

ELECTRICAL RESPONSES OF HUMAN MUSCLES
DURING FATIGUE AND RECOVERY

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ABSTRACT

The studies composing this thesis were designed to address the question of changes in muscle excitability during fatigue and recovery: the indirectly-evoked muscle compound action potential (M-wave) was used as an index of excitability. Earlier studies from this laboratory indicated that the rate of fatigue in human skeletal muscle depends on the frequency of excitation. The present studies have extended these findings by using a wide range of stimulating frequencies (0 - 30 Hz) and by comparing the change in muscle excitability in fast- versus slow-twitch muscles; the effect of ischaemia was also studied as was recovery from fatigue. Ten subjects (out of a total of fifteen) each successfully completed five experiments, spaced at least one week apart, in which intermittent tetanic trains at different frequencies were used to fatigue the ischaemic ankle dorsiflexors. The effects of ischaemia were studied by repeating one experiment under non-ischaemic conditions. Five out of the ten subjects also volunteered for the experiments comparing changes in muscle excitability in soleus, versus those in tibialis anterior, in response to intermittent fatiguing stimulation.

It was found that maintenance of excitability was possible for one minute regardless of stimulus frequency; thereafter stimulation at the highest frequencies induced the greatest change in the amplitude of the (M-wave). The amplitude

decline was also dependent on the position of the M-wave within the train of potentials; thus, at 30 Hz stimulation, the first, fourth and seventh responses within the train decreased by 50%, 80% and 95% respectively ($p < .01$). The decline in M-wave amplitude was always greater than the decline in the area of the compound action potential, indicating an increase in duration due to dispersion of single fibre action potentials. At 30 Hz stimulation the areas of the first, fourth and seventh responses decreased by 33%, 56% and 82% respectively.

On the basis of animal studies, it was hypothesized that muscle excitability would be preferentially retained in the soleus muscle; however, no significant differences emerged in M-wave changes between soleus and tibialis anterior although the onset of the decline was delayed in soleus. It is proposed that this delay was due to the early potentiating mechanisms observed in soleus but not in tibialis anterior. The presence of ischaemia significantly ($p < .01$) accelerated the decline in both amplitude and area of the tibialis anterior M-wave. Recovery of the M-wave was limited when tetanic stimulation ceased but progressed rapidly after the circulation was restored. M-wave failure occurred at firing rates not normally associated with neuromuscular blockade, implying propagation failure along the sarcolemmal membrane.

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List of Abbreviations

ACh	=	acetylcholine
ATP	=	adenosine triphosphate
ADP	=	adenosine diphosphate
Ca ⁺⁺	=	calcium ion
Cl ⁻	=	chloride ion
E _{DB}	=	extensor digitorum brevis
EDL	=	extensor digitorum longus
E _K	=	potassium equilibrium potential
E _m	=	resting membrane potential
F	=	Faraday constant
H ₂ PO ₄ ⁻	=	diprotanated form of inorganic phosphate
Hz	=	hertz (cycles per second)
K ⁺	=	potassium ion
ln	=	natural logarithm
mM	=	millimolar
M-wave	=	muscle compound action potential
min	=	minute
ms	=	millisecond
mV	=	millivolt
Na ⁺	=	sodium ion
NaHCO ₃	=	sodium bicarbonate
Na ⁺ -K ⁺ pump	=	sodium-potassium pump
P _i	=	inorganic phosphate
P-P	=	peak-to-peak amplitude
R	=	universal gas constant
T	=	absolute temperature
μm	=	micron
μs	=	microsecond

INTRODUCTION

1.1 SCOPE OF THE INVESTIGATION

The present work was designed to investigate changes in muscle fibre excitability during the course of fatigue and recovery. In 1954, Merton observed that the ischaemic human adductor pollicis muscle could be fatigued without a reduction in the amplitude of the indirectly evoked muscle compound action potential. This led him to conclude that failure by the muscle to maintain the required force (Edwards, 1981) resulted from an impairment in excitation-contraction coupling or in the contractile process itself rather than from a loss of excitation. Marsden *et al.* (1983) employed indirect muscle stimulation of the adductor pollicis and found that a decline in force output was dependent on the numbers of stimuli delivered, but was independent of the frequency of stimulation. The marked decline of the muscle compound action potential at the higher rates of stimulation (80-200 Hz) led these authors to postulate that there was a failure of the "activation" of the contractile machinery at the lower frequencies (5-40 Hz) where there is a dissociation between the performance of the compound action potential, which does not decline, and force, which does decline; they concluded that fatigue was therefore "electrical" rather than chemical in nature (Merton, 1981).

Garland et al. (1988a), in testing this hypothesis, showed that not only did muscle fatigue occur after fewer stimuli at 15 Hz than at 30 Hz, but this could not be ascribed to failure of muscle fibre excitation. Recently, Binder-MacLeod and Barker (1991) manipulated frequency to test whether a more rapid rate of force development could be produced with a lower rate of fatigue than that experienced by high frequency constant, short duration trains. They also noted that fewer pulses were needed to fatigue the muscle when using lower frequencies than when using higher frequencies.

The body of this thesis is made up of the results of a series of experiments designed to address the question of changes in muscle excitability by determining the relationship between the time-course and extent of failure of the muscle compound action potential and the numbers and delivery frequencies of stimuli. The time-course of recovery of the muscle compound action potential and the effect of ischaemia on the development of excitability have also been assessed. The final series of experiments compared changes in the excitability of fast and slow-twitch muscles; this was achieved by comparing the human tibialis anterior and soleus muscles using physiological stimulation frequencies (Grimby et al., 1981). Although the two muscles show relatively modest differences in fibre type composition (Johnson et al., 1973), they do show major discrepancies in contractile properties (Belanger et al., 1983). The remaining sections of the Introduction include one defining muscle fatigue and a brief historical account of

human muscle fatigue with particular reference to changes in excitability. These are followed by an overview of the muscle compound action potential and its use and applicability to studies of fatigue and recovery; the information on this point is relatively sparse as most fatigue studies tend to be concerned with tension not excitability. Subsequent sections address the effect of frequency of firing on excitability, the physiological implications of ischaemia on the development of changes in excitability, and the changes in excitability of slow-twitch and fast-twitch muscles.

1.2 DEFINITION OF FATIGUE

The word "fatigue" has historically been the victim of an inordinate amount of abuse. So much so that in 1921 Muscio argued that the word fatigue was too general in meaning for scientific use and should be abandoned (Bartley, 1976). However, physiologists continued to use the term "fatigue" but Muscio's admonitions led them to subdivide the concept of it, an approach exemplified by Bills (1943) who suggested subdivision into three categories: a) subjective, b) objective and c) physiological. The first two categories addressed the phenomena observed as declines in psychological factors such as alertness (subjective fatigue) and declines in work output (objective fatigue). The third category, physiological fatigue, was characterized by changes in selected body processes during performance of a task. A subset of physiological fatigue was identified as that

induced by muscle contraction and was associated with an inability to maintain a desired force output.

Muscle fatigue has recently been defined as the "failure to maintain the required or expected force" (Edwards, 1981) or "the required or expected power output" (Edwards, 1983). Bigland-Ritchie *et al.* (1986) have argued that this definition has different implications for maximal versus submaximal effort, and have therefore defined fatigue as any reduction in the maximum force generating capacity.

Even though it is generally agreed that the generation of voluntary muscle activity involves a sequence of events linking the psyche, through the central and peripheral nervous systems, to the contractile elements within the myofilaments, there is still a tendency to target one element in the above chain of command as the cause of fatigue. In fact, any of the links in this chain could conceivably fail through such factors as motivation, the type of muscular activity, muscle fibre composition and the degree of training (Edwards, 1983; Gibson and Edwards, 1985). Central mechanisms of fatigue (those pertaining to brain and spinal cord) are certainly vulnerable to impairment during voluntary muscle activation but are bypassed during electrically stimulated contractions and, as such, are not as relevant to this discussion as peripheral mechanisms of fatigue.

Peripheral mechanisms of fatigue include: a) an inability to develop an action potential at the motor end plate, b) a failure of the sarcolemma to

regenerate an action potential, c) a loss of coupling of the excitation process between t-tubules and sarcoplasmic reticulum, d) a depression in Ca^{++} release from the sarcoplasmic reticulum, e) an impairment in regulatory contractile proteins, and f) a failure in the crossbridge cycle, possibly due to reduction of energy substrates (Green, 1987). Any of these mechanisms could result in a "failure to maintain the required or expected force" (Edwards, 1981). It is this last definition of fatigue that has been chosen for the purposes of this research because force loss is an accessible and testable measure.

1.3 HISTORY OF HUMAN MUSCLE FATIGUE STUDIES

The first to study human muscle fatigue objectively was Angelo Mosso. In his extensive work on fatigue (Mosso, 1915) Mosso introduced his "ergograph", an instrument that he modified from the "myograph" (perfected by Marey in the mid 1800's), with which the force production from repeated voluntary contractions of the flexors of the middle finger was recorded. With his ergograph, Mosso was able to demonstrate that, with the muscles in situ stimulated voluntarily through the intact nervous system, there may be two basic mechanisms of fatigue, central and peripheral. He observed that his colleague, a Professor Aducco, was able to perform considerably more work after his inaugural lecture as professor of physiology than just before the lecture. He ascribed this fact to increased nervous arousal what we would term today as

heightened motivation. Mosso also used indirect electrical stimulation of the finger flexors, after his subjects had fatigued through voluntary contractions, to distinguish between central and peripheral fatigue (Mosso, 1915).

Unfortunately, Mosso made no attempt to record the electrical signal from the muscle, even though he could have used the "Einthoven galvanometer" (a string galvanometer) which had been developed by Willem Einthoven in the late 1800's for investigations of the electrocardiogram. In 1791, Luigi Galvani, by depolarizing the muscles of a pair of frog legs, was able to show that muscle contraction was an electrical event. This observation was to be highly contested by Alessandro Volta which led to a controversy that delayed further research into this field until 1838, when Matteucci showed the first galvanometric evidence that electrical activity actually originated from the muscle (Needham, 1971; Oddson, 1990). In 1849, DuBois-Reymond described a way of detecting electrical muscle activity in humans by detecting a depolarization following tetanic stimulation (Needham, 1971), but it was not until 1907, when Piper (cited in Oddson, 1990) developed the first usable metal surface electrodes and Gasser and Erlanger (1925) exploited the cathode ray tube (the "Braun" tube developed by Ferdinand Braun in 1897) to observe the electrical response of a dog phrenic nerve preparation, that electromyographic recordings from human muscles seemed possible. Two classical publications by Adrian and Bronk (1928, 1929) on the use of indwelling concentric needle electrodes for the recording of single motor

unit action potentials from human muscle and from spinal and decerebrate cat preparations, established the systematic use of electromyography in the study of muscle function (Adrian and Bronk, 1929).

In the pioneering study of Merton (1954) electrical signals from human muscle were used to demonstrate that the site of fatigue was peripheral. Merton observed that there was no addition to the force elicited by a sustained maximal voluntary contraction of the adductor pollicis when single supramaximal shocks were delivered to the ulnar nerve. In addition, at a time when force had declined during the fatigue process, the muscle compound action potential was not decreased in amplitude. On the other hand, Ikai *et al.* (1967) (cited in Asmussen, 1979) found that force did increase with short bursts of tetanic stimulation interspersed between intermittent maximal voluntary contractions of the adductor pollicis, a finding interpreted as evidence for decreased muscle activation by the central nervous system during fatigue. The latter work has been criticized on methodological grounds since it is highly improbable that a full maximal voluntary contraction was generated within one second of effort (Bigland-Ritchie, 1984).

1.3.1 Recent muscle fatigue studies

At present, the general consensus seems to be that during the fatigue associated with voluntary contractions or with stimulation at low frequencies

(<30 Hz), the reduction in force exceeds that in muscle fibre excitability as tested by single evoked muscle compound action potentials (M-waves). Numerous reviews address this observation (e.g. Bigland-Ritchie and Woods, 1984; Edwards, 1983; Bigland-Ritchie, 1981). However, a reduction in muscle fibre excitation is clearly demonstrable when tested by paired stimuli (Stephens and Taylor, 1972) or short trains of stimuli at 20-70 Hz (Bellemare and Garzaniti, 1988). Enoka *et al.* (1988) found a systematic reduction of the M-wave during trains of stimuli to rat hindlimb muscles. They concluded that the response was due to a progressive elimination of single-fibre action potentials within each train.

Cooper *et al.* (1988) investigated the interrelationship between excitation and force generation during fatiguing activities. These studies were conducted on the human adductor pollicis using standard sequences of stimulated contractions at different frequencies. They concluded that, at high frequencies, a safety factor operates to maintain force in the presence of obvious loss of excitation. Low frequency stimulation resulted in marked potentiation of force in spite of unchanged excitation. In addition low frequency stimulation (20 Hz) under ischaemic conditions produced a slight potentiation of the mean M-wave amplitude during fatigue. Peak potentiation of M-wave amplitude occurred after approximately 6 minutes of repetitive application of intermittent trains of programmed stimulation (see their figure 2C). Since the authors measured only M-wave amplitude, it is difficult to determine unequivocally whether this was true

potentiation or hypersynchronization of the muscle fibre action potentials (Duchateau and Hainaut, 1984).

Recently, Enoka and co-workers (1992) studied single motor units from the cat tibialis posterior and showed that the time courses of failure for the motor unit action potential (MUAP) and for force output were dissociated during application of an extended version of a standard motor unit fatigue test (Burke *et al.*, 1973). Although they observed profound changes in MUAP, they found that the change did not occur until a substantial decline in force had occurred.

A major difficulty inherent in the interpretation of these studies is that all the stimulation protocols were different. A consistent testing protocol is needed to assess the degree of fatigue at the various stimulating frequencies, such as that employed by Garland *et al.* (1988a) and in this thesis. For these reasons there is still uncertainty regarding the contribution of changes in muscle fibre excitability toward progressive failure to maintain force output.

1.4 THE DETERMINANTS OF EXCITABILITY IN NORMAL MUSCLE

Excitability is defined as a readiness to respond to a stimulus, whereas excitation is an act of irritation or stimulation (Dorland's Medical Dictionary, 22nd edition, 1977). Muscle excitability is the preferred expression for this thesis and is defined operationally as the size of the muscle compound action potential (M-wave) evoked by a supramaximal stimulus to the motor nerve. There are

three factors contributing to the size of the M-wave: a) the number of nerve fibres excited by the stimulation, b) neuromuscular transmission and the integrity of presynaptic events, and c) muscle fibre excitability. In the case of a), since all the electrical stimuli used in the present experiments were supramaximal, we can assume that all the nerve fibres innervating the muscle were excited.

1.4.1 Neuromuscular transmission

The arrival of a motor nerve impulse at the presynaptic terminal of the neuromuscular junction, causes an influx of Ca^{++} into the axon terminal, thereby evoking the release of acetylcholine (ACh). Postsynaptically, ACh binding to endplate receptors triggers an endplate potential by increasing muscle fibre membrane permeability to sodium and potassium, thereby depolarizing the membrane. Endplate potentials exceeding threshold initiate action potentials which propagate, in both directions, along the muscle fibre. The compound nerve action potential seems to be quite resistant to failure, at least with repetitive stimulation at 30 Hz (Pagala et al., 1991). However, with higher frequencies of stimulation, there may be failure of the nerve action potential at axonal branch points where there is a lowered safety factor for onward propagation and invasion of the terminal region and the ensuing release of acetylcholine (Swadlow et al., 1980).

At the neuromuscular junction Grob et al. (1956) identified four possible

mechanisms which might result in failure of synaptic transmission; these were: 1) deficient release of ACh from the presynaptic terminal; 2) excessive removal of ACh from the synaptic cleft; 3) inhibition of the depolarizing action of ACh on the endplate; 4) prolonged depolarization of the endplate. These mechanisms were investigated in the rat diaphragm and gracilis muscles *in vitro* using microelectrode recordings of intracellular potentials (Krnjevic and Miledi, 1958; Thesleff, 1959). It appeared that depletion of transmitter in the motor nerve terminal was not a significant factor. By recording simultaneously from two muscle fibres belonging to the same motor unit, Krnjevic and Miledi (1958) showed that an end-plate potential was sometimes absent in one of the fibres, an observation which pointed to a failure of impulse propagation in a terminal motor axon. However, Thesleff (1959) also observed declines in endplate potential amplitude at frequencies above 20 Hz, and he postulated that an additional mechanism could be receptor desensitization, since externally applied ACh did not increase the membrane depolarization.

Synaptic failure could also be caused by reduced liberation of ACh from motor nerve terminals. For example, Brooks and Thies (1962) used an *in vitro* preparation of the guinea pig serratus anterior muscle to show that the number of ACh quanta was considerably depressed within seconds of the initiation of stimulation at frequencies as low as 2 Hz. However, the *in vitro* nature of the experiments by Krnjevic and Miledi (1958) and Brooks and Thies (1962) may

have compromised the function of the neuromuscular junction. Thus muscle fibres in vivo may not show significant decrement, even after thousands of stimuli, as in the investigations of fatigue in single motor units (e.g. Burke et al., 1973).

1.4.2 Muscle fibre excitability

The following section reviews the ionic events taking place during the propagation of action potentials along the surface of the muscle fibre and is relevant to the discussion of sarcolemmal inexcitability.

1.4.2.1 Resting membrane potential

In the resting state, most biologic membranes are permeable to Cl^- and K^+ and relatively impermeable to other ions (Hodgkin and Katz, 1949). Muscle fibre E_M (the resting membrane potential) is determined by the intra- and extracellular concentrations of these ions and their permeabilities. In 1949, Hodgkin and Katz modified Goldman's (Goldman, 1943) equation to predict the potential difference across a membrane that had different permeabilities for different ion species. The GHK equation, as it is commonly known, is used to predict the resting membrane potential and has recently been modified (Mullins and Noda, 1963) to include the possible contribution from the electrogenic Na^+/K^+ -ATPase. The equation is as follows:

$$E_m = \frac{RT}{F} \ln \frac{(r[K^+]_o + b[Na^+]_o)}{(r[K^+]_i + b[Na^+]_i)} \quad (1)$$

where r is 1.5 and is the $Na^+ : K^+$ ion exchange ratio for the sodium pump and b is the ratio of the membrane permeabilities for Na^+ and K^+ respectively. In the resting state, the membrane is more permeable to K^+ than to Na^+ , so that E_m approaches the potassium equilibrium potential (E_K) (Hodgkin and Katz, 1949). In mammalian muscle fibres in vivo the resting membrane potential is approximately -80 mV (Segal et al., 1986; Hicks and McComas, 1989). In contrast to nerve cells, mammalian muscle fibres are more permeable to Cl^- than to K^+ and it has been postulated that Cl^- ions play a significant role in the establishment of the membrane potential (Hutter and Noble, 1960).

1.4.2.2 The muscle fibre action potential

The action potential is a self-regenerative electrical event which traverses the sarcolemmal membrane, transiently reversing the sign of the membrane potential from approximately -80 mV to approximately +30 mV (Hodgkin and Huxley, 1952b). Like the resting potential, the action potential has an ionic basis and results from sequential movements of Na^+ (early inward current) and K^+ (late outward current) down their respective electrochemical gradients (Hodgkin and Huxley, 1952b). It has long been known that sodium ions played a very

important role in the generation of the action potential, since bathing nerve and muscle fibres in sodium-free media destroyed their excitability (Overton, 1902, cited in Kepner, 1979). However, it was not until 1952 that the ionic mechanisms underlying the action potential were revealed in a series of four classical publications by Hodgkin and Huxley (1952 a-d). A brief review of Hodgkin and Huxley's work with the squid giant axon is presented in the next section. Reference may be made to Hille (1984) for a more detailed discussion.

Briefly, Hodgkin and Huxley utilized the voltage clamp technique (Cole, 1949) on the squid giant axon to observe that a) an initial large inward current was followed by a fairly constant outward current which occurred when the membrane was depolarized to approximately 0 mV; b) the large inward current was carried by Na^+ ions, while K^+ was identified as the ionic species responsible for the maintained outward current; c) the change in conductance was more rapid for Na^+ than for K^+ ; d) maintained depolarizations inactivated the Na^+ conductance but not the K^+ conductance. Through these observations and by the application of equations describing the changes in the two conductances taking place with changes in time and membrane potential, Hodgkin and Huxley were able to reconstruct the rising and falling phases of the action potential. This model was the first to describe the ionic basis of excitation correctly and was to be the basis of numerous studies on the excitability of various cells, including those of striated muscle (Hille, 1984; Ruff, 1986a,b).

In the normal situation, muscle fibres are capable of conducting hundreds of action potentials even though there are significant changes in the concentrations of Na^+ and K^+ in the intracellular and extracellular spaces. These ionic changes will tend to make the membrane depolarize to the point where it may no longer conduct impulses. This situation is delayed by the Na^+/K^+ -ATPase in the muscle fibre membrane; this enzyme, in hydrolyzing one molecule of ATP, actively extrudes three sodium ions from the fibre in exchange for two potassium ions. Since more positive charges are lost than gained, the interior of the fibre becomes more negative; hence the pump is said to be "electrogenic" (for reviews see, Thomas, 1972; Skou, 1975; Clausen, 1986).

In summary, membrane excitability is determined by the intracellular and extracellular concentrations of Na^+ , K^+ , and Cl^- ions and by the permeability of the membrane to each of these species. Excitability is also dependent on the ability of the sodium pump to re-establish ionic gradients across the membrane and to generate intracellular negativity. In addition, since the action potential is initiated by the flow of Na^+ ions into the cell, anything that inactivates Na^+ channels will affect membrane excitability.

1.4.3 The muscle compound action potential (M-wave)

The muscle compound action potential is the algebraic summation of nearly synchronous single muscle fibre action potentials recorded from part or all

of a muscle. It is produced by stimulation of the nerve supplying the muscle. The M-wave is the compound action potential evoked from a muscle by a single supramaximal electric stimulus to its motor nerve (AAEE Glossary of Terms, 1987). Since all studies in this thesis relied on supramaximal activation, the term "M-wave" is used almost exclusively.

M-wave recordings are widely used in the clinical assessment of various neuropathies and myopathies. The amplitude of the response is proportional to the excitable muscle mass and is therefore diminished in diseases of both nerve and muscle, such as poliomyelitis and Duchenne muscular dystrophy. The M-wave is commonly used to indicate impulse conduction velocity in motor nerve fibres and, following repetitive stimulation, for the detection of neuromuscular transmission disorders (such as myasthenia gravis; Desmedt and Borenstein, 1976; Gilchrist and Sanders, 1987). The parameters of interest in the assessment of M-wave recordings are the latency, negative-peak amplitude, peak-to-peak amplitude, the duration of the waveform and the integral of either the negative peak or of the total waveform.

As noted above, the extracellularly recorded M-waves from whole muscle depend on the number of active muscle fibres and the temporal coincidence of their action potentials, resulting in the algebraic summation of positive and negative phases. In turn, the amplitudes of the individual fibre action potentials depend on the transmembrane potentials, the lengths of the

muscle fibres being depolarized, and the distances between the depolarized membrane and the surface electrodes. Repetitive activation of whole muscle changes the relative importance of these parameters.

In a series of studies on the rat soleus and extensor digitorum longus and on the cat tibialis posterior, it was found that fatiguable muscles demonstrated a decreased EMG amplitude and an increased area with repetitive stimulation (Burke *et al.*, 1973; Enoka *et al.*, 1988, 1989, 1992; Hamm *et al.*, 1989). Enoka *et al.* (1988) suggested that the data could be explained by considering the M-wave as a stochastic process that represents a composite of single-fibre events with variable probabilities of firing (or successful neuromuscular transmission), the range of probabilities being 0 to 1. M-waves were quantified with four measures of amplitude and duration, with one measure of area as the interaction term. The best single measure of whole muscle and motor unit M-wave behaviour was found to be area, and the latter gave the most accurate predictions of peak force in four of the six fatiguability groups into which the soleus and extensor digitorum longus muscles tested were placed (Enoka *et al.*, 1989).

1.4.4 Application of M-wave measurements to the study of fatigue and recovery

A loss of force during muscular contractions could be due to changes in any, or all, of the following: a) neuromuscular transmission, b) sarcolemmal

and t-tubular impulse propagation, c) excitation-contraction coupling; and d) cross-bridge function. Such changes could be due to compromised energy stores or to accumulation of metabolites and ions (Bigland-Ritchie et al., 1986). The analysis of these changes is greatly facilitated by the ability to distinguish between electric and metabolic events (Green, 1987; MacLaren et al., 1989; Jones and Round, 1989). The non-invasive nature of M-wave recordings has allowed this potential to be used extensively in studies of human muscle fatigue (cf. reviews by Edwards, 1983; Bigland-Ritchie and Woods, 1984; Jones and Round, 1989; Green, 1987; MacLaren et al., 1989).

Any changes in the evoked M-wave and the component motor unit action potentials (MUAP) during fatigue can be the outcome of a number of factors. Thus, the amplitude of the M-wave depends on the number and sizes of the individual muscle fibre action potentials. Various investigators have argued that a decline in M-wave amplitude is a sufficient index of transmission failure (Marsden et al., 1983; Miller et al., 1987; Bellemare and Garzaniti, 1988; Cooper et al., 1988; Gibson et al., 1988). However, measurements of amplitude alone do not distinguish between presynaptic failure of neuromuscular transmission and loss of muscle fibre excitability.

Various authors have measured both the amplitude and the duration of the M-wave (Naess and Storm-Matheson, 1955; Hultman and Sjöholm, 1983; Milner-Brown and Miller, 1986). The duration of individual muscle fibre action

potentials will depend on the rate of activation of outward potassium current channels and the rate of inactivation of the inward sodium current channels. The duration of the M-wave is also affected by the degree of synchronization of the muscle fibre action potentials; as the M-wave becomes dispersed the duration increases, and the amplitude of the M-wave declines. A corollary to the above consideration is that the length of depolarized muscle fibre membrane at any instant will depend on the duration of the action potential at any point on the membrane and the conduction velocity. Thus, if the duration were to increase and the velocity to remain the same, the length of depolarized fibre would increase; on the other hand, a reduction in velocity and an increase in duration would tend to counteract each other.

M-wave area (voltage-time integral) is proportional to amplitude only when duration remains constant (Milner-Brown and Miller, 1986). Some authors have suggested that area is the most appropriate index of transmission failure (Bigland-Ritchie et al., 1979, 1982; Stephens and Taylor, 1972; Pagala et al., 1984; Enoka et al., 1989). In point of fact, during the course of a fatiguing contraction, amplitude, duration and area of the evoked M-wave all change with time. Recently, Enoka et al., (1992) found that the measurement of waveform area provided the same information as the combined changes in MUAP amplitude and duration. They based this conclusion on the fact that the M-wave shape (calculated as a coefficient of proportionality between MUAP area and the

product of peak-to-peak amplitude and duration) did not change during this fatigue protocol. The lack of changes also suggested that MUAP shape depended primarily on the orientation of the electrode to the motor unit.

1.5 EFFECT OF FIRING FREQUENCY ON EXCITABILITY

The use of tetanic electrical stimulation of human muscle via the motor nerve trunk (Gibson et al., 1988; Cooper et al., 1988; Hicks et al., 1989) or the intramuscular nerve twigs (Edwards et al., 1977; Marsh et al., 1981; Sale et al., 1982) allows measurement of the frequency-force relationship as well as the size of the evoked action potential. Alterations in the excitability of muscle as a response to electrical stimulation during fatigue have been divided into two types, those due to repetitive stimulation at frequencies greater than 30 Hz and those due to stimulation at lower frequencies (MacLaren et al., 1989; Edwards, 1983; Gibson and Edwards, 1985).

1.5.1 Effects on excitability of stimulation at higher frequencies

When excitation frequencies employed in stimulated contractions exceed the range of firing rates observed during prolonged maximal voluntary contractions there are noticeable changes in the evoked M-waves. For example, severe declines in amplitude (to 10% of the control value) have been reported as a result of continuous stimulation at 80 Hz (Sandercock et al., 1985).

Concomitant with the amplitude decline is an increase in M-wave duration (Sandercock et al., 1985; Kugelberg and Lindegren, 1979; Metzger and Fitts, 1986; Naess and Storm-Mathesson, 1955; Stokes et al., 1989; Gibson et al., 1988; Cooper et al., 1988; Moritani et al., 1985). M-wave area also declines with high frequency stimulation (to 70% and 20% of control values with 50 and 80 Hz stimulation respectively, over a period of 60 s (Bigland-Ritchie et al., 1979, 1981; Jones, 1981). Excitability failures of this nature may be due to presynaptic or postsynaptic events (Grob et al., 1956; Swadlow et al., 1980; Krnjevic and Miledi, 1958; Thesleff, 1959) at the neuromuscular junction, but it is unlikely that neuromuscular block ever occurs in normal muscle contraction. A final potential site of failure of excitability could be the sarcolemma. High frequency stimulation raises the excitation threshold of the sarcolemma (Jones, 1979) to a degree sufficient to exceed the safety margin for neuromuscular transmission (Krnjevic and Miledi, 1958; Jones, 1979).

It has been observed that the decline in force production at a high frequency of stimulation is reversed if the frequency is reduced, for example from 80 to 20 Hz (Bigland-Ritchie et al., 1979; Jones et al., 1979). This observation suggests that failure of the contractile elements was not the limiting factor; fatigue could have resulted from failure of action potential propagation or from excitation-contraction uncoupling. At the onset of high frequency stimulation, the action potential increases in duration with little or no loss of amplitude, thereby

resulting in a substantial increase in area and conduction time (Naess and Storm-Mathessen, 1955 at 50 Hz; Bigland-Ritchie et al., 1979 at 50 and 80 Hz). Jones et al. (1979) suggested that slowing of fibre conduction velocity, possibly due to reduced $[Na^+]$ and elevated $[K^+]$ in the extracellular space would account for these changes in duration and area (see also, Jones, 1981). Shifts in the ionic equilibrium of the muscle fibre membranes are normally corrected through the action of the Na^+/K^+ -pump; however, during high-frequency stimulation, there may not be time between action potentials for the pump to restore the normal ionic gradients.

The changes occurring in the negative after-potential, reflect function in the T-tubular system (Adrian and Peachey, 1973). Although no direct information regarding T-tubular function is possible through simply observing the evoked M-waves from human muscle (MacLaren et al., 1989), cautious extrapolation to the human situation may be made from the animal literature on the subject. Studies using frog muscle indicate that the greatest accumulation of $[K^+]_i$ is in the T-tubules, where diffusion is restricted because of the high surface-to-volume ratio (Bezanilla et al., 1972; Adrian and Peachey, 1973).

In a series of studies using single fibres from lumbrical muscles of the African clawed frog, Lannergren and Westerblad (1986, 1987; Westerblad and Lannergren, 1986) showed considerable action potential fatigue (reduced amplitude and increased duration) during continuous or intermittent stimulation

at 70 Hz. The rate of decline was dependent on whether the fatiguing stimulation used was continuous or intermittent and on the fibre type. In the third study (Lannergren and Westerblad, 1987), the authors concluded that action potential failure was caused by failure of regenerative activity in the T-tubules. They based this conclusion on the observation that normal, rested fibres and fibres in which post-contractile depression was present (i.e. normal excitability with depressed force production) exhibited considerable action potential decline (more than a 50% decrease in amplitude and a four-fold increase in duration) during stimulation at 70 Hz while de-tubulated fibres exhibited considerable fatigue resistance (less than a 25% decrease in amplitude and only a two-fold increase in duration). The first two groups of fibres exhibited normal negative after-potentials, indicating regenerative activity in the T-tubules, while de-tubulated fibres exhibited no after-potentials. This is an extremely promising finding as there is close electrical coupling between the surface membrane and the T-tubules (Adrian *et al.*, 1969) allowing for the possibility of reflection of impaired T-tubular function in the shape of conventionally recorded action potentials.

An explanation of action potential decline based on the accumulation of $[K^+]_e$ has some shortcomings, however. Metzger and Fitts (1986) reported identical changes in the action potential during 5 and 75 Hz stimulation (for a duration of 1.5 and 1 min respectively) of a rat phrenic-nerve diaphragm preparation. From this they were obliged to conclude that events distal to the

sarcolemma were responsible for the higher force decline observed during 75 Hz stimulation. Firing frequencies of 5 Hz would provide ample time for the Na⁺/K⁺-pump to restore ionic gradients (Clausen, 1986). In addition, Pagala *et al.* (1984), using stimulation frequencies of 30 Hz, found that the action potential decline was slower than the fall in tension. Furthermore, they calculated an increase in [Na⁺]_i of only 10 mM, concentrations insufficient for sarcolemmal inexcitability. It would have been more instructive if the external [Na⁺] was calculated as activation of the contractile machinery is the result of a Na⁺-dependent action potential. The influx of Na⁺ ions is compromised at low extracellular Na⁺ concentrations (Venosa, 1979).

While the above studies have provided much data of physiological interest, their relevance to normal daily activities is probably limited. Thus, high rates of motor unit discharge (eg 150 Hz) have only been observed during the initial stages of maximal voluntary contractions (Marsden *et al.*, 1983) and during ballistic contractions (Desmedt and Godaux, 1977). These rates are only observed for very brief periods and normally slow down to < 30 Hz for the remainder of the contraction (Bigland-Ritchie *et al.*, 1986b).

1.5.2. Effects of low stimulus frequencies on excitability

The normal frequency of motor unit discharge during sustained effort is in the range of 10-30 Hz (Grimby *et al.*, 1981; Bellemare *et al.*, 1983). Both

human and animal studies have been used in this section to address the changes observed in muscle excitability as a result of low frequency stimulation.

Generally, muscle excitability is preferentially maintained as force declines during fatiguing stimulation at low frequencies. There is, however, a reduction in the M-wave during tetanic stimulation that is frequency dependent, such that stimulation at 1 Hz results in a very small or no change in excitability, whereas stimulation at 20-30 Hz results in considerable decrement. This was true for studies using the human adductor pollicis muscle (Gibson *et al.*, 1988; Wiles *et al.*, 1981; Bigland-Ritchie *et al.*, 1979; Stokes *et al.*, 1989), the human tibialis anterior (Garland *et al.*, 1988a), and the cat medial gastrocnemius and soleus (Sandercock *et al.*, 1985). Metzger and Fitts (1986), using a rat phrenic-nerve diaphragm preparation, disagreed with this view. They reported equal decrements in fibre action potentials following continuous 5 Hz and 75 Hz stimulation. The values reported by these authors are those after 10 s of recovery from stimulation; unfortunately, the values during or immediately following fatigue were not reported.

The complexity of action potential change is also dependent on whether stimulation is sustained or intermittent. Duchateau and Hainaut (1985) found that an identical decrease in tetanic force was associated with different electrical changes during 60 s sustained contractions at 30 Hz and 60 one-second isometric contractions (at 30 Hz) separated by either 0.5, 1.0, or 2.0 s intervals. Their

results suggested that mechanical failure must therefore be associated with processes beyond the sarcolemma. However, it is of interest to examine the time course of changes in muscle excitability during intermittent stimulation at low frequencies. Intermittent low frequency stimulation tends to be more physiologically representative of the natural state than sustained contraction. However, if the circulation is not returned completely for an appropriate period of time during the rest periods between intermittent contractions then the time course of force loss is similar to that observed in the presence of applied ischemia (Duchateau and Hainaut, 1985).

1.6 EARLY FATIGUE: POTENTIATION OF THE M-WAVE

During the early part of intermittent stimulation at low frequencies, the M-wave amplitude typically exhibits a slow potentiation (human tibialis anterior - Garland et al., 1988a,b; human adductor pollicis - Gibson et al., 1988; Cooper et al., 1988; Stokes et al., 1989; Duchateau and Hainaut, 1985; human triceps surae - Moritani et al., 1985; human quadriceps - Hultman and Sjöholm, 1983). M-wave area and duration were measured in only one of the above studies (Duchateau and Hainaut, 1985) and both showed progressive increases during early tetanic stimulation.

Animal studies also show slight increases in amplitude and larger increases in area and duration during tetanization (Pagala et al., 1984; Uramoto

et al., 1983). Enoka et al. (1988, 1989) demonstrated that the potentiation in M-wave area was more marked in the rat extensor digitorum longus than in the soleus. In a series of studies using the cat tibialis posterior muscle, Hamm et al. (1989) and Enoka et al. (1992) identified four different types of motor units in terms of their fatiguability. In these studies considerable potentiation of M-wave area was observed in fatiguable (FF) and intermediately fatiguable (Fint) motor units during the early stages of a fatigue test using intermittent 40 Hz tetani. By contrast to other reports (see above), the fatigue resistant (FR) and slow (S) motor units showed very slight changes during early fatigue; in fact, very little deviation from control values was observed for the duration of the fatigue test. Recently, Larsson (1992) demonstrated a small but significant variability in enzyme activities of rat tibialis anterior motor unit fibres. Clearly other factors than the motoneurone allow further differentiation of motor unit fibre properties, and throw further doubt on the assumption of fibre homogeneity of motor units.

Although these studies have reported M-wave potentiation, very little attempt has been made to explain it, and this is true for both the human and animal work. Duchateau and Hainaut (1985) explained the potentiation in amplitude and area as being due to slowing of conduction velocity along the muscle fibre membrane while Enoka et al. (1992) did not comment on it. Hicks et al. (1989) found significant enlargement of the M-wave during early fatigue during intermittent voluntary contractions of the human thenar muscle. Since the

circulation to the hand was intact, this potentiation could not have been due to ischaemia and since fatigue was by means of voluntary contraction, the potentiation could clearly occur under normal physiological circumstances. Using an *in vitro* rat soleus preparation, these authors subsequently went on to show that potentiation of the M-wave was due to increased activity of the electrogenic Na⁺/K⁺-pump (Hicks and McComas, 1989).

Despite many differences in experimental technique there seem to be characteristic changes during early fatigue which typically last 30-60 s (depending on the frequency). M-waves (or MUAP's) exhibit either no change or an increase in amplitude, area and duration. These changes are quite similar during both sustained and intermittent tetanic stimulation, but tend to be more prominent during sustained contractions (for human studies-Duchateau and Hainaut, 1985; Bigland-Ritchie *et al.*, 1979; Fitch and McComas, 1985; for animal studies - Sandercock *et al.*, 1985; Hanson and Persson, 1971). The real separation between the effects of intermittent and sustained contractile activity appears during late fatigue.

1.7 M-WAVE CHANGES DURING LATE FATIGUE

M-wave changes during late fatigue are dependent on the duration of fatiguing stimulation, on whether stimulation is intermittent or sustained, on frequency of tetanic stimulation, on whether the circulation is intact or occluded

and on muscle speed (i.e. fast-twitch vs. slow-twitch). The two latter parameters will be discussed in subsequent sections of the Introduction. Effects of intermittent versus sustained contractions will be discussed jointly with effects of stimulation frequency in two separate sections detailing observations in human studies and animal studies respectively.

1.7.1 Human studies

In a comparison between intermittent and sustained contractions for a period of one minute, at 30 Hz, Duchateau and Hainaut (1985) found that, during sustained contractions, M-wave area and amplitude both decreased in the adductor pollicis while duration continued to increase. These changes were significantly different from those during intermittent contraction in which M-wave amplitude and area both increased to a plateau after approximately 30 s of repetitive stimulation. In contrast, M-wave duration continued to increase during both sustained and intermittent contractions; however, the final value (140% of control) was much higher after sustained stimulation. In another study, in which intermittent tetani at various frequencies were applied to the adductor pollicis in the presence of an intact circulation, Cooper *et al.* (1988) found that during stimulation for ten minutes at both 10 and 20 Hz, M-wave amplitude increased to a plateau for the duration of fatiguing contraction. This was not so for higher frequencies (50 and 100 Hz), which caused the M-wave amplitude to decline

considerably over the same period of time. Similarly, Moritani et al. (1985), using sustained 20 Hz stimulation on the triceps surae, found that M-wave amplitude increased by 33% over 60 s and declined at higher frequencies. These authors were able to record M-waves selectively from gasirocnemius (lateral head) and soleus and observed a slight reduction in the former muscle but not in the latter. These results differed markedly from those of Duchateau and Hainaut (1985) although the only differences in experimental protocol for sustained contraction were the use of 30 Hz for stimulation and the adductor pollicis as the experimental muscle. Bigland-Ritchie et al. (1979), also using the adductor pollicis and 20 Hz stimulation, found that M-wave area continued to increase for the duration of 60 s of sustained contraction.

1.7.2 Animal studies

In the studies employing human muscle, the changes observed in late fatigue during sustained contractions with an intact circulation are similar to those reported for animal studies employing slow twitch muscles and frequencies of stimulation below 30 Hz (sustained - Hanson, 1974; Sandercock et al., 1985; Uramoto et al., 1983; Grabowski et al., 1972). This concordance was perhaps not surprising as a large proportion of the human studies have used the adductor pollicis which, like the soleus, has a large proportion of type I fibres (Johnson et al., 1973).

Most of the animal studies reporting changes in muscle excitability have used high frequencies of stimulation and both fast- and slow-twitch muscles or motor units (e.g. Kugelberg and Lindgren, 1979; Clamann and Robinson, 1985).

Few studies have used an intermittent stimulation protocol. Of those that did, Jami et al. (40 Hz - 1982, 1983) were the only ones reporting no changes by the end of fatigue in MUAP's of both fast and slow motor units of the cat peroneus tertius. Pagala et al. (1984, 1991) employed a 30 Hz intermittent protocol lasting five minutes. In in vitro preparations of the rat EDL, soleus and diaphragm, they observed declines in M-wave amplitude and increases in both area and duration for all muscles. Soleus exhibited the least amount of change and EDL the most. Intermittent stimulation of the rat EDL and soleus produced the same time course of fatigue as previously mentioned, however, the changes were of greater magnitude (Rankin et al., 1988; Enoka et al., 1988). Measurements of action potential amplitude (Hamm et al., 1989) and area (Hamm et al., 1989; Enoka et al., 1992) of cat tibialis posterior motor units, revealed that the fast-fatiguable and fast-intermediate-fatiguable units had declined in both amplitude and area by the end of fatigue. On the other hand, slow- and fast-fatigue resistant units showed only slight declines in amplitude, but increases in area.

1.7.3 Summary

In summary, two phases of M-wave behaviour can be delineated during fatiguing contractions. There is first a phase lasting between 30-60 s in which the response exhibits either no change or increases in amplitude, area and duration; these changes appear to be most marked in Type I fibres although, as previously discussed, Enoka and co-workers (1992) demonstrated greater potentiation in fatiguable and intermediately fatiguable motor units which, assuming motor unit fibre homogeneity (Larsson, 1992), would be composed primarily of Type II fibres. The late phase is characterized by reductions in amplitude and area that are frequency dependent and are present during both sustained and intermittent activity. These changes are exacerbated with ischaemia (see below).

1.8 EFFECTS OF ISCHAEMIA ON MUSCLE EXCITABILITY

The changes observed during intermittent fatiguing contractions with an intact circulation are accelerated and intensified during ischaemia (Cooper *et al.*, 1988). The situation then becomes similar to that during voluntary isometric contractions of moderate, or greater, intensity. The contraction force at which muscle blood flow is occluded varies from 20% of maximum (Barcroft and Millen, 1939); to 70% (Humphreys and Lind, 1963; see also Barnes, 1980). The use of arterial occlusion during repetitive stimulation ensures that the reduction of circulation is equal throughout the muscle, thereby circumventing the problem

of regional variation in intramuscular blood flow. Maintaining ischaemia throughout the period of intermittent stimulation renders the muscle a closed system, allowing diffusion of ions and metabolites between the interstitial spaces and the intramuscular capillaries. A further advantage of employing ischaemia is that fatigue can be maintained as long as the arterial cuff remains inflated; release of the cuff allows a well-defined starting point for studying the time-course of recovery.

An important issue is the extent to which ischaemia by itself can induce changes in muscle excitability. Jennische *et al.* (1982) reported that ischaemia alone caused a 30 mV drop in membrane potential with a 12.5 mmol/l rise in $[K^+]_e$ in the rabbit gastrocnemius after 3 hours. These changes were associated with a fourfold increase in intramuscular lactate concentration. In an accompanying study (Jennische, 1982), a 4 h ischaemic period caused membrane depolarizations of similar magnitudes in the cat soleus and gastrocnemius muscles, with high levels of intramuscular lactate. Both studies showed very small changes in intracellular ATP content in the gastrocnemius but a 40% decrease in the soleus. Hagberg (1985) also used the rabbit gastrocnemius and found that a 4 h period of ischaemia resulted in a six-fold increase in intracellular lactate and membrane depolarizations of 30 mV. Interestingly, intracellular pH altered very little but extracellular pH decreased from 7.30 to 6.36. The authors commented that the less negative intracellular milieu would favour H^+ diffusion

to the extracellular space.

It should be noted that even small decrements in whole cell ATP contents may reflect large changes in the supply available for maintenance of membrane potential. Thus, in avian skeletal muscle as much as 70% of ATP utilization is tied to cation transport through the Na^+/K^+ -ATPase (Fambrough *et al.*, 1987); although similar calculations are not available for mammalian skeletal muscle, it is likely that long periods of ischaemia may deplete sarcolemmal ATP supply, thus causing depolarization. Such an interpretation would be consistent with the observation that myophosphorylase-deficient patients exhibit unusually rapid declines in M-wave amplitude with repetitive stimulation, in the presence of ischaemia (Edwards and Wiles, 1981; Edwards *et al.*, 1982). This considerable depolarization of the sarcolemma occurred without high lactate levels and was presumably due to the complete block of anaerobic glycogenolysis.

In normal muscles, Cooper *et al.* (1988) showed that changes in M-wave amplitude during activity with ischaemia were in the same direction as those with the circulation intact but occurred three times as quickly. The studies cited above suggest that one of the main effects of ischaemia on muscle excitability is impairment of Na^+/K^+ -ATPase and that this is accelerated during fatiguing stimulation. Although the resulting failure in excitation has been viewed as a protective mechanism, protecting the muscle from driving itself into rigor (Edwards, 1983), the M-wave usually persists longer than the force (Cooper *et*

al., 1988; Gibson et al., 1988). This last observation indicates that ischaemia may promote uncoupling of excitation and contraction, or may accelerate contractile failure.

1.9 COMPARISON OF EXCITABILITY IN FAST-AND SLOW-TWITCH MUSCLES DURING FATIGUE

Evidence from animal experiments shows that the rate of failure of the electrical response of a motor unit corresponds closely to the force fatigability of that unit (Clamann and Robinson, 1985). Numerous studies report that slow-twitch muscle fibres (Hanson, 1974; Hanson and Persson, 1971), motor units (Sandercock et al., 1985; Hamm et al., 1989; Enoka et al., 1992) and whole muscles (Pagala et al., 1984; Uramoto et al., 1983; Rankin et al., 1988; Enoka et al., 1988; Enoka et al., 1989) maintain their action potentials longer than those with fast-twitches. The only human study in which an attempt was made to compare the electrical responses to repetitive stimulation of slow- and fast-twitch muscles was that of Moritani et al. (1985); these authors observed that the intramuscular M-wave of soleus was better maintained than that of gastrocnemius (lateral head) during continuous stimulation at 20 Hz. However, no attempt was made to control for crosstalk between these muscles even though indwelling recording electrodes were used; the close proximity of the recording sites throws some doubt on the validity of the results.

1.9.1 Contractile properties in fast-and slow-twitch muscles

To address the obvious need for human studies comparing slow and fast-twitch muscles, the tibialis anterior and soleus muscle M-waves were compared during fatigue with repetitive stimulation at low frequencies. There are considerable discrepancies in the contractile properties of the two muscles. For example, mean contraction times in the human tibialis anterior were reported as 81 ms in males and 82 ms in females, while the corresponding half-relaxation times were 94 ms and 98 ms (Belanger et al., 1983). The contractile properties of the plantarflexor muscles are dominated by those of soleus (Vandervoort and McComas, 1983) with mean contraction times of 122 ms in males and 136 ms in females (Belanger et al., 1983). Another striking difference between the tibialis anterior and the plantarflexor muscles, is the much greater post-activation potentiation of the twitch in the former (Vandervoort et al., 1983). In addition the human tibialis anterior exhibits more fatigue during voluntary contraction (Belanger et al., 1983). Despite these pronounced differences in contractile properties, the fibre type composition of the human tibialis anterior and soleus muscles are not dissimilar, both muscles having high proportions of Type I muscle fibres. For example, Johnson et al. (1973) found that the mean percentages of Type I fibres in the soleus and tibialis anterior muscles were 89 and 76% respectively (see also Moulds et al., 1977; Jakobsen et al., 1988). A more recent study (Henriksson-Larsen et al., 1985) revealed a rather lower

proportion of Type I fibres (34%) in tibialis anterior muscles from human cadavers. Not only did the proportion of Type I to Type II fibres vary depending on the depth of the sampling sites (see also Johnson *et al.*, 1973), but the cross-sectional areas of both types of fibres were found to be larger in the deeper part of the muscle than in the central and superficial regions (Henriksson-Larsen *et al.*, 1985). The results from the latter study would certainly substantiate a discrepancy in contractile properties of the human tibialis anterior and soleus muscles. There is however considerable disagreement between the results of the study by Henriksson-Larsen *et al.* (1985) and those studies finding considerably higher proportions of Type I fibres in tibialis anterior (see above). In this context the fibre type compositions of the two muscles are sufficiently different to explain the discrepancies in contractile properties.

The discrepancy in contractile properties is surprising because in non-human muscles studied under isometric conditions there is a strong correlation between twitch duration and the relative proportions of Type I and Type II fibres (for example, Clamann and Robinson, 1985). However, unlike the situation for other mammals, human studies must necessarily gain information from muscles which are a heterogeneous mixture of fast and slow fibre types with varying combinations of fatigable and fatigue-resistant motor units. To further complicate the issue, twitch duration depends primarily on the Ca^{++} -pumping capacity of the fibre sarcoplasmic reticulum (SR), and is related to the volume

density of the terminal cisternae (Schmalbruch, 1979; Kugelberg and Thornell, 1983). Fast-twitch fibres exhibit higher SR volumes (Briggs *et al.*, 1977; Schmalbruch, 1979) and Ca^{++} uptake capabilities (Briggs *et al.*, 1977), reflecting shorter active state durations than those of slow-twitch fibres. Kugelberg and Thornell (1983) found that the volume density of terminal cisternae was the same for some anterior tibial and soleus motor units in spite of the difference in histochemical types of myosin; this observation, coupled with an inverse relationship between the wide range of contraction times and the terminal cisternae volumes, led them to conclude that the SR properties of the muscle fibre confer a finely graded control of contraction times to match the frequency characteristics of the motoneuron. The authors were doubtful whether the different myosin structures permitted such an exquisite variation.

The fatiguability of muscle fibres and hence, of the motor unit, also depends on the metabolic properties of the fibres. It is beyond the scope of this investigation to discuss this aspect of fatigue resistance exhaustively, but examples of metabolic differentiation include the higher mitochondrial volumes (Schmalbruch, 1979) and mitochondrial enzyme activities of slow-twitch versus fast-twitch fibres (e.g. Kugelberg and Lindgren, 1979). Both of these properties contribute to the greater fatigue resistance of the slow-twitch fibres, at least during aerobic activity. It is interesting to note the continuous distribution of fatigue-resistance within the motor unit population of the rat tibialis anterior as

observed by Kugelberg and Lindgren (1979).

1.9.2 Neuromuscular excitability in fast- and slow-twitch muscle

In light of the poor correlation between histochemical fibre type and twitch duration in human muscle (Moulds *et al.*, 1977), it is appropriate to ask whether there is any correlation between the contractile properties and the capacity for neuromuscular transmission during sustained activity. In non-human mammals the neuromuscular junctions of fast- and slow-twitch motor units are tailored to match the functional demands of the motor unit. In support of this observation, there is evidence that facilitation and potentiation of end-plate potentials act to increase transmitter release to meet the required need for the frequency matching necessary for reliable transmission across the neuromuscular junction (Lev-Tov, 1987). Potentiation of end-plate potential amplitude during sustained stimulation was greater in the rat EDL, a fast-twitch muscle, than in soleus. Furthermore, the potentiation in EDL increased over the entire range of stimulation frequencies (5-100 Hz) while potentiation in soleus increased most steeply between 10 and 40 Hz and changed very little thereafter. Such a difference would be in keeping with the contrasting firing frequencies of fast- and slow-twitch muscles during natural activities. Potentiation has also been tested using paired stimulation and again was generally higher in EDL than in soleus (Lev-Tov, 1987).

Sterz et al. (1983) voltage-clamped motor endplates in rat EDL and soleus fibres and determined the equilibrium interactions between ACh and the ACh receptor from dose-response curves obtained by iontophoresis of ACh. These authors found that the maximum ACh-induced conductance per unit of endplate surface is significantly smaller at the soleus endplate than at the EDL endplate. In addition, the mean number of functional ACh receptors was 60% lower at the soleus endplate than at the EDL endplate (Sterz et al., 1983). In contrast to the above studies, Albuquerque et al. (1974) found no difference in ACh sensitivity of the junctional membrane between fast and slow muscle fibres. Nevertheless, the majority of investigations, including that by Sterz et al. (1983), indicate that slow twitch muscle has a lower safety margin for neuromuscular transmission than fast-twitch muscle. This conclusion is consistent with the higher susceptibility of slow-twitch muscle to neuromuscular block during high frequency stimulation (Clamann and Robinson, 1985).

The morphology of the neuromuscular junction also differs between fast- and slow-twitch muscle fibres. Ellisman et al. (1976) used freeze-fracture sectioning, histochemical staining and high voltage electron microscopy to compare rat soleus and EDL fibres. In the soleus the nerve terminal resembled a vesicle-rich flattened plaque, while the endplate itself was ovoid in appearance, some 30-50 μm long and 20-30 μm wide. The much larger EDL endplate (75-100 μm in length) was also ovoid and acquired its size from the many

anastomosing and bulblike terminal expansions of the overlying nerve terminal. In addition, junctional folds in soleus fibres were broader and more irregular than those in EDL fibres. When the two types of fibre were compared, the sizes of the respective neuromuscular junctions were seen to be proportional to the diameters of the respective fibres, as well as to the frequency of their endplate potentials. Microscopic structural differences were not apparent at the membrane molecular level. The putative transmitter release sites were identical in size in both fibre types. These authors emphasized the findings (discussed above) by Albuquerque *et al.* (1974), namely, that no differences existed in the ACh sensitivity of the respective junctional membranes (Ellisman *et al.*, 1976). In their remarkably all-encompassing study, Ellisman and co-workers (1976) went on to make observations of the nonjunctional sarcolemmal membrane. They found that, in contrast to the junctional membrane, microscopic morphological differences did not exist between fibres, but did exist at the molecular level. The major difference was the frequent presence of particle aggregates, termed "square arrays", in EDL but not in soleus fibres. Although the presence of the "square arrays" was highly correlated with the fibre contraction time, such that they could always be found in fast-twitch fibres, the authors could not attribute an active contractile role to these structures because they have also been found in inexcitable cells (for example, brain astrocytes; Landis *et al.*, 1974).

Studies of the electrical characteristics of the fibre membrane indicate

that fast-twitch fibres exhibit higher impulse conduction velocities and larger action potential amplitudes than do slow-twitch fibres (for example, cat flexor hallucis longus and soleus; Buller *et al.*, 1965). In addition, resting membrane potentials are as much as 10-15 mV greater in fast-twitch fibres (Albuquerque and Thesleff, 1966; Locke and Solomon, 1967; Yonemura, 1967; Leader *et al.*, 1984). Concomitant to those observations are slower rise times (266 versus 216 μsec) in the slow-fibre action potentials of the cat (Buller *et al.*, 1965). In the slow human soleus the extracellularly-recorded motor unit potentials are also slower in their rise-times than those in the faster triceps brachii muscle (lateral head) (190 versus 107 μsec ; Buchtal *et al.*, 1973). The lower conduction velocities of the soleus muscle fibres could not be entirely explained by their smaller diameters, as determined from needle biopsy, leading the authors to conclude that the membrane properties must be different from those of fast fibres. A further difference in the electrical properties of fast- and slow-twitch fibres is in the sizes of their respective negative after-potentials. These potentials, which are thought to reflect excitation in the transverse tubular system (Hanson and Persson, 1971), are significantly larger in fast-twitch fibres (Luff and Atwood, 1971).

The density of Na^+ , K^+ -pumps is for the most part higher in avian fast muscle fibres than in slow fibres (Fambrough *et al.*, 1987). In mammalian muscles, studies utilizing [^3H]ouabain binding, agree with the above observation

(Clausen et al., 1982). However, the slow avian muscle fibres are not the same as the 'slow' fibres in mammals, since only the former exhibit junctional potentials and graded contractions. Among mammalian muscles, the slow mouse soleus has been shown to contain significantly higher densities of pumps than the faster EDL (Clausen and Hanson, 1982; Clausen, 1986). This finding was confirmed in a later study by Everts et al. (1988) who also observed that the rat soleus exhibited a higher level of Na⁺, K⁺-pump activity than in the fast-twitch EDL. In human muscle, however, post-mortem studies have shown no significant differences in [³H]ouabain binding sites between muscles with high and low ratios of Type I to Type II fibres (e.g. soleus and rectus femoris respectively) (Dorup et al., 1988). Part of the reason for the species differences is the variation in the functional characteristics of mammalian tissue used for these observations. As observed by Moulds et al. (1977), the mouse soleus, consisting largely of slow fibres, was still approximately twice as fast as the fastest of their human in vitro sartorius preparations containing 65% Type II fibres; thus, what is fast for a human is distinctly slow for a mouse. In conclusion, although it is appropriate to attempt to correlate morphological and functional properties of fibres within a species, it is potentially misleading to apply results from the animal literature to the human situation.

1.9.3 Fatigue characteristics in slow- versus fast-muscles

It is with the above reservation in mind that the excitabilities of slow and fast muscles during fatigue are now discussed. In light of the fact that the physiological range of firing rates in motor units during sustained voluntary contractions is between 10-30 Hz, only those changes in response to prolonged stimulation at frequencies within this range will be discussed.

1.9.3.1 Physiological range of motor unit firing frequencies

A detailed discussion on the physiological range at which motor units fire during sustained voluntary contractions appears in Section 1.12.1. At this time, a brief introduction to published observations in human muscles will be presented. Motor unit discharge rates in the human biceps brachii, adductor pollicis and soleus were recorded by Bellemare et al. (1983) using tungsten microelectrodes. The mean firing rate for soleus was approximately 11 Hz with a range of 5-20 Hz during maximal voluntary contractions. This was in direct contrast to the other two muscles, both of which exhibited higher mean firing rates and larger ranges. Hannerz (1974) obtained firing rates of 30 to 60 Hz, which fell to between 15 and 20 Hz during sustained maximal voluntary contractions, from tibialis anterior. Motor units fire at their highest rates to rapidly build up tension at the initiation of a maximal voluntary contraction. The highest discharge frequencies recorded from human motor units were obtained by Marsden et al.

(1983) who reported initial frequencies as high as 150 Hz during contractions of the adductor pollicis.

1.9.3.2 Excitability changes in response to repetitive stimulation

In animal studies, the effects of repetitive stimulation, at low (10 Hz or less) frequencies, on single fibre action potentials are a decline in amplitude and an increase in duration in fast-twitch fibres (Hanson and Persson, 1971; Hanson, 1974; Grabowski, 1972) and no change in either property in slow-twitch fibres (Hanson, 1974). Motor unit action potentials reflect the overall changes in their fibre populations. Fast motor units typically show initial potentiation and then reductions in amplitude (Sandercock et al., 1985; Hamm et al., 1989), together with an increase in duration (Sandercock et al., 1985). The same trend is observed in potential area as in amplitude (Hamm et al., 1989; Enoka et al., 1992), though the decline in area is considerably less (Hamm et al., 1989). Conversely, action potentials of slow motor units exhibit considerable resistance to decrement that is dependent on the stimulation frequency (Sandercock et al., 1985). Fatigue changes in MUAP are insignificant with low frequency stimulation (10 Hz) and are more marked with intermittent tetani at 40 Hz (Sandercock et al., 1985). M-wave changes in animal muscles of relatively homogeneous fibre compositions exhibit the same trends in response to repetitive stimulation at low frequencies, with slow-twitch muscle exhibiting greater

maintenance of action potential variables throughout the entire fatigue protocol (Uramoto et al., 1983; Pagala et al., 1984; Rankin et al., 1988; Enoka et al., 1988 and 1989).

In response to prolonged excitation, the M-waves of human muscles, with their heterogeneous fibre population, will reflect the sum of the individual fibre action potentials and their corresponding resistance to decrement. At present, although there are some ultrastructural differences between the membranes of fast- and slow-twitch fibres in animals (see Section 1.9.2) these are unlikely to account for the difference in resistance to decrement. It is more likely that the latter is related to the ability of the fibre to withstand depolarization. Since the initiation of the action potential is driven by Na^+ , inexcitability would result if too few Na^+ channels were available, either because they failed to deactivate after an action potential had been established, or because the membrane was depolarized.

The accumulation of K^+ in the interstitial space will cause membrane depolarization (Sreter, 1963; Bezanilla et al., 1972). Potassium loss from muscle cells during fatiguing contraction has been well established (Sreter, 1963; Hnik et al., 1986; Sjøgaard, 1985, 1990) and there are recent reports suggesting that K^+ conductance may be altered by metabolic factors (Spruce et al., 1985). During high-intensity exercise the action of the Na^+, K^+ -pump is insufficient to maintain normal ionic gradients, as shown by the rise in $[\text{K}^+]$ in the venous blood draining

from the muscle (Lindinger et al., 1987; Sjøgaard, 1985). The studies of Clausen et al. (e.g. Clausen and Everts, 1988) have also demonstrated that, even during stimulation at 1 Hz, the K^+ and Na^+ fluxes exceed the maximum pumping capacity. Further evidence of inadequate Na^+ and K^+ pumping has come from measurements, with ion-sensitive electrodes, of interstitial $[K^+]$ during invoked or voluntary contractions. In one such study, performed on human forearm muscles, Vyskocil et al. (1983) found rises in interstitial K^+ concentrations to as much as 15mM during maximal voluntary contractions. Rises in interstitial K^+ concentrations have also been observed during stimulated contractions. Juel (1986) found that after intermittent stimulation of the mouse soleus at 40 Hz, the K^+ concentration in the interstitium doubled. Hirche et al. (1980) reported a rise in interstitial K^+ values of 7.4 mM in the dog gastrocnemius after 4 min of intermittent stimulation at 100 Hz. A rise in extracellular K^+ plays a critical role in reducing sarcolemmal excitability by decreasing the resting membrane potential and inactivating Na^+ channels. Failure of the Na^+ , K^+ -pump will therefore result in membrane depolarization and subsequent inexcitability (Jones and Round, 1989).

At rest, intracellular ionic concentrations of Na^+ and K^+ differ between fast- and slow-twitch fibres, with the former having the higher K^+ values (eg. $[K^+] = 175\text{mM}$ (fast), 163mM (slow); $[Na^+] = 9\text{mM}$ (fast), 13mM (slow), Sreter, 1963). These differences would affect both the continued propagation of

the Na^+ dependent action potential and the magnitude of the resting membrane potential. The resting potential is approximately -10 mV higher in fast fibres (Leader *et al.*, 1984) and this would result in a larger action potential, as observed experimentally (Buller *et al.*, 1965).

A tantalizing observation pertinent to the maintenance of fibre action potentials during repetitive stimulation has been made in hypothyroid patients, in whom the compound action potential amplitude was maintained at its initial value after 2400 impulses (at 20 Hz) while euthyroid (normal) subjects showed a 60% decline in amplitude over the same time period (Edwards and Wiles, 1981). These results suggested that hypothyroid patients were able to maintain muscle excitability because of their improved energy economy (secondary to the reduction in resting metabolic rate). It is becoming more apparent that the limiting step in maintenance of muscle excitability is the critical requirement for energy for continued action of the Na^+/K^+ -ATPase. It is possible that slow muscle is better able to maintain excitation because of greater production of ATP through oxidative processes, thereby keeping up the supply for the membrane Na^+/K^+ -pumps. Such a difference between the two types of fibre would be exaggerated by the larger blood and capillary volume of the slow-twitch fibres (Folkow and Halicka, 1968), features which would allow the removal of metabolites and the delivery of oxygen and energy fuels to proceed more rapidly. It is, however, noteworthy that the findings on hypothyroid patients, referred to

above, were made under ischaemic conditions.

1.10 RECOVERY OF MUSCLE EXCITABILITY

In a keynote address highlighting the biochemical basis of fatigue during exercise, Richard Edwards applied catastrophe theory, to explain muscular fatigue and recovery. He proposed four pathways whereby fatigue could be caused as a result of depletion of energy stores, excitation or activation failure or combinations of the above. He observed that since the aerobic recovery of excitation from ischaemic fatigue occurs very rapidly, suggesting a critical readjustment of electrolyte balance, the recovery pathway had to be different from the fatigue pathway. Edwards proposed a "recovery hysteresis" (see Figure 10, Edwards, 1983), where there is a restitution of the action potential with little or no force generation. The recovery of excitation-contraction coupling then completes the loop. However, it should be noted that there is less hysteresis in experiments employing low frequencies of stimulation, in which excitation and force may also be uncoupled (Edwards, 1983). The recovery of muscle excitation has been reported in very few studies, since the majority have been concerned with the restitution of force (see below).

1.10.1 Time course of recovery of the M-wave

The energy dependence of the recovery of the muscle compound action potential is evident in studies of patients with myophosphorylase deficiency who

exhibit rapid fading of the action potential under ischaemic conditions (Edwards and Wiles, 1981; Edwards et al., 1982). With continuing ischaemia at rest, normal subjects recovered up to 90% of the control value of their adductor pollicis M-wave while no recovery of the action potential was observed in the myophosphorylase patients until the circulation was restored, suggesting that the energy supplied by glycolysis is normally instrumental in maintaining excitation (Edwards et al., 1982). This study is one of the few dealing with the recovery of excitability during maintained ischaemia, although observations have been cited in unpublished studies by Edwards and co-workers (see Edwards et al., 1982, Edwards, 1983) and, very recently, by ourselves in ischaemic recovery of the brachial biceps (Galea and McComas, 1992).

In other investigations, the recovery of M-wave amplitude in the human adductor pollicis was always complete by two minutes post cuff release (Cooper et al., 1988; Gibson et al., 1988) and within the first two minutes following both sustained and intermittent voluntary contractions with an intact circulation (Duchateau and Hainaut, 1985). The rapidity of recovery of M-wave amplitude depends to a certain degree on the frequency of the stimuli used for testing (Gibson et al., 1988; Cooper et al., 1988). Single M-waves decline very little and therefore recovery changes are negligible (Garland et al., 1988b).

The few animal studies, in which recovery of excitation was followed, yielded similar results. For example in the experiments by Sandercock et al.

(1985) and by Metzger and Fitts (1986), complete recovery of muscle excitability after low frequency stimulation took place within two minutes of termination of stimulation, while recovery following 80 Hz stimulation had achieved 90% of the control value by one minute but was not complete by 30 minutes (Sandercock et al., 1985).

The experiments on single amphibian muscle fibres by Luttgau (1965) raised the possibility that the recovery of the M-wave is due to metabolic mechanisms involving the contractile machinery. Luttgau found that stimulation of single fibres (in vitro) at a 100 Hz caused some action potentials to drop out within 2 s. If the stimuli were applied at lower rates and maintained until the fibre became exhausted, the action potentials recovered. Abolishing the contractile responses by metabolic inhibitors such as iodoacetate and sodium cyanide, or by immersion of the preparation in hypertonic bathing solutions also resulted in better maintenance of membrane excitability. Caution must be exercised in extending observations made on an amphibian in vitro preparation to the human in vivo situation. Significant changes in the composition of the extracellular space would essentially be eliminated because of the large volume of fluid bathing the muscle.

Partial recovery of the M-wave can occur when ischaemia is maintained (Edwards et al., 1982), a condition in which little or no production of PCr occurs (Harris et al., 1976). Further support for this observation lies in the study by

Miller et al. (1987) in which the maximal voluntary contractile force of the adductor pollicis recovered with the same time course (15-20 minutes) as intracellular pH and phosphocreatine while the M-waves recovered faster. The restoration of excitability cannot be linked to intracellular pH either, as patients with myophosphorylase deficiency exhibit a decline and subsequent recovery of the M-wave without any drop in pH (Edwards and Wiles, 1981; Wiles et al., 1981).

1.10.2 Recovery of force

In most published studies, recovery of contraction was delayed following low frequency stimulation. However, Cooper et al. (1988) found that, at 10 Hz stimulation, the force exerted by the human adductor pollicis potentiated to 160% of its control value by the third minute after cuff release and then declined to approximately 60% of its value by 15 minutes; the increased tetanic tension was accompanied by a small potentiation of the twitch. The potentiation in tetanic force could not be explained by an increased relaxation time, allowing greater summation of the individual responses, because the relaxation time was decreasing at the same time as the force was potentiating.

It is likely that the delay in force recovery reflects a failure of excitation-contraction coupling due to impairment of either the Ca^{++} release mechanism or impulse transmission in the transverse tubular system. The subject of excitation-

contraction coupling is vast and beyond the scope of this work. For excellent reviews of the possible mechanisms underlying the dissociation between excitation and contraction the reader is referred to Donaldson (1986), Eisenberg (1987) and Martonosi and Beeler (1983).

In an attempt to elucidate the metabolic basis for the recovery of force after fatigue, Cady and co-workers (Cady *et al.*, 1989 a and b) used NMR spectroscopy to measure metabolic changes during fatigue and during recovery in the human first dorsal interosseus. They found that the relationship between force and the concentration of a monobasic series of intracellular Pi (H_2PO_4^-) was essentially linear during fatigue, but not during recovery. The same relationship between force and H_2PO_4^- was recently reported by Boska *et al.* (1990) in the human adductor pollicis and tibialis anterior muscles. The changes in H_2PO_4^- were more pronounced in the adductor pollicis, in keeping with the greater fatigue in this muscle. One criticism of the study is that it did not include excitability measurements. Earlier observations by this group (Miller *et al.*, 1987) indicate that complete recovery of M-waves from the human adductor pollicis occurred by 6 min post fatigue. M-wave recovery was not related to the recovery of pH or the high-energy phosphates as measured using NMR spectroscopy.

1.11 SUMMARY

The preceding sections of the Introduction indicate that the changes in muscle membrane excitability, reflected in changes of the temporal characteristics of the muscle compound action potential, are dependent on stimulus frequency during fatigue induced by repetitive stimulation, and to a lesser extent during recovery from that fatigue. Furthermore, slow-twitch fibres exhibit greater resistance to decrement in that there is a better maintenance of excitation in slow rather than fast-twitch fibres.

1.12 VALIDATION OF EXPERIMENTAL TECHNIQUES

The majority of the experiments in this thesis were conducted using tetanic stimulation of the ischaemic ankle dorsiflexors. The following sections provide validation for the use of tetanic stimulation, ischaemia, and ankle dorsiflexor muscles to observe changes in muscle excitability.

1.12.1 Validity of using tetanic stimulation and ischaemia

The use of tetanic stimulation as an experimental tool would receive some justification if the frequencies of stimulation were similar to those of voluntary motor unit excitation. However, quantifying the discharge rates of motor units during maximal voluntary (isometric) contractions presents severe methodological problems because of difficulties in identifying single motor unit

discharges. The difficulty arises largely because of the background impulse activity generated by other motor units, and also because of electrode movement, which alters the appearance of the potentials discharged by the units under study (Bigland-Ritchie et al., 1983; Bellemare et al., 1983; Bigland-Ritchie et al., 1986). Grimby and co-workers (Grimby et al., 1979, 1981a; Grimby and Hannerz, 1977; Borg et al., 1978; Hannerz, 1974) overcame some of this difficulty by: 1) reducing the number of motor units in the short extensor of the big toe by progressive lesioning of the terminal nerve twigs; at the same time, the motor unit potentials became larger through collateral sprouting; 2) performing Lidocaine blockade of the main deep peroneal nerve on subjects with accessory deep peroneal nerves; the latter nerve innervates only a few motor units of the short toe extensor; 3) obtaining tibialis anterior motor unit potentials from intact muscles by using high impedance wire electrodes and "considerable patience" (Hannerz, 1974; Grimby et al., 1981b). Use of the first two techniques probably created physiological abnormalities. Neuromuscular transmission may have been impaired through collateral sprouting (Ribchester, 1988; Klingman et al., 1988; Thomas et al., 1987; Dengler et al., 1989) and the contractile properties and proprioceptive inflow could also have been disturbed. The lower selectivity of the third technique precluded the identification of single motor unit potentials on supramaximal electrical stimulation of the peroneal nerve, yet was sufficiently selective to allow observation of firing rates of 30 to

65 Hz, which fell to between 15 and 20 Hz during sustained maximal voluntary contractions (Hannerz, 1974). These authors (Borg et al., 1978) concluded that motor units with axonal conduction velocities higher than 45 m/s and contraction times shorter than 50 ms fired in the range of 20-50 Hz. Those units with conduction velocities lower than 40 m/s and with contraction times greater than 80 ms fired in the range of 10-30 Hz, although firing rates lower than 10 Hz were often observed. At the initiation of maximal voluntary contractions, all motor units fired at their highest rates in order to rapidly build up tension.

Motor unit discharge rates in the human biceps brachii, adductor pollicis and soleus were recorded by Bellemare et al. (1983) using high impedance (tungsten) microelectrodes. The mean firing rate for soleus was approximately 11 Hz with a range of 5-20 Hz during maximal voluntary contractions. This was much slower than the other two muscles, both of which exhibited higher mean firing rates and larger ranges. Being sure of the maximality of each contraction, these authors suggested that the range of discharge rate of each motor-unit pool was just sufficient to produce maximum force in each motor unit; this conclusion was based on the relationship between the firing frequencies and contraction times of the motor units. The highest discharge frequencies recorded from human motor units were obtained by Marsden et al. (1983) who reported initial frequencies as high as 150 Hz during contractions of the adductor pollicis.

A very recent study by Bigland-Ritchie et al. (1992) deserves mention

because of the presently held belief that higher excitation rates are required for tetanic fusion when a muscle is maximally activated at shortened lengths (Rack and Westbury, 1969; Marsh et al., 1981). Bigland-Ritchie et al. (1992) found no difference between discharge rates at a control length of tibialis anterior (28.2 ± 9.9 Hz; ankle at 90°), and at a shortened length (27.7 ± 9.8 Hz; ankle at approximately 75°) during maximal contractions. There was also no difference between firing rates during 50% and 75% MVC's at short and control lengths. Recruitment of additional motor units at short lengths, to augment the reduced force output of individual motor units, could have explained the consistent firing frequencies at different lengths. However, no reason could be given for the similarity of firing frequencies during maximal contractions at different lengths.

During sustained maximal voluntary contractions, the mean motor unit firing rate declines within the first 60 s (Bigland-Ritchie et al., 1983, 1986a; Bigland-Ritchie and Lippold, 1979; Grimby et al., 1981a; Hannerz, 1974; Freund et al., 1974). Possible mechanisms causing this decline may reside in excitability changes in either the descending motor pathways or at the level of the motoneuron (Kernell and Monster, 1982). The favoured hypothesis seems to be reflex inhibition of the α -motoneuron by afferent signals from the fatiguing muscle (Bigland-Ritchie et al., 1986b; Garland et al., 1988; Garland and McComas, 1990). In light of the preceding evidence, it is therefore reasonable to state that the range of fatiguing stimulus rates (5, 10, 20 and 30 Hz) used in this

work are physiological for both tibialis anterior and soleus.

1.12.2 Validity of employing ischaemia

The use of ischaemia in the experimental protocol simulates the condition encountered during maximal sustained voluntary contractions. Thus, blood flow in the human plantar flexors is arrested with contractions as low as 20-30% of maximal voluntary effort (Barcroft and Millen, 1939; Sjøgaard, 1990). By contrast, Humphreys and Lind (1963) found that considerably higher levels of contraction (70% maximal voluntary contraction) were needed to arrest blood flow completely in muscles of the forearm during hand-grip contraction. The large discrepancy between these results and those from Barcroft and Millen (1939) was attributed to the shortening of the calf muscles as the subjects stood on tiptoe. There is the further complication that blood pressure may rise during effort, and by different amounts in different subjects (MacDougall et al., 1985, 1992). In the present study maximal activation of both the plantar- and dorsiflexors at 10-30 Hz results in intensities of contraction within the ranges quoted in the above studies by virtue of the force/frequency curves of the respective muscles (plantarflexors - Sale et al., 1982; dorsiflexors - Marsh et al., 1981). However, if a cuff had not been used, circulation would have been resumed in the intervals between successive stimulus trains (see Methods).

1.12.3 Use of ankle dorsiflexors for repetitive stimulation

The bulk of the experiments in this dissertation were performed on the human tibialis anterior. The low stimulus threshold of the common peroneal nerve at the fibula allowed relatively painless supramaximal stimulation, which could be tolerated for several minutes.

Another advantage of choosing tibialis anterior is that earlier work performed on this muscle has yielded useful information; the latter includes observations on the force/frequency and length/tension curves (Marsh *et al.*, 1981), twitch potentiation (Vandervoort *et al.*, 1983) and the effects of muscle length and excitation frequency on fatigue (Fitch and McComas, 1985; Garland *et al.*, 1988). The only major disadvantage of using tibialis anterior is that peroneal nerve stimulation also activates the peroneus longus and brevis, both of which are plantarflexors; thus the force recordings are unreliable in terms of absolute tension.

In summary, the advantages of using tibialis anterior for observation on the effects of different frequencies of stimulation on muscle excitability were considered to outweigh the disadvantages.

METHODOLOGY

2.1 INTRODUCTION

Changes in muscle excitability during fatigue induced by tetanic stimulation are dependent on the stimulus frequency used. In order to determine the related changes in muscle excitability the rates of onset of action potential failure in muscle fibres were investigated during fatigue induced by different frequencies of stimulation and during recovery from that fatigue. The fatigue in fast-twitch muscles (prime dorsiflexor) was compared with that in slow-twitch ones (prime plantarflexor) in order to determine whether or not slow-twitch fibres actually exhibit greater resistance to decrement than fast-twitch fibres as is presently believed. Ischaemia was artificially imposed throughout all testing procedures in order to maintain fatigue and to establish a definite starting point for studying the time course of recovery. Even though circulatory occlusion of the muscle naturally occurs as intramuscular pressure increases with contraction, the resulting ischaemia is relieved as the muscle fatigues because of decreasing mechanical compression of the vasculature. Because ischaemia was maintained in these studies it was necessary to study its effects during fatigue and recovery of the ankle dorsiflexors.

2.2 Time course and extent of excitation failure

2.2.1 Ankle dorsiflexor studies

The ten volunteers who completed all test runs (4 men and 6 women) are described in Table 1; none of the subjects had any neurological disease and all were quite active. A few were involved in varsity athletics and are so identified in Table 1. The subjects were paid for their participation and the study carried the approval of the University Ethics Committee.

2.2.2 Torque measurement

All runs were performed on the right tibialis anterior (TA) muscle. Net isometric dorsiflexor torque was measured with the ankle plantarflexed by 15°, an angle corresponding to an optimal length of tibialis anterior for both twitch and tetanic force production (Marsh et al., 1981). The subject sat in a chair of adjustable height with the right knee braced in a steel frame at 90°. The foot was strapped on a thin rotating aluminum plate which, for the purposes of this experiment, was locked in 15° of plantarflexion. Dorsiflexor torque was detected by strain gauges mounted on a short metal tongue projecting from the

Table 1. Description of subjects

Subject	Sex	Age (yrs.)	Occupation	Sport
SM	M	20	Undg. Student	Swimming
LA	F	20	"	
KD	F	21	"	Soccer
GY	M	20	"	
WB	M	27	"	
LM	F	19	"	
JT	F	20	"	
TD	F	20	"	
JR	F	21	"	
JV	M	22	"	Swimming

rear of the foot-plate which abutted against one of the two horizontal rods fixed between metal side plates (Figure 1). The original mass of the foot plate (1.51 kg) was slightly increased by a restrictive strapping system which reduced torque error due to strap compliance. The resonant frequency of the system was 70 Hz with the subject in position and foot strapped to the plate. Calibration of the apparatus was performed by measuring the DC voltage developed when known weights were suspended from the footplate. Figure 2 shows the relationship between the calibration weight and torque development. The conversion to units of torque was performed by multiplying each weight by the distance between the axis of rotation and the point of weight attachment. Deviation of the response from linearity was less than 0.1%. A complete description of this system appears in Marsh et al. (1981).

2.2.3 Recording system

EMG's were all recorded with chlorided silver cup electrodes, 9 mm in diameter, filled with conducting gel; recordings were bipolar with the reference electrode being placed on the skin over the middle one third of the tibialis anterior.

Pilot studies, in which a stationary reference electrode was coupled with a stigmatic electrode in various positions, revealed that the optimal placement of the latter was the lower end of the middle third of the muscle. Figure 3

Figure 1 Schematic representation of foot holder used for torque measurement (from March et al., 1981).

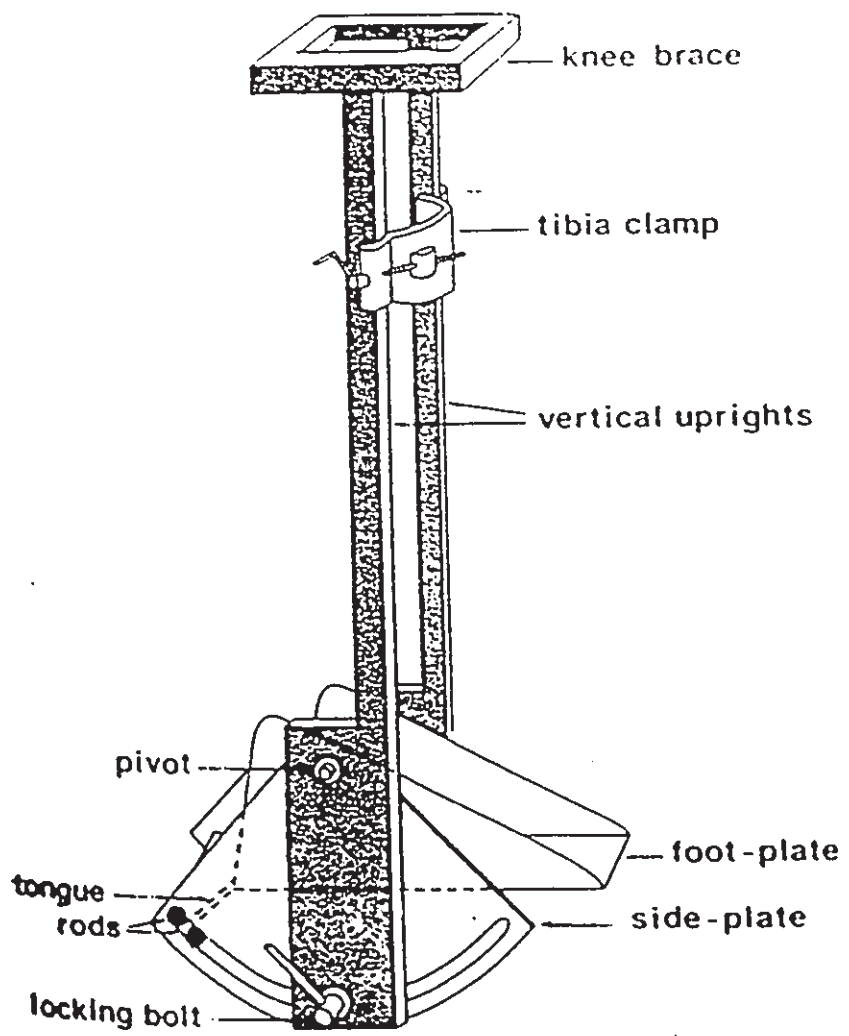
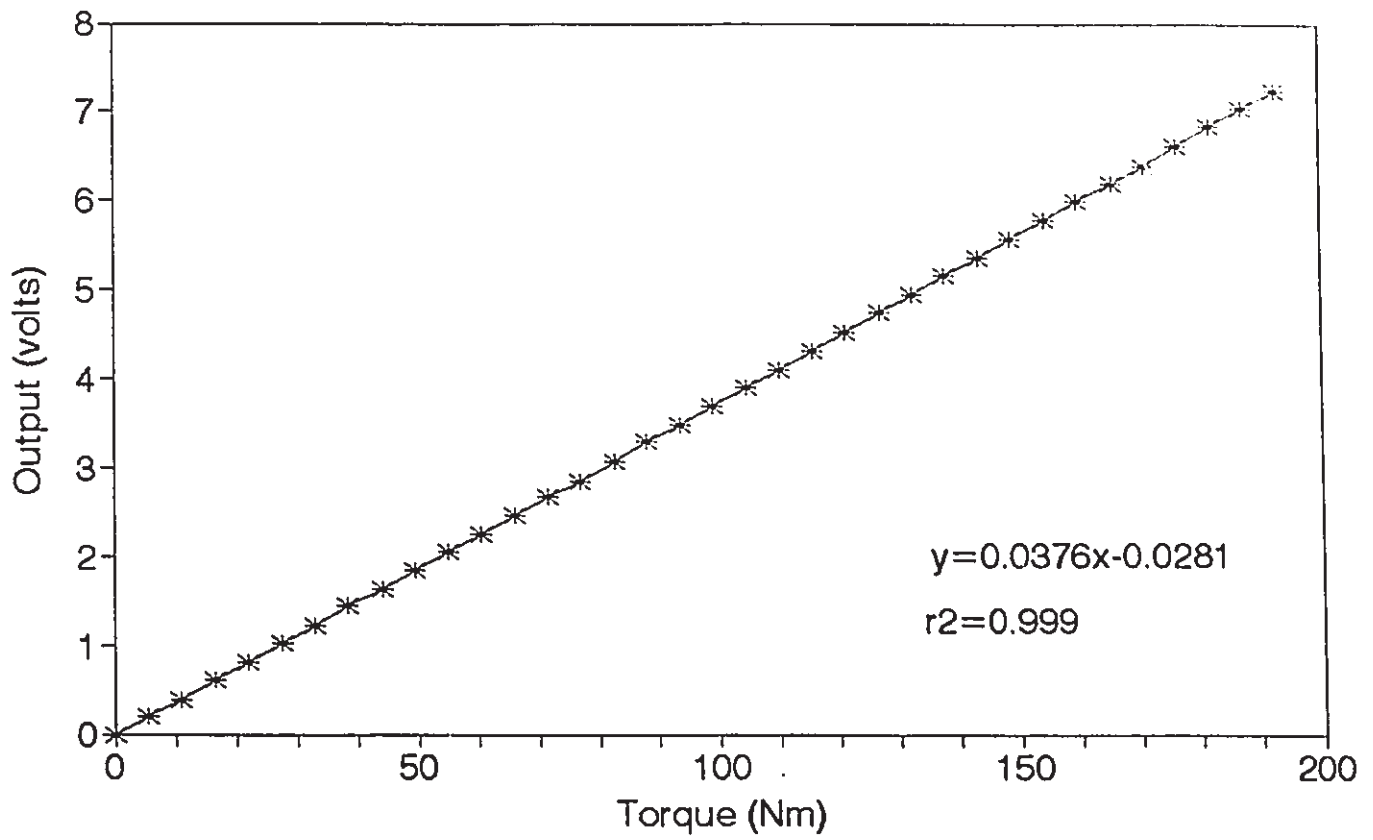


Figure 2 Force transducer calibration curve and linearity check. The equation of the regression line of output/torque was $y=0.0376x-0.0281$. Regression analysis yielded an r^2 value of 0.999

Force Transducer Calibration Curve



shows the results from two of the pilot studies. The middle of the proximal one third of tibialis anterior was designated as location 0, further locations for the stigmatic electrode were then marked off in 1 cm increments proximal and distal to 0. This mapping technique was repeated for every session by means of a manipulable electrode. The criterion for acceptance was initial negativity of the recorded M-wave, indicating that the stigmatic electrode was situated over the motor innervation zone. Only biphasic potentials were acceptable. The ground electrode was a silver strip (6 cm x 0.5 cm) placed anterolaterally and inferiorly to the tibial tuberosity. Electrodes were secured by 12.5 mm wide adhesive tape (Blenderm No. 1525). A schematic representation of recording and stimulating systems for the ankle dorsiflexor studies appears in Figure 4.

2.2.4 Stimulating system

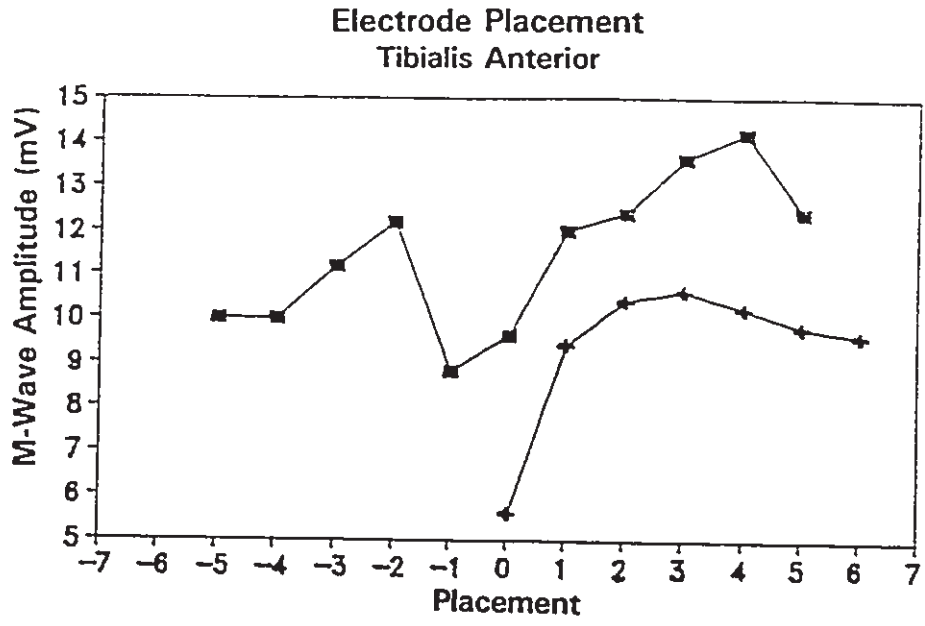
The stimuli for all runs were applied to the common peroneal nerve. The stimulating electrodes were lead plates (4.0 cm x 2.0 cm) which fitted over the optimal location on the common peroneal nerve. This site was found by applying supramaximal 100 μ s pulses delivered from a high-voltage stimulator (Model 3072, Devices Ltd.) through a hand-held bipolar stimulator (the stimulating terminals were brass prongs approximately 3 mm in diameter and 2 cm apart). Criteria for the optimal location were pure dorsiflexion of the unrestrained foot and the elicitation of an M-wave of suitable amplitude and

Figure 3a) Optimal stigmatic recording electrode placement for tibialis anterior in two subjects. The scale on the abscissa represents locations (at 1 cm increments), distal (+) and proximal (-) to the middle of the proximal one third of the muscle.

■-■ - subject #1; +-+ - Subject #2.

Figure 3b) Diagram of leg indicating the location for placement numbers 3 and 4 located in the middle one third of the muscle. These placements were usually optimal.

a.



b.

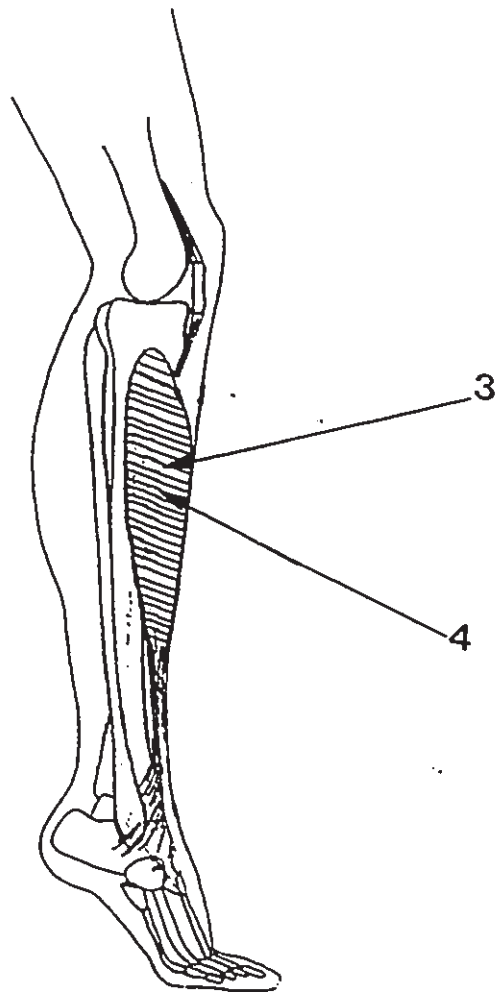
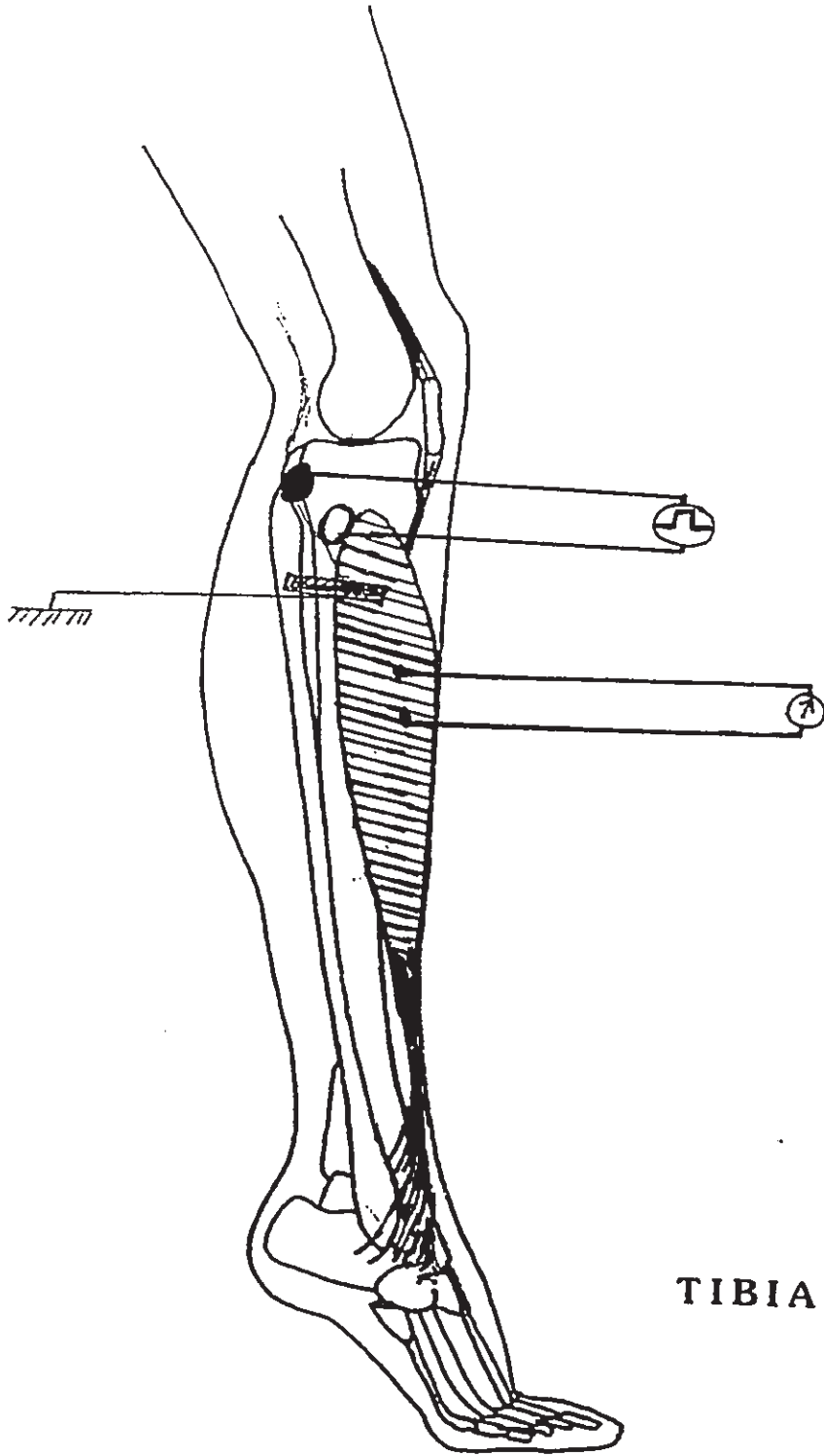


Figure 4 Schematic representation of recording and stimulation systems for the ankle dorsiflexor studies. Filled and open stimulating electrodes represent cathode and anode respectively.



TIBIALIS ANTERIOR

negative-positive polarity. The cathode was then placed over this location; the anode was placed slightly lower and anterior, since this arrangement yielded the lowest stimulus thresholds and minimized the activation of the peroneal muscles. Although anodal block was a theoretical complication, it was not observed with this arrangement. Stimulating electrodes were secured by 25 mm wide paper, adhesive tape (Micropore, No. 1530) and with 25 mm wide velcro belts. The stimulus pulse width was then set to 50 μ s, and the M-waves were measured following both single shocks and short 500 ms tetani at 10 Hz. The unrestrained foot and ankle were carefully observed at this time to ensure that the stimulation evoked a dorsiflexor moment and not plantarflexion. The stimuli were delivered from a high voltage stimulator which was triggered by a digital timing device (Digitimer Model 3290, Devices Ltd.) through a gated pulse generator (Model 2521, Devices Ltd.). In addition to using the briefest available stimulation pulse (50 μ s), it was found that the discomfort of tetanic stimulation was somewhat alleviated by using a biphasic stimulus pulse; the latter was achieved by inserting a capacitor ($.25 \pm 10\%$ 400 DC) in series with the stimulating electrodes (Baker *et al.*, 1988).

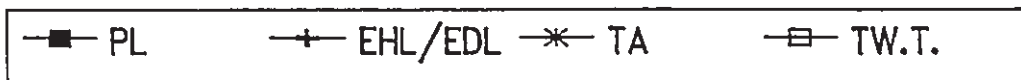
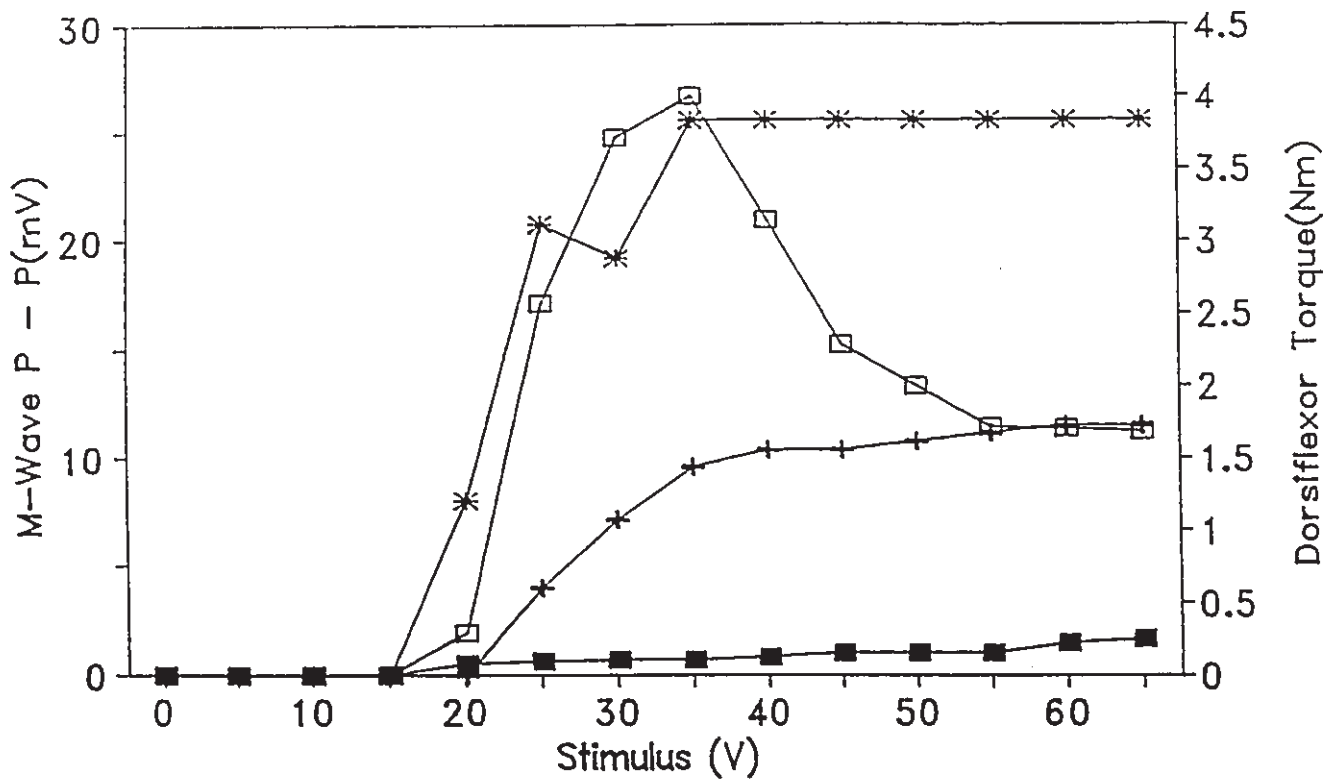
Common peroneal nerve stimulation evokes a net dorsiflexion torque, contributed to by tibialis anterior, extensor hallucis longus, extensor digitorum longus and peroneus tertius and opposed by peroneus longus and brevis. The contribution of the peroneal muscles was minimized by correct cathodal placement

and by conducting all experiments with the ankle in 15° of plantarflexion; this angle coincided with the optimum length of the tibialis anterior and would have caused the plantarflexor muscles to shorten and to become less effective mechanically (Marsh et al., 1981). In spite of the considerable care taken to minimize the opposing component, some subjects still exhibited a strong peroneal contraction. Although it was never a component of twitch torque, the contamination of the dorsiflexor tetanic torque was particularly in evidence towards the end of fatigue and therefore, subjects who exhibited this trend were eliminated from the study.

Pilot studies using simultaneous recordings of evoked potentials from the peroneus longus, extensor hallucis, tibialis anterior and extensor digitorum showed that the contribution of these muscles to the tibialis anterior recording electrodes was minimal. Figure 5 also shows that at the point of maximal tibialis anterior M-wave amplitude and subsequent plateau, peroneus longus M-wave amplitude increased in size while twitch torque declined. This suggests that at higher intensities of stimulation the contribution of peroneus longus (a plantar flexor) reduces the net dorsiflexor output. It was therefore gratifying to observe that the tibialis anterior M-wave peaked at similar stimulus intensities as the twitch torque suggesting that the tibialis anterior contribution supervened over that of the other three muscles. True EMG cross-correlation studies of the respective muscle responses were beyond the scope of this work. It was judged sufficient

Figure 5 Simultaneous responses, from a single subject, of Tibialis anterior (TA), Extensor hallucis longus/Extensor digitorum longus (EHL/EDL), Peroneus longus (PL) and net dorsiflexor torque (TW.T.).

Dorsiflexor Crosstalk
Subject:AH



to make qualitative observations on the responses to single stimuli.

2.2.5 Recording equipment

It was necessary to record the electrical response, the torque response and the timing and duration of the stimulus pulses. Electromyographic signals were amplified (bandwidth 10 Hz - 1 kHz) and displayed, together with twitch and tetanic torque, on a 4-trace storage oscilloscope (Model 141B, Hewlett-Packard Ltd.). Amplified EMG and torque data was also stored on magnetic tape (Model 3960 recorder, Hewlett-Packard Ltd.) for subsequent analysis using specifically designed software. The individual Digitimer pulses were stored, on a separate channel, to enable the computer software to locate specific points during the tetanic trains and to trigger digital data acquisition. A detailed description of the data analysis technique appears later in this chapter.

2.2.6 Experimental protocol

It was necessary to induce fatigue using stimulus frequencies commensurate with physiological motor unit firing rates observed in fatiguing voluntary contractions. The effects of the different frequencies of stimulation were observed by invoking a single stimulus followed by a standard 10 Hz tetanus lasting 2 s. The fatiguing stimuli were given in 4 s epochs and were followed by 3 s rest periods (see Figure 6). Thus, in each 10 s cycle there were, in sequence,

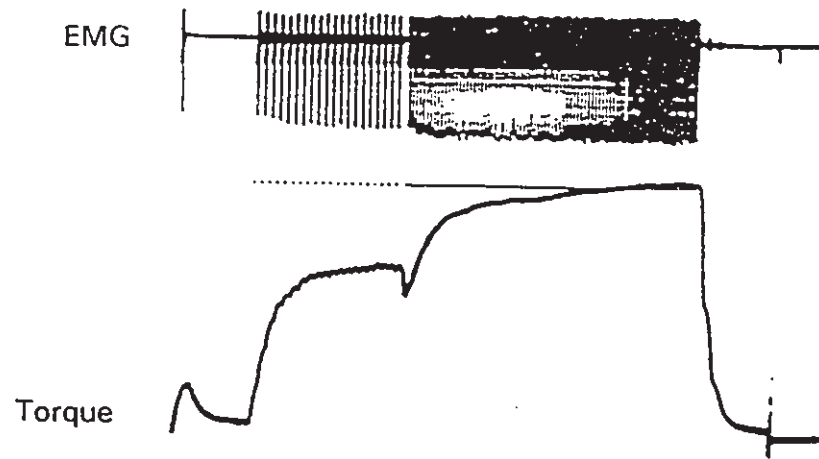
Figure 6a) Oscilloscope trace of EMG and torque output from one cycle of stimulation. In this example, the 10/30 Hz pair were used.

Figure 6b) Schematic of fatigue and recovery protocol. Fatigue was induced by changing the frequency of stimulation (fatiguing tetanus: 4 s bar) for each different run.

a.

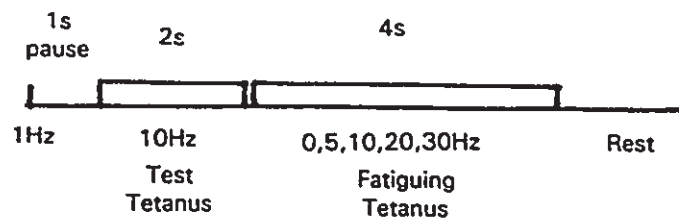
Stimulation Cycle

73



b.

Fatigue Protocol



Recovery Protocol



a single stimulus, a 1 s pause, a 2 s "testing" tetanus at 10 Hz, a 4 s "fatiguing" tetanus, and a 3 s pause. Pilot experiments established that, when 20 Hz was employed as the fatiguing stimulus frequency, complete abolition of twitch and tetanic torque could be achieved within 20 cycles, corresponding to a total time of 200 s. From the fatigue protocol shown in Figure 6 b) it can be seen that the only component changed for each session was the fatiguing frequency; a single stimulus initiated the cycle allowing observation of the single M-wave and twitch amplitude, contraction time and half-relaxation time. Fatiguing tetani were then applied at either 5, 10, 20 or 30 Hz. Only the single stimulus and 2 s "testing" tetanus were applied in the control run. Averaged frequencies over the 10 s, for each cycle, were 2 (for the control condition), 4, 6, 10 and 14 Hz respectively. The number of stimuli over the entire fatiguing period (3 min. 20 s) were 420 (control), 820, 1220, 2020 and 2820 respectively.

2.2.6.1 Session protocol

Subjects underwent five sessions, separated by at least one week to eliminate residual effects. In each session the skin over the recording and stimulation areas was prepared and optimal positioning of the recording and stimulating electrodes was located. The leg was secured in the torque measuring apparatus and the maximal stimulation voltage was established by slowly increasing the intensities of the single stimuli until there were no further

increments in M-wave peak-to-peak amplitude. This was followed by a short tetanic burst at the same voltage to ensure that there had been no movement of the stimulus electrodes and that optimal dorsiflexor torque was still being elicited. Supramaximal stimulation was ensured by increasing the stimulus amplitude by a further 20%. A 10 s stimulus cycle was applied to the fresh muscle so as to determine the control (non-fatigued) twitch and 10 Hz tetanic response. An arterial cuff was then wrapped around the thigh and inflated to above mean arterial pressure to render the leg ischaemic. Fatiguing stimulation was then applied as described above. The single twitch and test tetanus were continued under ischaemia for a further four cycles (40 s). Total fatigue time under ischaemia was four minutes.

2.3 M-wave and torque during recovery

The recovery of muscles after fatiguing stimulation and ischemia was monitored by measuring the response to single and test tetanic stimuli. Two recovery periods were of interest: 1) recovery following cessation of tetanic stimulation, but with maintenance of ischaemia, which occurred after 3 min 20 s after the initiation of stimulation and 2) recovery following cuff release which occurred 40 s later. A 10 s cycle was repeated continuously for 10 min during which time the cycle consisted of a single stimulus, a 1 s pause, 2 s of 10 Hz stimulation and 7 s of rest. After 10 min, the recovery cycles were evoked once

every 30 s for the next 6 min. The total recovery time was 16 min.

2.4 Effect of ischaemia on fatigue development

Subjects were asked to return for a sixth session in which the effects of ischaemia were determined by repeating one run with an intact circulation. One fatiguing stimulus frequency (20 Hz) was chosen for these studies. The protocol was identical to that used previously with the exception of arterial occlusion.

2.5 Muscle excitation changes in fast-twitch vs. slow-twitch muscle

Animal experiments suggest that the rate of action potential failure in a motor unit appears to correspond to the force fatiguability of the unit (Hamm *et al.*, 1989; Clamann and Robinson, 1985; Kugelberg and Lindgren, 1979). In order to determine whether a slow-twitch muscle maintained excitability longer than a fast-twitch muscle, the electrical responses of soleus muscles were compared with those of tibialis anterior to electrical stimulation using the same stimulation frequency.

It was necessary to stimulate both muscles maximally or supramaximally. This was not difficult for tibialis anterior since the threshold for supramaximal peroneal nerve stimulation is low (90-140 V with a 50 μ s stimulus pulse) and usually well within the pain tolerance of the subject. With soleus, however, tetanic stimulation of the tibial nerve tends to be unbearable and is dangerous,

since there is the possibility of the Achilles tendon rupturing. Percutaneous stimulation of soleus at the level of the calf also proved to be extremely uncomfortable. It was therefore necessary to develop a method which would permit subjects to tolerate, at best, maximal stimulation of soleus with the cathode over the calf. As before, the level of activation was based on the peak-to-peak amplitude of the M-wave response.

2.5.1 Pilot projects for minimizing pain during soleus stimulation

Three different procedures were attempted to reduce the sharp cutaneous pain associated with tetanic stimulation of the soleus through the skin of the calf. On the basis of human and animal studies (Kelly, 1981), the pain was thought to be mediated through cutaneous A, δ and C fibre afferents. C fibres are also widely distributed deep in muscle (Kelly, 1981) and probably mediate the pain responses to muscle contraction. However, since the cutaneous response seemed to be the prime limitation to maximal activation, efforts were concentrated on minimizing it rather than the deep pain due to muscle contraction.

Studies conducted on tibialis anterior indicated that use of a biphasic stimulus pulse increased the comfort level of peroneal nerve stimulation. This was achieved by placing a capacitor in series with the stimulating electrodes (Baker et al., 1988). This necessitated that the voltage be almost doubled to produce the same level of muscle activation achieved without the capacitor. The

low threshold required for peroneal nerve stimulation (90-140 V at 50 μ s) meant that the higher voltage levels were still within the range of the (Devices Ltd.) high-voltage stimulator (400 V max). By contrast, the threshold for percutaneous stimulation of the soleus was much higher (e.g. 200-350 V at 50 μ s) and the maximum output of the stimulator was reached before the soleus could be maximally excited. Hence biphasic pulse stimulation could not be used to reduce the discomfort in the soleus experiments.

A second approach was attempted on two subjects using transcutaneous electrical nerve stimulation (TENS). TENS was developed for clinical control of pain based on the gate control theory of Melzack and Wall (1965). A complete discussion of the theory is beyond the scope of this work; suffice it to say that it is based on the presence of a gating mechanism in the substantia gelatinosa, within the dorsal horn of the spinal cord. Interneurones in the substantia gelatinosa regulate the flow of sensory information to the higher centres in the brain where the sensation of pain is interpreted. TENS is thought to mediate its action through stimulation of large afferent myelinated fibres. The impulses in these fibres, on reaching the substantia gelatinosa, close a biological gate to incoming impulses from small diameter afferent fibres. The stimulator used for this pilot study was an Eclipse TENS (Medtronic, Inc.). TENS units are pulse generators capable of producing a current of a particular wave form at variable frequencies and intensities. In the present experiments, carbon impregnated

rubber electrodes were attached to the skin, approximately 10 cm on either side of the stimulating cathode. The unit was set to a frequency of 125 Hz, at a pulse width of 250 μ s for a duration of 10 to 15 minutes. These were the suggested settings for use during painful procedures such as wound debridement, friction massages and joint mobilization, procedures which mimicked the brief intense nature of the pain due to electrical stimulation. Maximal tetanic excitation of soleus was attempted during TENS stimulation in the hope that any gating of cutaneous afferent fibres would extend to those supplying the area under the stimulating cathode for soleus. The immediate subjective response was that this intervention was completely unsuccessful in increasing comfort or tolerance.

The third attempt to increase tolerance to electrical stimulation involved a combination of submaximal and supramaximal stimulations within the tetanic trains. A system was developed whereby the single stimulus and a 100 ms burst at the end of the fatiguing tetanus were delivered through a second high-voltage stimulator set at supramaximal activation while the rest of the tetanic train was delivered using a tolerable stimulation level through the first high voltage stimulator. The rationale behind these studies was that submaximal stimulation would fatigue a constant fraction of the muscle; the remainder of the muscle would be unaffected and would be expected to generate the same torque during the 100 ms bursts throughout the experiment. It became apparent, however, that submaximal stimulation was probably activating changing fibre populations in the

course of the fatiguing run. It was also possible that the non-fatigued fibres might have been affected by potassium ions and metabolites released by the fatigued fibres.

The fourth approach to reducing pain to a tolerable level was quite successful. The best objective was to anaesthetize the area of skin where the pain was felt under the stimulating cathode without affecting the contractile response. This was achieved by using a cold cathodal electrode, which consisted of a 5 cm × 3 cm × 2 cm rectangular brass box, composed of two machined plates enclosing three interconnected chambers. Ethanol, cooled to -40°C or lower, was perfused through the chambers at a flow rate of 6 ml/min. Gravity was sufficient to provide the force needed to drive the coolant through the chambers; the connecting tubing was insulated to minimize equilibration with room temperature and the flow was regulated by an adjustable clamp. A schematic representation of the system is shown in Figure 7. The coolant was collected through a tube connected to the exit orifice of the electrode and reused. A thermocouple, placed between the electrode and the skin, provided constant monitoring of the interface temperature. This temperature was maintained between 7°C and 10°C, to allow sufficient cold anaesthesia without causing frostbite in the ischaemic experiments.

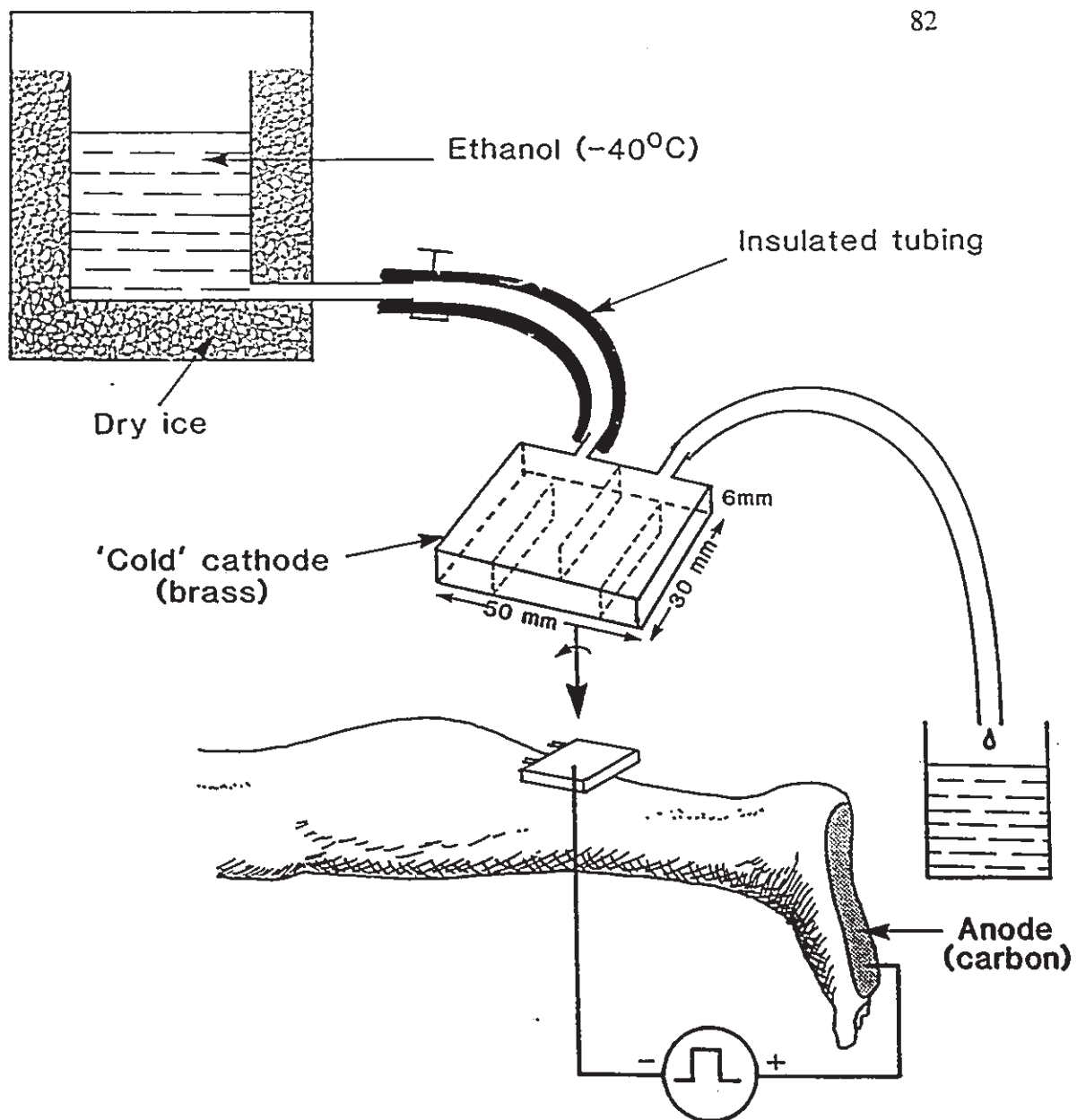
It was possible to tetanize the soleus muscle using maximal activation with this system. The cathode was placed just inferior to the V-shaped notch

formed by the two bellies of gastrocnemius. Given the thermoinsulating properties of the skin, subcutaneous fat and fascia covering the soleus muscle, it was unlikely that deep muscle temperature was significantly lowered. This assumption was tested on two subjects by inserting an intramuscular temperature probe (YSI #524) into the muscle, deep to the cold cathode placement. Intramuscular temperature was monitored in these subjects at the same time as the temperature at the skin/electrode interface through use of a surface probe (YSI #409A) over a four minute period of ischaemia. All of these measurements were performed on resting muscle as contraction was not possible with the intramuscular probe in place. With the surface temperature maintained between 7°C and 10°C, intramuscular temperature immediately below the cathode fell to 16°C but was essentially unaffected elsewhere (temperature recordings at the level of the recording electrodes fell by only 3°C). It became evident that the temperature gradient created by the cooling produced sufficient anaesthesia to enable the subject to tolerate maximal electrical (tetanic) stimulation.

2.5.2 Stimulating and recording electrode placement: Plantarflexors

Electromyography of soleus presented a different set of problems from those encountered in tibialis anterior. Maximal percutaneous stimulation was used because tetanic stimulation through the tibial nerve was not possible and in fact, placed the subject in danger of Achilles tendon rupture.

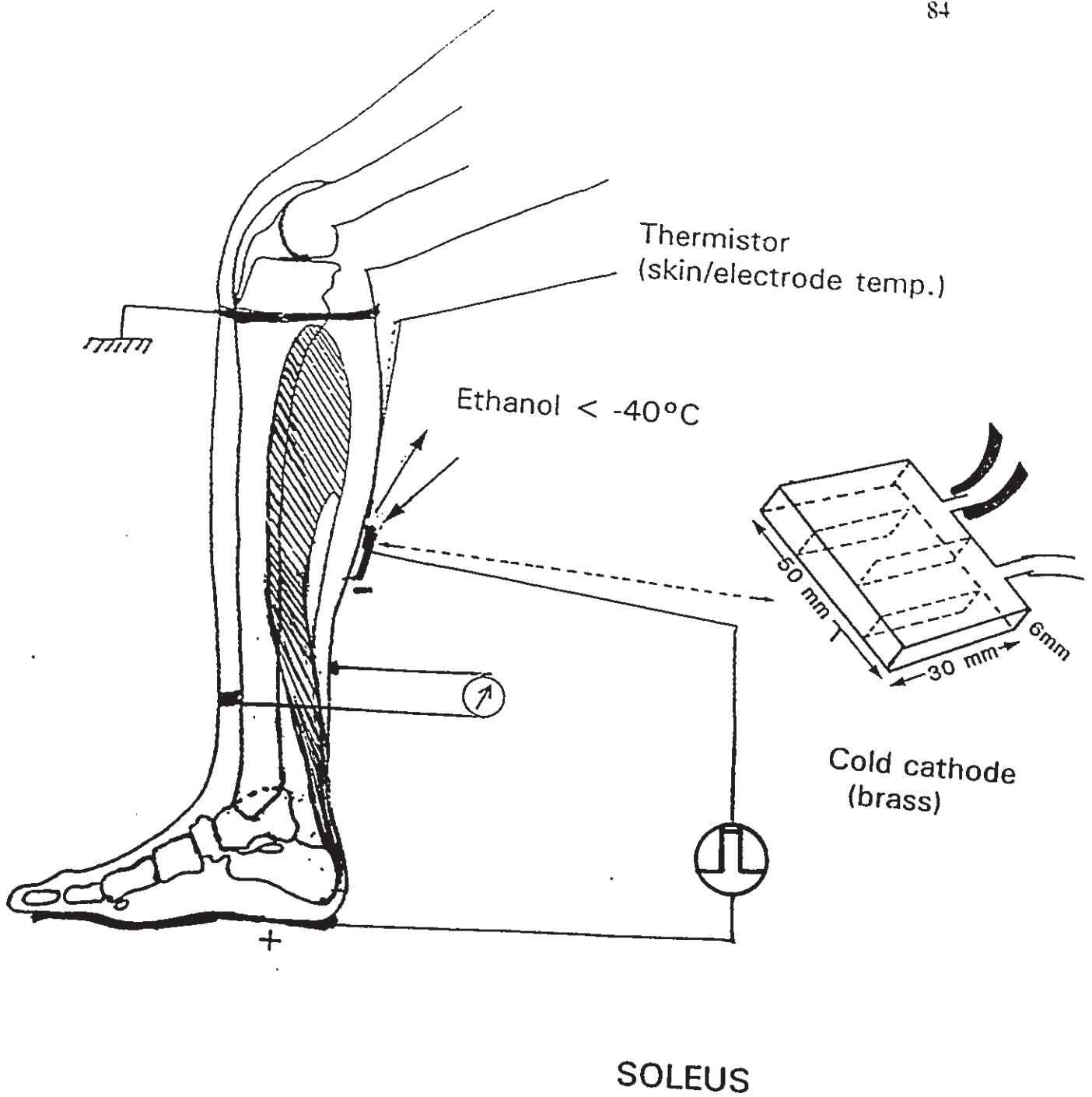
Figure 7 Schematic of system used to cool cold cathode. Ethanol was cooled to -40°C or lower.



The pain of this type of stimulation was alleviated using cold anaesthesia (see previous Section). The best stimulating arrangement was to place the cold cathode just inferior to the V-shaped notch formed by the medial and lateral gastrocnemii, the soleus muscle being quite superficial and therefore accessible at this point. To avoid significant simultaneous activation of the medial or lateral gastrocnemius muscles, the anode was placed on the sole of the foot so as to direct the current flow away from these muscles. Figure 8 shows the location of the cathodal and anodal electrodes. The latter was composed of a series of interconnected, 4.7 cm by 10.2 cm carbon-impregnated rubber electrodes (Medtronic, Inc.). Pilot studies were undertaken to determine how much of soleus was being stimulated with this arrangement. Comparison of peak-to-peak M-wave responses using single shocks applied to the tibial nerve and through the percutaneous circuit, indicated that 30 to 50% of the total soleus muscle mass was being activated by the latter method. Since the tibial nerve stimulation would have evoked responses in the gastrocnemii, tibialis posterior and flexor digitorum longus, all of which might have contributed to the M-wave recorded over soleus, the percentage of soleus activated by percutaneous stimulation was probably underestimated. The muscle mass activated percutaneously remained constant.

The proximity of the cathodal electrode to the recording circuit led to contamination of the M-wave response by stimulus artifact. Various recording

Figure 8 Schematic representation of stimulation and recording location for plantarflexor studies. A sketch of the cold cathode is shown in the insert.



arrangements were tried, including the generation of a compensating pulse by feeding the stimulus pulse through a differentiating R-C circuit (Hultman and Sjöholm, 1983). This circuit was intended to nullify the stimulus artifact in the electrical recording trace; the procedure turned out to be unsuccessful. An acceptable response was obtained, however, by using monopolar recording, the active (or stigmatic) electrode being placed distal to the cathode and just superior to the soleus musculotendinous junction. The reference electrode was placed on the skin over the lower one-third of the tibial shaft. The rationale was that current would flow between the stimulating electrodes in the longitudinal axis of the leg, the stigmatic and reference recording electrodes, lying on the same transverse axis, would be on the same isopotential contour and the stimulus artifact would be reduced. Both electrodes were chlorided silver cups filled with conducting cream and had d.c. impedances of 50 to 60 K Ω . The circuit was completed with a 33 cm x 0.5 cm silver strip ground electrode, covered with conducting cream; various positions were tried for this electrode and the most satisfactory one was around the leg at the level of the tibial tuberosity. A schematic representation of the recording circuit is shown in Figure 8.

2.5.3 Protocol for plantarflexors

Single shocks of increasing amplitude were applied until a plateau was reached in the peak-to-peak amplitude of the soleus M-wave. If the plateau was

maintained when the stimulus was increased by 10 to 15 volts, then the upper value was accepted as supramaximal activation for the distal part of the muscle. The leg was secured in the torque measuring system described previously, with the knee at 90° and the ankle at 15° of dorsiflexion. The latter angle was chosen on the basis of the length-tension curve for the ankle plantarflexors (Sale et al., 1982) and corresponded to maximal tension. A solid heel cup was constructed out of hardened plasticized material (Sansplint), padded with high density foam and fixed to the foot plate in order to secure the heel. The heel was held in the heel cup by Velcro strapping which was riveted to the Sansplint.

The same stimulation cycle was used for soleus as for tibialis anterior. Since the tibialis anterior response decremented most after stimulation with the 10/30 Hz combination, this was used for comparison with soleus. Five subjects from the initial ten volunteered for the soleus studies. One run, under ischaemic conditions, was performed on each of the subjects.

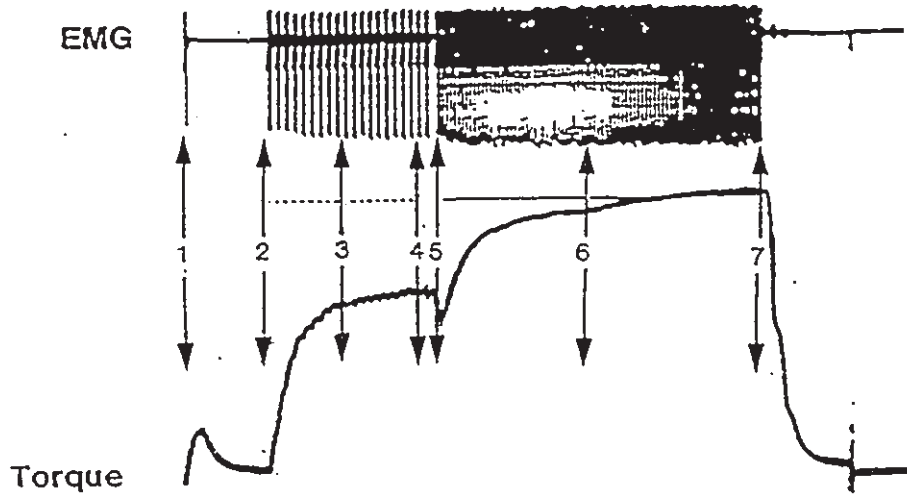
2.6 M-wave and torque sampling and analysis

The purpose of this study was to observe differences in the response of muscles to various excitation frequencies under various conditions. It was considered that the accuracy and objectivity of the measurements would be enhanced if they could be made and analyzed by computer. To do this, a program was developed which permitted sampling and analysis at seven different

points of both the EMG and force signals during one 10 s cycle. Computer software was developed on a Texas Instruments microcomputer (Texas Instruments, Inc.), interfaced with a Tecmar LAB Master board containing a 12 bit A/D convertor capable of maximum sampling frequencies of 40 KHz. Signals were analyzed off-tape.

The points at which the fatigue and recovery cycles were sampled and analyzed are shown in Figure 9. Identification of the sampling loci was possible through the simultaneous storage of the Digitimer pulses on a separate tape-recorder channel. Different software counters were written, such that the computer recognized the appropriate number of pulses and then triggered sampling and storage of the appropriate EMG and torque responses. The sampling rate used was 2.5 kHz (400 μ s/sample) and the numbers of samples collected were determined by calculating the different stimulus intervals for each run. As an example, for 10 Hz tetani, the stimulus interval would be 100 ms. and therefore, 250 samples would be collected. Aliasing errors occur if a signal is sampled at too low a frequency. Aliasing errors were prevented through the development of another algorithm in which wave-forms were sampled at different rates. The peak-to-peak amplitude and area of the responses were then compared with those obtained with a sampling rate of 10 kHz (100 μ s/sample). Figure 10 indicates that the sampling rate could have been as low as 800 Hz (1250 μ s/sample) before the wave-form was significantly affected as evidenced by the

Figure 9 M-Wave and Torque points sampled and analyzed during fatigue and recovery.

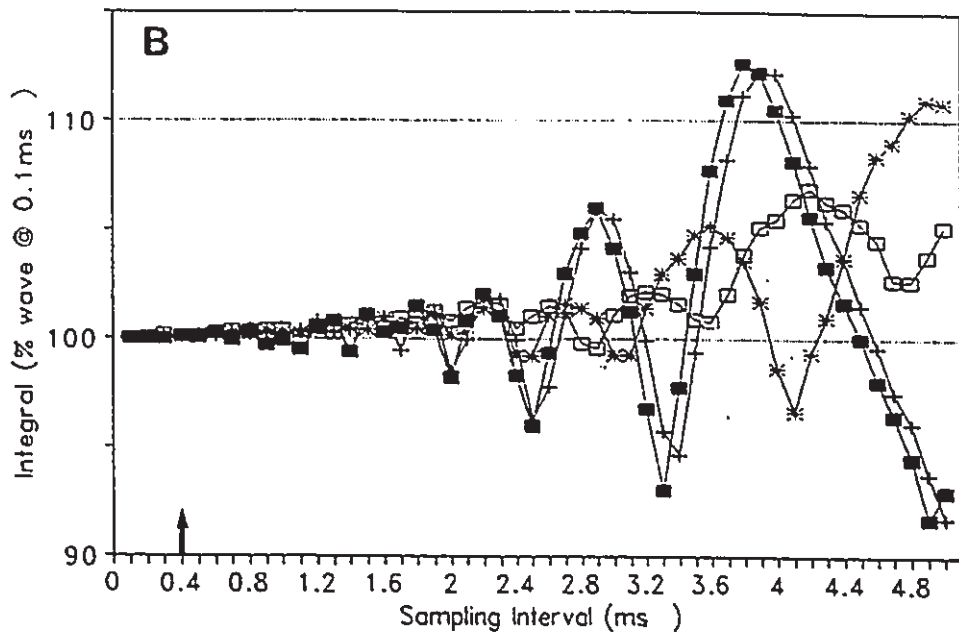
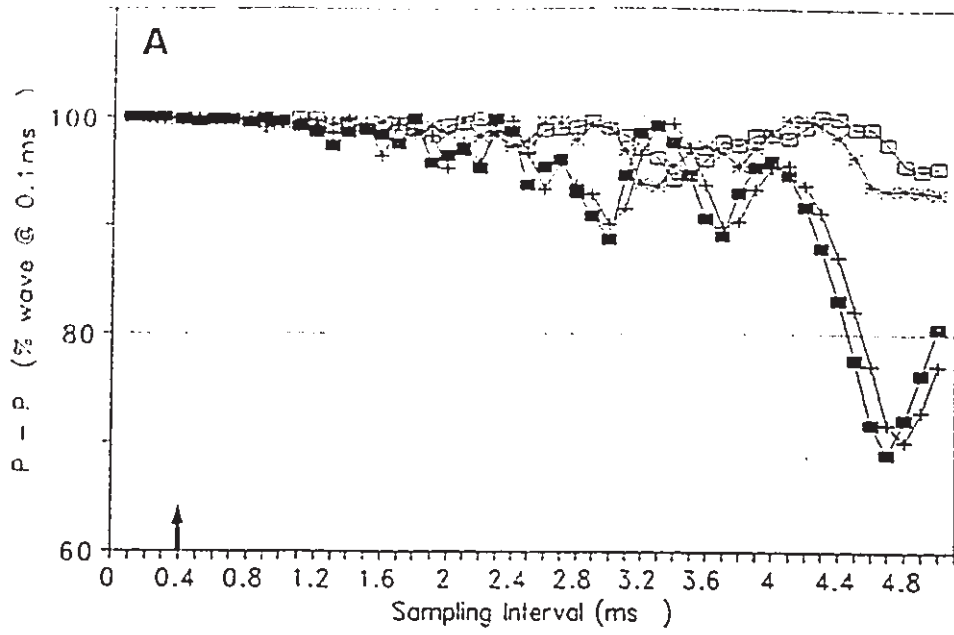


Sampling Points During Fatigue Cycle



Sampling Points During Recovery Cycle

Figure 10 Effect of increasing sampling interval (and therefore decreasing the sampling rate) on wave-form distortion. The P-P (A) and Integral (B) were compared with those analyzed using the shortest sampling interval ($100 \mu\text{s}/\text{sample}$). The arrows indicate the sampling interval which was selected for this study. The four graphs represent sampling periods at the middle and end of the test and fatiguing tetani.



—■— Beg. test tet. —+— Mid. test tet. —x— Mid. fat. tet. —□— End fat. tet.

increasing variability in the waveforms as the sampling rate decreased. Only seven periods in each cycle were chosen for sampling and analysis. The reasons for this were twofold: 1) the unavailability of direct memory access precluded sampling periods longer than six seconds; anything longer than that exceeded the buffer capacity available; 2) the acquired files had to be sufficiently small to allow the analysis to take place during the three second rest period (in the fatigue cycles), sampling could then continue without starting and stopping the tape recorder for every cycle.

M-wave peak-to-peak amplitude and area (voltage-time integral) were analyzed together with torque amplitude following sample collection and storage of each cycle. Twitch torque amplitude, contraction time and half-relaxation time were also calculated. Results were calculated both in absolute terms and as a percentage of the control value. The latter were used for all figures and statistical analyses. A complete printout of the software program is given in Appendix I.

2.7 Statistical treatment

Repeated measures analysis of variance designs were used, in which M-wave changes at selected time points were averaged and compared. A two-way ANOVA was used for most of the treatments. Post-hoc Tukey A tests were performed in instances of significant F-Ratios to test selected differences between means.

RESULTS

3.1 Introduction

The results obtained in this study fall naturally into four sections. The first of these deals with the effects of fatiguing stimulation at different frequencies on the size of the compound action potential evoked in the tibialis anterior.

The second section describes the time-course of the recovery of the compound action potential following cessation of tetanic stimulation at each of the different frequencies.

The third section evaluates the contribution of ischaemia to the observed changes in the compound action potential during and following tetanic stimulation at given frequencies.

The fourth section compares the changes in the compound action potential, during tetanic stimulation, between a fast-twitch muscle, the tibialis anterior, and the soleus, a slow-twitch muscle.

Although the primary object of the study was the excitability of the muscle fibres, as reflected in the amplitude and area of the compound action potential, observations were also made on the forces generated by the muscle. The purpose of the latter observations was to provide an estimate of the degree of fatigue which was present at any given instant during the course of the

experiment. In this sense, the force (torque) measurements served as a biological reference for any changes in the action potential.

Seven time points were sampled in each stimulus cycle during fatigue. Briefly, the time points occurred at the beginning of the cycle (M-wave 1), at the beginning of the testing tetanus (M-wave 2), the middle and end of the testing tetanus (M-waves 3 and 4 respectively), the beginning of the fatiguing tetanus (M-wave 5) and at the middle and end of the fatiguing tetanus (M-waves 6 and 7 respectively). Measurements were made of both action potential area and amplitude of each action potential and thus, a large amount of data was collected in each experiment. In fact, regardless of the fatiguing stimulus frequency used, over 1,300 measurements of action potential and torque were made in each run. To simplify matters, the responses at some time points have been given greater weight than at others. Thus, the action potential at time point 2 (start of the testing tetanus) would be expected to be similar in amplitude and area to that at time point 1 (single shock at start of the cycle). Similarly, the action potential at time point 5 (start of the fatiguing tetanus) would be expected to resemble that at time point 4 (end of the testing tetanus). In practice, these predictions were fulfilled (see, for example, Figures 13 & 14) and consequently the responses at time points 2 and 5 have been eliminated from reporting and discussion. The responses at time points 3 and 6 were taken midway through the testing and fatiguing tetani and were often different to those at the start of the respective

trains. However, any changes in response were always more marked at the end of each train and for this reason, greater emphasis has been given to the responses at time points 4 and 7.

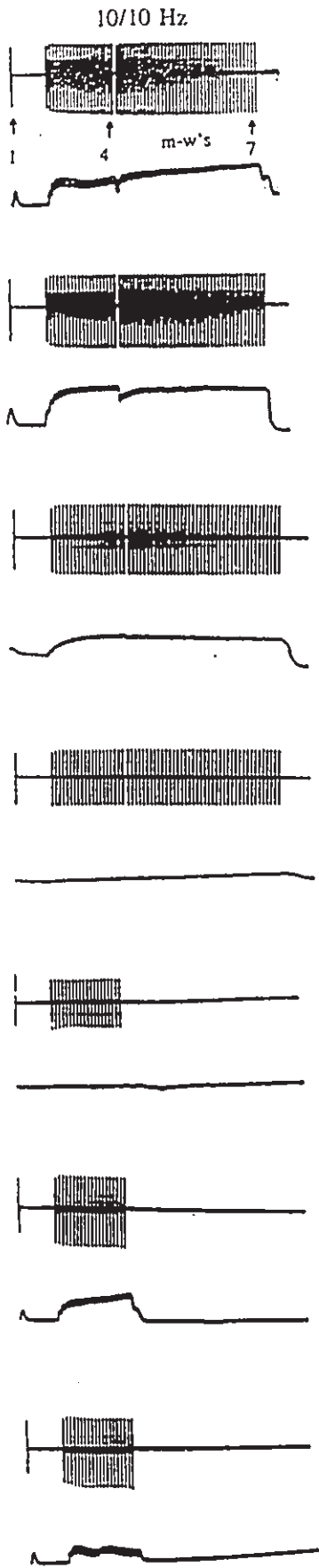
It will also be noted that certain figures appear more than once in Results; this is because a given experiment served more than one purpose. For example, 20 Hz was employed as one of the frequencies of stimulation to be investigated, and also as the stimulus frequency for the observations on the effects of ischaemia.

In view of the complexity of the data, a simplifying statement concerning the results of a particular experiment is given before the detailed presentation and analysis of the results. In addition, oscilloscope traces of a typical experiment have been provided at the outset, together with a qualitative description of the changes in muscle compound action potential and torque, so as to provide a sense of perspective for the remainder of Results.

3.2 Qualitative changes in electrical and mechanical responses during fatigue

Figure 11 compares the results of two fatiguing experiments made on the same subject on different days. In each case, the testing stimuli were identical, consisting of a single shock followed by a 2s tetanus at 10 Hz. In the

Figure 11 Oscilloscope traces at different points of the fatigue and recovery runs. A comparison between M-wave and torque behaviour during stimulation using the 10/10 Hz pair and 10/30 Hz pair. Top traces: EMG; bottom traces: torque (Subject LA ♀, Age: 20)



CONTROL

"CUFF ON"

↓ ↓

30 s

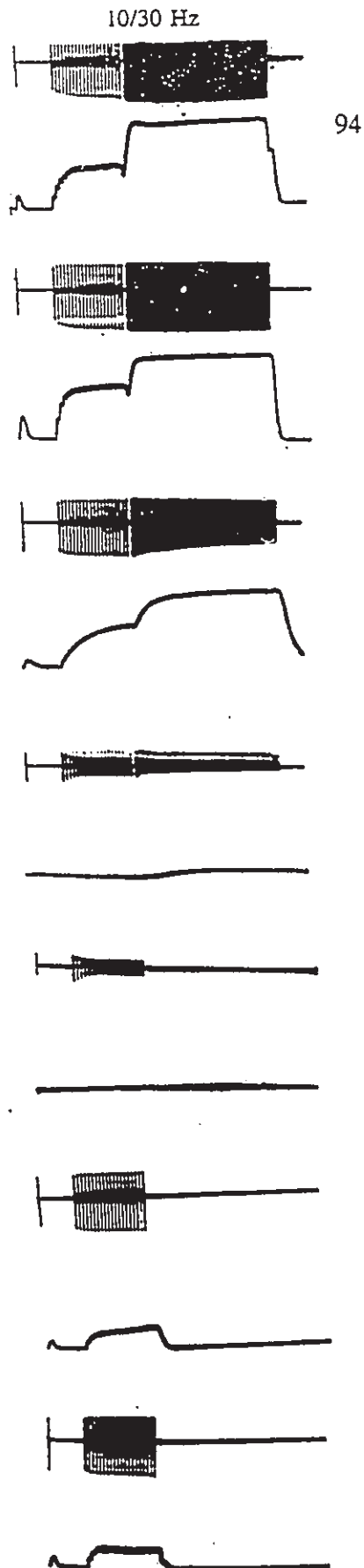
2'0"

3'20"

4'00"

5'0"

10'00"



experiment shown on the left, the fatiguing stimuli were given at 10 Hz for 4 s, while in the experiment depicted on the right, the fatiguing stimuli were delivered at 30 Hz for 4 s. A small break in the EMG responses (top traces) marked the transition from the testing tetanus to the fatiguing tetanus.

The control responses were not always identical on different days. For example, if the results shown at the top of the left and right columns are compared, it can be seen that, although the twitches were similar, the 10 Hz testing tetani developed appreciably greater torque in the results on the right than on the left. The cause of this variability was not obvious since every attempt was made to ensure that the stimulating, recording, and leg fixation arrangements were identical on both occasions. Possibly the use of the leg before the experiment was a factor. Such variations in the control data reinforced the need to normalize the responses of each experiment rather than to present the data as absolute values.

In the control results at top right, there is an increment in the EMG responses during the testing tetanus which continued after the force had reached a plateau. This could not have been a mechanical artifact; rather, it is an example of the M-wave potentiation noted in some experiments.

The record at top right shows that the torque developed during the 30 Hz tetanus was approximately twice that generated at 10 Hz and this would be expected from the shape of the force/stimulus frequency curve of the ankle

dorsiflexor muscles (see, for example, Marsh et al., 1981). In both the top left and top right records the torque declined rapidly at the end of the fatiguing tetani; the notch in the record is attributed to a flexor reflex.

As the two experiments proceeded, there was initially potentiation of the twitch, shown best in record at 30 s (right), in which the force developed was approximately twice that in the control period. In the 30 s record at left, the responses during the 10 Hz testing stimuli became more fused, allowing greater tetanic torque to be developed than in the control period; this change reflects the slowing of the twitch relaxation phase (Bigland-Ritchie and Woods, 1984; Fitch and McComas, 1985; Cooper et al., 1988)

By 2 min, fatigue had begun in both experiments causing reduction in the torques generated during the twitch and in the testing and fatiguing tetani. In addition, the rate of rise of torque at the onset of each tetanus was reduced; the rate of relaxation at the end of the fatiguing train was also decreased. These changes in the time course of force development and decline were typical of fatiguing muscles and have been well described (for reviews see Edwards, 1981, 1983; Bigland-Ritchie, 1981, 1986; Gibson and Edwards, 1985; Green, 1987; MacLaren et al., 1989). The preservation of EMG responses in the records at 2 min (left), despite the decline in torque, suggested either excitation-contraction uncoupling or failure of the contractile apparatus. This also is a well recognized feature of muscle fatigue (Eberstein and Sandow, 1986; Cooper et

al., 1988; Gibson et al., 1988; Stokes et al., 1989). In the records at 2 min (right), the EMG responses during the fatiguing stimulation at 30 Hz are beginning to decline.

As the experiments proceeded the twitch and tetanic torque disappeared completely although EMG responses were still present, as shown in the left and right records at 4 min. Although the fatiguing tetani had stopped by this time, there was no recovery of torque. Recovery began, however, after the ischaemic cuff was released and as can be seen in the left and right records at 5 min. At 10 min., however, a further decline in torque is evident; this was an example of the low-frequency fatigue which can persist for up to 24 hrs (Edwards et al., 1977). The EMG responses, by contrast, had recovered to control values.

3.3 Effects of different fatiguing stimulus frequencies on the M-wave of ankle dorsiflexors during ischaemia

3.3.1 Conclusions

Enlargement of the M-wave at the initiation of fatiguing stimulation was absent at low frequencies of fatiguing tetanic stimulation (5 Hz, 10 Hz), and was only 110% of the control value at higher frequencies (20 Hz, 30 Hz). As fatiguing stimulation proceeded, the M-wave showed no significant change at 5 Hz and declined in both amplitude and area at higher frequencies. The decline

was greater in amplitude than in area and was more marked at the higher frequencies.

3.3.2 Initial Changes in the M-wave

Potentiation of the M-wave during the first minute of tetanic stimulation has been reported previously in human ankle dorsiflexor muscles (Fitch and McComas, 1985; Garland *et al.*, 1988a) and was therefore looked for in the present experiments. In addition to measuring the peak-to-peak amplitudes of the M-wave responses, the voltage time areas were also studied. Simultaneous increases of amplitude and area would be expected in true potentiation while increases in amplitude, but not in area, would suggest better synchronization of the muscle fibre action potentials. Conversely, a reduction in amplitude but not in area would suggest asynchrony of muscle fibre action potentials and/or changes in the threshold of individual fibres.

The results for one subject are shown in Figures 12 and 13 for fatiguing stimuli of 10 Hz and 30 Hz respectively; this subject was the one from whom the results shown in Figure 11 were obtained. During 10 Hz fatiguing stimulation, all M-waves declined in amplitude, though not in area, after the first minute. At a higher frequency of fatiguing stimulation (30 Hz: Figure 13) there were considerable increases in the areas of M-waves 6 and 7 during the first 2 min, but not of M-waves 1, 3 and 4.

Figure 12 Time course of M-waves 1, 3, 4, 6 and 7 (see METHODS) during fatigue and recovery of 10/10 Hz run. Top figures are P-P and area of the respective M-waves followed over time. Bottom figure shows the corresponding torque value during fatigue and recovery. The first arrow marks the end of fatiguing stimulation: the second - end of ischaemia. (Subject LA ♀, Age: 20).

10 Hz Fatigue Run
Subject: LA

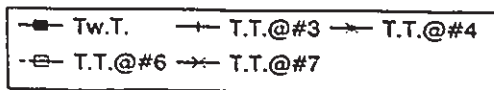
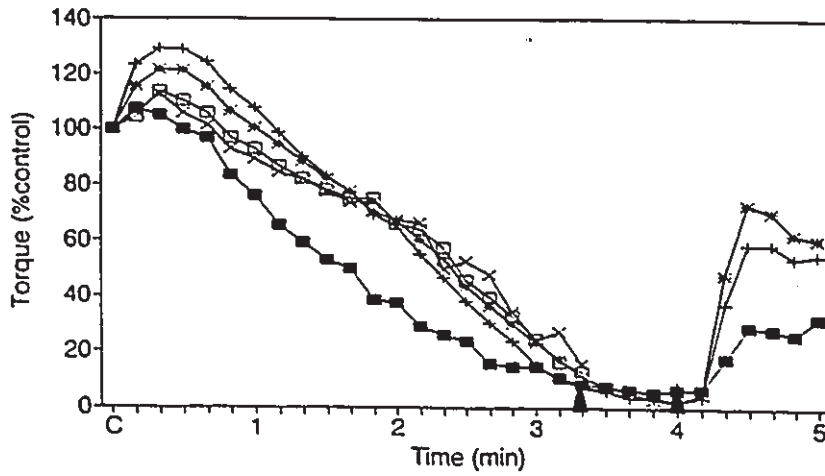
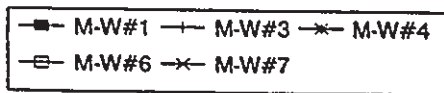
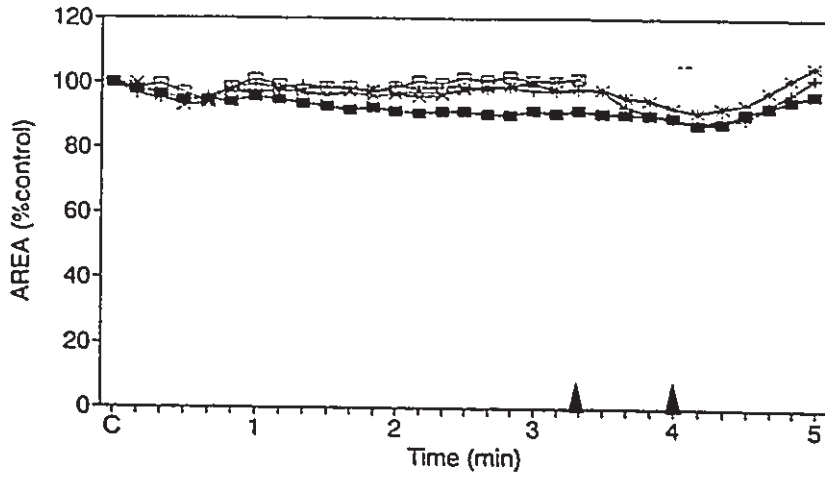
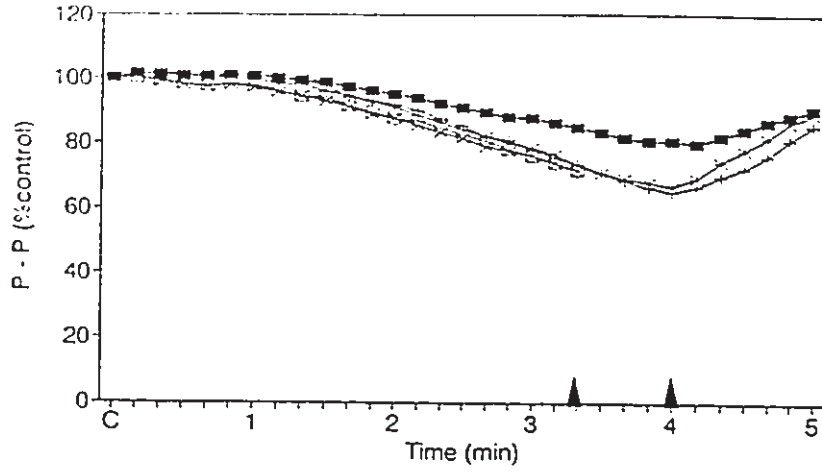
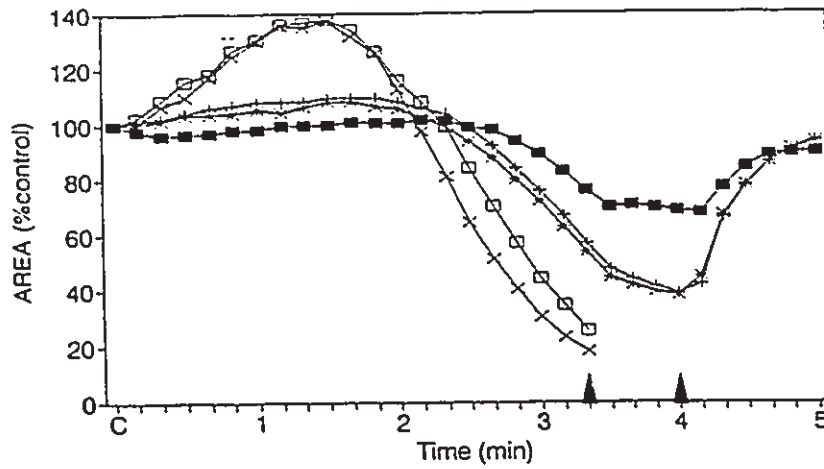
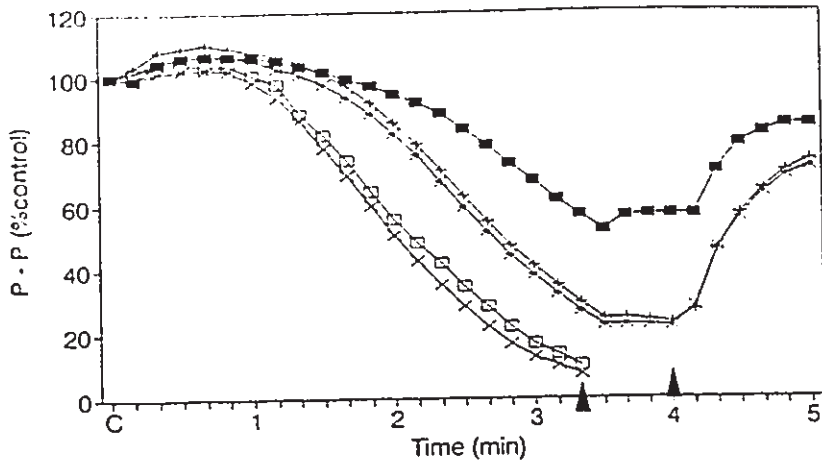


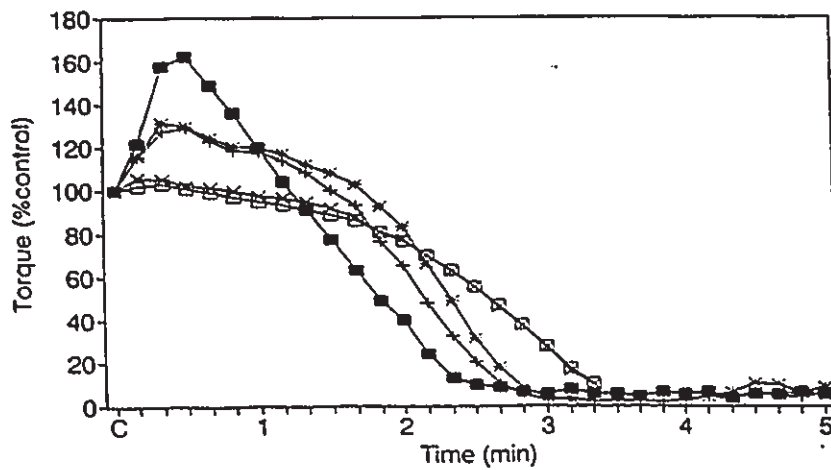
Figure 13 Time course of M-waves 1, 3, 4, 6 and 7 during fatigue and recovery of 10/30 Hz run. See Figure 12 for further explanation. (Subject LA ♀ Age:20).

30Hz Fatigue Run Subject: LA

100



■ M-W#1	— M-W#3	× M-W#4
□ M-W#6	— M-W#7	



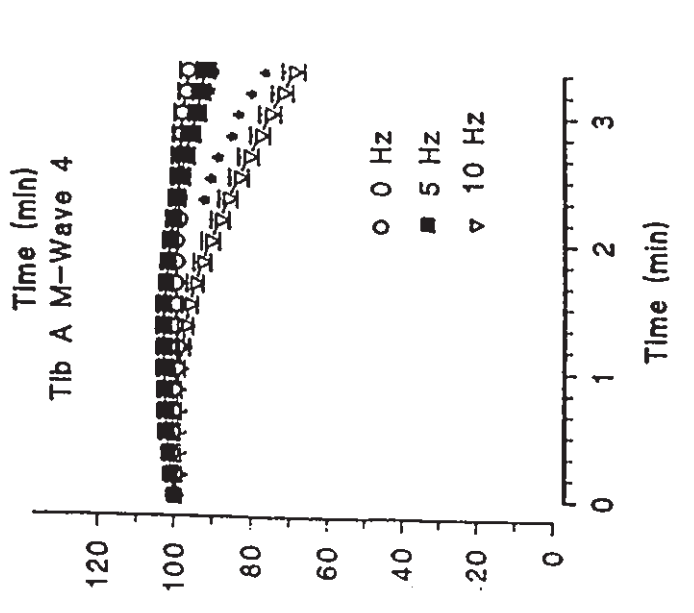
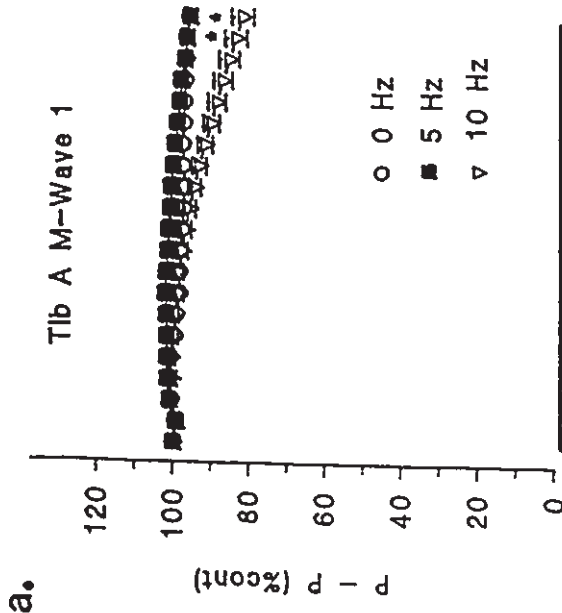
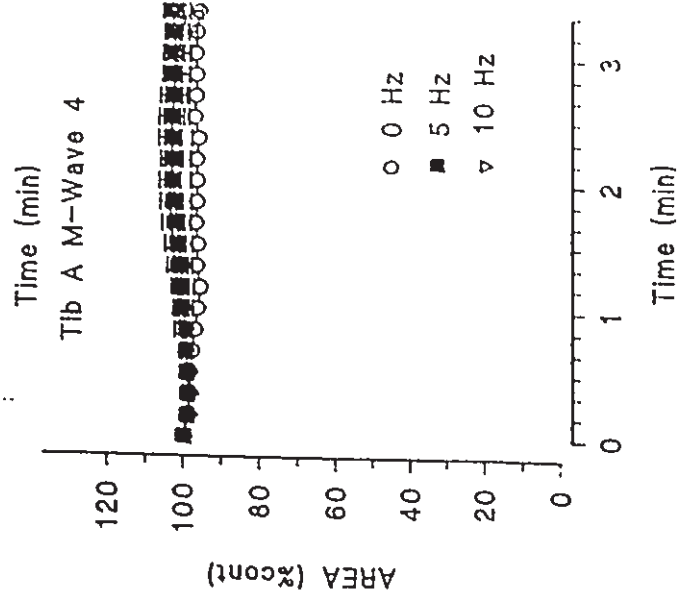
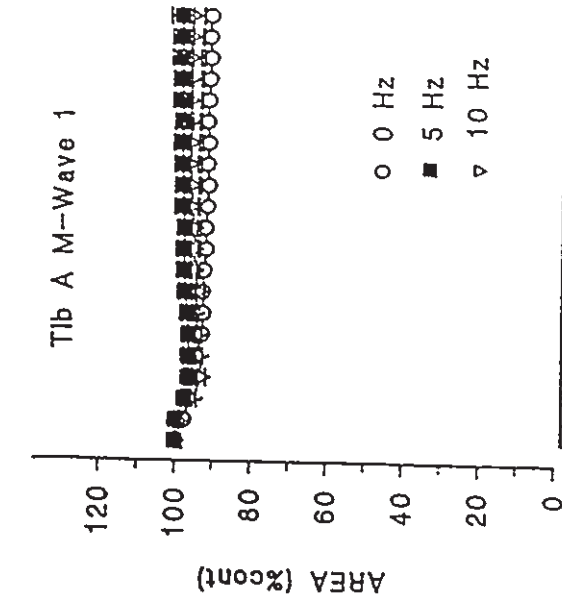
■ Tw.T.	— T.T.@#3	× T.T.@#4
□ T.T.@#6	— T.T.@#7	

The mean values of M-wave parameters for all the subjects are shown for the first 3 min 20 s of stimulation in Figures 14 and 15. As in the results for the single subject, the mean M-wave amplitude was not increased at any of the stimulus frequencies. The mean area of M-wave 4, however, was increased by up to 6.8% at 1-1½ min during 30 Hz stimulation and by rather less at 20 Hz stimulation (Figure 15b). Although individual means were not significantly different from control means, the genuine nature of the increase in area was indicated by the consistency of changes at successive time points during the critical period and by the similarity of the curves for 20 Hz and 30 Hz stimulation. As in the results for the single subject given in Figures 12 and 13, the mean changes in M-wave 7 (at the end of the fatiguing tetanus) were still larger than those in M-wave 4. These are shown in Figure 16. The increase in area was greater during stimulation at 30 Hz than at 20 Hz and was not significant at low frequencies. However, since M-wave 7 was elicited during the fatiguing tetanus rather than the testing tetanus, its properties cannot be justifiably compared between one stimulating frequency and another. Its value lies in emphasizing that significant increases in M-wave area can follow repetitive stimulation. For this reason, the maximal changes in M-wave amplitude and area for each fatiguing stimulus frequency are given in Figure 17 for M-wave 1 only. The increased M-wave areas could have been due to individual muscle fibre action potentials becoming broader possibly due to

Figure 14a P-P and area values for M-wave #1 plotted every 10 secs over the course of ischaemic fatigue. The results for 10/5 Hz (■) and 10/10 Hz (▽) stimulation are plotted in combination with those from the control run where there was no fatiguing tetanus (0 Hz). Values are means \pm standard error.

*Significant difference from resting value ($p < .01$).

Figure 14b P-P and area values for M-wave #4. See Figure 15a for legend.



a.

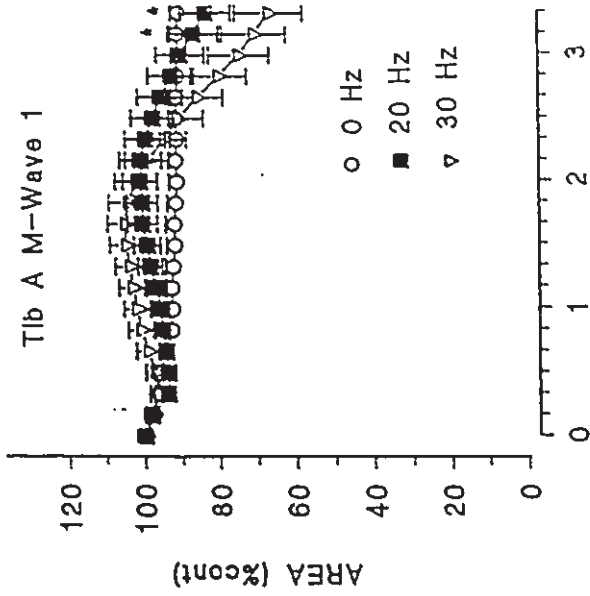
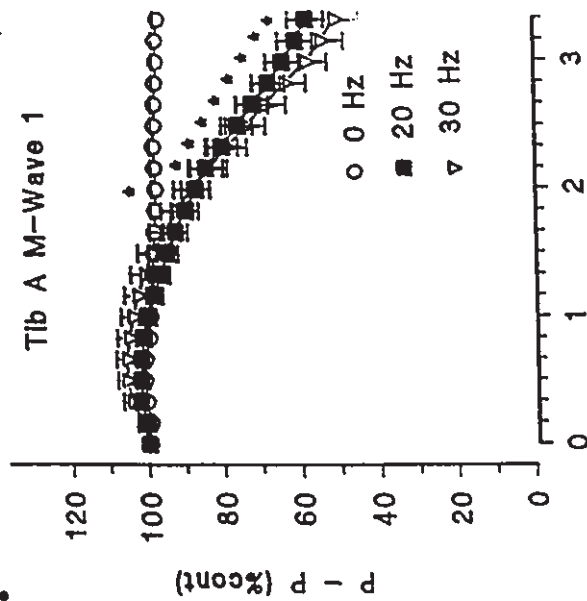
b.

Figure 15a P-P and area values for M-wave #1 plotted every 10 sec over the course of ischaemic fatigue. The results from 10/20 (■) and 10/30 Hz (▽) stimulation are plotted in combination with those from the control run at 0 Hz (○). Values are means \pm standard error.

*Significant differences from resting and control values ($p < .01$).

Figure 15b P-P and area values for M-wave #4. See Figure 15a for legend.

a.



b.

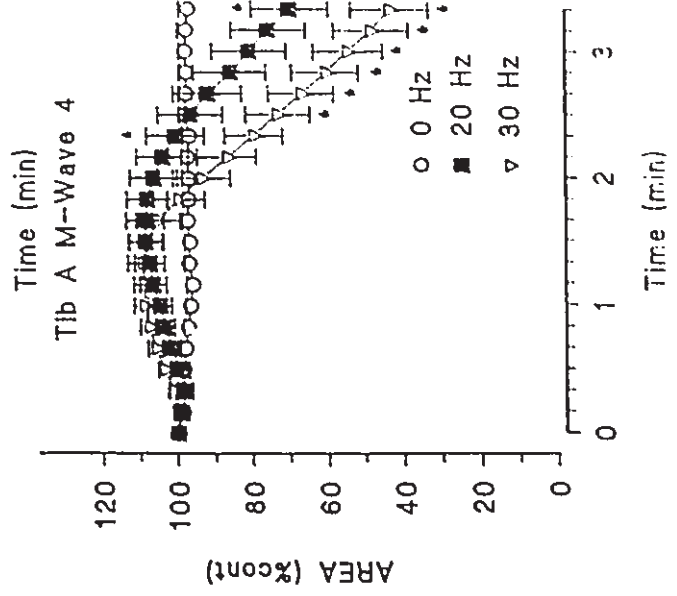
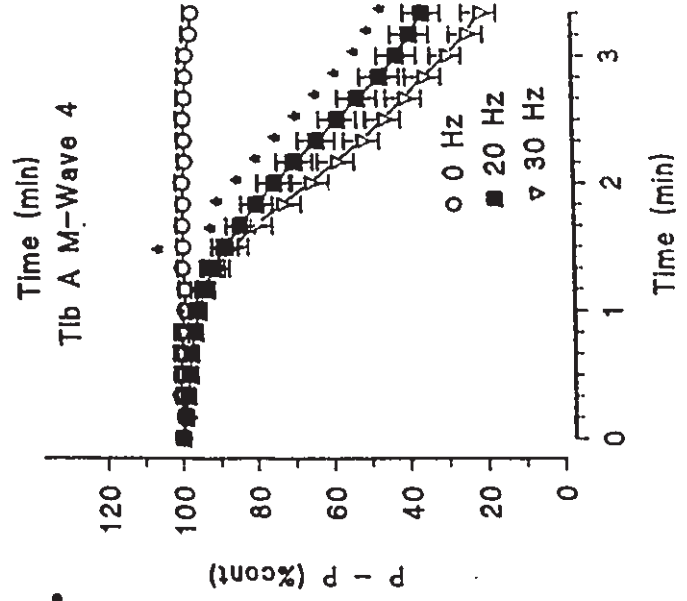
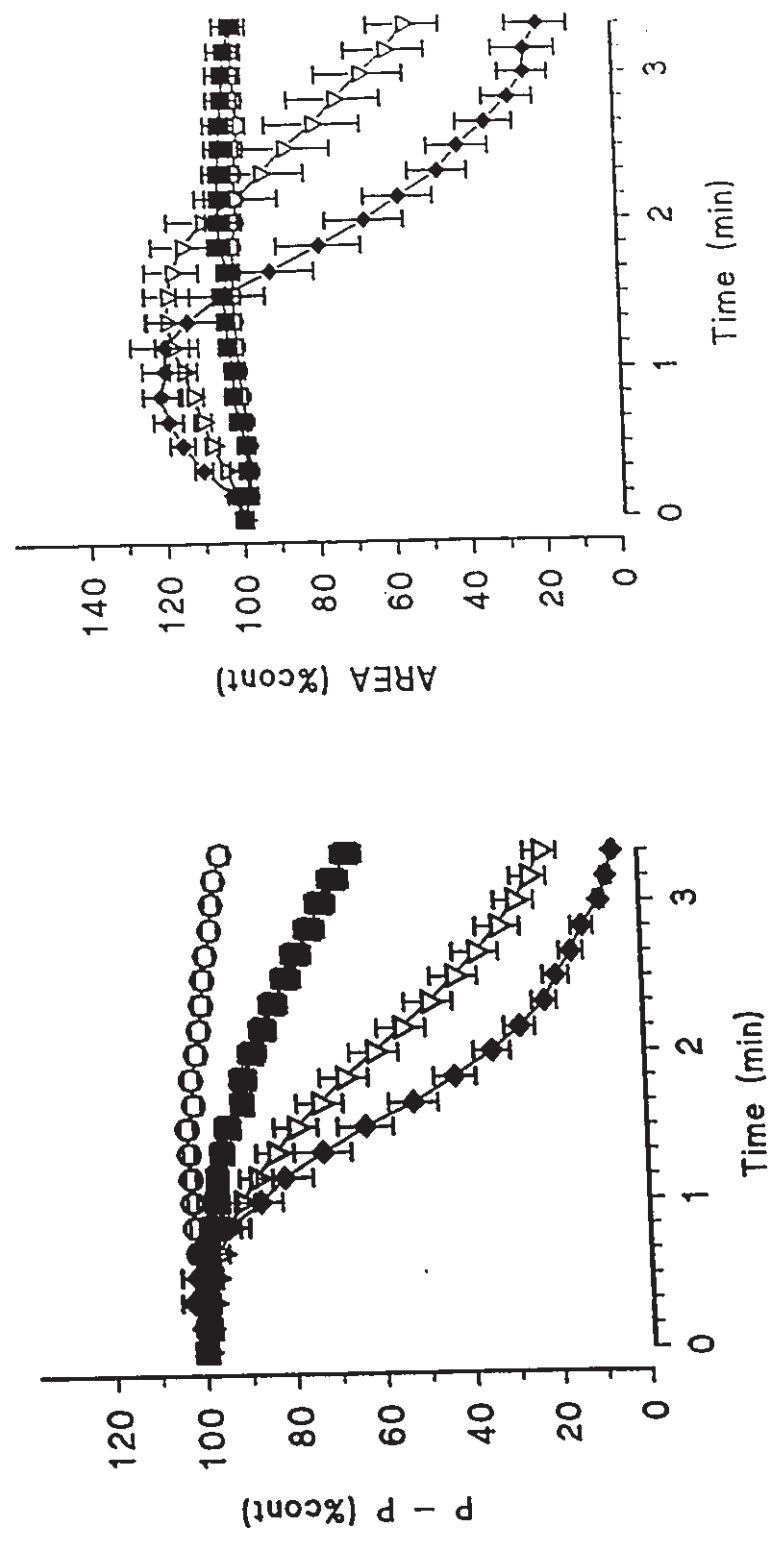


Figure 16 P-P and area values for M-wave #7 plotted every 10 s over the course of ischaemic fatigue. The results for 10/5 Hz (○), 10/10 Hz (■), 10/20 Hz (▽), and 10/30 Hz (◆) are plotted. Values are means \pm standard error.

Tib A M-Wave 7



○ 5 Hz ■ 10 Hz ▽ 20 Hz ◆ 30 Hz ♀

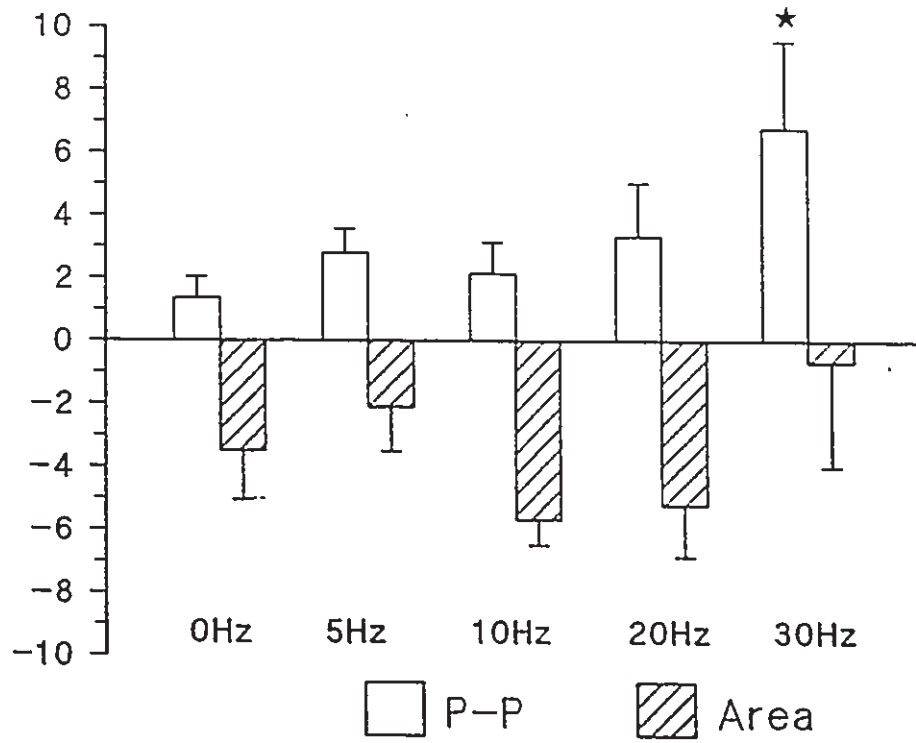
Figure 17a Maximum peak-to-peak potentiation, beyond control (100%), of M-wave #1 observed during the first 2 minutes of ischaemic fatigue, related to the frequency of the fatiguing tetanus. Area values of M-wave #1, at the same time point, are means \pm standard error.

*Significant difference from resting value ($p < .05$).

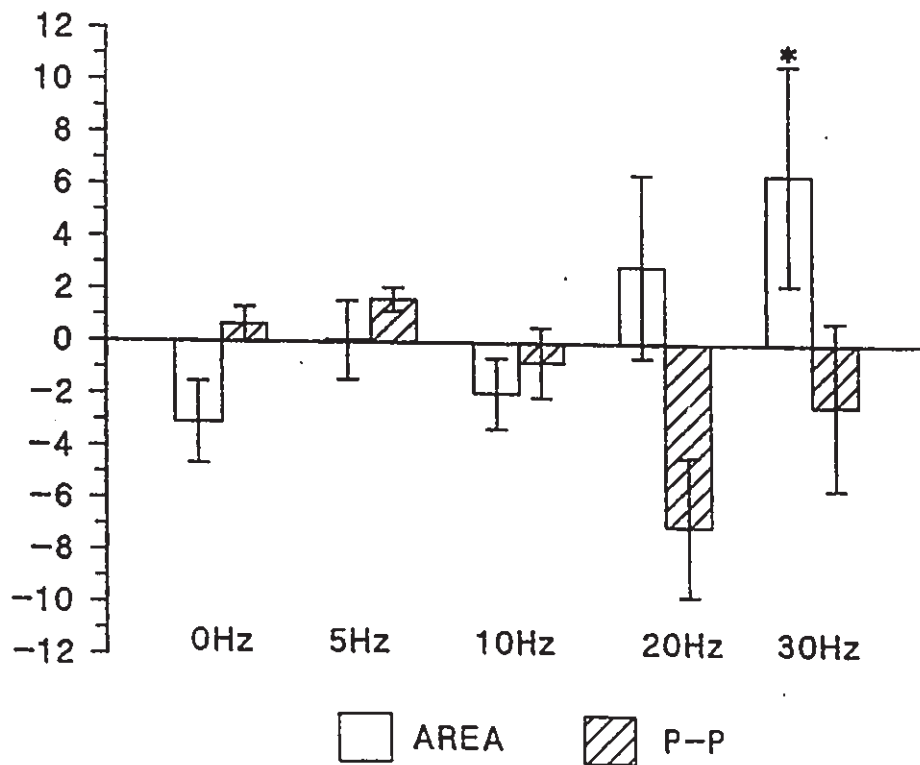
Figure 17b Maximum area potentiation, beyond control (100%), of M-wave #1 observed during the first 2 minutes of ischaemic fatigue. Peak-to-peak values of M-wave #1, at the same time point, are plotted using the shaded bars. Values are means \pm standard error.

*Significant difference from resting value ($p < .05$).

a.



b.



decreases in individual fibre conduction velocities, or to a combination of temporal dispersion and potential loss within the population of muscle fibre action potentials.

Regardless of the frequency of the fatiguing stimulation, the M-wave did not decrease for at least one minute. Indeed, in the case of M-wave 4 during 30 Hz stimulation, the area of the response was maintained at, or above, the control value for two minutes. By this time, almost 1600 action potentials would have been fired in each of the muscle fibres in the presence of ischaemia.

3.3.3 Late changes in the M-wave

Beyond 1 min, fatiguing stimuli at 10, 20 and 30 Hz decreased the amplitude of the M-wave. The decline in M-wave area occurred almost a full minute later than that in amplitude possibly due to the different time courses of the mechanisms causing the initial increase. For example, the loss of individual fibre action potentials began to dominate over fibre asynchrony thereby initiating the decline. The mean changes are shown for the first 3 min 20 s in the entire population of subjects in Figures 14 and 15, and for the complete duration of the experiments in Figures 18 and 19. Although the reductions were greater for M-waves 6 and 7 (as is evident from Figures 12 and 13), the changes in M-waves 1 and 4 were those of greater analytical value, for the reasons given in the previous section, and have been presented in Figures 14, 17, 18 and 19. From

Figure 18a M-wave #1, P-P and Area values during fatigue and recovery with 10/5 and 10/10 Hz stimulation compared to the control run (10/10 Hz). Values are means \pm standard error and are calculated as percentages of the resting value. The two arrows demarcate the period of ischaemic recovery. The second arrow signifies the point of cuff release.

*Significant difference from resting and control values ($p < .01$).

Figure 18b P-P and Area values for M-wave #4. See Figure 18a for explanation.

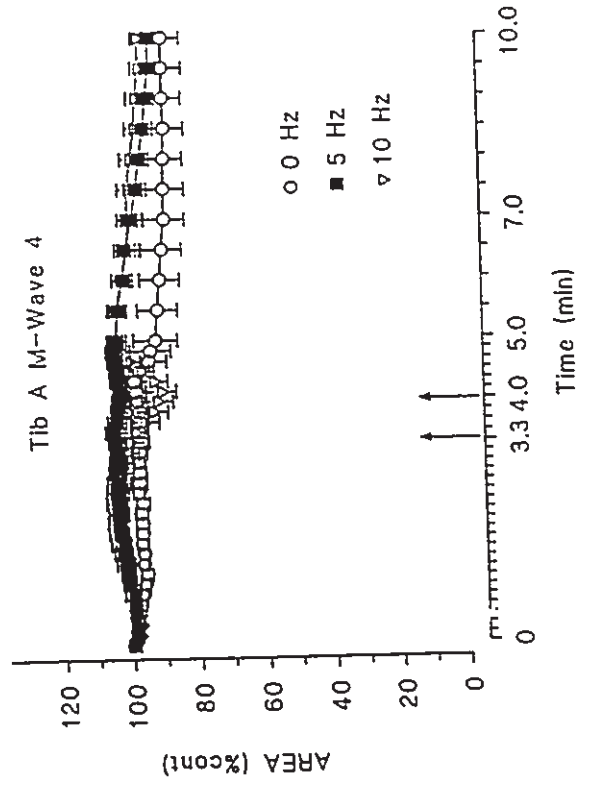
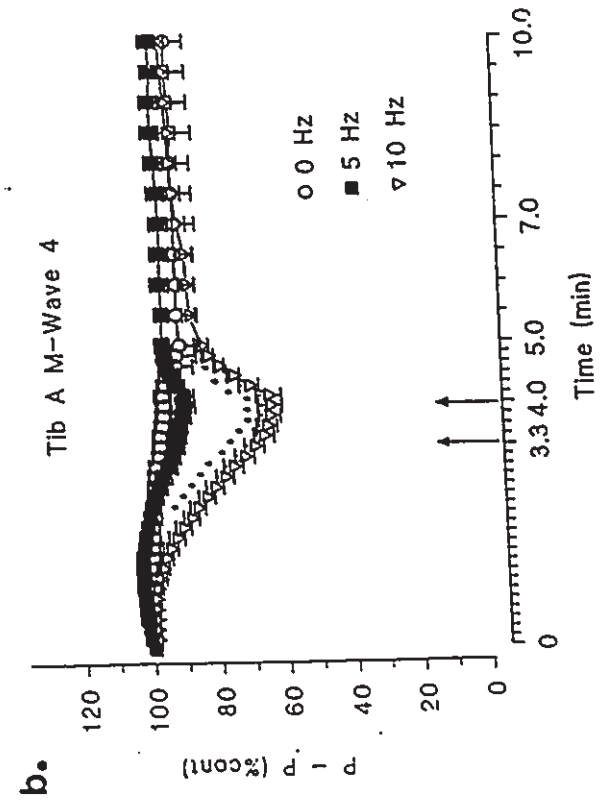
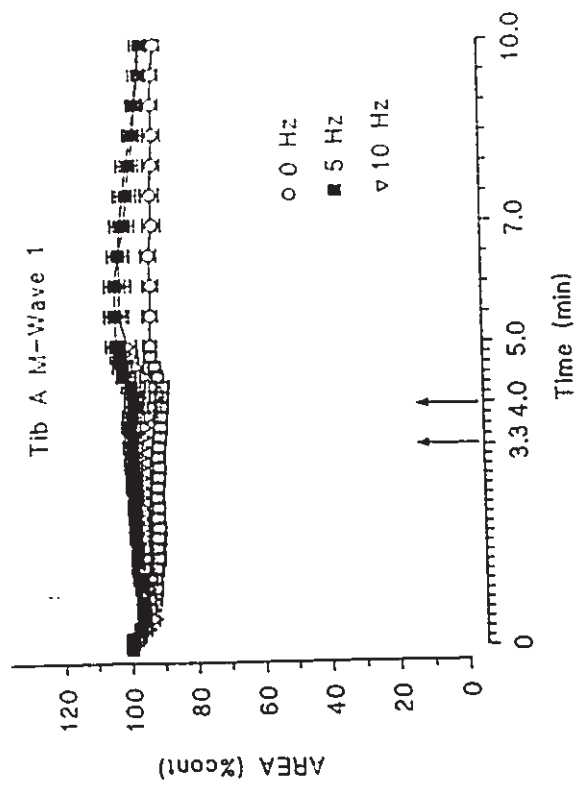
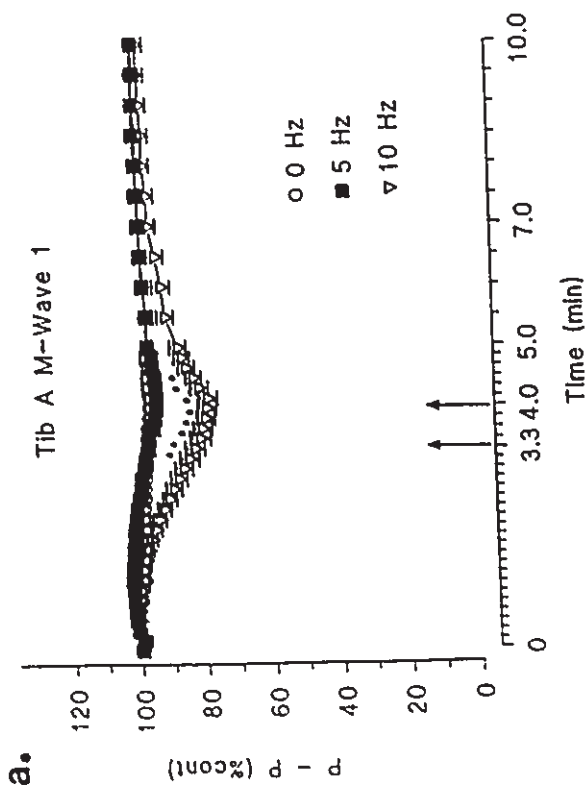
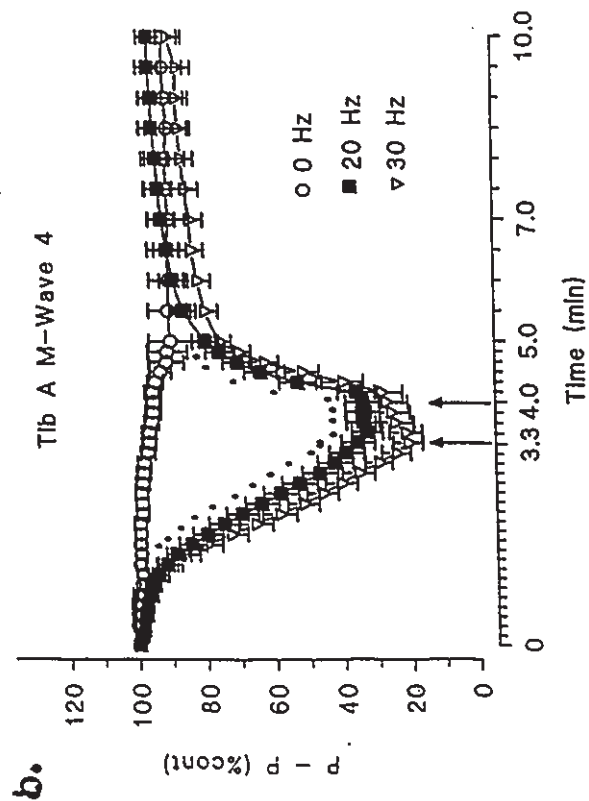
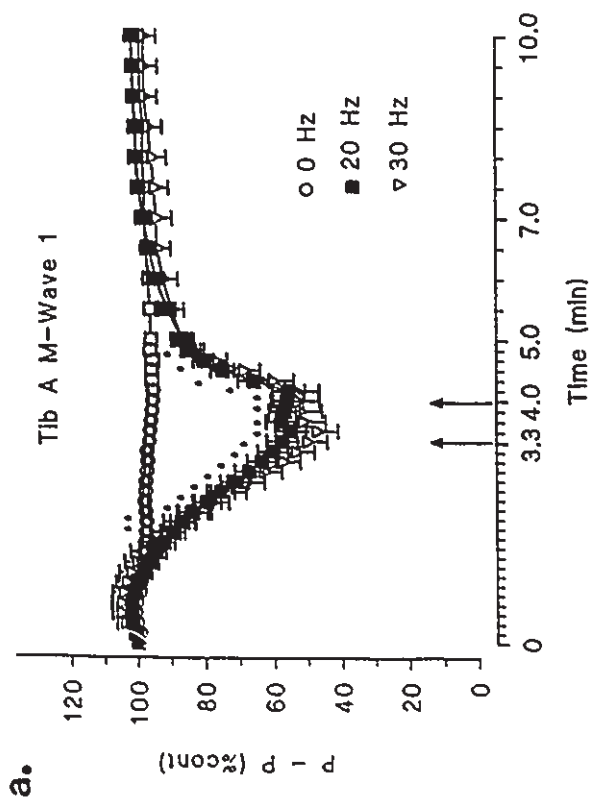
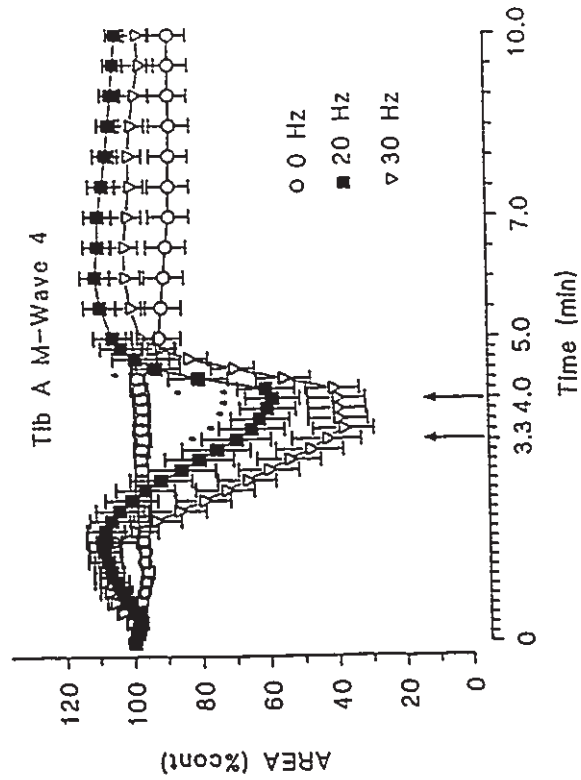
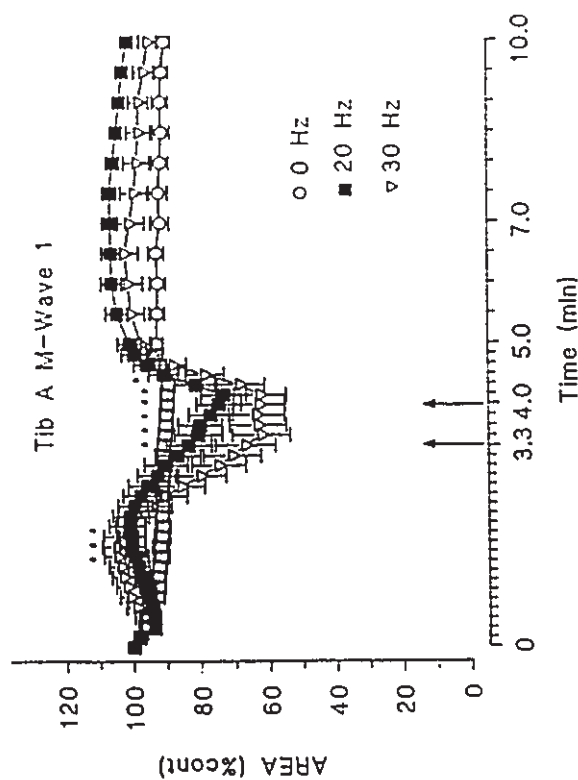


Figure 19a M-wave #1, P-P and Area values during fatigue and recovery with 10/20 and 10/30 Hz stimulation compared to the control run. See Figure 18a for explanation.

Figure 19b P-P and Area values for M-wave #4. See Figure 19a for explanation.



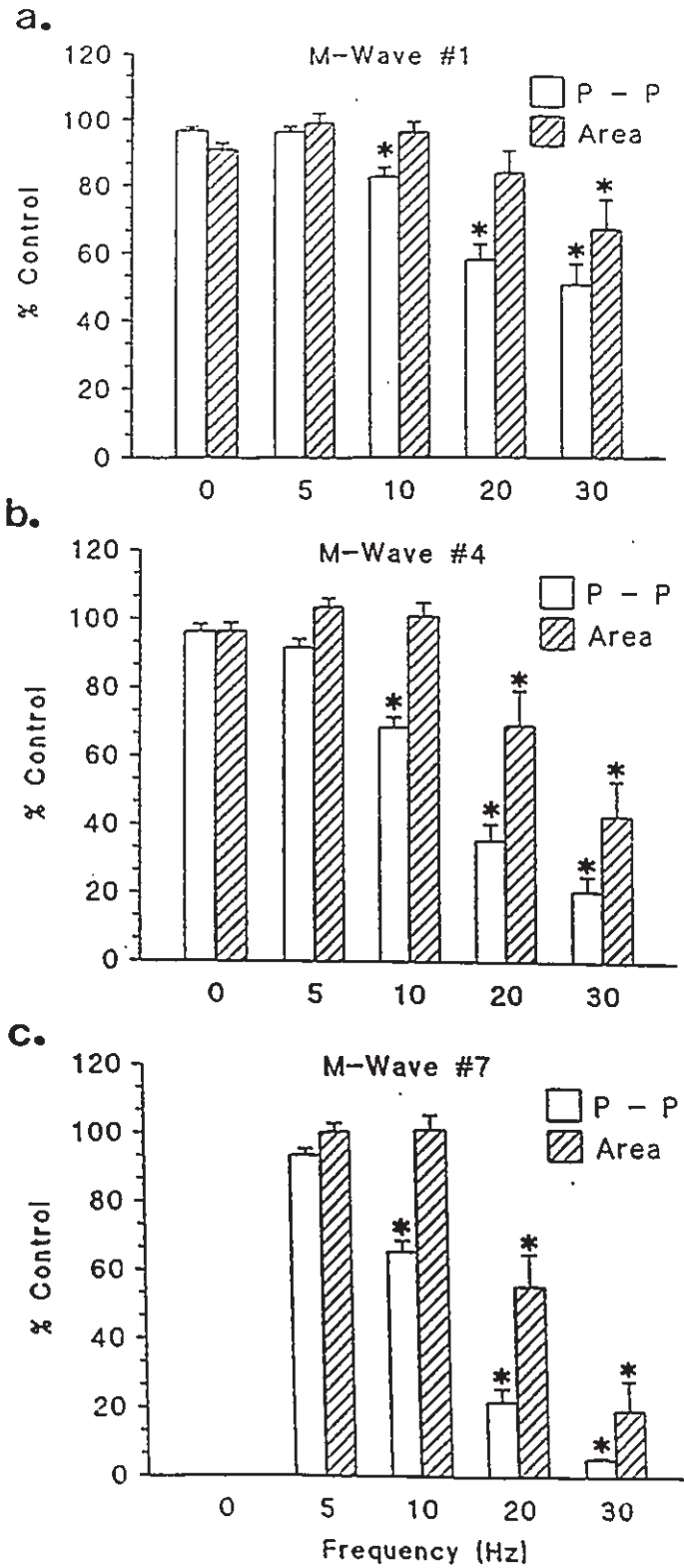
a.

b.

Figure 20a M-wave #1 P-P and area at the end of the fatigue run plotted against fatiguing frequency. Open bars P-P; shaded bars area. Values are means \pm standard error. *Significant difference from resting value ($p < .01$).

Figure 20b M-wave #4 P-P and area at the end of the fatigue run. See legend for 21a.

Figure 20c M-wave #7 (end action potential in fatiguing tetanus) P-P and area at the end of the fatigue run. See legend for 21a.

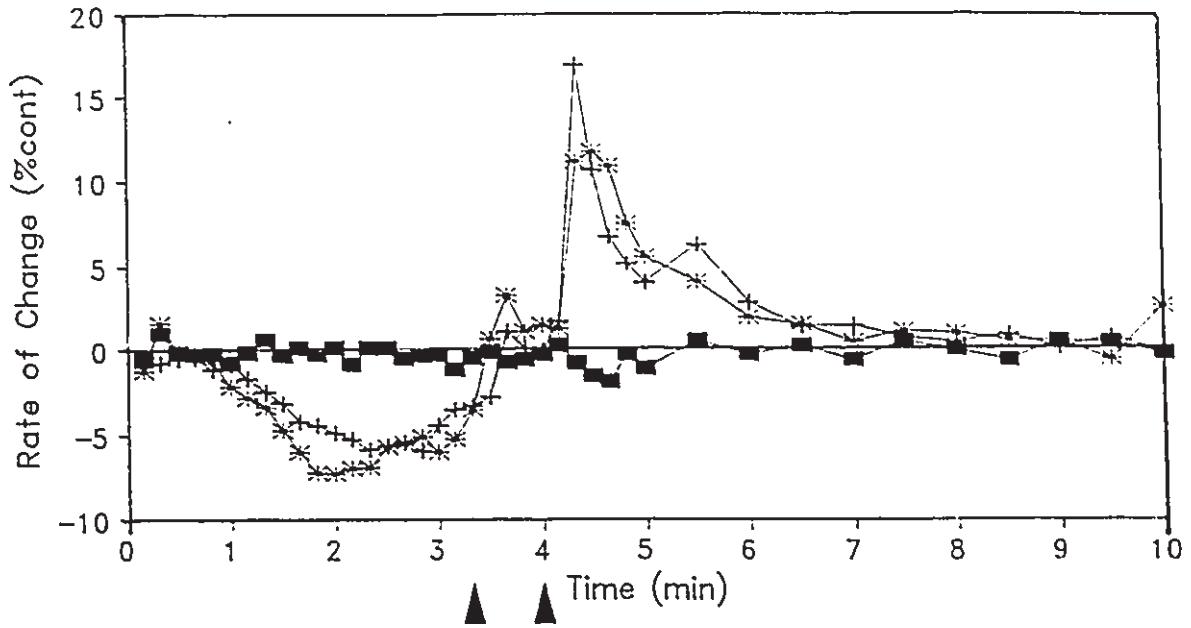


these figures, it appears that the decrease was greater for M-wave 4 than for M-wave 1 and for amplitude than for area. Figure 20 shows that the declines in M-wave amplitude and area were frequency dependent, being greatest when the fatiguing frequency was 30 Hz and least when it was 10 Hz; a frequency of 5 Hz was without effect. Figure 20 also shows that the declines were dependent on the position of the M-wave in the stimulus train. Declines were greatest for M-wave #7 and least for M-wave #1.

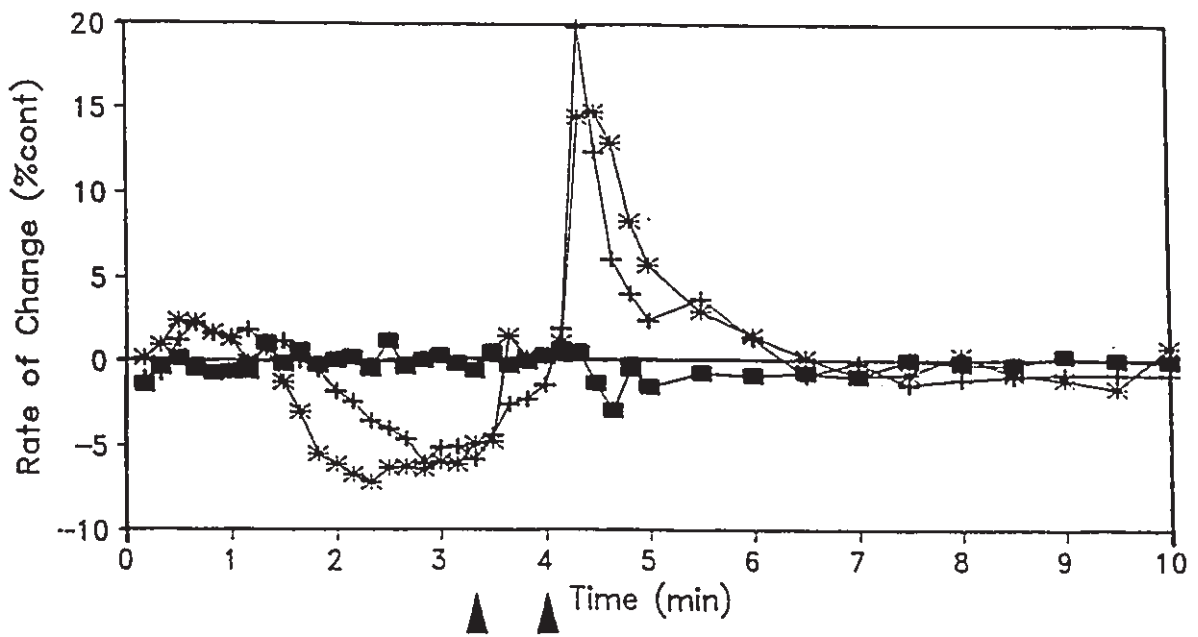
Figure 19 shows that, once it had started, the decline of the M-wave remained nearly linear until the fatiguing stimuli were stopped; the declines were steeper for 30 Hz stimulation than for 20 Hz, especially when area was plotted rather than amplitude. The implication of linearity was largely confirmed by differentiating the M-wave areas and amplitudes with respect to time. The results of 20 Hz and 30 Hz stimulation on M-wave 4 are given in Figure 21. It can be seen that for each experiment there was a period of more than one minute, usually corresponding to the interval between 2 and 3 min on the abscissa, in which the differentiated curve remained nearly horizontal - indicating no change in the rate of decline. It was of interest that the mean amplitude of M-wave 4 was only 21% of control when the fatiguing stimulation ceased, suggesting that the responses might have been completely abolished had the stimulation continued. Indeed, the final value for M-wave 7, following 30 Hz stimulation was only 5% of its initial value (Table 2: for area values see Table 3).

Figure 21 Rate of change (% control) of M-wave #4 P-P and Area during 10/20 and 10/30 Hz stimulation. Both fatigue and recovery values are plotted. The control run (10/0 Hz) was added for comparison purposes. Values are means of the individual subject derived P-P's and areas (n=10). Arrows demarcate the period of ischaemic recovery.

M-Wave #4
P - P



M-Wave #4
AREA



■ 0Hz + 20Hz * 30Hz

Table 2. Extent of M-wave P-P failure for the first, fourth and seventh potential. Significant differences from resting values (Column 3), control values (Column 4) and position (Columns 5 and 6) in tetanic trains are listed (n = 10). Values are mean \pm standard error (*p < .01.).

1	2	3	4	5	6	
Frequency of SE Fatiguing Stimulation	X \pm	(% resting) (100%)	Significance from Rest	Significantly Different from Control (0 Hz)	M-wave #4	M-Wave #7
<u>M-Wave #1</u>						
0 Hz	96.8 \pm 1.0	-	N/A	-	-	-
5 Hz	96.3 \pm 1.6	-	-	-	-	-
10 Hz	82.4 \pm 2.8	*	-	-	-	*
20 Hz	57.9 \pm 4.3	*	*	*	-	*
30 Hz	50.7 \pm 5.8	*	*	*	-	*
<u>M-Wave #4</u>						
					M-wave #1	M-wave #7
0 Hz	96.4 \pm 2.0	N/A	N/A	-	-	-
5 H	91.9 \pm 2.4	-	-	-	-	-
10 Hz	68.8 \pm 2.8	*	*	-	-	*
20 Hz	35.7 \pm 4.6	*	*	*	-	-
30 Hz	20.9 \pm 4.1	*	*	*	-	*
<u>M-Wave #7</u>						
					M-wave #1	M-wave #4
5 Hz	93.7 \pm 1.8	-	-	-	-	-
10 Hz	65.5 \pm 3.1	*	N/A	*	-	-
20 Hz	21.9 \pm 3.6	*	N/A	*	-	-
30 Hz	5.4 \pm 0.5	*	N/A	*	-	*

Table 3. Extent of M-wave Area decrement for the first, fourth and seventh potential. Significant differences from resting values (Column 3), control values (Column 4) and position (Columns 5 and 6) in tetanic train are listed (n=10). Values are mean \pm standard error (*p < .01).

1	2	3	4	5	6	
Frequency of SE Fatiguing Stimulation	X \pm	(% resting) (100%)	Significance from Rest	Significantly Different from Control (0 Hz)	M-wave #4	M-Wave #7
<u>M-Wave #1</u>						
0 Hz	91.1 \pm 1.9	-	N/A	-	-	-
5 Hz	99.1 \pm 2.8	-	-	-	-	-
10 Hz	96.1 \pm 3.0	-	-	-	-	-
20 Hz	83.7 \pm 6.4	-	-	-	-	-
30 Hz	67.1 \pm 8.4	*	-	-	-	*
<u>M-Wave #4</u>						
				M-wave #1	M-wave #7	
0 Hz	96.7 \pm 2.3	-	N/A	-	-	-
5 Hz	103.8 \pm 2.4	-	-	-	-	-
10 Hz	101.2 \pm 3.8	-	-	-	-	-
20 Hz	69.5 \pm 9.7	*	*	-	-	-
30 Hz	43.0 \pm 9.7	*	*	-	-	-
<u>M-Wave #7</u>						
				M-wave #1	M-wave #4	
5 Hz	100.5 \pm 2.4	-	N/A	-	-	-
10 Hz	101.0 \pm 4.1	-	N/A	-	-	-
20 Hz	55.2 \pm 9.0	*	N/A	*	-	-
30 Hz	17.7 \pm 5.5	*	N/A	*	-	-

3.4 Effect of different fatiguing stimulus frequencies on torque (ankle dorsiflexor muscles) during ischaemia

3.4.1 Conclusions

The changes in twitch and tetanic torque were more pronounced than those affecting the M-wave; during the initial period of fatiguing stimulation twitch and tetanic torque were potentiated by as much as 40% and 93% (respectively) of the control value, while in later stages torque could be completely abolished.

3.4.2 Initial changes in torque

The first effect of fatiguing stimulation on torque was a marked potentiation of the twitch which was greater than that exhibited by the tetanic responses. In Figure 11, potentiation of the twitch is evident in the oscilloscope traces at 30 s, when 30 Hz was the fatiguing stimulus frequency; the individual values for this subject have been plotted in Figure 13. From this figure, it is evident that the twitch could increase in amplitude (by 60%) without any significant changes having taken place in the corresponding M-wave (M-wave 1). Conversely, at the time when the area of M-wave 7 had enlarged maximally (at 1.5 min during 30 Hz stimulation), the corresponding tetanic torque was already decreasing.

The mean changes in torque for the entire population of subjects are shown in Figures 22 and 23. The potentiation of the twitch was greatest in the control experiments ($37 \pm 8\%$) and was evidently caused by the testing stimuli. By contrast, the increase in tetanic torque amplitude was most evident in the 5 Hz experiments, reaching a maximal value of $93 \pm 22\%$. Although not as large, potentiation of the twitch and tetanic torque were present in the 10, 20 and 30 Hz stimulation experiments, and reached maximal values earlier than in the low-frequency experiments (compare the tetanic torque for the 5 Hz and 10 Hz experiments).

As the fatiguing stimulation proceeded, the twitch and tetanic torques declined; the rate of decline was always greater for the twitch than the tetanic torque. The extent of the decline depended on the fatiguing stimulus frequency. Thus, twitch and tetanic torques were completely abolished by 20 Hz and 30 Hz stimulation and markedly reduced by 10 Hz stimulation. By contrast, the tetanic torque was still at the control level at the end of 5 Hz stimulation.

3.4.3 Final changes in torque during fatigue

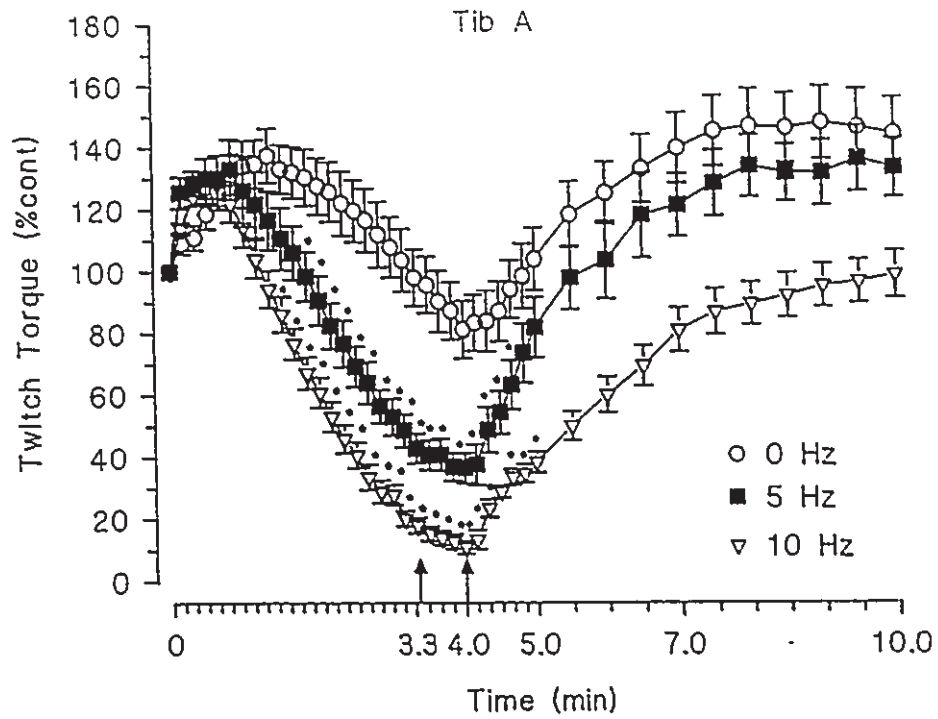
Torque was much more susceptible to fatiguing stimulation than was the M-wave. The difference was especially evident when low fatiguing frequencies of stimulation were employed. For example, when subject LA was stimulated at 10 Hz, the M-wave areas remained constant (Figure 12, middle). By contrast,

Figure 22a Twitch torque amplitude values (% cont) during fatigue and recovery following 10/5 and 10/10 Hz fatiguing stimulation. Torque values for the control run (10/0 Hz) are plotted for comparison. Arrows demarcate the period of ischaemic recovery. Values are means \pm standard error.

*Significant difference from resting and control values ($p < .01$).

Figure 22b Tetanic torque amplitude values (% cont) during fatigue and recovery. See Figure 22a for explanation.

a.



b.

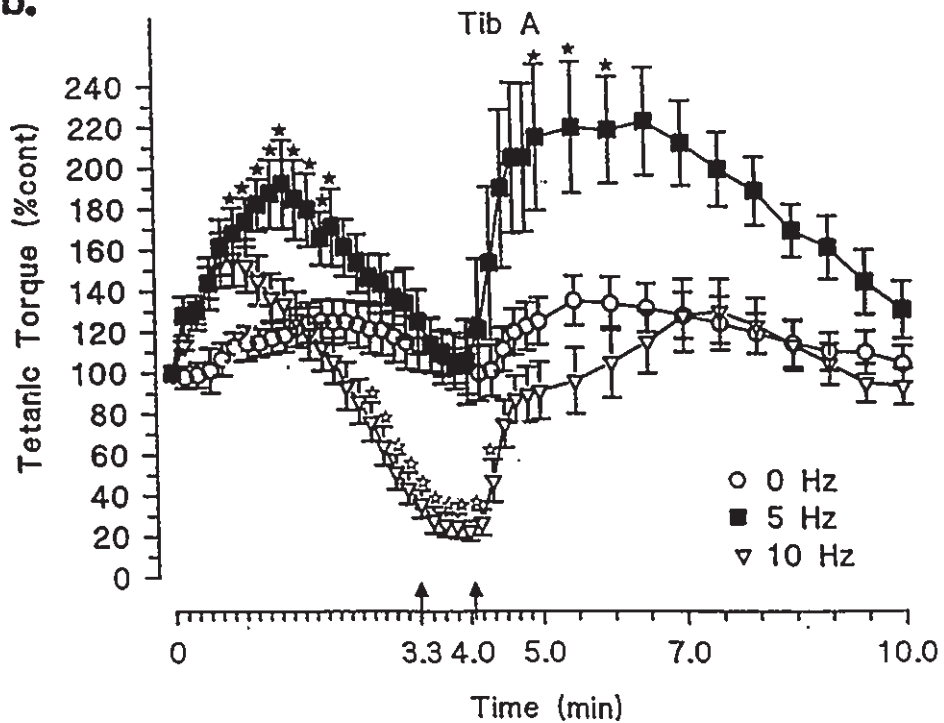
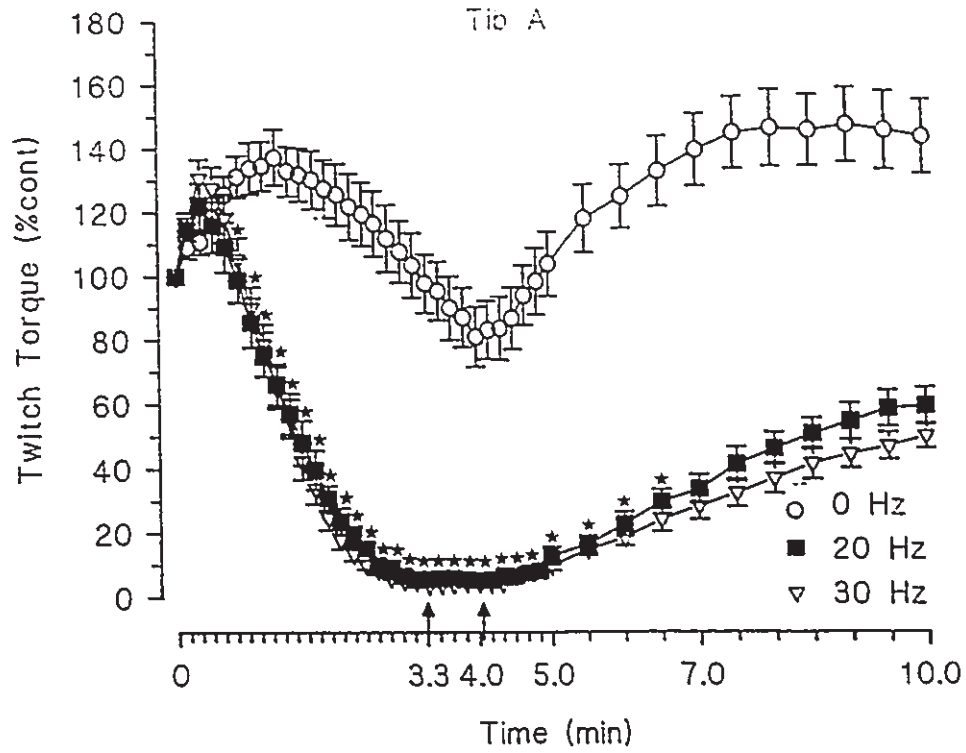


Figure 23a Twitch torque amplitude values (% resting) during fatigue and recovery following 10/20 and 10/30 Hz fatiguing stimulation. Torque values for the control run (10/10 Hz) are plotted for comparison. Arrows demarcate the period of ischaemic recovery. Values are means \pm standard error.

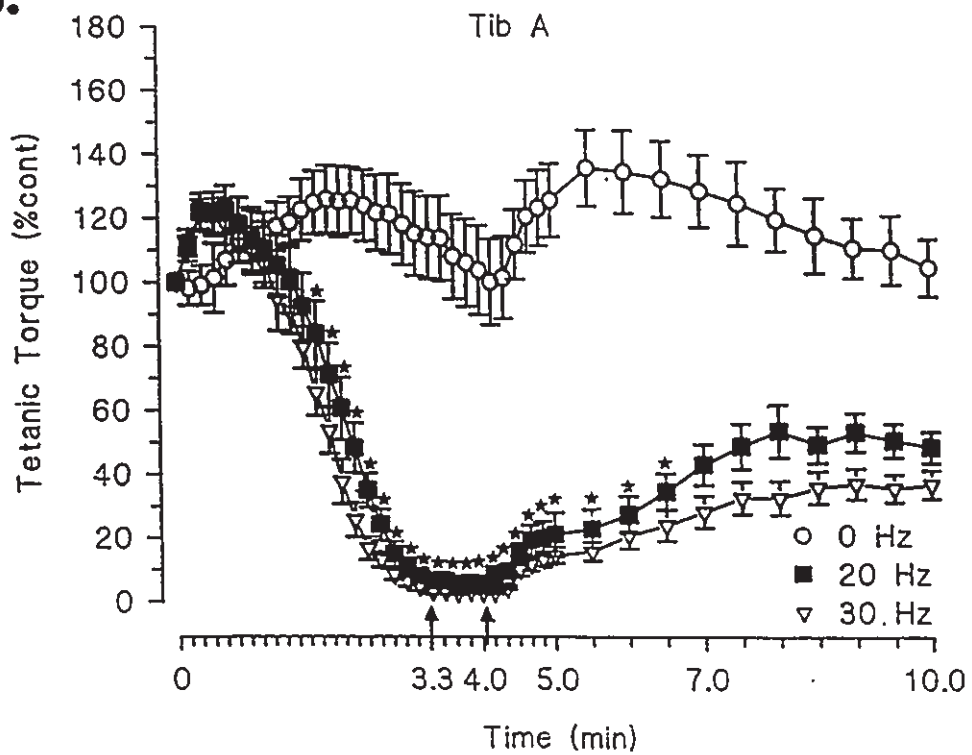
*Significant difference from resting and control values ($p < .01$).

Figure 23b Tetanic torque amplitude values (% resting) during fatigue and recovery. See Figure 23a for explanation.

a.



b.



the twitch and tetanic torques essentially disappeared by the time that the fatiguing stimulation was stopped (Figure 12, bottom). This dissociation between electrical and mechanical events suggested a failure of excitation-contraction coupling or of the contractile apparatus. Higher rates of fatiguing stimulation caused torque to diminish more rapidly than lower ones (compare Figures 23 and 24 bottoms).

3.4.4 Changes in twitch duration

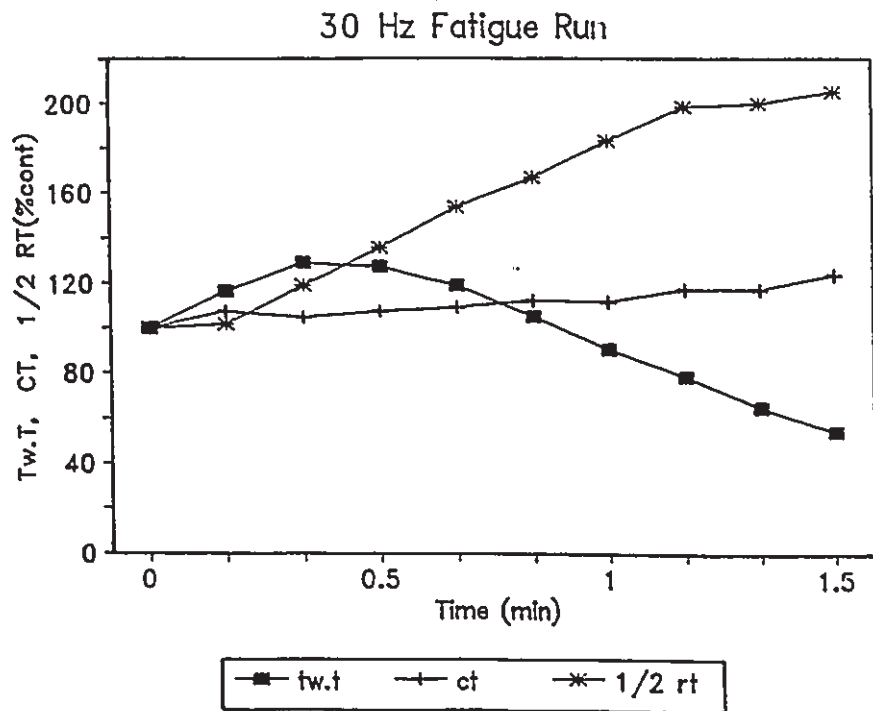
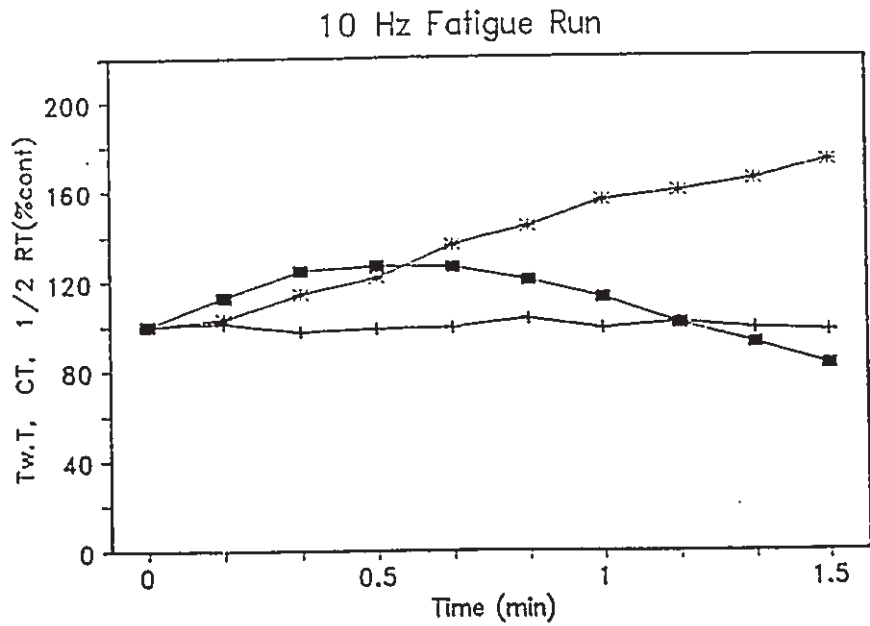
The computer software programme was used to measure the contraction and half-relaxation times of the twitch during fatiguing stimulation. Only the first 1.5 min of fatiguing stimulation have been analyzed in Figure 24 because, as the muscle fatigued, the signal-to-noise ratio decreased and measurements became increasingly unreliable. During both 10 Hz and 30 Hz stimulation the contraction times did not change significantly but the half-relaxation times increased by 70-90%.

3.5 Recovery of the M-wave after fatiguing stimulation

3.5.1 Summary

When the fatiguing stimulation was discontinued the amplitudes and areas of the M-waves remained diminished below control values until the arterial cuff

Figure 24 Twitch torque (Tw.T) Contraction Time (C.T.) and Half-Relaxation Time ($\frac{1}{2}$ RT) during 10/10 and 10/30 Hz fatigue runs. Values are plotted for both fatigue and recovery. Results obtained from a single subject (LA: ♀ Age: 20).



was deflated. There was rapid recovery of the M-wave to control values during the next minute, with a slight overshoot in the area values of some responses.

3.5.2 Results after cessation of fatiguing stimulation, with maintenance of ischaemia

After the fatiguing stimulation was discontinued the arterial cuff remained inflated for a further 40 s. During this period the muscle fibres would be denied oxygen and the concentrations of metabolites produced during stimulation would be maintained. Testing stimuli, consisting of a single shock and a 2 s tetanus at 10 Hz, were delivered every 10 s.

At the lower rates of fatiguing stimulation (5 Hz and 10 Hz), the areas of M-waves 1 and 4 remained normal during this further period of ischaemia (Figure 18). The peak-to-peak amplitudes, which had been reduced during fatiguing stimulation, remained depressed.

At the higher rates of fatiguing stimulation (20 Hz and 30 Hz), the amplitude of M-waves 1 and 4 showed little change, and the same was true for the areas of the M-waves following 30 Hz stimulation. The areas of the M-wave, following 20 Hz stimulation decreased further (Figure 19).

3.5.3 Results after cuff deflation

Once the arterial cuff was deflated there was a rapid increase in both the

amplitudes and the areas of the diminished M-waves. The changes were present within the 10 s between cuff release and the next testing cycle. Within 1 min of cuff release, the areas of M-waves 1 and 4 had fully recovered and were slightly enlarged above control values for several minutes (Figure 19); these measurements were not statistically significant. The recovery of M-wave amplitude was slower, reaching 80% at the end of the first minute and gradually approaching the control values over the next 5 min (Figure 19).

3.6 Recovery of torque after fatiguing stimulation

3.6.1 Summary

Torque did not start to recover until the arterial circulation was restored. In the 5 Hz stimulation experiment there was then a rapid recovery of tetanic torque with potentiation of the signal reaching values as high as 124% of control values. With higher fatiguing frequencies recovery of twitch and tetanic torque was slower and usually was incomplete after 6 min.

3.6.2 Results with fatiguing frequencies lower than 10 Hz

After a rate of fatiguing stimulation of 5 Hz and in the control experiments, the previously potentiated torque values continued to decline (Figure 22). On releasing the arterial cuff, there was an immediate rise in tetanic torque

in the 5 Hz experiments, followed by a marked potentiation (mean $124 \pm 26\%$); the potentiation gradually declined but was still detectable 6 min after cuff release. This potentiation occurred even though the repetitive stimulation had not caused any fatigue during the first 200s of the experiment. Figure 22 shows that, even in the control experiments, the testing stimuli were sufficient to cause appreciable potentiation of the twitch. The 5 Hz experiments also caused potentiation.

3.6.3 Results with fatiguing frequencies of 10-30 Hz

With higher rates of fatiguing stimulation (10-30 Hz) the twitch and tetanic torques remained depressed during the period of additional ischaemia. The rate of recovery then depended on the prior frequency of stimulation. Thus, recovery of twitch and tetanic torque was slowest and least complete for 30 Hz and was fastest and most pronounced for 10 Hz (Figure 22 and 23). The results of the 10 Hz experiments suggest that the recovery of tetanic torque took place in two phases - an early rapid phase followed by a slower phase. The two phases are illustrated in Figure 22b which shows that the tetanic torque recovered to more than half the control value in the first 20 s after cuff release; it then stabilized before slowly increasing over the next 5 min. In the 10 Hz experiments tetanic torque recovered faster than twitch torque.

With 30 Hz stimulation, even after 6 min of recovery with a restored

circulation, the twitch and tetanic torque were only 50% and 37% respectively of the control values. Tables 4, 5, and 6 summarize the maximal potentiation and maximal decline in twitch torque (Table 4) and tetanic torque (Tables 5 and 6). The time (from initiation of fatiguing stimulation) at which these changes occurred is given to the right of each value. It can be seen that at lower fatiguing frequencies (0 Hz and 5 Hz), twitch values were potentiated mainly during recovery (from ischaemia) while at higher frequencies (10, 20, and 30 Hz) they were potentiated mainly during fatiguing stimulation. Maximal twitch reductions occurred before cuff release, with the exception of 0 Hz where the twitch torque continued to decline for a further 10 s. Tetanic torque sampled at the end of the 10 Hz testing train (Table 5) reflected the same pattern. Tetanic torque values at the end of the fatiguing trains (Table 6) showed no potentiation with high frequency stimulation (20 Hz and 30 Hz) but were potentiated earlier during fatiguing stimulation at 5 Hz and 10 Hz. Maximal declines all occurred towards the end of fatiguing stimulation.

3.7 Contribution of Ischaemia to changes in M-wave and torque during repetitive stimulation at 20 Hz

3.7.1 Summary

The decreases in M-wave amplitude and area, previously noted during

Table 4. Summary of maximum twitch potentiation and decline. Times quoted are from the initiation of fatiguing stimulation. Values are means \pm standard error (n=10). *Significantly different from resting values (p < .01).

Frequency of Fatiguing Stimulation	Maximum Twitch Potentiation (% Control)	Time (Min)	Maximum Twitch Decline (% Control)	Time (Min)
0 Hz	147.6 \pm 11.8*	9	83.3 \pm 9.1	4.10s
5 Hz	136.0 \pm 10.6*	9.30s	36.4 \pm 4.9	4
10 Hz	128.1 \pm 4.5*	40s	11.0 \pm 2.1	4
20 Hz	121.7 \pm 7.6*	20s	4.6 \pm 0.6	3.20s
30 Hz	130.9 \pm 6.0*	20s	3.8 \pm 0.6	3.20s

Table 5. Summary of maximum tetanic potentiation and decline sampled at the end of the 10 Hz testing trains. Times quoted are from the initiation of fatiguing stimulation. Values are means \pm standard error (n=10). *Significantly different from resting values (p < .01).

Frequency of Fatiguing Stimulation	Maximum Tetanic Potentiation (% Control)	Time (Min)	Maximum Tetanic Decline (% Control)	Time (Min)
0 Hz	136.1 \pm 12*	6.30s	100.5 \pm 13.4	4.10s
5 Hz	223.7 \pm 26.4*	7.30s	103.9 \pm 8.6	3.50s
10 Hz	156.4 \pm 13.6*	50s	23.1 \pm 4.7*	4
20 Hz	120.9 \pm 7.5*	40s	5.7 \pm 1.9*	4
30 Hz	120.8 \pm 5.0*	30s	2.9 \pm 0.6*	3.40s

Table 6. Summary of maximum tetanic potentiation and decline sampled at the end of the fatiguing trains. Times quoted are from the initiation of fatiguing stimulation. Values are means \pm standard error. *Significantly different from resting values ($p < .01$).

Frequency of Fatiguing Stimulation	Maximum Tetanic Potentiation (% Control)	Time (Min)	Maximum Tetanic Decline (% Control)	Time (Min)
5 Hz	131.9 \pm 6.6*	40s	85.7 \pm 15.3	2.30s
10 Hz	131.8 \pm 12.8*	1	40.9 \pm 9.9*	3.20s
20 Hz	-	-	13.8 \pm 4.8*	3.20s
30 Hz	-	-	4.6 \pm 1.6*	3.20s

fatiguing stimulation at 20 Hz in the presence of ischaemia, were absent when the stimulation was repeated with an uninterrupted circulation on a further occasion. Preservation of the circulation also changed the contractile responses to the same stimulation; the decrease in twitch torque was much smaller while the tetanic torque remained potentiated.

3.7.2 M-wave changes

As noted already (Section 3.3.2), fatiguing stimulation at 20 Hz caused a reduction in the amplitude of M-waves 1 and 4 to 42% and 64% respectively. The corresponding reductions in M-wave areas were 16% and 31% (see Tables 2 and 3).

When the experiments were repeated using the same fatiguing stimulus frequency but with the arterial circulation intact, the M-wave areas did not change significantly, nor did the amplitude of M-wave 1. There was, however, an 11% decline in the amplitude of M-wave 4 (Figures 25 and 27).

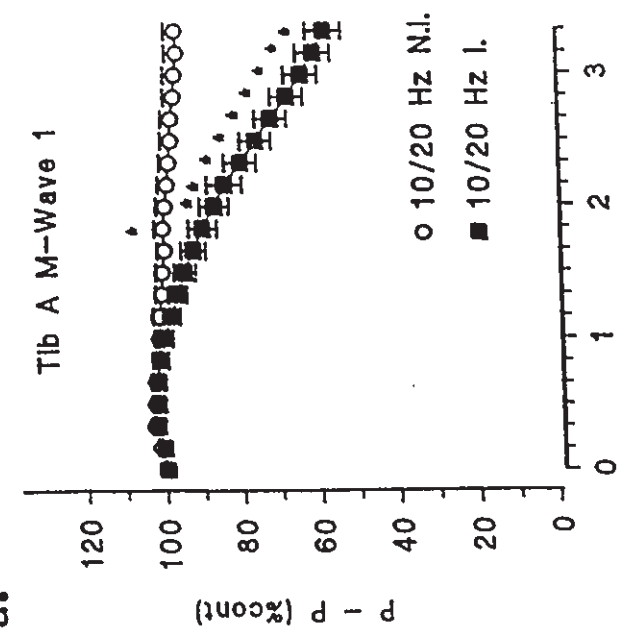
The amplitudes and areas of M-wave 7 during the ischaemic and non-ischaemic runs were also compared. It will be recalled that this response was the last compound action potential to be evoked in each fatiguing tetanus. From Figure 26a, it can be seen that the effect of ischaemia on this response was considerably greater than on M-waves 1 and 4. By the end of the fatiguing phase in the ischaemic experiments the mean amplitudes and areas of M-wave 7 had

Figure 25a Fatigue of M-wave #1 P-P and Area following ischaemic (■) and non-ischaemic (○) stimulation using 10/20Hz tetani. Values are means \pm standard error.

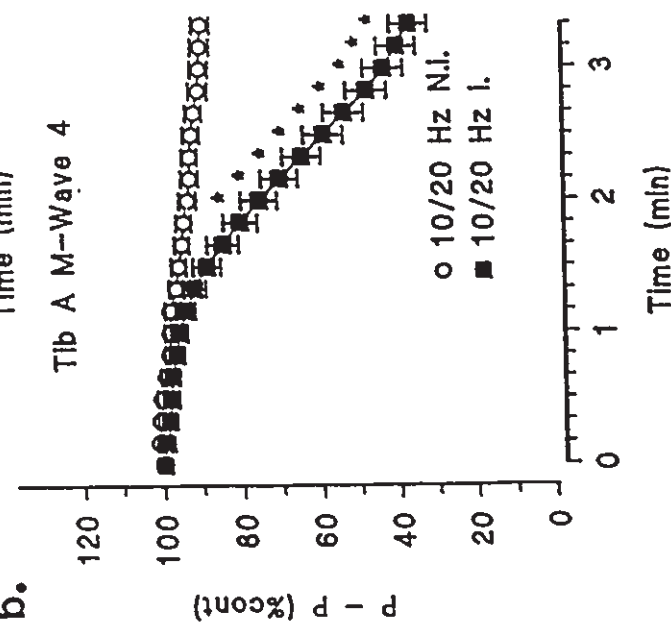
*Significant difference between ischaemic and non-ischaemic values ($p < .01$).

Figure 25b Fatigue behaviour of M-wave #4. See Figure 25a legend for explanation.

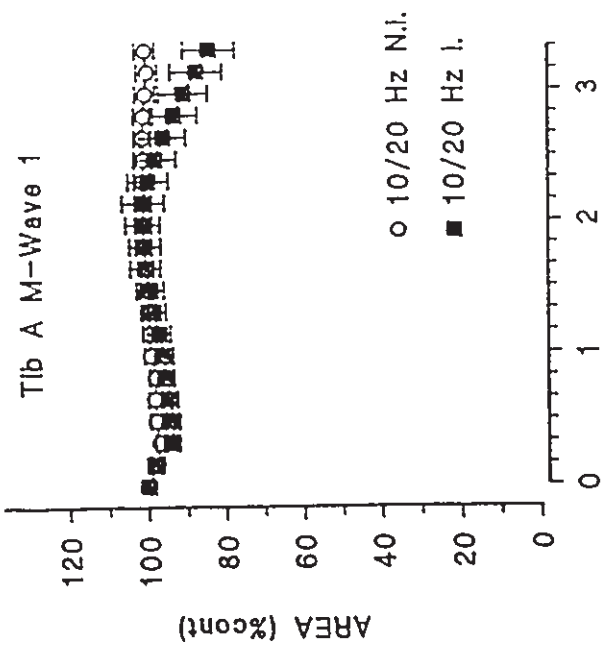
a.



b.



Tib A M-Wave 1



Tib A M-Wave 4

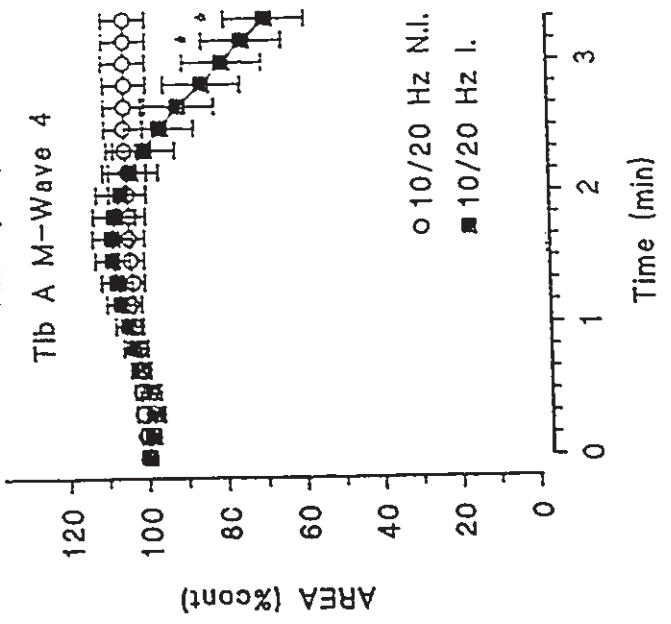


Figure 26a P-P and Area behaviour of M-wave #7 following ischaemic (■) and non-ischaemic (○) stimulation using the 10/20 Hz protocol. Values are means \pm standard error.

Figure 26b Tetanic torque values at the end of the fatiguing tetani following ischaemic (■) and non-ischaemic (○) stimulation using the 10/20 Hz protocol. Values are means \pm standard error.

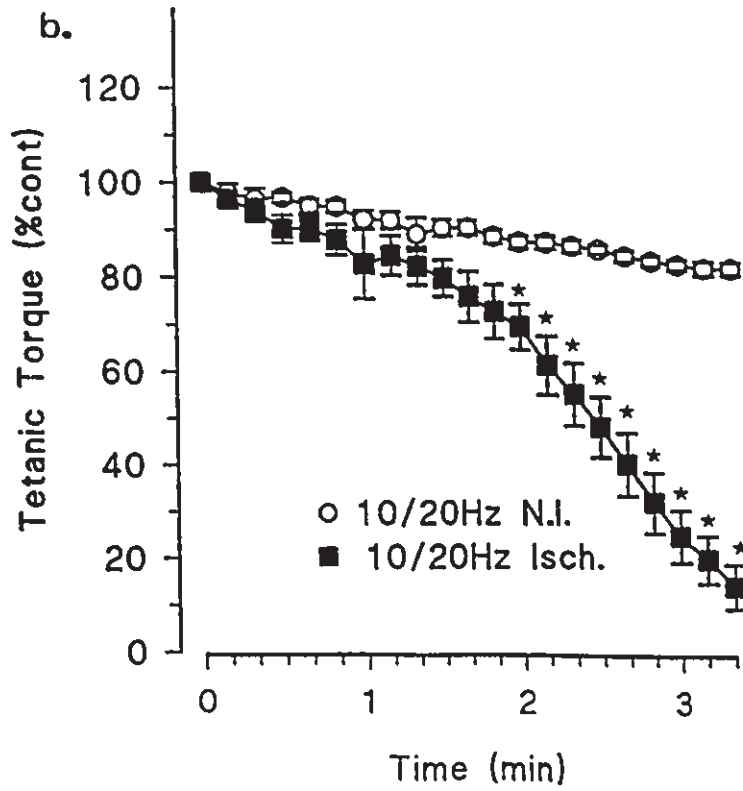
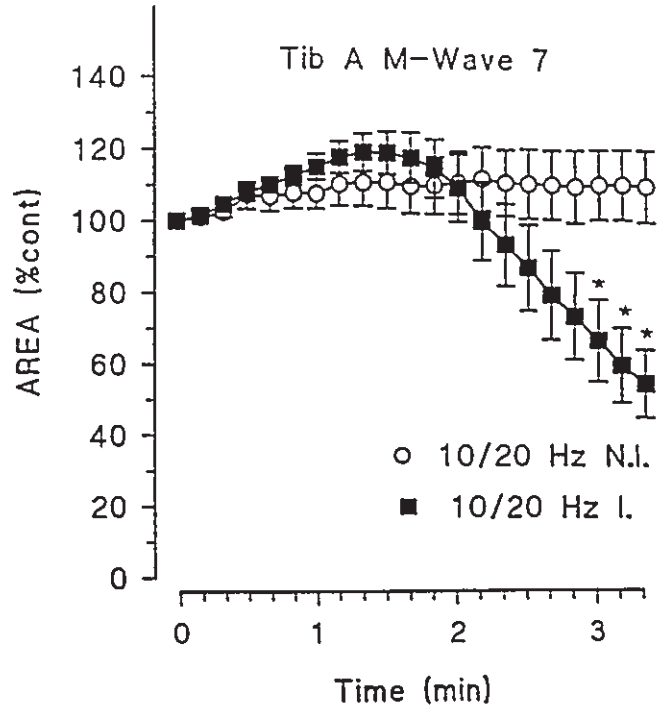
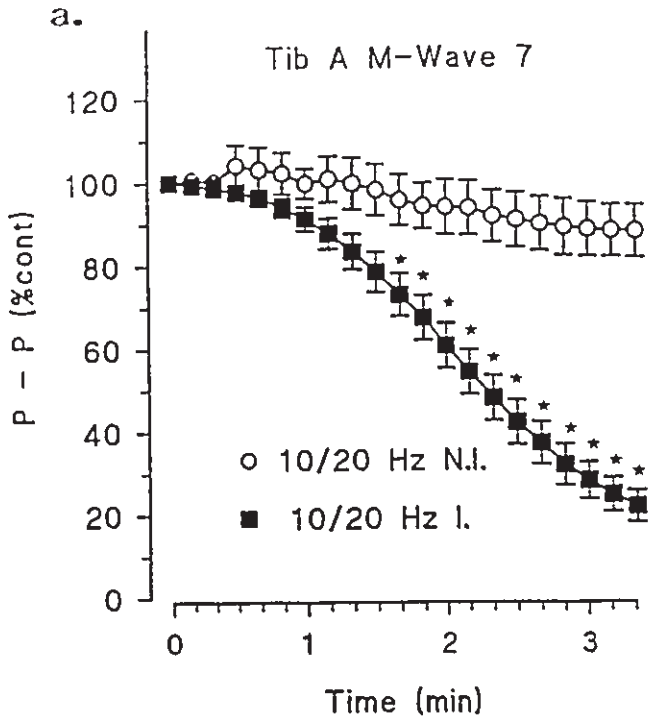
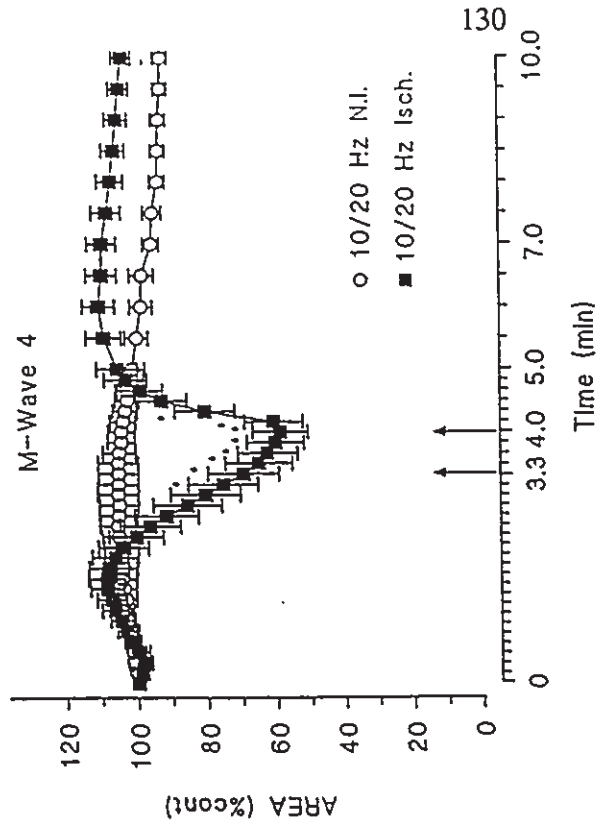
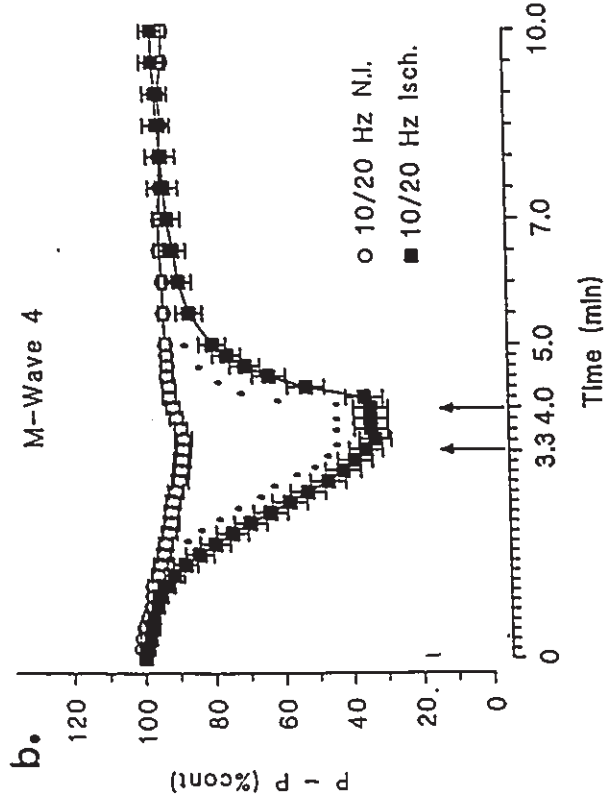
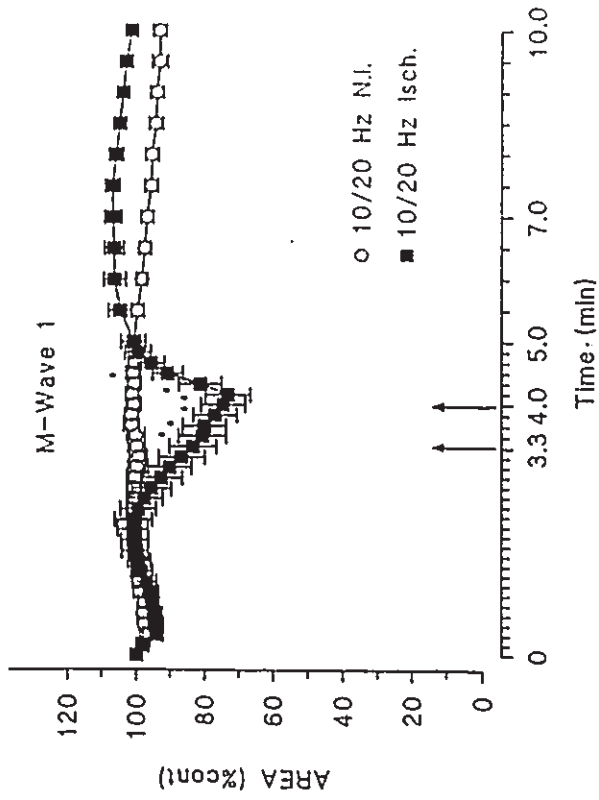
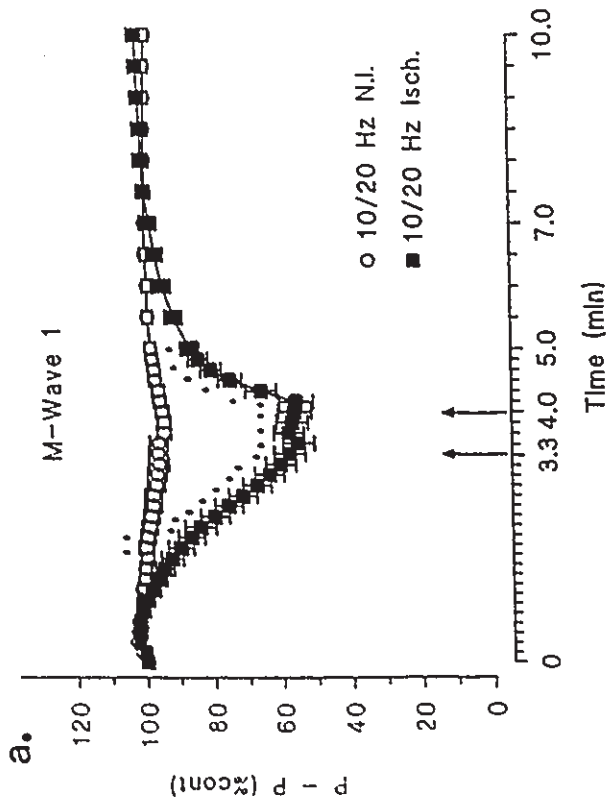


Figure 27a M-wave #1, P-P and Area values during fatigue and recovery following ischaemic and non-ischaemic fatiguing stimulation using the 10/20 Hz protocol. Values are means \pm standard error. The two arrows demarcate the period of ischaemic recovery, the second arrow signifying the point of cuff release.

*Significant differences between ischaemic and non-ischaemic values ($p < .01$).

Figure 27b M-wave #4, P-P and area values during fatigue and recovery. See Figure 27a for explanation.



declined to 22% and 55% of the resting values. In the non-ischaemic experiments, the mean amplitudes had only fallen to 90% of the resting values and the mean area had increased by 10%.

Differences between the ischaemic and non-ischaemic results were also evident in the recovery phase. Whereas the areas of M-waves 1 and 4 increased above the resting values following cuff release, the non-ischaemic values showed a nonsignificant decline at the same time points (Figure 27).

3.7.3 Torque changes

The early potentiation of twitch torque was unaffected by the presence or absence of ischaemia, the respective mean values being 21% and 18% (Figure 28a). Ischaemia had an effect thereafter, however, abolishing the twitch entirely before the end of the fatiguing phase (Figure 28a). In the experiments with intact circulation fatigue was also present, though it was significantly less than in the ischaemic experiments amounting to a 30% reduction. Following the fatiguing tetani the twitch torque had reversed fully within one minute and then fell away during the remainder of the recovery phase.

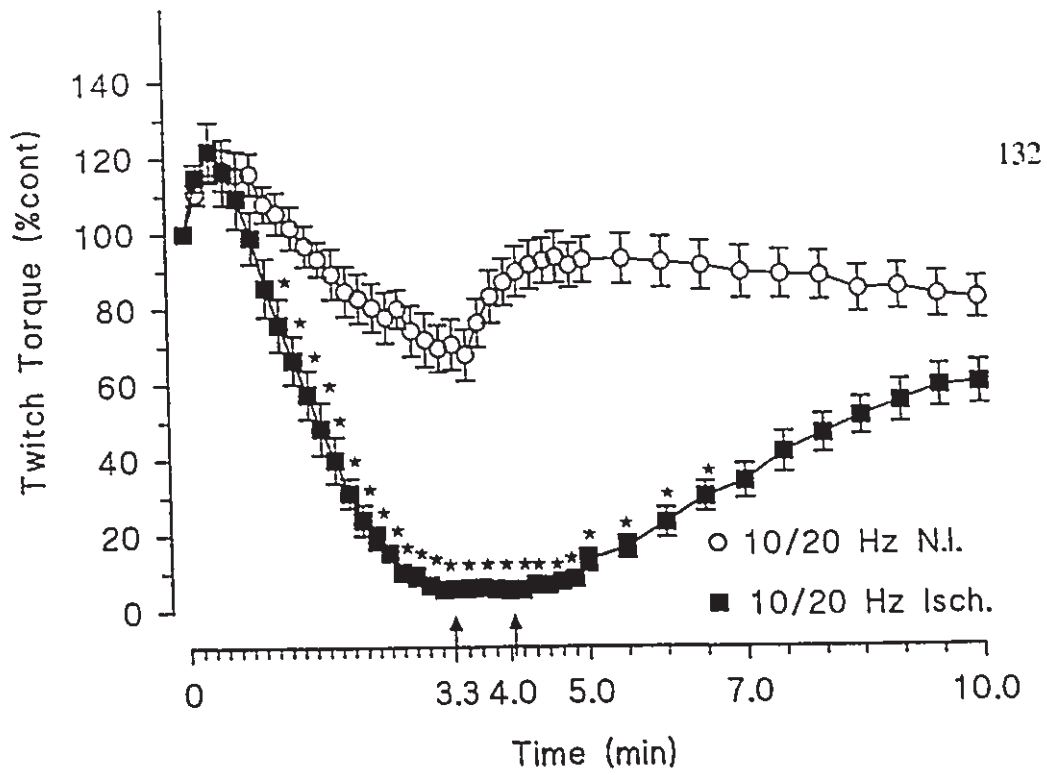
The comparison of ischaemic and non-ischaemic effects was even more striking in the case of tetanic torque. It was completely abolished in the ischaemic experiments whereas, in the non-ischaemic ones, the torque underwent greater potentiation and remained potentiated throughout the duration of the

Figure 28a Twitch torque amplitude following both ischaemic (■) and non-ischaemic (○) stimulation using the 10/20 Hz protocol. Values are means \pm standard error. The two arrows demarcate the period of ischaemic recovery, the second arrow signifying the point of cuff release.

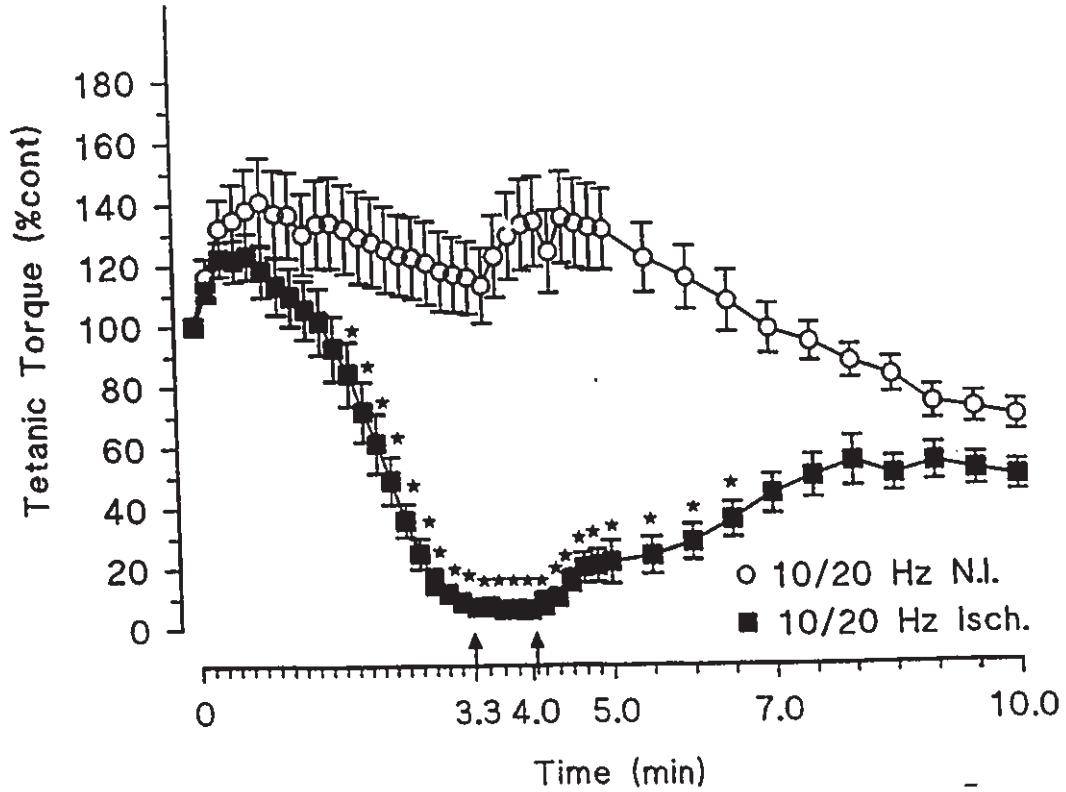
*Significant differences between conditions ($p < .01$).

Figure 28b Tetanic torque amplitude following both ischaemic (■) and non-ischaemic (○) stimulation. See Figure 28a for explanation.

a.



b.



fatiguing stimulation. Termination of the latter caused further potentiation, followed by a steady decline to 69% of the resting value (Figure 28b). In the ischaemic experiments the recovery of tetanic torque was also incomplete the mean values at the end of the experiment being 49% (Figure 23b).

3.8 Comparison of ankle dorsiflexors (fast-twitch) and soleus muscle (slow-twitch)

3.8.1. Summary

The M-waves evoked by single shocks and testing stimulation at 10 Hz were maintained longer in the soleus than in the ankle dorsiflexors, due to appreciable potentiation in the former muscle. The rates of decline, however, were similar in the two muscles. Higher frequency stimulation (30 Hz) showed no differences between the two muscles. Twitch and tetanic torques decreased more rapidly, and to a greater extent, in the dorsiflexor muscles; recovery of torque was incomplete in both muscles, especially so in the dorsiflexors.

3.8.2 Effect of fatiguing stimulation on M-waves

The last component of this research was an investigation into whether any differences in the maintenance of excitability and force production exist between slow-twitch and fast-twitch muscles. Five subjects volunteered to return

for this series of experiments. The electrical and torque responses of the soleus muscle were compared to those of tibialis anterior during ischaemic electrical stimulation at 10/30 Hz; this protocol was chosen because it was expected to produce the greatest changes in excitability and torque in the two muscles.

Before embarking on the fatigue experiments, the twitch responses of the two muscles were compared in each subject to confirm that differences in twitch duration were present. Table 7 shows the mean values for the two muscles; both the contraction and half-relaxation times were significantly longer in the soleus than in the dorsiflexors. The absence of any difference in twitch torque was presumably a consequence of stimulating only part of the soleus muscle belly.

Figure 29 shows that the main difference between the two muscles during fatiguing stimulation was the potentiation of both the amplitude and area of M-waves 1 and 4 which occurred only in the soleus. It began between 10 and 20 s of fatiguing stimulation and was more in evidence for area than for amplitude; the increase in area was also more prolonged. The potentiated values failed to reach statistical significance as compared with the control values; this may be ascribed to the large standard errors at those points of the fatiguing run.

Because of the extent of their potentiation, the areas of M-waves 1 and 4 did not begin to decline in the soleus muscle until 2 min of fatiguing stimulation had elapsed; this was approximately 80 s later than in the ankle dorsiflexors.

Table 7. Contractile properties of ankle plantarflexors (soleus) and ankle dorsiflexors (TIB.A) at rest. Values are means \pm standard deviation (n=5). *Significant difference between muscle groups ($p < .01$). TW.T = Twitch torque amplitude (Nm); CT = Contraction time (ms); $\frac{1}{2}$ RT = Half-Relaxation Time (ms).

Muscle	TW.T	CT	$\frac{1}{2}$ RT
SOLEUS	7.84 \pm 3.24	124.4 \pm 21.54*	142.24 \pm 16.66*
TIB.A	5.28 \pm 2.15	71.44 \pm 11.27	68.06 \pm 9.07

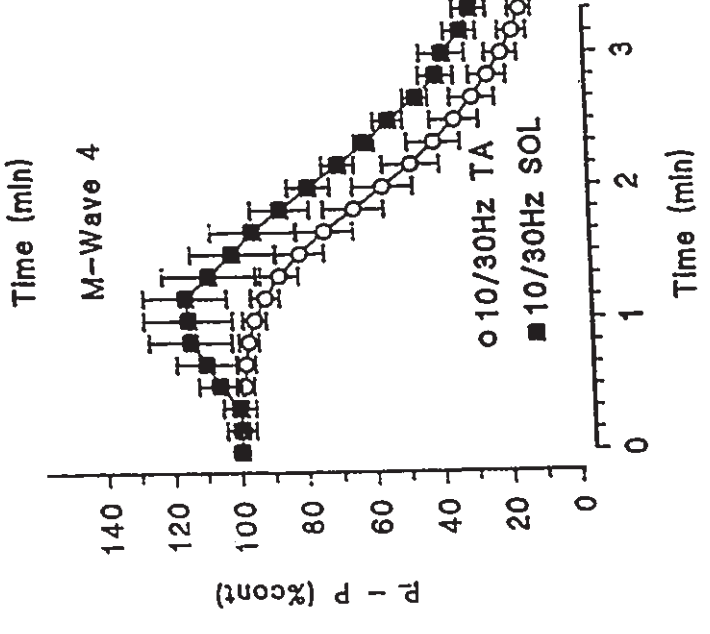
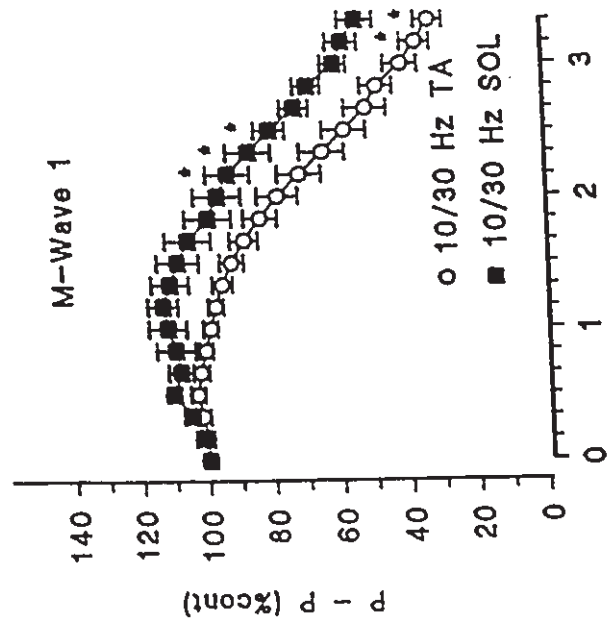
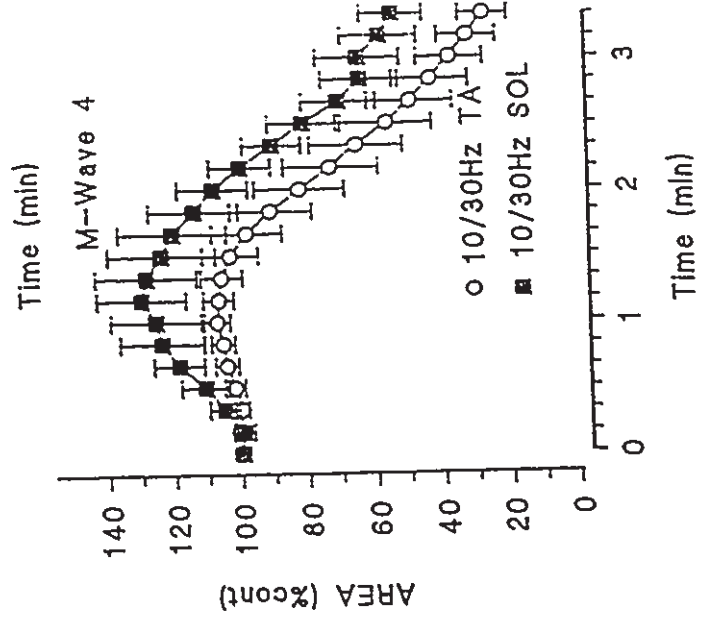
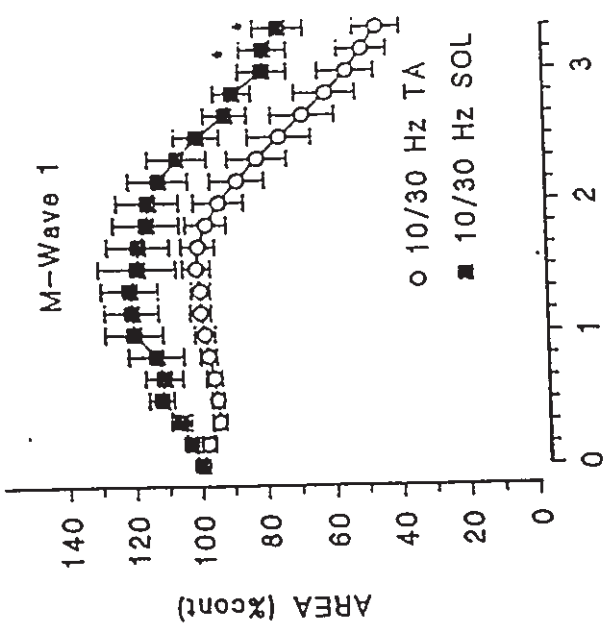
Figure 29a M-wave #1 P-P and area values for Tibialis Anterior (○) and Soleus (■) following fatiguing stimulation using the 10/30 Hz protocol.

Values are means \pm standard error (n=5).

*Significant differences between muscles (p < .01).

Figure 29b M-wave #4 P-P and area values for Tibialis Anterior (○) and Soleus (■) following fatiguing stimulation. See Figure 29a for explanation.

a.



b.

Once started, however, the rates of decline were very similar (Figure 29 and the differentiated curves in Figure 31). As a consequence of the different delays but similar rates of decline, there were greater reductions, by the end of the fatiguing stimulation, in the amplitudes and areas of M-waves 1 and 4 in the soleus than in the ankle dorsiflexors. In both muscles, the reductions were greater for amplitude than for area (Table 6).

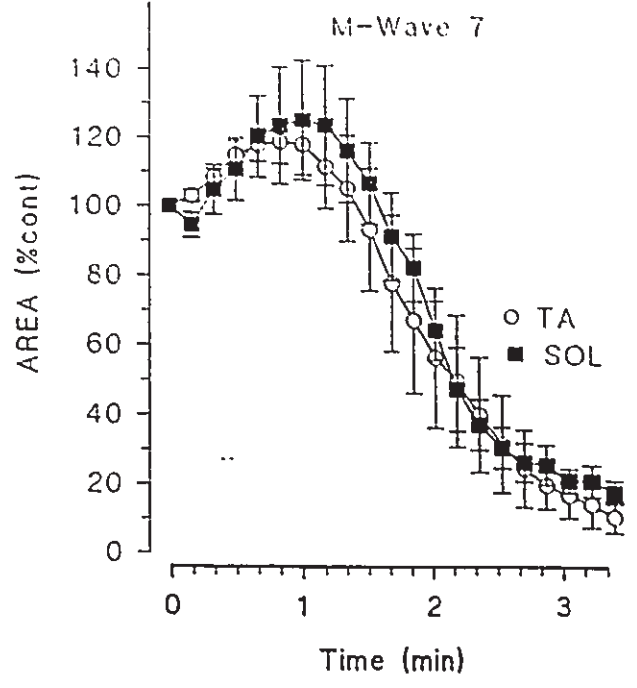
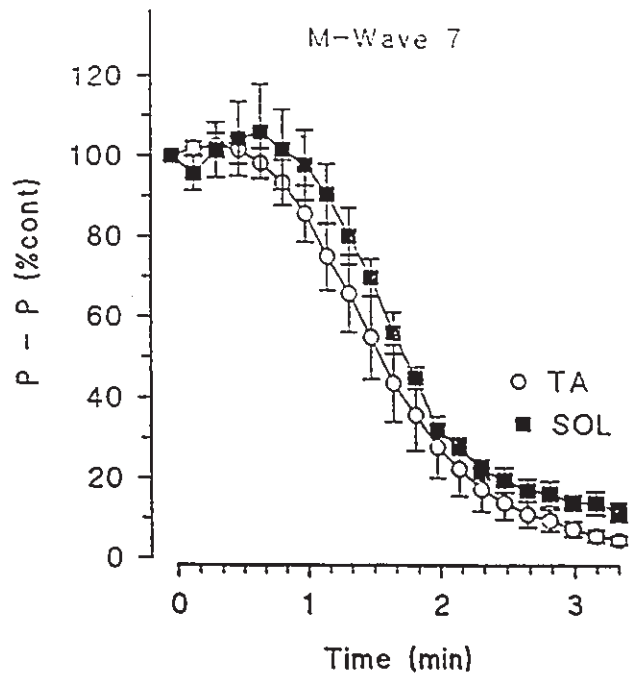
By contrast, changes in M-wave 7, evoked at the end of each 30 Hz fatiguing train, showed no significant differences between the two muscles during fatiguing stimulation; this was because of the similar potentiation which occurred in the first 90 s (Figures 29 and 30).

The recovery of M-waves 1 and 4 also differed between the two muscles. In soleus there was appreciable improvement in the mean amplitude and area of M-wave 4 after the fatiguing stimulation had been stopped but before cuff release (Figure 32). In fact, the restoration of the arterial circulation made no difference to the rate of recovery of M-wave 4 in soleus, whereas it was followed by rapid increases in the amplitude and area of the response evoked in the dorsiflexors (Figure 33). The ultimate recovery of M-wave 4 area was also greater in the dorsiflexors.

Figure 30a P-P and area changes M-wave #7, located at the end of the fatiguing trains during stimulation using the 10/30 Hz protocol. Soleus (■) behaviour is compared to that of Tibialis Anterior (○). Values are means \pm standard error (n=5). *Significant differences between muscles ($p < .01$).

Figure 30b Tetanic torque, followed over fatigue, at the end of the fatiguing tetani. See Figure 30a for explanation.

a.



b.

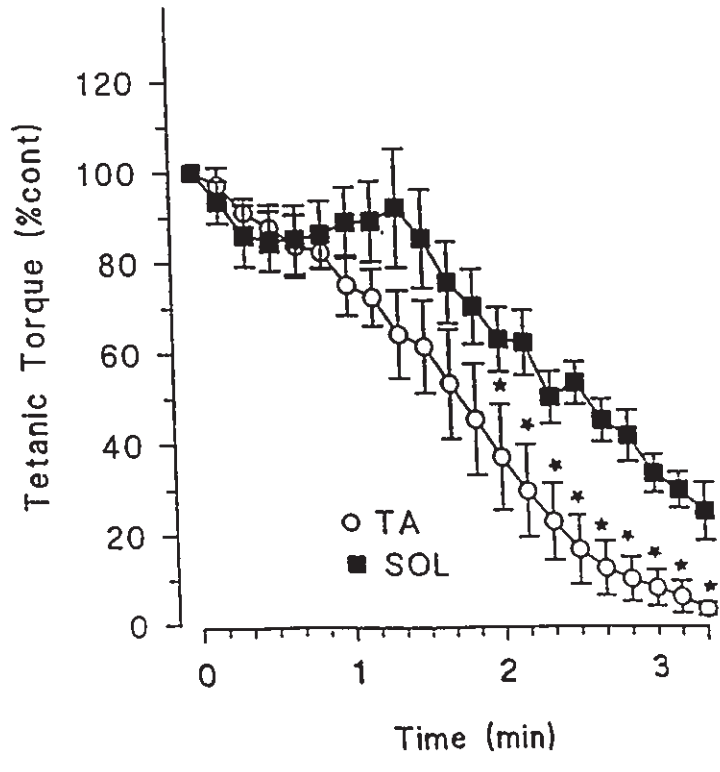
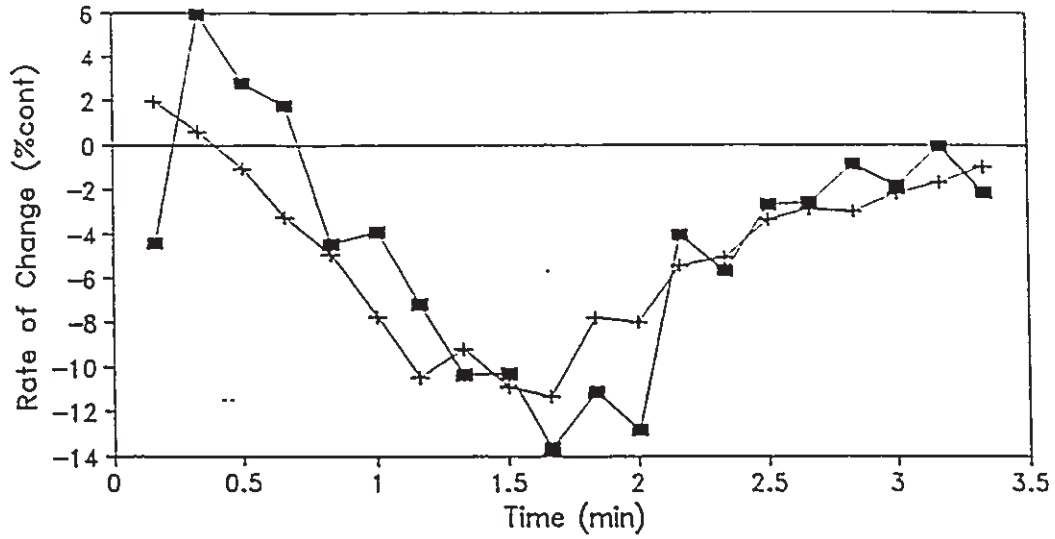
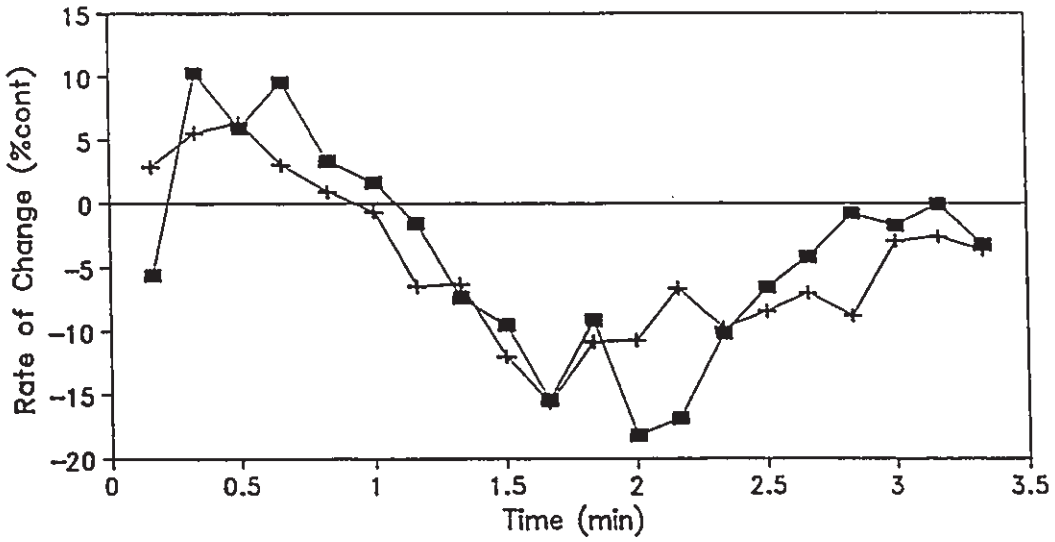


Figure 31 Rate of change of P-P and area in M-wave #7 during fatiguing stimulation using the 10/30 Hz protocol. The rate of change of soleus behaviour (■) is compared to that of tibialis anterior (+). Values are subject means (n=5).

M-Wave #7
P - P



M-Wave #7
AREA



■ 10/30Hz SOL + 10/30Hz TA

Figure 32a P-P and area values for M-wave #1 during fatigue and recovery following ischaemic stimulation using the 10/30 Hz protocol. The soleus muscle (■) is compared to tibialis anterior (○). The arrows demarcate the period of ischaemic recovery. Values are means \pm standard error (n=5). *Significant differences between muscles (p < .01).

Figure 32b P-P and area values for M-wave #4. See Figure 32a for explanation.

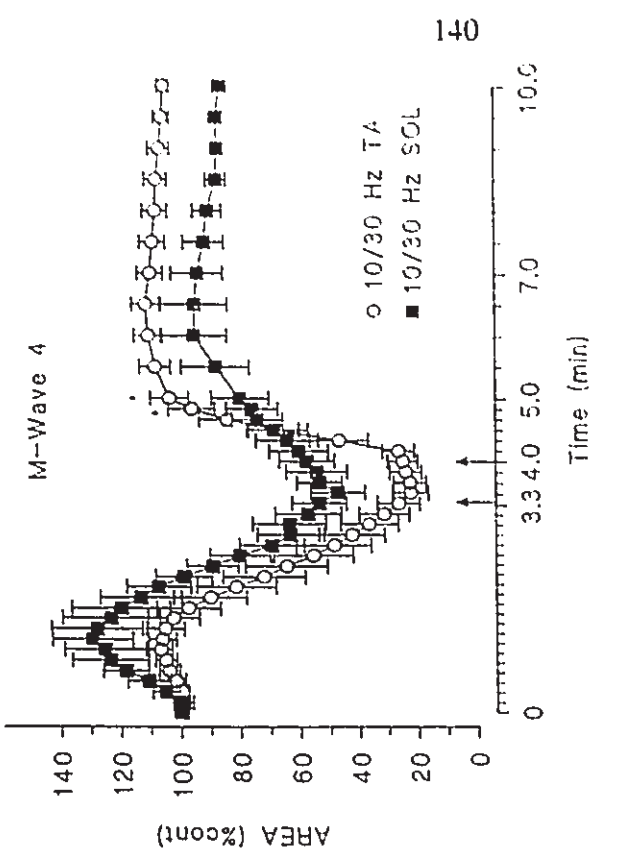
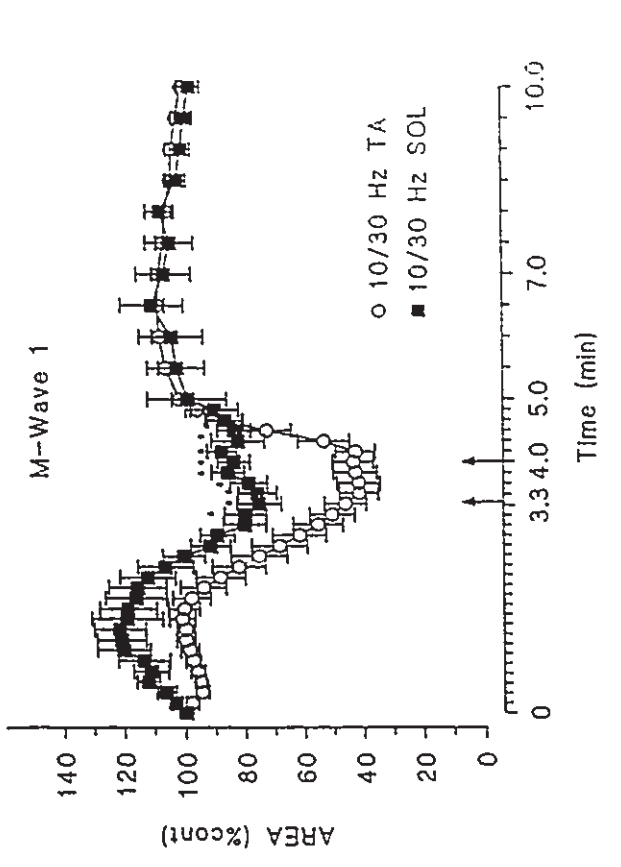
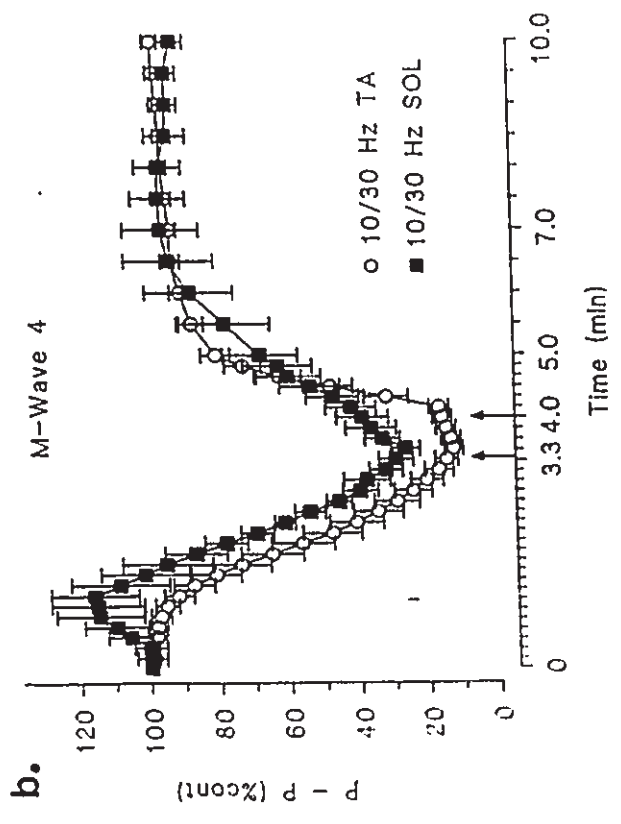
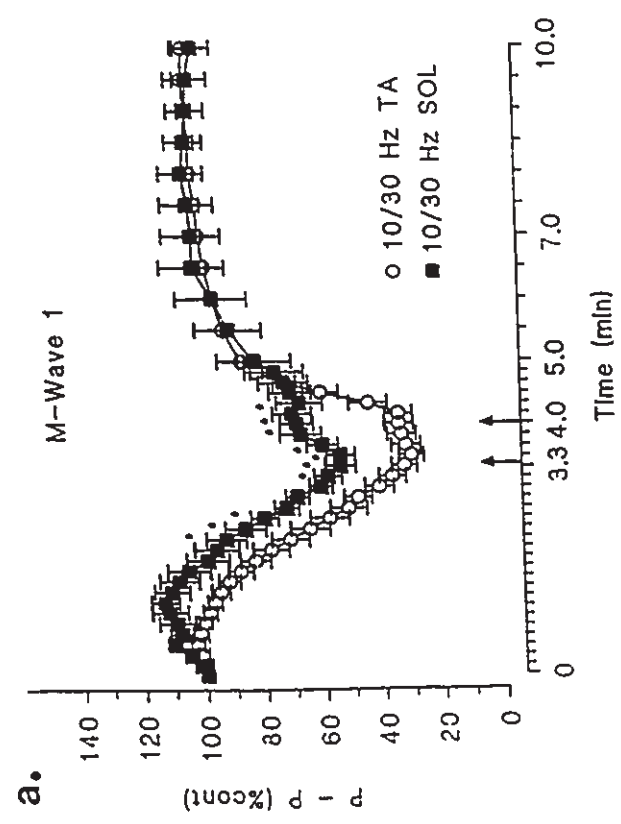
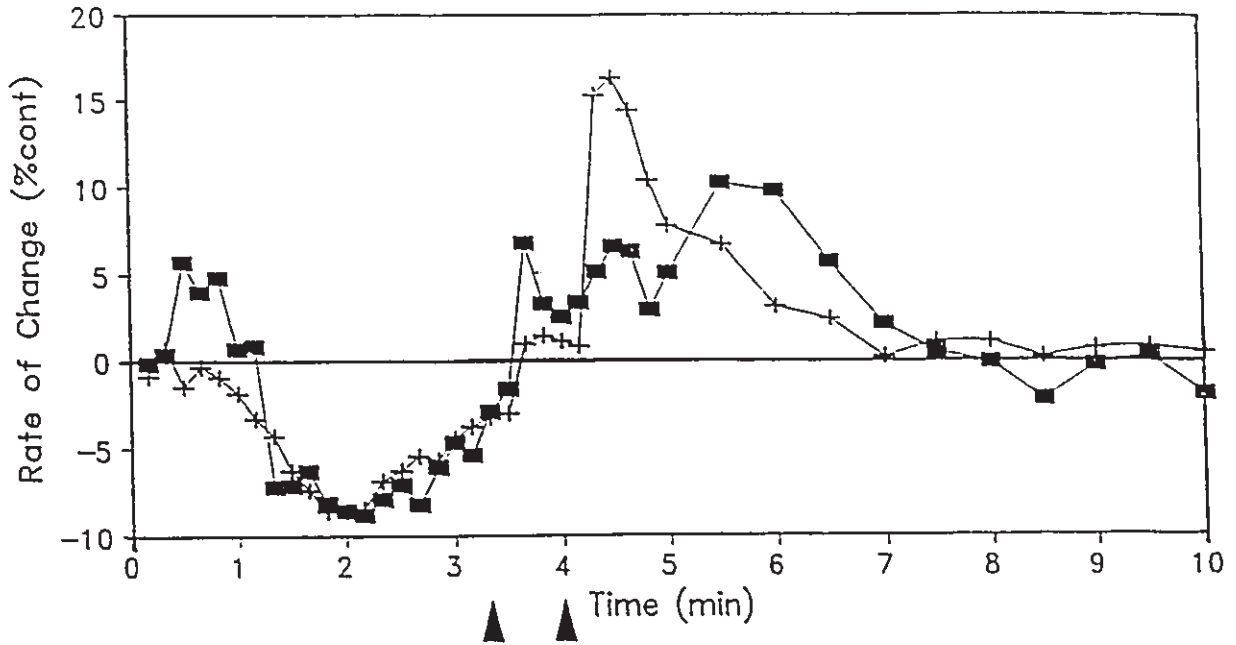


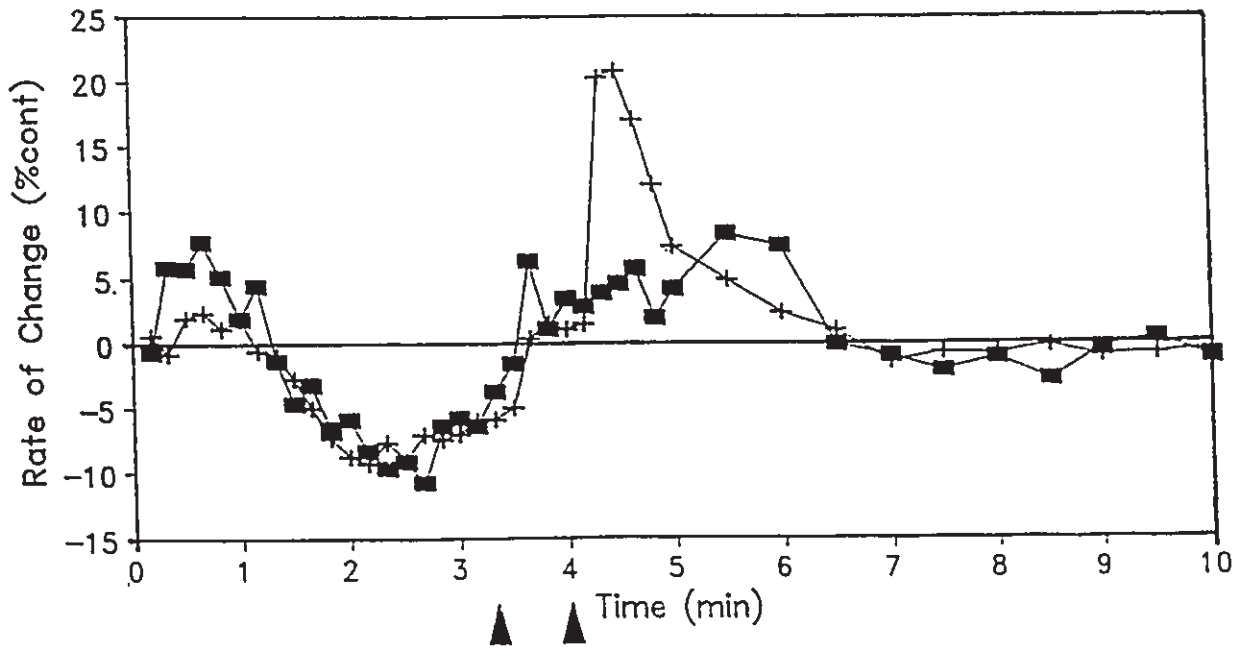
Figure 33 P-P and area rates of change for soleus M-wave #4 (■) and tibialis anterior M-wave #4 (+) during fatigue and recovery after fatiguing stimulation using the 10/30 Hz protocol. Values are subject means (n=5).

M-Wave #4
P - P

141



M-Wave #4
AREA



■ 10/30Hz SOL + 10/30Hz TA

3.8.3 Effects of fatiguing stimulation on torque

In both the soleus and the ankle dorsiflexor muscles, there was potentiation of the twitch following the onset of fatiguing stimulation (17% and 27% respectively; Figure 34a & b). Subsequently, however, twitch torque decreased more rapidly in the dorsiflexors and was reduced to 5% of resting values by the end of the fatiguing stimulation. At each time point the normalized twitch torque was significantly greater in the soleus, the final value being 30% (Figure 34a). Torque did not begin to recover in either muscle until the arterial cuff was released; the rates of recovery were similar in the two muscles, but the greater depression of torque in the dorsiflexors during fatigue caused the extent of recovery at 10 minutes to be less than that in soleus; the mean values were 55% and 74% respectively.

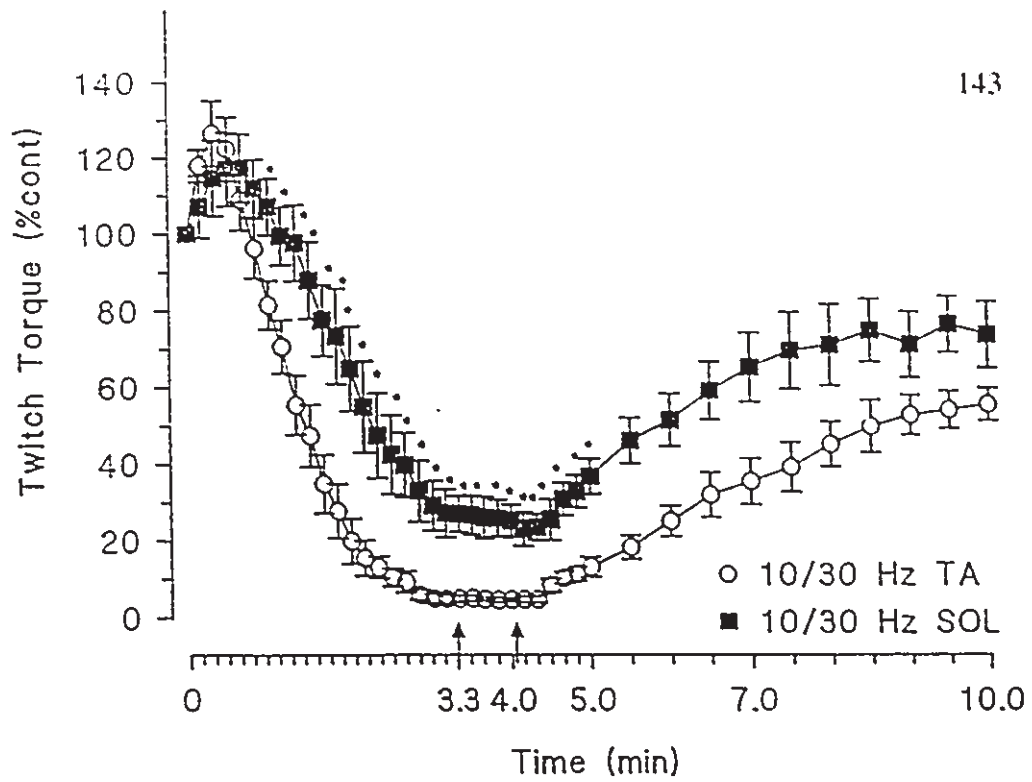
Tetanic torque also declined significantly more slowly in the soleus than in the ankle dorsiflexors during fatiguing stimulation although early potentiation was absent in the former muscles (Figure 34b). The mean tetanic torques at the end of the fatiguing period were 4% in the dorsiflexors and 20% in the soleus (Table 8). Recovery of torque began in both muscles after release of the arterial cuff; it was greater, although incomplete, in the soleus by the end of the experiment (Figure 34b). The mean torques at the end of the recovery period, expressed in terms of the resting values, were 43% and 68% respectively for the ankle dorsiflexors and soleus.

Figure 34a Twitch torque values following fatiguing stimulation using the 10/30 Hz protocol. Plantarflexor twitch torque (■) is compared to dorsiflexor twitch torque (○). The arrows demarcate the period of ischaemic recovery. Values are means \pm standard error (n=5).

*Significant differences between muscles ($p < .01$).

Figure 34b Tetanic torque values (end of control tetanus) following fatiguing stimulation using the 10/30 Hz protocol. See Figure 34a for explanation.

a.



b.

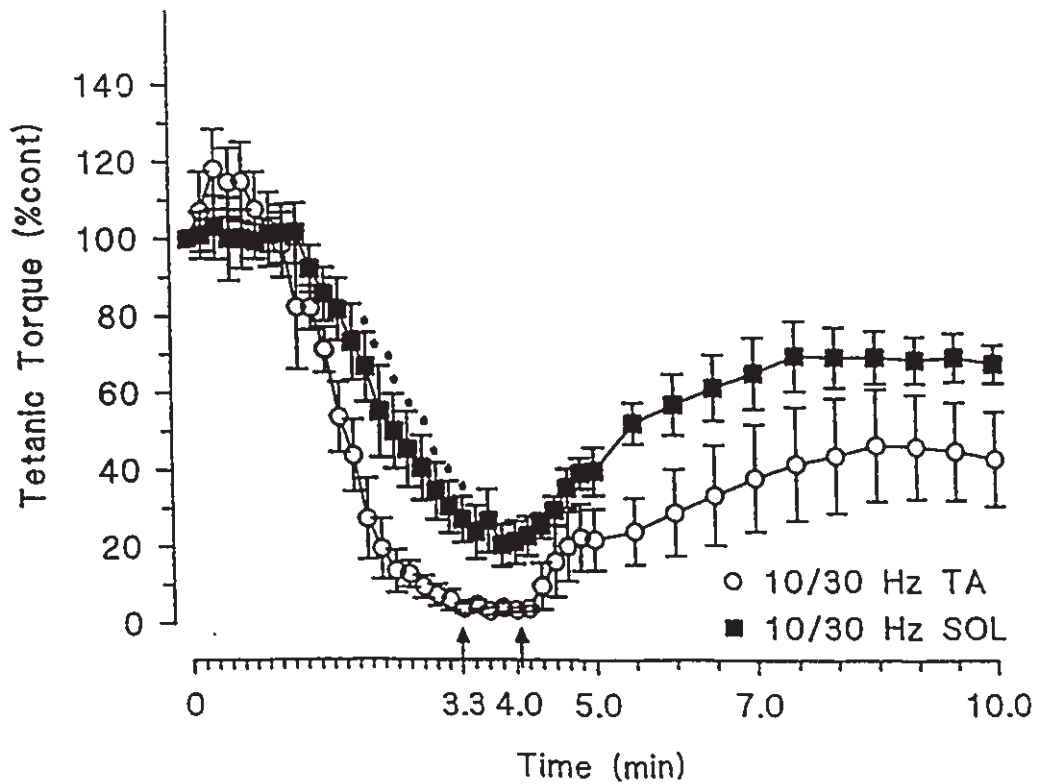


Table 8. Comparison of M-wave and torque decline between soleus and dorsiflexors. Values are percentages of control and are given as means \pm standard error (n=5). Asterisks indicate soleus values significantly higher than dorsiflexors (p < .01).

Extent of M-wave 1 decline and twitch failure

	P-P	Area	Twitch T
Soleus	53.9 \pm 4.9*	75.6 \pm 7.5*	23.0 \pm 4.5*
Tib. A	32.5 \pm 4.2	41.5 \pm 6.5	4.5 \pm 0.6

Extent of M-wave 4 decline and (testing) tetanic torque

	P-P	Area	Tetanic T
Soleus	26.2 \pm 4.2	47.1 \pm 9.2	20.4 \pm 5.6*
Tib. A	12.2 \pm 2.6	22.3 \pm 6.1	3.1 \pm 1.1

Extent of M-wave 7 decline and (fatiguing) tetanic torque

	P-P	Area	Tetanic T
Soleus	11.7 \pm 2.5	18.4 \pm 3.5	24.8 \pm 6.5*
Tib. A	4.7 \pm 1.1	11.1 \pm 4.6	3.1 \pm 1.5

DISCUSSION

4.1 Suitability of ankle dorsiflexors for fatigue studies: Present experiments

The use of tibialis anterior turned out to be eminently suitable for the present experiments. Supramaximal stimulation applied to the common peroneal nerve elicited stable biphasic M-wave recordings during both single and tetanic stimulation with very little discomfort to the subjects. Even with the use of a biphasic stimulus pulse and a short stimulus duration (50 μ s), thresholds were low (90-140V) for supramaximal stimulation, thus allowing the subjects to tolerate 4 minutes of intermittent tetanic stimulation. The ischaemic cuff seemed to be more difficult to withstand and those subjects able to tolerate the cuff were also able to accept the stimulation without difficulty.

The M-wave recordings, which were the main focus, of this thesis, were readily analysed by the software created for this study; the programme enabled selected M-waves within the tetanic trains to be analysed. Inconsistencies in the electrical recordings would have resulted in appreciable errors around the mean curves but this was not the case.

4.2 M-wave and force responses during fatigue

In general, the changes in muscle excitability exhibited during fatiguing

stimulation fell into two phases. The early phase lasted for approximately the first two minutes of fatiguing stimulation, while the remaining minute and twenty seconds comprised the second phase. At all frequencies of stimulation used, the changes in the M-wave response were out of phase with changes in twitch torque.

4.2.1 M-wave responses during early phase of fatigue

The most striking observation from these experiments was that both M-wave amplitude and area were very well maintained for the first one to two minutes of fatiguing stimulation, during which time up to 2000 stimuli had been delivered. The early phase in these studies was longer than that usually observed in other studies (for example Cooper *et al.*, 1988). This difference was probably due to the different muscles used in the studies as the frequencies of stimulation were similar. M-wave area was maintained preferentially to amplitude, suggesting that the duration was increasing; moreover, this trend was completely independent of frequency. This increase in duration was due to the broadening of the individual muscle fibre action potentials (Bigland-Ritchie *et al.*, 1979), due to slowing of conduction velocity and the temporal dispersion of the population of potentials, which, in turn, resulted from reduced impulse conduction velocities (Bigland-Ritchie *et al.*, 1979; Jones *et al.*, 1979). The increase in M-wave duration is a common occurrence in stimulated contractions (Bigland-Ritchie and Woods, 1984) and in M-waves interspersed between intermittent voluntary

contractions (Hicks et al., 1989; Milner-Brown and Miller, 1986; Miller et al., 1987). Frequency analyses of the voluntary (surface) electromyographic signal reveal shifts of the mean power frequency to lower values as the muscle fatigues. Decreasing action potential velocities contribute to this shift (Lindstrom et al., 1977; Lindstrom et al., 1970) which probably occurs because of the accumulation of ions and metabolites in the extracellular space (Bigland-Ritchie et al., 1979; Moxham et al., 1982).

Unlike earlier studies from this laboratory (Fitch and McComas, 1985; Garland et al., 1988a, b; Hicks et al., 1989) and others (Gibson et al., 1988; Cooper et al., 1988; Stokes et al., 1989), potentiation of M-wave amplitude was not evident in any of the experiments during this segment of fatiguing stimulation. The fatiguing runs utilizing 20 and 30 Hz tetani did result in increased areas of M-wave 1 and 4 (Figures 19 a and b); however, there was no concomitant gain in amplitude, indicating that the M-waves were simply increasing in duration (see Figure 17 in Results).

Although the M-wave did not potentiate, at least in amplitude, it is extremely significant that the size was maintained during repeated excitation of the muscle fibres. The preservation of muscle fibre excitability during early fatigue is achieved through the intervention of the Na⁺, K⁺-pump (Hicks and McComas, 1989; McComas et al., 1992); the pump counteracts the depolarizing effect of the rise in interstitial K⁺ ([K⁺]_i) that occurs as a result of impulse

activity in the muscle fibres (Hicks and McComas, 1989). Mean maximum interstitial K^+ values as high as 10 mM (Juel, 1986) have been reported in isolated mammalian muscles and even higher values, to 15mM, appear in the study of the human brachioradialis muscle by Vyskocil et al. (1983) (see their Figure 2). A recent review by McComas et al. (1992) points out that if one takes into account K^+ efflux per impulse, a firing rate at the onset of contraction of 25 Hz and the fact that all motor units are recruited during maximal contractions, then one second of contractile activity would raise $[K^+]_e$ by 5 mM, to approximately 9 mM. Calculation of the membrane potential using this value for $[K^+]_e$ reveals that a depolarization of approximately 14 mV would occur, thereby causing impulse block. Clearly, early muscle paralysis did not occur in the present experiments, even in the presence of ischaemia - in fact, muscle action potentials were maintained for over a minute during physiological rates of firing.

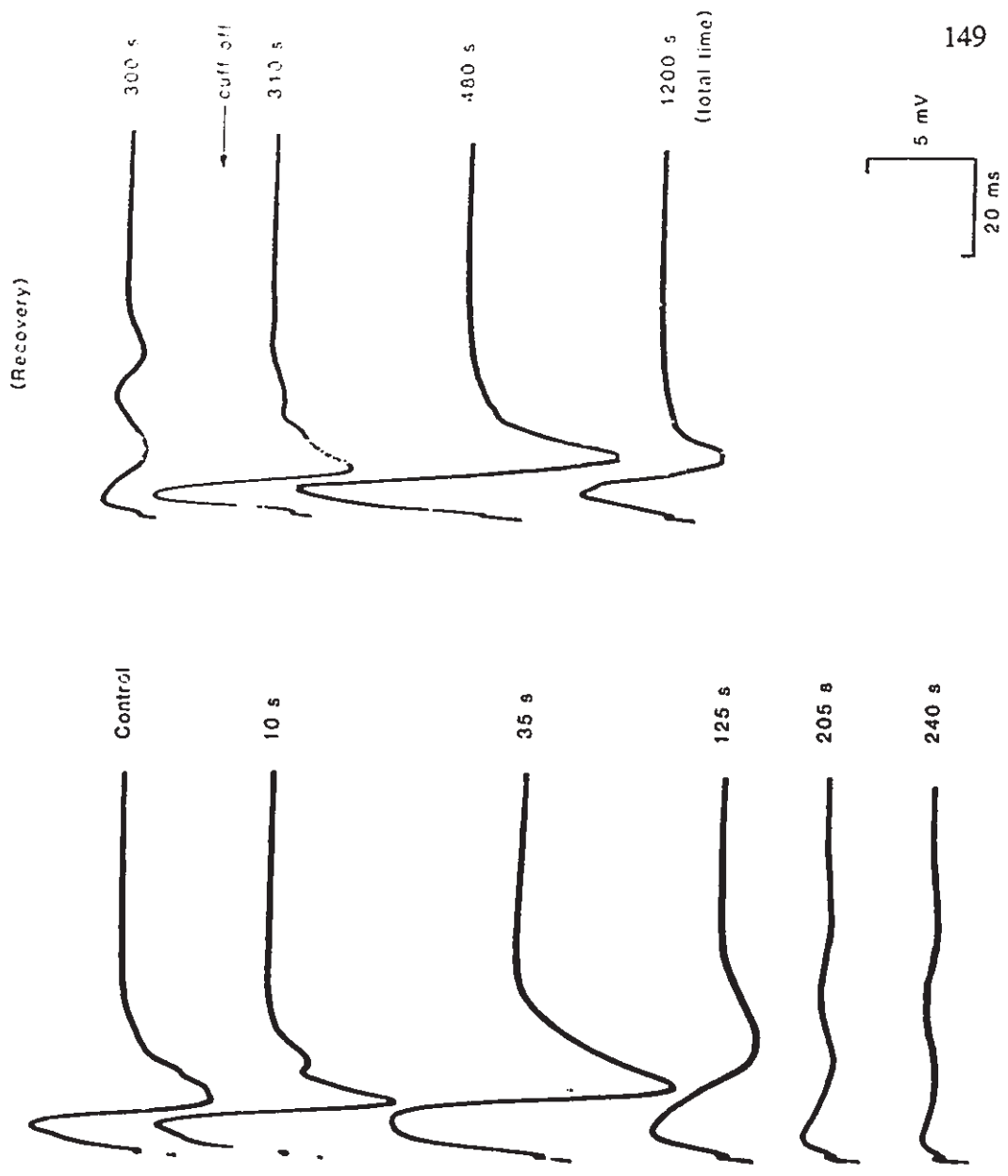
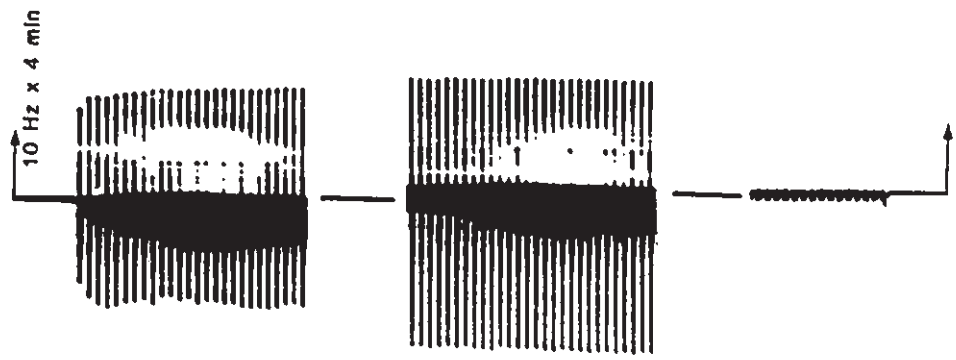
Although potentiation of the muscle compound action potential amplitude did not occur in the present investigation, it is a true phenomenon nonetheless. In fact, it is often in evidence in muscle excitation studies, but tends to be ignored, or mentioned but not explained (see Enoka et al., 1992). More recent studies from this laboratory suggest that potentiation varies among muscles, since we have consistently observed enhancement of the M-wave in the biceps brachii muscle, using stimulus frequencies and aggregates similar to those employed in this thesis for tibialis anterior and soleus. Figure 35 is an example of potentiation

Figure 35

Fatigue and recovery of M-wave amplitude during and after ischaemic repetitive (continuous) stimulation at 10 Hz. The results from this subject show considerable potentiation of both amplitude and area early in fatigue and during early recovery.

(Galea and McComas, unpublished results).

Hi m, 20 yr, biceps brachii



in the biceps brachii of a 20 yr old male volunteer. Not only did the amplitude (and area) of the M-wave increase during the first minute of tetanic stimulation, but the potentiation reappeared during the recovery phase also (see Galea and McComas, 1992). The biceps experiments thus provide an excellent human model to study potentiation. Since it has been shown that this phenomenon is due to acceleration of the Na^+ , K^+ -pump (Hicks and McComas, 1989), this model, by extension, will be suitable for the *in vivo* study of pump dynamics in human muscle for the first time.

In contrast to upper limb muscles, tibialis anterior is very often shown to exhibit modest changes in excitability (Milner-Brown and Miller, 1986; Hicks *et al.*, 1992). A lessened susceptibility to fatigue, as exemplified by a reduced metabolic response, was observed by Boska *et al.* (1990). These findings are consistent with the fact that tibialis anterior is a postural muscle. The results of animal studies, in which denervated muscles were chronically stimulated, suggest that postural muscles are characteristically slower and more fatigue resistant than upper limb muscles because they are much more active throughout the day (Kernell *et al.*, 1987). The effect of increased muscle activity on the Na^+ , K^+ -pump has been investigated by Knochel *et al.* (1985), who trained dogs to run on a treadmill for six weeks. They observed a definite increase in Na^+ , K^+ -ATPase activity in the trained animals. Enhanced Na^+ , K^+ -pump activity was also reflected by an appreciable hyperpolarization of the muscle fibres at rest. The

electrogenic component of the membrane potential during fatigue may be considerable.

In an *in vivo* study of tetanically-stimulated rat soleus muscles, Hicks and McComas (1989) have calculated that the Na⁺, K⁺-pump may contribute as much as -20 mV. It should be noted that the hyperpolarization is directly responsible for the increased size of the muscle fibre action potential, and hence of the M-wave. It is possible that increased usage increases the electrogenicity of human muscles also, as has been suggested by Hicks *et al.* (1992), on the basis of a training study on senior citizens.

The fact that the M-wave was preserved for the first minute or so of tetanic stimulation, regardless of the frequency employed, throws light on the modulation of the Na⁺, K⁺-pump during muscle activity. Thus, at the higher frequencies of stimulation a more pronounced rise in interstitial [K⁺] would be anticipated (see, for example, Hirche *et al.*, 1980) and this, in turn, would be expected to cause greater depolarization and loss of action potential amplitude. The observation that the M-wave is maintained, equally as well as at lower frequencies of stimulation, strongly suggests that the enhancement of pump activity is strongly frequency dependent; such a conclusion would be consistent with the animal experiments of Everts *et al.* (1988). Also in studies on the human biceps brachii, greater potentiation of the M-wave was seen during stimulation at 10 Hz than at 3 Hz (Galea and McComas, unpublished

observation). Beyond 1-2 minutes, in the presence of ischaemia, the force output will begin to fall and it is then no longer advantageous to maintain the M-wave.

4.2.1 M-wave responses during the late phase of fatigue

In agreement with the literature cited in Chapter 1, the muscle excitability changes in this study were more pronounced during the late phase of fatigue. The changes in the M-wave were dependent both on the frequency of stimulation used and on the position of the waveform within the tetanic train.

During fatiguing stimulation at 5 and 10 Hz, the single waveform (M-wave 1) exhibited the least amount of decrement. Even under ischaemic 5 Hz stimulation there was no change in either amplitude or area of M-wave 1, while fatiguing stimulation at 10 Hz caused a 20% decline in amplitude and no change in area for the same waveform. The M-wave located at the end of the 10 Hz test tetani (M-wave 4) declined considerably more in amplitude than M-wave 1, but there was no decline in area. The greatest amount of decline by the end of fatiguing stimulation was expected to occur in the last potential in the fatiguing trains (M-wave 7), but again the 5 Hz waveform was completely maintained, while the 10 Hz potential exhibited an 18% decline in amplitude and a 4% decline in area. It is clear that during stimulation with very low frequencies, the Na⁺, K⁺-pump has ample time to maintain excitability of the muscle fibre for long periods.

As anticipated, there were larger effects on muscle excitability during 20 and 30 Hz stimulation; at these frequencies there were considerable declines in both M-wave amplitude and area. The amount of decrement increased as later waveforms were considered in the train of potentials; thus the decline was greater in M-wave 7 than in M-wave 4 while M-wave 1 was affected least. These observations underscore the importance of testing sarcolemmal excitability with a consistent protocol which includes repetitive stimuli as well as single shocks. Thus, the muscle may respond fully to single stimuli but may exhibit considerably different characteristics in response to a train of stimuli.

Once the M-wave began to decline, the decreases in amplitude and area during 20 and 30 Hz stimulation were approximately linear with time. Furthermore, greater rates of decline were observed for the 30 Hz stimuli than for 20 Hz stimulation. This last finding was attributed to the fact that the greater number of stimuli during 30 Hz stimulation, would cause higher K^+ efflux and hence, more depolarization. The observed effect of stimulus frequency was consistent with the findings of Garland *et al.* (1988a), that the frequency, as well as the number, of stimuli determine decrement. If, because of depletion of energy stores the Na^+ , K^+ -pump was rendered ineffective, then M-wave failure should have started sooner at 30 Hz than at 20 Hz and this was indeed suggested by the M-wave measurements. The above observations agree with the studies by Cooper *et al.* (1988) and Gibson *et al.* (1988) in which increasing stimulus

frequencies were associated with steeper declines in M-wave amplitudes.

With 10, 20 and 30 Hz stimulation, the rate of decline for amplitude was always greater than that of area. As noted previously, the increased duration of the M-wave, due to temporal dispersion and broadening of the single fibre potentials, has been observed previously in human studies (Bigland-Ritchie *et al.*, 1979; Duchateau and Hainaut, 1985; Bigland-Ritchie and Woods, 1984; Milner-Brown and Miller, 1986; Hicks *et al.*, 1989; Miller *et al.*, 1987) and in animal studies (Hanson and Persson, 1971; Hanson, 1974; Grabowski *et al.*, 1972; Sandercock *et al.*, 1985; Pagala *et al.*, 1984; Rankin *et al.*, 1988). Sandercock *et al.* (1985) used a stainless steel extracellular microelectrode to record from single muscle fibres (as single motor units from the cat medial gastrocnemius were stimulated) and observed that as the frequency of stimulation was increased from 10 Hz to 40 Hz to 80 Hz, single fibres suddenly "dropped out". That finding suggested that excitation was failing at the neuromuscular junction or in the motor nerve twigs. Thus the decline in the amplitudes of the motor unit potentials in the above study was attributable to both decreased amplitudes of the constituent fibre action potentials and to complete block of the fibre action potentials, or to a combination of both. Although single unit recordings were not made in the present study, it is likely that both factors contributed to the decline in the M-wave, particularly at the highest frequencies of stimulation employed.

In summary, the decline in muscle excitability during the second phase of stimulated muscle fatigue is postulated to depend on two factors. The first factor, pump failure, is a consequence of the diminished ATP hydrolysis and, in turn, is the outcome of the metabolic changes in the fibre during ischaemic muscle contraction; this factor determines the moment at which the size of the M-wave starts to diminish. The changes in ATP hydrolysis are considered in the next section. The second factor is the accumulation of K^+ in the interstitial spaces of the muscle; by depolarizing the muscle fibres, it determines the rate of decline of the M-wave.

4.2.2 Force response during late fatigue

At all frequencies of fatiguing stimulation used, the changes in the M-wave response were out of phase with changes in twitch and tetanic torque. Thus the potentiation of twitch and tetanic torque was maximal before that of the M-wave; similarly both torques began to decrease before the M-wave started to diminish. The lack of temporal correlation between the electrical and mechanical responses clearly showed that the greater force production, during potentiation, did not depend on a larger transmembrane action potential. Current thinking regarding the cellular mechanism of force potentiation is that the latter results from myosin light chain phosphorylation (Sweeney and Stull, 1986; Houston *et al.*, 1985; Klug *et al.*, 1982; Houston and Grange, 1991) or increased

intracellular Ca^{++} release (Vergara et al., 1977; MacIntosh and Gardiner, 1987; Duchateau and Hainaut, 1986).

The extent of the decline in force was dependent on the fatiguing stimulus frequency between 10 - 30 Hz. During 5 Hz stimulation, however, the tetanic torque remained undiminished throughout the 4 min period. The belief that there is a single cause of muscle fatigue has many adherents. An alternative view is that there are a number of potential factors, each acting at a different stage in the chain of command for muscular contraction (Edwards, 1983). Also, one or other factors will become critical, depending on the type of muscle activity undertaken (e.g. maximal or submaximal, voluntary or stimulated, ischaemic or non-ischaemic). One such factor is certainly the rate at which ATP can be hydrolysed (Dawson et al., 1978, 1980), since this energy compound is required both for Na^+ , K^+ -pumping (see above) and for myosin cross-bridge detachment (Kushmerick, 1983). Declines in the free energy of ATP hydrolysis occur because the reaction products (ADP, P_i , and H^+) are all increasing with fatigue and therefore splitting of ATP would not occur thus reducing the availability of free energy. Although Dawson et al. (1980) were unable to find a satisfactory relationship between the rate of ATP hydrolysis and force, they did find a good correlation with the maximal relaxation rate, indicating defective Ca^{++} uptake by the sarcoplasmic reticulum brought on by insufficient free energy from the Ca^{++} -ATPase.

Attempts have been made to partition the total ATP turnover rate between the major ATPases: Na^+ , K^+ -ATPase, Ca^{2+} ATPase and actomyosin ATPase. Calculation of maximal rates from experiments with frog skeletal muscle indicate that actomyosin ATPase requires the largest amounts of energy supply (Kushmerick, 1983). This is in contrast to a reference made by Fambrough *et al.* (1987) in which, in avian skeletal muscle, the Na^+ , K^+ -ATPase was responsible for the higher ATP consumption. An alternative viewpoint was recently proposed by Thompson and Fitts (1992) who suggested that the energy demands of frog single skeletal muscle fibres were reduced by the inhibiting effects of elevated H^+ (see also Thompson *et al.*, 1992) and inorganic phosphate (P_i) concentrations before ATP availability or free energy of ATP hydrolysis became limiting.

A point of fundamental importance is the extent to which ATP is mobile within the muscle fibre sarcoplasm. If there is compartmentalization, perhaps in the form of a tight linkage between the mitochondria and the ATPase, then it would be possible for one type of ATPase to fail before another. Indeed, such a mechanism might explain the earlier failure of force than of excitation during the present study.

Human studies employing exhaustive exercise (Hultman and Spriet, 1986; Hultman and Sjöholm, 1983; Karlsson and Saltin, 1970) report very small changes in intracellular ATP concentrations, but definite declines in ATP

turnover rates. This has been attributed to the observation that the fraction of energy supplied by anaerobic metabolism, as opposed to aerobic metabolism, decreases during prolonged ischaemic activity. When ATP production from aerobic sources eventually falls, there is a simultaneous reduction in force output (Hultman and Spriet, 1986).

4.3 Recovery of muscle excitability and torque

After fatigue has been induced, the muscle compound action potential will stabilize at a reduced amplitude, provided the tetanization is terminated and the ischaemia is maintained (see below).

4.3.1 Ischaemic recovery

The present study showed only limited recovery of the M-wave during the minute of maintained ischaemia after the fatiguing stimulation had been terminated; in some cases there was further decline of the response (cf. 20 Hz experiments: area measurements). In the study by Edwards *et al.* (1982) there was a rapid recovery of M-wave amplitude, during maintained ischaemia, in the adductor pollicis muscle of the normal subject in contrast to the total lack of recovery exhibited by a patient with myophosphorylase deficiency. These last results should be treated with caution since only one subject and one patient were tested; however, the results in the present investigation were means of 10 and 5

subjects respectively and therefore provide more reliable data on this point.

It is probable that, had muscle excitability been tested in the ischaemic recovery period by single shocks, greater restoration of the M-wave might have occurred. As it was, the short testing tetani (2 s at 10 Hz) may well have added to the adverse situation, by causing greater K^+ efflux from the muscle fibres. However, in experiments on the human biceps brachii, in which single stimuli rather than brief tetani were used to assess fatigue, only modest recovery of the M-wave occurred, so long as ischaemia was maintained (Galea and McComas, 1992).

The most plausible interpretation of the continuing depression of the M-wave is that it resulted from the persistently high accumulation of K^+ in the interstitial spaces, coupled with the inability of the Na^+ , K^+ -pump to restore the normal ionic gradients across the membrane and to develop an electrogenic potential. The rapid rise in M-wave amplitude, once the cuff had been released, suggests that K^+ accumulation had indeed been a factor (see next section).

4.3.2 Recovery after cuff release

Muscle excitability recovered very rapidly after cuff release, in agreement with the observations by Cooper *et al.* (1988) and Gibson *et al.* (1988) on fatigue produced by low frequency stimulation. In the present study recovery of both M-wave area and amplitude was usually complete 2 to 3 minutes after

cuff release. The most obvious explanation of the rapidity of the change in the M-wave is that, as K^+ was washed out of the interstitial spaces by the reactivated capillary circulation, the transmembrane concentration gradient for K^+ would have risen immediately. Since the resting potential is dominated by the K^+ equilibrium potential (Hodgkin and Huxley, 1949) there would have been an immediate increase in the former, and this, in turn, would have produced a commensurate enlargement of the muscle fibre action potential and hence, of the M-wave.

Early recovery is also characterized by enhanced Na^+ , K^+ -pump activity which acts to restore the Na^+ and K^+ concentration gradients as well as increasing the electrogenic component of the resting potential (Hicks and McComas, 1989). Potentiation was not evident in the present experiments, possibly due to the fact that tibialis anterior, by virtue of its function as a postural muscle, could be close to its maximal pumping capacity and would be expected to exhibit very little potentiation (see Section 4.2). The lack of potentiation may have been further compounded by the fact that recovery of excitation was tested by using multiple stimuli. The study by Cooper *et al.* (1988), in which repetitive stimulus trains were delivered at different time points of recovery, also failed to show any potentiation of muscle compound action potential during recovery in the adductor pollicis.

4.3.3 Recovery of twitch and tetanic force

During the period of observation, both twitch and tetanic torque failed to recover fully after cessation of tetanic stimulation; furthermore, the recovery that did take place was at a much lower rate than the recovery of the compound action potential. These results were true for recovery from fatiguing stimulation at 10, 20 and 30 Hz. Following 5 Hz stimulation, there was no demonstrable fatigue since the mean tetanic torque was 104% of the control value. It should be noted that although 5 Hz is within the physiological range of motor unit firing rates for slow-twitch muscle (Bellemare *et al.*, 1983), it is a sufficiently low frequency to cause unfused tetani in the tibialis anterior. The potentiation of tetanic torque, admittedly slight, might have been due to slowing of the maximal relaxation rate (in this study reflected in an increase in twitch half relaxation times), allowing some summation of the twitch responses to occur. In addition, myosin light chain phosphorylation and increased intracellular Ca^{++} concentrations are likely to have been contributing factors (see page 156).

Force output following low frequency stimulation to fatigue is characterized by extremely long recovery periods, as much as 24 hours (Edwards, 1981; Gibson and Edwards, 1985; Edwards *et al.*, 1977). In the present study, twitch torque recovered to 98% of the control value within 7 min of terminating 10 Hz stimulation; when 20 Hz and 30 Hz were used as the fatiguing frequencies, the corresponding recovery was only half. Tetanic torque recovered

less than twitch torque after 20 and 30 Hz stimulation; after 10 Hz stimulation, however, it was close to the control value by the end of the recovery period. Since recovery of muscle excitation was complete by the first minute after cuff release, the persisting fatigue must have been due to changes occurring beyond the sarcolemma (Edwards *et al.*, 1977; Edwards, 1981). There are two possible reasons for force depression: (a) a continuing impairment of excitation-contraction coupling; and (b) contractile machinery failure due to metabolic factors.

4.3.3.1 Excitation-contraction coupling

The sarcolemmal action potential propagates to the interior of the muscle fibre through the t-tubular system and, through intermediary molecular events, initiates the contraction. The action potentials travelling down the t-tubules trigger voltage-sensing receptors (the dihydropyridine (DHP) receptors) located in the t-tubular walls. In turn the DHP receptor activates the ryanodine receptor and completes the linkage between the t-tubules and the sarcoplasmic reticulum (S-R). The end result is that an efflux of Ca^{++} from the S-R into the cytosol occurs, following probable conformational changes in the ryanodine receptors; the intracellular free Ca^{++} then combines with the troponin molecules on the actin filaments and initiates cross-bridge interaction. The active reuptake of Ca^{++} by the S-R through the action of Ca^{++} -ATPases located in the S-R membrane

terminates cross-bridge cycling, thereby allowing relaxation of the contractile elements in anticipation of the next excitation (for reviews see Donaldson, 1986; Eisenberg, 1987; Martonosi and Beeler, 1983).

There is considerable evidence for excitation-contraction uncoupling during fatigue. For example, low frequency (1 Hz) stimulation of isolated amphibian fibres produced fatigue of the twitch that could not be related to action potential failure or to failure of contractile elements. Thus, the action potential was maintained and tension could be restored using high K^+ solutions to produce contractures (thereby maintaining membrane depolarization of the t-tubules). Contractures could also be induced in the fatigued muscle by the application of caffeine; this compound caused release of Ca^{++} from the S-R (Eberstein and Sandow, 1963; Grabowski *et al.*, 1972; Nassar-Gentina *et al.*, 1981; Edman and Lou, 1992). In the present experiments also, twitch force began to decline at a time when the surface action potential, as reflected in the M-wave, was still of normal amplitude. These various experiments established the fact that, although the muscle fibre was still capable of generating force, it was unable to, during fatigue, because of a lack of activation (Edman and Lou, 1992). Although it has not yet been possible to make reliable recordings of the inwardly-propagated action potential in the T-tubules in rested or fatigued fibres, it remains likely that the action potential mechanism is compromised in fatigue. Thus it is predictable that diffusion of K^+ from the tubules would be delayed because of

the high surface-to-volume ratio (Bezanilla *et al.*, 1972), and further, the perfusion experiments of Hodgkin and Horewicz (1959) are indicative of appreciable diffusion delays. Another factor which would tend to maintain a high tubular $[K^+]$ is that the density of Na^+ , K^+ -pumps in the transverse tubules is relatively low (Fambrough *et al.*, 1987). The combination of these factors could result in continued depolarization of the t-tubular membrane and failure of action potential propagation. Adrian *et al.* (1969) voltage-clamped the surface membranes of isolated amphibian fibres and used tetrodotoxin to block the inwardly propagating action potentials. They found that there was just sufficient electrotonic spread of the action potential from the surface of the fibre to the interior to activate the innermost myofibrils. It is perhaps functionally significant that, in those human muscle fibres which show M-wave potentiation, the electrotonically-conducted signal to the interior would be enhanced. However, as muscle excitation is continued, the declining action potential at the surface may fall below the safety-level for myofibrillar activation. In fact failure of inward spread of activation through freeze fracture analysis has recently been shown by Edman and Lou (1992). They showed that with severe fatigue only the most peripheral layers of myofibrils were activated producing the paradoxical situation that a correspondingly larger proportion of the myofibrils are in fact resting because of failure of activation. Recent experiments by Allen *et al.* (1989) and Lee *et al.* (1991), using fluorescent indicators (aequorin and Fura-2) to measure

the cytosolic Ca^{++} concentration in isolated amphibian muscle fibres, have indeed shown that progressively less Ca^{++} is released as the fibre fatigues. A failing t-tubular action potential, and diminished electrotonic conduction, would be consistent with the observed reduction in Ca^{++} release.

It is possible that uncoupling of excitation and contraction also occurs late in the recovery process. For example, it has also been shown in humans that force output in response to low frequency stimuli remains depressed, at a time when the muscle compound action potential and muscle energy stores were normal. The fact that full force can be restored by high frequency tetanic stimulation and maximal voluntary effort is further, strong, evidence of E-C uncoupling (Edwards *et al.*, 1977; Hultman and Sjöholm, 1983; Jami *et al.*, 1983; Bigland-Ritchie *et al.*, 1986).

4.4 Effects of ischaemia on muscle excitability

As discussed previously, it is probable that the maintenance of muscle excitability is highly dependent on oxidative mechanisms to supply energy for the Na^+ , K^+ -pump, and on the removal of extracellular K^+ . In the present study, this dependence was brought out by comparing the results of the ischaemic and non-ischaemic experiments. In the latter, and not the former, there was very good preservation of both M-wave area and amplitude throughout fatiguing stimulation at 20 Hz. A further indicator of the important role of the muscle

capillary blood supply was the rapid recovery of muscle excitability that occurred upon cuff release. These results were in agreement with those described by Cooper *et al.* (1988), who increased the time period of fatigue in order to cause tetanic force to decline to the same degree as their ischaemic experiments. In the present study, the same protocol was used with and without ischaemic intervention. If, in the present experiments without ischaemia, the stimulation had been allowed to continue, there would eventually have been a decline in force in keeping with the results of Cooper *et al.*, (1988). Contributing to the better preservation of the M-wave in the present study was the intermittent nature of the stimulating cycles, within which the 3 s of rest would have allowed muscle perfusion to resume. Sustained stimulation with an intact circulation invariably results in a potentiation of M-wave amplitude that is maintained throughout the fatiguing period (see Duchateau and Hainaut, 1985).

It is clear from the above results that ischaemia contributes to the decline in muscle excitability. As has already been discussed, ischaemia for very short periods (3-4 min) produces negligible changes in metabolites in non-contracting muscles of animals (Jennische *et al.*, 1982). Similarly, during ischaemia in relaxed human quadriceps muscles no decrement in ATP concentrations was observed over a period of approximately 15 minutes (Harris *et al.*, 1975). Coupled with stimulation, however, ischaemia produced marked acidosis, with intracellular pH decreasing from 7.0 to 6.4 (Sahlin, 1986; Boska

et al., 1990). This condition is observed during very intense isometric or concentric exercise sustained to fatigue, in which occlusion of the circulation is present (Sjøgaard, 1990). The increased H^+ accumulation occurs because of the complete dissociation of lactic acid, which is a by-product of anaerobic glycolysis. H^+ accumulation will compromise cellular excitation and contraction processes and is discussed below (Westerblad et al., 1991). An excellent discussion of the metabolite "accumulation" hypothesis of fatigue has been given by MacLaren et al. (1989). In addition to fatigue possibly being caused by the accumulation of H^+ , metabolites such as ammonia (NH_3), the ammonium ion (NH_4^+) and inorganic phosphate (P_i) are considered. The accumulation of the first three metabolites affects muscle excitability and will be discussed in the next section.

4.4.1 Effect of metabolite accumulation on muscle excitability

The effects of prolonged ischaemia are consistent with the accumulation of metabolites and with restoration of the normal concentrations upon return of circulation. H^+ ion accumulation affects excitation by causing inhibition of action potential generation during fatigue (DeLuca, 1984). It has been suggested that decreased pH causes conformational changes in the sarcolemmal membrane of proteins controlling ionic permeability (Bass and Moore, 1973); such changes cumulatively cause depolarization of the membrane and result in depression of the

compound action potential, both during stimulated and maximum voluntary contraction (Bigland-Ritchie *et al.*, 1979; Milner-Brown and Miller, 1986; Miller *et al.*, 1987). In exercising humans artificially induced acidosis is associated with an increased rate of decline of initial force, a decreased glycolytic rate and lower rate of La^- release (Sutton *et al.*, 1981). Conversely, La^- release from contracting muscle seems to be enhanced in conditions of extracellular alkalosis induced by the ingestion of NaHCO_3 (Sutton, *et al.*, 1981).

Of course, the effects of changes in H^+ are usually refuted on the basis that myophosphorylase deficient patients do not accumulate this ion, but do exhibit considerable declines in membrane excitability (Edwards *et al.*, 1981; Edwards and Wiles, 1982; Wiles *et al.*, 1981), observations that cannot be ignored. It is interesting to note, however, that if H^+ accumulation causes conformational changes in membrane proteins then it should also affect fibre conduction velocity, thereby causing a decrease with increasing acidosis. It is therefore significant that EMG power spectral shifts to low frequencies, concomitant with muscle fatigue (Mills, 1982), are observed in myophosphorylase deficient patients as well as in normals (Mills and Edwards, 1984).

Ammonia ion (NH_4^+) accumulation has been studied on the frog sartorius muscle. Heald (1975) made the observation that NH_4^+ depolarized individual muscle fibres in proportion to its concentration. Resting membrane depolarizations as high as 33 mV occurred in muscle equilibrated in 120 mM

ammonium Ringer for a period of 30 min. It was observed that the depression in tension was directly related to the loss of membrane excitability (Heald, 1975). The direct effect of ammonium ions on human muscle excitability has not been addressed, although large amounts of this ion are released from muscle during intense or prolonged exercise (Allen and Cohn, 1960). Ammonia is a by-product of amino-acid metabolism and is therefore raised during periods of starvation (Newsholme and Leech, 1983), where the body reverts to metabolizing protein in order to survive. One human model in which the effect of ammonium ions on muscle excitability may be studied is anorexia nervosa. Patients with this condition exhibit a constant state of protein calorie deprivation and, although it has not been studied directly, probably exhibit elevated levels of ammonia.

Another human model would be those patients with chronic renal failure; studies of muscle fibre excitability could be made before and after dialysis. Perhaps the most simple model, however, would be to administer an ammonium salt to healthy subjects, and to examine muscle excitability in relation to plasma NH_4 levels, in the vein of the experiments by Sutton *et al.* (1981) referred to above.

4.4.2 Effects of ischaemia on force

During the ischaemic 20 Hz experiments, there were considerable declines in both twitch and tetanic torque output. Non-ischaemic stimulation at

20 Hz produced only a slight decline in twitch torque (to 70% of control value). Conversely, tetanic torque potentiated above control values and remained so for the entire duration of fatiguing stimulation. Upon termination of fatiguing stimulation, there was concomitant recovery of both twitch and tetanic torques. Twitch and tetanic torque output remained depressed during the one minute of ischaemic recovery in the related experiments. Comparison of that time period to the non-ischaemic studies revealed that both twitch and tetanic torque continued to decline (albeit by very small amounts). Recovery of non-ischaemic twitch torque achieved control values within two minutes after termination of fatiguing stimulation and then hovered about control values for the remainder of the recovery period. Tetanic torque potentiated, peaking at approximately one and a half minutes after termination of stimulation, and then proceeded to decline to 80% of control values by the end of the recovery period. It should be noted that both twitch and tetanic torques potentiated in early fatigue independently of ischaemia; furthermore, tetanic torque potentiated in the absence of fatigue in the non-ischaemic experiments. This phenomenon, termed post-tetanic potentiation, has been observed in animal fast-fatiguing motor units (Enoka *et al.*, 1992) and in earlier studies from this laboratory (Vandervoort *et al.*, 1983; Garner *et al.*, 1989).

It is clear from the above results that ischaemia also affects the decline in torque. Accumulation of metabolites also applies in this case. The

accumulation of H^+ affects the production of force by: 1) competing with Ca^{++} for troponin and thereby preventing engagement of the myosin cross-bridges with actin (Katz and Hecht, 1969); 2) reducing the sensitivity of troponin and thereby increasing the Ca^{++} requirement for development of the same tension pre-acidosis (Donaldson et al., 1978; Fabiato and Fabiato, 1978; Metzger and Moss, 1990a,b). Recently, Thompson et al. (1992) proposed that the recovery of force was biphasic with elevated H^+ being partially linked to the slow phase. However, observations made by Sahlin (1986) and Bertocci et al. (1992) support the view that fatigue can occur without an elevation in H^+ concentration. Rather they propose that skeletal muscle fatigue is highly correlated with increases in ADP, possibly due to insufficient rates of rephosphorylation of this metabolite (Sahlin, 1986).

Further evidence refuting a role for elevated H^+ in fatigue is based on the observation that myophosphorylase deficient patients fatigue rapidly but do not become acidotic (Edwards and Wiles, 1982). However, muscles fatigue rather rapidly in these patients. Since, the glycolytic blockade prevents the formation of ATP, it is appropriate to ask whether the lack of this energy substrate precedes any effects of intracellular acidosis in causing the failure in force production.

An accumulation of inorganic phosphate has been found to occur in ischaemic, non-contracting, muscles (Edwards et al., 1982) and in muscular exercise in both amphibian (Dawson et al., 1978) and human muscles (Miller et

al., 1987, 1988). P_i acts on the myosin head by reducing the opportunity for engagement with actin thereby reducing force output by increasing the forward rate of cross-bridge cycling (Brandt et al., 1982; Hibberd et al., 1985). However, the increase in inorganic phosphate occurs early in fatigue (within 30 seconds) when there is very little reduction in force (cf. Cady et al., 1989), so that, the importance of this particular metabolite is difficult to estimate at present.

There is a second type of change in phosphate involving the levels of a diprotonated phosphate ($H_2PO_4^-$) formed from the buffering of the monoprotonated phosphate (HPO_4^{2-}) with H^+ . Skinned fibre preparations have been used to show that in acidic conditions (pH 6.5) twice as much of the diprotonated form exists as the monoprotonated form. Furthermore, concentrations of 20 mM diprotonated phosphate were found to reduce force by half (Nosek et al., 1987). Miller et al. (1988) found good correspondence between the changes in protonated phosphates and force loss in the human adductor pollicis as examined by NMR spectroscopy. Thompson and Fitts (1992) observed a 32% increase in $H_2PO_4^-$ with fatigue and found that it significantly correlated with force after 3 min of recovery. Once again, the observations made in myophosphorylase deficient patients are important because the lack of intracellular acidosis also prevents the rise in diprotonated phosphates. As discussed above, the generation of muscle force may be limited in these patients due to the unavailability of ATP for hydrolysis.

The effects of metabolite accumulation during ischaemia cannot be separated from the anoxic effects. In order to test this issue adequately, it is necessary to differentiate between the effects due to anoxia and those due to metabolite accumulation. An excellent *in vivo* animal model for this purpose is the perfused rat hindlimb (Lindinger and Heigenhauser, 1987), in which the arterial pO_2 could be reduced and muscle excitability could be measured concomitantly to force output. Unfortunately, the M-wave and force output must necessarily be measured from the triceps surae, consisting of both fast- (gastrocnemius lateral head), intermediate muscle (gastrocnemius medial head and plantaris) and slow-twitch (soleus). Although there does not seem to be a reliable way of isolating one of these muscles in terms of their arterial supply and venous return, single fibre measurements of excitability would still be possible using intracellular microelectrode techniques (cf. Hicks and McComas, 1989).

Attempting to differentiate between the influences of anoxia and metabolite accumulation in the human is difficult, primarily because of ethical considerations. However, it is possible to ask human volunteers to inhale gas mixtures where the pO_2 is lowered by replacing O_2 with another gas (nitrogen is often used). Monitoring of arterial pO_2 using serial plasma samples from an arterial line in the brachial artery would indicate when gas inhalation results in a lowered arterial pO_2 and very short lasting stimulation (of biceps brachii) could then be performed. Muscle excitability and torque responses would then be

compared with those measured during ischaemic trials allowing for the differentiation between metabolite accumulation and anoxia.

4.5 Muscle excitability in fast- versus slow-twitch muscles

As reviewed in section 1.9 of the Introduction, the large majority of investigative studies comparing the response of fast- versus slow-twitch muscle have been performed on animal rather than human muscle. The only attempt to differentiate electrical response between a slow- and a fast- human muscle was the study by Moritani et al. (1985) where the soleus M-wave during continuous stimulation at 20 Hz was better maintained than that of gastrocnemius (lateral head) as measured by intramuscular electrodes. The recording sites in this study would have been fairly close and therefore, susceptible to cross contamination because of volume conduction. The reasons that human studies are not often attempted are twofold: 1) human muscle is for the most part heterogenous in nature and does not exhibit separation based on fibre type (Johnson et al., 1973; Brooke and Engell, 1969). Therefore, attempting to differentiate fast and slow responses based on the histochemical nature of the muscle is usually unrewarding. The second problem lies in finding appropriate recording and stimulation sites that would provide consistently reliable EMG responses as well as adequate comfort for the subject. Subject tolerance, particularly in stimulated contractions, is of prime importance for completion of experiments. Even in patients who reach the

end of the experiment there often is a tendency to "fight" the stimulation, as their pain tolerance decreases; this results in contamination of the evoked response by background muscle activity.

In this study the human soleus and tibialis anterior muscles were successfully compared during fatigue with repetitive stimulation at a moderate frequency (30 Hz). Fibre type analyses reveal that soleus is composed almost entirely of Type I fibres (89%: Johnson et al., 1973), while tibialis anterior exhibits 73-76% Type I fibres (Johnson et al., 1973; Moulds et al., 1977; Jakobsen et al., 1988). Recently, Henriksson-Larsen et al. (1985) revealed lower proportions of Type I fibres in tibialis anterior (66%) in deeper regions of the tibialis anterior. In addition, fibre cross-sectional areas in both types of fibre were found to be larger in the deeper regions than in the central and superficial regions.

Although the two muscles contain appreciable proportions of Type I fibres, soleus and tibialis anterior display considerable discrepancies in their twitch durations. In the tibialis anterior the mean contraction times were reported as 81-82 ms and the half-relaxation times as 94-98 ms, depending on the sex of the subject (Belanger et al., 1983). These values agree quite well with those reported by Marsh et al., 1981. Plantarflexor muscle contractile properties are dominated by soleus (Vandervoort and McComas, 1983) and are considerably slower with contraction times of 122-136 ms and half-relaxation times of 115-129

ms (Belanger *et al.*, 1983); see also Sale *et al.* (1982) and Vandervoort and McComas 1983). The two muscles also differ in their post activation potentiation, with the tibialis anterior exhibiting considerably more potentiation than the soleus (Vandervoort *et al.*, 1983).

The two muscles were compared using intermittent stimulation at 10/30 Hz. The higher frequency was chosen because it showed the greatest effects in the tibialis anterior experiments. Although 30 Hz is within the physiological range of motor unit firing frequencies (Grimby *et al.*, 1981b), it is beyond the range observed in soleus during voluntary contraction (Bellemare *et al.*, 1983). Considerable attention was given to differentiate this muscle from the rest of the plantarflexors. Percutaneous stimulation of the soleus intramuscular nerve twigs was utilized in order to eliminate the effects of the gastrocnemius muscles, which would have been present had tibial nerve stimulation been employed. Tetani could be tolerated by the subjects through the use of cold anaesthesia; induced by a specially-built cathodal electrode. Cold anaesthesia induces a temperature gradient whereby the skin and subcutaneous tissue would be cooler than the contracting muscle. This is particularly true of the larger lower limb muscles (Asmussen *et al.*, 1976). Cooling alterations in muscle temperature have been induced by the immersion of the limb in cold water (at 15°C). The effect on muscle excitability of lowering the muscle temperature, is a rapid fade of the action potential of the human adductor pollicis at high frequencies of stimulation

but no effect at lower frequencies (10, 20 Hz) (Edwards and Wiles, 1981; Wiles and Edwards, 1982); in addition there is an increase in twitch tension in fast-twitch muscle and a decrease in slow-twitch muscle. Intramuscular temperature measurements showed that soleus possessed a sufficient temperature gradient to maximize the effect of the cooling anesthesia to the cutaneous afferents rather than to the muscle fibres. Physiological evidence that cooling could not have affected the bulk of the soleus muscle fibres significantly was the potentiation of M-wave amplitudes and areas; therefore it is unlikely that sodium pump activity could have been comprised (see below).

The extent of M-wave decline was less in soleus than in the tibialis anterior. During early stimulation of the soleus, there was potentiation of both the amplitudes and the areas of M-waves 1 and M-wave 4 whereas potentiation was absent in all tibialis anterior experiments. It is possible that the higher Na⁺, K⁺ pumping capacity observed in slow-twitch fibres (Clausen and Hansen, 1982; Clausen, 1986; Enoka et al., 1988) may have been responsible for this difference in potentiation, but unfortunately, results from post-mortem human data do not show a relationship between fibre type and Na⁺, K⁺-pump density (Dorup et al., 1988). It should be pointed out that once the M-wave started to decline, the rates were similar for soleus and tibialis anterior. It is possible that, in both muscles the frequency of stimulation caused failure of the action-potential in the nerve twigs or neuromuscular block, as discussed earlier (Bigland-Ritchie et al., 1979;

Jones, 1981). Other factors diminishing the M-wave would be the temporal dispersion and attenuation of the muscle fibre action potentials.

It is evident that slow-twitch muscle exhibits a lower safety margin for neuromuscular transmission during high frequency stimulation than fast-twitch muscle (Clamann and Robinson, 1985); at low frequencies of stimulation , however, declines of this magnitude in slow muscle are not observed (Hanson, 1974; Sandercock et al., 1985; Hamm et al., 1989; Enoka et al., 1992).

Torque output did decline in soleus, but was far less than the decline observed in tibialis anterior; these findings were consistent with the fatigue resistance observed in animal studies (Enoka et al., 1989; Pagala et al., 1984). It is probable that in slow muscles stimulated at high frequencies, the limiting step in the maintenance of force output and of muscle excitability is the energy supply at the level of sarcolemma.

SUMMARY OF RESULTS

1. During repetitive stimulation of the ischaemic tibialis anterior, the decline in the M-wave was greater for amplitude than for area. This difference indicated that the duration of the response had increased due to temporal dispersion and dropout of single fibre action potentials.
2. Maintenance of muscle excitability was possible for at least one minute, regardless of the stimulus frequency employed. On the basis of this work it was suggested that the maintenance of the muscle action potential was made possible by the electrogenic Na^+ , K^+ -pump, and that the pump activity was geared to the excitation frequency of the muscle fibres.
3. Once the M-wave began to diminish in ischaemic muscles, the rate of decline was proportional to the frequency of the fatiguing stimuli. The decline was attributed to failure of the electrogenic Na^+ , K^+ -pump and to the accumulation of K^+ in the interstitial spaces of the muscle.
4. Experiments on the tibialis anterior, without ischaemia, revealed minimal M-wave decline.

5. The decline in the M-wave started later in the ischaemic soleus than in tibialis anterior, but then proceeded at a similar rate. This delay in the soleus response was due to the early potentiating mechanisms.
6. The rapid increase in the M-wave of the fatigued tibialis anterior muscles, following release of the arterial cuff, was attributed to the washing out of K^+ from the interstitial spaces.
7. It is envisaged that the resulting observations on the effect of stimulus frequency on muscle excitability will be of importance to clinicians using repetitive stimulation of muscle for rehabilitation and those concerned with functional electric stimulation of paralysed muscle.
8. The use of a cold cathode as a stimulating electrode may have other applications in situations in which pain from electrical stimulation is a deterrent.
9. The software created to analyse the potentials of interest in this study enabled the sampling and complete analysis of M-waves within tetanic trains. It was apparent that analysis of a single potential alone was not likely to provide a true reflection of the functional status of the muscle.

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Appendix I: Listing of analysis software.

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10 'TO MEASURE EMG M-WAVE (PEAK TO PEAK AND INTEGRAL)
20 DEFINT D: LOADER=3:ZAP=6:DO=9
30 DEFINT A,X,Y
40 I=1:H=1:T=0:TT=0:II=0:SUM=0:TOTL=0
45 DCTR=0
46 IPFF=0
47 IPTF=0
48 N1=0
49 N2=0
50 AMP.CAL=2 ' 2 VOLTS/DIV OUTPUT
60 SMP.TME=400 ' IN USECS. SEE ADSET
70 TQ.C.F = .143 'TORQUE TRANSDUCER CALIBRATION FACTOR
80 DIM DA(3000)
90 DIM DB(3000)
100 DIM DC(25)
110 DIM AA(200,2)
120 DIM E(30) ' FILTER CO-EFFICIENTS
130 DIM B(25,7,7)
140 DIM C(130,4,7)
160 GOSUB 3340 ' LOAD SALT AND PLOT ROUTINES
170 CLS
180 INPUT "WOULD YOU LIKE TO DETERMINE INTER-PULSE-INTERVAL Y/N";P$
190 IF P$="Y" THEN GOTO 200 ELSE GOTO 460
200 CALL LOADER(DR.8,TIME.2.11000.3)
210 CALL LOADER(DR.9,COUNT.2,D.CNT)
220 CALL LOADER(DR.10,RHA.0,RLA.0)
230 N=0
240 LOCATE 22,1:INPUT"PRESS RETURN TO ARM TRIGGER";E
250 CALL DR.8
260 FOR PP=1 TO 50:NEXT
270 CALL DR.9
280 WHILE D.CNT < 10990
290 CALL DR.10
300 CALL DR.9
310 AA(N,0)=D.CNT
320 N=N+1
330 WEND
340 FOR IP=0 TO N
350 AA(IP+1,1)=AA(IP,0)-AA(IP+1,0):PRINT AA(IP+1,1);
360 NEXT IP
370 N1=0
380 N2=0
390 FPI=AA(35,1)
400 FOR IP=1 TO N-2
410 IF AA(IP,1)>90 AND AA(IP,1)<160 THEN N1=N1+1
420 IF AA(IP,1)>FPI-10 AND AA(IP,1)<FPI+10 THEN N2=N2+1
430 NEXT IP
440 PRINT N1
450 PRINT N2
460 INPUT "ENTER E M G AMP SETTING IN microVOLTS/DIV";A.S
470 PRINT:PRINT
480 INPUT "ENTER TORQUE AMPLIFIER SETTING (?mg)";D.TORQUE
490 PRINT:PRINT
500 PRINT "FATIGUING FREQUENCY IS ";(1/FPI)*1000

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510 FLG$="F":Z=7
520 CALL DO(IOINIT) 'INITIALIZE TECMAR BOARD
530 CALL LOADER(DR.1, ADSET.14.3000.400.0) ' SET UP BUFFER
540 CALL
LOADER(DR.3,RHA.0,RLA.0,VDA.1.1000,TIME.4.10.2,WAIT.4,VDA.1.0,AD30.900)
' WAIT FOR TRIGGER & COLLECT DATA
550 CALL
LOADER(DR.11,RHA.0,RLA.0,VDA.1.1000,TIME.4.10.2,WAIT.4,VDA.1.0,AD30V,D.S
AMPTF)
560 CALL
LOADER(DR.12,RHA.0,RLA.0,VDA.1.1000,TIME.4.10.2,WAIT.4,VDA.1.0,AD30V,D.S
AMPFF)
570 CALL LOADER(DR.2,FETCH,D.CHAN,D.AP) ' TRANSFER DATA TO BASIC
ARRAY
580 CALL LOADER(DR.5,SUBV.15,D.OFFST) ' ADJUST EMG OFFSET
590 CALL LOADER(DR.14,SUBV.14,D.TQOFST) 'ADJUST TQ OFFSET
600 CALL LOADER(DR.6,RHA.0,RLA.0) 'FOR COUNTING TRIGGER PULSES
610 CALL LOADER(DR.7,TIME.2.100.3,WAIT.2,VAD.15,D.OFFST) 'GET OFFSET
SAMPLE
612 CALL LOADER(DR.15,RHA.0,RLA.0,VDA.1.500,TIME.4.10.2,WAIT.4,VDA.1.0)
620 AD.G=2
630 AD.GAIN=AD.G
632 IPTF=INT(N1/2)
634 D.SAMPTF=(AA(IPTF,1)*2.5)*.9
636 IPFF=INT(N2/2)
638 D.SAMPFF=(AA(IPFF,1)*2.5)*.9
640 XMIN=200:XMAX=XMIN+500
650 YMIN=10: YMAX=YMIN+206
660 CALL DR.1
670 C=1
680 AX1=YMIN+(YMAX-YMIN)/3
690 AX2=YMAX-10
700 OUT 65476!,INT(LOG(AD.GAIN)/LOG(2))
710 CLS ' DRAW GRAPH OUTLINE
720 LINE (XMIN-1,YMIN-1)-(XMAX+1,YMAX+1),5,B
730 LINE (XMIN,AX1)-(XMAX-1,AX1),2
740 LINE (XMIN,AX2)-(XMAX-1,AX2),2
750 D.CHAN=15
760 LOCATE 22,1:INPUT "PRESS RETURN TO ARM TRIGGER";E
770 DA(0)=1
780 CALL DR.3 'AQUIRE DATA
790 CALL DR.7
800 D.OFFST=D.OFFST*AD.GAIN
810 OUT 65476!,INT(LOG(AD.GAIN)/LOG(2))
852 CALL DR.11
860 FOR DCTR = 1 TO 8
865 CALL DR.15
880 NEXT DCTR
890 CALL DR.11
900 FOR DCTR = 1 TO 9
910 CALL DR.15
920 NEXT DCTR
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930 CALL DR.11
940 IF FLG$="F" THEN GOSUB 4528
950 CALL DR.5
960 D.CHAN=14
970 DC(0)=0
980 D.AP=VARPTR(DC(0))
990 CALL DR.2 'TRANSFER TQ OFFSET SAMPLES
1000 FOR OFST=1 TO 25
1010 D.TQOFST=D.TQOFST+DC(OFST)
1020 NEXT OFST
1030 D.TQOFST=D.TQOFST/25
1040 CALL DR.14
1050 D.CHAN=15
1060 D.AP=VARPTR(DA(0))
1070 CALL DR.2 'TRANSFER EMG DATA TO BASIC ARRAY
1080 D.CHAN=14
1090 DB(0)=0
1100 D.AP=VARPTR(DB(0))
1110 CALL DR.2 'TRANSFER TORQUE DATA TO BASIC ARRAY
1120 DT(1)=901
1130 DT(2)=DT(1)+D.SAMPTF
1140 DT(3)=DT(2)+D.SAMPTF
1150 DT(4)=DT(3)+D.SAMPTF
1160 DT(5)=DT(4)+D.SAMPFF
1162 DT(6)=DT(5)+D.SAMPFF
1164 DT(7)=DT(6)+D.SAMPFF
1170 XXX=1
1180 FOR V=1 TO Z
1190 LOCATE 22,1:PRINT "I AM PLOTTING M-WAVE AND TORQUE SO DON'T TOUCH
ANYTHING!!"
1200 ON V GOSUB 1510,1720,1720,1720,1930,1930,1930
1210 FL=0
1220 PRINT
1230 LOCATE 22,1:PRINT "
ACCEPT Y/N ?":M$=INPUT$(1)
1240 IF M$ <> "Y" GOTO 640
1250 LOCATE 22,1
1260 INPUT"                                ADJUST INT. POINTS
Y/N";CR$
1270 IF CR$="Y" OR CR$="y" THEN GOSUB 3770
1280 GOSUB 2910 'CALCULATES M-WAVE VARIABLES
1290 ON V GOSUB 2140,2570,2570,2570,2740,2740,2740 'CALCULATES TORQUE
VARIABLES
1300 PRINT "KEEP ?Y/N":K$=INPUT$(1):IF K$="N" AND FLG$="F" THEN
I=I-1:V=V+1 ELSE IF K$="N" AND FLG$="R" THEN H=H-1:V=V+1
1310 IF K$ <> "Y" AND K$ <> "N" THEN GOTO 1300
1320 XXX=DT(V)
1330 NEXT V
1340 PRINT "CHANGE AD.GAIN Y/N":G$=INPUT$(1)
1350 IF G$="Y" THEN INPUT "AD.GAIN OF 2 OR 4";AD.G:GOTO 1370
1360 IF G$ <> "N" AND G$ <> "n" THEN 1340
1370 IF FLG$="F" THEN PRINT "MORE FATIGUING Y/N":M$=INPUT$(1)

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1380 IF FLG$="R" THEN PRINT "MORE RECOVERY Y/N":M$=INPUT$(1)
1390 IF G$="Y" AND M$="Y" AND FLG$="F" THEN I=I+1:GOTO 630 ELSE IF
G$="Y" AND M$="Y" AND FLG$="R" THEN H=H+1:GOTO 630
1400 IF G$="N" AND M$="Y" AND FLG$="F" THEN I=I+1:GOTO 630 ELSE IF
G$="N" AND M$="Y" AND FLG$="R" THEN H=H+1:GOTO 630
1410 IF M$ <> "N" AND M$ <> "n" THEN 1370
1420 IF FLG$="F" THEN INPUT "ANY RECOVERY CYCLES Y/N":REC$
1430 IF REC$="Y" AND FLG$="F" THEN FLG$="R":Z=4:GOTO 630
1440 LOCATE 22,1
1450 INPUT "SAVE PEAK AND INTEGRAL ARRAY TO DISK      ,      Y/N      ";P$
1460 IF P$="Y" OR P$="y" THEN GOSUB 3510
1470 LOCATE 22,1
1480 INPUT "DO YOU WISH TO PRINT PK & INT PARAMETERS ? ,      Y/N
";P$
1490 IF P$="Y" OR P$="y" THEN GOSUB 4230
1500 CLS: SYSTEM
1510 PSET (XMIN,AX1-DA(XXX)/20) ' PLOT 1ST. M-WAVE
1520 FOR T=0 TO 125
1530 LINE -(T*4+XMIN,AX1-DA(T+XXX)/20),C
1540 NEXT T
1550 FOR TIC=XMIN TO XMAX STEP (XMAX-XMIN)/10
1560 LINE (TIC,AX1) - (TIC,AX1+5),5
1570 LOCATE INT(AX1/12)+2,INT(TIC/9)-1
1580 PRINT INT((TIC-XMIN)/10)
1590 NEXT TIC
1600 LOCATE INT(AX1/12)+3,47:PRINT"milliSEC."
1610 PSET (XMIN,AX2-DB(XXX)),C 'PLOT TW.TRQ. DATA
1620 FOR F=0 TO 500
1630 LINE -(F+XMIN,AX2-DB((F*2)+XXX)/2),C
1640 NEXT F
1650 FOR TIC=XMIN TO XMAX STEP (XMAX-XMIN)/10
1660 LINE (TIC,YMAX) - (TIC,YMAX+5),5
1670 LOCATE INT(YMAX/12)+2,INT(TIC/9)-1
1680 PRINT INT((TIC-XMIN)/25)*20
1690 NEXT TIC
1700 LOCATE INT(YMAX/12)+3,47:PRINT"milliSEC."
1710 RETURN
1720 PSET (XMIN,AX1-DA(XXX)/20) 'PLOT 2ND, 3RD $ 4TH M-WAVE
1730 FOR T=0 TO 125
1740 LINE -(T*4+XMIN,AX1-DA(T+XXX)/20),C
1750 NEXT T
1760 FOR TIC=XMIN TO XMAX STEP (XMAX-XMIN)/10
1770 LINE (TIC,AX1) - (TIC,AX1+5),5
1780 LOCATE INT(AX1/12)+2,INT(TIC/9)-1
1790 PRINT INT((TIC-XMIN)/10)
1800 NEXT TIC
1810 LOCATE INT(AX1/12)+3,47:PRINT"milliSEC."
1811 FOR T=5 TO D.SAMPTF-6
1812 SUM=DB(XXX+T)
1813 FOR TT=1 TO 5:SUM=SUM+DB(XXX+T+TT)+DB(XXX+T-TT):NEXT TT
1814 DB(XXX+T)=SUM/11
1815 NEXT T

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1820 PSET (XMIN,AX2-DB(XXX)),C 'PLOT TEST F. TT/TRQ.
1830 FOR F=0 TO D.SAMPTF-1
1840 LINE -(F*2+XMIN,AX2-DB(F+XXX)/10),C
1850 NEXT F
1860 FOR TIC=XMIN TO XMAX STEP (XMAX-XMIN)/10
1870 LINE (TIC,YMAX) - (TIC,YMAX+5),5
1880 LOCATE INT(YMAX/12)+2,INT(TIC/9)-1
1890 PRINT INT((TIC-XMIN)/5)
1900 NEXT TIC
1910 LOCATE INT(YMAX/12)+3,47:PRINT"milliSEC."
1920 RETURN
1930 PSET (XMIN,AX1-DA(XXX)/20) 'PLOT 5TH,6TH & 7TH M-WAVE
1940 FOR T=0 TO D.SAMPFF-2
1950 LINE -(T*4+XMIN,AX1-DA(T+XXX)/20),C
1960 NEXT T
1970 FOR TIC=XMIN TO XMAX STEP (XMAX-XMIN)/10
1980 LINE (TIC,AX1) - (TIC,AX1+5),5
1990 LOCATE INT(AX1/12)+2,INT(TIC/9)-1
2000 PRINT INT((TIC-XMIN)/10)
2010 NEXT TIC
2020 LOCATE INT(AX1/12)+3,47:PRINT"milliSEC."
2021 FOR T=5 TO D.SAMPFF-6
2022 SUM=DB(XXX+T)
2023 FOR TT=1 TO 5:SUM=SUM+DB(XXX+T+TT)+DB(XXX+T-TT):NEXT TT
2024 DB(XXX+T)=SUM/11
2025 NEXT T
2030 PSET (XMIN,AX2-DB(XXX)),C 'PLOT FAT. TT/TRQ
2040 FOR F=0 TO D.SAMPFF-1
2050 LINE -(F*2+XMIN,AX2-DB(F+XXX)/10),C
2060 NEXT F
2070 FOR TIC=XMIN TO XMAX STEP (XMAX-XMIN)/10
2080 LINE (TIC,YMAX) - (TIC,YMAX+5),5
2090 LOCATE INT(YMAX/12)+2,INT(TIC/9)-1
2100 PRINT INT((TIC-XMIN)/5)
2110 NEXT TIC
2120 LOCATE INT(YMAX/12)+3,47:PRINT"milliSEC."
2130 RETURN
2140 LOCATE 22,1:PRINT "FILTERING TWITCH TQ. DATA. PLEASE WAIT"
2142 FOR II=15 TO 884
2144
DB(II)=(DB(II-12)+DB(II+12)+DB(II-11)+DB(II+11)+DB(II-10)+DB(II+10)+DB(II-9)+DB(II+9)+DB(II-8)+DB(II+8)+DB(II-7)+DB(II+7)+DB(II-6)+DB(II+6)+DB(II-5)+DB(II+5)+DB(II-4)+DB(II+4)+DB(II-3)+DB(II+3)+DB(II-2)+DB(II+2)+DB(II-1)+DB(II+1)+DB(II))/25
2150 NEXT II
2172 PSET (XMIN,AX2-DB(XXX)),6 'PLOT FILTERED TW.TQ.DATA
2174 FOR F= 0 TO 440
2176 LINE -(F+XMIN,AX2-DB((F*2)+XXX)/2),6
2178 NEXT F
2180 LOCATE 22,1:PRINT "I AM CALCULATING TORQUE VALUES. PLEASE WAIT"
2190 PT=6: COUNTER=1 'FIND TAKE-OFF POINT
2200 WHILE COUNTER < 11 AND PT < 150

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2210 IF DB(PT+COUNTER) > DB(PT) THEN COUNTER = COUNTER + 1 ELSE PT=PT+1:
COUNTER = 1
2220 WEND
2230 IF PT < 150 THEN D.TOFFP = PT ELSE D.TOFFP=0
2240 D.PK.TQ=0      'FIND PEAK TORQUE
2250 FOR T=6 TO 700
2260 IF ABS(DB(T)) > D.PK.TQ THEN D.PK.TQ = ABS(DB(T)):D.T=T
2270 NEXT T
2280 REM          FIND TIME TO PEAK
2290 IF D.TOFFP <> 0 THEN D.TIME.PK.TQ= D.T-D.TOFFP ELSE D.TIME.PK.TQ =
0
2300 TRU.TIME.PK.TQ=D.TIME.PK.TQ * 400 * .001
2310 REM          FIND HALF RELAXATION TIME
2320 IF D.TOFFP <> 0 THEN D.PK.TQ = D.PK.TQ - DB(D.TOFFP) ELSE D.PK.TQ =
D.PK.TQ-DB(6)
2330 TRU.PK.TQ = D.PK.TQ/2048 * 10/ AD.GAIN * D.TORQUE * TQ.C.F
2340 IF D.TOFFP <> 0 THEN HR = (DB(D.T) - DB(D.TOFFP))/2 ELSE HR =
(DB(D.T) - DB(6))/2
2350 T=D.T
2360 WHILE DB(T) > HR AND T < 800
2370 T = T+1
2380 WEND
2390 D.HF.RX.TM = T-D.T
2400 TRU.HF.RX.TM = D.HF.RX.TM * 400 *.001
2410 LOCATE 22,1:PRINT"
2420 LOCATE 2,1
2430 FOR Q=1 TO 20
2440 PRINT"
2450 NEXT Q
2460 LOCATE 2,1
2470 PRINT" CONTRACTION":PRINT" TIME =":PRINT
TRU.TIME.PK.TQ:PRINT"msec."
2480 PRINT
2490 PRINT" PEAK":PRINT" TORQUE=":PRINT TRU.PK.TQ:PRINT"Nm."
2500 PRINT
2510 PRINT"HALF RELAXATION":PRINT" TIME=":PRINT
TRU.HF.RX.TM:PRINT"msec."
2520 PRINT"TAKE-OFF PT=":PRINT D.TOFFP*400*.001
2530 IF FLG$="F" THEN PRINT"N=";I,V ELSE PRINT"N=";H,V
2540 IF FLG$="F" THEN
B(I,V,5)=TRU.TIME.PK.TQ:B(I,V,6)=TRU.PK.TQ:B(I,V,7)=TRU.HF.RX.TM
2550 IF FLG$="R" THEN
C(H,V,5)=TRU.TIME.PK.TQ:C(H,V,6)=TRU.PK.TQ:C(H,V,7)=TRU.HF.RX.TM
2560 RETURN
2570 LOCATE 22,1:PRINT"I AM CALCULATING TORQUE VALUES.PLEASE WAIT"
2580 D.PK.TQ=0      'FIND TEST TETANIC PEAK TORQUE
2590 FOR T=0 TO D.SAMPTF-1
2600 IF ABS(DB(T+XXX)) > D.PK.TQ THEN D.PK.TQ=ABS(DB(T+XXX))
2610 NEXT T
2620 IF D.TOFFP <> 0 THEN D.PK.TQ=D.PK.TQ-DB(D.TOFFP) ELSE
D.PK.TQ=D.PK.TQ-DB(6)
2630 TRU.PK.TQ=D.PK.TQ/2048 *10/AD.GAIN * D.TORQUE *TQ.C.F

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2640 LOCATE 22,1:PRINT"
"
2650 LOCATE 2,1
2660 FOR Q=1 TO 20
2670 PRINT"
"
2680 NEXT Q
2690 LOCATE 2,1
2700 PRINT"TETANIC TORQUE=":PRINT TRU.PK.TQ:PRINT" Nm."
2710 IF FLG$="F" THEN B(I,V,6)=TRU.PK.TQ
2720 IF FLG$="R" THEN C(H,V,6)=TRU.PK.TQ
2730 RETURN
2740 LOCATE 22,1:PRINT"I AM CALCULATING TORQUE VALUES.PLEASE WAIT"
2750 D.PK.TQ=0 'FIND FAT.TETANIC PEAK TORQUE
2760 FOR T=0 TO D.SAMPFF-1
2770 IF ABS(DB(T+XXX)) > D.PK.TQ THEN D.PK.TQ=ABS(DB(T+XXX))
2780 NEXT T
2790 IF D.TOFFP < 0 THEN D.PK.TQ=D.PK.TQ-DB(D.TOFFP) ELSE
D.PK.TQ=D.PK.TQ-DB(6)
2800 TRU.PK.TQ=D.PK.TQ/2048*10/AD.GAIN * D.TORQUE * TQ.C.F
2810 LOCATE 22,1:PRINT"
"
2820 LOCATE 2,1
2830 FOR Q=1 TO 20
2840 PRINT"
"
2850 NEXT Q
2860 LOCATE 2,1
2870 PRINT"TETANIC TORQUE=":PRINT TRU.PK.TQ:PRINT" Nm."
2880 IF FLG$="F" THEN B(I,V,6)=TRU.PK.TQ
2890 IF FLG$="R" THEN C(H,V,6)=TRU.PK.TQ
2900 RETURN
2910 LOCATE 22,1
2920 PRINT" CALCULATING INTEGRAL + PEAK TO PEAK , PLEASE WAIT
"
2930 T1=5:IF D.SAMPFF < 125 THEN T2=D.SAMPFF-2 ELSE T2=125
2940 DPP=0:DNP=0 'FIND PEAKS & TIME OF PEAKS
2950 FOR T=T1 TO T2
2960 IF DA(T+XXX)>DPP THEN DPP=DA(T+XXX):DPT=T
2970 IF DA(T+XXX)<DNP THEN DNP=DA(T+XXX):DNT=T
2980 NEXT T
2990 IF FL=1 THEN TB=T1:CO=T2:GOTO 3110
3000 TB=5:CNTR=0
3010 WHILE CNTR <11 AND TB <DPT
3020 IF DA(TB+1)>DA(TB) THEN CNTR=CNTR+1 ELSE CNTR=0
3030 TB=TB+1
3040 WEND
3050 IF TB >DPT THEN TB=25 ELSE TB=TB-10
3060 PK=-(DPP-DNP)/5 ' FIND UPPER INT. POINT
3070 CO=T2
3080 FOR T=DNT TO 120
3090 IF DA(T+XXX+1)>PK THEN CO=T:T=120:GOTO 3100
3100 NEXT T
3110 INTGL=0 ' CALCULATE INTEGRAL
3120 FOR T=TB TO CO

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3130 INTGL=INTGL+ABS(DA(T+XXX))
3140 NEXT T
3150 TRUEINTGL=INTGL/2048*10/AD.GAIN*A.S/1000/AMP.CAL*SMP.TME/1000
3160 PTOP=(DPP-DNP)/2048*10/AD.GAIN*A.S/1000/AMP.CAL
3170 LOCATE 22,1:PRINT"
"
3180 LOCATE 2,1
3190 FOR Q=1 TO 20
3200 PRINT"
"
3210 NEXT Q
3220 LOCATE 2,1
3230 PRINT" INTEGRATED":PRINT" E M G = ":PRINT TRUEINTGL: PRINT"
mVOLT-mSEC."
3240 PRINT
3250 PRINT"PEAK TO PEAK":PRINT" E M G= ":PRINT PTOP: PRINT" millivolts"
3260 PRINT
3270 PRINT"INT FROM ":PRINT TB*SMP.TME/1000:PRINT" mSEC."
3280 PRINT
3290 PRINT"INT TO ":PRINT CO*SMP.TME/1000:PRINT" mSEC."
3300 IF FLG$="F" THEN PRINT"N=";I,V ELSE PRINT"N=";H,V
3310 IF FLG$="F" THEN
B(I,V,2)=TRUEINTGL:B(I,V,1)=PTOP:B(I,V,3)=TB*SMP.TME/1000:B(I,V,4)=CO*SM
P.TME/1000
3320 IF FLG$="R" THEN
C(H,V,2)=TRUEINTGL:C(H,V,1)=PTOP:C(H,V,3)=TB*SMP.TME/1000:C(H,V,4)=CO*SM
P.TME/1000
3330 RETURN
3340 OPEN "SALT." AS #1
3350 FIELD #1, 1 AS DUM$, 2 AS SEG$
3360 GET #1,1 :SEG=CVI (SEG$) :CLOSE
3370 DEF SEG = SEG
3380 BLOAD "SALT."
3390 DEF SEG =&H2AB0
3400 BLOAD "TP11.RUN"
3410 DEF SEG = SEG
3420 FOR T=1 TO 3:PRINT:NEXT
3430 FOR T=4 TO 24:PRINT:NEXT
3440 KEY OFF
3450 KEY 7,"CR1 L"
3460 KEY 8,"CR1 R"
3470 KEY 9,"CR2 L"
3480 KEY 10,"CR2 R"
3490 KEY ON
3500 RETURN
3510 ON ERROR GOTO 3750
3520 INPUT "FILE NAME=";N$
3530 N$=N$+".DAT"
3540 OPEN "I",#1,N$
3550 LOCATE 22,1
3560 PRINT N$ " ALREADY EXISTS
"
3570 PRINT"CHOOSE ANOTHER NAME"
3580 CLOSE #1

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3590 GOTO 3520
3600 OPEN "O",#1,N$
3610 PRINT #1,I
3630 FOR ZZ=1 TO 7
3632 FOR XX=1 TO I
3640 PRINT
#1,B(XX,1,ZZ);", ";B(XX,2,ZZ);", ";B(XX,3,ZZ);", ";B(XX,4,ZZ);", ";B(XX,5,ZZ
);", ";B(XX,6,ZZ);", "B(XX,7,ZZ)
3648 NEXT XX
3650 NEXT ZZ
3670 IF FLG$ <> "R" THEN GOTO 3730
3690 FOR ZZ=1 TO 7
3692 FOR XX=1 TO H
3700 PRINT #1,C(XX,1,ZZ);", ";C(XX,2,ZZ);", ";C(XX,3,ZZ);", ";C(XX,4,ZZ)
3708 NEXT XX
3710 NEXT ZZ
3730 CLOSE #1
3740 RETURN
3750 IF ERR =53 THEN CLOSE #1:RESUME 3600
3760 GOTO 1500
3770 KEY(7) ON:KEY(8) ON:KEY(9) ON:KEY(10) ON
3780 LOCATE 22,1
3790 PRINT"USE F7-F8 FOR LEFT CURSOR & F9-F10 FOR RIGHT CURSOR"
3800 PRINT"PRESS RETURN WHEN DONE"
3810 T1=20:T2=300
3820 ON KEY(7) GOSUB 3900
3830 ON KEY(8) GOSUB 3980
3840 ON KEY(9) GOSUB 4060
3850 ON KEY(10) GOSUB 4140
3860 B$=INKEY$
3870 IF B$="" THEN 3860
3880 IF ASC(B$)=13 THEN LOCATE 23,1:PRINT "
":RETURN
3890 GOTO 3820
3900 X=T1-1:IF X<1 THEN X=1
3910 XP=X+XMIN
3920 LINE (XP,AX1)-(XP,AX1+5),1
3930 LINE (XP+1,AX1)-(XP+1,AX1+5),5
3940 PSET (XP+1,AX1-DA(X+1)/20),1
3950 PSET (XP+1,AX1),2
3960 T1=X:FL=1:LOCATE 22,56:PRINT"T1=";T1
3970 RETURN
3980 X=T1+1:IF X>500 THEN X=500
3990 XP=X+XMIN
4000 LINE (XP,AX1)-(XP,AX1+5),1
4010 LINE (XP-1,AX1)-(XP-1,AX1+5),5
4020 PSET (XP-1,AX1-DA(X-1)/20),1
4030 PSET (XP-1,AX1),2
4040 T1=X:FL=1:LOCATE 22,56:PRINT"T1=";T1
4050 RETURN
4060 X=T2-1:IF X<1 THEN X=1
4070 XP=X+XMIN

```



```

4080 LINE (XP,AX1)-(XP,AX1+5),1
4090 LINE (XP+1,AX1)-(XP+1,AX1+5),5
4100 PSET (XP+1,AX1-DA(X+1)/20),1
4110 PSET (XP+1,AX1),2
4120 T2=X:FL=1:LOCATE 22,70:PRINT"T2=";T2
4130 RETURN
4140 X=T2+1:IF X>500 THEN X=500
4150 XP=X+XMIN
4160 LINE (XP,AX1)-(XP,AX1+5),1
4170 LINE (XP-1,AX1)-(XP-1,AX1+5),5
4180 PSET (XP-1,AX1-DA(X-1)/20),1
4190 PSET (XP-1,AX1),2
4200 T2=X:FL=1:LOCATE 22,70:PRINT"T2=";T2
4210 RETURN
4220 'PRINT FILE TO PRINTER
4230 INPUT" INPUT FILE HEADER"; F$
4240 PRINT "CHECK THAT RS-232 CABLE IS CONNECTED TO PRINTER. PRESS ANY
KEY WHEN READY"
4250 X$=INKEY$:IF X$ ="" THEN 4250
4260 OPEN "LPT1" FOR OUTPUT AS #3
4270 PRINT #3," "
4280 PRINT #3,"                EMG ANALYSIS"
4290 PRINT #3," "
4300 PRINT #3,"                ";F$
4310 PRINT #3," "
4320 PRINT #3,"FATIGUE"
4330 PRINT #3," "
4340 PRINT #3,"NO.      PTOP(mv)  INT(mv-ms)  AMP/INT  T1  T2          CT(ms)
      PTQ(Nm)      HRT(ms)"
4350 PRINT #3," "
4360 A$="###  ##.##_(##.##_) ###.##_(##.##_)  .##  ##.##  ##.##
###.##_(##.##_) ###.##_(##.##) ###.##"
4370 FOR T=1 TO I
4380 FOR V=1 TO 7
4390 PRINT #3,USING
A$;T,B(T,V,1),B(T,V,1)/B(1,V,1),B(T,V,2),B(T,V,2)/B(1,V,2),B(T,V,1)/B(T,
V,2),B(T,V,3),B(T,V,4),B(T,V,5),B(T,1,5)/B(1,1,5),B(T,V,6),B(T,V,6)/B(1,
V,6),B(T,V,7)
4400 NEXT V
4410 NEXT T
4420 IF FLG$ = "F" THEN RETURN
4430 PRINT #3," "
4440 PRINT:PRINT:PRINT #3,"RECOVERY":PRINT
4450 PRINT #3," "
4460 FOR T=1 TO H
4470 FOR V=1 TO 4
4480 PRINT #3,USING
A$;T,C(T,V,1),C(T,V,1)/B(1,V,1),C(T,V,2),C(T,V,2)/B(1,V,2),C(T,V,1)/C(T,
V,2),C(T,V,3),C(T,V,4),C(T,V,5),C(T,1,5)/B(1,1,5),C(T,V,6),C(T,V,6)/B(1,
V,6),C(T,V,7)
4490 NEXT V
4500 NEXT T

```

```
4510 CLOSE #3
4520 RETURN
4528 CALL DR.12
4530 FOR DCTR = 1 TO IPFF-1
4540 CALL DR.15
4550 NEXT DCTR
4560 CALL DR.12
4570 FOR DCTR = 1 TO IPFF-1
4580 CALL DR.15
4590 NEXT DCTR
4600 CALL DR.12
4610 RETURN
```