and Wing (1972) counted the myelinated nerves in 3-4 month old anterior tibialis of normal and dystrophic 129 ReJ mice. They divided their nerve fibres into two groups; large diameter myelinated axons including alpha motor nerves and large afferents (Type I) and small myelinated axons including Type II afferents and gamma efferent fibres. A significant reduction in axon number was shown for dystrophic nerves and suggested a failure of development in the peripheral nervous system. Based on

the decrease in both groups of axons they concluded that both sensory and motor nerves were affected. Bradley and Jenkison (1973) confirmed this loss of myelinated axons for the sciatic nerve of dy mice and several of its branches.

Montgomery and Swenarchuk (1977, 1978) studied both muscle fibre counts and myelinated axon counts in C57 and ReJ mice. They compared normal and dystrophic soleus, plantaris, medial gastrocnemius and EDL in mice aged between 4 weeks and 72 weeks. Muscle fibre loss was progressive although the rate was faster for dy mice than dy2J animals. 129 ReJ dy/dy mice did not exhibit a progressive loss of axons as did C57BL/6J dy2J/dy2J mice, but showed a significant loss of nerves already present at 4 weeks that remained unaltered through 72 weeks.

It would therefore appear that the observed loss of motor units in dy2J EDL reported in this thesis is consistent with a neurogenic etiology for dystrophy and this is further supported by the observations of reduced HRP-stained motorneurones, progressive loss of myelinated axons and by the indirect physiological estimates of motor unit numbers reported by others.

However, with no method such as the glycogen depletion technique to identify the muscle fibres of a specific unit one cannot directly test for the etiology of dystrophy and must settle for conclusions that are consistent with its implications.

We have seen in Chapter VII the theoretical frequency distributions for absolute tension. Figure 9 and 10 show the observed distribution for motor unit twitch tension and tetanic tension, respectively. This discussion will deal with the distribution of motor unit tetanic tension (refer to Chapter VIII for the basis of this). A similar discussion could be applied to the unit twitch tension distribution and consequently will not be included.

The mean unit absolute tetanic tension (Figure 10) shows a significant decrease (1438 mgm to 840 mgm) and a shift towards lower values of tension. This is not consistent with the predictions for a pure (acute) neuropathy. It is however consistent with a random myopathy.

It would therefore appear that we are not dealing solely with an acute neuropathy or a pure myopathy but some intermediate state or combination of the two. This might arise in two ways.

Firstly if dys directly affects the muscle with this having an indirect effect on the motorneurone or, dystrophy has a direct effect on a common denominator in both muscle and nerve.

The weakening of units in dystrophic mice is not the result of an altered degree of activation of the twitch. In motor units and in whole muscle the twitch/tetanus ratio was not significantly different between normal and dystrophic animals. This would suggest that despite any loss of muscle fibres and their replacement with connective tissue this does not alter the degree of activation of dystrophic motor units.

The reduced mean twitch tension for motor units of dystrophic EDL may be due to either a decreased tension output per muscle fibre or may arise from a loss of functional muscle fibres in the affected "sick" motor units.

Law and Caccia (1975) claimed that in dy soleus the 40% reduced mean unit Pt they estimated from graded threshold nerve stimulation of motor units represented a "functional denervation" of 40% of the muscle fibres in dystrophic soleus. Parry (1977) has questioned this suggesting that because they detected no difference between the sizes of dystrophic and normal motor unit action potentials (presumably indicative of the number of fibres producing action potentials) it would seem simpler to explain the decreased unit tension as due to functional fibres becoming relatively weaker.

Briefly, when compared to normal soleus, Law and Caccia (1975) observed the following:

decrease in whole muscle Pt
decrease in motor unit Pt

decrease in whole muscle AP
no change in motor unit AP

The combination of decreased motor unit Pt and no change in motor unit action potential must mean that while these units have no decrease in their number of muscle fibres the fibres have become weaker. The observation of a decreased whole muscle AP but no change in the motor unit AP clearly means that some units have been lost.

The following scenario could explain these observations and be consistent with a "sick" motorneurone hypothesis. Suppose a motorneurone becomes non-functional through a loss of its trophic influence on the muscle fibres of that unit. The unit would become weaker. But, since the axon and neuromuscular junction (NMJ) may still be intact there would be no change in the motor unit AP. Further, suppose that the fibres continue to weaken and that at some critical point either the muscle fibres, the NMJ or the axon itself becomes electricaly inexcitable. At this point this motor unit's AP would go to zero. If the axon were the site of this electrical inexcitability, there would be an

instantaneous transition from a 100% MUAP to 0% MUAP. If the fault lay within the muscle fibre or the NMJ such an instantaneous transition might not be expected. However, if this transition state were relatively short one might still not expect to observe this brief phase of decreased MUAP.

In such an interpretation the observations of Law and Caccia are accounted for, as well as being consistent with a neuropathy involving "sick" motorneurones. This hypothesis can also account for the observations made in this thesis and may represent the most plausible explanation. As the trophic influence is withdrawn and muscle fibres become weaker the mean unit Po will decrease, as observed. As some units would be unaffected and some more affected, this would account for the observed relative unit size distribution and the presence of some relatively large units. Similarily, unaffected units would still exhibit fast CT, as shown in Figure 13, while the mean CT is shifted towards greater CT (assuming the altered trophic influence causes an increased CT, which seems reasonable). At some critical stage whole motor units drop out and therefore we see a decreased number of motor units in the dystrophic EDL.

Such an interpretation would also be consistent with previous observations on the functional innervation ratio of dystrophic EDL (Parry 1977) and the decreased counts of myelinated

nerve axons (Montgomery and Swenarchuk 1977, 1978).

Interestingly, Law and Caccias' observation of a 40% decrease in mean motor unit twitch tension for dy soleus and Harris and Wilson's (1971) observation of a 44% reduction in dy anterior tibialis mean motor unit twitch tension are very similar to the 43% reduced motor unit Pt for dy2J EDL reported in this thesis.

The frequency distribution of normal EDL muscle motor unit twitch times to peak tension was seen to be quite narrow, clearly unimodal and normally distributed (Figure 13 and 14). This distribution is remarkably close to that reported by Luff and Atwood (1972) for single muscle fibres in mice. Close (1967) showed a similar histogram for EDL motor units in rat. Both the unit data reported here and that by Close are reflected in a unimodal narrow frequency distribution of whole muscle CT.

Such a narrow unimodal distribution of CT in normal mice might arise in either of two ways. Firstly there may be no difference between the CT in Type II oxidative and Type II glycolytic units. However, in view of data from cat, rat and mouse (Lewis et al 1982) this seems highly unlikely. Alternatively, there might be two normal populations whose distributions so overlap as to yield a single unimodal distribution. This seems the more probable.

In dystrophic EDL the unit twitch time to peak distribution is considerably different from that for normal EDL units. A definite shift towards increased CT is shown. This is reflected in the significantly increased mean unit time to peak 11.9 msec vs 8.7 msec, respectively. While there appears to be an almost biomodal distribution, and some distortion of the probability line does occur in the region of units of less than 8.0 msec CT (Figure 13), cumulative probability plotting (Figure 14) and linear regression analysis were unable to detect this as significant and indicated a normal unimodal distribution for dystrophic EDL.

In the case of a random myopathy the predicted distribution would be shifted towards an increased mean CT. In an acute neuropathy no change in distribution of CT or mean CT should occur. From the theoretical distributions for time to peak (sequence Figure 2c) for CT the results observed are consistent only with the case in which some units are affected (slowed). This is in agreement with the previous discussion of results for absolute tensions and relative unit size and the proposed explanation of the data.

Two methods by which a selective neuropathy might act have been previously proposed. Parry and Parslow (1981) demonstrated that in the EDL of dystrophic C57 mice there is a

very marked decrease in the proportion of Type II glycolytic fibres with the reduction of total fibre numbers in dystrophic EDL being almost identical to the reduction in Type II glycolytic fibres. This would initially suggest a selective loss of Type II glycolytic fibres. If this were the case then one would predict a slowing of the whole muscle because the remaining motor units would be predominantly Type II oxidative. In terms of motor unit CT distribution one would expect a skewed distribution composed of those glycolytic units that have not yet been affected and no change in the distribution of those oxidative units that are unaffected. This does not seem to be the case (Figure 13). Parry warns however that their histological findings may be indicative of a progressive shift in fibre type from II glycolytic to intermediate to II oxidative, possibly due, in part, to the hindlimb spontaneous activity, followed by death of the II oxidative fibres due to dystrophy. Were this the situation in dystrophy, motor unit distribution of CT would appear as observed. This latter interpretation would also be consistent with the previous expressed hypothesis (page 138) since withdrawal of the trophic influence and spontaneous activity would allow fibres to shift to an intermediate state.

The dystrophic EDL CT histogram shows some motor units still exist which are as fast as the fastest normal EDL motor

units. In a neurogenic etiology this would be predicted and it would be reasonable to assume that these correspond to unaffected motor units. These unaffected units would be expected to have identical parameters to their normal counterparts. By grouping these units (CT less than 8.0 msec) from dystrophic mice and comparing them to their corresponding normal EDL units it can be seen in Table VIII that these may in fact be identical populations. The presence of units in the dystrophic EDL that exhibit mechanical parameters similar to those units from normal muscle is consistent with a neurogenic etiology for dystrophy since a principle prediction of this hypothesis is the presence of such units.

Table IX compares these "fast" units from dystrophic EDL with the remaining unit population from the dystrophic EDL and shows them to be significantly different, having greater tensions, faster CT and shorter ½RT as well as contributing a larger % of the whole muscle tension. This too is to be expected in a neuropathy.

Neither of these tables present data totally consistent with a primary myopathy or an acute neuropathy.

Thus it would appear that the mechanical parameters of EDL motor units from dystrophic mice when compared to EDL motor

TABLE VIII

A COMPARISON OF MOTOR UNITS FROM DYSTROPHIC EDL WHOSE CT ARE LESS THAN 8.0 MSEC WITH THEIR CORRESPONDING MOTOR UNITS FROM NORMAL EDL.

MOTOR UNIT DESCRIPTION	Pt (mgm)	Po (mgm)	CT (msec)	1/2RT (msec)	Pt/Po	UNIT Po x 100/EDL %
C57 (18)	310.3 ± 112.5	1354.6 ± 460.7	7.3 ± 0.7	8.0 ± 1.5	0.23 ± 0.03	5.0 ± 2.0
dy2J (7)	238.7 ± 113.0	1215.0 ± 310.2	7.0 ± 0.6	6.6 ± 1.2	0.19 ± 0.08	8.8 ± 2.5
P	NS	NS	NS	< 0.05	NS	< 0.05

a) Number in parenthesis is N = number of motor units studied.

b) Values are Mean ± standard deviation (SD).

c) P indicates levels of significance using the Student's t test for unpaired data.

TABLE IX

A COMPARISON OF MOTOR UNITS FROM DYSTROPHIC EDL WITH CT LESS THAN 8.0 MSEC (FAST) WITH THOSE MOTOR UNITS WITH CT GREATER THAN 8.0 MSEC (SLOW).

MOTOR UNIT DESCRIPTION	Pt (mgm)	Po (mgm)	CT (msec)	RT (msec)	Pt/Po	UNIT Po x 100/EDL %
fast dy2J (7)	238.7 ± 113.0	1215 ± 310.2	7.0 ± 0.6	6.6 ± 1.2	0.19 ± 0.08	8.8 ± 2.5
slow dy2J (53)	176.0 ± 66.7	853.6 ± 354.6	12.6 ± 2.0	14.7 ± 3.2	0.22 ± 0.07	6.2 ± 3.1
P	< 0.005	< 0.005	< 0.005	< 0.005	NS	< 0.05

a) Number in parenthesis is N = number of motor units studied.

b) Values are Mean ± standard deviation (SD).

c) P indicates levels of significance using the Student's t test for unpaired data.

units from normal mice are consistent with a neurogenic etiology for murine muscular dystrophy. It also appears that the effects of dystrophy and the superimposed effects of the spontaneous hindlimb activity serve to convert unit types towards progressively more oxidative ones which are then being lost in a way that would be predicted by the neurogenic hypothesis.

While other experiments suggest this may be the case, and there is evidence for the involvement of Type II glycolytic conversion to Type II oxidative with comcomitant loss, until a direct method of identifying specific unit fibres is developed this is only surmize.

Also the suggestion that the "fast" units in dystrophic mouse are Type II glycolytic is also surmize. Still there is evidence in addition to the theoretical considerations dealt with to support this.

Comparing the normal CT distribution with that of Lewis et al (1982) we have seen that the slowest EDL units correspond to the fastest soleus units. Knowing the fibre type distribution of these muscles (EDL: II glycolytic and II oxidative, soleus: II oxidative and Type I) and assuming that the situation in mouse is the same as that in the cat (Burke et al 1973) ie. that glycolytic units are faster than oxidative units which are faster than Type I units, it seems reasonable to assume that the faster units in EDL

are glycolytic.

Another time related parameter, mean axonal conduction velocity, was seen to be significantly faster for normal EDL motor units than that of dystrophic EDL motor units. The range of conduction velocity reported for normal units (52 to 88 M/sec with the exception of one unit of 110 M/sec) was faster than that found by Lewis & Parry in a series of experiments on normal soleus (32 to 70 M/sec, Parry personal communication). However, in view of the known fibre type composition of EDL compared to soleus this would be expected.

In dystrophic EDL units, the axonal conduction velocity was significantly less than that of control animals. As well, the conduction time was significantly longer. With the known amyelination of portions of the sciatic nerve and axons within the ventral and dorsal roots this observation is of no surprise. Various workers have reported on this amyelination and the increased conduction time and reduced conduction velocity associated with it (Huizar, Kuno and Miyata 1975, Biscoe et al 1977, Rasminsky et al 1978).

Since Buller, Eccles and Eccles (1960) described the results of cross-innervation studies and demonstrated that contractile properties of muscles were controlled by the motor innervation they received, it has been accepted that specific

influences are exerted by the motorneurone on the muscle fibres it innervates. This, coupled with Henneman's proposed size principle (Henneman et al 1965), has led to various investigations of the relationships between motorneurone properties and muscle parameters.

Bessou, Emonet-Dénand and Laporte (1963) showed that fast conducting axons innervated large motor units with short contraction times whereas slowly conducting motor axons innervate weaker motor units having longer contraction times. If the motorneurone is responsible for establishing such a relationship then one would expect this to be universal. However, this does not appear to be the case.

In their pioneering work Emonet-Dénand and co-workers (Bessou, Emonet-Dénand and Laporte 1965, Apelberg and Emonet-Dénand 1967, Emonet-Dénand, Laporte and Proske 1971) presented such relationships in cat lumbrical muscles and correlated unit CT and unit Po with the conduction velocities of their innervating axons. However, the existence of such relationships in the larger cat hindlimb muscles remains controversial.

McPhedran et al (1965) working with cat medial gastrocnemius found "an approximately linear relationship between axonal conduction velocity and motor unit tension for units

producing 20 gms or less tension." No such relationship could be shown for units producing greater than 20 gms. Olson and Swett (1966) could demonstrate a linear relationship between conduction velocity and unit Po only for units producing less than 20 gms tension in cat flexor digitorum longus. Bagust (1974) using cat soleus (a homogeneous Type I muscle) demonstrated a clear linear relationship between axonal conduction velocity and motor unit CT and Po for this muscle. This work suggests that for Type I units and units producing small tensions the size of an individual motor unit is determined by its motor innervation as reported for cat lumbricals.

This work however was not confirmed by Mosher et al (1972) studying anterior tibialis (a mixed fast muscle). They could show no relationships as previously described in either individual experiments or in their data as a whole. Bagust et al (1973) claimed that linear relationships between axon conduction velocity, unit CT and unit Po did exist for cat FDL at all tensions. They suggested that the relationship was obscured in the experiments of Olson and Swett's due to their pooling of data from several muscles. They maintained that by using only individual experiments from which sufficient units have been isolated, relationships were not masked. Still, in experiments dealing with large numbers of units per muscle, Gerlach et al 1975

were unable to show any relationships betwen axonal conduction velocity and CT or Po in cat plantaris, flexor hallucis longus and flexor digitorum brevis. Clearly any such correlations cannot be the rule for large cat hindlimb muscles. Similarly these workers have reported such controversial findings for relationships between time to peak tension and contraction strength and time to peak tension and the twitch/tetanus ratio.

In only one report have these relationships been examined in mice. Lewis, Parry and Rowlerson (1982) demonstrated in C57 soleus motor units a clear relationship between axonal conduction velocity and unit CT and a less obvious relationship between unit tetanic tension and reciprocal conduction time. Despite these no relationship between unit CT and unit Po could be shown.

In view of the controversial findings for cat hindlimb muscles and the limited data available for mice, including an absence of any reports regarding fast hindlimb muscles of mice, these relationships relating axonal propeties and muscle parameters have been examined in the EDL of both normal and dystrophic animals. In accordance with the proposition that fast conducting axons innervate large tension producing units which in turn have fast contraction times the relationships between axonal conduction velocity and motor unit CT and Po were examined. No

obvious relationships have been consistently seen for either normal or dystrophic units.

Ridge (1967) has shown that after early post-natal development conduction times of peripheral motor nerves remain constant despite increasing length and therefore increasing conduction velocity. The conduction time is therefore independent of age in mature animals and is characteristic of a particular nerve. For this reason the parameters of unit CT and Po were examined against reciprocal conduction time. Here also no linear relationship could be shown.

Various workers have reported opposing results regarding the relationship between unit Po and unit CT (Stephens and Stuart 1975, Bagust, Knott, Lewis, Luck and Westerman 1973). In motor units from both normal and dystrophic animals no relationship could be shown between these parameters.

Bagust and co-workers (1973, 1974) have shown a direct relationship between time to peak twitch tension and the unit twitch/tetanus ratio. They proposed that the active state of a muscle might be determined by the motor innervation. They reasoned that, all things being equal, fibres with a brief active state would have a fast twitch and produce an associated smaller twitch tension compared to their tetanus tension (i.e. with decreasing CT a decreasing Pt/Po would be predicted). In my

experiments using normal EDL units no such relationship could be shown. Similarly no such relationship existed for dystrophic mice.

One of the basic problems encountered in these studies, and a probable explanation for the discrepancies, is that motor units have been treated without regard to their specific histochemical makeup. With the exception of cat soleus, which is a homogeneous muscle (Type I), and small tension producing units in medial gastrocnemius and FDL, no clear relationships devoid of controversy have been shown for other cat hindlimb muscles.

Burke et al (1973, 1974) have shown that the tension produced by a motor unit depends on the histochemical type of its fibres. They noted some gastrocnemius units, histochemically identified as Type S (Type I), had a greater number of innervated fibres but produced less tension than other gastrocnemius units, either Type FF (Type II glycolytic) or Type FR (Type II oxidative), having a lesser degree of muscle fibre innervation. In these unit types it is possible to rank them in order of descending tension output and ascending unit time to peak tension such that: FF > FR > S. Clearly in view of the findings of Burke and co-workers unit data must be considered with regards to their specific fibre types. However, even in light of this, Burke et al (1973, 1974) could only demonstrate a relationship between motor

unit Po and axonal conduction velocity and between motor unit CT and unit Po in the case of Type S units. No obvious relationships were seen for Type FF or FR units.

Proske and Waite (1976) have reconsidered their data in view of this delineation of motor unit types and have shown a linear relationship between unit Po and axon conduction velocity for Type S units. A weak relationship for Type FR units (lesser tension producing units) with no relationship for Type FF units was reported.

From this work it seems probable that relationships exist for Type S units and for units that produce small tensions.

It would appear therefore that earlier work in which the distinction of unit type was not made could only by chance demonstrate relationships of the kind that have been proposed.

This lack of histochemical definition may well explain the lack of correlations observed in this thesis for the EDL motor units of both normal and dystrophic mice.

In normal EDL units the differences between Type II glycolytic and Type II oxidative units would be significant if the tendency seen in cats is applicable to mice. One would therefore expect that a random sample, as used in this thesis, would contain both unit types and therefore the inability to specifically identify the unit type would obscure relationships specific for an

individual unit type.

Similarly in dystrophy, unbiased sampling would yield units of three possible types: glycolytic, intermediate and oxidative. A linear relationship between nerve and motor unit parameters would not necessarily be expected.

This analysis brings forward the next step in this area of study, that of identifying specific motor unit types. The conventional technique of labelling motor unit fibres has already been mentioned, glycogen depletion (Kugelberg 1968). The technique or labelling with 3_H - deoxyglucose (Rapoport et al 1978) has yet to be developed for the mouse. Preliminary glycogen depletion experiments by Parry (personal communication) on soleus motor units have been unsuccessful. If this or a comparable technique can be developed followed by the defining of units physiologically (after Burke et al 1973) the question of fibre type susceptibility in dystrophy can be directly examined along with the aforementioned relationships between axonal conduction velocity and muscle properties.

Burke et al (1973) examined the innervation ratios of cat medial gastrocnemius motor units. It appears that when Type S units from medial gastrocnemius are compared to Type FF and FR units no support was obtained for the premise that large axons with fast conduction velocities innervate large numbers of muscle

fibres and produce large tensions. Type S units had a greater innervation ratio than Type FR units but produced significantly less tension and exhibited significantly slower axonal conduction velocities. It would therefore seem apparent that motor unit tension cannot be accounted for by a simple relationship between axon diameter and unit innervation ratio. To expect simple linear relationships betwen axonal properties and muscle parameters with mixed motor unit data is naïve. Several factors apparently must contribute to the ultimate determination of size of a motor unit; only one of which is the degree of axonal branching and muscle fibre innervation.

It may be that in Type S units however, axonal branching is the predominant factor for unit size determination, since clear relationships between axon properties and various parameters in Type I units have been demonstrated. The vague relationships for Type FR units and the lack of relationships for Type FF units (in terms of conduction velocity versus unit CT or unit Po as well as unit CT versus unit Po) would suggest other factors dominate the innervation of these motor units.

Work by Gordon et al (1976) has shown that slow motorneurones innervate and activate muscle fibres several days earlier than fast motorneurones (in chick embryos). This then leaves the remaining uninnervated muscle fibres to be competed for

by the later arriving axons of motorneurones destined to become either Type FR or FF. In this way Type S motorneurones are able to establish a motor unit size directly related to their axon size. This may enable them to exhibit more distinct relationships between axonal conduction velocity and motor unit time to peak tension and unit tetanic tension.

The later arriving "fast" axons must compete on perhaps a functional demand basis for the remaining muscle fibres. Since these "fast" axons arrive rather simultaneously (Gordon et al 1976) this same competition may exist in a muscle devoid of Type S units, for example the EDL of the mouse. Oxidative units may have an advantage over glycolytic units during developmental innervation (suggested by the linear relationships seen in Type S units and the partial relationships seen in Type FR units). The latter observations regarding Type FR units by Proske and Waite (1976) could be interpreted this way.

This would suggest possible relationships might arise in the dystrophic EDL whose fibre composition, and presumably unit type, is shifting towards a more oxidative state. If motor units could be identified histochemically in the mouse it would be interesting to see if this hypothesis would hold. Still, in view of the myelination abnormalities in dystrophic mice any relationships regarding conduction velocity and conduction time

might be expected to be very complex if they exist at all.

The fact that Lewis et al (1982) did observe a significant relationship betwen axonal conduction velocity and unit CT and between reciprocal conduction time and unit Po with C57 soleus units might be expected. If lesser tension producing oxidative units have an advantage over glycolytic units during innervation, a muscle composed of Type I and Type II oxidative units (soleus) might be expected to yield such relationships as seen for cat soleus (a pure Type I muscle).

In addition, due to the differing functional roles of muscles it does not necessarily follow that Type II oxidative units in soleus are identical with those of a fast muscle ie. medial gastrocnemius or EDL. Burke et al (1973, 1974) have shown that the Type S (Type I) units of soleus are significantly different (greater CT, greater Po, larger estimated specific tension, smaller innervation ratio and a PTD) than Type S units from medial gastrocnemius (lesser CT, Po, specific tension output, greater innervation ratio and unit PTP). Muscle fibre length and orientation and the related mechanical stresses and demands imposed on fibres may play a role in determining the ultimate characteristics of the motor unit types. It is therefore possible that Type II oxidative units in soleus are also significantly different from Type II oxidative units of fast muscle. If soleus

II oxidative units more closely resemble Type I units the relationships Lewis et al (1982) observed might be expected. In EDL however, the diversity between II oxidative and II glycolytic units would preclude any such relationships existing.

It would seem then that the lack of correlation between the parameters examined in normal and dystrophic EDL motor units are due probably to the pooling of unit data within experiments without regard to the specific unit type. If unit types are identified then grouping within experiments and possibly between animals using functionally like muscles may well expose the correlations that have been proposed and examined.

Again, the necessity to be able to identify specific motor unit types and their associated muscle fibres is of paramount importance not only in examining normal units but in determining how motor units in dystrophic muscle are being altered.

SUMMARY

The mechanical properties of motor units of the fast twitch EDL have been described for both normal and dystrophic mice of the C57 strain.

The frequency distribution of unit twitch tensions was compared to theoretical distributions and found to be consistent with a neurogenic etiology. This was further supported by the distribution of motor unit times to peak tension. An estimate of the motor unit number based on the average motor unit size, determined from the unit tetanic tension expressed as a percent of whole EDL tetanic tension, supported a neurogenic etiology by indicating a significant loss of motor units with dystrophy (22 units for normal EDL versus 15 for dystrophic EDL). A subpopulation of units from dystrophic EDL were shown to be identical to units of normal mice, as would be predicted in a neuropathy. It was suggested these units were Type II glycolytic units. Such a subpopulation is what would be predicted if muscular dystrophy were a neuropathy involving a conversion of Type II glycolytic units to Type II oxidative units with their subsequent loss. A hypothesis is advanced which might explain how this situation arises.

An additional factor to be considered with the "pure" effects of dystrophy is that of the spontaneous hindlimb

activity. It has been proposed that this may contribute to be responsible for the conversion of fibre types. This is consistent with the hypothesis advanced to explain the data of this thesis and other works.

Various relationships involving motor axon conduction velocity and conduction time with unit time to peak tension and unit tetanic tension were examined. Interrelationships of motor unit parameters CT, Po and Pt/Po were also studied. No simple relationships were observed. These findings are discussed in comparison with the literature and a possible explanation for a lack of correlation was made.

In conclusion the results described are consistent with a neurogenic etiology in murine muscular dystrophy. The methods utilized here for motor unit isolation are devoid of the criticisms previously cited for other motor unit estimates in both man and mouse and therefore represent an important contribution towards our understanding of murine muscular dystrophy as well as providing significant intrinsic physiological data with regards to the properties of motor units from both normal and dystrophic fast muscle (EDL).

APPENDIX 1

Hindlimb Spontaneous Activity

While conducting preliminary experiments on dystrophic mice I spent some time studying the phenomenon of spontaneous activity seen in the hindlimb. While the results of these experiments do not directly bear upon this thesis work it has been shown that this phenomenon may be important in assessing the effects of dystrophy on muscle fibres. For this reason a brief summary has been included as an appendix.

Dystrophic mice exhibit a continuous spontaneous twitching of their hindlimb musculature. This adds a degree of complexity to studying muscle and motor units in dystrophics not present in normal mice. This phenomenon may also play a part in modifying the properties of muscle in addition to the effects of dystrophy per se.

Because of the amyelinated spinal rootlets (Bradley and Jenkison 1973, Bray, Rasminsky and Aguayo 1974, Stirling 1975a) single stimuli to a muscle nerve will elicit cross-talk and amplification within the spinal roots (Huizar et al 1975, Biscoe et al 1977, Rasminsky 1977, 1978) resulting in multiple twitches and/or a partial tetanic-like discharge of the muscle (personal observation).

Douglas (1975) claimed that both procaine

anesthetisation of sensory roots or dorsal rhizotomy at levels L4, L5 and L6 (which contain the afferent fibres of murine sciatic nerves) abolished this spontaneous activity, suggesting an afferent component was involved. In addition, it was also reported that the dynamic and static responses of muscle spindles were abnormal thereby implicating again afferent input as instrumental in this spastic twitching.

Since forearm muscles of dystrophic mice are clearly dystrophic histochemically (Dr. D.J. Parry personal communication) and it is known that the forearm cervical roots are also amyelinated (Bradley and Jenkison 1975, Okada et al 1976) it seems reasonable to expect spontaneous forearm activity analagous to the activity so obvious in the hindlimb musculature. I have clearly shown (unpublished) no such spontaneous EMG forearm activity (via intramuscular recordings with both mono and bipolar needle electrodes). (However, this is not necessarily conclusive since the amyelinated regions in these roots are extremely small and therefore there exists less chance for ephaptic transmission to occur.) One might suspect the source or trigger for this spontaneous activity to reside not within the amyelinated region of the spinal roots (these acting only as amplifiers) but to be associated with the afferent side of the reflex arc.

An attempt was made to corroberate Douglas' findings.

Animals were set up as described in Chapter VIII. Electromyographic activity was recorded from hindlimb muscles before and after dorsal rhizotomy. If abnormal afferent input were the trigger for this spontaneous activity then dorsal rhizotomy should abolish it. If not then by sequentially transecting the dorsal root as it entered the spinal canal, then as it entered the spinal cord followed by transection of the ventral root leaving the spinal cord then just prior to leaving the spinal canal the ectopic generating sites would be sequentially removed. In this way one could elucidate the trigger or site of generation of action potentials responsible for this spontaneous twitching.

By far the majority of cases showed no alteration in activity patterns after peripheral dorsal root section. In almost all the cases in which the spontaneous firing was lost no successful reflex loop could subsequently be initiated by stimulation of the central end of the dorsal root stumps. It was concluded that Douglas' results might be explained in terms of ventral root injury.

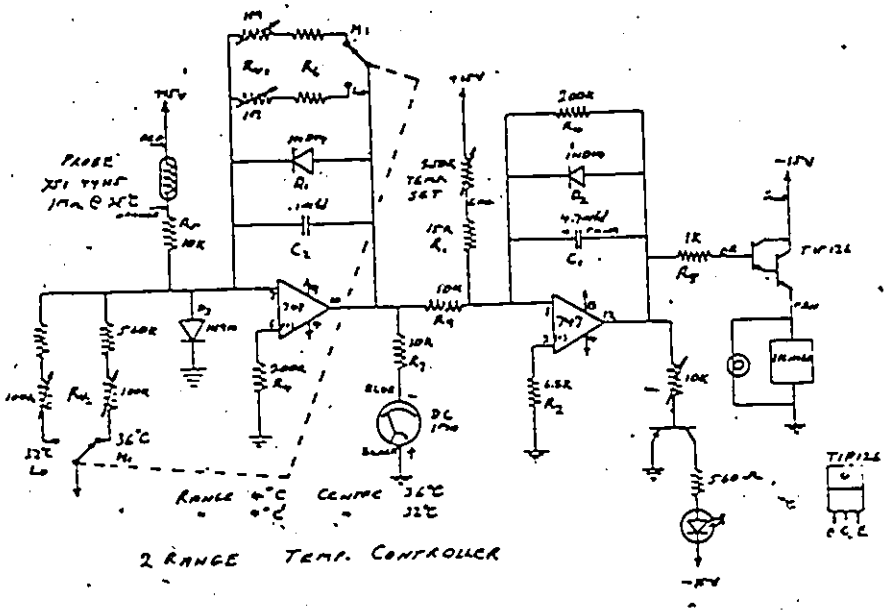
Rasminsky's work (1976, 1977, 1978) has elegantly demonstrated the amyelinated regions to be a source of spontaneous generation of action potentials. The general impression from my work was that though spindle output may be abnormal (and this has

not been confirmed or further examined) this serves only as an additional source of stimulus for spinal root cross-talk and amplification, while the primary site of stimulus generation is ectopic action potentials in the amyelinated region of the spinal roots (as Rasminsky suggests).

APPENDIX 2

Temperature Feedback Circuitry

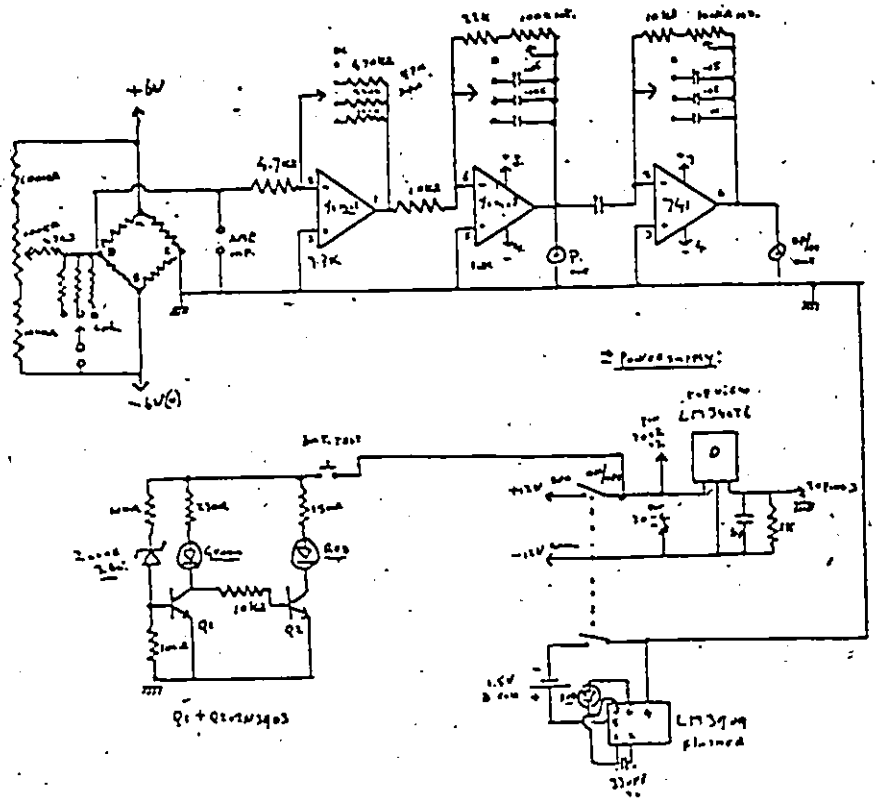
The temperature for both the heating blanket and the leg pool were maintained at 36.5 $\pm$ 0.5° C. Thermistor probes and heating coils were linked through a feedback circuit as shown.



APPENDIX 3

Transducer Amplifier Circuitry

The transducer amplifier circuitry is shown. This unit enabled both transducer function as well as calibration pulse output.



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