PERIPHERAL EXCITATORY AND CONTRACTILE MECHANISMS UNDERLYING FATIGUE RESISTANCE OF HUMAN SKELETAL MUSCLE

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September, 1988

This thesis is submitted in accordance with the

requirements of the University of Liverpool

for the degree of Doctor of Philosophy in the

Faculty of Medicine by

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Experiments have been designed to investigate the physiological factors influencing the interrelationship between excitation and force generation that may counteract the processes leading to a decline in force (fatigue) during stimulated isometric contractions of the human adductor pollicis *in vivo*. Indices of isometric force, relaxation and contraction rates and evoked compound muscle action potentials (CMAP) were measured during defined patterns of stimulated activity (via the motor nerve). A computerized stimulator controller for precise generation of trains of electrical impulses was developed for this purpose. Forces generated at different frequencies were reproducible on separate occasions.

Using an ascending frequency stimulation protocol (1-100Hz) the relationship between force decline and excitation (measured as the amplitude of the surface evoked CMAP) appeared to be dependent on stimulation frequency during ischaemic and non-occluded activity. At high frequencies (50-100Hz), a 'safety factor' was apparent, allowing preservation of force despite a marked fall in excitation, whereas at low frequencies (1-10Hz) force initially potentiated and then declined in excess of excitation. Maximum relaxation rate was reduced at all stimulation frequencies and was independent of stimulation frequency.

Contractile activity performed was shown to be linearly related to maximum relaxation rate over a frequency range of 20-100Hz for up to 30max.seconds. Contractile activity performed was therefore used as a measure of the metabolic cost of a contraction. Force failure appeared to depend upon the numbers of stimuli delivered, independent of frequency, rather than on contractile activity performed, suggesting that electrophysiological factors are of importance in contributing to fatigue.

Further studies on CMAP characteristics demonstrated a broadening of the action potential, reflecting a slowing of conduction velocity, which is thought to lead to 'run-in' of action potentials, and hence the reduction of CMAP amplitude associated with the high-frequency 'safety factor'. The broadening of the action potential recovered immediately during ischaemic conditions at 100Hz following 2400 stimuli but did not recover following prolonged activity at 20Hz until circulation was restored, whereas CMAP amplitude recovered immediately at both frequencies, suggesting that slowing of conduction velocity may be dependent on metabolic factors at low stimulation frequencies which in turn may depend on the contractile history of the muscle. Patients with myophosphorylase deficiency (and thus unable to utilize glycogen), were studied to investigate the importance of energy supply. A failure of ischaemic recovery of the CMAP amplitude and no broadening of the CMAP after stimulated activity at 20Hz was observed, suggesting a failure of excitation of individual muscle cells occurs resulting in force failure in these individuals.

Reversing the pattern of stimulation resulted in an initial enhancement of low-frequency (10Hz) force and a prolonged maintenance of this force throughout the period of contraction studied. This was independent of slowing of relaxation or excitation. The initial force enhancement may result from the increased slowing of relaxation, and in addition, a form of post-tetanic twitch potentiation operates to counteract the decline in force despite a loss in excitation.

In conclusion, during stimulated contractile activity of the adductor pollicis, mechanisms act to maintain or increase force generated per action potential distal to the

sarcolemmal membrane, at both high and low frequencies of stimulation, thereby counteracting mechanisms that lead to fatigue. It is postulated that the alterations in intramuscular processes may allow voluntary isometrically contracting muscle to optimize force production at the onset of a contraction where high motor unit discharge rates are initially developed, delaying or eliminating the influence of excitation failure which would lead to contractile failure once maximal force is achieved, and subsequently to optimize contractile activation in the light of possible excitation failure as motor unit discharge rates decline.

These findings may have important functional implications and may form the basis of physiological strategies for optimizing force production in the development of stimulation regimes for 'functional electrical stimulation' or to any area of skeletal muscle research in which fatigue resistance is of importance.

ACKNOWLEDGEMENTS

I am extremely grateful to Professor R.H.T. Edwards, my supervisor, for this opportunity and for his expert advice and criticism during the course of this study.

I am indebted to Drs. R.G. Cooper and Maria Stokes for their constant advice, criticism, encouragement and all their valuable time for assistance and discussion.

My thanks go to all the members of the Department of Medicine for putting up with my efforts to recruit (!) subjects for experiments and to the postgraduate students of the Department of Physics who were always prepared to act as subjects for my studies. In particular, I would like to thank Drs. Alan Laine, Howard Cranmer-Gordon and Mr. Ian Collins.

I would also like to thank Dr. G. Kidd of the Department of Physiology for the details of the electronics necessary for computer control of stimulation and Dr. R. Gayton for the loan of the Unilab BBC microprocessor interface. Also to Dr. D. Ashby of community health for advice on statistical methods.

Undue thanks must also go to all the members of the University of Liverpool Orchestra for their support, encouragement and light relief.

Finally, I would like to thank Councillor Dr. Pauline McKibbin (Kibby), my landlady, for offering me shelter on my return to Liverpool in 1984 and for all her encouragement,criticismand antagonism during the course of this study.

This study was made possible by a postgraduate studentship of the University of Liverpool and additional support from ICI pharmaceuticals.

The initial suggestion for using the Programmed Stimulation Electro-Myogram (PSEM) in chapter 3, part III came from Drs. M. Stokes and R.G. Cooper. Some of the experiments described in chapter 3, part III were performed jointly with Drs. M. Stokes & R.G. Cooper.

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1. Physiological

CMAP	- Compound muscle action potential
MRR	- Maximum relaxation rate (maximum percent plateau force loss per 10 msec).
-dF/dT	- First differential of force relaxation to obtain MRR.
MCR	- Maximum contraction rate (maximum percent plateau force gain per 10 msec).
dF/dT	- First differential of force to obtain MCR.
Force x Time	- Product of force and time (Newton.secs) This is the force integral (with respect to time) during an isometric contraction and is also expressed as Max.seconds (the equivalent force x time held at maximum tetanic force).
E	- Excitation. Measured from peak negative phase to peak positive phase of the CMAP.
F	- Force.
PSEM	- Programmed Stimulation Electro-Myogram.
NMR Spectroscopy	- Nuclear Magnetic Resonance Spectroscopy
FES	- Functional Electrical Stimulation

2. Biochemical

ATP, ADP, AMP	- adenosine tri-, di- mono-phosphate
PCr	- phosphoryl creatine
Ca^{2+}	- calcium ions
Na^{+}	- sodium ions
K^{+}	- potassium ions

H^+	- hydrogen ions
Pi	- inorganic phosphate
FFA	- free fatty acid

3. Structural/histochemical

Type I fibre	- Slow twitch, oxidative fibre.
Type II fibre	- Fast twitch, oxidative/glycolytic fibres.
T-system	- Transverse tubular system
SR	- sarcoplasmic reticulum
NMJ	- neuromuscular junction

4. Statistical

SD	- Standard deviation
SEM	- standard error of mean
CV	- coefficient of variation
CI	- confidence interval
ANOVA	- analysis of variance

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1. GIBSON H & EDWARDS RHT. (1985) Muscular exercise and Fatigue. Sports Medicine, **2**: 120-132.

2. COOPER RG, EDWARDS RHT, GIBSON H & STOKES M. (1987) Frequency dependence of excitation and force generation in human adductor pollicis. Journal of Physiology, **386**: 113P.

3. COOPER RG, EDWARDS RHT, GIBSON H & STOKES M. (1987) Human muscle fatigue: effects of ischaemia, perfusion and B-adrenergic blockade. Journal of Physiology, **390**: 156P.

4. COOPER RG, EDWARDS RHT, GIBSON H & STOKES M. (1988) Human muscle fatigue: frequency dependence of excitation and force generation. Journal of Physiology, **397**: 585-599.

5. GIBSON H, COOPER RG, STOKES M & EDWARDS RHT. (1988) Human muscle fatigue: Frequency independence of slowing of relaxation rate with stimulated isometric contractions. European Journal of Clinical Investigation, **18**: A36.

6. GIBSON H & EDWARDS RHT. (1988) No ischaemic recovery of the evoked compound muscle action potential in McArdles disease. Clinical Science. In press.

7. COOPER RG, EDWARDS RHT, GIBSON H & STOKES M. (1987) Human muscle fatigue: Post-tetanic potentiation of low-frequency tetani is not related to altered excitation or slowing of relaxation. Journal of Physiology, **394**: 141P.

8. GIBSON H, COOPER RG, STOKES M & EDWARDS RHT. (1988) Mechanisms resisting fatigue in isometrically contracting human skeletal muscle. Quarterly Journal of Experimental Physiology. **73**: In press.

INTRODUCTION

An inevitable consequence experienced by all individuals undergoing some form of muscular activity is the eventual deterioration of performance, no matter how hard an attempt is made to maintain it. This phenomenon is commonly described by the non-specific term 'fatigue'. Fatigue may mean various things to different individuals, and is commonly used to describe the perception associated with muscular performance as well as a true impairment of force generation. For the purpose of this thesis fatigue is defined as the failure to maintain the required or expected force (Edwards, 1981).

An understanding of the processes contributing to the development of fatigue in man is of both theoretical and practical importance, not only in relation to athletic performance, but also in medicine. Here, it may have particular relevance to the understanding and treatment of human myopathies, in electrical stimulation rehabilitation strategies and in the development of skeletal muscle 'functional electrical stimulation' techniques where fatigue may present a problem.

Since the last century, much controversy has surrounded the underlying mechanisms of skeletal muscle fatigue. These are complex in nature, due to the many physiological and psychological factors contributing to it. The many investigations that have been carried out have led to divisions of those factors pertaining to 'motivation' or 'drive' (central fatigue) and those originating in the muscle itself (peripheral fatigue). The majority of literature concerns peripheral fatigue, reflecting its relative importance, and much controversy still exists as to the cause of the latter, whether a consequence of metabolic or electrophysiological factors or both.

The study of skeletal muscle fatigue in man, although hampered by ethical and practical considerations, offers an alternative and applicable approach to counterpart animal studies. It is not possible to analyse in detail the different mechanisms leading to fatigue because of the inaccessibility of the tissues. However, the changes in muscle function associated with fatigue may be identified: loss of force

or power output (the defining features of fatigue), slowing of relaxation, changes in contractile characteristics and alterations in electrical properties. Advantage of these changes may be taken to permit the analysis of how changes in contractile properties of muscle and electrophysiological factors may interrelate, depending upon the circumstances the muscle is measured and how the muscle is fatigued. This approach has been applied in the present studies.

This thesis discusses the contribution of endogenous physiological processes that alter the mechanical-electrophysiological interrelationship during stimulated activity of the human adductor pollicis. These investigations may have important implications in areas of research where human skeletal muscle is to be electrically stimulated for the purpose of overcoming physical, locomotor or respiratory disability in patients.

CHAPTER 1: LITERATURE REVIEW

CHAPTER 1 - LITERATURE REVIEW

MECHANISMS OF SKELETAL MUSCLE FATIGUE

1.1 DEFINITIONS OF FATIGUE

Similar views exist as to the definition of fatigue. Simonsen (1971) has defined fatigue as a reversible state of decreased physical and mental work capacity resulting from preceding work. Similarly, Asmussen (1979) stated that fatigue applied to situations where a transient decrease in working capacity resulted from previous activity, whilst Edwards (1981) defined fatigue as a failure to maintain the required force or power output. Much evidence in the literature suggests that the onset of the mechanisms that contribute to force failure occur before any reduction in force is seen. Subsequently Bigland-Ritchie (1984) has defined fatigue as any reduction in the force generating capacity of the total neuromuscular system, regardless of the force required at any period.

1.2 MECHANISMS OF FATIGUE

The present understanding of the chain of command for voluntary muscular activity involves many steps, from the brain, to the formation of actin-myosin cross bridges within the muscle (Figure 1.1) (Edwards, 1981). Fatigue may occur as a result of failure of any one or more links in this chain.

Early studies classified fatigue as either 'central' (a failure of neural drive from the brain) or 'peripheral' (impairment of force generation by the muscle) and was the source of much controversy. The work of Waller (1891) and Mosso (1915) considered central mechanisms to be more important than peripheral. This was based on comparisons of voluntary effort and electrically stimulated contractions, the latter demonstrating less fatigue than during voluntary activity, although the experimental stimulation techniques employed by these early workers were not specific to the study of individual muscle groups and hence the conclusions drawn may be questioned. The comparison of voluntary to supramaximally stimulated contractions has been used in more recent studies; Merton (1954), who found no difference in stimulated and voluntary force generation in the adductor pollicis muscle concluded that fatigue was

peripheral in origin, but Ikai *et al.*, (1967) demonstrated in the same muscle greater contractile force with supramaximally stimulated activity than with voluntary activity, implying that central fatigue was operating. Other studies employing a twitch interpolation technique to assess the proportion of muscle not centrally activated (Belanger & McComas, 1981) have confirmed a central component to fatigue (Woods *et al.*, 1987), but its contribution is thought to become significant later on in a fatiguing voluntary contraction (Bigland-Ritchie, 1984) unless there is an impairment of motor unit-recruitment at the start of a voluntary contraction.

1.2.1 Central fatigue

The work of Mosso (1915) suggested that nervous arousal was of importance in influencing central fatigue. Motivation clearly is an important factor e.g., running after a train or exercising for reward (Schwab, 1953). Such observations have led to the suggestion that central fatigue may occur because of malfunction of nerve cells or inhibition of voluntary drive; the action of sensory pathways on the reticular formation has been suggested to be critical (Asmussen, 1979). Afferent impulses from free nerve endings or receptors (probably a form of chemoreceptor) in the fatigued muscle may be of importance (Bellemare & Bigland-Ritchie, 1987; Woods *et al.*, 1987).

Metabolic factors may have a role in central fatigue but there is little evidence to support this. Ammonia released from the muscle during exercise, as a product of many biochemical reactions, is thought to have central toxic effects (Barnes *et al.*, 1964). In man CNS dysfunction, as a result of ammonia toxicity, may impair dynamic exercise performance although there is little evidence to support this role in fatigue. Moreover, its contribution to fatigue in contractions of small muscle groups seems unlikely. Acidosis in the brain is unlikely to occur except in pathological conditions and exercise lactacidosis is thought to have a negligible effect on brain function (Siesjo, 1982).

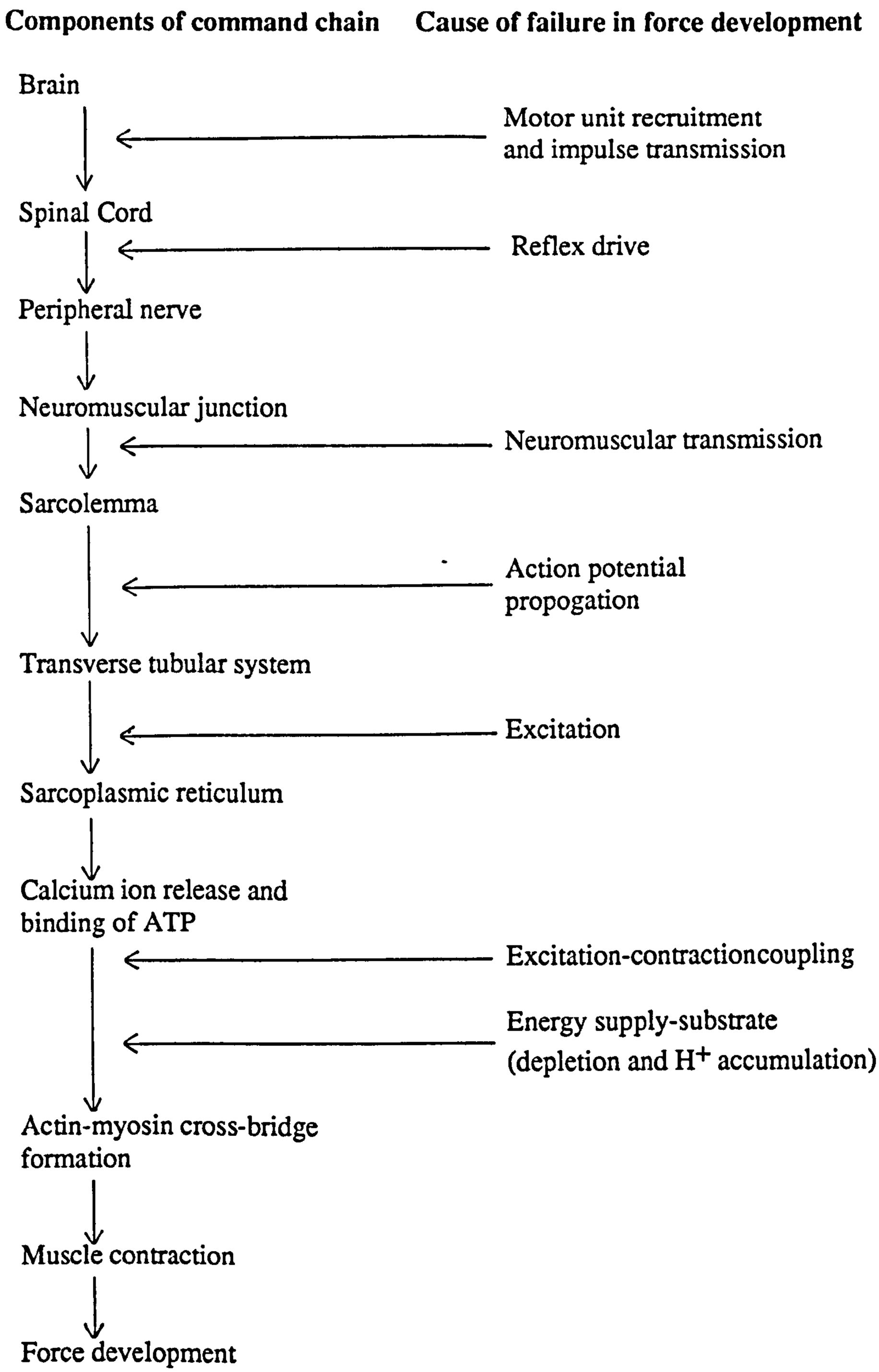


Figure 1.1 Illustration of the command chain for muscular contraction and the major causes of fatigue (after Edwards (1983)).

A psychological component in fatigue is an obvious possibility, e.g. athletes who learn to ignore painful or inhibitory sensory inputs and approach performance limits set by the motor pathways and muscle fibres. However, its presence should not be considered to diminish the importance of clear evidence of peripheral fatigue.

1.2.2 Peripheral fatigue

Fatigue during tetanic electrical stimulation of *in vitro* and *in situ* denervated muscle preparations has long been of interest (Briscoe, 1931; Davies & Davies, 1932; Naess & Storm-Mathisen, 1955). The importance of the contribution of peripheral fatigue mechanisms in man was not realised, however, until Merton (1954) showed that electrically stimulated contractions, superimposed upon voluntary contractions, could not produce any additional force once severe fatigue had been induced.

Two general schools of thought underly the present understanding of the mechanisms of peripheral fatigue. Historically, tissue acidosis and lactic acid accumulation have occupied central positions in many models of fatigue. The early work of Hill and co-workers (1924) proposed that H^+ ions were of importance since studies in isolated preparations of amphibian muscle showed a consistant correlation between the rate of recovery from fatigue and the rate of disappearance of lactic acid. At about the same time, studies on the relation between electrophysiological factors and force generation in frog gastrocnemius indicated that the two were intimately related (Fulton, 1925). Moreover, it was believed H^+ ions affected both, contractile and electrophysiological factors simultaneously. Subsequently, the H^+ ion hypothesis has dominated the thinking of muscle physiologists for many years.

The early work of Assmusen (1934), in which fatigue was demonstrated to occur during indirect tetanic stimulation of isolated lizard intercostal muscle, suggested that a failure of the 'transmission mechanism' contributed to peripheral fatigue i.e., the neuromuscular junction and muscle cell membrane. When followed by direct stimulation, an initial increase in force occured which was followed by a further reduction in force. This was attributed to a failure of the contractile mechanism. These findings were supported by similar experiments on the tibialis

muscle of decerebrate cats (Brown & Burns, 1949). Such studies demonstrated that fatigue could occur at several sites and clearly demonstrated that electrophysiological factors were also of importance. The latter has since occupied the minds of many workers.

1.3 THE ROLE OF METABOLIC FACTORS IN PERIPHERAL FATIGUE

Peripheral fatigue may obviously be affected by metabolic factors in muscle. The fuel for muscular contraction is adenosine triphosphate (ATP) which is regenerated by anaerobic glycolysis or oxidative phosphorylation (Table 1.1). It may be seen from Table 1.1 that metabolic products other than ATP are generated during these reactions and that the source of energy substrates may be diminished. Subsequently, Simonson (1971) has proposed two hypotheses for the cause of fatigue; the 'accumulation' hypothesis, related to the accumulation of metabolites which may impair force generation, and the 'depletion' hypothesis, a result of a depletion of essential metabolites necessary for energy supply.

Reports of the first of these hypotheses dates back to 1904, when Weichardt suggested an accumulation of a substance, which he called 'Kenotoxin' was responsible for fatigue. Support for this hypothesis came from Muller (1935) who demonstrated a faster onset of fatigue with local circulatory occlusion. Hill *et al.*, (1924) proposed that lactic acid was the 'fatigue substance'. Since these early studies other metabolites, namely ammonia (NH_3) and inorganic phosphate (Pi) have also been shown to impair muscle force generation (see later).

1.3.1 Hydrogen ion accumulation

During intense, short-duration muscular activity, lactate (and hence H^+ ions) is produced predominantly from anaerobic glycolysis. The fall in intracellular pH is paralleled with impaired force production and is well supported by many studies in the literature; as evidenced from iodoacetate poisoned or unpoisoned isolated muscle preparations (Sahlin, 1983), skinned mammalian fibres (Donaldson & Hermansen, 1978), as well as nuclear magnetic resonance studies during isometric contraction of

TABLE 1.1 Energy exchange in muscle and ammonia production

Energy supply from ATP:

$$H_2O + ATP \xrightarrow[\text{actomyosin ATPase}]{} ADP + Pi + H^+ + ENERGY$$

ATP is regenerated from:

1) Creatine kinase

$$ADP + PCr + H^+ \rightleftharpoons ATP + Cr$$

2) Glycogenolysis

$$1/n\ (C_6H_{10}O_5)_n + 3ADP + 3Pi \longrightarrow 2C_3H_6O_3 + 3ATP$$

glycogen — lactic acid

3) Oxidative phosphorylation - pyruvic acid

$$C_3H_4O_3 + 2.5O_2 + 18ADP + 18Pi \longrightarrow 3CO_2 + 20H_2O + 18ATP$$

pyruvic acid

4) Oxidative phosphorylation - fatty acids

Ammonia production

1) $ADP + ADP \xrightarrow[\text{myokinase}]{} ATP + AMP$

2) $AMP + H_2O \xrightarrow[\text{adenylate deaminase}]{} IMP + NH_3$

frog muscle (Dawson *et al.*, 1978) and muscle biopsy studies after dynamic and isometric activity in man (Sahlin *et al.*, 1975).

Accumulation of hydrogen ions has been shown to have profound affects on glycolysis which may subsequently influence force generation. For example, phosphofructokinase activity is almost completely inhibited at pH 6.5 (Ui, 1966). Inhibition of PFK as a limiting factor in fatigue is supported by the reported inverse relationship between lactic acid accumulation and the ratio fructose 1,6-diphosphate:fructose-6-phosphate (Bergstrom *et al.*, 1971). Hexokinase activity is also inhibited by physiological concentrations of H^+ (Sjodin, 1976) and moreover, the accumulation of H^+ may also influence the reactions which involve the production or consumption of H^+ (Table 1.1). Clearly, during anaerobic activity inhibition of glycolysis as a result of H^+ accumulation may influence the adequate supply of energy for contraction, although there is a body of evidence which suggests the supply of ATP is not depleted (see below 1.3.4).

The introduction of the *in vitro* 'skinned fibre' technique in which the sarcolemmal membrane is removed or disrupted was a significant development in the study of the effect of pH on the contractile apparatus since it allows control of substrate and Ca^{2+} concentrations in the solutions bathing the myofilaments (Donaldson & Hermansen, 1978). A reduction in pH was found to reduce Ca^{2+} induced force development which required an increase in Ca^{2+} concentration in the bathing medium to achieve a similar tension (Fabiato & Fabiato, 1978; Donaldson & Hermansen, 1978; Metzger & Moss, 1987) thus demonstrating a direct effect of H^+ ions on contractile function. It is unclear, however, whether H^+ ions decrease (Blanchard *et al.*, 1984) or leave unchanged (Fuchs, 1979) the affinity of Ca^{2+} binding sites on troponin or whether H^+ may directly disrupt cross-bridge formation between actin and myosin (Fabiato & Fabiato, 1978). Interestingly, it has been shown recently that a reduction in pH may have different effects on force generation in muscle fibre types (Metzger & Moss, 1987), where the force of type II fibres is depressed more than that seen for an equivalent decrease in pH in type I fibres. This

difference may further explain the differential fatigue properties of the two fibre types.

1.3.2 Ammonia accumulation

Ammonia production was first linked to fatigue by Tashiro in 1922. Muscle is a major source of ammonia during exercise (Lowenstein, 1972) and may be produced from an increase in the myokinase reaction and the enhancement of the purine-nucleotide cycle (Lowenstein, 1972), converting adenosine monophosphate (AMP) to inosine monophosphate (IMP) with the formation of ammonia (Table 1.1). Ammonia has been found to stimulate PFK activity (Lowenstein, 1972), inhibit the Krebs Cycle (Katanuma *et al.*, 1966) and reduce mitochondrial oxidation (Worcel & Erecinska, 1964), which overall may result in a larger lactic acid production and faster glycogen depletion. It has been suggested that ammonium ions may also affect excitatory membrane function (Heald, 1975) and thus contribute to fatigue. This latter point is taken up further in section 1.5.

In contrast to the deleterious affects of ammonia accumulation in muscle, it has been suggested that ammonia may buffer H^+ ions and at low levels potentiate twitch force thereby delaying the onset of fatigue (Heald, 1975; Sahlin *et al.*, 1975). However, it still remains to be shown what role ammonia accumulation plays in the development of peripheral fatigue.

1.3.3 Inorganic Phosphate (Pi) Accumulation

Using NMR spectroscopy techniques, it has been shown that Pi is present in muscle cells at a concentration of 4.4mmol.Kg^{-1} (Wilkie *et al.*, 1983). Four-fold increases in Pi have been demonstrated during maximal voluntary isometric contractions in man (Dawson *et al.*, 1983) and similarly in isolated frog sartorius muscle preparations (Dawson *et al.*, 1978). Direct evidence of Pi induced force reduction has been obtained from skinned muscle fibre preparations (Brandt *et al.*, 1982; Hibberd *et al.*, 1985) and it has been suggested that Pi may bind to myosin in such a way so as to increase the forward rate of cross-bridge cycling and thereby to reduce force output (Cooke & Pate, 1985; Kawai *et al.*, 1985). At pH 7, Pi may exist

as $H_2PO_4^-$ and HPO_4^{2-} (Gadian, 1982). From the work of Dawson *et al.*, (1978) and other similar work, Wilkie (1986) has proposed that the acid form of Pi ($H_2PO_4^-$) may be produced as a consequence of the high concentrations of H^+ developed which may result in force reduction. This is supported by the good correlation between fatigue and this form of Pi during fatiguing activity of the adductor pollicis (Miller *et al.*, 1988). More recent work in patients with myophosphorylase deficiency (McArdle's disease) has demonstrated greater fatiguability than normal individuals (Cooper *et al.*, 1987) and a concomitantly larger increase in Pi accumulation as indicated by NMR studies (Lewis *et al.*, 1985). This further substantiates a plausible role for Pi in fatigue, but these studies may not support the hypothesis of Wilkie (1986) since H^+ accumulation is not a problem in these individuals. Furthermore, recent work by Cady *et al.*, (1988) employing NMR techniques indicates that less Pi^- is produced in these individuals compared to normal subjects in the first dorsal interosseous, again suggesting that Pi may not be of importance in the development of fatigue, although it was not clear from that study whether the reduced Pi^- could have been due failure of individual fibres as a result of excitation failure (Edwards & Wiles, 1981), since no EMG was recorded during stimulated activity. This raises the question of whether force loss in these individuals is necessarily due to the same mechanisms as in normal muscle. At present, it can only be argued that there is a good correlation between Pi^- and force loss in normal muscle. Further studies are indicated for the role of Pi^- in fatigue.

1.3.4 ATP, PCr and glycogen depletion

During work which is predominantly anaerobic, muscle contraction may be impaired by the depletion of the short-term energy substrates ATP and PCr, and also by reduced levels of glycogen (Gollnick *et al.*, 1973) (Table 1.1).

Low levels of ATP have been recorded at exhaustion following dynamic exercise (Sacks, 1960; Piper *et al.*, 1968; Boobis, 1982), yet the role of ATP depletion remains equivocal. The use of needle biopsy techniques (Edwards *et al.*, 1972a; Jansson *et al.*, 1987) and NMR spectroscopy (Dawson *et al.*, 1978) have

shown only small changes in total ATP, ADP and AMP levels after activity, apart from a large change in free ADP (Dawson *et al.*, 1978), although it is possible to reduce ATP levels during aerobic intermittent activity (e.g., Taylor *et al.*, 1986). In contrast, the reduction in PCr is more marked after 1-2 minutes of maximal dynamic activity (Hultman *et al.*, 1967) and only after 50 seconds of stimulated isometric activity of the quadriceps at a frequency of 20Hz (Hultman & Sjoholm, 1983). The reduction in PCr and isometric force in mouse muscle has been shown to be well correlated, suggesting that fatigue was dependent on PCr levels. However, Dawson *et al.*, (1978) observed no proportional relationship between PCr and force development. Edwards *et al.*, (1972a) have further demonstrated that during conditions of local ischaemia, three succesive contractions of the quadriceps each held to fatigue is possible, even when PCr levels are nearly depleted following the first contraction. More recent studies have shown that during prolonged intermittent stimulated activity of the quadriceps, PCr recovers to 60% of fresh control values by 45 minutes of activity at a time when force has decreased to less than 30% (Hultman & Spriet, 1986).

The depletion of glycogen during brief maximal and submaximal anaerobic exercise is thought to be unlikely and could not account for fatigue (Hermansen, 1981; Vollestad *et al.*, 1984; Bigland-Ritchie *et al.*, 1986a), although it is possible that early depletion in type II fibres may result in a reduction in force (Gollnick *et al.*, 1974). During long-term sustained submaximal dynamic exercise, however, glycogen depletion is observed to be closely related to fatigue (Bergstrom *et al.*, 1967a), but only in those cells recruited, depending on exercise intensity and duration (Vollestad & Blom, 1985).

These studies demonstrate that no simple relationship exists between fatigue and changes in high energy phosphates and glycogen. It has been suggested, however, that fatigue may not be due to the absolute level of any one substrate, but to the relative kinetics of supply and demand, as determined by a few key enzymes (Hultman & Bergstrom, 1971).

1.3.5 Glucose and free fatty acid availability during aerobic exercise

During submaximal activity energy may be supplied predominantly from glucose and free fatty acids which are stored in the form of glycogen and triglycerides respectively (Newsholme, 1981). Once muscle glycogen stores are exhausted, blood glucose becomes more important as a fuel for aerobic metabolism. Hypoglycaemia is thought to develop during long term exercise (Ahlborg & Felig, 1982) which may stimulate glycogenolysis and deplete hepatic glycogen stores (Baldwin *et al.*, 1973). In addition, central nervous function, which is dependent on blood glucose as a fuel, may be impaired. Further evidence suggesting muscle glycogen availability limits performance has come from glycogen loading studies in which high glycogen diets consumed for three days before exercise offsets fatigue during long-term exercise (Bergstrom *et al.*, 1967b).

Free fatty acid (FFA) oxidation (Gollnick *et al.*, 1972) is thought to have a glycogen sparing effect (Hickson *et al.*, 1977), thereby delaying the onset of fatigue. Hermansen *et al.*, (1976) have challenged the suggestion that FFA oxidation becomes increasingly more important as carbohydrate stores become depleted since during 1.5 hours of exercise, the respiratory exchange ratio (which is dependent on the fuels being oxidized) remained above 0.9, indicating that carbohydrate stores are predominantly oxidized. More prolonged activity (24 hours), however, has been shown to reduce the respiratory exchange ratio (Davies & Thompson, 1979).

1.4 THE ROLE OF ELECTROPHYSIOLOGICAL FACTORS IN PERIPHERAL FATIGUE

Much physiological evidence is available suggesting that a failure of excitation processes (other than a reduction in motor drive from the CNS), in both anaerobic and aerobic activity may override the influences of metabolism in the onset of fatigue (Edwards, 1981). For example, fatigue occurs more rapidly during high frequency electrical stimulation than during voluntary contractions (Naess & Storm-

Mathisen, 1955; Jones *et al.*, 1979; Marsden *et al.*, 1983) thus resulting in premature force failure at a time when potential depletion of energy is not a problem.

1.4.1 Fatigue during maximal voluntary isometric contractions

The loss of force from sustained maximal voluntary ischaemic isometric contractions is accompanied by a similar decrement in the smooth rectified (integrated) EMG from surface and intramuscular recordings (Stephens & Taylor, 1972; Komi & Rusko, 1974; Jones *et al.*, 1979; Bigland-Ritchie *et al.*, 1983a). These studies appear to indicate that force loss during voluntary contractions is a consequence of excitation failure.

1.4.1.1 Failure of neuromuscular transmission

An obvious site was the neuromuscular junction (NMJ), the failure of which was initially thought to be the cause of excitation failure (Naess & Storm-Mathisen, 1955). Considered in more detail, possible sites of failure include the inhibition of pre-synaptic nerve terminals, depletion of transmitter or to decreased post-synaptic end plate excitability (Krnjevic & Miledi, 1958).

The involvement of the NMJ in peripheral fatigue is disputed by the work of Merton (1954), who demonstrated that the evoked surface measured action potential amplitude was not reduced at a time when force could no longer be generated. Later studies by Bigland-Ritchie *et al.*, (1982) using both surface and intramuscular recordings of EMG, similarly showed no decline in amplitude or area of the evoked signal for up to 60 seconds in a sustained MVC, even though the smooth rectified EMG signal declined. These observations, however, were not consistent with reports by Stephens & Taylor (1972) of a reduction in the evoked EMG signal from the first dorsal interosseous muscle and later by Marsden *et al.*, (1983) employing the adductor pollicis. The reason for these differences remains unclear since similar changes were observed using paired impulses (to remove the effects of interference from voluntary motor impulses) and single impulses (Bigland-Ritchie *et al.*, 1982). Bigland-Ritchie *et al.*, (1982) attributed the differences in the EMG measured by other workers to the methods employed to analyse the EMG signal.

Direct high-frequency stimulation of curarized mouse muscle, by-passing the NMJ, has been shown to produce a similar force loss as indirect high-frequency stimulation of the human adductor pollicis (Jones *et al.*, 1979). This most important observation suggests certain post-synaptic factors lead to a decline in EMG amplitude and area and force. However, no means of *in vivo* measurement of NMJ function during muscular fatigue is as yet possible.

1.4.1.2 Changes in motor unit firing frequency

During sustained voluntary contractions, motor units discharge asynchronously at different rates with a mean discharge rate of up to 30Hz, depending on the muscle group contracting and the force held (Bigland & Lippold, 1954; Bellemare *et al.*, 1983). The asynchronous discharge of motor units allow full tetanic tension to be achieved at a lower rate than would be required if the units discharged synchronously as demonstrated by multi-electrode stimulation techniques in cat muscle (Rack & Westbury, 1969). Intramuscular EMG recordings have demonstrated a decline in mean firing frequency of motor units and of single units during a sustained contraction (Marsden *et al.*, 1971; Bigland-Ritchie *et al.*, 1983b; Grimby *et al.*, 1981) and peak frequencies as high as 150 Hz have been recorded (Marsden *et al.*, 1983) at the onset of ballistic contractions, indicating that an accomodation of the motor unit discharge rate occurs during a sustained maximal contraction. Interestingly, force failure during a maximum voluntary contraction (MVC) can be simulated during stimulated contractions by gradually reducing the stimulation frequency (Jones *et al.*, 1979; Marsden *et al.*, 1983) and has been termed "artificial wisdom" (Marsden *et al.*, 1983). This observation has an important role in the study of fatigue, since it permits investigation of the whole muscle as if it were a single cell. However, such a model has been considered too simple, since single unit EMG recordings indicate optimal frequencies differ between motor units (Marsden *et al.*, 1983).

It can be argued that a decline in motor neurone firing rate would cause a loss of force since low-frequency stimulation of muscle produces less force than that

obtained at higher stimulation frequencies (Bigland & Lippold, 1954; Merton, 1954; Edwards *et al.*, 1977a). A concomitant reduction in relaxation rate accompanies the changes in motor neurone discharge rate (Bigland-Ritchie *et al.*, 1983b), which is thought to be sufficient to allow full activation of the muscle. It has been suggested that the decline of the discharge rate may alter in response to the slowing of relaxation with fatigue (Bigland-Ritchie & Woods, 1984). This might be advantageous to motor control, and may further explain a matching of motor neurone discharge rates and the contractile properties of human muscles with different fibre composition (biceps brachii and soleus) (Bellamere *et al.*, 1983).

It is thought that the reduction in motor unit discharge rates during a sustained voluntary contraction would also minimize the tendency to fatigue at high frequencies, thus protecting against possible action potential failure at the peripheral nerve, NMJ and/or the sarcolemmal membrane itself (Marsden *et al.*, 1983; Jones & Edwards, 1986).

The origin of the decline in motorneurone firing rate is thought to be a fatigue-induced reflex, although it is not clear whether this reflex originates in the central nervous system or within the muscle (Bigland-Ritchie *et al.*, 1985). A lack of recovery of motor unit discharge rates during ischaemia of the quadriceps muscle (Woods *et al.*, 1987) suggests a peripheral inhibitory reflex may act to reduce motoneurone firing rate without impairment of neuromuscular transmission or voluntary effort. Thus it would appear that central mechanisms adapt in response to changes within the peripheral muscle. Bigland-Ritchie *et al.*, (1986b) have suggested that the afferent limb of the reflex described arises from receptors, possibly, Golgi tendon organs, muscle spindles, or group III and IV nerve endings, which may respond to either changes in muscle contractile properties or the metabolic state of the muscle.

Synchronization of motor unit discharge rates (defined as the tendency of motor units to discharge regularly at or near a time that other motor units discharge (De Luca *et al.*, 1982)) also occurs during prolonged activity (Lippold *et al.*, 1960;

Mori & Ishiada, 1976; De Luca *et al.*, 1982a). Grouping of motor unit discharge rates occurs at a rate of approximately nine bursts a second (Lippold *et al.*, 1958) and is thought to be due to interaction of motor units as a result of a feedback mechanism (Mori & Ishiada, 1976). A consequence of this would be to reduce force generation by reducing fusion, but the contribution synchronization of motor unit discharge rate to fatigue is probably small in view of other electrophysiological changes that occur.

1.4.2 Fatigue during stimulated contractions

1.4.2.1 Electrical stimulation of human skeletal muscle

The use of electrical stimulation of muscle, as well as surface and intramuscular EMG recording of evoked myoelectrical activity, has been widely employed in the study of fatigue. Tetanic electrical stimulation of muscle via the motor nerve trunk (Merton, 1954), or via intramuscular motor end nerves by percutaneous stimulation (Edwards *et al.*, 1977a) has frequently been applied in studies on man. Unlike voluntary contractions, tetanic supramaximal stimulation is synchronous and all motor units are recruited independent of stimulation frequency. Consequently at low stimulation frequencies, where fusion is incomplete, force development is small whereas with increasing frequency fusion is more evident resulting in still greater force generation (Bigland-Ritchie, 1981). The frequency at which plateau force develops is thus dependent on muscle fibre composition owing to differences in fusion frequency of the fast and slow constituant fibre types of muscle.

In a small hand muscle, the adductor pollicis, application of a series of frequency trains has been used to document the frequency:force relationship in fresh and fatigued muscle (Bigland & Lippold, 1954; Merton, 1954; Ikai *et al.*, 1967; Edwards *et al.*, 1977a). In larger muscles of the leg the validity of percutaneous stimulation to obtain this has been questioned since it has been suggested the frequency:force relationship obtained in this way is voltage dependent (Davies & White, 1982). Further work on the quadriceps muscle has shown this is not the case except at low voltages (Edwards & Newham, 1984). Indirect stimulation via the

femoral motor nerve trunk is apparently discomforting and unsafe due to the high tensions that can be developed (Bigland-Ritchie *et al.*, 1978).

Direct stimulation of the human adductor pollicis has also been employed in the investigation of fatigue, involving stimulation voltages of 1000 volts or more (Hill et al, 1979). Unlike indirect stimulation which is safe and not particularly painful, direct stimulation of muscle is dramatically painful (Edwards, 1981). Furthermore, repetitive stimulation of muscle by this technique is not possible due to potential damage of tissue as well as the discomfort associated with this form of stimulation.

1.4.3 The interrelationship between force generation and excitation during stimulated contractions

In contrast to the similar declines in smooth rectified EMG and force observed in voluntary contractions, during stimulated contractions force and excitation do not always decline together. In some studies the evoked action potential amplitude and force decline in a similar fashion during stimulated fatiguing exercise eg., in the quadriceps (Hultman & Sjoholm, 1983) and anterior tibialis of man (Fitch & McComas, 1985). In contrast, twitch force and the evoked action potential amplitude have been shown to dissociate when twitch stimuli were superimposed on voluntary contractions during near complete force failure (Merton, 1954) and similarly, during ischaemic intermittent voluntary contractions (Mills, 1982). Similar results have been reported for isolated preparations of rat muscle (Kugelberg & Lindegren, 1979). Furthermore, during high frequency stimulation of single fibre preparations of amphibian muscle, action potential amplitude appears to decline in advance of force failure (Luttgau, 1965), demonstrating that some degree of change of the action potential is possible without affect on force.

Clearly, some form of frequency dependence of fatigue exists during stimulated contractions which suggests that force failure may result from several mechanisms acting at different sites. The application of high and low frequency stimulation trains to fatigue skeletal muscle (Edwards *et al.*, 1977b) have subsequently led to two types of peripheral fatigue being defined; high-frequency

fatigue (HFF): a reduction of force with high frequency tetanic stimulation which recovers fairly rapidly, and low frequency fatigue (LFF): a reduction of force with low-frequency tetanic stimulation (Edwards *et al.*, 1977b; Davies & White, 1982) which recovers with a much slower time course (Edwards *et al.*, 1977b). The mechanisms of these forms of fatigue appear to differ and are therefore considered below under their respective headings.

1.4.3.1 Mechanisms contributing to High-Frequency Fatigue (HFF)

High frequency stimulation (80-100Hz) of skeletal musle leads to a rapid decline in force with a concomitant decline in surface evoked action potential amplitude which may be reversed by reducing the frequency of stimulation (Davies & Davies, 1932; Jones *et al.*, 1979; Bigland-Ritchie et al, 1979). This rapid reversal is important in differentiating the influences of excitatory and metabolic factors on force reduction, since it is suggestive of redistribution of electrolytes across the sarcolemmal membrane. PCr recovery (half-time about 30 seconds, Harris *et al.*, 1976) is too slow to account for the rapid recovery of excitation or force. Accompanied with the decline in action potential amplitude is a broadening in shape which suggests a slowing of conduction velocity (Stalberg, 1966; Bigland-Ritchie *et al.*, 1979). Such studies have indicated that HFF is due to impaired propagation of the action potential along the surface membrane of the muscle fibre. Fatigue of directly stimulated isolated muscle can be overcome by increasing stimulus intensity or duration indicating that a change in excitation threshold occurs (Krnjevic & Miledi, 1958; Jones, 1979), further supporting the suggestion that membrane properties are altered in HFF.

Both, a reduction of Na^+ concentration and an increase in K^+ concentration in the bathing medium of isolated preparations further hastens the onset of HFF and also produces broadening of the action potential waveform (Jones *et al.*, 1979). Calculations by Adrian and Peachey (1973) indicate that in frog sartorius muscle, for each muscle action potential, the Na^+ concentration in the T-tubules may decline by 0.5mM and K^+ concentration increase by 0.28mM. Such changes may dramatically

alter both resting membrane potentials and Na^+/K^+ conductances, hence affecting the amplitude and propagation of the action potential. An increase in K^+ conductance is thought to explain the reports of low membrane resistance observed in metabolically fatigued single muscle fibres (Grabowski *et al.*, 1972). More recently an ATP dependent K^+ channel has been identified in skeletal muscle (Spruce *et al.*, 1985) further suggesting that K^+ conductance may be altered by metabolic factors. In addition, 1-2nM caffeine added to the bathing medium of normal frog sartorius muscle fibres (thereby elevating free internal Ca^{2+}) also results in a reduction in membrane resistance (Fink & Luttgau, 1976) indicating a role for Ca^{2+} in the activation of K^+ channels. Indeed, Ca^{2+} sensitive K^+ channels have recently been identified in rat muscle (Pallotta, 1985); which may be influenced by accumulation of Ca^{2+} in the T-tubular space during high-frequency stimulation (Bianchi *et al.*, 1982). Of course, it is possible that the accumulation of Ca^{2+} in the T-tubules may have other implications on excitation-contraction coupling.

The accumulation of extracellular K^+ has been suggested to be greatest in the T-tubules where diffusion is restricted because of the high surface-to-volume ratio (Adrian & Peachey, 1973; Bezanilla *et al.*, 1972). This may additionally account for the failure of contractile elements within the fiber where loss of the sarcolemmal action potential is not observed (Bezanilla *et al.*, 1972). Recent studies involving the measurement of T-tubule action potentials in amphibian muscle (penetrating the surface membrane), do not support this proposition since changes in the T-tubular action potential appear similar to those of the sarcolemmal membrane (Deleze *et al.*, 1986). It is, however, a distinct disadvantage that no direct information about T-tubular function is possible using EMG techniques in human muscle.

Alterations in extracellular K^+ concentration may not be the sole determinant of HFF, however. Accumulation of K^+ in the extracellular space would result in an expected alteration in resting membrane potential. Several studies in amphibia and mammalian muscle seem to indicate that little change in the resting membrane potential occurs during fatigue (Krnjevic & Miledi, 1958; Grabowski *et al.*, 1972;

Khan & Bengtsson, 1985; Metzger & Fitts, 1986), suggesting that an alternative mechanism may be responsible for excitation failure.

The hypothesis that HFF results from electrical pertubations of the action potential has also recently been challenged. Metzger & Fitts (1986) have postulated events distal to the sarcolemma are responsible for fatigue at both high and low-frequencies of stimulation. This conclusion was based on rat phrenic nerve-diaphragm preparations stimulated at 5 and 75Hz which showed a marked difference in the tetanic response during recovery (more fatigue exhibited at high stimulation frequency) despite identical changes in recovery of the action potential. Further evidence to suggest that changes in sarcolemmal properties are not responsible for fatigue has been obtained from observations of the length dependency of muscle to fatigue (Fitch & McComas, 1985) in which shortened muscle fatigues to a lesser degree than optimum length muscle indicating a possible energy dependent phenomenon related to the number of cross-bridge interactions. Shortened muscle length may result in deformation of the T-tubules, preventing the propagation of excitation to all parts of the cell thereby resulting in some parts of the contractile apparatus not being equally fatigued. This means that studies of fatigue with muscles contracting at short length are difficult to interpret.

1.4.3.2 Mechanisms contributing to Low-frequency fatigue

Despite the changes in electrical function of the sarcolemmal membrane during high-frequency stimulation which are thought to result in fatigue, it is still not clear as to what causes fatigue at lower stimulation frequencies. Low-frequency fatigue is characterized by a selective loss of force at low-stimulation frequency (lasting several hours) whereas following fatiguing activity, force generation at high frequency appears to return rapidly to normal (Edwards *et al.*, 1977b). This form of fatigue can be demonstrated following a series of contractions made under anaerobic conditions (Edwards *et al.*, 1977b) and also following specific forms of voluntary dynamic contractions (Davies & White, 1981; 1982; Edwards *et al.*, 1977b). Since the mean firing frequency of a sustained maximal contraction may be 10-30Hz

(Bellemare *et al.*, 1983), it is likely that this type of fatigue may result in significant force reduction unless a compensatory increase in firing frequency can be achieved, or there is a concomitant recruitment of further motor units in parallel.

The cause of LFF is probably located further down the command chain, in a failure of excitation-contraction coupling (Edwards, 1981; Figure 1.1). This may be in part due to a reduction in Ca^{2+} release or impaired transmission in the transverse tubular system. This suggestion is further supported by work carried out on rats which, in addition, appear to suggest that type II fibres are more likely to demonstrate LFF than type I fibres (Kugelberg & Lindegren, 1979). Low frequency fatigue is not simply due to lactic acid accumulation as a consequence of activity, since it may be demonstrated in conditions in which there is no lactate (and hence H^+) production with exercise eg., patients who lack myophosphorylase or phosphofructokinase (Wiles et al, 1981). Nor can it be due to accumulation of Pi induced myofibrillar Ca^{2+} insensitivity as a result of activity (Dawson *et al.*, 1980) since NMR studies indicate Pi levels return to normal within minutes on cessation of exercise (Edwards *et al.*, 1985), not hours.

The comparatively slow recovery of low-frequency stimulated force after exercise (a day or more (Edwards *et al.*, 1977b; Newham *et al.*, 1983a)) indicates that LFF may be due to structural damage of the sarcoplasmic reticulum or tubular system, but this has not been demonstrated microscopically. LFF is more pronounced following eccentric contractions, in which the muscle is stretched during activity (resulting in greater force generation per unit fiber cross-sectional area than that obtained in concentric contractions) which does produce sarcomere disruption, further supporting the suggestion that some form of cellular damage may contribute to LFF (Newham *et al.*, 1983b).

That energetic factors alone may be responsible in development of LFF is unlikely. The energetic cost of eccentric contractions is about one sixth less than of concentric contractions as indicated by oxygen uptake measurements during cycle ergometry (Bigland-Ritchie & Woods, 1976). The slow recovery rate also dismisses

regeneration of high-energy phosphates as the cause of LFF since rates of recovery are markedly different. The time course of glycogen recovery following depletion does follow a similar time scale to long-term LFF (Piehl, 1974; Bergstrom & Hultman, 1976). It is unlikely, however, that glycogen depletion has occured in following short term intermittent ischaemic exercise (Edwards, 1983), or following eccentric exercise where a similarly worked concentrically contracting muscle shows less LFF (Edwards *et al.*, 1981). Furthermore, muscles which have contracted eccentrically have been shown to have a delayed recovery of glycogen possibly lasting 10 days or more (O'Reilly *et al.*, 1987), indicating that the recovery of glycogen may be related to sarcomere disruption.

1.5 INTERELATION BETWEEN METABOLIC AND ELECTRO-PHYSIOLOGICAL FACTORS IN FATIGUE

Undoubtedly there is a close relationship between energy metabolism and excitation processes; failure of one will affect the extent of the other. Of significance is the study by Luttgau (1965) in which iodoacetate/cyanide poisoned amphibian muscle (inhibiting oxidative phophorylation and glycolysis) could still conduct action potentials without a reduction in action potential amplitude for over 1000 impulses, whereas muscle still able to contract demonstrated a relatively rapid reduction in action potential amplitude when stimulated at 100Hz. It was concluded that action potential failure was the consequence of products produced by the contractile process itself.

Accumulation of lactate and hence hydrogen ions may have important effects on membrane function, inhibiting generation of action potentials in excitable membranes (De Luca, 1984) as pH decreases (Orchardson, 1978), possibly due to conformational changes in the arrangement of membrane proteins or due to the electric field generated by their charge (Bass & Moore, 1973). However, recent studies indicate little correlation between venous lactate concentrations and CMAP amplitude recovery following sustained and intermittent contractions of the human flexor carpi ulnaris (Duchateau *et al.*, 1987) and studies in isolated preparations of

mouse soleus and extensor digitum longus (Jeul, 1988) indicate that while slowing of conduction velocity may occur, a reduction in intracellular pH in the physiological range does not result in inexicatability of membranes. Other metabolites generated during activity such as ammonium ions have been suggested to reduce membrane resting potential and excitability (Heald, 1975) and so may contribute to fatigue.

Much has been learned from patients with selected enzyme defects of metabolism in muscle, providing alternative models for the investigation of fatigue and understanding of possible interactions of excitation processes and energy metabolism. Already discussed are patients who are unable to utilize glycogen due to myophosphorylase deficiency. An important observation in these patients is that the the surface recorded evoked action potential amplitude declines rapidly during stimulated activity (Edwards & Wiles, 1981) and moreover, fails to recover during maintained local ischaemia following an ischaemic stimulated contraction at 20Hz (Wiles, 1980), which in normal subjects recovers rapidly. Conversely, hypothyroid patients are able to sustain force for longer periods than normal subjects at presumably at a lower ATP cost (Wiles *et al.*, 1979). In these individuals, an improved preservation of excitation is also noted, possibly accounting for their improved endurance. Clearly, energy appears to play an important role influencing excitation and electrolyte balance within the cell. However, the relationship between energy and excitation is not firmly established.

To highlight the possible interaction between 'energy' and 'electricity' a three-dimensional model, based on the 'catastrophe theory' (Zeeman, 1977) has been used to illustrate the interaction between the two principle factors involved in the onset of fatigue (Edwards, 1983). The theory, applied to many biological and sociological phenomena (Zeeman, 1977), describes the sudden discontinuities that occur in what would otherwise be a continuous system as a consequence of the interaction of one or more factors. Thus, two controlling axes, 'energy' and 'excitation/activation', together, affect the third axis, force (Figure 1.2). Failure of excitation/activation clearly leads to a fall in force without energy loss, whereas a reduction in energy supply without

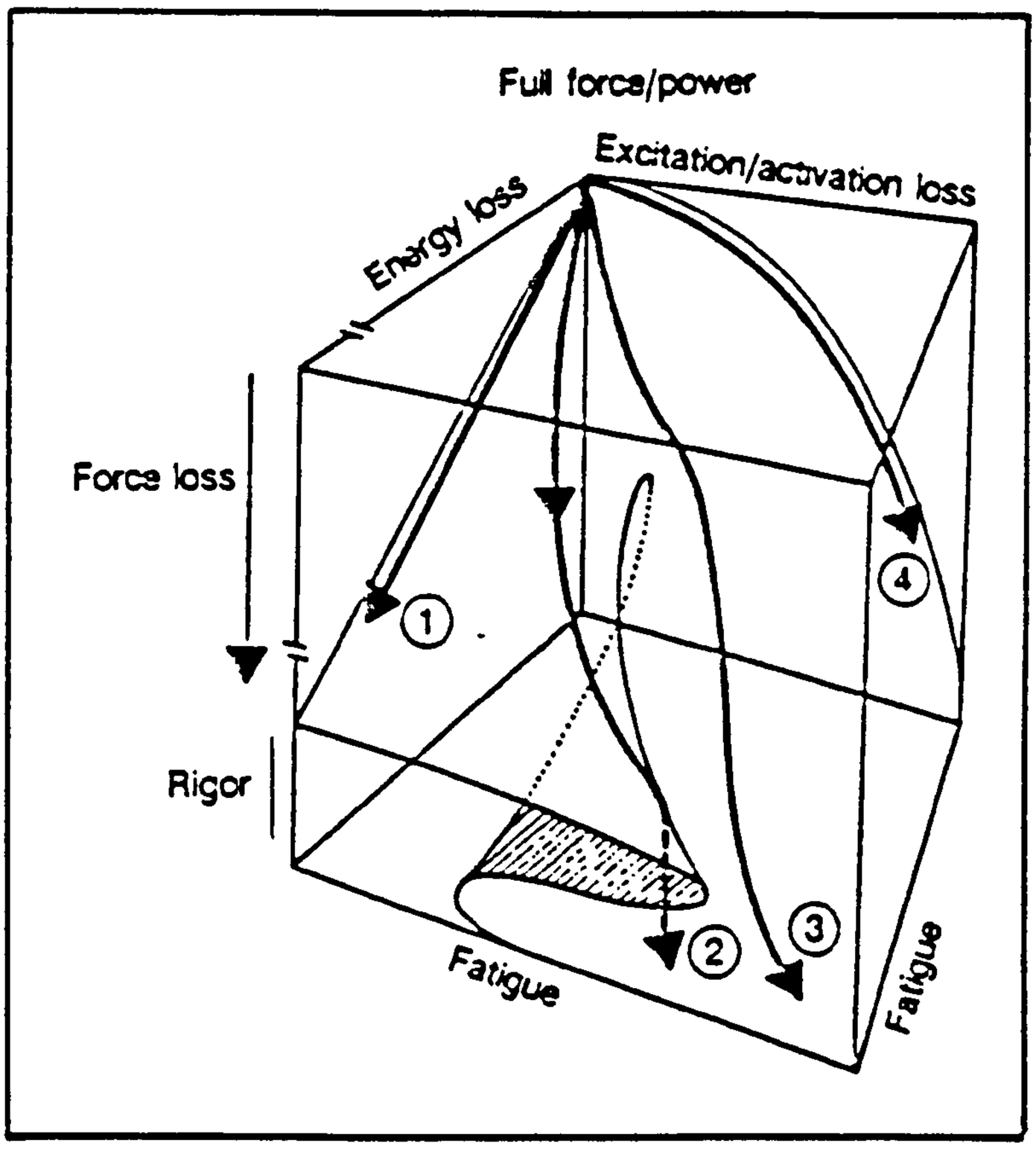


Figure 1.2 Catastrophe theory: Illustration of the interrelationships between energy loss and excitation/activation loss in the development of muscular fatigue. Pathway 1 shows a hypothetical 'pure' loss of energy (under conditions of optimal excitation and activation) with the risk of developing rigor; 4 is pure excitation failure; 3 could occur with dynamic exercise while 2 is the 'safety-factor' provided by failure of excitation in association with energy loss as seen with isometric contractions and glycolytic disorders (after Edwards, 1983).

failure of activation would also prove to limit force production. Rigor does not arise with the depletion of ATP, and hence irrepairable damage to the muscle cell, due to excitation/activation loss as illustrated by the fold of the catastrophe cusp, whereupon the stability of the system is only maintained with a sudden decline in force generation thus reducing contractile activity and hence ATP demand. The catastrophe theory has also been used to explain contractile activation and relaxation within the muscle cell (Alesso, 1973).

1.6 OTHER FACTORS INFLUENCING FORCE GENERATION DURING MUSCLE ACTIVITY

Already mentioned is the slowing of relaxation during fatiguing activity. The interplay of the electromechanical changes that occur during fatigue (slowing of relaxation, reduction in motor unit discharge rates) appears to minimize fatigue and optimize force production (Marsden *et al.*, 1983; Bigland-Ritchie, 1984).

Slowing of relaxation of muscle has long been recognized as a feature of fatigue (Mosso 1915; Feng, 1931). Slowing of relaxation will potentiate the frequency:force curve at low frequencies by increasing fusion of tetani, as has been demonstrated by cooling of fresh muscle (Edwards, 1980). However, following the development of low-frequency fatigue, slowing of relaxation does not overcome this form of fatigue since relaxation rate recovers in a much shorter time period (half-time of slow componant 83 seconds, Wiles, 1980) than force generation. In this respect, slowing of relaxation does not appear protect against all forms of fatigue.

The mechanisms that lead to slowing of relaxation have not been resolved, although there is a strong indication that metabolic factors are primarily involved as indicated by metabolic heat studies (Wiles & Edwards, 1982a) and a parallel time course of recovery of PCr (Sjoholm *et al.*, 1983). Further evidence is shown by the association of the failure of recovery of slowing or PCr following fatiguing contractions under anaerobic conditions (Harris *et al.*, 1976).

Potentiation of twitch force is a further mechanical change associated with muscle contraction which has been proposed to influence fatigue resistance (Hanson

& Person, 1971; Green, 1987). Potentiation of twitch force has long been identified during low-frequency (2-3Hz) stimulated isometric contractions ('staircase' potentiation) in both human and rodent muscle (Desmedt & Hainuat, 1968; Krarup, 1981) and following brief tetanic stimultion (Brown & von Euler, 1938; Connolly *et al.*, 1971; Takamori *et al.*, 1971; Blinks *et al.*, 1978). Twitch potentiation also occurs after short (5-10sec) maximal voluntary contractions (Belanger & Quinlan, 1982; Vandervoort *et al.*, 1983), presumably as a result of preceeding endogenous tetanic stimulated activation.

It was suggested by Hanson and Person (1971) who observed twitch potentiation in their studies on frog muscle that twitch potentiation may contribute to force maintenance at low stimulation frequencies. As yet, it appears no work has been reported in the literature to test this hypothesis. Clearly, more work in this area is indicated.

1.7 AIMS OF STUDY

An understanding of the mechanisms that alter the contractile properties of skeletal muscle associated with voluntary and stimulated activity is of considerable importance, not only in sports performance, but also in medicine. In this regard, the processes altering the interrelationship between mechanical and electrophysiological factors may be of considerable importance to the clinical investigation of patients with disease-related fatigue and may also have important theoretical and practical implications in rehabilitation techniques or in any circumstances where skeletal muscle is to be electrically stimulated e.g., in functional electrical stimulation (FES). Examples of where FES is employed are shown in Table 1.2, and may range from control of posture and movement of proximal limbs (Susak *et al.*, 1986), phrenic nerve stimulation for the assistance of respiratory disorders (Glen *et al.*, 1977), to the development of skeletal muscle cardiac assistance devices in which skeletal muscle may be used to assist cardiac function (Acker *et al.*, 1987).

Before electrical stimulation of muscle can be employed for the purposes of FES, it is necessary to characterize and understand the processes that alter the

TABLE 1.2 Examples where Functional Electrical Stimulation may be applied in medicine.

condition	**reference**
1. Paraplegia	Susak *et al.*, (1985)
i. conditioning exercise	
ii. testing procedures	
iii. mobility assistance	
2. Breathing	
i. phrenic nerve stimulation	Glen *et al.*, (1977)
3. Physiotherapy regimes	
i. Bell's palsy	Farragher *et al.*, (1987)
ii. rehabilitation in arthritis	Oldham, (1987)
4. Foot drop in stroke victims	Waters, (1977)
5. Cardiac assistance devices	Acker *et al.*, (1987)

function of the 'contractile machine'. A review of the literature of the physiological processes that contribute to fatigue of skeletal muscle has revealed that the interrelationship between electrophysiological factors and force generation is still poorly understood. There appears to be a need to clarify the frequency dependence of this relationship and, in addition, to assess the contribution of other mechanical changes associated with muscular activity, namely changes in relaxation rate and force potentiating mechanisms which may serve to *offset* the processes that lead to force failure.

The work described in this thesis was therefore undertaken to investigate the factors influencing the mechanical-electrophysiological interrelationship during stimulated activity of human skeletal muscle which may counteract the processes that lead to fatigue.

Each experimental chapter is arranged in the form of: introduction, methods, results and discussion. In chapter 2 the details of the experimental methods used for measurement of contractile properties of muscle are presented. A computerized stimulator controller was developed specifically for these studies and is descibed in more detail in appendix 4. Chapter 3 addresses the question of 'fatiguability' of skeletal muscle stimulated at various frequencies and the frequency dependence of the interrelationship between force generation and excitation in a single fatiguing procedure. The dependence of this interrelationship on numbers of stimuli delivered and activity performed is investigated in chapter 4 to assess the contribution of metabolic and excitatory factors in fatigue development. The changes in action potential characteristics during fatiguing activity in normal and myophosphorylase deficient patients are reported in chapters 4 and 5 to investigate the role of energy supply on changes in excitation. The contribution of slowing of relaxation and twitch post-tetanic potentiation to resisting fatigue is described in chapter 6. The

implications of the results are considered in relation to voluntary contractions together with functional implications in rehabilitation and FES in chapter 7.

CHAPTER 2: GENERAL METHODS

CHAPTER 2 - GENERAL METHODS

2.1 INTRODUCTION

All experimental investigations were carried out on the human adductor pollicis 'in situ'. The technique employed to measure contractile characteristics of the adductor pollicis is similar to that described by Merton (1954) and was subsequently developed and used by Edwards *et al.*, (1977a). A similar experimental arrangement described by Edwards *et al.*, (1977a) was used for the present investigations. For the purposes of the following investigations, the following parameters were measured;

a)Isometric force,

b)First differential of force (with respect to time) for determination of relaxation characteristics,

c)Integrated force (with respect to time) to obtain a measure of activity undergone during contraction,

d)Surface electromyography, and

e)Muscle surface temperature measured on the skin surface over the belly of the adductor pollicis.

Experimental protocols are described seperately for each investigation under their respective headings.

2.2 CHOICE OF MUSCLE

The adductor pollicis is a small muscle of the hand, its function being to press the thumb against the side of the index finger. The site and accessibility of this muscle make it an ideal choice for study, which is borne out by the numerous studies already carried out by other workers (eg., Merton, 1954; Edwards *et al.*, 1977a; Bigland-Ritchie *et al.*, 1979; Wiles, 1980).

Anatomy: Generally, the adductor pollicis has two heads, oblique and transverse which are attached separately medially, but are united laterally. The oblique head is attached from the sheath of the tendon of the flexor carpi radialis, the bases of the second, third and fourth metacarpal bases and the anterior aspects of the trapezoid and

capitulate bones. The transverse head comes from the longitudinal ridge on the front of the shaft of the third metacarpal. The two heads converge and are inserted into the ulnar side of the base of the proximal phalanx of the thumb, thus appearing as a flat, 'fan' shaped muscle. Round *et al.*, (1984) have shown that there are three common variants of the anatomical arrangement of the adductor pollicis: a) there may be no clear division between the transverse and oblique heads, b) a major transverse head may be apparent with a smaller oblique head and c) a subdivision of the oblique head may be apparent giving the whole muscle an appearance of three heads.

Nerve supply: The ulnar nerve supplies the muscle and its accessibility at the wrist permits electrical stimulation via surface electrodes placed on the skin surface at a superficial site.

Situation: The situation of the muscle permits measurement of evoked action potentials and control of external influencing factors such as temperature and muscle length. Precise measurement of contractile function can be made without much difficulty.

Physiology: Post-mortem studies (Johnson *et al.*, 1973; Round *et al.*, 1984) have shown that the adductor pollicis is composed mainly of type I fibres (approximately 80%) with a smaller proportion of type II fibres (approximately 20%). Studies of contractile properties have shown the adductor pollicis muscle to behave in a paradoxical fashion, unexpectedly demonstrating similar contractile characteristics to muscle groups containing similar proportions of type I and II fibres, for example, quadriceps (Edwards *et al.*, 1977a), diaphragm (Moxham *et al.*, 1980a) and sternomastoid (Moxham *et al.*, 1980b) rather than to the soleus which has a similar proportion of type I fibres (Round *et al.*, 1984). The small size of this muscle does not permit investigation of fibre type composition using current biopsy and histological techniques as is possible with larger muscle groups.

2.3 SUBJECTS AND PATIENTS

The normal volunteers studied were postgraduate students and members of staff of the faculties of Science and Medicine in the University of Liverpool.

Subjects were selected on the basis of having no history of muscle or joint problems, nor of ingestion of drugs at the time of study.

Informed consent was obtained before experimental procedures were performed. All subjects were informed of the nature of the studies to be performed and of their right to withdraw from any experimental procedure at any time.

Patients studied were under the clinical care of Professor R.H.T. Edwards, Department of Medicine, University of Liverpool. Informed consent was obtained as for normal subjects.

Ethical approval for all procedures used was obtained from the ethics committee of the Royal Liverpool Hospital.

2.4 MEASUREMENT OF PHYSIOLOGICAL CHARACTERISTICS OF MUSCLE FUNCTION

2.4.1 Preparation of hand

The left hand was used for all studies. This was generally the non-dominant hand for most individuals. Experience indicated that those studied perceived less subjective discomfort during electrical stimulation than with the right hand (the reason for this was not evident).

Before preparing the hand for force and EMG measurement (muscle (the temperature was standardized. Temperature markedly alters muscle contractile properties (Edwards, 1980) and may be influenced by ambient conditions due to its peripheral situation, poor insulation by fat and small size. Mechanical and electrophysiological factors may consequently be altered by change in muscle temperature eg., relaxation rate (Wiles & Edwards, 1982a) and action potential conduction velocity (Bigland-Ritchie *et al.*, 1981). The forearm and hand were therefore immersed in a water bath maintained at a temperature of approximately 45°C for 10 minutes and then thoroughly dried before each procedure. When possible, sleeving of clothing was kept on around the forearm to help insulate from heat loss by radiation. Hand temperature was maintained constant during an experimental procedure by warming the hand with a 100 watt electric light bulb

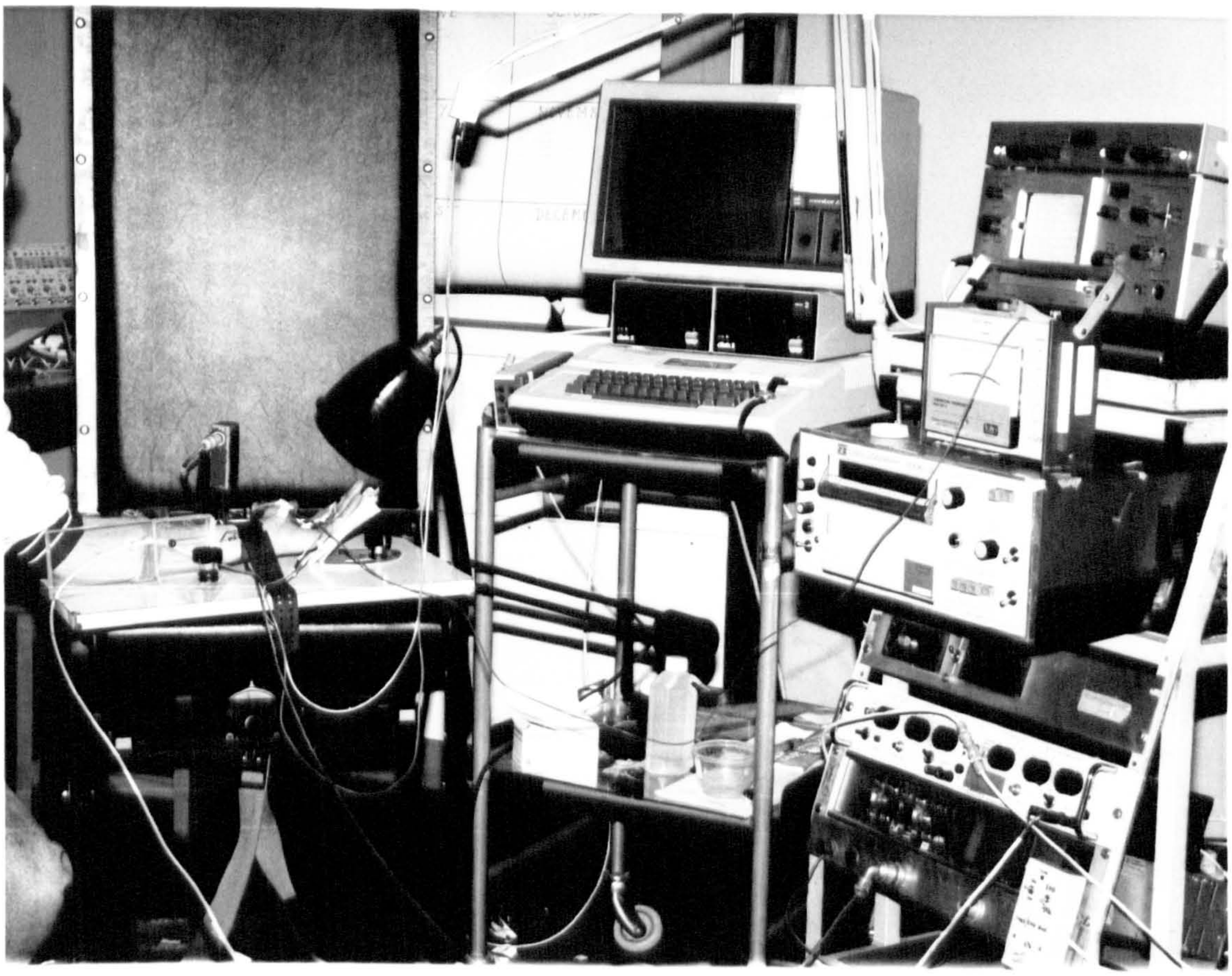


Figure 2.1 Photograph of handboard and apparatus for stimulation of adductor pollicis and recording of force and EMG.

(Edwards *et al.*, 1977a) placed 10 cm above the adductor pollicis. This procedure has been shown to maintain the temperature of the adductor pollicis over the range 35-37°C for up to three hours (Wiles, 1980). Skin surface temperature, measured above the belly of the adductor pollicis, was monitored throughout experimental procedures using an electronic analogue thermometer (Bailey Instruments, BAT 4) incorporating a gold plated surface thermistor probe which was taped to the skin. This was protected from warming by the lamp by securing a small piece of polystyrene (1cm x 2cm x 0.5cm) over the thermistor. Skin surface temperature was found to be well maintained throughout each experimental procedure. The intramuscular temperature of the adductor pollicis was not determined in the present studies. Since hand temperature was kept constant throughout an experimental procedure, it is unlikely muscle temperature varied to a considerable degree. This is supported by the good correlations between intramuscular and surface temperature in the adductor pollicis (Lennmarken *et al.*, 1985) and first dorsal interrosseus (Ranatunga *et al.*, 1987).

In order to measure isometric force of the adductor pollicis the forearm and hand were first immobilized in a specially constructed handboard, shown in Figure 2.1. The subject sat in a comfortable chair with the elbow extended to about 130° and with the forearm immobilized in a horizontal position by adjustable perspex supports either side of the forearm mounted on a wooden board. The hand was positioned in a metal support at an angle of 30° so that all digits, except for the thumb, were supported. In some individuals rubber foam and paper tissues were placed behind the hand for comfort and to pack the hand more firmly. Care was taken to prevent circulatory occlusion. This procedure ensured total immobility of the hand.

In several early experiments a material strap was positioned around the elbow and fixed to the perspex supports to further prevent the arm sliding back during strong contractions. However, this was found to be unecessary provided the forearm and hand were firmly secured.

2.4.2 Force Recording

Isometric force of the adductor pollicis was measured by means of a strain gauge mounted on a steel cantilever. The assembly was rigidly fixed to the hand board via two adjustable bolts. The strain gauge was attached to the proximal phalanx of the thumb via a perpendicular inextensible band (Figure 2.2). A slight resting tension was applied to the thumb by adjusting the position of the strain gauge. An error in the angle of attachement of up to 10^{o} was considered negligable since the degree of error in force measurement is dependent on the cosine of the angle of attachment .

The Transducer formed part of a bridge circuit. The output was amplified (Fylde 215GA) and recorded on an UV oscillograph (S.E. Labs 3006/DL) via a galvanometer (S.E.Labs B.450) (frequency response 450Hz) onto Kodak Linograph direct print paper (type 1895). The amplified force signal was also fed to electronic differential and integrating circuits.

Specifications of force transducer: Static and dynamic properties of the force transducer were established to ensure suitability for force measurement under the conditions used. These are summarized in Table 2.1.

Measurement of static properties:

1). Linearity and calibration. The strain gauge was regularly calibrated using known weights (0.5-12.5kg) over a range of 0-120 Newtons (Figure 2.3). The transducer response was linear (r=0.999) over the range of calibration.

ii). Compliance. Compliance of the steel lever was determined by attaching a 250mm pointer to the lever (supported in a horizontal manner) and applying known weights at the point of attachement. The deviation of the steel lever was determined from the displacement of the pointer and the compliance calculated in $mm.Newton^{-1}$.

Measurement of dynamic properties: Resonance frequency and the 90%-10% duration of damped oscillations were determined by suspending a weight from the transducer via a nylon line and initiating a sudden force displacement by melting the

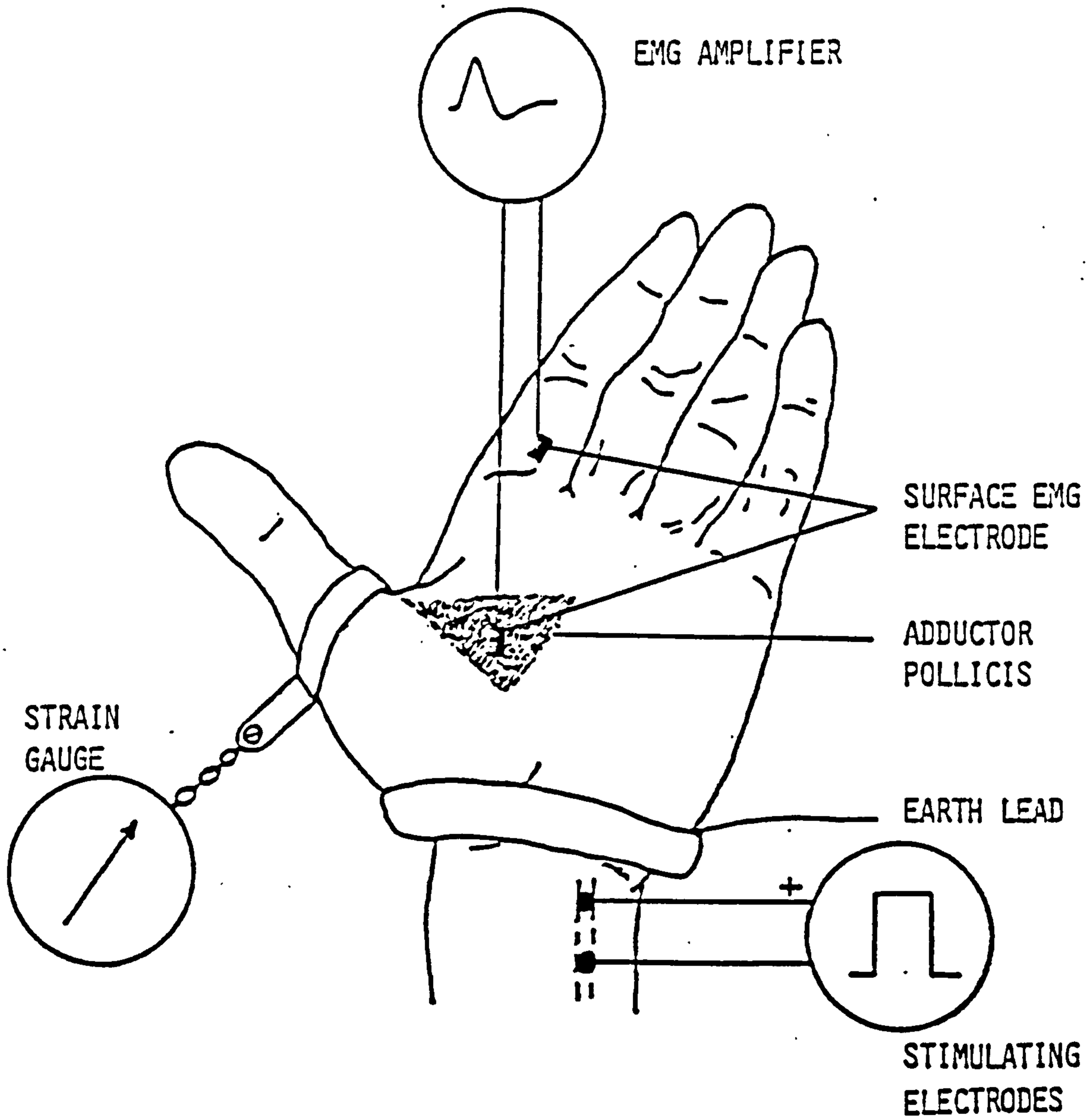


Figure 2.2 Diagrammatic figure of hand showing site of the adductor pollicis muscle and positioning of strain gauge strap of force transducer, EMG electodes, earth strap and stimulating electrodes.

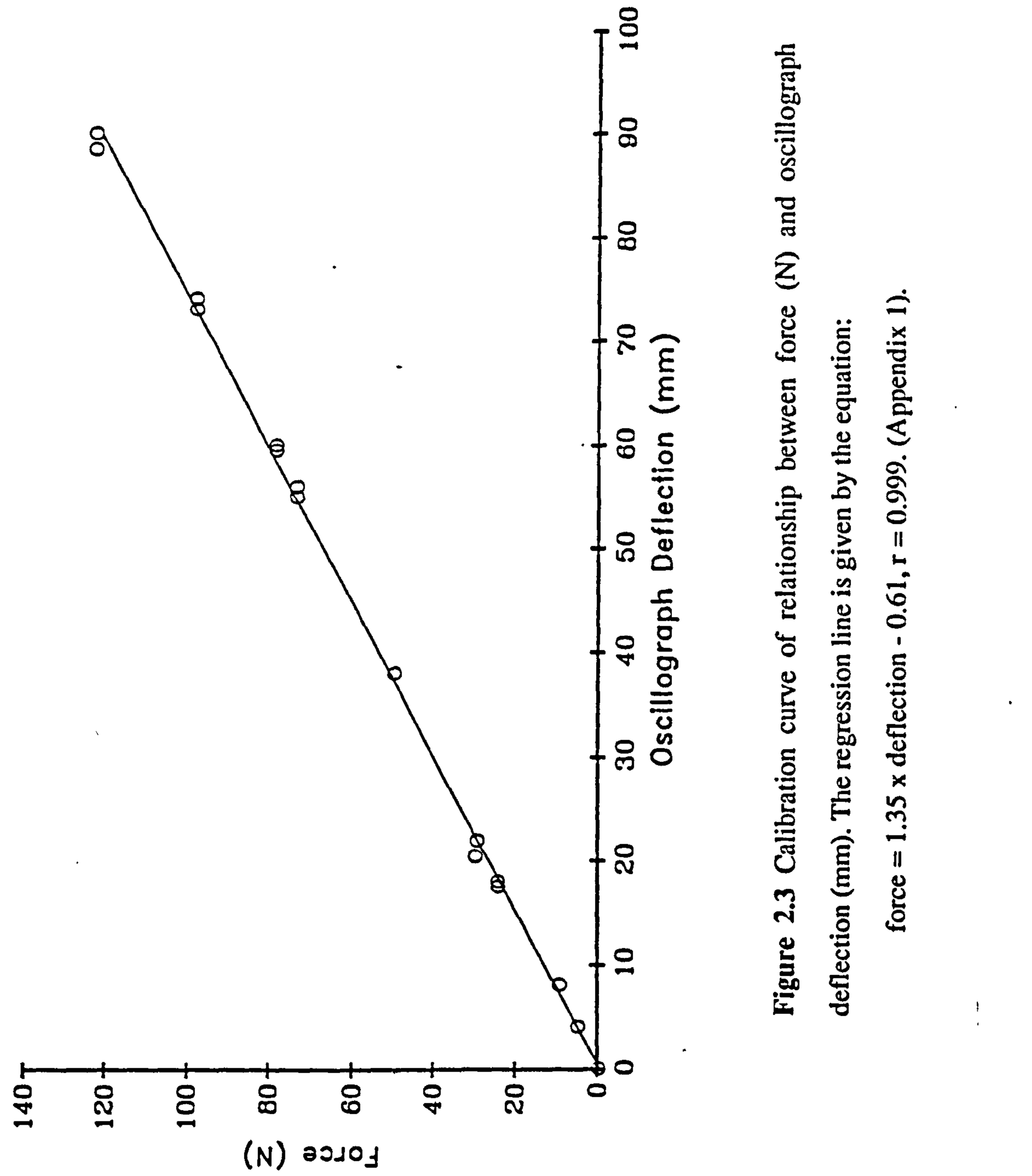


Figure 2.3 Calibration curve of relationship between force (N) and oscillograph deflection (mm). The regression line is given by the equation:

force = 1.35 x deflection - 0.61, r = 0.999. (Appendix 1).

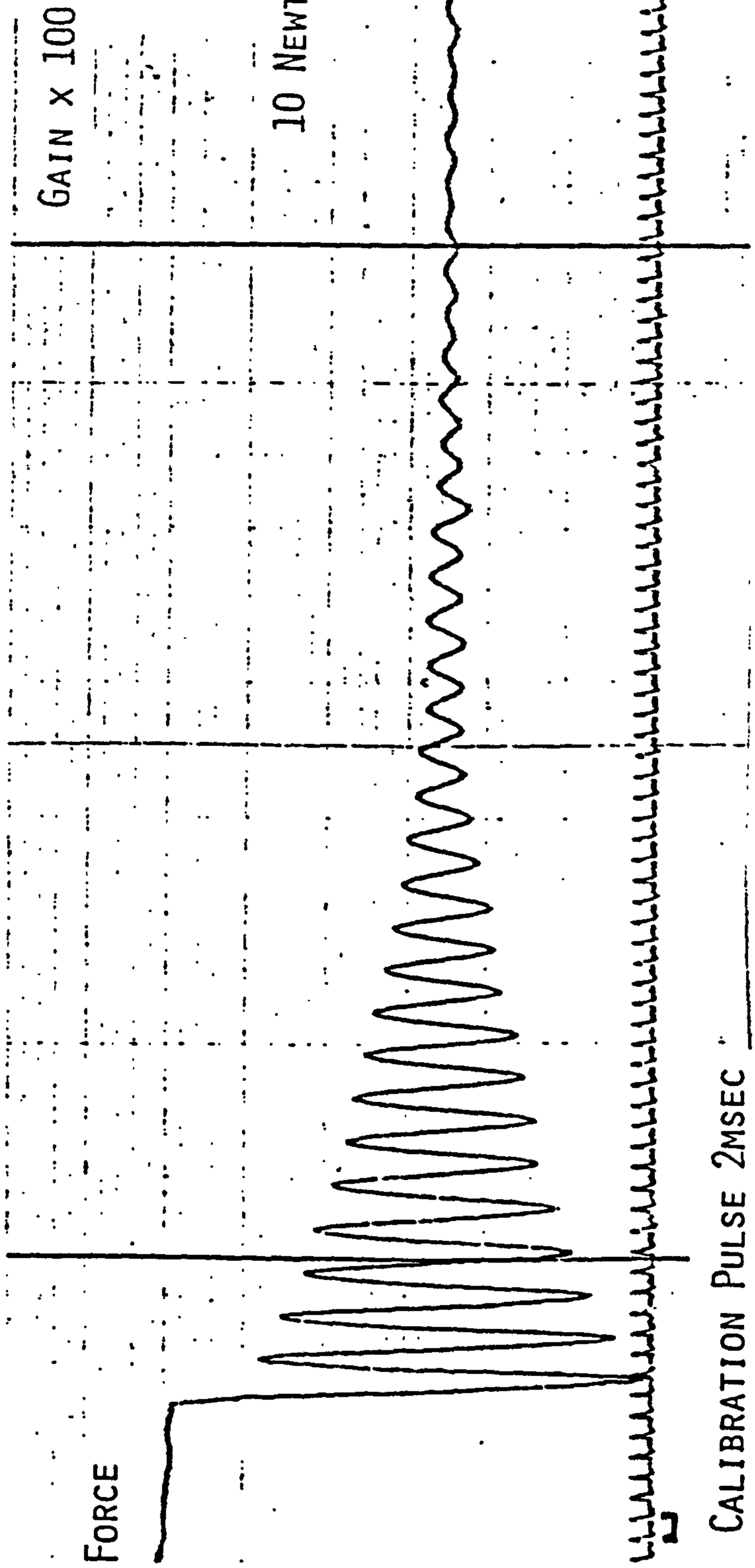


Figure 2.4 Oscillograph tracing of dynamic properties of force transducer. A weight was suspended from the strap attachment point of the transducer via a nylon line. A sudden force displacement was induced by melting the nylon cord attachment and recorded on an U.V. oscillograph.

Table 2.1 Strain gauge specifications

1. Static properties

Regression equation:	Force = -0.610 + 1.35 x Deflection
Regression coefficient:	1.35 $N.mm.^{-1}$
Linear correlation coefficient:	0.999
Compliance:	$8.8 \times 10^{-4} \pm 7.8 \times 10^{-5}$ $mm.N^{-1}$. n=9
Calculated Max. strain gauge displacement (122.6N):	1.09mm.

2. Dynamic properties

Resonant frequency:	238Hz
Duration of undamped oscillations (90%-10%):	60msec.

nylon attachement. Force displacement was recorded on a UV oscillograph for permanent record (Figure 2.4).

2.4.3 Measurement of total contractile activity performed

In order to obtain a measure of total activity performed, the force signal was electronically integrated (integrator constructed in the department of medicine) with respect to time and displayed on the UV oscillograph via a galvanometer (S.E. Labs A.1600).

Drift of the integrated signal (resulting from resting tension of the adductor pollicis) was corrected once the initial resting tension was applied.

Integrated force was standardized between individuals by expressing total integrated activity as a fraction of maximum tetanic force at 100Hz (max.second). Hence,

1 max.second = (force x time) / maximum tetanic force of fresh muscle.

Calibration: Calibration was carried out at regular intervals by applying a series of known weights suspended vertically from the force transducer for different durations. A typical calibration curve is shown in Figure 2.5.

2.4.4 Measurement of maximum relaxation and contraction rates

Maximum relaxation rate was derived from the first differential of the force signal. Several studies have shown that the half-time of the exponential decline in force is well correlated to the differential signal (Edwards *et al.*, 1972b; Wiles, 1980; Bigland-Ritchie *et al.*, 1983a).

Calibration: The differential signal was calibrated against known electrical ramp functions derived from a triangular wave-form output of a frequency generater (LF.141, Servomex controls Ltd.). The signal output of the frequency generator was substituted for the transducer bridge-circuit input to the force amplifier. 5Hz and 10Hz triangular wave functions were applied over a range of input voltages of 0-6mV to alter the slope of the wave form. A calibration curve was plotted from the displacement of the differential trace and slope of the ramp function for both rising

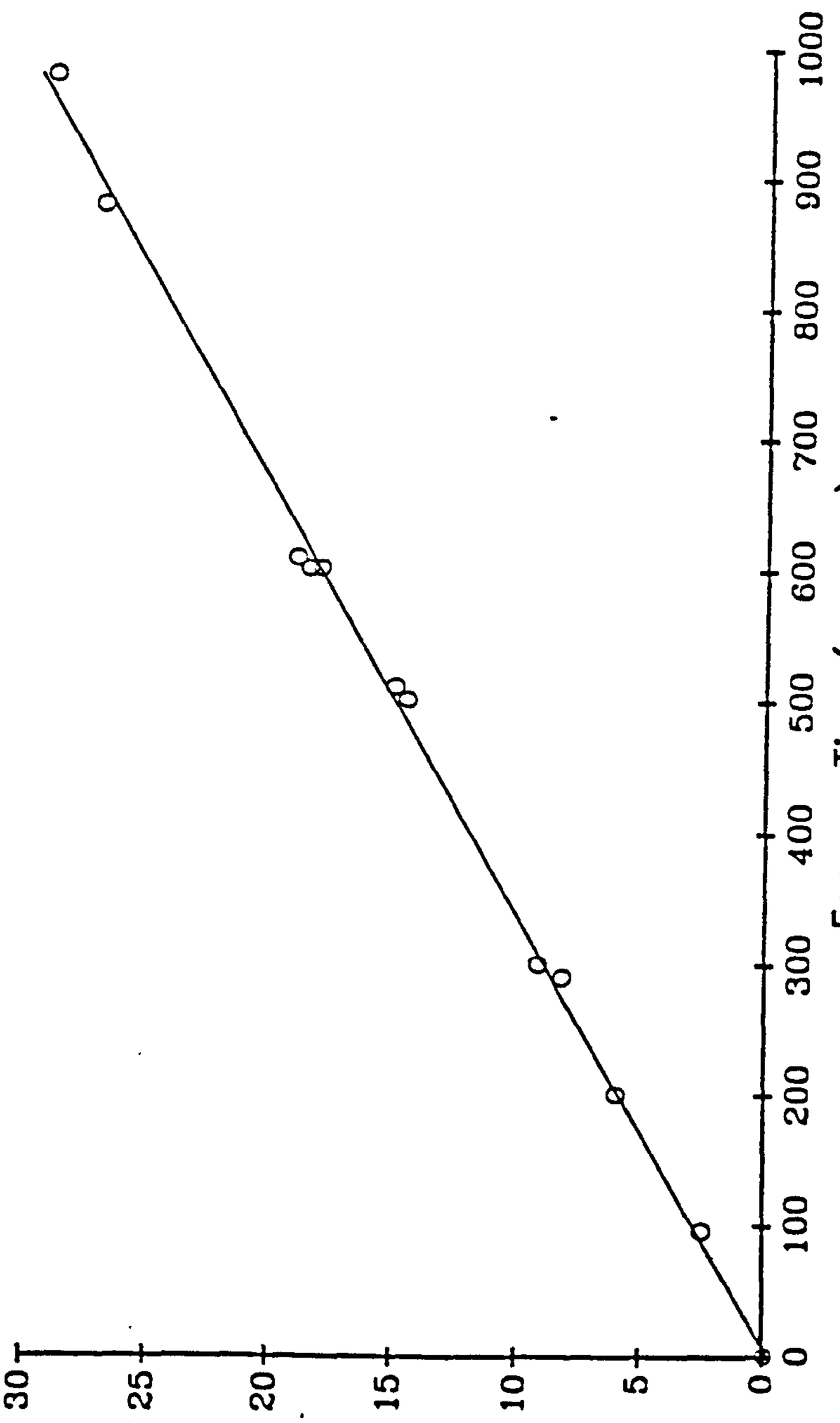


Figure 2.5 Calibration curve of relationship between integrated force (force x time) and oscillograph deflection (mm). The regression line is given by the equation:

force x time = 0.0305 x deflection - 0.093, r = 0.998 (Appendix 2).

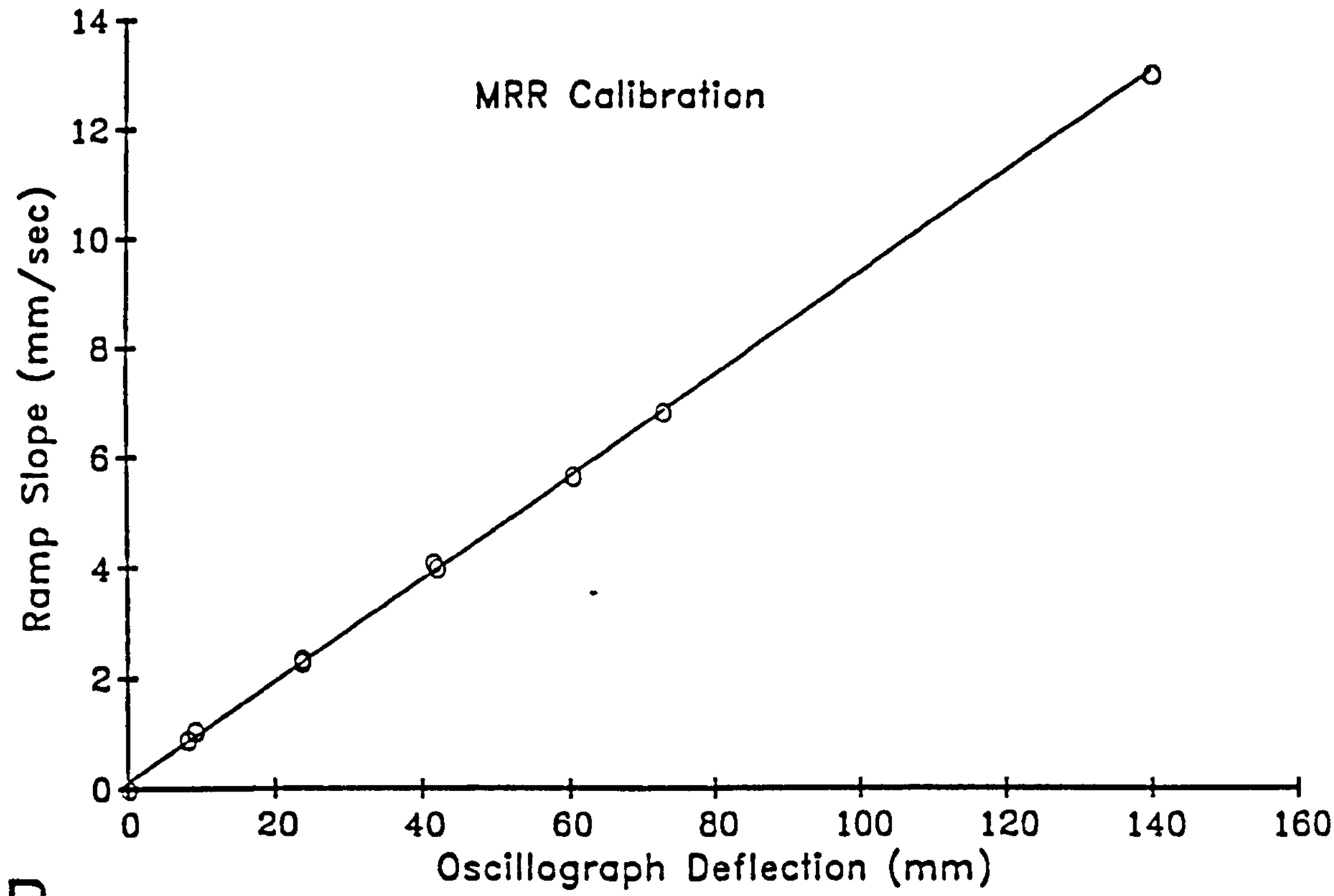
Figure 2.6 Calibration curves for force differentiator (Appendix 2). Relationships between slopes ($mm.s^{-1}$) of standard ramps delivered by the signal generator and the oscillograph deflection of the differentiated signal (mm). A. Negative-going ramp slopes (deflection upwards on oscillograph trace) for determination of maximum relaxation rate. Equation of line:

ramp slope = 9.99 x deflection - 4.27, r = 0.999.

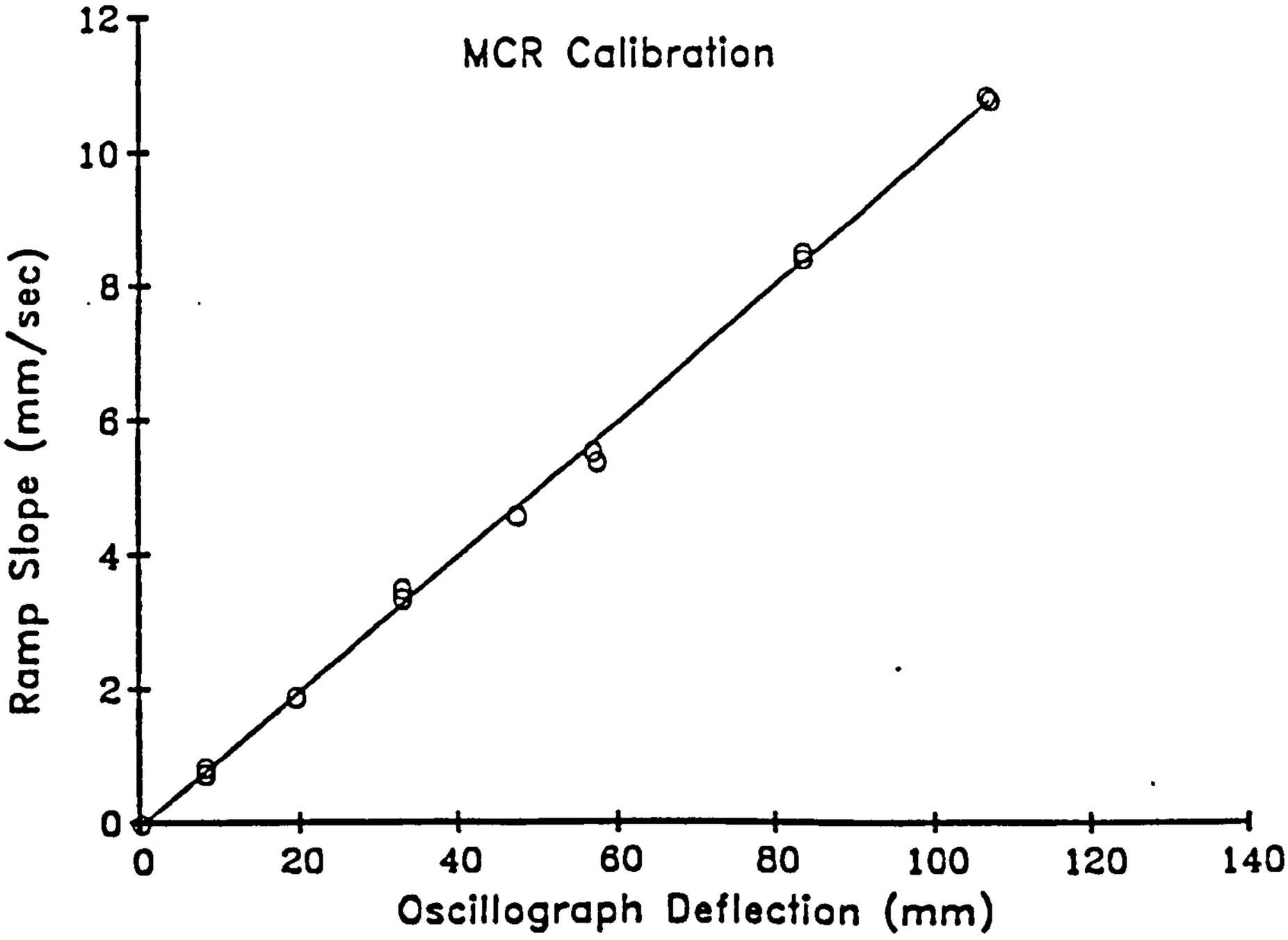
B. Positive-going ramp slopes for determination of maximum contraction rate. Equation of line:

ramp slope = 9.17 x deflection + 14.2, r = 1.000.

A


MRR Calibration
Ramp Slope (mm/sec)
0
2
4
6
8
10
12
14
0
20
40
60
80
100
120
140
160
Oscillograph Deflection (mm)

B


MCR Calibration
Ramp Slope (mm/sec)
0
2
4
6
8
10
12
0
20
40
60
80
100
120
140
Oscillograph Deflection (mm)

and falling slopes. Typical calibration curves are shown in Figure 2.6. A detailed procedure for calibration is given in appendix 3.

2.3.5 Electromyography (EMG)

Self-adhesive disposable Ag/AgCl surface electrodes (Disa) or 5mm cup electrodes were used to detect electrical activity over the adductor pollicis. The electrodes were positioned after a fashion described by Wiles (1980) and Slomic *et al.*, (1968) which allowed minimal contribution of the EMG signal by the 1st dorsal interosseous (<10%) . The 'active' electrode was placed above the belly of the adductor pollicis on the midpoint of a line delineating the thenar eminence distally. The 'inactive' electrode was positioned on the distal phalanx of the index finger.

The skin above the adductor pollicis was immediately prepared after warming by cleansing with isopropyl alcohol. Electrode gel (Dracard Ltd.) was applied to the electrodes and the electrodes taped into position to prevent movement artefact or dislodgement. A felt covered earth conductor, previously soaked in water for 10 minutes, was placed around the wrist between the stimulating and EMG electrodes. The EMG signal was filtered (bandwidth 2-2000Hz), amplified and displayed on a Disa electromyograph (type 14 A 11). The output signal was passed to a further channel of the UV oscillograph via a galvanometer of frequency response 1600Hz (S.E. labs, type A.1600). Negative deflection of the evoked potential was recorded as an upward deflection.

The amplified EMG output was further channelled to a MED80 Nicolet mini-computer (NicMed 80) or BBC microcomputer (via an A to D interface, Unilab, Blackburn) for further analysis of the EMG signal. The signal was digitized at 16KHz and 8KHz with 12 and 8 bit resolution respectively, via an A/D converter to obtain a 63 msec sweep (1024 and 512 points respectively) and stored on disc for subsequent analysis. The percentage error for an 8 bit resolution (256 points) is 0.39% and 12 bit, 0.02%. For a 63 msec sweep of 512 and 1024 points the percentage errors in measurement are 0.195% and 0.098% respectively. Acquisition was triggered by an eight channel event controller under computer control (Apple II).

Calibration: The amplitude of the signal (in mV) was calibrated against a calibration signal derived from the Disa amplifier.

2.4.6 Electrical stimulation

Initial experiments using a single button electrode placed over the superficial site of the ulnar nerve at the wrist and anode plate placed on the underside of the hand (Wiles, 1980; Bigland-Ritchie *et al.*, 1983a) were found to be unsatisfactory. Problems were experienced in maintaining stability of the cathode position and furthermore, large depolarizing voltage artefacts were apparent on the EMG signal. An alternative stimulating arrangement was therefore used.

Stimulation electrode: A modified bipolar MEDELEC electrode (Figure 2.7) was employed which could be conveniently strapped in place over the site of the ulnar nerve at the wrist. Lint pads were constructed around the exposed metal electrodes to provide a 'button' area of approximately 5mm x 10mm (Figure 2.7). Exposed metal was insulated with silicon gel. Before use, the lint pads were soaked in 0.83% saline for approximately 20 minutes and gelled with electrode gel (Dracard) to aid electrical contact. The electrode was strapped over the superficial site of the ulnar nerve with the anode distal.

Stimulation: Supramaximal square-wave, 50μsecond impulses (Devices, type 3702) were delivered via the ulnar nerve to contract the adductor pollicis. Supramaximal voltage was determined by obtaining the maximal peak-to peak evoked compound muscle action potential (CMAP) and increasing stimulation voltage approximately 20% further. Beyond this some individuals experienced some discomfort. During the progression of some experiments, the stimulation voltage was increased further to ensure electrical stimulation remained supramaximal and that no more force could be produced in this way.

2.5 PRECISE CONTROL OF STIMULATION AND EMG DATA AQUISITION

An eight channel computer controlled pulse generator, based around an Apple II microcomputer, was developed to control precisely the pattern of stimulation trains

Table 2.2 Specifications of computer controller (Appendix 4)

Computer:	Apple II
Maximum number of frequency trains per channel:	20
Frequency range:	0 - 500Hz
Duration of impulse train:	0 - 255 seconds
Number of programmable channels:	PULG12 = 1 NSTIM = 8

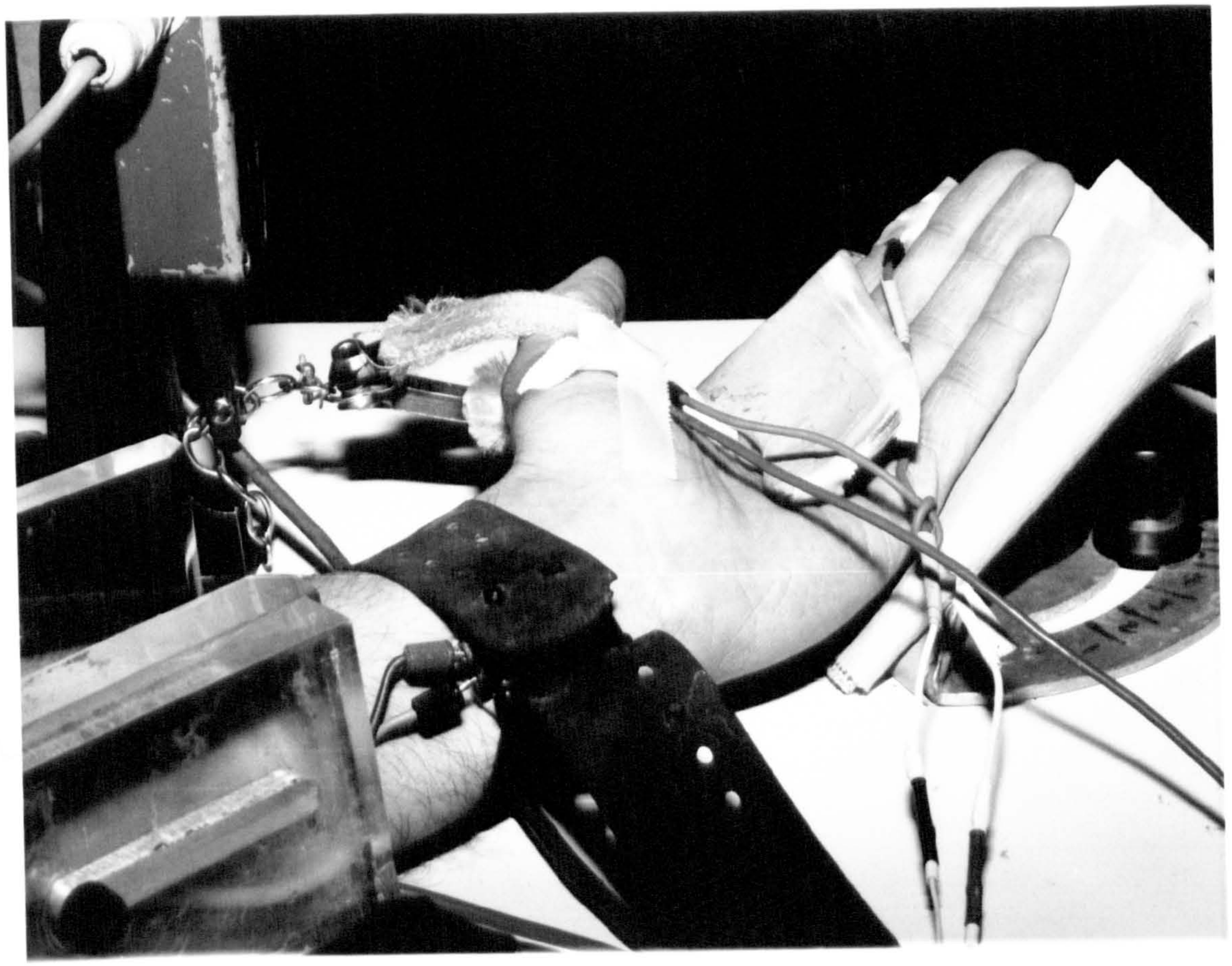


Figure 2.7 Close up photograph of stimulating electrode arrangement.

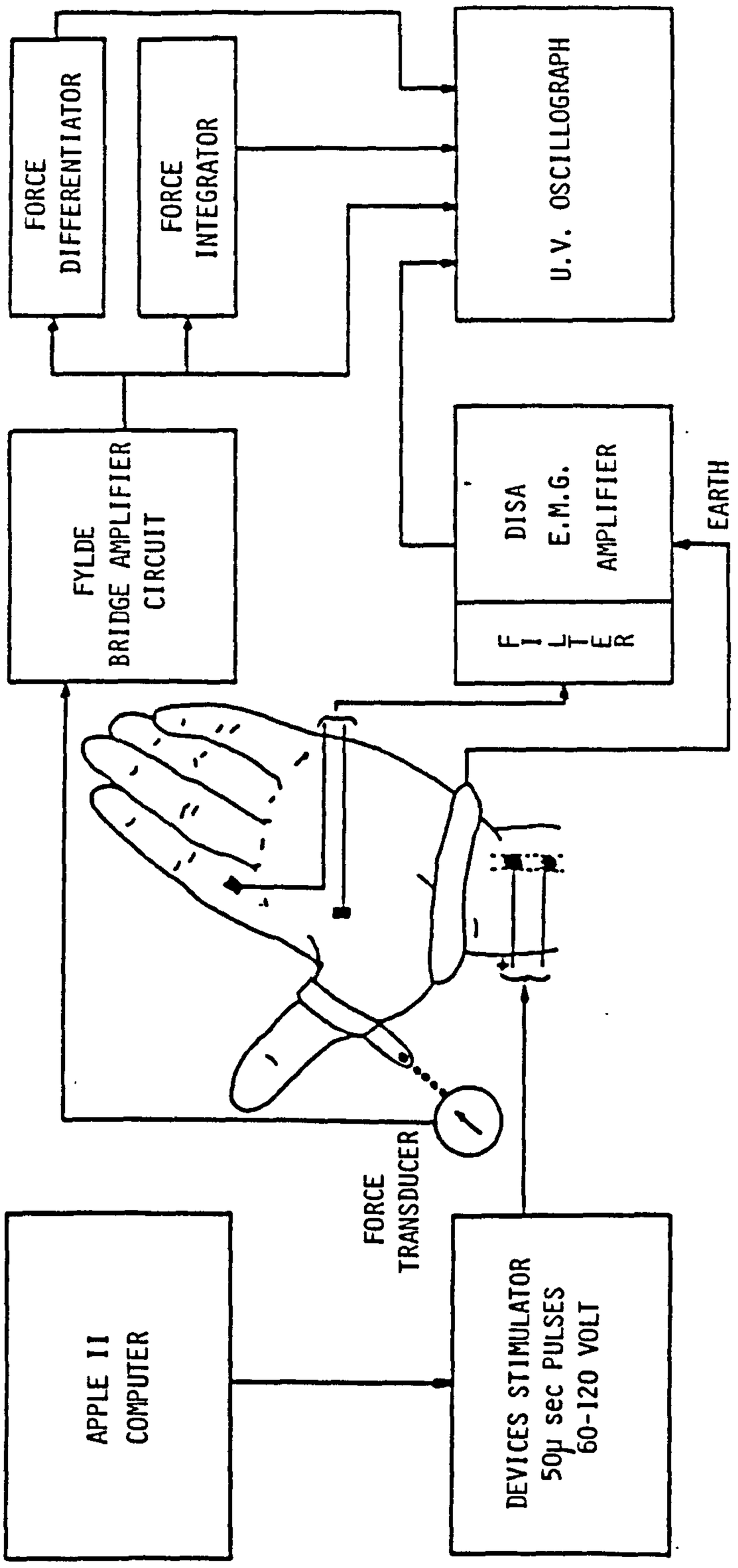


Figure 2.8 Diagrammatic representation of stimulation arrangement and apparatus.

delivered to the adductor pollicis and control sampling of the EMG signal for subsequent analysis. Negative going impulses were delivered to the muscle stimulator and trigger input of the EMG aquisition computer. Specifications of the controller are shown in Table 2.2. The details of development are given in appendix 4. A diagrammatic representation of the complete stimulating and recording arrangement is shown in Figure 2.8.

2.6 STATISTICAL METHODS

Statistical analyses were made using a Minitab Statistical Package (Minitab Inc.) running on an IBM personal computer and IBM3083 mainframe computer. Parametric tests were employed throughout the study for comparisons between groups where a normal distribution was observed and where sufficient numbers of individuals were used. The tests employed are described under each experimental sub-heading.

CHAPTER 3: FREQUENCY DEPENDENCE OF FORCE GENERATION DURING INTERMITTENT ELECTRICAL STIMULATED ACTIVITY AND RECOVERY

PART 3: FREQUENCY DEPENDENCE OF EXCITATION AND FORCE GENERATION DURING INTERMMITENT ELECTRICAL STIMULATED ACTIVITY AND RECOVERY

CHAPTER 3: FREQUENCY DEPENDENCE OF EXCITATION AND FORCE GENERATION DURING INTERMITTENT ELECTRICAL STIMULATED ACTIVITY AND RECOVERY

General Introduction

Indications for the differences in opinion of the interrelationship between force generation and excitation as reviewed in chapter one appear to result from the various methodologies used to fatigue muscle and how fatigue was determined; whether voluntary or stimulated contractions were used, during ischaemic or non-occluded conditions with continuous or interrupted contractions, and if contractile characteristics were tested using single high or low frequencies of stimulation. Clearly, there is a need to establish further the relationship between these two factors during fatiguing activity.

Preliminary experiments were therefore designed to demonstrate the frequency dependence of force generation at a high (non-physiological) and low (physiological) stimulation frequency (Part I) (Bellemare *et al.*, 1983). In order to investigate the frequency dependence of the interrelationship between force and excitation, a means of fatiguing muscle and simultaneously monitoring muscle function characteristics throughout a fatiguing procedure during local occluded and non-occluded conditions, at low and high frequencies of stimulation was developed and validated. This permitted measurement of the frequency:force characteristics, changes in MRR and altered excitation of the adductor pollicis muscle, and was based on a method employed by Edwards (1981) (Part II). This was subsequently applied to investigate the frequency dependence of force and excitation during fatiguing activity and recovery in a single fatiguing contraction (Part III).

PART I: FREQUENCY DEPENDENCE OF FORCE GENERATION

INTRODUCTION

Skeletal muscle can be maximally activated by voluntary effort and electrical stimulation. The changes that occur in force generation during prolonged stimulated contractions have been shown to differ markedly according to frequency of stimulation (Davies & Davies, 1932; Naess & Storm-Mathisen, 1955; Jones *et al.*, 1979). To demonstrate these differences in human adductor pollicis, a single subject was stimulated via the wrist to contract adductor pollicis at a high stimulation frequency to evoke full contractile activation (Edwards *et al.*, 1977a; Marsden *et al.*, 1983) and at a low-stimulation frequency which encompasses the natural motor unit discharge rates during prolonged maximal contractions in this muscle (Bigland-Ritchie *et al.*, 1983b; Bellemare *et al.*, 1983).

METHODS

The adductor pollicis of the left hand in one subject was prepared and set up for electrical stimulation and electrophysiological recording of force, MRR and CMAP as described in chapter 2.

Stimulation

Fatigue of the adductor pollicis was induced by continous supramaximal stimulation at 100Hz and 20Hz for 60 seconds on separate occasions separated by two days to allow full recovery of the adductor pollicis from the long term effects of possible low-frequency fatigue (Edwards *et al.*, 1977b).

RESULTS

The changes in force, MRR and CMAP amplitude are shown in Figure 3.1. Continuous stimulation at 100Hz resulted in a rapid reduction in force to 50% of its initial value in 14 seconds, which continued to decline to 10% after 35 seconds, thereafter plateauing at this level until the end of stimulated activity. CMAP amplitude declined rapidly from the onset of stimulation, declining to 28.6% by 14 seconds and finally to 12% at the end of tetanic activity.

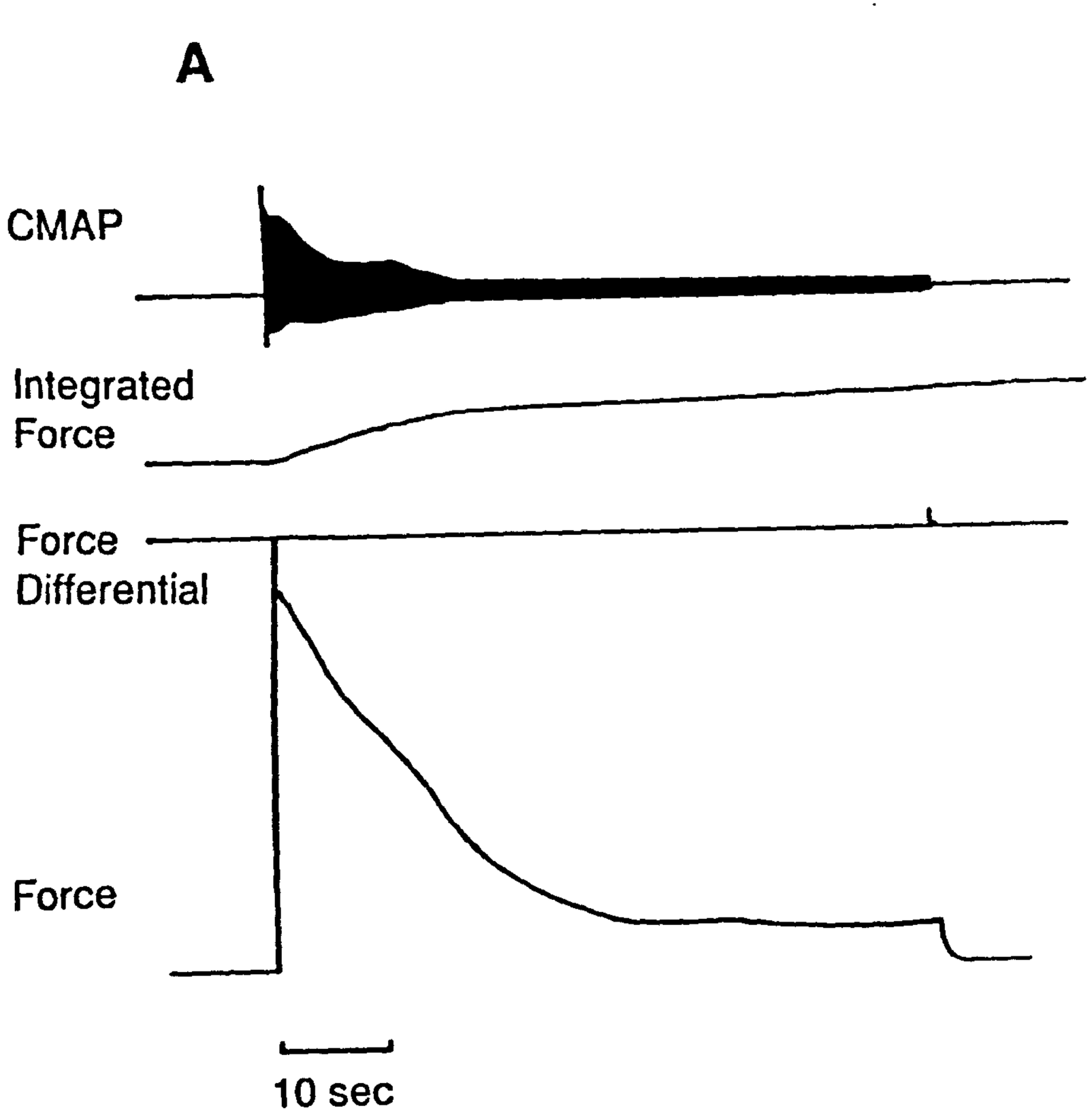
Stimulation at 20Hz produced a force that was 70.5% of the value at 100Hz in fresh muscle. Continuous stimulation resulted in a much slower decline in force compared to that observed at 100Hz and declined by only 20% of its initial value after 30 seconds, reaching a final value of 60.5% at the end of stimulated activity. CMAP amplitude increased slightly during this period by 15.8% after 30 seconds of stimulation and subsequently declined to 63.2% of initial amplitude at the end of stimulated activity (initial amplitude was measured 1 second into the contraction).

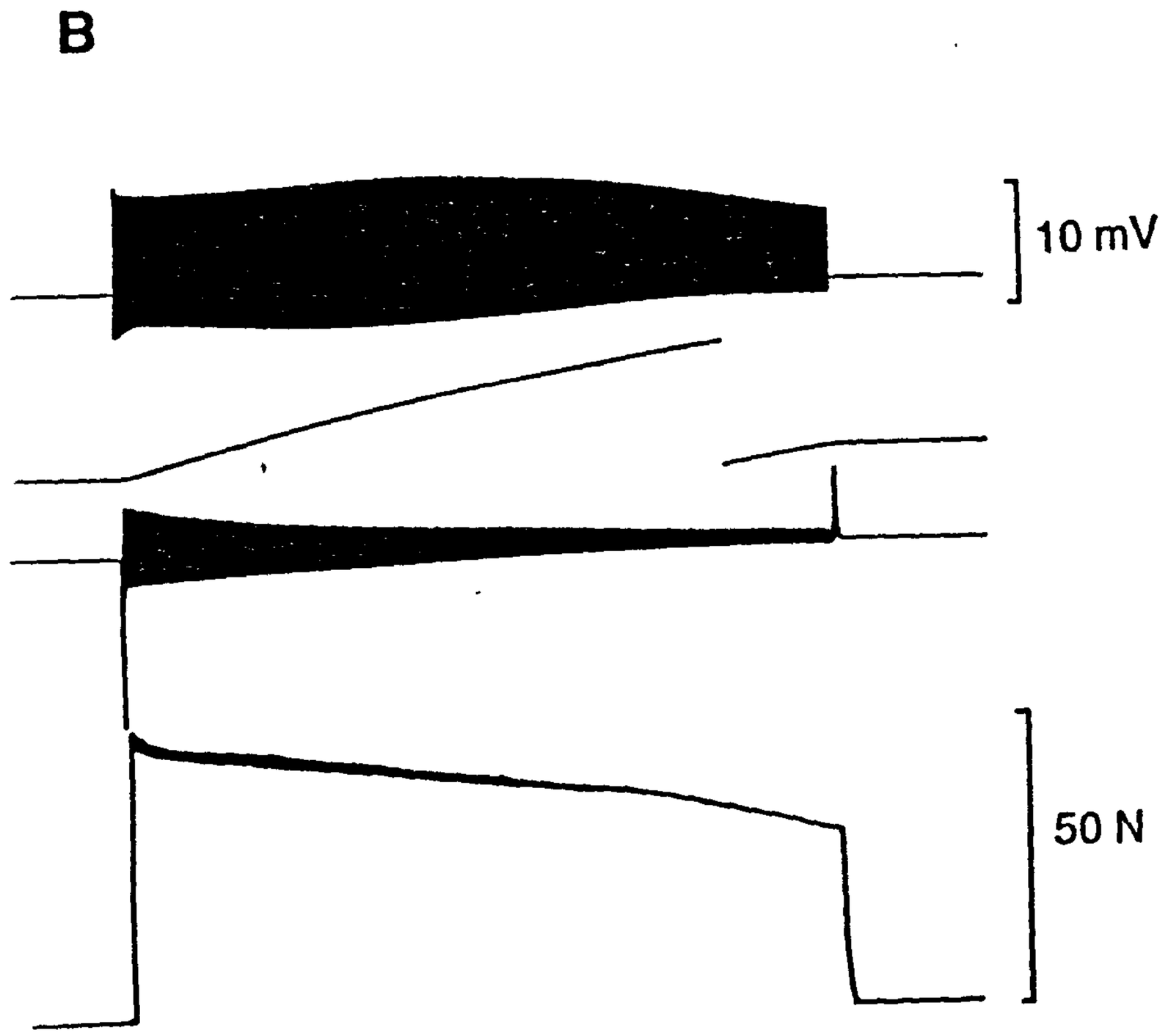
The area under the force-time curve (i.e., the force integral with respect to time) was greater for the tetani elicited by the 20Hz stimulation than that elicited by 100Hz. Thus, at 20Hz, the force-time integral was 2160 N.sec compared to 1050 N.sec at 100Hz.

DISCUSSION

This preliminary experiment serves as an example of the frequency dependency of the changes of force and excitation (measured as the amplitude of the CMAP) that occur during low and high-frequency stimulation of the adductor pollicis. It confirms the observations of several investigators working on the same muscle (Naess & Storm-Mathisen, 1955; Jones *et al.*, 1979; Marsden *et al.*, 1983) and demonstrates that fatigue at a high stimulation frequency occurs rapidly with a concomitant reduction in CMAP amplitude, whereas at low frequencies of stimulation, fatiguability of muscle is markedly less than that observed at high frequencies, and is associated with only a small decrement in the action potential amplitude. Consequently over twice the amount of activity could be performed by the low-frequency contraction compared to the high-frequency contraction. Such findings have stimulated much debate as to the factors that contribute to fatigue (see chapter 1, section 1.4.2). Further discussion of the mechanisms influencing the frequency dependence of force generation is reserved until the final discussion of this chapter.

Figure 3.1 Changes in force, maximal relaxation rate (MRR) and CMAP amplitude during A) high (100Hz) and B) low (20Hz) frequency stimulation of the adductor pollicis. Contractile activity performed was obtained from the electrical integration of the force signal and displayed alongside. The reduction of force was greatest at the high frequency with a concomitant reduction in CMAP amplitude. Twice as much contractile activity was performed with 20Hz stimulation than at 100Hz.


A
CMAP
Integrated
Force
Force
Differential
Force
10 sec


B
10 mV
50 N

PART II: DOCUMENTATION OF FREQUENCY:FORCE CHARACTERISTICS OF MUSCLE AND VALIDATION

INTRODUCTION

Early methods of obtaining frequency:force characteristics in human skeletal muscle have involved stimulation of a muscle group either indirectly via the motor nerve or percutaneously with single brief trains of stimuli at various frequencies (Merton, 1954; Edwards *et al.*, 1977a). An alternative method to obtain muscle frequency:force characteristics, developed by Edwards and colleagues (Cambell *et al.*, 1976), involved stimulation with a programmed series of impulse trains at frequencies of 1, 10, 20, 40 & 80Hz. This was termed the programmed stimulation myogram (Edwards et 1978). Other workers have subsequently employed this technique in the sternomastoid (Moxham *et al.*, 1980b), diaphragm (Moxham *et al.*, 1980a) and quadriceps muscles (Davies & White, 1981). This technique was therefore considered for investigation of the frequency dependence of the relationship between force generation and excitation.

Preliminary studies to investigate the relationship between force generation and excitation during fatigue and subsequent recovery employed an identical stimulation pattern as used by Edwards (1980,1981), incorporating sequential 2 second trains of stimuli at 1, 10, 20, 50 and 100Hz to evoke fatigue. However, the long duration of tetanic activity and large numbers of impulses delivered was thought to markedly influence the studies of recovery following a fatiguing contraction since the testing procedure may itself induce fatigue. Hence, a briefer testing procedure was developed, which incorporated a 5 second period of tetanic stimulation. The influence of the duration of the two testing procedures described above on the frequency:force characteristics and maximal relaxation rate were therefore compared in fresh muscle. The reproducibility of the frequency:force characteristics were established for repeated contractions on the same, and separate, visits. A comparison of the frequency:force characteristics and maximal relaxation rate in males and females was also made since differences are apparent in maximal voluntary grip strength

(Lenmarken *et al.*, 1985). Furthermore, it was necessary to establish the influence of the differences of sex on muscle functional characteristics since the relation between electrophysiological and mechanical factors was studied in both males and females.

METHODS

Measurement of muscle contractile properties

The left hand was prepared for electrical stimulation and measurement of force, MRR and CMAP as described in chapter 2.

Stimulation protocols

One of two stimulation procedures was undertaken by each subject: the adductor pollicis was stimulated via the ulnar nerve at the wrist with either an ascending series of frequency trains at 1Hz (for 5 seconds), 10Hz, 20Hz, 50Hz, 100Hz (2 seconds each) and 1Hz (5 seconds) (Edwards, 1980), or 1Hz (1 second), 10Hz (2 seconds in order to obtain a force plateau), 20Hz, 50Hz, 100Hz and 1Hz (1 second each) to produce the 'Programmed Stimulation Electro-Myogram' (PSEM). Typical oscillographic records are shown in Figure 3.2. These frequencies were selected on the basis of encompassing the physiological discharge rates of motorneurones in adductor pollicis (during voluntary activity) (Bellemare *et al.*, 1983) and those necessary to achieve near full activation (Merton, 1954; Edwards *et al.*, 1977). In addition, these frequencies are the same as those used by other workers (Moxham *et al.*, 1980a,b; Edwards *et al.*, 1977,1980) thus allowing direct comparison of data between experiments and different muscle groups.

Validation

Inter- and intra-day coefficients of variation and two-way analysis of variance was undertaken for the shorter PSEM. Each subject undertook a series of 3 contractions separated by 5 minutes to obtain inter-day coefficients of variation. Intra-day coefficients of variation were obtained from the first contraction of two visits on separate days.

A comparison of frequency:force characteristics and relaxation rate obtained from the shorter PSEM between 10 males and 10 females was also made. In addition,

a comparison of the two testing procedures was made to assess the influence of test duration on muscle contractile properties.

The possible influences of resting tension and ambient temperature on the frequency:force characteristics of muscle were not determined, since these factors were standardized during the setting up procedure (Chapter 2, section 2.4.1).

Criteria for acceptance of the PSEM

Only successful contractions were accepted for analysis, as defined by the following criteria:

1. Force and CMAP traces appeared 'clean', showing no gaps or uneveness in either the force plateau or CMAP amplitude.
2. There was no evidence of voluntary activity in addition to that obtained by electrical stimulation. This was generally identified by a marked slowed relaxation phase and a sustained submaximal contraction after cessation of tetanic activity. This problem was particularly noted in subjects unaccustomed to this form of activity.
3. The pre-tetanic and post-tetanic force baselines were similar.

Analysis

On each contraction, the force was measured and the MRR from 100Hz plateau force calculated as described in chapter 2 (section 2.4.4). Where oscillation of force was apparent the mean value of force was taken. Intra- and inter-day coefficients of variation were determined from the formula:

$$C.V. = \frac{S.D.}{\text{mean } x} \times 100$$

and the mean and one standard deviation were determined for all the subjects.

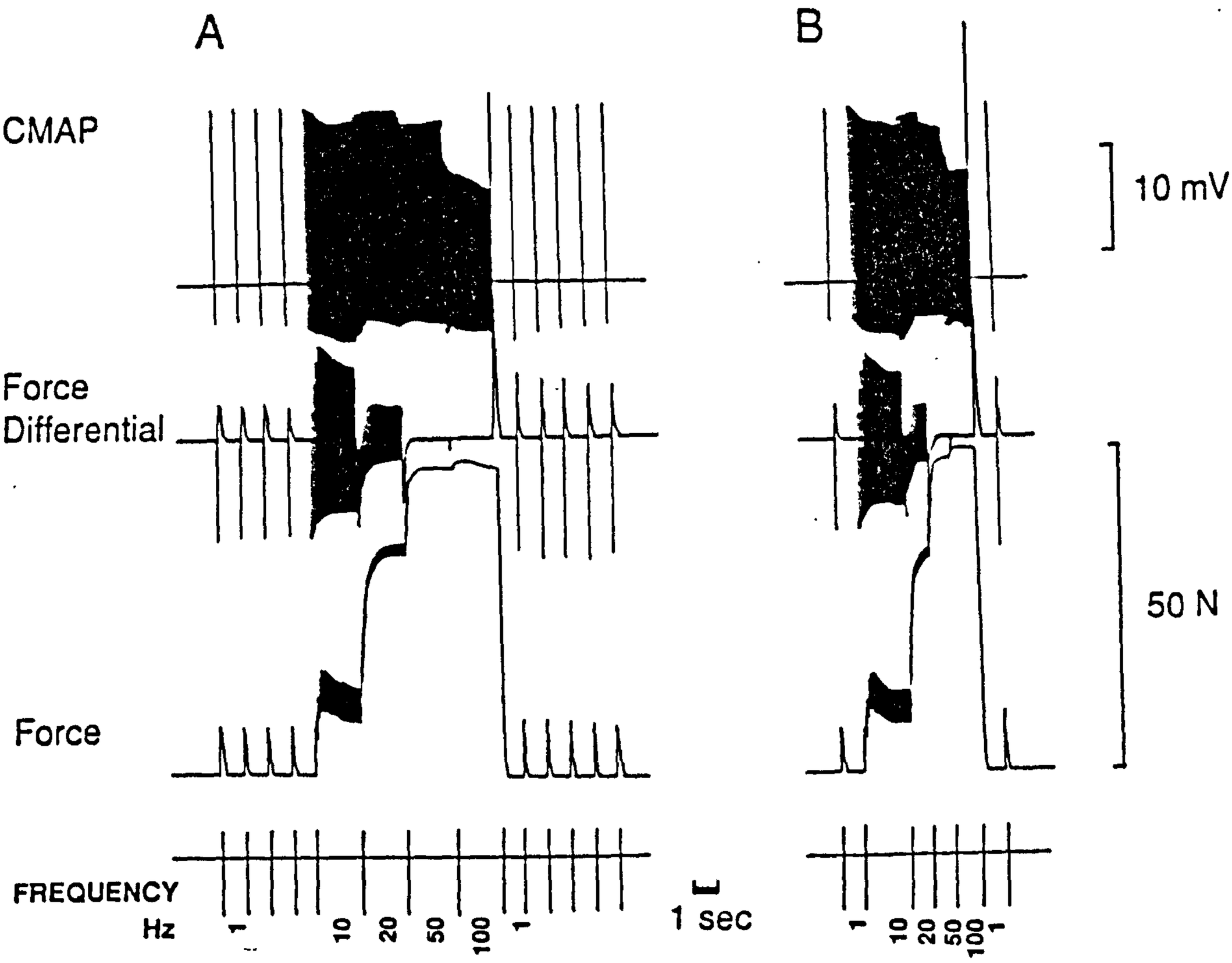


Figure 3.2 Tracings of example oscillograph records of A) 8 second Programmed Stimulation Electro-Myogram (PSEM) and B) 5 second PSEM, showing simulataneous recordings of compound muscle action potential (CMAP), force and force differential. Subject H.G.

Two-way analysis of variance was performed to test for the significance of variability as an influencing factor of the inter- and intra-day variation. In some instances where PSEMs were unacceptable or it was not possible to measure a particular force, missing values were predicted for the two-way analysis of variation by the equation:

mean value of 1st and 2nd determination + correction factor.

The correction factor was calculated as:

$$\text{For 1st missing value, } a - \frac{(b + c)}{a}$$

$$\text{For 2nd missing value, } b - \frac{(a + c)}{c}$$

$$\text{For 3rd missing value, } c - \frac{(a + b)}{b}$$

where, a = mean value of 1st determination
b = mean value of 2nd determination
c = mean value of 3rd determination

One degree of freedom was subtracted from the total for each missing value before looking up significance tables.

Differences in frequency:force characteristics and MRR in males and females were assessed using Students t-test. A p value of < 0.05 was taken to be significant. Frequency:force curves were calculated by expressing all force values as a percentage of that obtained at 100Hz in fresh muscle. All results reported are expressed as mean and 95% confidence intervals of the *difference of the mean* and those used to construct figures are expressed as mean $\pm$ 1 S.D..

RESULTS

1. Influence of frequency on force generation

The forces generated for a range of stimulation frequencies and the associated force:frequency curve is shown in Figure 3.3 a and b respectively for brief 1 second

contractions of the adductor pollicis in a single individual to illustrate the influence of stimulation frequency on force generation. Typically, the degree of oscillation decreased as stimulation frequency was increased and hence increased fusion.

2. Repeatability of measurements

The coefficients of variation for the measurements of MRR and frequency:force characteristics for three consecutive contractions and on two separate occasions measured from the shorter PSEM are shown in Table 3.1. Two-way ANOVA showed no significant influences of contraction position or visit for the variables presented in Table 3.1, although there was a significantly large variation between subjects ($p < 0.05$).

3. Influence of sex

No significant differences of relative force generation (expressed as a percentage of maximum tetanic force at 100Hz) or MRR were observed in a comparison of muscle contractile characteristics between males and females (T-test) (Table 3.2). Maximum tetanic force was generally greater in males than in females (75.8 Newtons compared to 60.6 Newtons, 95% C.I. of difference -32.1, 1.6) and was highly significant ($p < 0.0001$).

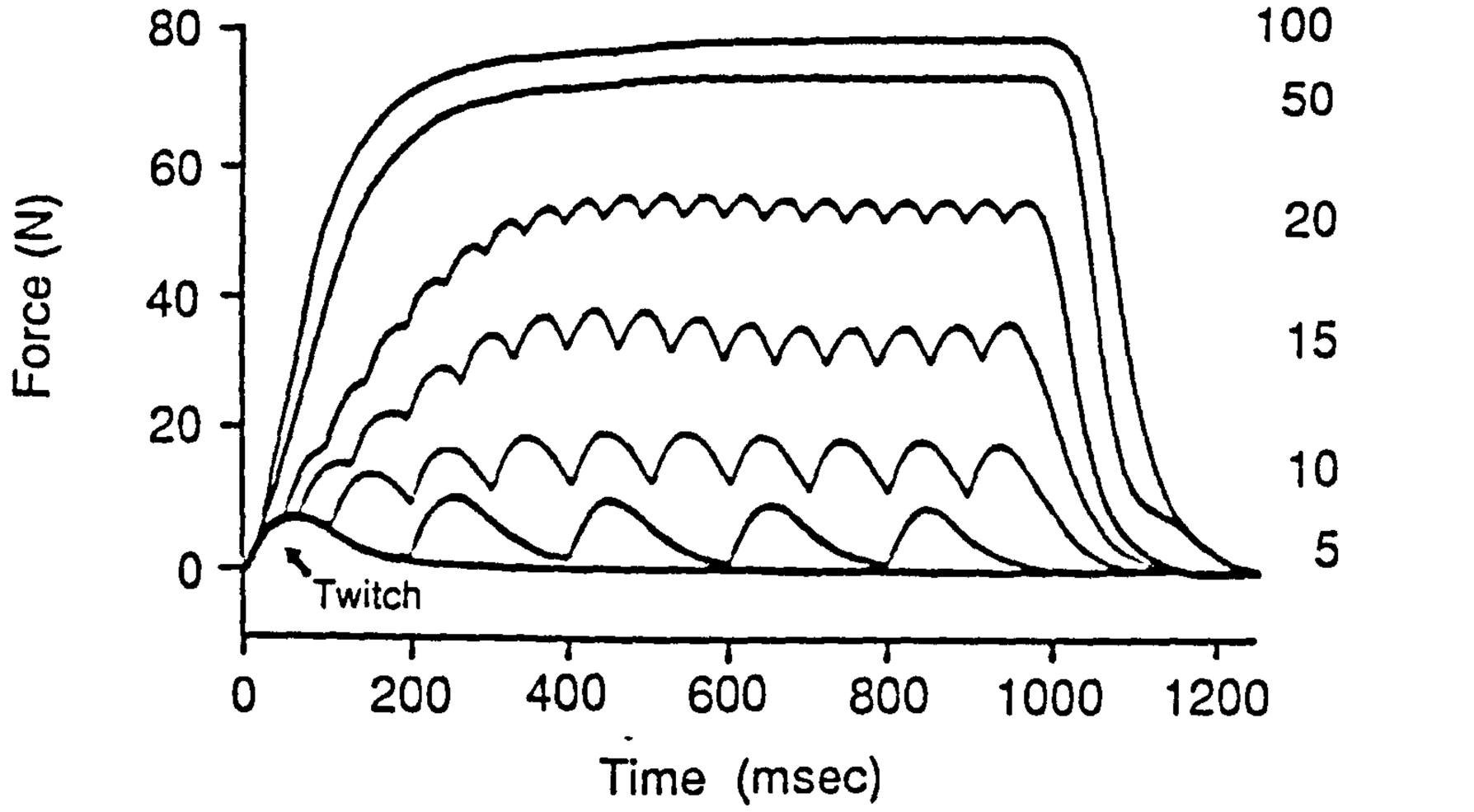
4. Influence of duration of contraction

A comparison of maximal tetanic force obtained from both test procedures and from a single 100Hz tetanic contraction in 11 subjects showed a clear fatiguing effect on maximal force generation presumably as a result of the preceding activity at the lower frequencies. This was greatest for the longer PSEM (mean: 52.1N compared to 59.3N for a 100Hz tetanus, 95% C.I. of difference -7.3, -4.6) ($p < 0.0001$, paired t-test) and less, but still significant, for the shorter PSEM (55.2N compared to 59.3N for a 100Hz tetanus, 95% C.I. of difference -5.3, -2.9). This had obvious implications on the respective frequency:force curves (Figure 3.4, Table 3.3), resulting in a relative increase in force at low frequencies and a greater F20/50 ratio.

A comparison of the results obtained by others with the present results are shown in Table 3.4.

Figure 3.3 Generation of a tetanus. A) 1 second contractions of adductor pollicis at various frequencies from a single twitch to 100Hz leading to the formation of a fused contraction. B) Frequency:force curve showing mean force (solid line) and oscillation of force (dashed lines). Note that oscillation is greatest at low frequencies where fusion is incomplete.

A


Frequency Hz
100
50
20
15
10
5
Force (N)
80
60
40
20
0
Twitch
0
200
400
600
800
1000
1200
Time (msec)

B

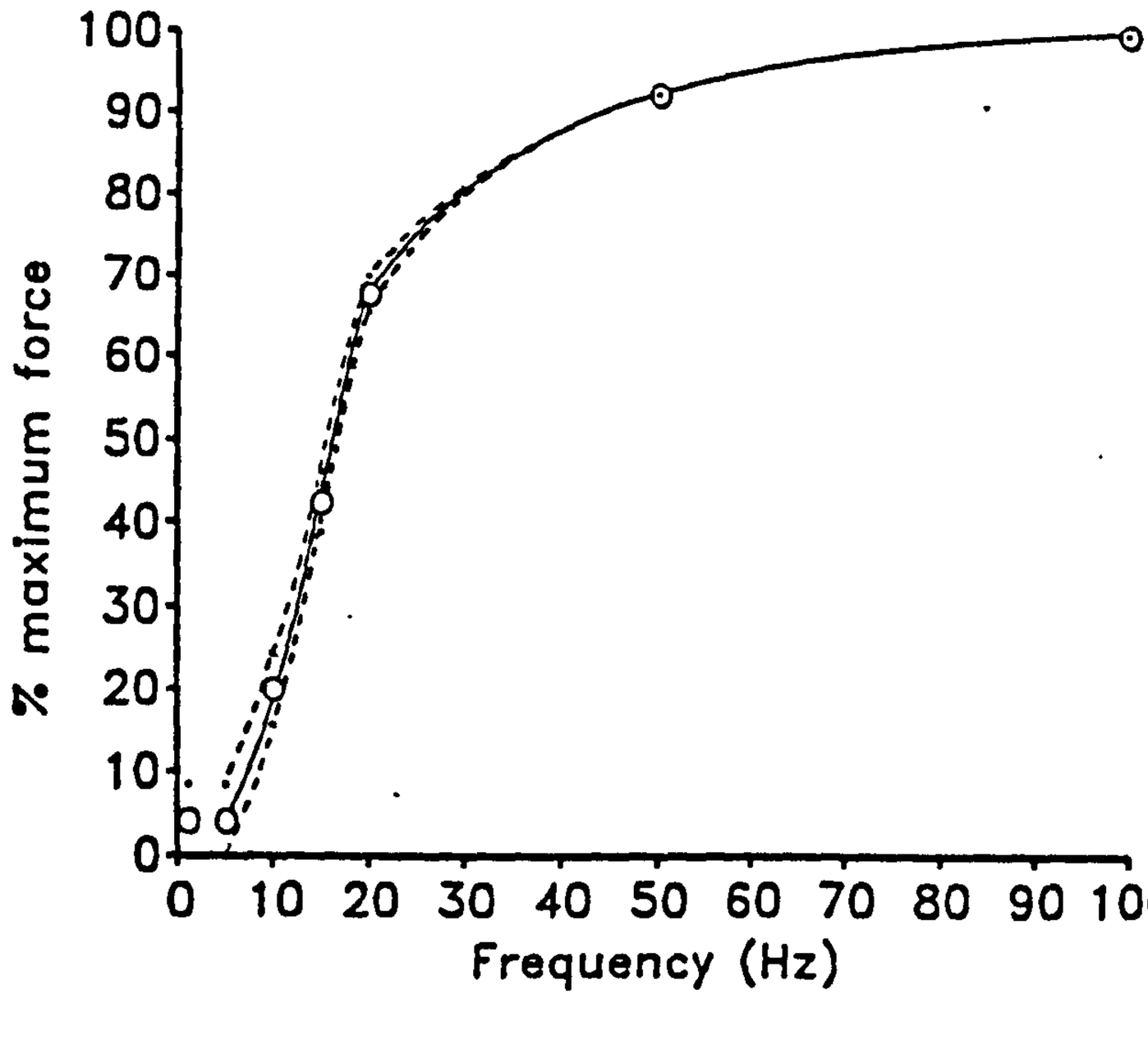

% maximum force
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Frequency (Hz)

Table 3.1 Mean and 1 S.D. of coefficients of variation for reproducibility of the five second PSEM in fresh muscle between two visits and on a single occasion (three PSEMs separated by five minutes) for 9 and 20 subjects respectively.

Inter-day

	% Coefficient of Variation	
	mean ± 1 S.D.	range
Maximum Relaxation Rate.	5.82 ± 3.76	1.86 - 12.51
Twitch force as % of max.	11.34 ± 6.96	0.60 - 5.00
Force at 10Hz as % of max.	16.15 ± 12.57	1.90 - 36.15
Force at 20Hz as % of max.	2.50 ± 1.47	0.51 - 4.75
Force at 50Hz as % of max.	0.86 ± 0.85	0.09 - 2.16
Post-tetanic force as % of max.	4.79 ± 3.76	0.98 - 17.20

n = 9, mean age 29.7 (range 25-36) yrs.

Table 3.1 continued

	Intra-day	
	% Coefficient of Variation	
	mean ± 1 S.D.	range
Maximum Relaxation Rate.	3.79 ± 2.29	0.98 - 8.72
Twitch force as % of max.	7.50 ± 4.58	0.88 - 20.70
Force at 10Hz as % of max.	8.41 ± 5.84	1.14 - 19.48
Force at 20Hz as % of max.	2.23 ± 1.55	0.83 - 6.12
Force at 50Hz as % of max.	0.84 ± 0.64	0.05 - 2.76
Post-tetanic force as % of max.	5.96 ± 3.54	1.16 - 14.6

n = 20, mean age 29.2 (range 21-39) yrs.

Table 3.2. Influence of sex on frequency:force characteristics and MRR obtained from five second PSEM. n=10

	Males Mean(SD)	**Females Mean(SD)**	**95% confidence interval of difference of mean**
Age range (yrs)	21-36	22-39	
Maximum relax. rate (%loss $10msec^{-1}$)	11.07(1.21)	11.27(1.33)	-1.48, 1.08
Force of twitch as % max.force	12.49(2.92)	11.54(3.49)	-1.99, 4.10
Force at 10Hz as % max.force	22.85(6.29)	22.50(11.8)	-8.70, 9.50
Force at 20Hz as % max.force	68.62(4.41)	68.60(7.10)	-5.60, 5.70
Force at 50Hz as % max.force	95.41(1.13)	96.51(1.50)	-2.39, 0.18
Force of post-tetanic twitch as % of max.force	17.58(3.10)	19.52(5.57)	-6.51, 2.60
Ratio of force at 20Hz to 50Hz	72.58(3.89)	71.05(7.00)	-4.00, 7.00

Note that all values were not significantly different (t-test).

Table 3.3 Frequency:force characteristics of adductor pollicis expressed as a percentage of maximum tetanic force at 100Hz, MRR and F20/F50 ratio obtained from eight and five second PESMs in 10 subjects. Relative low frequency force is greater in the eight second PSEM presumably due to a fatiguing effect at 100Hz. Mean ± 1 S.D.

	8 second	**5 second**	**p (paired t-test)**
MRR (% loss $10msec^{-1}$)	10.1±0.9	11.5±1.4	<0.0001
%Force twitch of maximum	16.5±4.8	15.0±3.9	NS
%Force at 10Hz of maximum	27.8±7.9	22.6±8.2	<0.0001
%Force at 20Hz of maximum	74.9±3.7	70.3±4.6	<0.0001
%Force at 50Hz of maximum	96.6±1.9	95.9±1.3	<0.05
Ratio of force at 20Hz to 50Hz (%).	77.6±3.8	73.3±4.7	<0.0001

Table 3.4 Comparison of muscle contractile characteristics determined by other workers on adductor pollicis using 8second PSEM and of the shorter 5 second PSEM.

Source	Contraction	10/100 %	20/50 %	MRR %Force loss $10msec^{-1}$	n
Edwards *et al.*, 1977	Single frequencies	*	73.1±2.8	*	10
Newham (personal communication)	8s PSEM	28.7±9.5	*	10.1±1.1	20
Present study	8s PSEM	24.4±7.2	74.4±4.6	10.2±0.9	21
Present study	5s PSEM	21.9±8.6	71.5±5.4	11.4±1.3	24

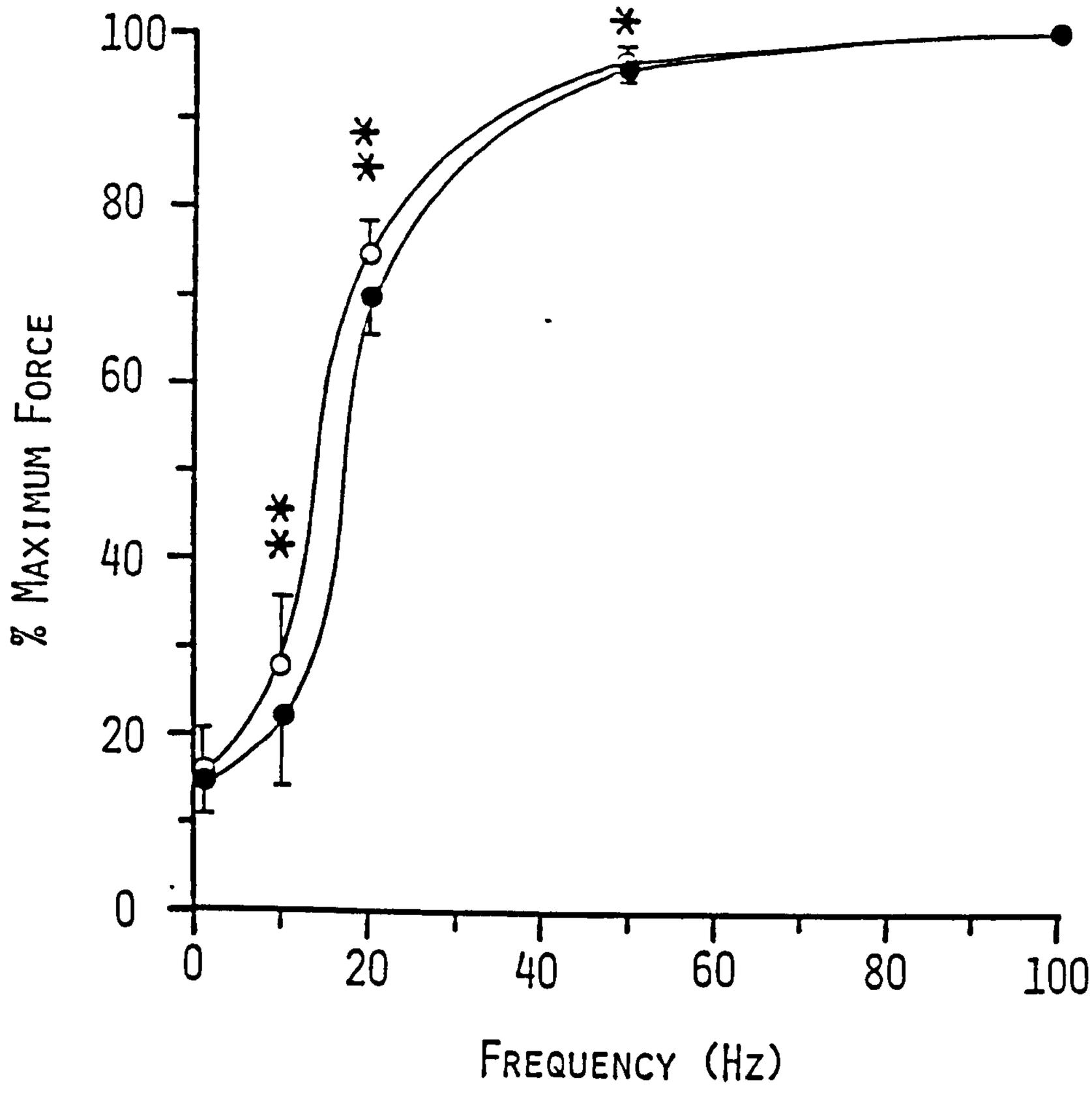


Figure 3.4 Frequency:force curves for adductor pollicis produced in fresh muscle from five second (●) and eight second (o) PSEMs. The resultant fatigue at high frequency during the longer PSEM produces a shift of the curve towards the right. Mean $\pm$ 1 S.D. where n=10. * $p < 0.05$, ** $p < 0.0001$ (paired t-test) (Appendix 6).

DISCUSSION

The present results indicate that stimulation of the adductor pollicis to obtain frequency:force characteristics and MRR is remarkably reproducible on the same occasion and on subsequent separate occasions. The determinations of C.V. were not done on the absolute measurements since only relative forces (ratios) are used in the present studies. The results compare favourably with other workers (Table 3.4). The larger variation at low stimulation frequencies observed in the inter-day visit measurements are likely to be due to several factors, particularly those dependent on the setting up procedure, for example, muscle length, temperature, resting tension, although every precaution was taken to minimize this source of error.

The influences of sex on muscle functional characteristics were only obvious in the absolute strength of the adductor pollicis, a finding which has been noted by several workers in other muscle groups (Lenmarken *et al.*, 1985) possibly reflecting a differential size aspect of the muscle in males and females (Bruce *et al.*, 1985). This was not investigated further since relative measurements were not influenced by sex.

The design of the testing procedure was found markedly to influence the maximal tetanic force elicited by 100Hz stimulation and MRR, both of which are likely to have been influenced by the preceding tetanic activity resulting in fatigue to a small degree. The implication of this is reflected in the frequency:force curves which show a small shift in force to the left for the longer testing procedure compared to that obtained for the briefer contraction (Figure 3.4). The greater fatiguing affect on the muscle under test was considered to be undesirable to investigate the frequency dependence of the interrelationship between excitation and force generation during subsequent recovery from fatigue, although the greater fatigue induced by the longer testing procedure clearly would require less contractions to induce marked fatigue than the shorter testing procedure. The subsequent studies reported in this chapter were therefore designed around the shorter PSEM.

SUMMARY

1. Measurement of contractile characteristics of adductor pollicis is repeatable and reproducable on separate occasions.

2. There are no significant differences in relative muscle characteristics between males and females.

3. The use of a long testing procedure to obtain frequency:force characteristics may induce fatigue at high-frequency and consequently shift the frequency:force curve to the left. A shorter testing procedure, consisting of 5 seconds tetanic activity was found to influence force generation at high-frequency to a lesser degree, although the high-frequency force was still reduced by the preceding tetanic activity.

PART III: FREQUENCY DEPENDENCE OF EXCITATION AND FORCE GENERATION DURING INTERMITTENT ELECTRICAL STIMULATED ACTIVITY AND RECOVERY

INTRODUCTION

The frequency dependence of the interelationship of force generation and excitation was studied in single fatiguing intermmitent contractions utilizing the PSEM described in part II (Cooper *et al.*, 1988).

METHODS

Experimental subjects

Nine normal subjects (8 male, 1 female) aged 25-34 years participated in this study.

Measurement of muscle contractile properties and electromyography

The left hand was prepared for stimulation and recording of force, MRR and CMAP as described in chapter 2. In addition, the CMAP signal of the pre-tetanic twitch of the PSEM was captured under computer control (Apple II) and stored on floppy disc for subsequent analysis (Med 80) as described in chapter 2, section 2.4.5.

Experimental fatigue protocols

In each subject, two activity protocols were undertaken to fatigue the adductor pollicis; i.e., during conditions of a) local ischaemia, thereby providing a 'closed system' preventing the supply of oxygen for oxidative phosphorylation and the transport of metabolic waste products away from the muscle, and b) with intact circulation, thus permitting some degree of supply of oxygen and removal of metabolic by-products.

1. Activity with arterial occlusion

In each subject, a control PSEM was performed in fresh muscle. After a two minute period for recovery, a sphygmomanometer cuff placed around the upper arm was inflated to 220 mmHg to occlude arterial circulation. This was followed by a three minute period of ischaemic rest to minimize local oxygen availability for oxidative metabolism (Harris et al., 1975). Fatiguing activity then followed and

consisted of 15 PSEMs at intervals of 37 seconds (30 second rest periods were allowed between each PSEM). At the end of activity the cuff was deflated to allow return of circulation. Aerobic recovery was followed using PSEMs at intervals of 0.5, 1, 2, 5, 10 and 15 minutes

2. Activity with intact circulation

A control PSEM was performed in fresh muscle before commencement of activity. After a 3 minute rest period, fatiguing activity commenced and consisted of 50 PSEM's at intervals of 12 seconds (5 second rest periods were allowed between contractions). Preliminary studies indicated that the greater number of contractions and the shorter period of duration between contractions were necessary to induce force failure. Aerobic recovery was monitored at intervals of 0.5, 1, 2, 3, 5, 10 and 15 minutes.

Frequency dependence of change in relaxation rate

An additional 3 series of occluded contractions with modified PSEMs were carried out to examine the influence of frequency on change in MRR and hence force generation. Five subjects from the above group volunteered for this study. The fatiguing procedure was identical to that used for occluded contractions above, except intervals of 5 second rest were allowed between each contraction. Preliminary studies indicated that the different periods of ischaemic rest between contractions did not influence the changes in force reduction. Furthermore, this procedure reduced the total time of ischaemia which was described as extremely discomforting by most subjects. The PSEM was split by a 0.5 second pause after either 10Hz, 20Hz or 50Hz to allow relaxation of force to baseline levels to obtain the MRR.

These protocols were performed in random order and at least one week apart to ensure full recovery of the adductor pollicis.

Analysis

The force and CMAP amplitude were measured at the final impulse of each frequency train. MRR was determined from the 100Hz force plateau except in the case of interrupted PSEMs, where MRR was determined from the frequency train

under investigation. Each variable was expressed as a percentage of the equivalent part from fresh muscle. Measurements were made on every PSEM during activity with occlusion and on every 5th PSEM during activity with intact circulation due to the larger numbers of contractions performed. Frequency:force curves were constructed for fresh muscle and at intervals during recovery by expressing force values as a percentage of that at 100Hz in fresh muscle. The interrelation between force and CMAP was assessed as the 'force per excitation' (F/E) ratio, calculated for each frequency as: percent force of fresh muscle / percent excitation (CMAP amplitude) of fresh muscle. Changes in CMAP duration, which may be used as an indirect measure of the changes in conduction velocity, were measured from the stimulus artifact to the peak of the negative phase of depolarization (Mortimer *et al.*, 1971; Bigland-Ritchie *et al.*, 1981) and expressed as a percentage of the fresh value. The results reported in the text and used to construct figures are expressed as mean ± 1 SD and for n=9 except where stated. Paired t-tests were employed to test for differences between control and fatigued muscle.

RESULTS

1. Activity with Arterial occlusion

Influence of frequency on force generation

Typical oscillograph records are shown in Figure 3.5. The time course of the changes in force is shown in Figure 3.6. During stimulated activity, force loss occured at all frequencies. The initial rates of decline in force for 20-100Hz tetani up to the 8th PSEM were similar and initially linear, with a reduction in force of 2.52% per contraction (arithmetic mean of slopes). Thereafter, the reduction in force was more rapid and frequency dependent. Final force loss was greatest at low stimulation frequencies compared to high frequency stimulation (Table 3.5). However, low-frequency 10Hz tetanic force initially *increased* up to the 8th PSEM. Pre-tetanic twitch force also increased up to the 3rd PSEM before subsequently

Figure 3.5 Tracings of oscillograph records of simultaneous recordings of CMAP, force and force differential producing the PSEM in fresh muscle, during ischaemic fatiguing activity and subsequent non-occluded recovery.

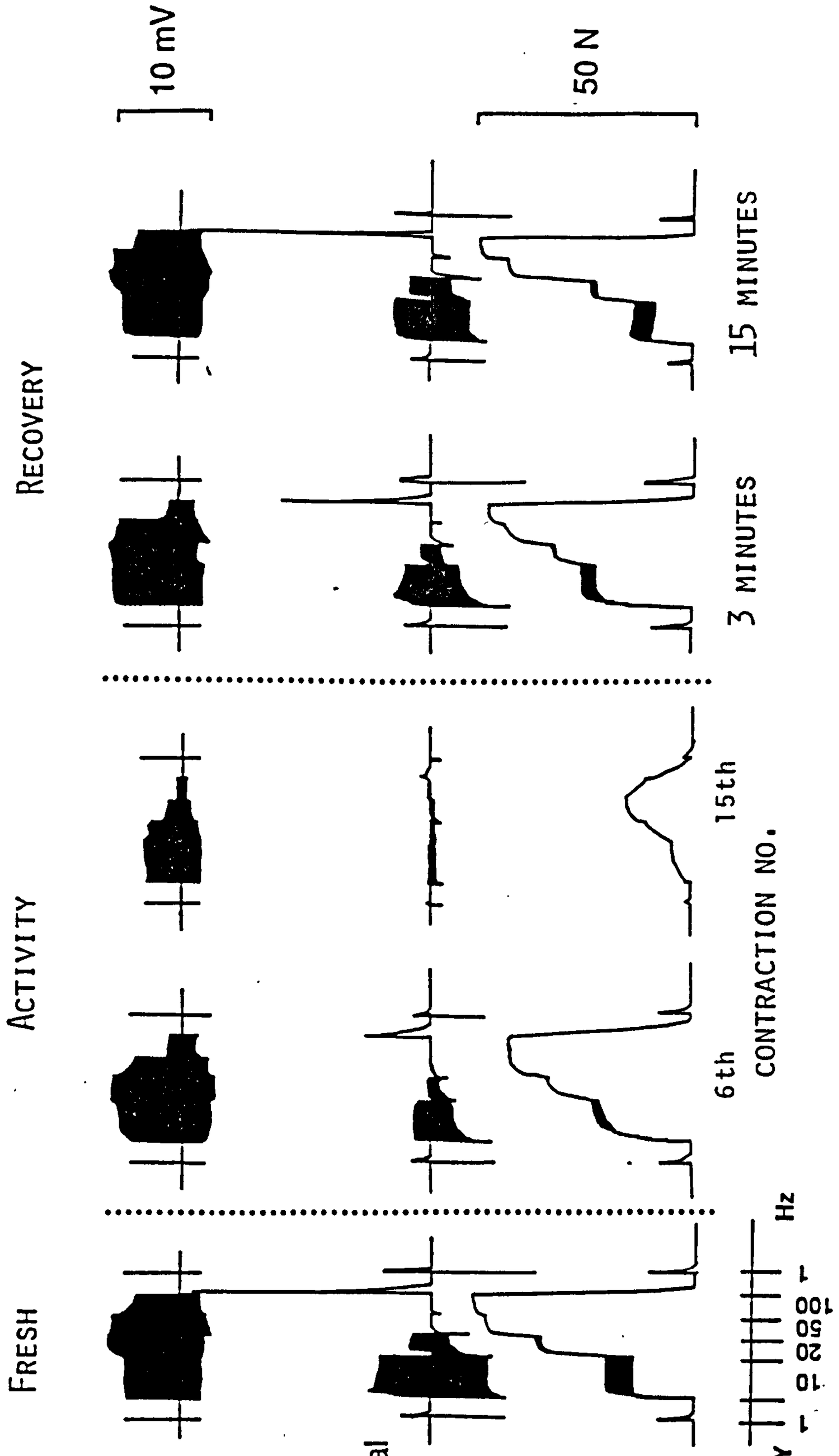
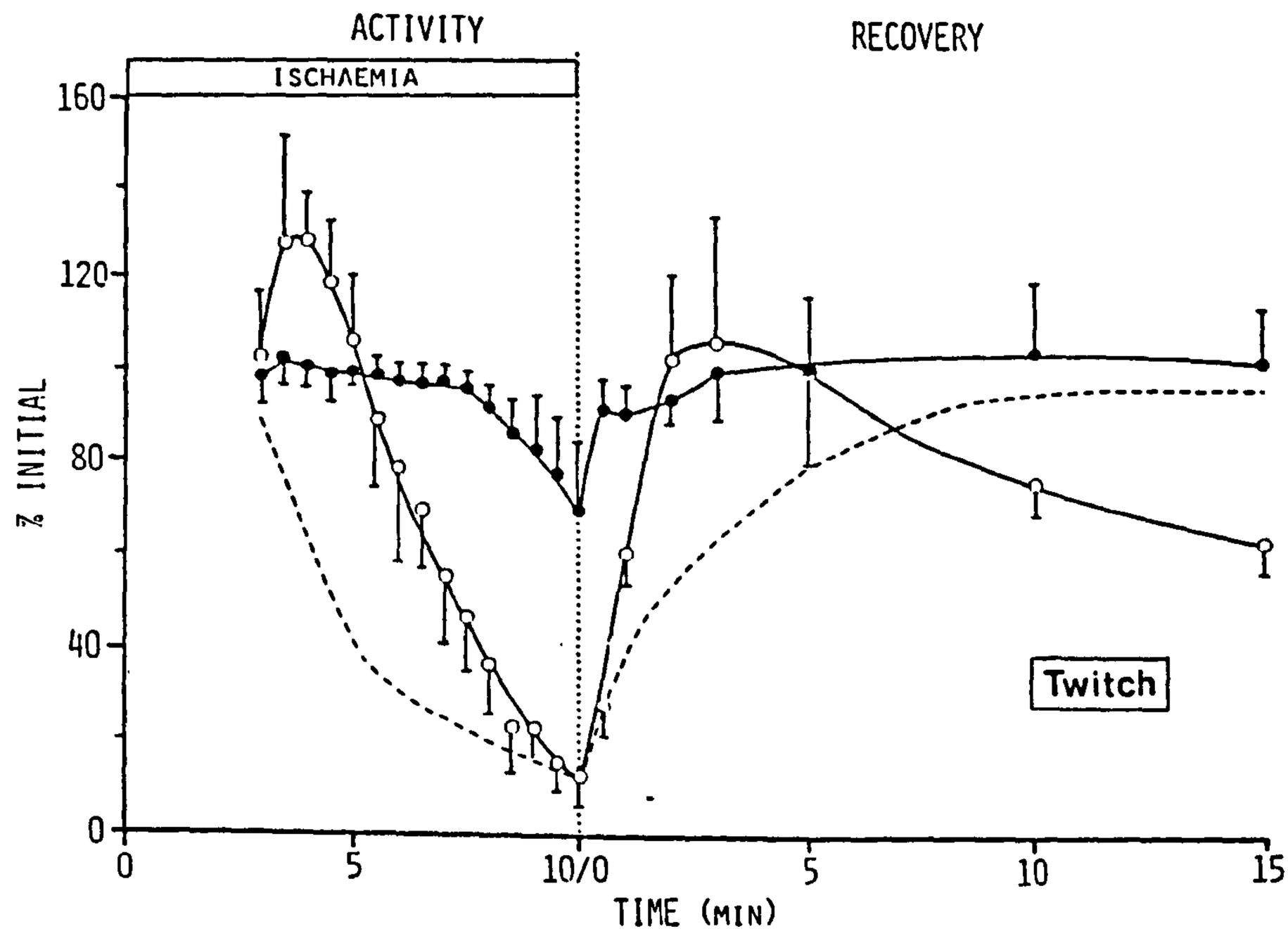
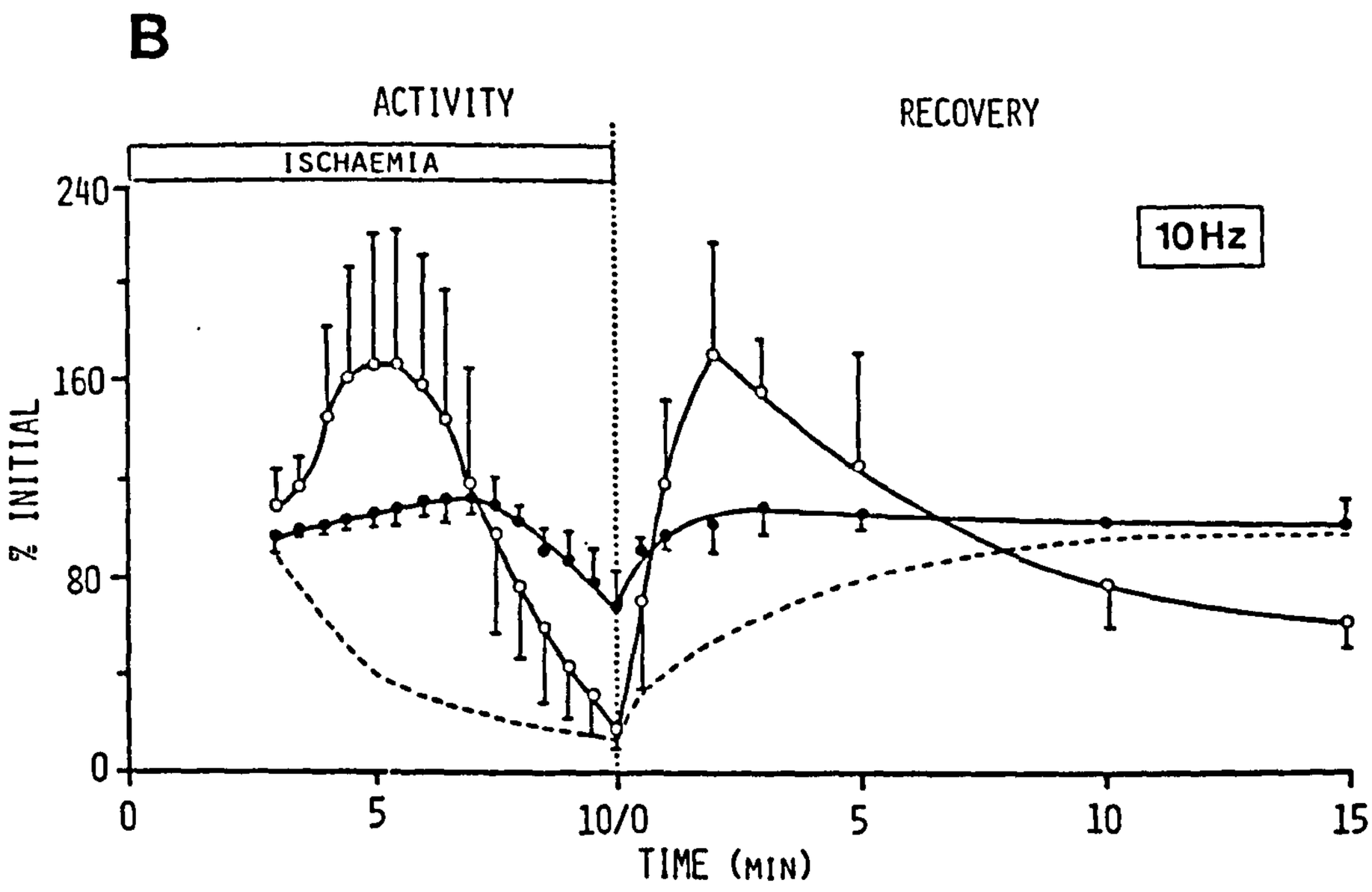
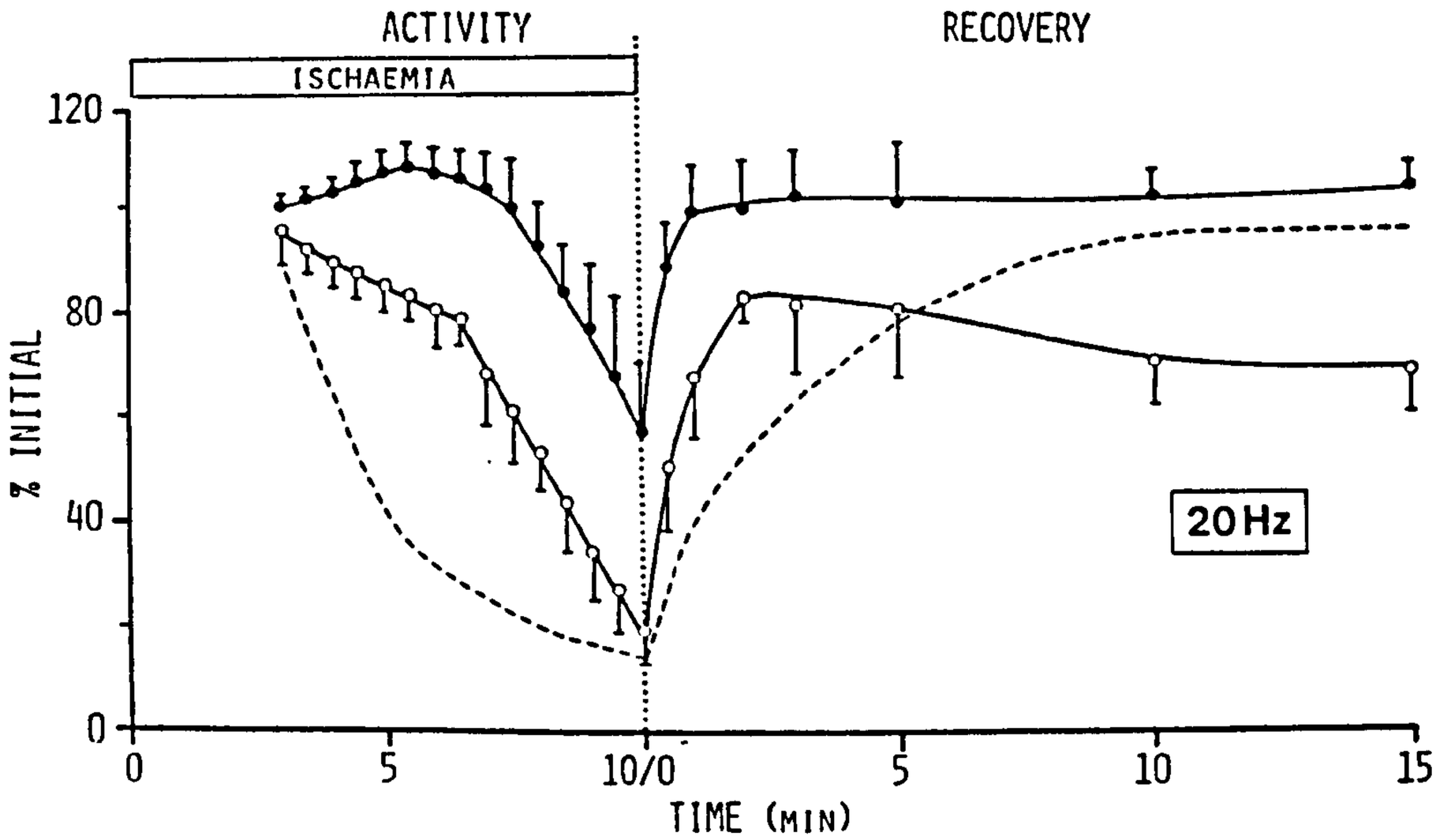

10 mV
50 N
15 MINUTES
3 MINUTES
15th
6th
CONTRACTION NO.
Hz
100
50
20
10
1
FRESH
RECOVERY
ACTIVITY
CMAP
Force
Differential
Force
FREQUENCY

Figure 3.6 Time courses of changes of force (o) and CMAP amplitude (●) during activity with arterial occlusion (fifteen PSEMs at 30s intervals) and recovery with intact circulation at various stimulation frequencies: A) twitch, B) 10Hz, C) 20Hz, D) 50Hz, E) 100Hz and F) post-tetanic twitch. Mean relative changes in MRR at 100Hz are represented by a dotted line on each range, except at 100Hz where mean $\pm$ 1 S.D. are shown.

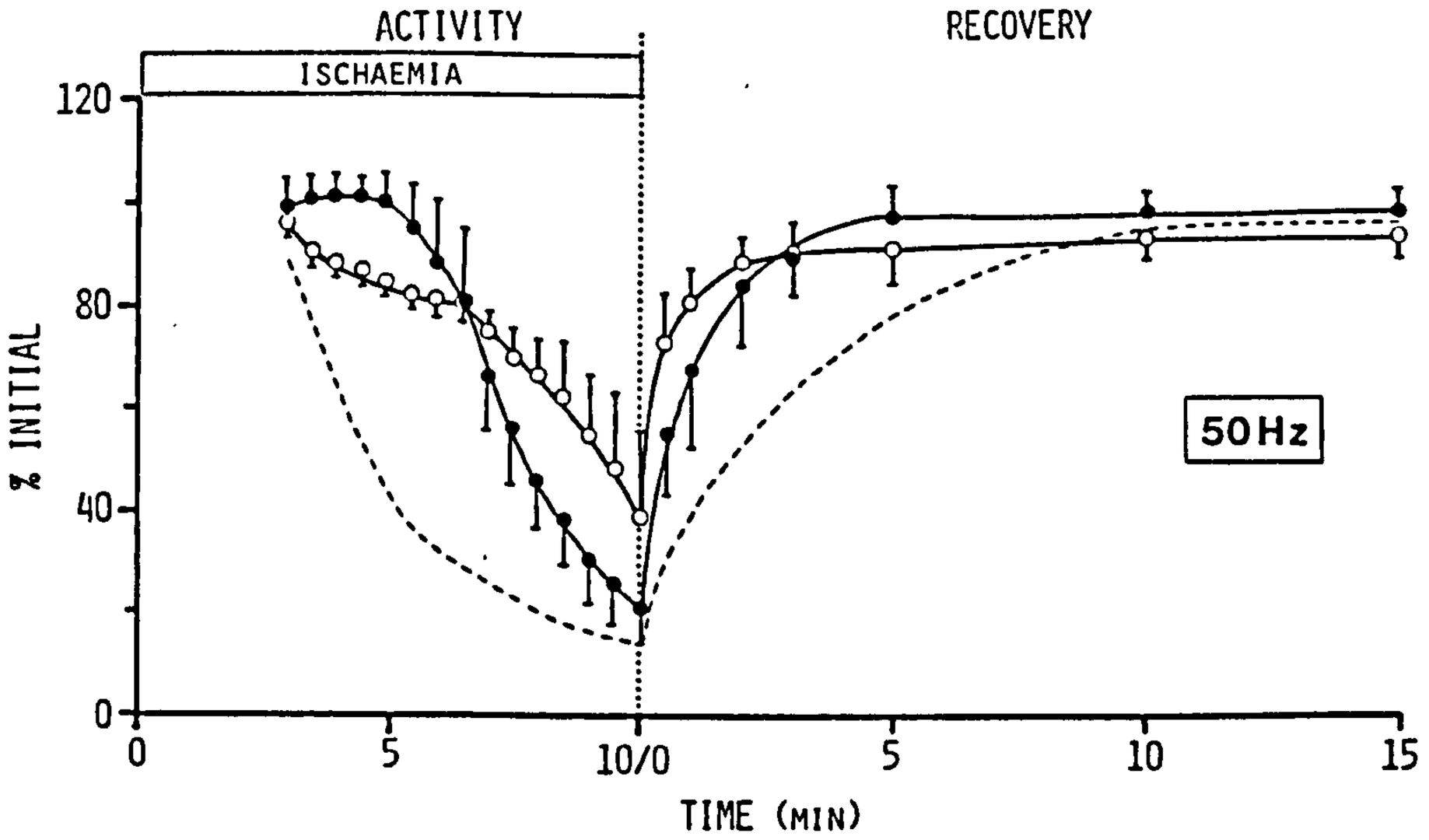

A
ACTIVITY
RECOVERY
ISCHAEMIA
160
120
80
40
0
% INITIAL
Twitch
0
5
10/0
5
10
15
TIME (MIN)


B
ACTIVITY
RECOVERY
ISCHAEMIA
240
160
80
0
% INITIAL
10Hz
0
5
10/0
5
10
15
TIME (MIN)

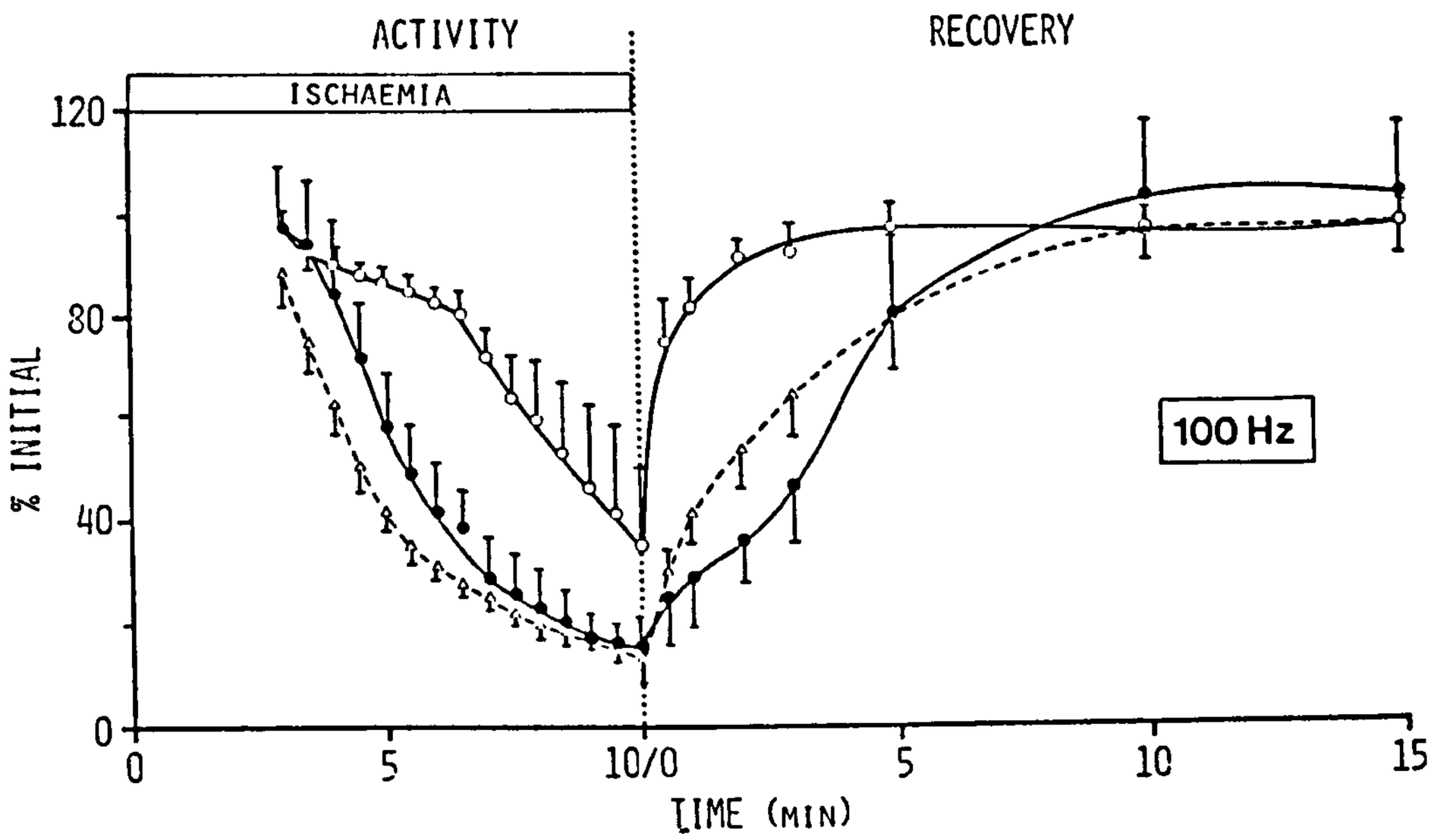
C


ACTIVITY
RECOVERY
ISCHAEMIA
120
80
40
0
% INITIAL
0
5
10/0
5
10
15
TIME (MIN)
20Hz

D


ACTIVITY
RECOVERY
ISCHAEMIA
120
80
40
0
% INITIAL
0
5
10/0
5
10
15
TIME (MIN)
50Hz

E


ACTIVITY
RECOVERY
ISCHAEMIA
120
80
40
0
% INITIAL
100 Hz
0
5
10/0
5
10
15
TIME (MIN)

F

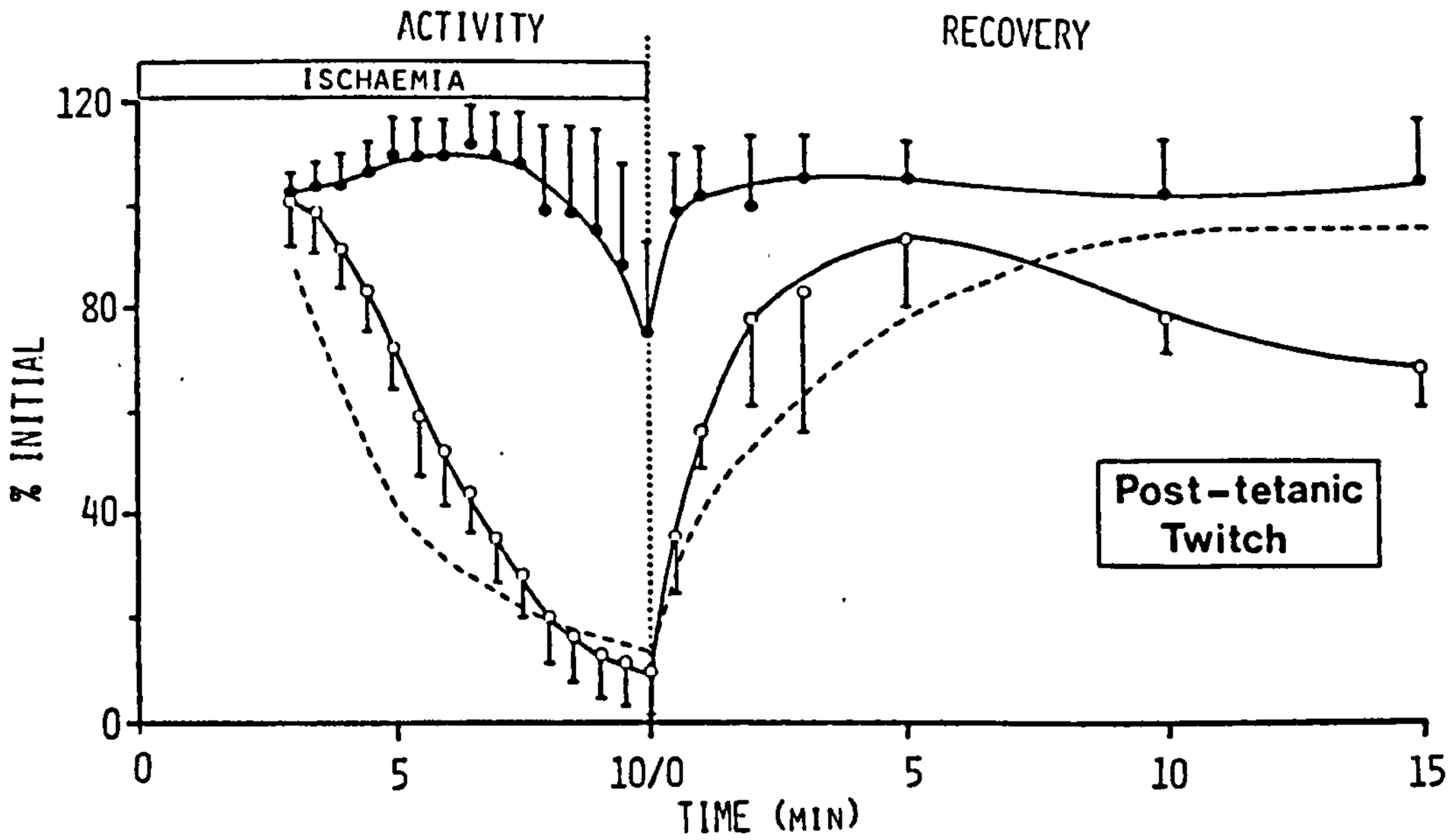

ACTIVITY
RECOVERY
ISCHAEMIA
120
80
40
0
% INITIAL
Post-tetanic Twitch
0
5
10/0
5
10
15
TIME (MIN)

Table 3.5 Force and CMAP amplitude (as percentage of fresh muscle and F/E ratios at each frequency at the end of activity with and without circulatory occlusion.

Activity	Force (%)	CMAP Amplitude (%)	F/E ratio
Frequency (Hz)			
		With arterial occlusion	
1	13±4	70±15	0.21±0.05
10	18±7	68±15	0.26±0.17
20	19±7	57±15	0.32±0.09
50	40±16	21±8	1.78±0.48
100	35±17	15±6	2.40±0.96
1	10±5	75±18	0.14±0.05
		Without arterial occlusion	
1	71±15	81±22	0.96±0.24
10	93±31	90±18	1.03±0.36
20	66±7	91±11	0.73±0.11
50	76±5	68±19	1.19±0.34
100	72±7	42±9	1.59±0.44
1	49±9	73±19	0.67±0.16

Mean ± 1 S.D., n=9

declining with continued activity. Post-tetanic twitch force declined immediately and demonstrated the greatest fall in force.

The influence of stimulation frequency on force generation during activity is expressed by the frequency:force curves of Figure 3.7. These demonstrate a shift in the force:frequency curve to the left at low frequencies early on during activity, reflecting low-frequency force potentiation, but by the 10th PSEM the curve is moved to the right. Progressive high frequency fatigue results in the upper segment of the curve shifting downwards.

Changes in CMAP

The decline in force was not associated with an equivalent reduction in CMAP amplitude (Table 3.5, Figure 3.6). In contrast to force, the final CMAP had declined more at high frequencies than at low frequencies of stimulation. Moreover, at low stimulation frequencies (1, 10 & 20Hz), CMAP amplitude initially increased by a small degree before declining (Figure 3.6a-c). The decline in CMAP amplitude was not immediate, but was dependent on stimulation frequency. Thus, for the twitch, CMAP amplitude did not decline until the 10th contraction, whereas for 10Hz, 20Hz, 50Hz and 100Hz, CMAP amplitude declined after the 9th, 9th, 5th and 1st PSEM respectively.

At the highest frequencies alternate action potential amplitudes appeared to become reduced, which became more marked as fatigue progressed. Unfortunately, it was not possible to document this since it was not possible to capture and store data of this form using the experimental design and equipment available.

The duration of the action potential from stimulus artifact to peak negative phase of depolarization of the pre-PSEM twitch increased linearly and proportionally to contraction number (Figure 3.15a). The individual records for each subject are shown since, for technical reasons, it was only possible to obtain data for five subjects. The absolute increases at the end of activity are shown in Table 3.6.

Table 3.6 Change in CMAP duration of the twitch at the end of fatiguing activity with and without arterial occlusion. Duration measured from stimulus artifact to peak of the negative phase of depolarization. Data for each subject shown.

Subject	**Control (fresh muscle) msec**	**End activity (fatigued) msec**	**Difference msec**
	With arterial occlusion		
N.P.	5.48	6.78	1.30
A.W.	5.97	7.81	1.84
J.C.	5.29	6.21	0.92
P.S.	6.46	7.57	1.11
M.J.	5.54	7.14	1.16
Mean $\pm$ 1SD	5.75 $\pm$ 0.47	7.10 $\pm$ 0.63	1.27 $\pm$ 0.35
	Without arterial occlusion		
M.J.	5.29	5.60	0.31
H.G.	6.15	6.52	0.37

Table 3.7 Absolute values of MRR at various frequencies in fresh muscle (% force loss per 10 msec).

Subject	**10Hz**	**20Hz**	**50Hz**	**100Hz**
M.S.	10.8	13.4	12.4	11.3
A.W.	10.0	14.6	14.0	11.6
H.G.	8.2	11.5	13.5	10.9
B.C.	11.1	12.6	11.8	10.2
J.C.	9.6	11.5	12.3	11.2
Mean (S.D.)	9.9(1.1)	12.7(1.3)	12.8(0.9)	11.0(0.5)

Frequency dependence of the relationship between force and excitation

Changes in the ratio F/E may result from changes in either force or excitation (measured as the amplitude of the CMAP). The time course of changes in mean values of F/E are shown in Figure 3.8. During activity with high frequency stimulation F/E ratio was > 1 due to greater excitation failure in excess of force failure, whereas at low frequencies F/E < 1 as force declined in excess of excitation. However, an initial increase in F/E to > 1 was observed for the pre-twitch and 10Hz tetanic stimulation which subsequently declined to <1 with continued activity. This was probably due to the increase in force at these frequencies.

The dependence of force on the absolute values of CMAP amplitude at various frequencies of stimulation is shown for one subject in Figure 3.9a. This figure clearly demonstrates that at high frequencies of stimulation force generation is well maintained despite a marked loss in CMAP amplitude. In contrast, at low frequencies there is marked force loss with only a small reduction in CMAP amplitude.

Influence of frequency on relaxation rate

Due to the design of the PSEM, the MRR could only be determined from the end of the 100 Hz tetani. The time course of changes in MRR has been plotted as a dashed line on each panel of Figure 3.6. MRR declined more rapidly than either force or CMAP amplitude to 13±5% of its fresh value by the 7th PSEM after which the reduction in MRR was reduced.

Absolute values of MRR in fresh muscle were dependent on frequency of stimulation (Table 3.7). However, the changes in the relative rates of slowing during fatiguing activity and recovery were similar at all frequencies and are shown in Figure 3.10. The frequency dependence of force loss may therefore not be attributed to changes in MRR.

Aerobic recovery

Reperfusion of the muscle resulted in recovery of MRR, CMAP amplitude and force (Figure 3.6) at all frequencies. Recovery of force was almost complete by 2 minutes at all frequencies. However, twitch and mean 10Hz tetanic force first

potentiated, peaking after 2-3 minutes, and then declined to levels less than that obtained for fresh muscle.

Recovery of CMAP amplitude was dependent on stimulation. At low frequencies (1, 10 & 20Hz) recovery was almost complete within 1 minute whereas at higher frequencies recovery was more prolonged, being complete after 5 and 10 minutes for 50Hz and 100Hz tetani respectively. CMAP duration increased with a slow time course in an exponential fashion similar to that of MRR.

Recovery of MRR appeared independent of stimulation frequency (Figure 3.10). MRR recovered in an exponential fashion and at a slower rate than either force or CMAP amplitude, except at 100Hz. Recovery of MRR was complete by 10 minutes.

The dependence of force on CMAP amplitude followed a similar pattern to that obtained during fatiguing activity (Figure 3.9b).

2. Activity without arterial occlusion

Influence of frequency on force generation

The time course of the decline in force during activity without arterial occlusion at each stimulation frequency is shown in Figure 3.11. Force loss occured at all stimulation frequencies except for 10Hz and showed a similar frequency dependence to that seen during occluded activity. However, the changes in force were clearly less marked compared to occluded activity (Table 3.5). Consequently, the frequency:force curve was shifted to the left at lower frequencies with only a small depression of high frequency force (Figure 3.12).

Changes in CMAP

Changes in CMAP amplitude contrasted with that of force. CMAP amplitude declined more at high than at low frequencies (Table 3.5, Figure 3.11). The greater part of the decline in CMAP amplitude at high frequencies (50 and 100Hz) occured in the first 20 contractions after which the CMAP amplitude plateaued. The CMAP duration was only measured in two individuals for technical reasons. Figure 3.15b shows that by the 16th contraction, CMAP duration had increased by 5.7% and 7.5%

respectively, which thereafter plateaued. The absolute values of CMAP duration in fresh and fatigued muscle are shown in Table 3.6.

Frequency dependence of the relationship between force and excitation

The time courses of the changes in the F/E ratio are shown in Figure 3.13. At low stimulation frequencies (pre-tetanic twitch and 10Hz) the F/E ratio initially increased to > 1 presumably due to potentation of force, which then declined as activity proceeded. The F/E ratio increased at the higher frequencies (50 and 100Hz). In contrast, post-tetanic twitch and 20Hz values of F/E decreased to < 1 during activity.

The dependence of force on the absolute values of CMAP amplitude at various frequencies is shown for one subject in Figure 3.14. Similar changes were apparent to that observed for ischaemic activity in the same subject (Figure 3.9). Thus, force was well maintained at high frequency despite a marked reduction in CMAP amplitude, whereas at low stimulation frequency force declined markedly for only a small decrement in CMAP amplitude.

Changes in relaxation rate

MRR (from 100Hz tetani) initially declined to 49.0 $\pm$ 5.8% after 26 PSEMs and then increased slightly to 54.0 $\pm$ 5.2% of initial unfatigued control values by the end of fatiguing activity (Figure 3.11).

Aerobic recovery

At low stimulation frequencies (1, 10 & 20Hz), force did not recover. At high stimulation frequencies (50 and 100Hz) recovery of force was immediate, but incomplete by 15 minutes. CMAP amplitude had completely recovered after 5 minutes at the higher frequencies and duration by 2 minutes. Consequently the F/E ratio was < 1 at all frequencies by the end of the 15 minute recovery period.

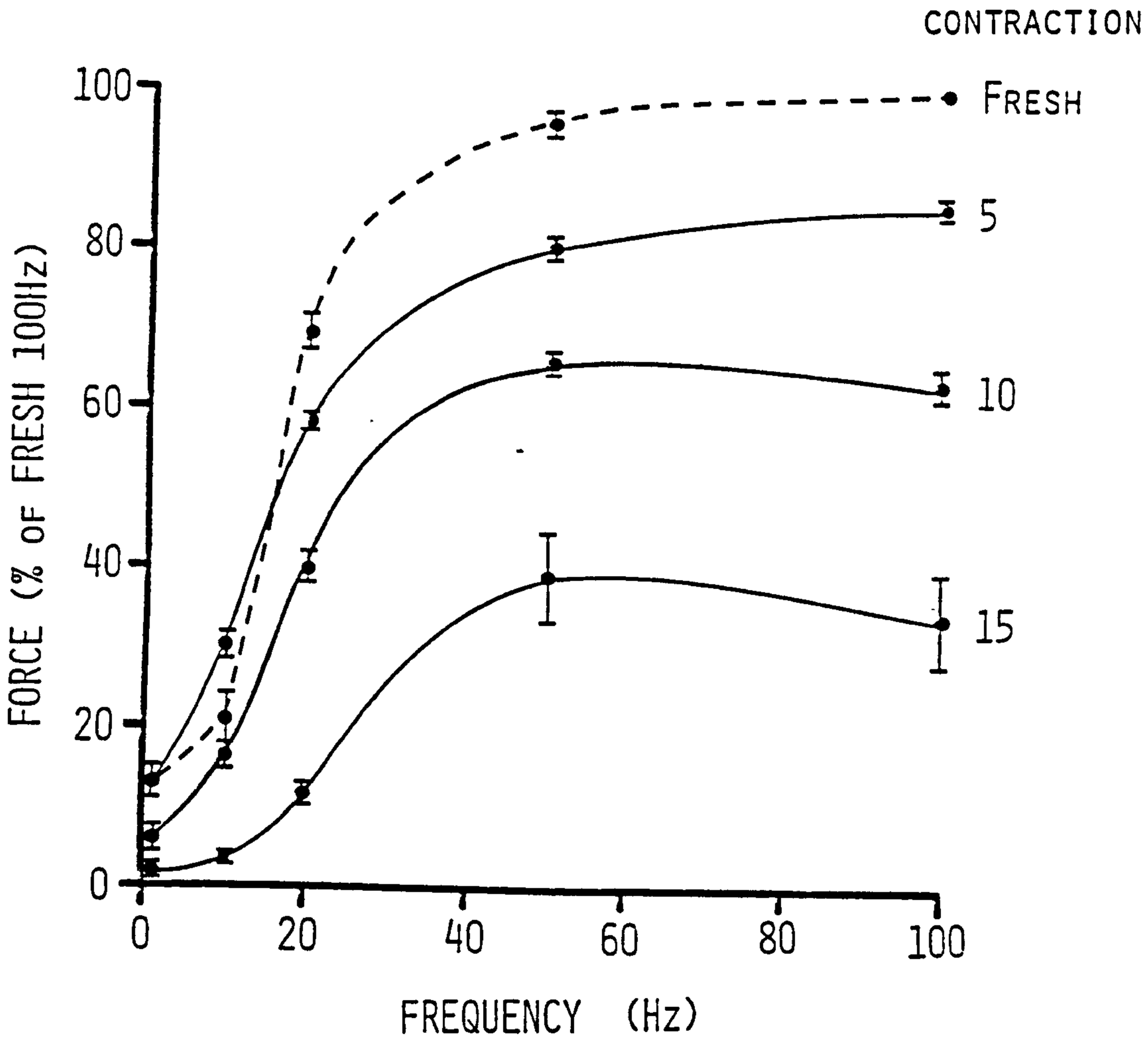
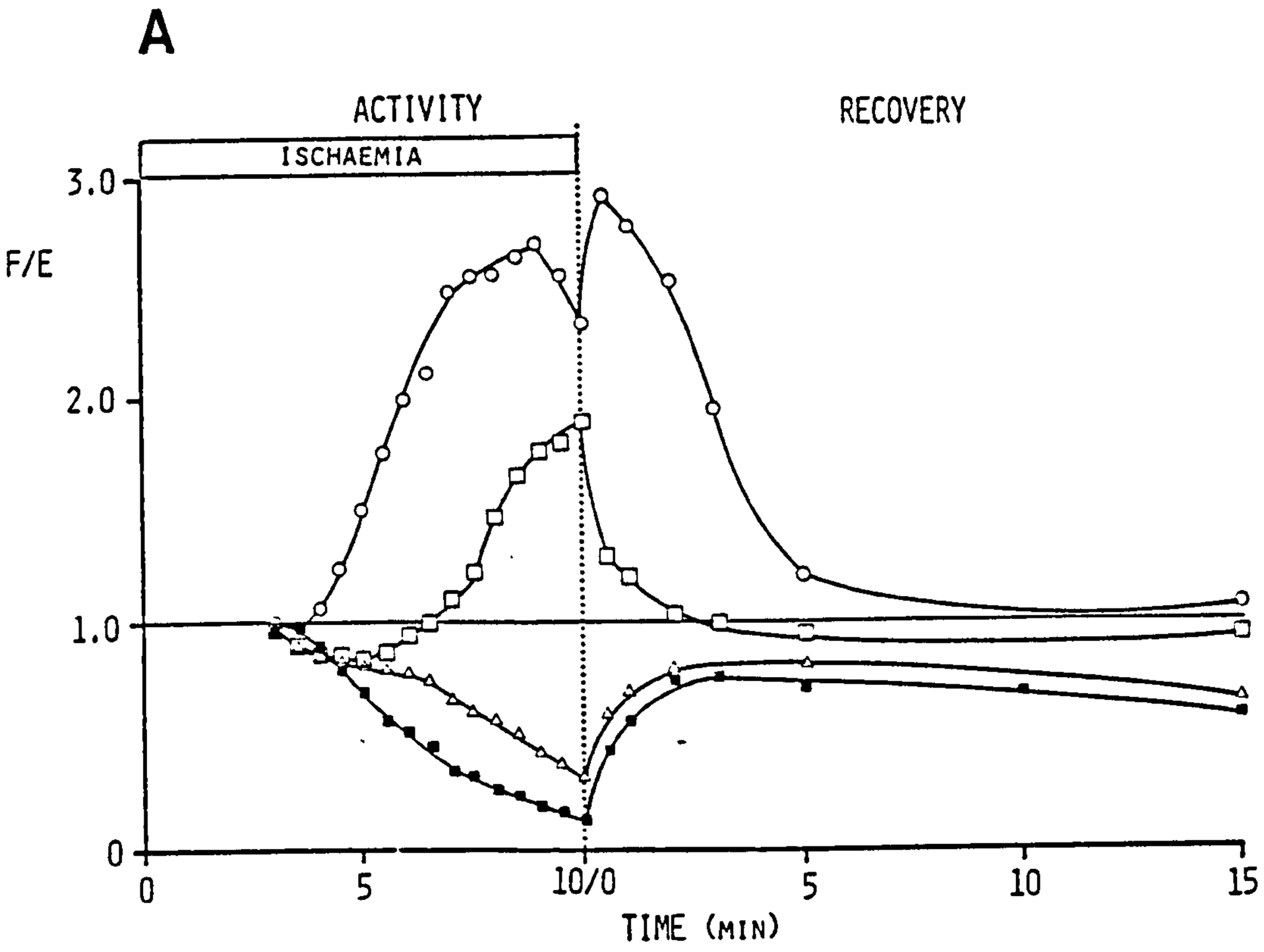


Figure 3.7 Frequency:force curves during ischaemic fatiguing activity. Potentiation at low frequency moves curve to left by 5th PSEM; subsequent low-frequency fatigue moves curve to right by 10th PSEM. Progressive high-frequency fatigue moves upper segment of curve progressively downwards.

Figure 3.8 Mean F/E ratios, during activity with arterial occlusion and recovery with intact circulation. A) during activity, the F/E ratio is > 1 at high frequency due to excitation failure in excess of force failure but is < 1 at low frequencies, due to force failure in excess of excitation failure. B) during activity at 1Hz pre-twitch and 10Hz, however, the F/E ratio is < 1 due to potentiation of force, and then becomes < 1 due to low-frequency fatigue. Similar potentiation occurs during recovery but there is eventual low-frequency fatigue. Symbols: (▲) pre-tetanic twitch, (●) 10Hz, (△) 20Hz, (□) 50Hz, (○) 100Hz, (■) post-tetanic twitch.


A
ACTIVITY
RECOVERY
ISCHAEMIA
3.0
F/E
2.0
1.0
0
0
5
10/0
5
10
15
TIME (MIN)

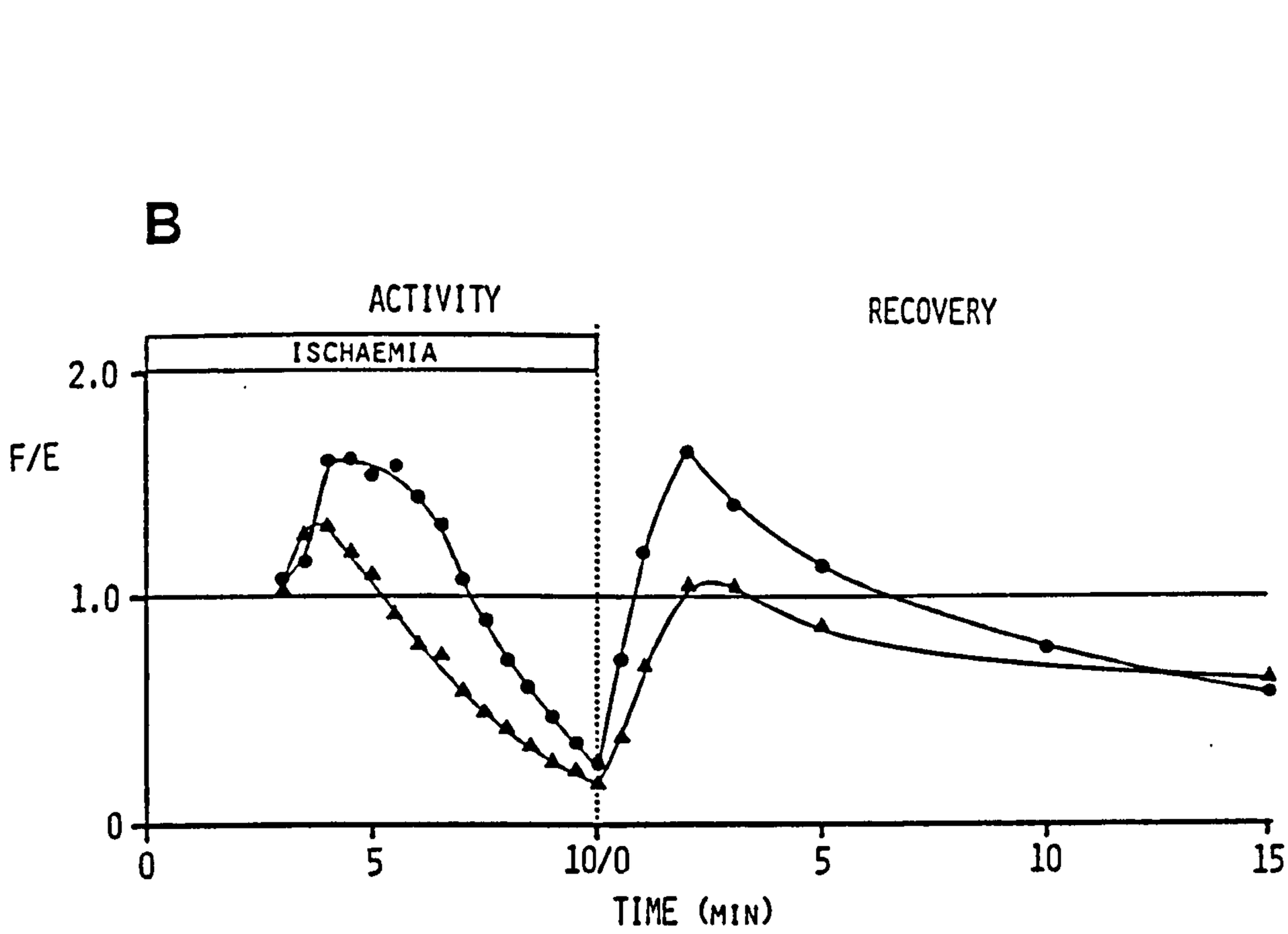
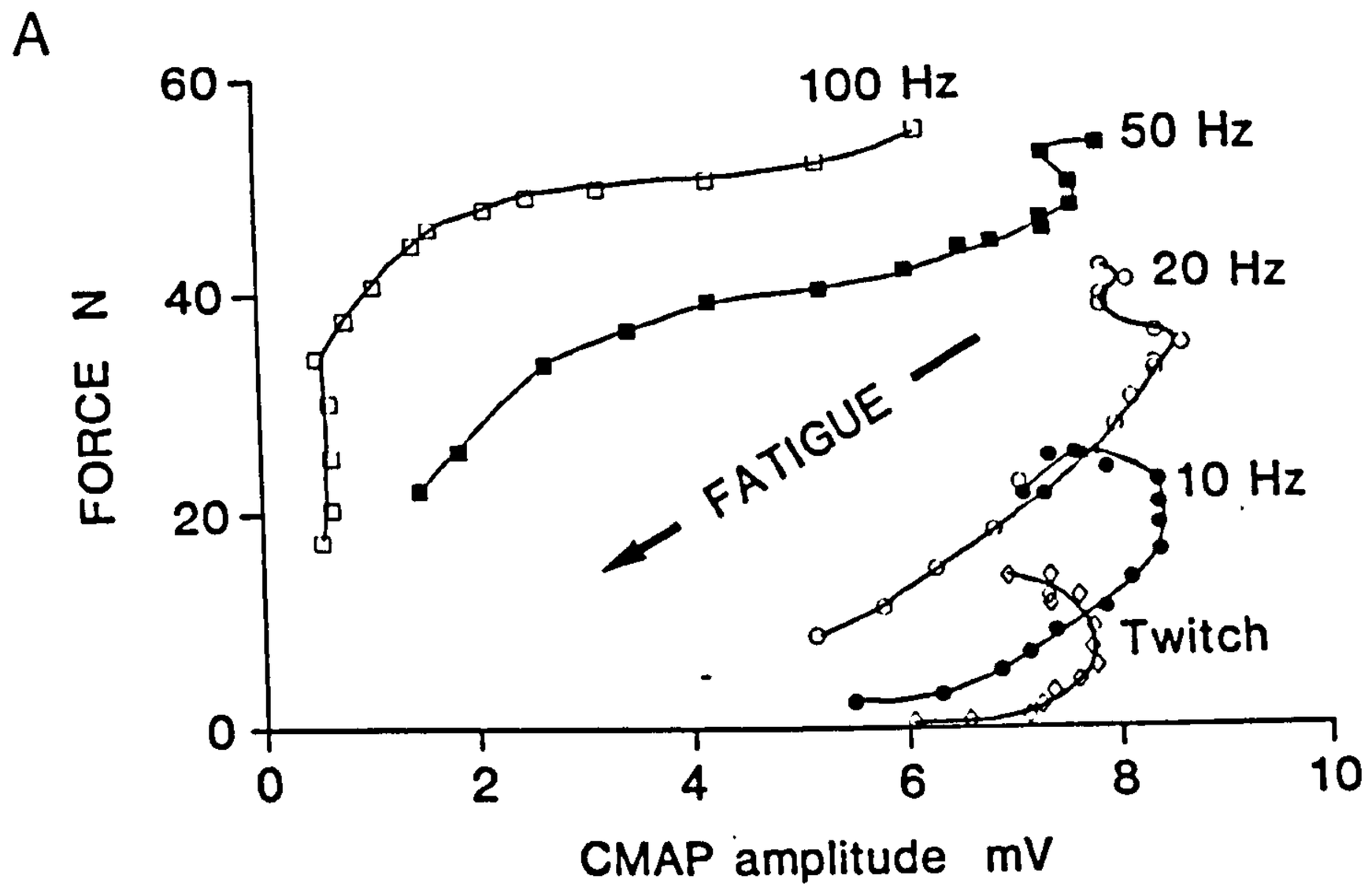
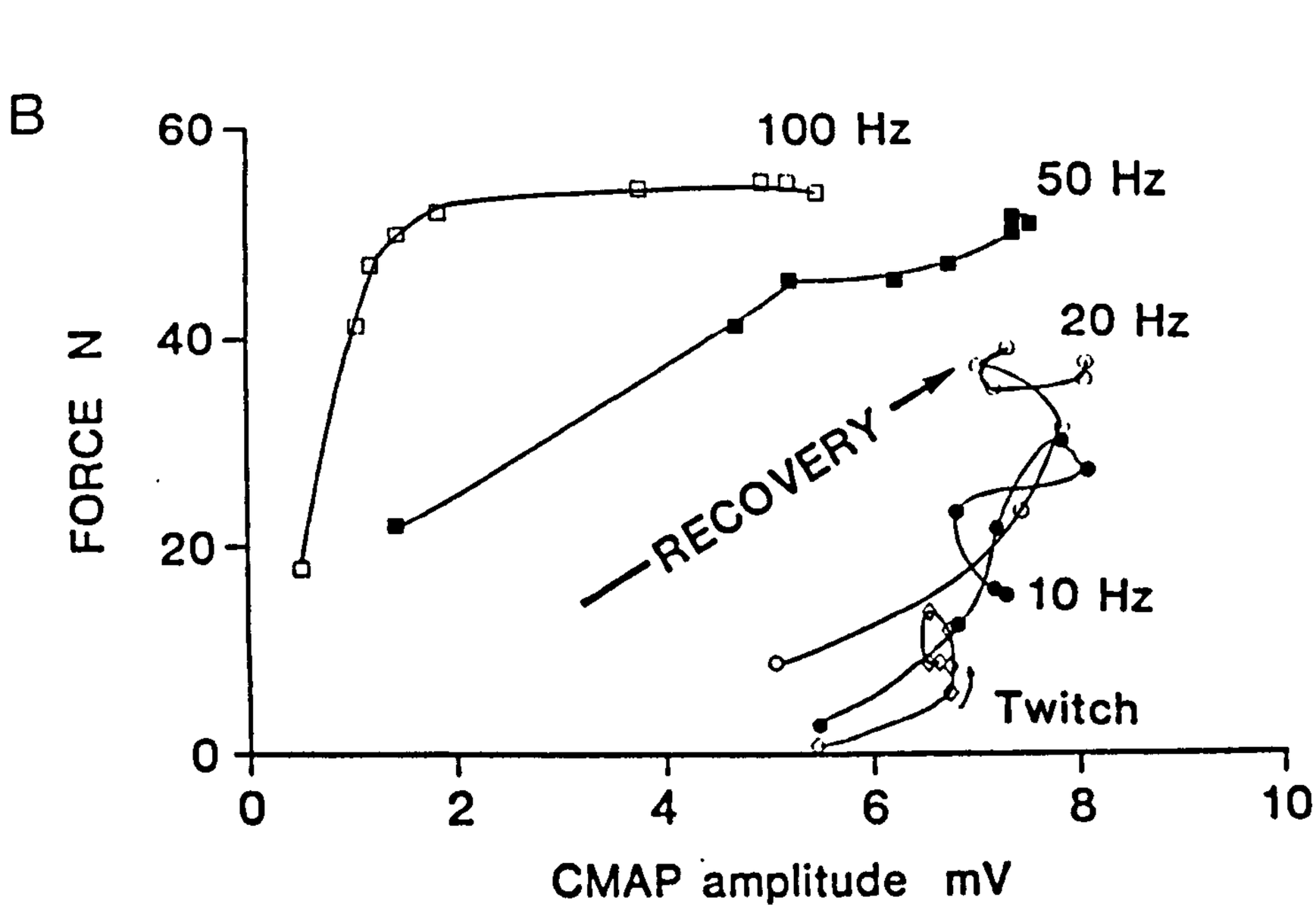

B
ACTIVITY
RECOVERY
ISCHAEMIA
2.0
F/E
1.0
0
0
5
10/0
5
10
15
TIME (MIN)

Figure 3.9 Dependence of force generation of CMAP amplitude at various frequencies during A) activity with arterial occlusion and B) non-occluded recovery shown for 1 subject (subject B.C.). At the highest frequencies (50 and 100Hz) a safety-factor is apparent within which action potential amplitude declines markedly with little effect on force generation. At lower frequencies force declines without loss in CMAP amplitude.


A
60
40
20
0
FORCE N
100 Hz
50 Hz
20 Hz
10 Hz
Twitch
FATIGUE
0
2
4
6
8
10
CMAP amplitude mV


B
60
40
20
0
FORCE N
100 Hz
50 Hz
20 Hz
10 Hz
Twitch
RECOVERY
0
2
4
6
8
10
CMAP amplitude mV

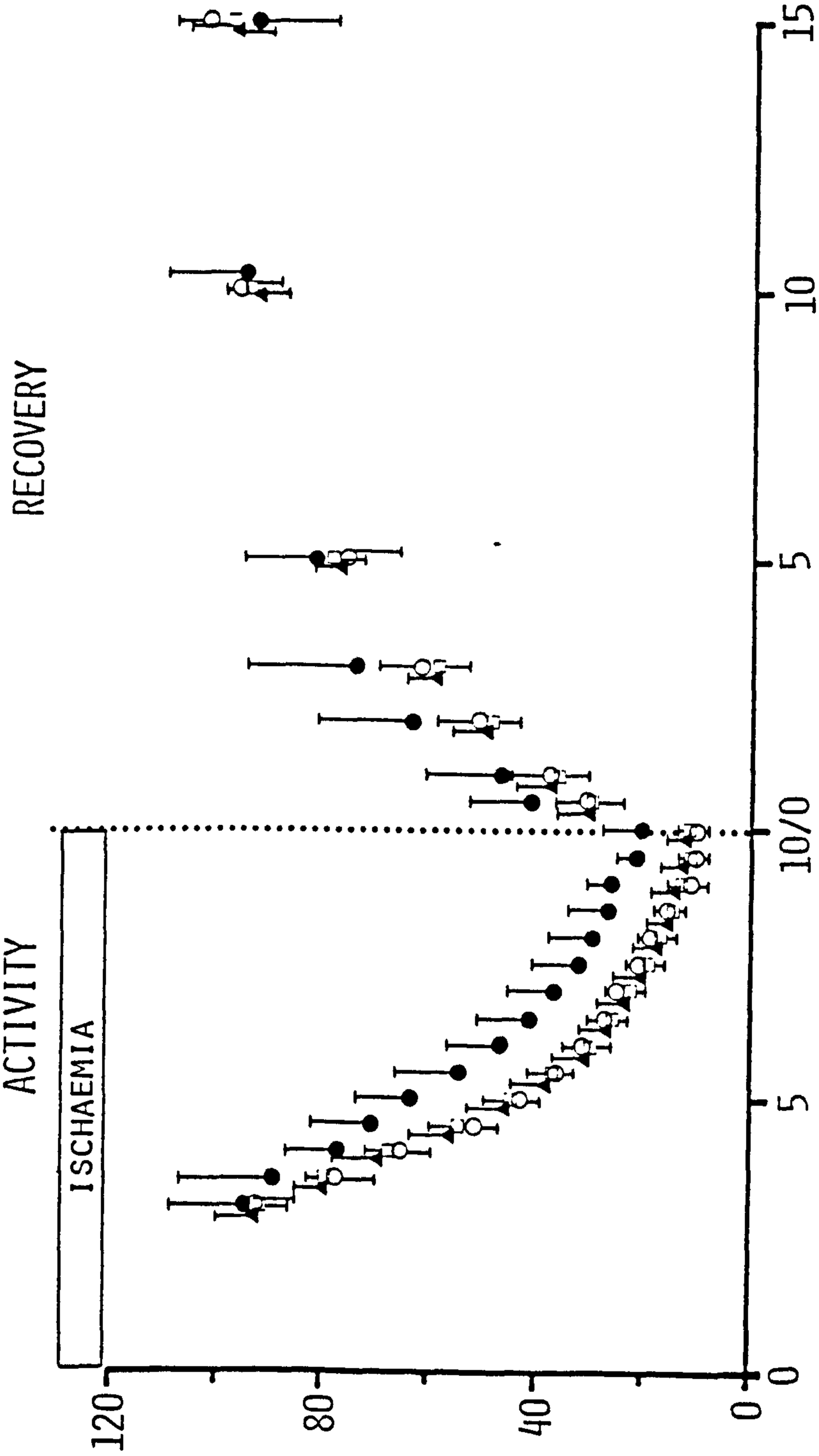
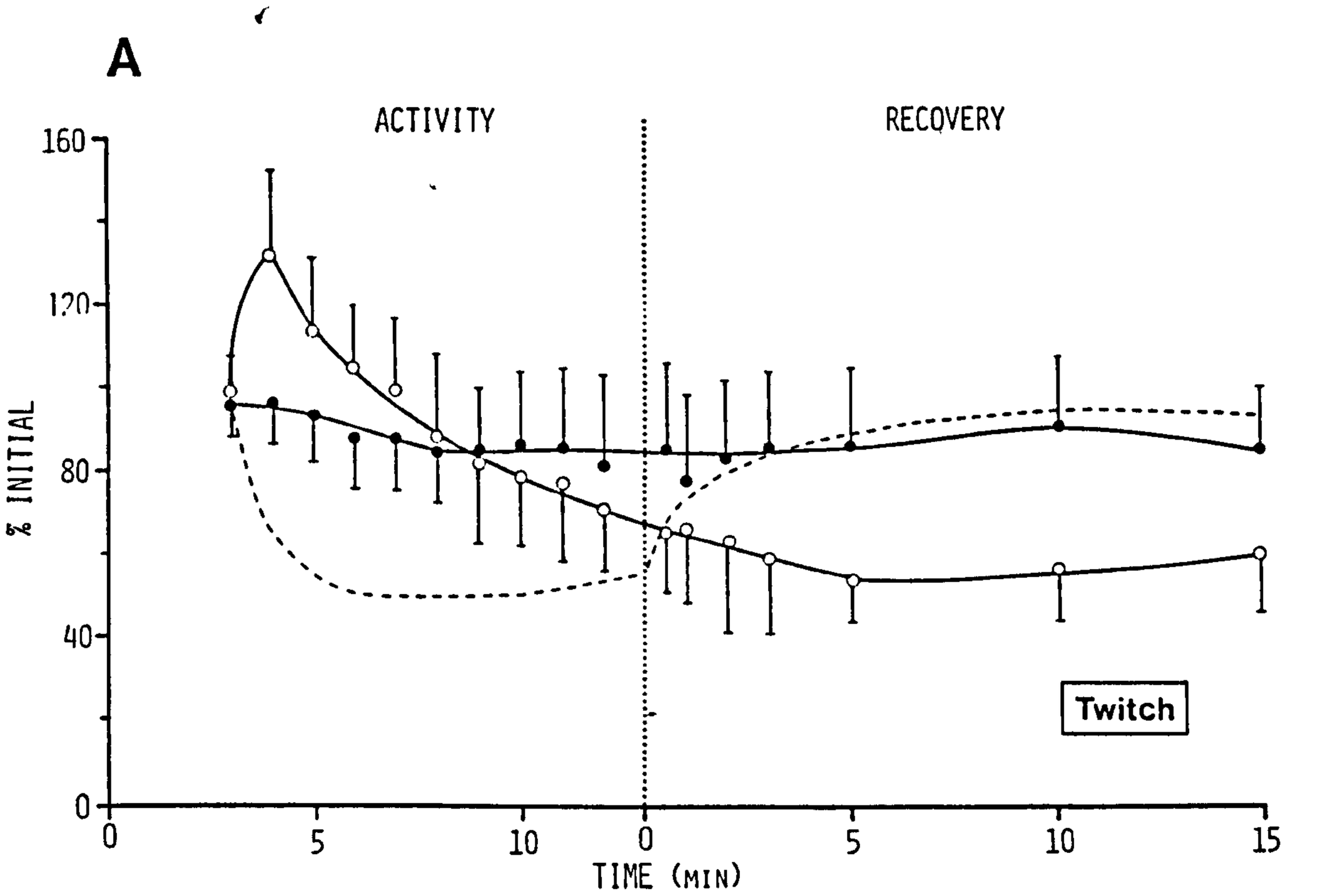
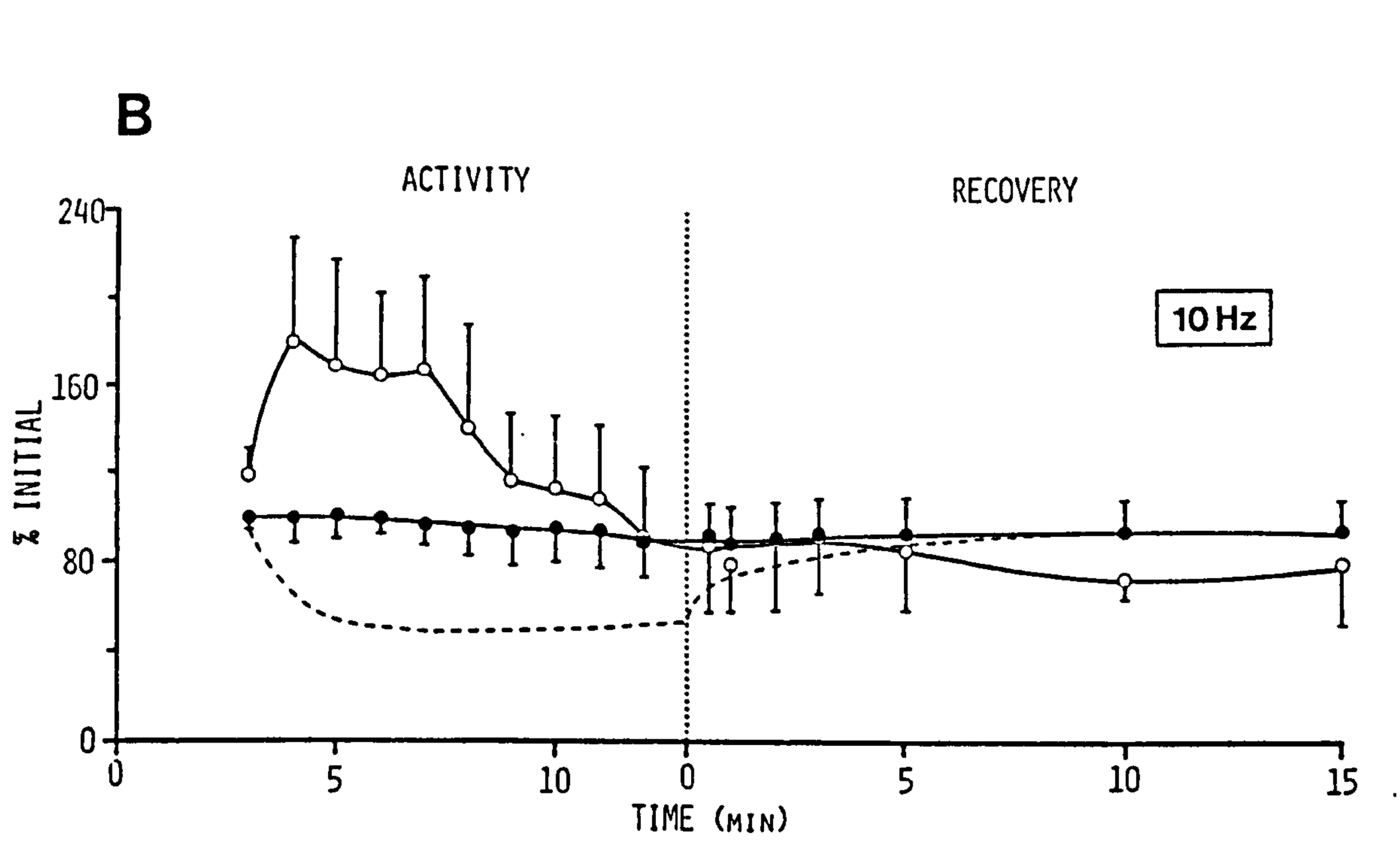


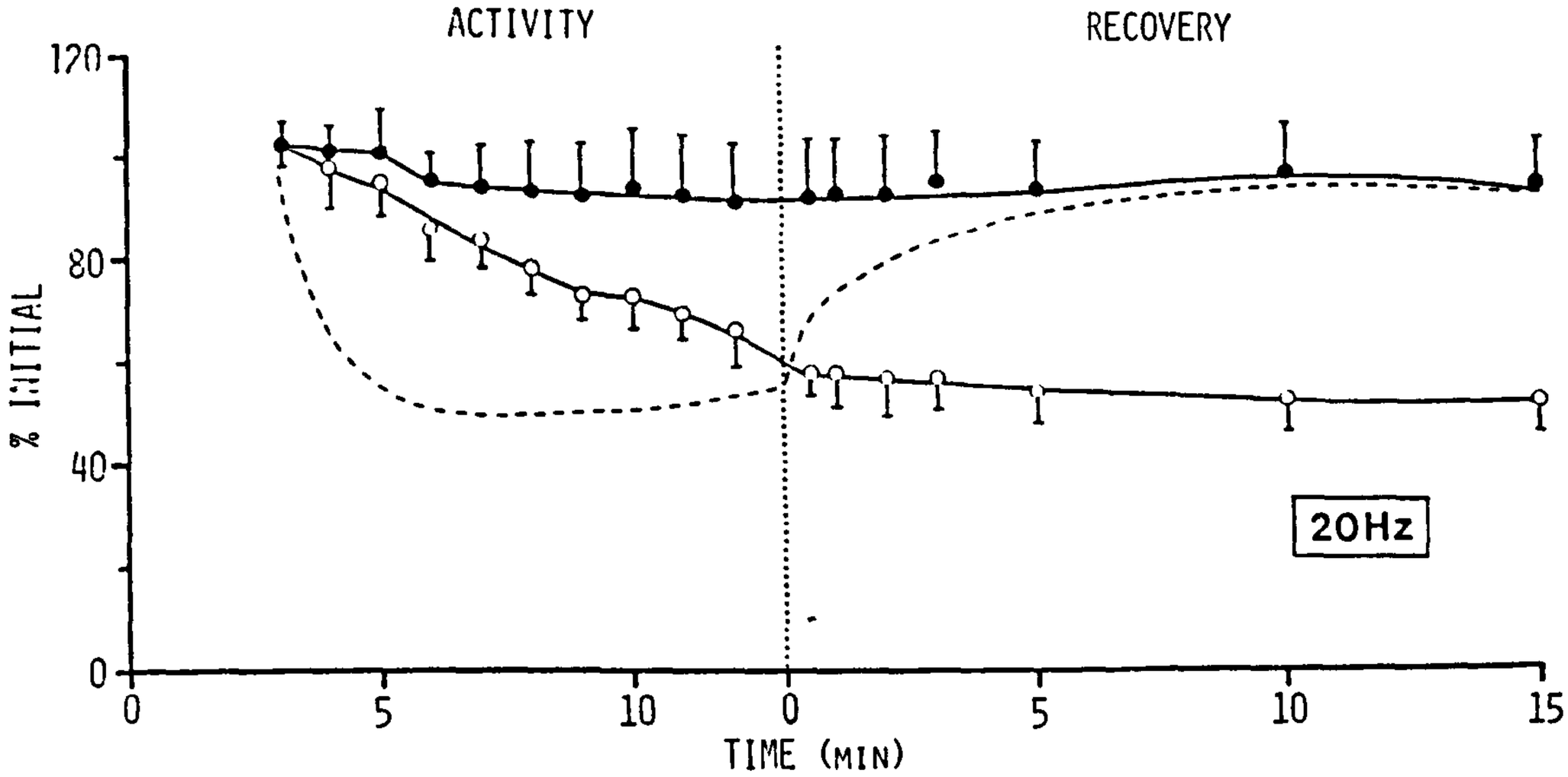
Figure 3.10 Declines in MRR at various frequencies during modified PSEMs. Each PSEM had a 0.5 second gap placed after the test frequency to allow relaxation of force to baseline. PSEMs were delivered at intervals of 12s with arterial occlusion and recovery followed with intact circulation.
Symbols: (●) 10Hz, (▲) 20Hz, (□) 50Hz, (○) 100Hz.

Figure 3.11 Time courses of change in force (o) and CMAP amplitude (●) at various frequencies without circulatory occlusion (fifty PSEMs at intervals of 12 s) and recovery without occlusion: A) twitch, B) 10Hz, C) 20Hz, D) 50Hz, E) 100Hz and F) post-tetanic twitch. Mean changes in MRR at 100Hz are represented by a dotted line on each panel, except at 100Hz where mean ± 1 S.D. are shown. The 1st and every 5th subsequent PSEM were measured.

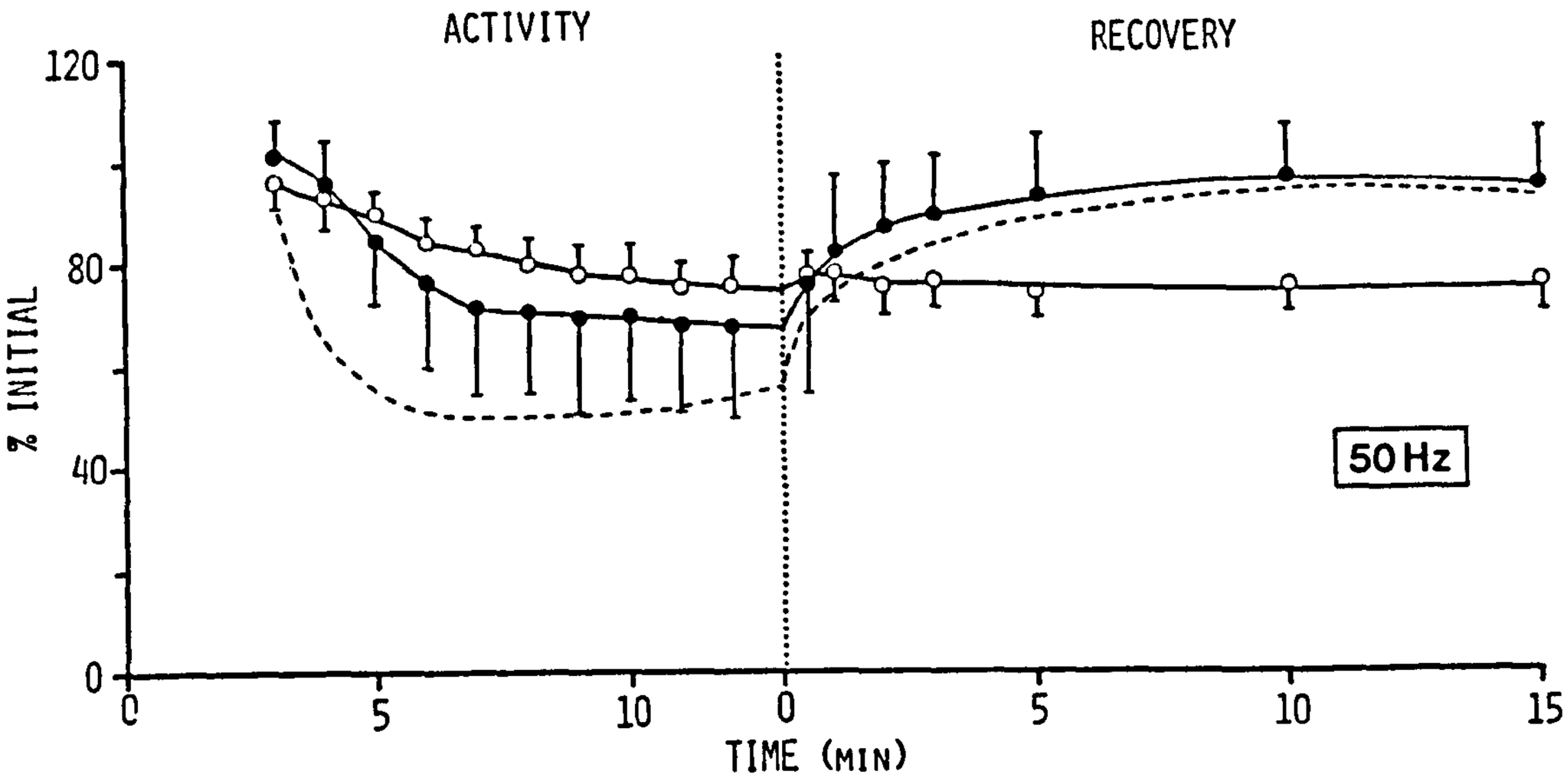

A
ACTIVITY
RECOVERY
160
120
80
40
0
% INITIAL
Twitch
0
5
10
0
5
10
15
TIME (MIN)

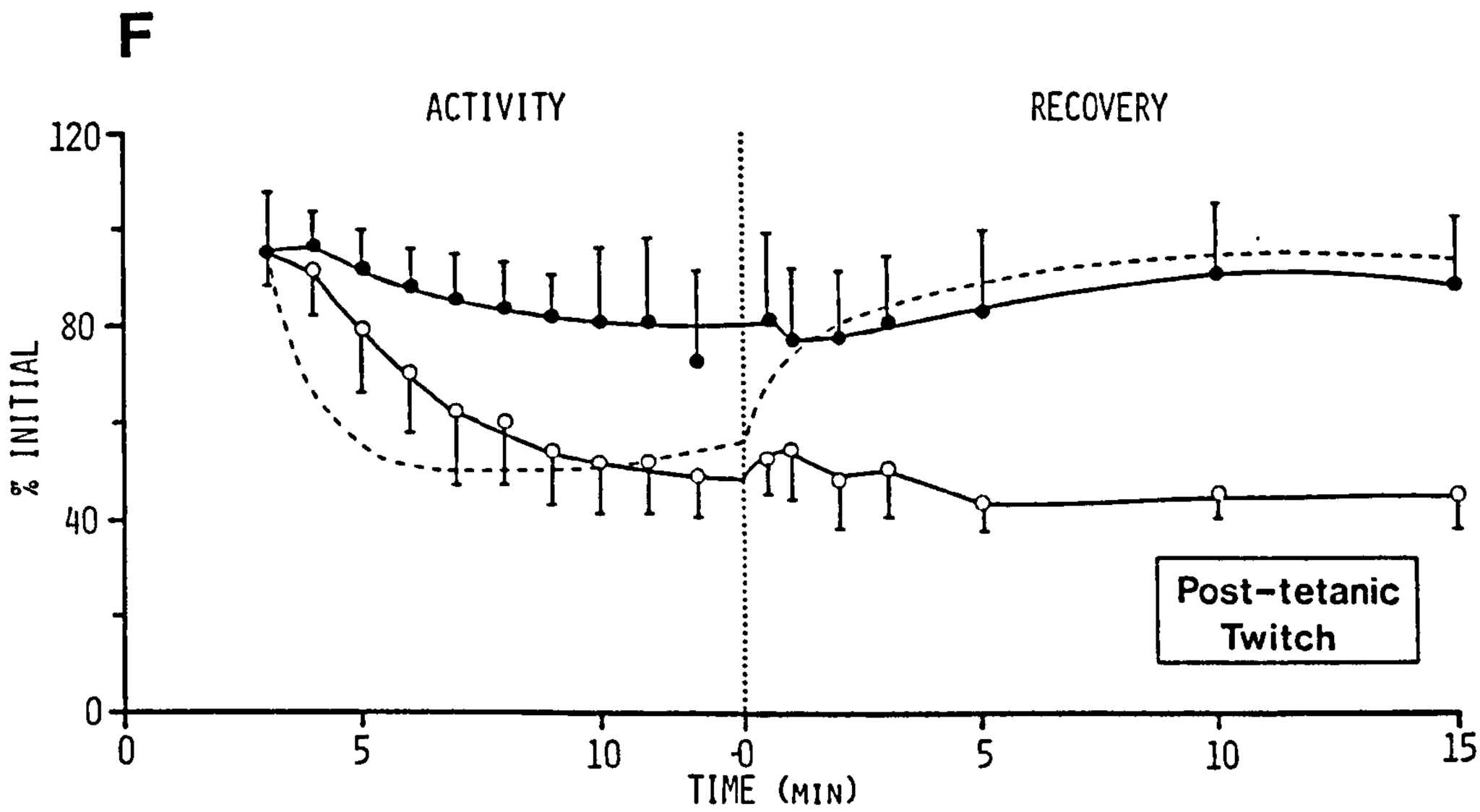

B
ACTIVITY
RECOVERY
240
160
80
0
% INITIAL
10 Hz
0
5
10
0
5
10
15
TIME (MIN)

C


ACTIVITY
RECOVERY
120
80
40
0
% INITIAL
0
5
10
0
5
10
15
TIME (MIN)
20Hz

D


ACTIVITY
RECOVERY
120
80
40
0
% INITIAL
0
5
10
0
5
10
15
TIME (MIN)
50Hz


F
ACTIVITY
RECOVERY
120
80
40
0
% INITIAL
0
5
10
-0
5
10
15
TIME (MIN)
Post-tetanic Twitch

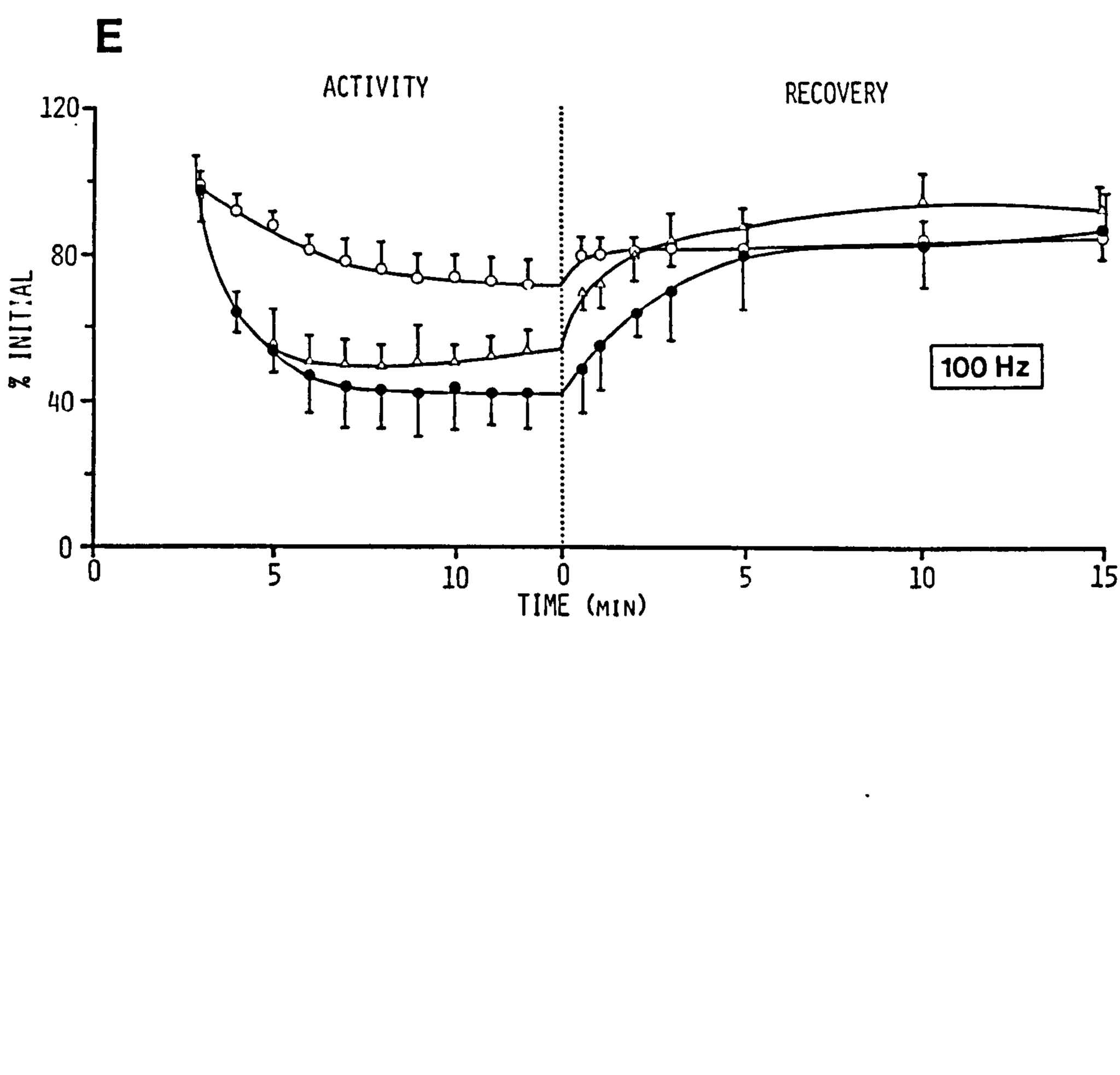

E
ACTIVITY
RECOVERY
120
80
40
0
% INITIAL
0
5
10
0
5
10
15
TIME (MIN)
100 Hz

Figure 3.12 Frequency:force curves during non-occlude fatiguing activity. Note similarity to frequency:force curves during ischaemic activity (Figure 3.7), although the changes observed are not as dramatic as seen during fatiguing activity. Potentiation at low frequency moves curve to left by 16th PSEM; subsequent low-frequency fatigue moves curve to right by 49th PSEM. Progressive high-frequency fatigue moves upper segment of curve progressively downwards.

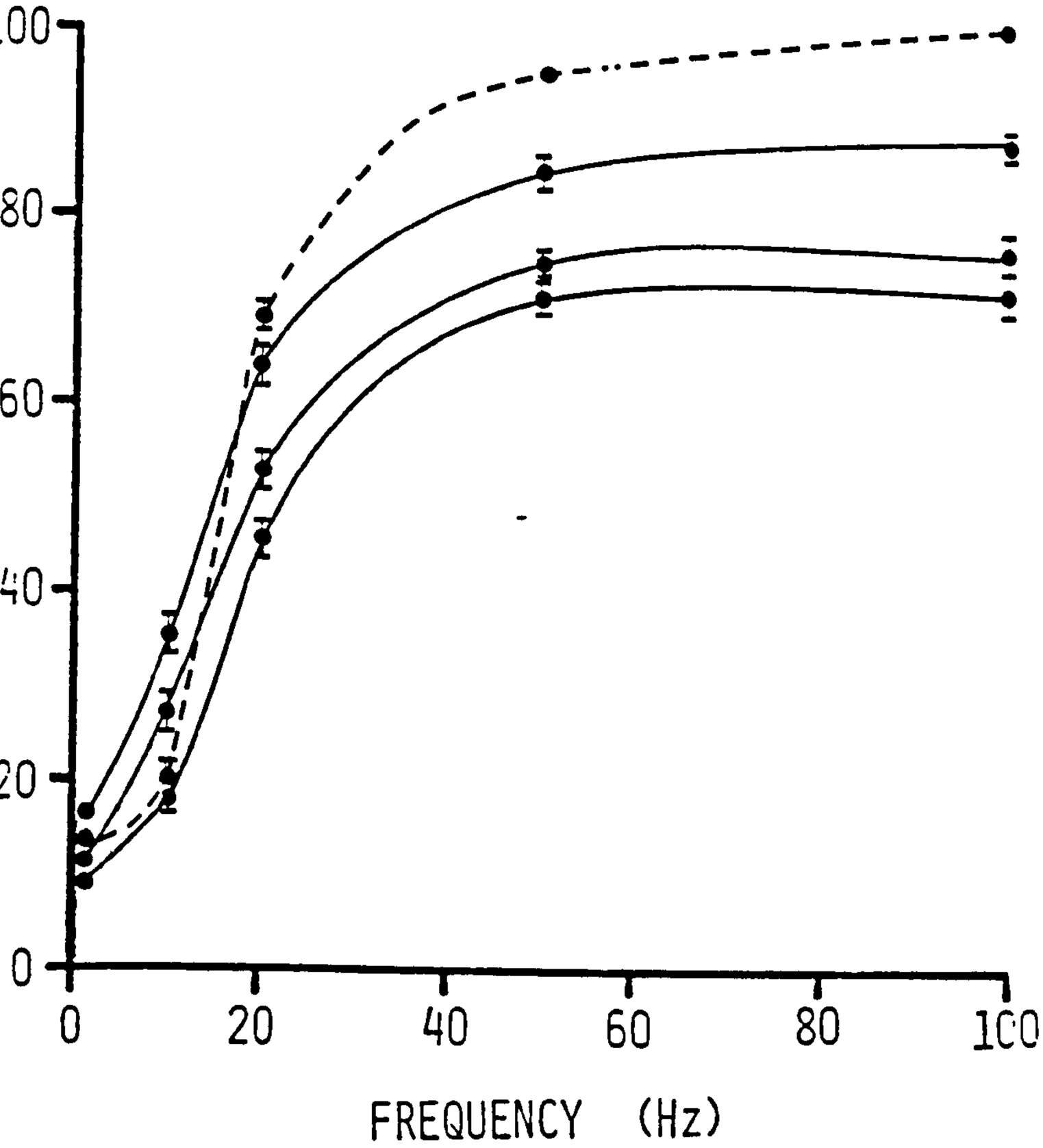
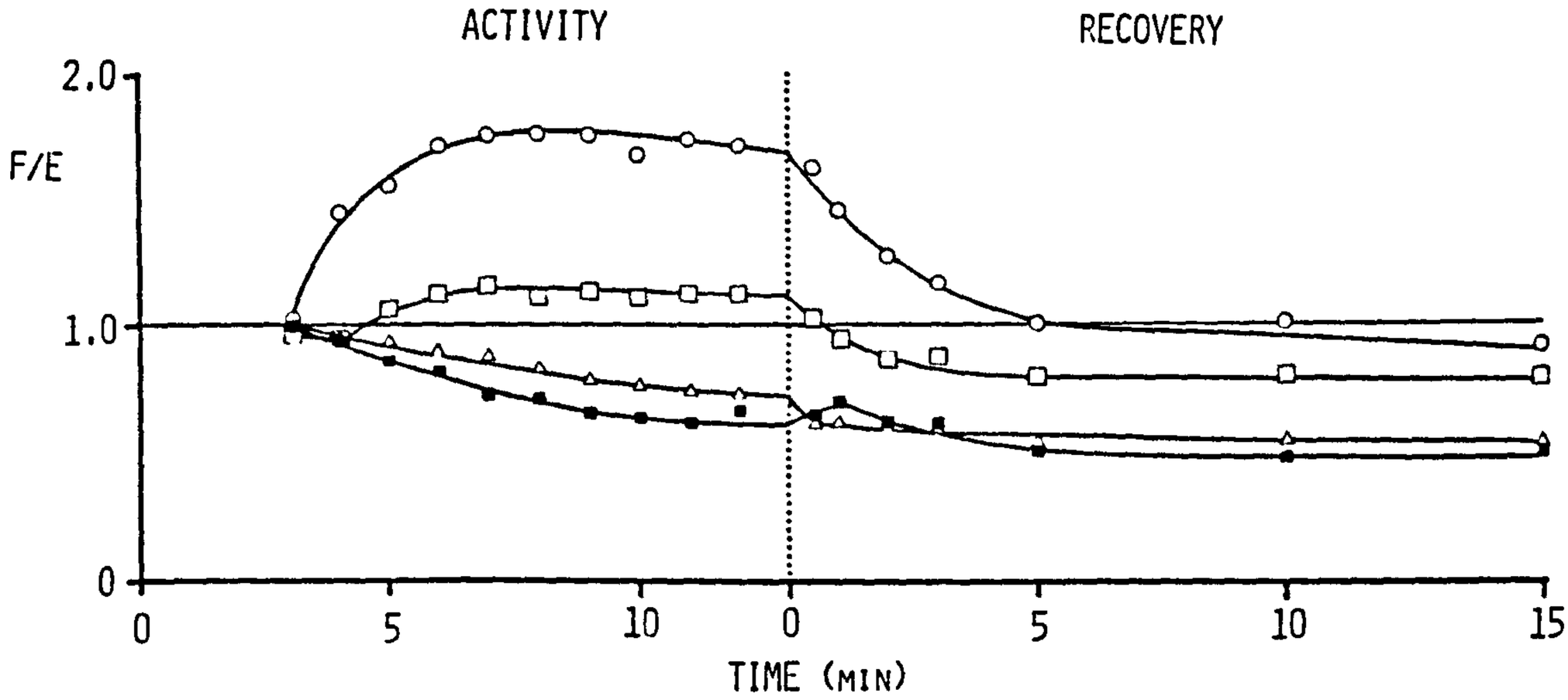

CONTRACTION
FRESH
16
31
49
FORCE (% OF FRESH 100Hz)
100
80
60
40
20
0
0
20
40
60
80
100
FREQUENCY (Hz)

Figure 3.13 Mean F/E ratios during activity and recovery without arterial occlusion. A) The F/E ratio was > 1 at high frequencies due to reduced excitation, while at some lower frequencies the ratio was < 1 due to excess force failure. Symbols: (△) 20Hz, (□) 50Hz, (○) 100Hz, (■) post-tetanic twitch. B) For the 1Hz pre-tetanic twitch (▲) and 10Hz (●), the ratio was initially > 1 due to potentiation of force which was not seen during recovery when there was force failure despite normal excitation (low-frequency fatigue).

A


ACTIVITY
RECOVERY
2.0
F/E
1.0
0
0
5
10
0
5
10
15
TIME (MIN)

B

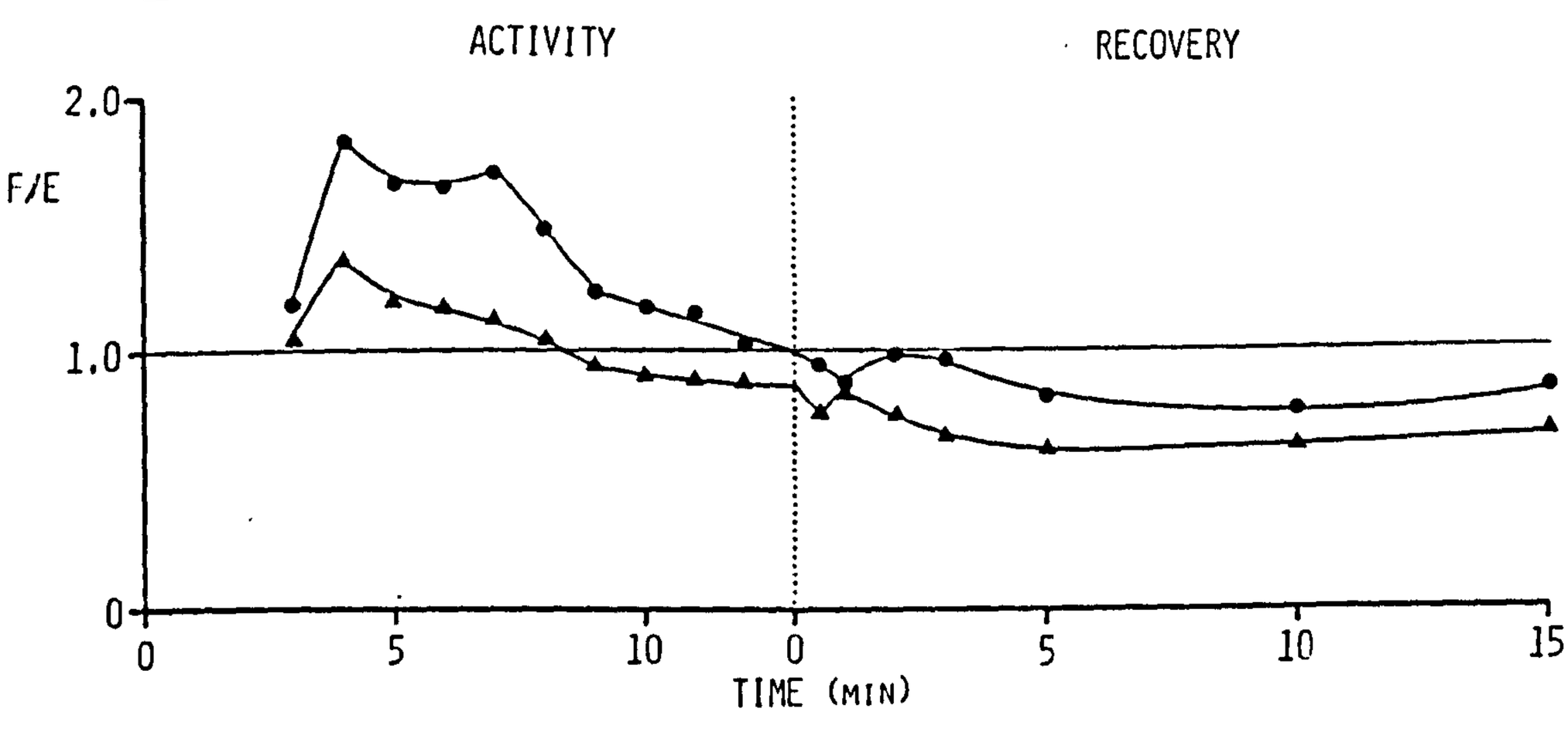
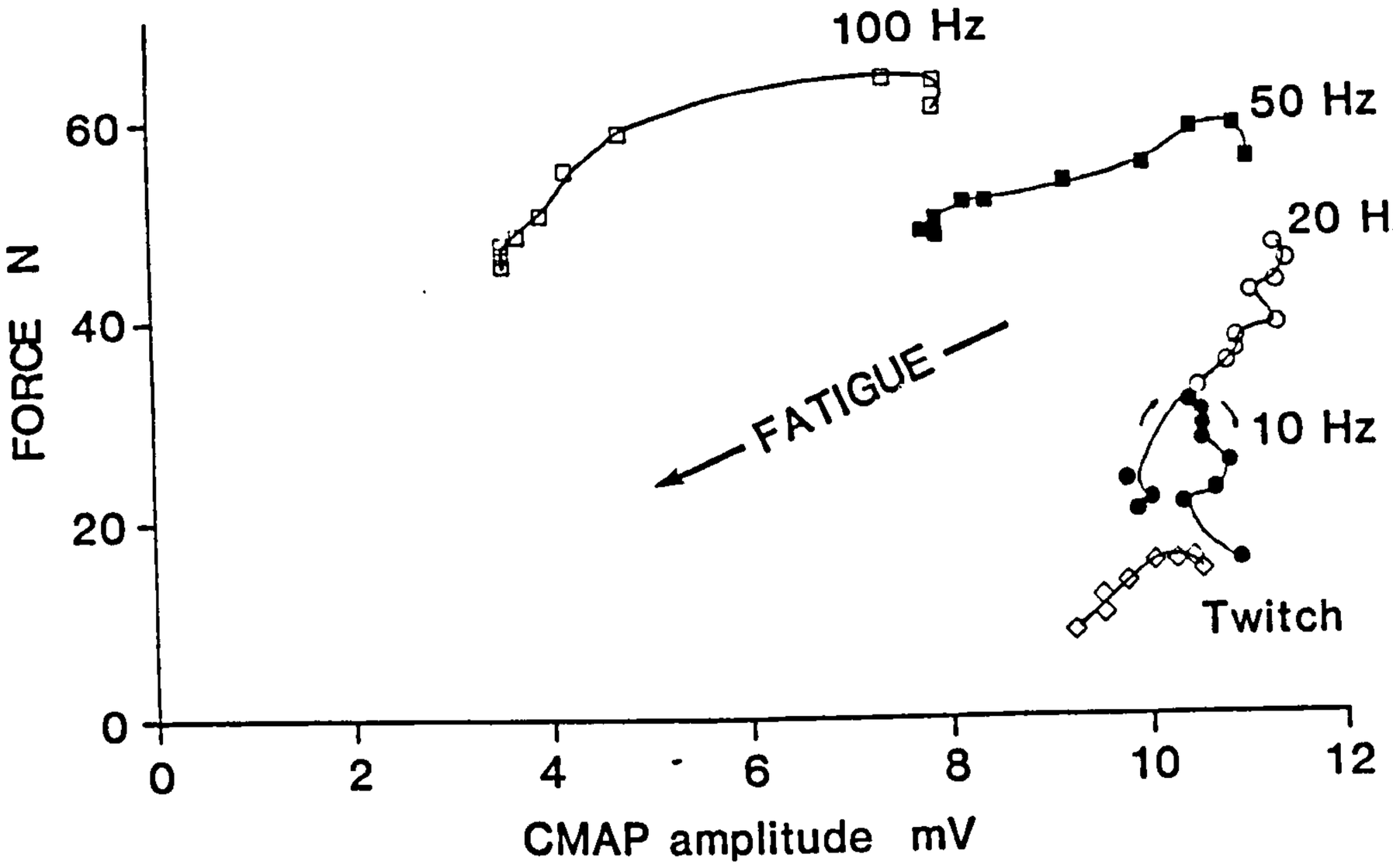

ACTIVITY
RECOVERY
2.0
F/E
1.0
0
0
5
10
0
5
10
15
TIME (MIN)

Figure 3.14 Dependence of force generation of CMAP amplitude at various frequencies during A) non-occluded fatiging activity and B) recovery shown for 1 subject (subject B.C.). Note similarity to fatiguing activity during ischaemic conditions (Figure 3.6).

A


100 Hz
50 Hz
20 Hz
10 Hz
Twitch
FATIGUE
FORCE N
60
40
20
0
0
2
4
6
8
10
12
CMAP amplitude mV

B

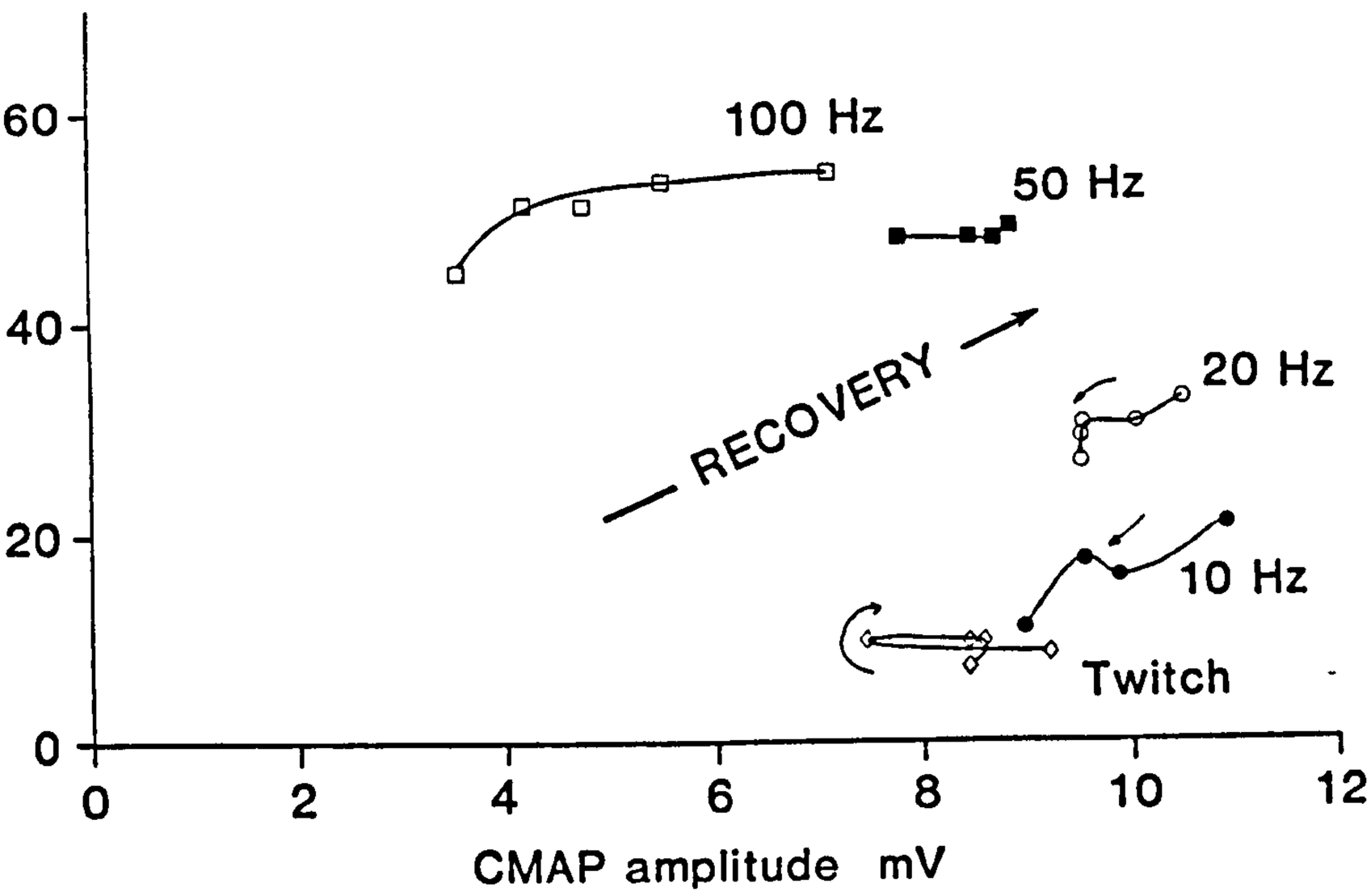
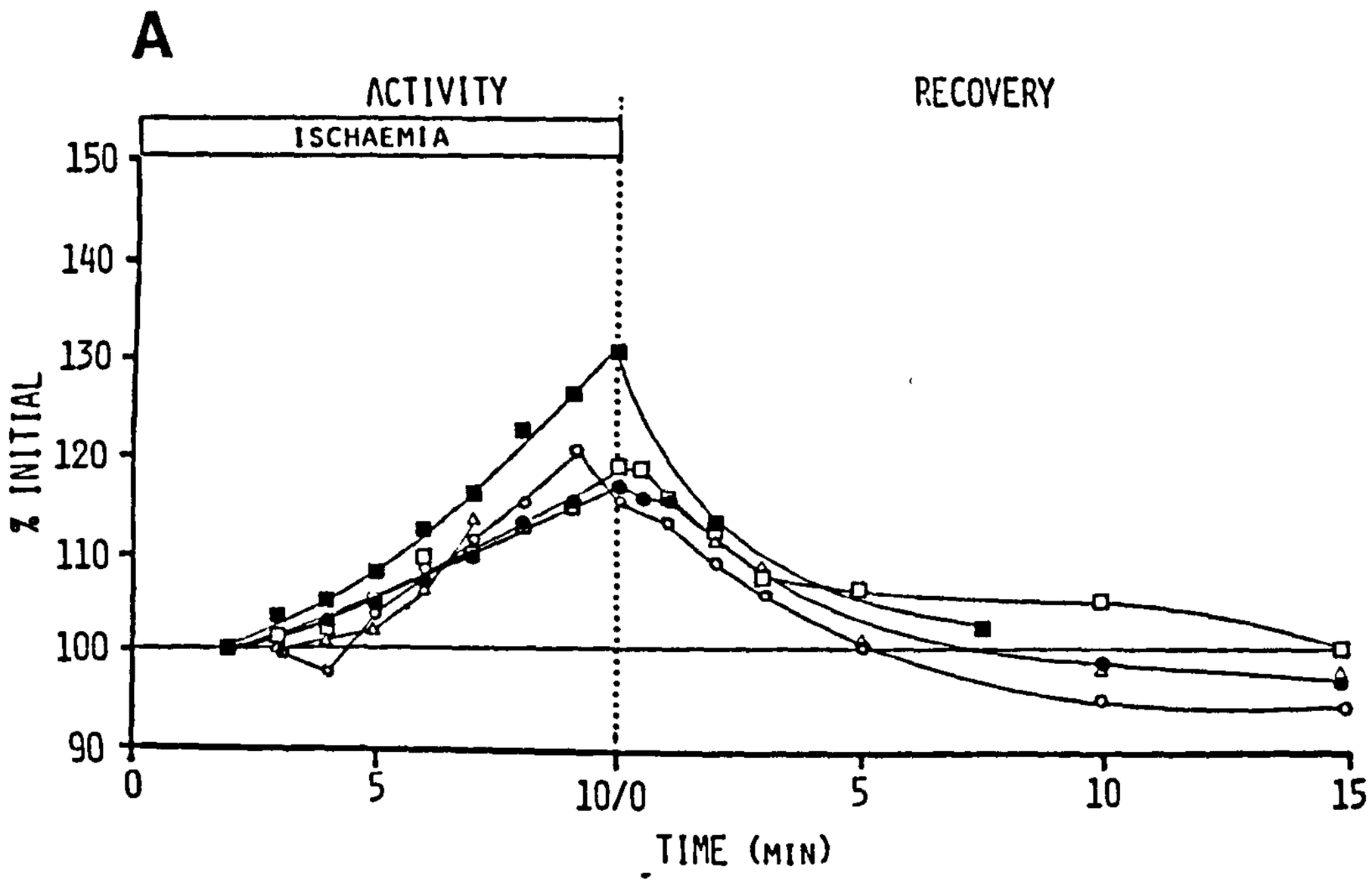
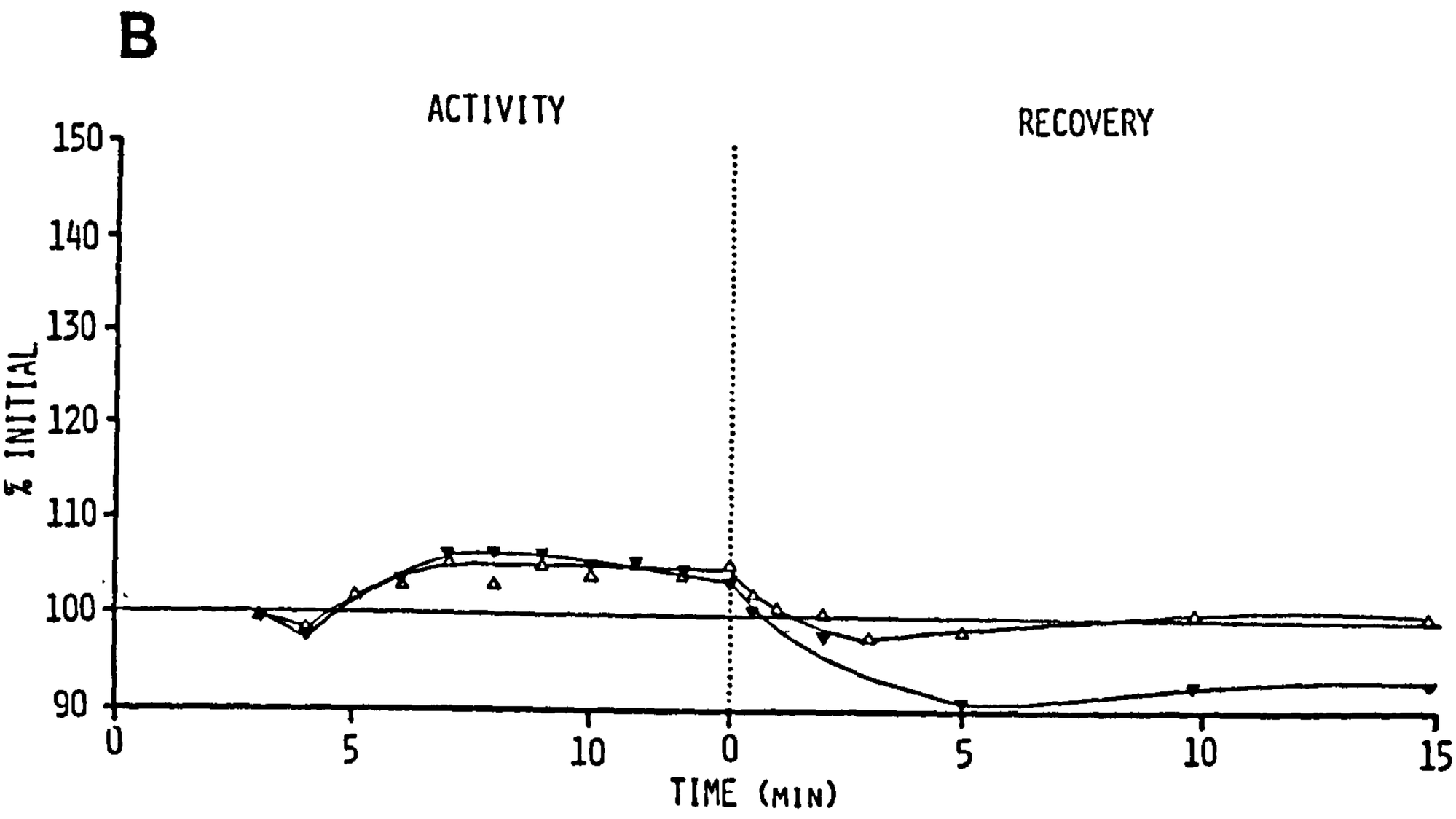

100 Hz
50 Hz
RECOVERY
20 Hz
10 Hz
Twitch
FORCE N
60
40
20
0
0
2
4
6
8
10
12
CMAP amplitude mV

Figure 3.15 Relative changes in CMAP duration of the pre-tetanic twitch of the PSEM as a percentage of control (measured from stimulus artifact to peak of the negative phase of depolarization) during A) ischaemic activity and non-occluded recovery and B) non-occluded activity and recovery. Symbols: (○) N.P., (■) A.W., (□) J.C., (●)P.S., (△) M.J., (▼)H.G.


A
ACTIVITY
RECOVERY
ISCHAEMIA
150
140
130
120
110
100
90
% INITIAL
0
5
10/0
5
10
15
TIME (MIN)


B
ACTIVITY
RECOVERY
150
140
130
120
110
100
90
% INITIAL
0
5
10
0
5
10
15
TIME (MIN)

DISCUSSION

1. Frequency dependence of force and excitation

The observations in part I of this study have shown that force and CMAP amplitude appear to decline in a similar fashion, the rate of decline appearing to depend on stimulation frequency. This confirms many observations in the literature (Naess & Storm-Mathisen, 1955; Marsden *et al.*, 1983; Jones *et al.*, 1979). Many studies of the interrelationship between force generation and excitation have been reported, these have generally involved single fatiguing contractions at only one frequency. In the present study, application of a programmed package of a series of impulse trains at various frequencies to fatigue the adductor pollicis and follow recovery from fatigue has been used in a single fatiguing procedure. This has shown that the relationship between force generation and excitation (measured as the amplitude of the CMAP) is not a simple one, differing markedly according to stimulation frequency and the degree of fatigue or recovery.

At the end of activity and during recovery obvious reductions in force at low frequencies (1, 10 and 20Hz) with little loss in excitation (F/E < 1) suggests a dissociation between sarcolemmal action potential propagation and myofibrillar force generation. This is in aggreement with many reports in the literature (Merton, 1954; Mills, 1982) where it has been termed 'low frequency fatigue' (Edwards *et al.*, 1977b). This could result from impairment of sarcoplasmic Ca^{2+} release or myofibrillar Ca^{2+} sensitivity, although the exact site of impairement of excitation-contraction coupling in fatigue is not yet known. Recent evidence suggests that the former mechanism may be of importance (MacIntosh & Gardiner, 1987) since the contraction time of fatigued twitches in rat gastrocnemius muscle is reduced at a time when that stimulated with caffeine (which triggers release of Ca^{2+} from the sarcoplasmic reticulum) is not.

In contrast to the observations at low frequency, a major finding of this study was the initial maintenance of force despite an obvious relative loss of excitation at the highest stimulation frequencies (50 & 100Hz), hence resulting in the F/E ratio

increasing to > 1. Clearly, force and excitation (CMAP amplitude) do not decline in parallel at high frequency, although the CMAP does decline markedly. This observation indicates the operation of a high-frequency 'safety-factor' within which the action potential amplitude declines markedly without effect on force generation (Figure 3.9 & 3.14). Other investigators have considered the possible role of a safety factor in fatigue (Sandow, 1952; Metzger & Fitts, 1986) in which the action potential can vary without influencing force generation. These findings raise the question as to what changes in the action potential may occur before force generation is affected.

The shape of the surface measured compound muscle action potential is dependent on the numbers and shape of the individual fibre action potentials that make up the summated action potential within the detection range of the recording electrode (Bigland-Ritchie, 1981). The simplest interpretation of a decline in CMAP amplitude is a reduction in the number of functioning parallel action potentials, possibly due to failure of excitability of the sarcolemmal membrane or at the NMJ (Naess & Storm-Mathisen, 1955; Krnjevic & Miledi, 1958). This would be expected to result in a concomitant reduction in force, but only a small degree of force failure was observed up to the seventh PSEM during ischaemic conditions and the 26th contraction during non-occluded conditions and thus cannot explain the observed 'safety-factor'.

The reduction of alternate action potentials, which has similarly been reported to occur in amphibian muscle stimulated at 100Hz (Luttgau, 1965), cannot acount for a reduction in CMAP amplitude either since only every alternate action potential is reduced. This may result in an effective reduction in stimulation frequency, however, thereby maintaining force (viz. frequency:force curve, Figure 3.4) with an apparent sparing effect on the CMAP. It is therefore possible that such a phenomenon may serve to resist fatigue and warrants further research.

A more plausable explanation for the decline in the CMAP amplitude is that action potentials run into each other before repolarization is complete. This seems reasonable since the duration of an action potential may be of the order of 25msec

from initiation to complete recovery, as measured using surface electrodes (Marsden *et al.*, 1983). Since at 100Hz the interstimulus interval is only 10msec, repolarization cannot possibly be completed by the time the next impulse arrives. Hence CMAP amplitude is reduced. Accumulation of electrolytes, such as K^+ in the interfibre spaces would result, leading to a reduction in excitability (Jones *et al.*, 1979; Jeul, 1988) and this may also explain the prolongation of the action potential of the pre-PSEM twitch (Figure 3.15, Table 3.6). The prolongation of the action potential may also account for the increasing tendancy of fading of the action potential at high stimulation frequency observed in each subsequent PSEM during ischaemic fatiguing activity (Figure 3.6). This may also lead to phase changes in different fibre populations which would also result in an apparent reduction in CMAP amplitude.

Despite the reduction in CMAP amplitude and a reduction in twitch force, high frequency tetanic force is still maintained. As mentioned previously, the reduction in twitch force is probably a result of a reduced release of Ca^{2+} (MacIntosh & Gardiner, 1987). It would therefore appear that a reduction in force should occur. However, studies in single amphibian muscle fibres, using the bioluminescent protein aquorin to detect free Ca^{2+}, have indicated that in the course of a tetanus myoplasmic Ca^{2+} concentrations increase to levels greater than that necessary to achieve full mechanical activation (Blinks *et al.*, 1978). It is thus probable that at high frequencies of stimulation, Ca^{2+} still saturates the contractile apparatus thereby leading to maximal activation, despite a possible reduction in Ca^{2+} release per impulse. Furthermore, this rise in Ca^{2+} concentration may overcome changes in myofibrillar Ca^{2+} sensitivity as a result of generation of metabolites such as H+ (Metzger & Moss, 1987) or Pi (Brandt *et al.*, 1982). A similar explanation has been proposed to account for maximal force generation at high stimulation frequencies when long-term low-frequency fatigue is apparent, where it is thought that excitation-contraction coupling is impaired at low frequency (Edwards *et al.*, 1977b). It is unlikely that sufficient myoplasmic Ca^{2+} concentrations are achieved at lower

stimulation frequencies, however, and so the high-frequency safety factor is lost. Consequently, force declines.

The biphasic reduction in force observed for the near-fused tetanic contractions (20-100Hz) during ischaemic conditions in this study is in accord with observations of other workers (Duchateau & Hainaut, 1985; Marsden *et al.*, 1976). The similarity of these force curves (Figure 3.6 b-f) suggest that a similar fatiguing mechanism is responsible for the rapid reduction in force at each frequency following the eighth PSEM, whereupon the high-frequency safety-factor is also lost. With the differences in F/E for each frequency in mind, it is not readily apparent what is responsible for the decline in force, but such a mechanism may 'protect' the muscle against being driven into harmful rigor, as previously hypothesized (Edwards, 1983).

Clearly, a reduction in CMAP amplitude, and hence excitability, cannot be responsible for the sudden decline in force after the eighth PSEM during ischaemic conditions. Since similar amounts of activity will have been performed, it may be argued that metabolic factors, possibly metabolites such as H^+ or Pi generated as a consequence of activity (Table 1.1), may reduce myofibrillar sensitivity to Ca^{2+} (Fabiato & Fabiato, 1978; Donaldson & Hermansen, 1978; Brandt *et al.*, 1982; Metzger & Moss, 1987) and hence result in force loss, independent of excitation failure. Such a view was recently proposed by Duchateau & Hainaut (1985) who attributed the initial slow phase of force reduction during stimulated activity of the biceps brachii muscle to anaerobic alactic processes and the latter, faster phase of force decline to lactic metabolic processes resulting in the generation of H^+ ions. Precautions were taken to reduce available oxygen stores within the muscle in the present study by making the forearm and hand ischaemic for 3 minutes before activity and thus prevent the supply of energy from oxidative metabolism. Furthermore, the amount of activity performed by a contraction at high frequency is markedly less than that at low frequency before fatigue occurs suggesting a greater metabolic cost occurs in the latter contraction (Figure 3.1). It is therefore difficult to conceive that metabolic factors are responsible for fatigue at both, the high and low stimulation

frequency. The observations of Marsden *et al.*, (1976, 1983), who showed that force generation was dependent on numbers of stimuli delivered regardless of the frequency of stimulation or the amount of contractile activity performed would further argue against such a proposition. However, there remains some controversy as to the findings of Marsden *et al.*, (1976, 1983) since it has been shown for the anterior tibialis muscle that fatigue during stimulation at 15 and 30Hz occurs after similar amounts of contractile activity performed while the CMAP amplitude is only modestly reduced (Garland *et al.*, 1986). The reduction in force could be explained by action potential propagation failure within the T-tubular structures (Bezanilla *et al.*, 1972; Jones *et al.*, 1979) since the events of excitation measured at the cell surface may not reflect those deep within the cell.

2. Potentiation of force at low stimulation frequency

During both ischaemic and non-occluded activity, pre-tetanic twitch force and 10Hz tetani became potentiated. This may serve as a protective mechanism resisting fatigue since the F/E ratio was increased to > 1. The immediate potentiation of the pre-tetanic twitch was not surprising, in view of the many observations of twitch potentiation following brief stimulated activity of the adductor pollicis (Bothelo & Cander, 1953; Desmedt & Hainaut, 1968; Takamori *et al.*, 1971). Potentiation of the twitch was not associated with an equivalent increase in CMAP amplitude and thus is unlikely to be attributed to altered excitation. This finding is in keeping with other workers, who have similarly noted a lack of association between twitch force potentiation and action potential characteristics (Brown & von Euler, 1938; Botelho & Cander, 1953; Desmedt & Hainaut, 1968).

Potentiation of low-frequency tetani is likely to occur as a result of an increase in the fusion of successive twitch contractions as the duration of the twitch increases (Bigland-Ritchie *et al.*, 1983a). This will depend on the duration of contraction and relaxation. It was not possible to determine accurately the changes in twitch MCR and MRR throughout ischaemic fatiguing activity since measurement of the twitch force

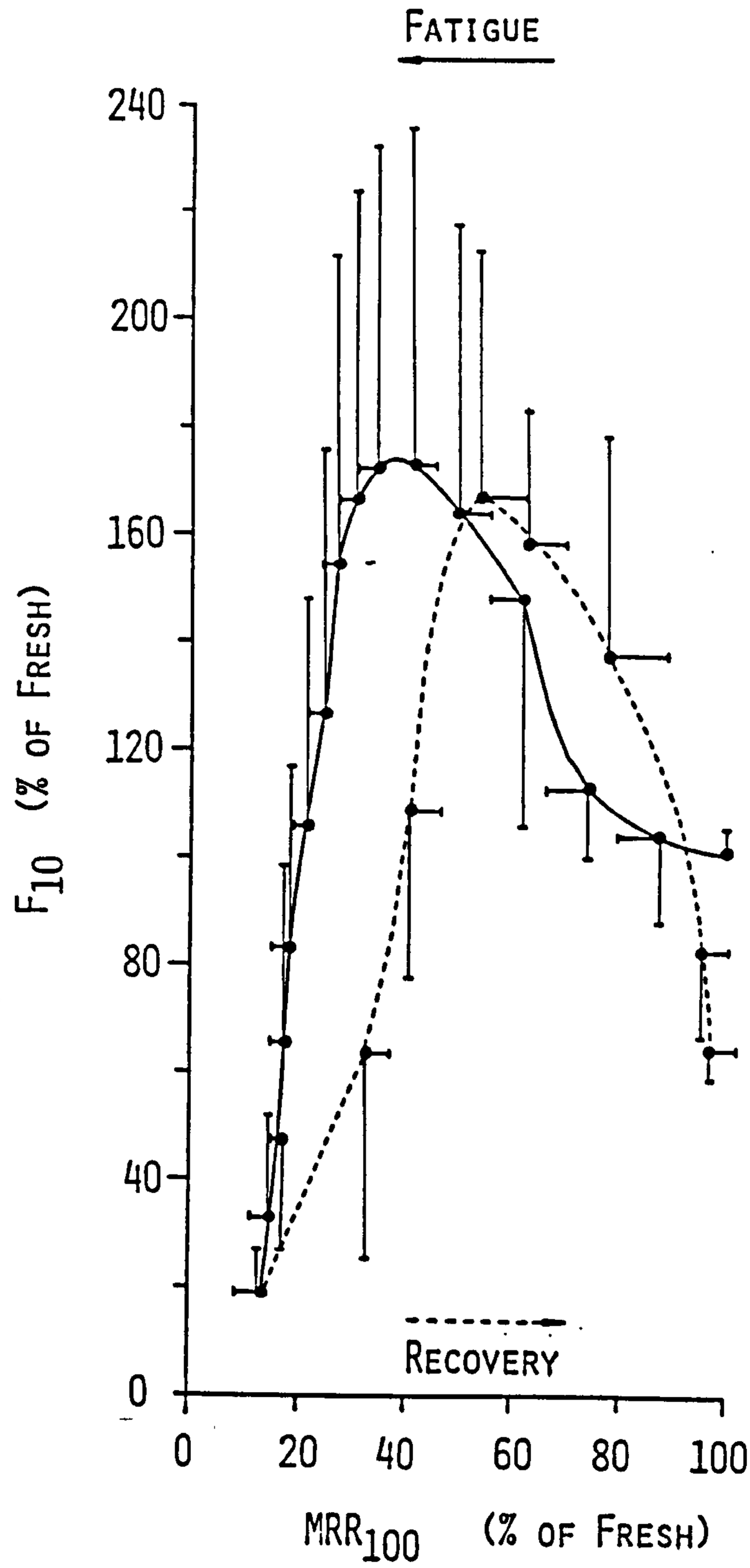


Figure 3.16 10Hz force (F_{10}) plotted as a function of MRR at 100Hz (MRR_{100}, % of initial) during activity with arterial occlusion (solid line) and subsequent recovery (dashed line). During recovery the curve is shifted to the right indicating a further factor other than MRR influences low-frequency force potentiation.

became unreliable as it decreased. However, the findings of Bigland-Ritchie *et al.*, (1983a) suggest that MRR increases markedly more than MCR. Since the reduction in MRR was similar at all frequencies of stimulation investigated during ischaemic conditions (Figure 3.10), it would appear that in the present study, low-frequency force potentiation is due to a reduction in MRR. A more detailed analysis of the time course of the summation of twitches by MacIntosh *et al.*, (1985) working on dog gastrocnemius supports this hypothesis.

However, during recovery following ischaemic activity, when the MRR was **increasing**, there was again marked potentiation of force at 10Hz. Thus, slowing of the MRR as the sole cause of low-frequency potentiation appears unlikely. This is shown more clearly in Figure 3.16 in which the mean 10Hz force has been plotted against MRR (% of initial) during fatigue with ischeamia and subsequent non-occluded recovery. Since low-frequency force is dependent on the summation of twitches, it is possible that twitch potentiation may contribute to low-frequency force potentiation.

3. Effect of circulation on fatiguability

The changes in force, CMAP and MRR were in the same direction during activity with ischaemia and non-occluded circulation at all frequencies, but were usually of a greater degree during ischaemic conditions. This confirms similar observations made by other workers (Merton, 1954; Stephens & Taylor, 1972; Duchateau & Hainaut, 1985).

The application of the sphygmomanometer cuff to invoke ischaemia was designed to parallel conditions during sustained isometric contractions. Early studies indicated that above 20% of a maximal voluntary contraction, blood flow through a muscle is prevented (Barcroft & Millen, 1939). Edwards *et al.*, (1972c) found that blood flow was progressively arrested when force became greater than 20% MVC in the vastus lateralis, although the point at which blood flow is prevented has been shown to vary considerably, ranging from 20-70% (Barnes, 1980). This may be due to, in part, a rise in mean systemic blood presssure. Clearly, during maximal

voluntary isometric contractions, ischaemic conditions prevail, until such time as fatigue reduces contractility sufficiently to allow return of blood flow (Stephens & Taylor, 1972). It is likely oxidative recovery processes then become quantitatively significant. This proposition is supported by studies of metabolism during intermittent stimulated contractions of the quadriceps rectus femoris in which aerobic pathways appear to provide an increasing fraction of the energy supply for contraction compared to anaerobic pathways (Hultman & Spriet, 1986). The small degree of recovery of MRR during non-occluded activity may also be explained by this. It is unlikely that in normal everyday activities only sustained maximal contractions are generated. The non-occluded model of muscle contraction may therefore help to further explain the mechanisms leading to fatigue which undoubtedly differ depending on the type of contraction performed.

Thus, it would appear that the greater fatigue observed during ischaemic conditions is the result of the differences in energy supply and metabolite removal between the two conditions. The more severe long-term low-frequency fatigue and a degree of persistent high-frequency fatigue may then represent a metabolic price paid for the greater activity, and may explain the lack of force potentiation at 1 and 10Hz during recovery. Since CMAP amplitude rapidly returned to normal, this form of fatigue was due to changes in excitation and activation of the contractile apparatus beyond the sarcolemmal membrane. During activity without occlusion, continued supply of oxygen and substrates and disposal of metabolic products probably allow effective excitation and activation to proceed for longer. This is further supported by the observation of only a small increase in CMAP duration during non-occluded conditions compared to during ischaemic conditions (Figure 3.15). Consequently, the high-frequency 'safety-factor' appears to function for a greater number of contractions during non-occluded conditions compared to that than seen in ischaemic conditions.

CONCLUSIONS

By employing a programmed pattern of stimulation trains of various frequencies, it has been possible to investigate the frequency dependence of force generation and excitation in a single fatiguing procedure of the adductor pollicis during occluded and non-occluded conditions. The discrepancies of force and excitation in previous reports have arisen because these studies only examined single frequencies, since they all agree with the present findings. The present study has, in addition, identified two mechanisms which could permit resistance to fatigue: At high frequency, a safety-factor operates within which force is maintained despite a reduction in CMAP amplitude, while at low frequencies (1 and 10Hz) there is marked potentiation of force with normal excitation. The first of these may be of limited importance during sustained maximal voluntary contractions since high motor unit discharge rates are not maintained. However, it is possible that the high-frequency safety-factor may operate at the onset of a contraction where high motor-unit discharge rates are encountered (Marsden *et al.*, 1971; Bigland-Ritchie *et al.*, 1983b), thereby preventing the loss of the force generating capacity of muscle whereas potentiation mechanisms may operate at lower discharge rates later on in a sustained contraction. Further studies are indicated to determine the contribution of these factors to fatigue resistance.

SUMMARY

1. Human adductor pollicis was fatigued using intermittent trains of programmed stimulation at 1, 10, 20, 50, 100 and 1 Hz, during activity with and without circulatory occlusion, to investigate the relationships between force generation, excitation and maximal relaxation rate (MRR).

2. The relationship between force generation and excitation was markedly dependent on stimulation frequency. Force loss was greatest at low frequencies, with little reduction in excitation. As frequency increased force was well maintained despite marked loss of excitation.

3. Changes in MRR during activity and recovery were independent of stimulation frequency.

4. Two mechanisms resisting fatigue were identified. At high frequency, a 'safety-factor' operates to maintain force despite marked loss of excitation, while at low frequency a marked potentiation of force occurs despite unchanged excitation. The contribution of these mechanisms to resisting fatigue warrants further investigation.

CHAPTER 4: RELATION OF FORCE AND EXCITATION TO NUMBERS OF IMPULSES, CONTRACTILE ACTIVITY PERFORMED AND DURATION OF CONTRACTION

Page

CHAPTER 4: RELATION OF FORCE AND EXCITATION TO NUMBERS OF IMPULSES, CONTRACTILE ACTIVITY PERFORMED AND DURATION OF CONTRACTION

4.1 INTRODUCTION

On the basis of the results of chapter 3 (part III), in which it was shown that force declined at all frequencies, except for the twitch, following the eighth PSEM, it was suggested that the reduction in force could be attributed to either metabolic factors or failure of excitation. A study was therefore designed to identify, if possible, which factor was responsible for force decline.

The suggestion that excitation propagation failure is a primary cause of fatigue is supported by the studies of Marsden *et al.*, (1983) in which similar force curves were obtained when the adductor pollicis was stimulated continuously over a frequency range of 5-200Hz and plotted against impulse numbers delivered. It was concluded that metabolic factors could not be responsible for force failure since the amount of contractile activity performed at the high and low frequencies differed. However, the results of Marsden *et al.*, (1983) have been disputed by Garland *et al.*, (1986) who concluded from their studies, in which the human dorsiflexor was fatigued with 15Hz and 30Hz stimuli, that the reduction in force was dependent on contractile events rather than on the excitatory mechanism since similar amounts of contractile activity were performed at the two frequencies at the point of fatigue.

Owing to the design of the PSEM employed in the previous study (chapter 3, part III), it was not possible to differentiate between total numbers of impulses delivered and activity undergone at each frequency since each PSEM was composed of trains of stimuli at various frequencies. Hence, the contribution of these factors to fatigue could not be separated. However, it is unclear whether the total contractile activity performed during muscular activity necessarily reflects the total energy utilized during a contraction and hence the use of this measure raises doubt as to the validity of the work of Marsden *et al.*, (1983) and Garland *et al.*, (1986). To obtain an independent measure of the metabolic cost of a contraction using a non-invasive

technique, the relationship between the maximal relaxation rate (MRR), which is thought to be dependent on metabolic status of the muscle (Edwards *et al.*, 1972; Wiles & Edwards, 1982a & b; Hultman *et al.*, 1983), and the contractile activity performed was also investigated.

Therefore, in this study, single frequency stimulated trains of impulses over a range of 20 to 100Hz were used to fatigue the adductor pollicis. The contractions were briefly interrupted to allow documentation of the relaxation characteristics and the frequency dependence of force and excitation in relation to impulse numbers delivered, contractile activity and duration was determined. The design of contraction also permitted the investigation of the changes in CMAP duration which is suggested to reflect conduction velocity (Mortimer et al., 1970; Bigland-Ritchie et al., 1981; Jones & Bigland-Ritchie, 1986), in relation to numbers of stimuli, contractile activity performed and duration of contraction, but this could only be studied in one subject due to availability of equipment and limitation of time. It was therefore possible to investigate the significance of prolongation of the action potential in relation to changes in excitation and therefore force loss, i.e., fatigue.

4.2 METHODS

4.2.1 Experimental subjects

Six normal subjects (4 male and 2 female), aged 24 - 33 years participated in this study.

4.2.2 Measurement of muscle contractile properties

Isometric force, MRR, MCR, integrated force, and electromyography of the prepared adductor pollicis were recorded on a multichannel UV oscillograph as described in general methods, chapter 2, for all six subjects. In addition, in one subject, the evoked action potential was digitized via an analogue to digital convertor at intervals during stimulation and stored on floppy disc for subsequent detailed analysis to measure changes in prolongation of the action potential. Both, Nicolet (MED 80) and BBC B computers were used for this purpose.

A series of 2 second impulse trains consisting of either 20, 40 60, 80 or 100Hz separated by 0.5 second were used to fatigue the adductor pollicis in all the subjects. A further series of contractions at 15 Hz and 30 Hz was carried out in the one subject in which the CMAP was investigated in more detail.

4.2.3 Experimental fatigue protocols

In each subject, five activity protocols were undertaken. These were carried out on separate occasions in random order and separated by at least two days to allow full recovery of function of the adductor pollicis. A control 2 second, 100Hz impulse train was obtained before each fatiguing procedure to obtain a maximal force plateau for subsequent calculation of activity performed. Following 5 minutes of rest (to minimize influences from potentiating mechanisms (Edwards *et al.*, 1977a)) a control contraction was performed in the fresh muscle. After a two minute rest period for recovery, a sphygmomanometer cuff was inflated around the upper arm and maintained at 220mmHg to induce ischaemia. This was followed by a further 3 minutes ischaemic rest to reduce oxygen reserves available for oxidative metabolism as described in the previous chapter, after which fatiguing activity commenced. This consisted of repeated stimulated contractions for up to 3600 impulses in total or until

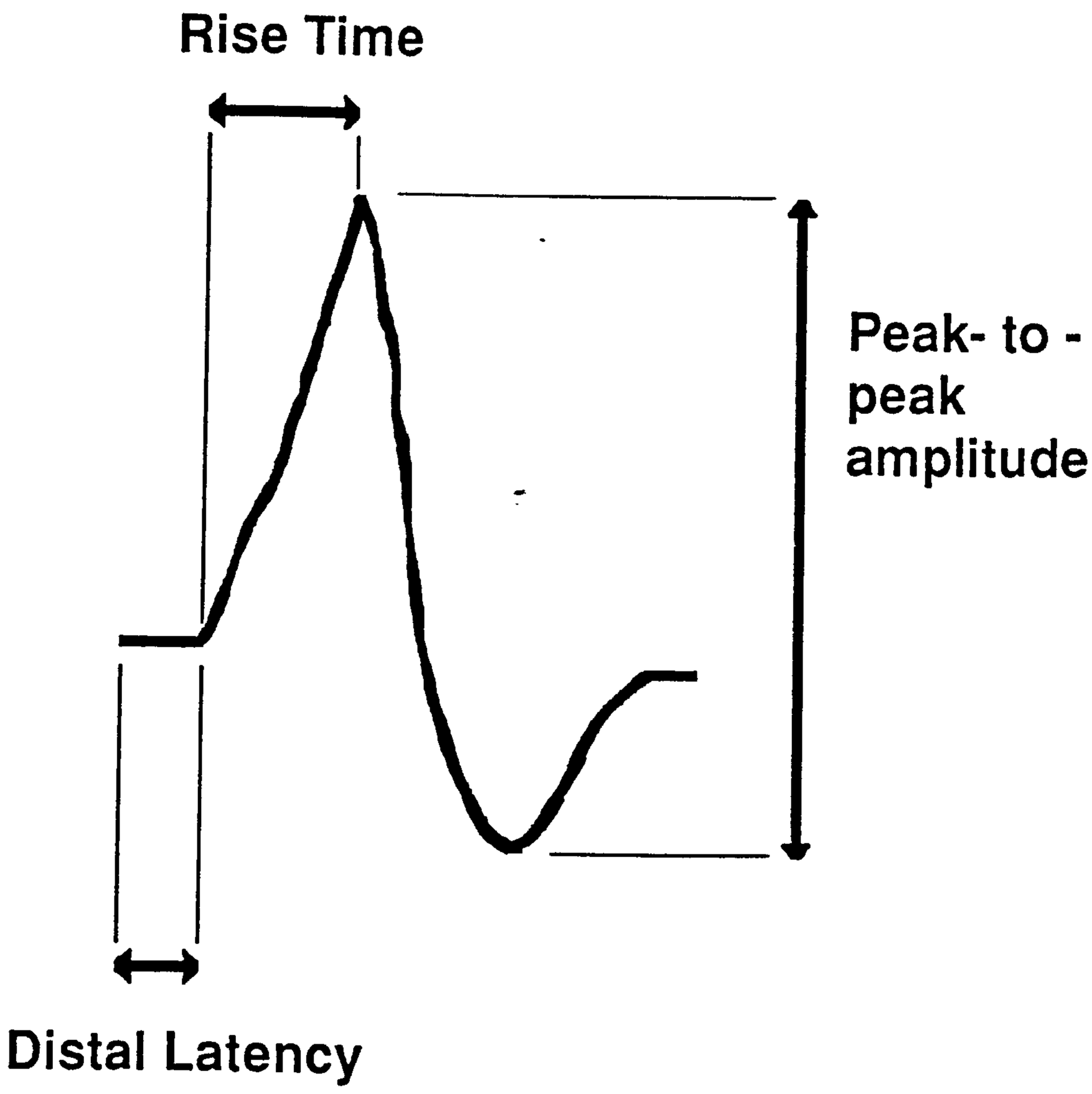


Figure 4.1 Measurement of CMAP characteristics. Excitability was measured as the peak-to-peak amplitude of the negative and positive phases of the action potential. Distal latency was measured from the stimulus artifact to the start of depolarization. Rise time was measured from the start of depolarization to the peak negative phase of the action potential.

discomfort became limiting. In addition, the last evoked action potential of each two second train in one subject was also recorded as described above.

4.2.4 Analysis

The force measured at the end of each impulse train was expressed as a percentage of maximum force generated by the 100Hz tetanus in fresh muscle and the control tetanus generated in the fresh muscle. MRR and MCR was calculated as described in chapter 2 and expressed as a percentage of the value obtained in fresh muscle. At low stimulation frequencies every fourth contraction was measured due to the large numbers of contractions developed.

CMAP amplitude was measured at the final impulse of each impulse train. In the case of the stored digitized data, the distal latency (measured from the stimulus artifact to start of rise of the negative phase) and rise time (measured from the start of the first negative phase to peak) were determined to obtain an indirect measure of the changes in propagation characteristics of the CMAP (Figure 4.1). The two measurements were carried out in preference to the single measurement used in chapter 3, part III, since the distal latency probably reflects serial changes in excitation propagation whereas the CMAP rise time probably reflects changes in excitation propagation of parallel fibres that make up the summated CMAP. Each was expressed as a percentage of the control value obtained in fresh muscle.

Figures were constructed of the change in force, MRR, MCR and CMAP parameters related to impulse number, activity undergone and duration of contraction. Where it has been necessary, error bars have been ommited from the figures for clarity.

4.3 RESULTS

The changes in MRR, force and CMAP amplitude as a function of impulse number, contractile activity and duration are illustrated in Figures 4.2, 4.3 and 4.4 respectively. The changes of force and CMAP amplitude expressed as a function of contractile activity have been plotted individually since the differences between subjects varied considerably, although the changes within an individual are consistent.

The changes in MRR, however, appears similar between all subjects and hence all the grouped data is shown (Figure 4.2b). Due to the discomfort experienced by some individuals during activity at the lower stimulation frequencies (20 & 40Hz) not all the stimulation procedures were completed. The results presented in the figures and tables are therefore expressed as the mean for 6 subjects unless otherwise stated. The degree of variation of MRR, force and CMAP amplitude following 1120 or 1200 impulses and 30 or 32 seconds of activity (the selection of which was dependent on the contractions measured for analysis) is shown in Tables 4.1, 4.2 and 4.3 and have been ommited from the figures for the purposes of clarity.

4.3.1 Relation of MRR to impulse number, contractile activity performed and duration of contraction

Maximum relaxation rate slowed at all frequencies, although in some subjects MRR increased after the first contraction before declining. The reduction in MRR was frequency dependent when expressed as a function of impulse number (Figure 4.2a), but frequency independent when expressed as a function of contractile activity performed, and was linear and inversely related for up to 30 max.seconds (Figure 4.2b). Thereafter, the reduction in MRR became less steep and began to plateau. Similarly, MRR appeared to be frequency independent when expressed as a function of the duration of the contraction.

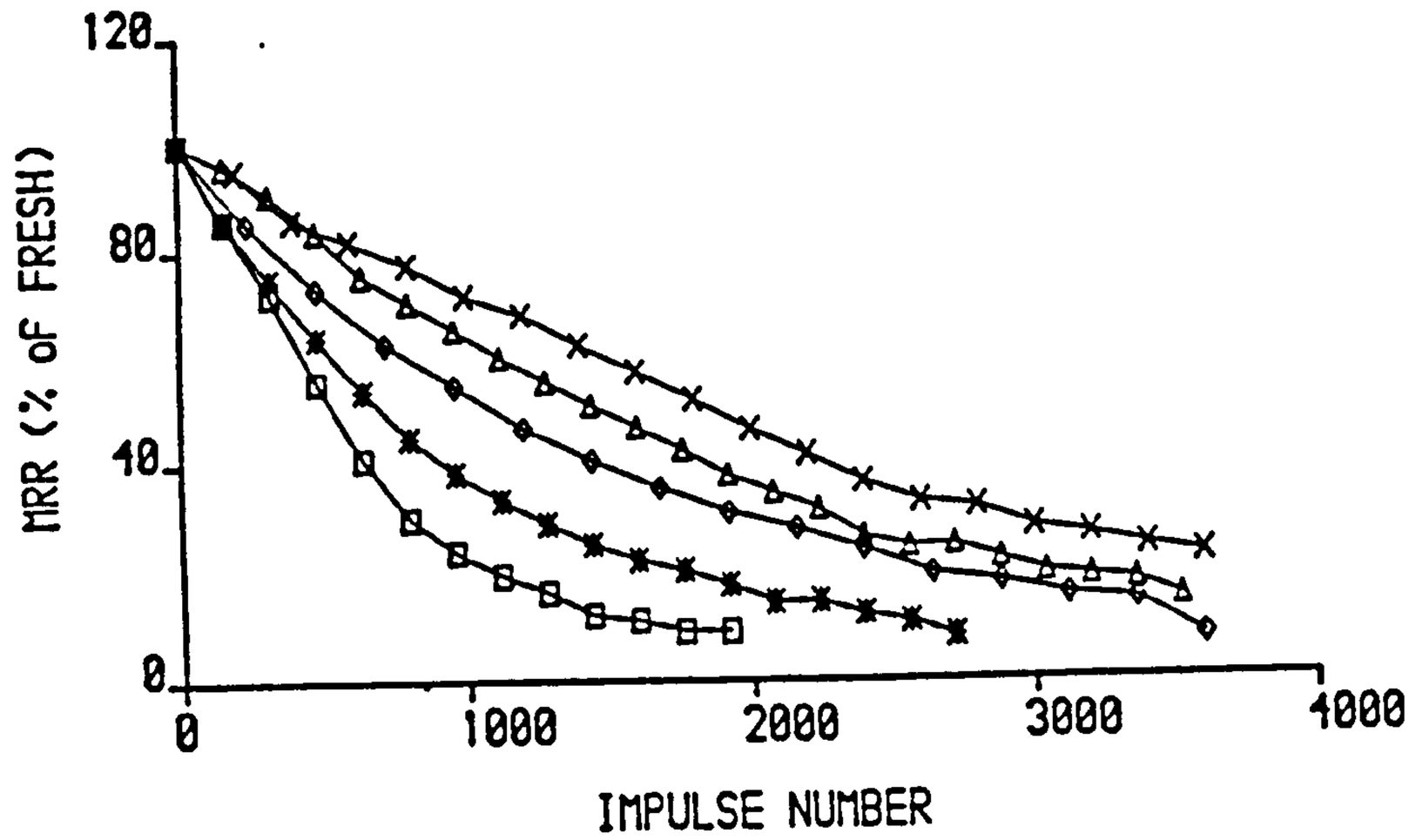
The linear portion of the decline in MRR as a function of contractile activity is expressed by the equation:

$$\%MRR = 100 - 2.38 \times \text{max.seconds} \qquad r=-0.979$$

In contrast, the MCR obtained from the 100Hz tetani did not alter in proportion to the MRR as shown in Figure 4.5, indicating its independence to MRR slowing.

Figure 4.2 Relation of MRR during interrupted stimulated activity (2 seconds activity, 0.5 seconds rest upto 3600 impulses) to A) numbers of impulses, B) contractile activity performed (force x time) and C) duration of contraction. MRR is expressed as the mean % of fresh value for six subjects. Error bars are ommited for clarity, the details of which may be found in Table 4.1 and appendix 10. Note that the relationship between MRR and contractile activity performed is linear up to approximately 30 max.seconds for all frequencies of stimulation. Symbols as follows: (□) 20Hz, (✳) 40Hz, (◇) 60Hz, (△) 80Hz and (×) 100Hz.

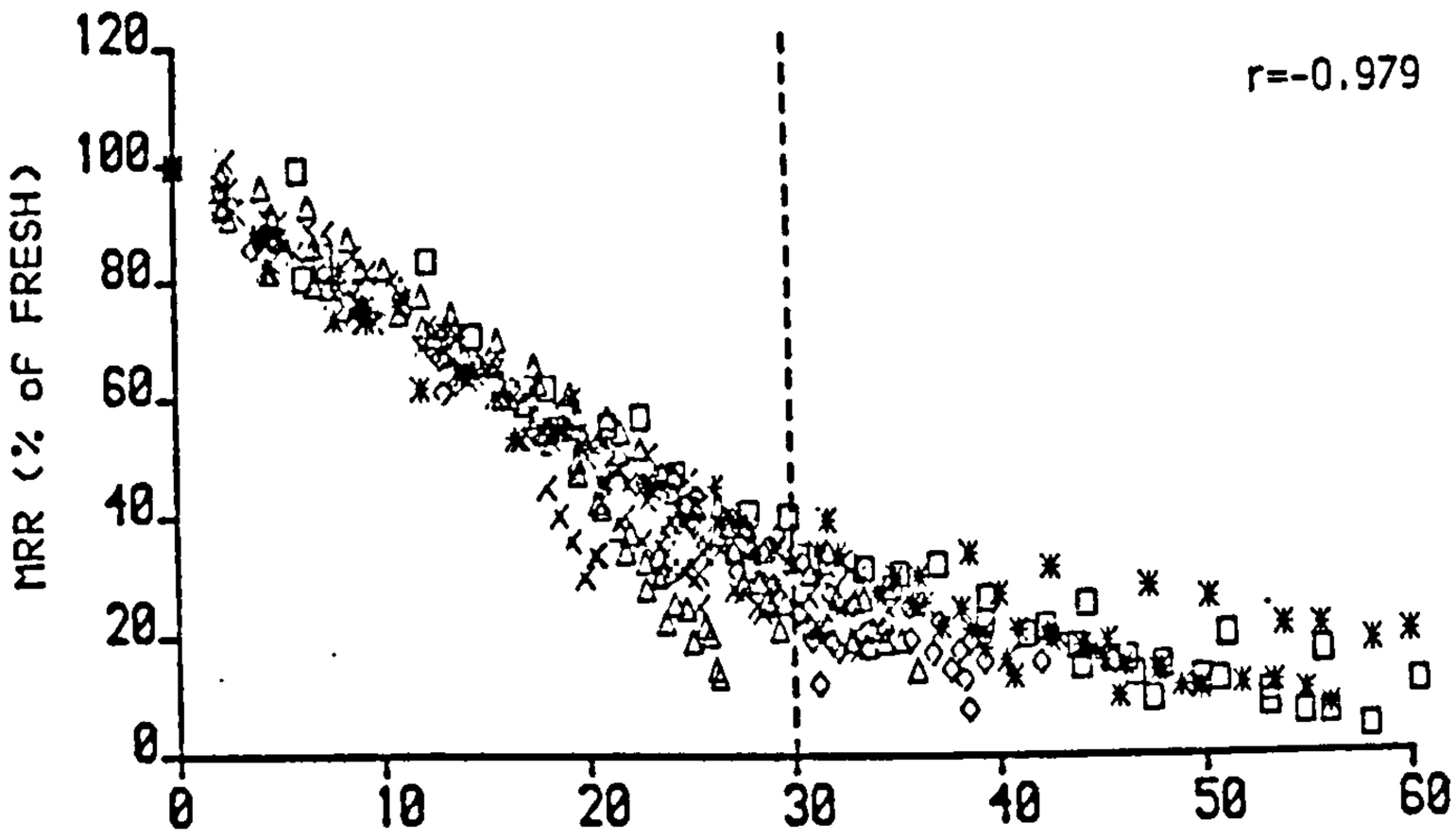
A



B

%MRR = 100 - 2.38 x max.seconds

r=-0.979



C

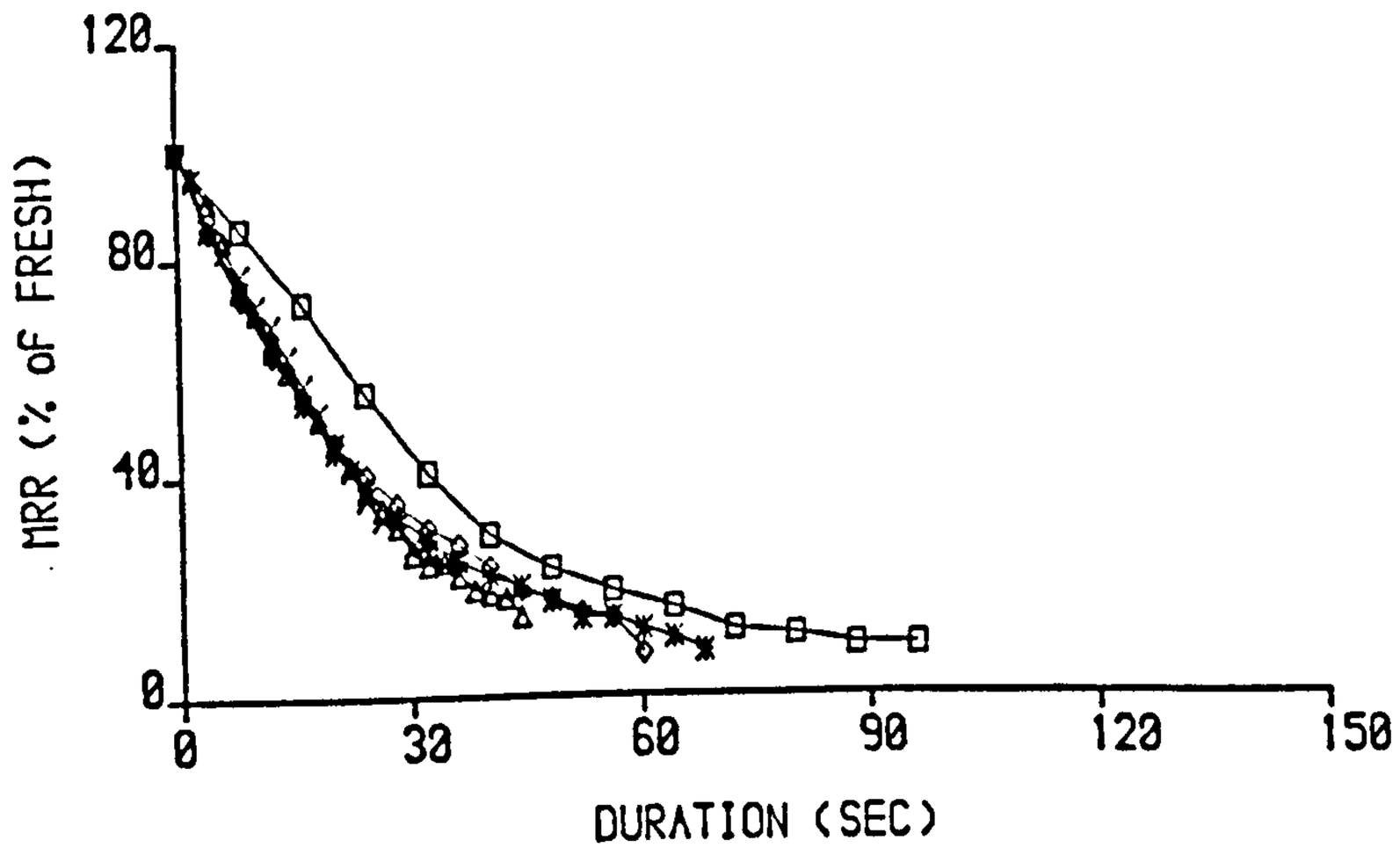


Table 4.1 Representative values of mean ± 1 S.D. of maximal relaxation rate at various frequencies following a) a total of 1120 impulses or 1200 impulses and b) a total of 30 or 32 seconds of activity during stimulation of the adductor pollicis.

A

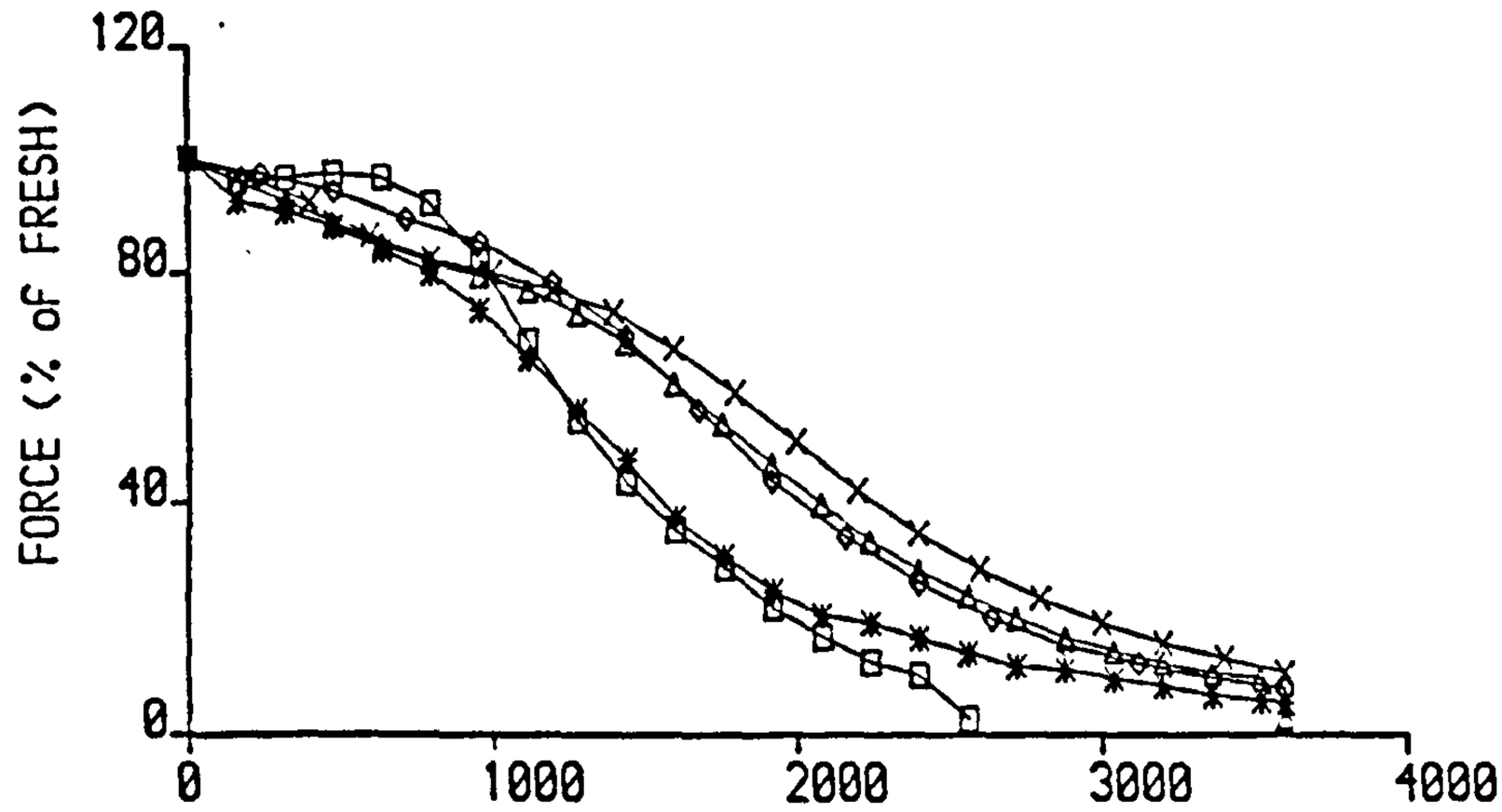
Frequency (Hz)	Number of impulses delivered	Mean value (%)	SD	n
20	1120	19.3	4.3	5
40	1120	33.2	1.8	5
60	1200	46.7	8.3	6
80	1120	67.9	4.5	5
100	1200	68.1	5.0	6

B

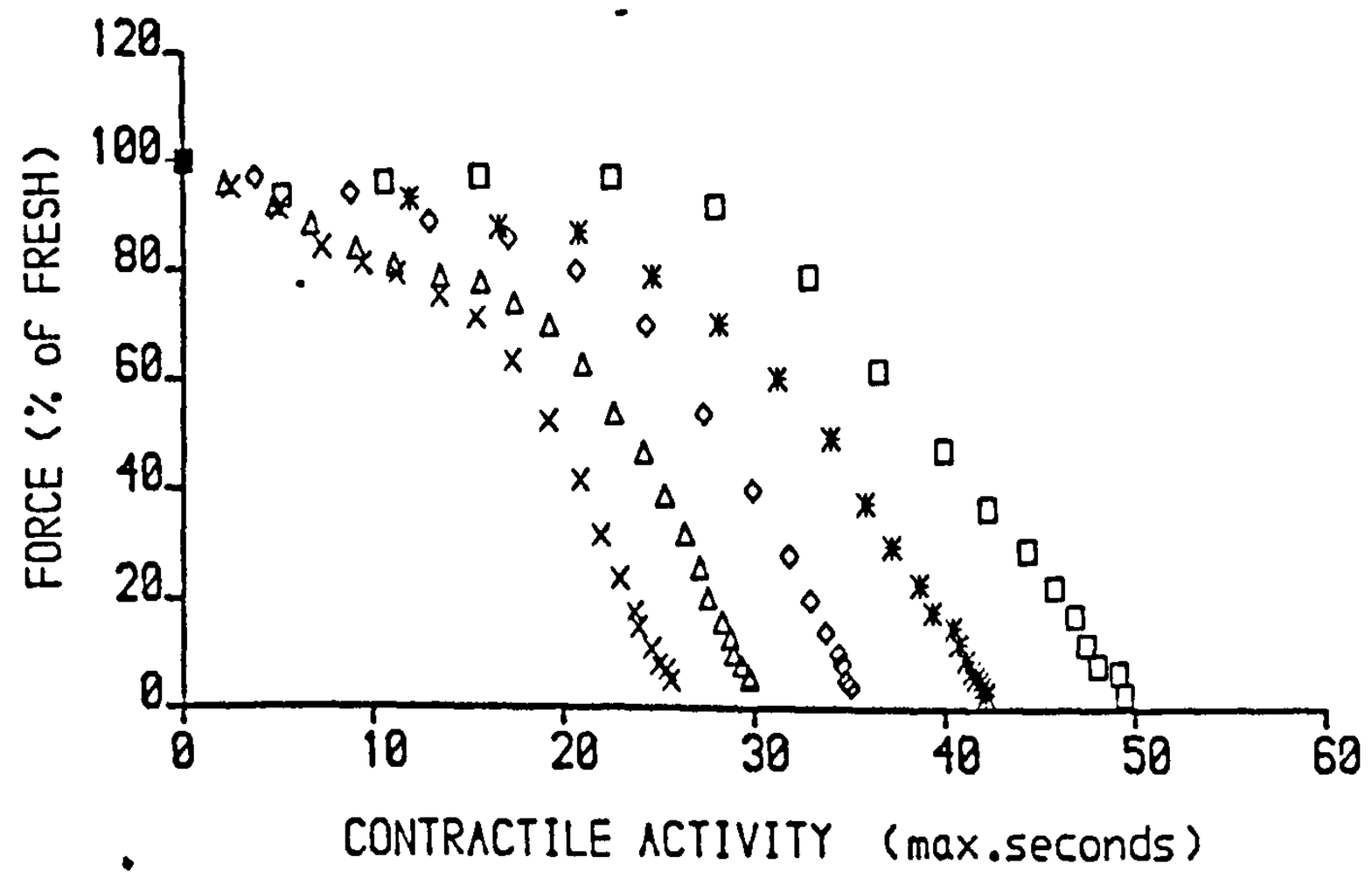
Frequency (Hz)	Time (minutes)	Mean value (%)	SD	n
20	32	40.9	3.8	5
40	32	28.8	2.0	5
60	32	30.8	2.3	6
80	30	30.8	4.4	5
100	30	28.0	5.1	6

Figure 4.3 Relation of Force generated during interrupted stimulated activity (see Figure 4.2) to A) numbers of impulses, B) contractile activity performed (force x time) and C) duration of contraction. Force is expressed as mean % of fresh value for six subjects except in (B) where the data presented is for one subject only for clarity and is typical of all subjects (Appendix 9). Error bars are ommited for clarity, the details of which may be found in Table 4.2 and appendix 10. Note that force appears frequency independent when plotted as a function of impulses delivered and declines in a biphasic fashion: the first phase is gradual and is followed by the second more rapid phase after approximately 1200 impulses. Symbols as for Figure 4.2.

A


120
80
40
0
FORCE (% oF FRESH)
0
1000
2000
3000
4000
IMPULSE NUMBER

B


120
100
80
60
40
20
0
FORCE (% oF FRESH)
0
10
20
30
40
50
60
CONTRACTILE ACTIVITY (max.seconds)

C

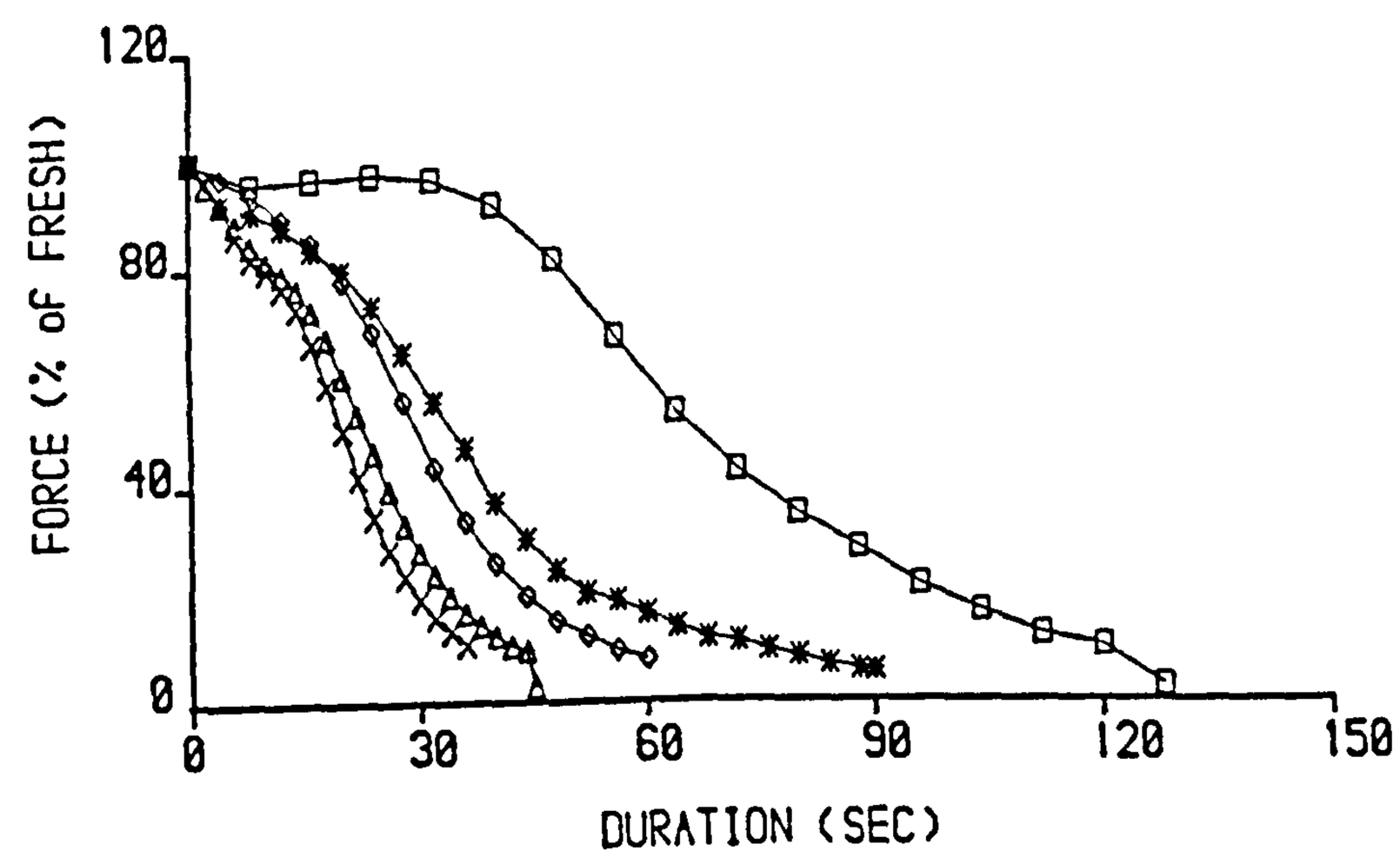

120
80
40
0
FORCE (% oF FRESH)
0
30
60
90
120
150
DURATION (SEC)

Table 4.2 Representative values of mean ± 1 S.D. of force at various frequencies following a) a total of 1120 impulses or 1200 impulses and b) a total of 30 or 32 seconds of activity during stimulation of the adductor pollicis.

A

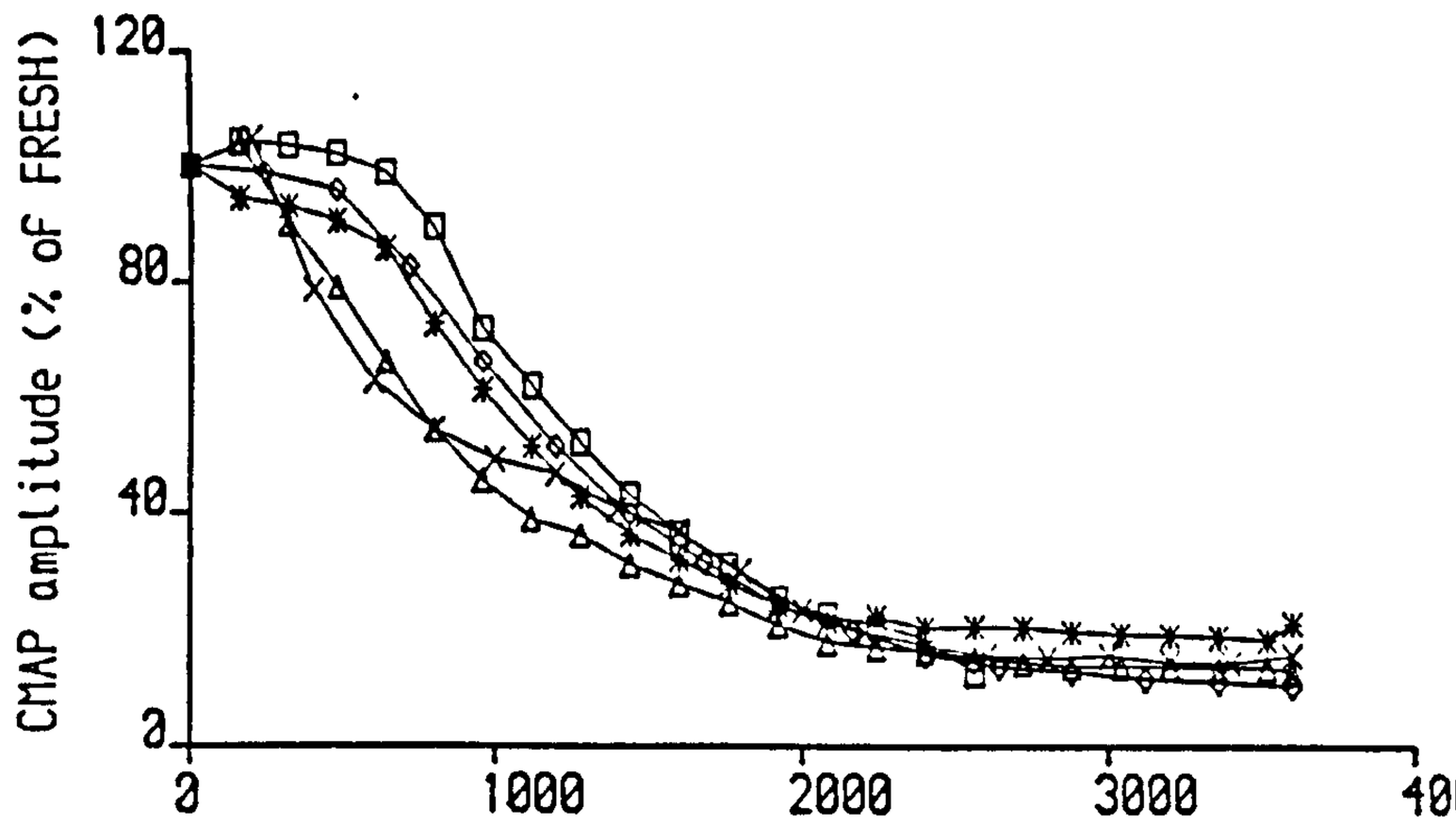
Frequency (Hz)	Number of impulses delivered	Mean value (%)	SD	n
20	1120	67.8	17.9	5
40	1120	64.7	17.8	5
60	1200	78.0	4.7	6
80	1120	76.6	4.9	5
100	1200	76.6	5.8	6

B

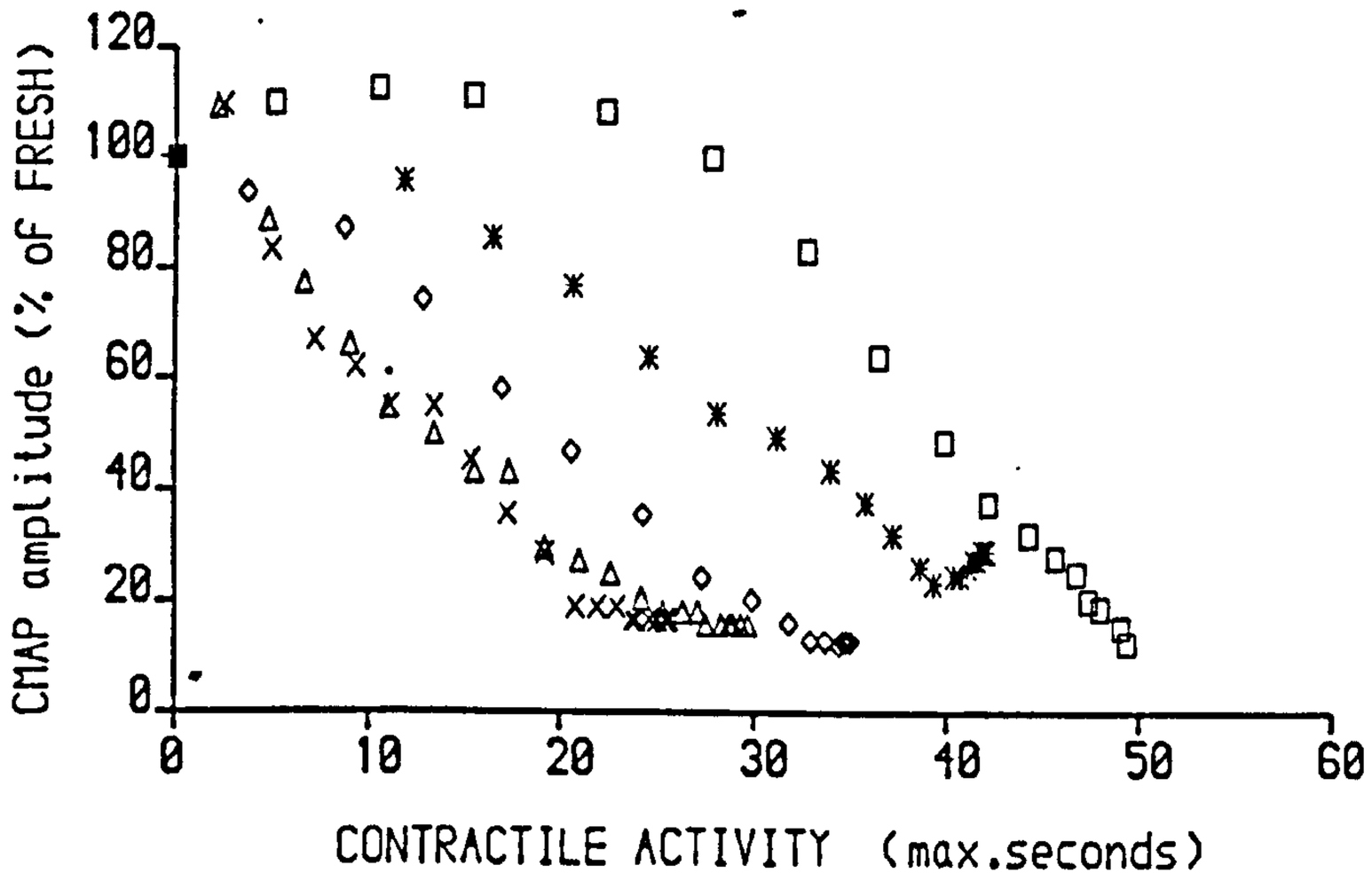
Frequency (Hz)	Time (minutes)	Mean value (%)	SD	n
20	32	96.5	9.3	5
40	32	55.6	15.1	5
60	32	43.5	11.7	6
80	30	28.0	11.7	5
100	30	19.0	8.7	6

Figure 4.4 Relation of CMAP amplitude during interrupted stimulated activity (see Figure 4.2) to A) numbers of impulses, B) contractile activity performed (force x time) and C) duration of contraction. CMAP amplitude is expressed as the mean % of fresh value for six subjects except in (A) where the data is presented is for one subject only for clarity and is typical of all subjects (Appendix 9). Error bars are ommited for clarity, the details of which may be found in Table 4.3 and appendix 10. Symbols as for Figure 4.2.

A


CMAP amplitude (% of FRESH)
120
80
40
0
0
1000
2000
3000
4000
IMPULSE NUMBER

B


CMAP amplitude (% of FRESH)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
CONTRACTILE ACTIVITY (max.seconds)

C

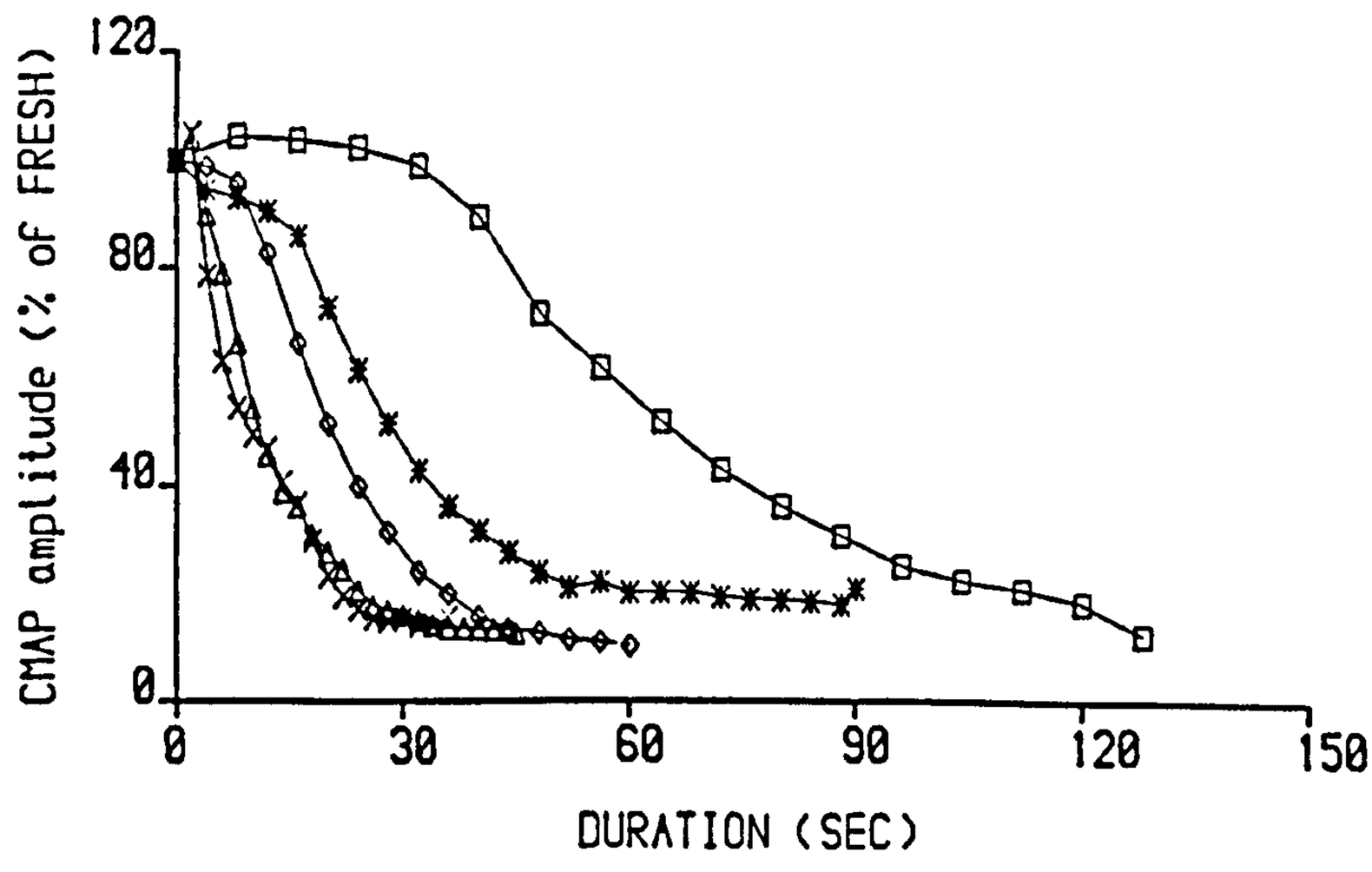

CMAP amplitude (% of FRESH)
120
80
40
0
0
30
60
90
120
150
DURATION (SEC)

Table 4.3 Representative values of mean ± 1 S.D. of CMAP amplitude at various frequencies following a) a total of 1120 impulses or 1200 impulses and b) a total of 30 or 32 seconds of activity during stimulation of the adductor pollicis.

A

Frequency (Hz)	Number of impulses delivered	Mean value (%)	SD	n
20	1120	61.9	19.2	5
40	1120	51.5	6.3	5
60	1200	51.5	13.3	6
80	1120	39.0	6.6	5
100	1200	46.9	6.0	6

B

Frequency (Hz)	Time (minutes)	Mean value (%)	SD	n
20	32	98.9	13.3	5
40	32	42.9	8.3	5
60	32	24.1	6.0	6
80	30	16.2	4.9	5
100	30	15.5	4.6	6

Figure 4.5 Relation of MCR and MRR changes measured for 100Hz tetani during intermittent stimulated activity (2 seconds activity, 0.5 second rest for up to 3600 impulses) in four subjects. Ischaemia was induced 3 minutes before activity commenced.

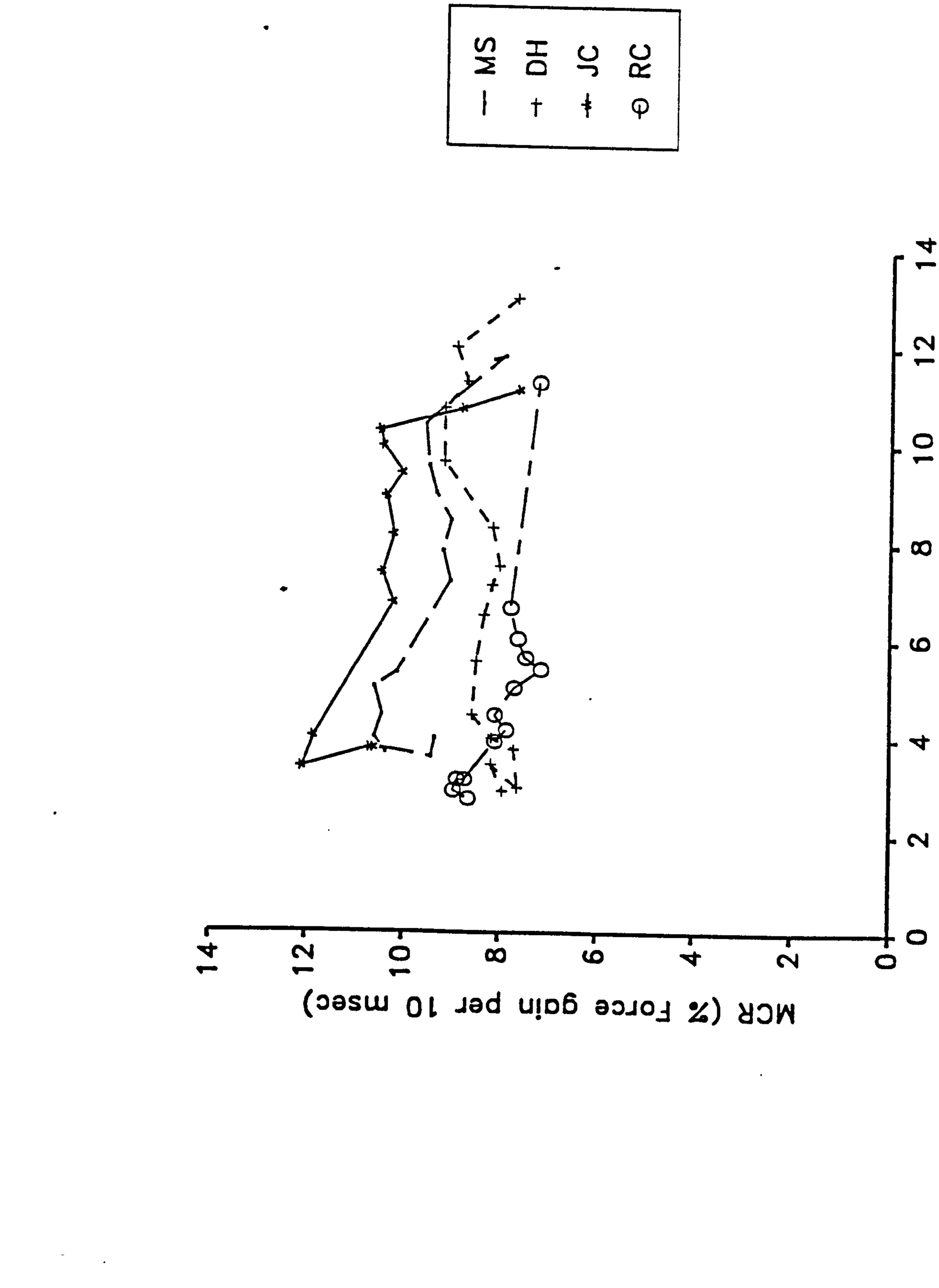
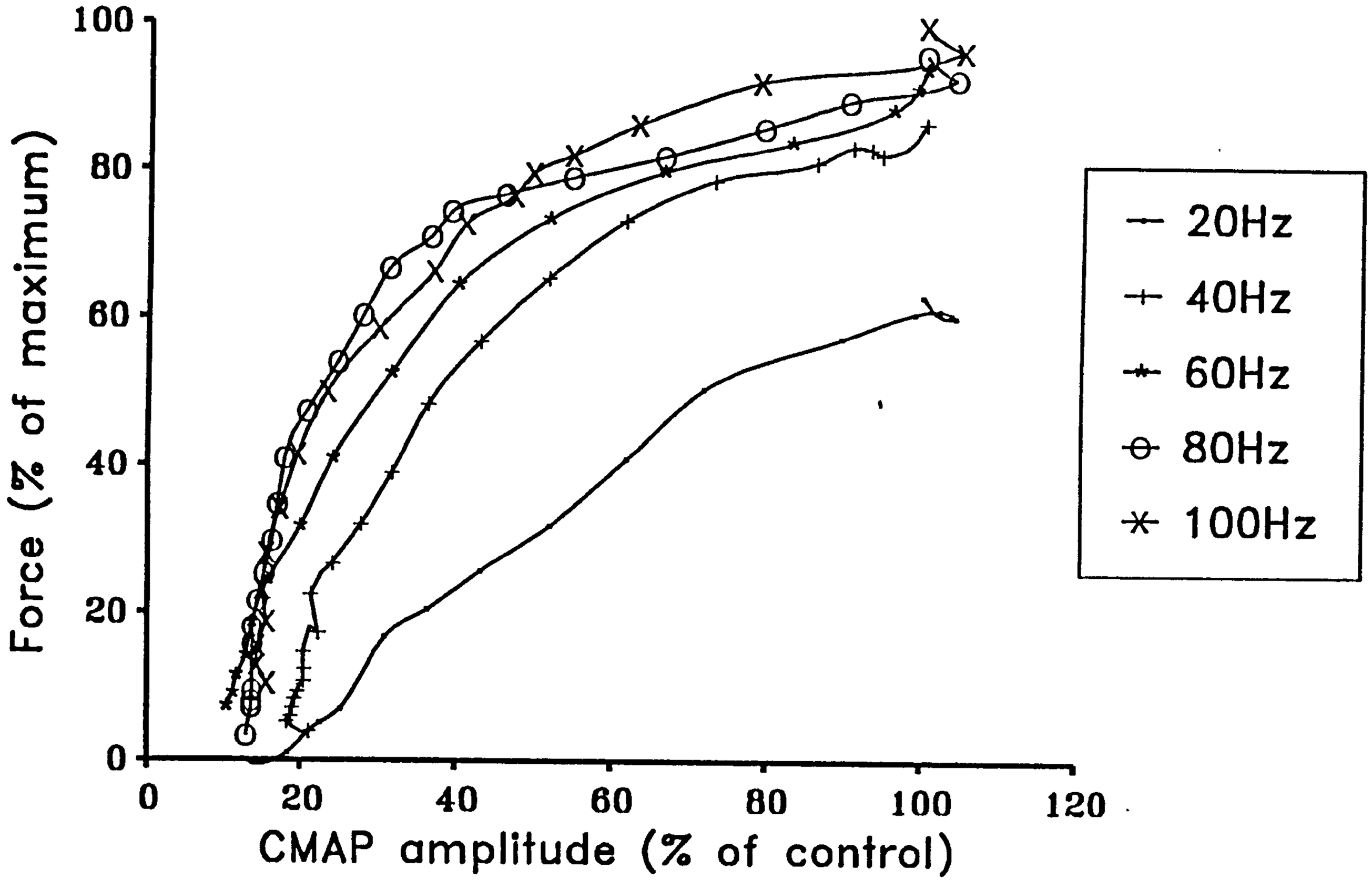

MS
DH
JC
RC
MRR (% Force loss per 10 msec)
MCR (% Force gain per 10 msec)
0
2
4
6
8
10
12
14

Figure 4.6 Dependence of force generation on CMAP amplitude at various frequencies during intermittent stimulated activity (2 seconds activity, 0.5 seconds rest for up to 3600 impulses). Ischaemia was induced 3 minutes before activity and maintained throughout the stimulation period. Note the similarity of the curves to those obtained in chapter 3, Figure 3.9. Mean data for 6 subjects. Error bars ommited for clarity (see Appendix 11 details).


100
80
60
40
20
0
Force (% of maximum)
0
20
40
60
80
100
120
CMAP amplitude (% of control)
20Hz
40Hz
60Hz
80Hz
100Hz

4.3.2 Relation of force and CMAP amplitude to impulse numbers, contractile activity performed and duration of contraction

The decline in force in relation to impulse number was similar at the highest frequencies (60-100Hz) and biphasic (Figure 4.3a). Force declined to approximately 80% following 1200 impulses and then declined more precipitously. Similarly, at 20 and 40Hz, the decline in force was biphasic, but the second more rapid phase of force decline occured after 800 impulses. The similarity of these curves indicate that the decline in force is independent of frequency. When expressed as a function of contractile activity performed, the rapid phase of force decline was clearly frequency dependent (Figure 4.3b). The changes in force expressed as a function of duration of contraction was similarly frequency dependent (Figure 4.3c).

The changes in CMAP amplitude appeared similar to those of force. However, when expressed as a function of impulse number (Figure 4.4a), the initial decline in CMAP amplitude at 80 & 100Hz was more rapid than that observed at 20-60Hz. The changes in CMAP amplitude were clearly frequency dependent when expressed as a function of contractile activity performed (Figure 4.4b) and duration of contraction (Figure 4.4c).

The implication of the changes in CMAP amplitude on force generation is shown in Figure 4.6. Force was well maintained at the highest frequencies despite a marked decline in CMAP amplitude whereas at lower stimulation frequencies force and CMAP amplitude declined in a similar fashion.

4.3.3 Changes in CMAP characteristics

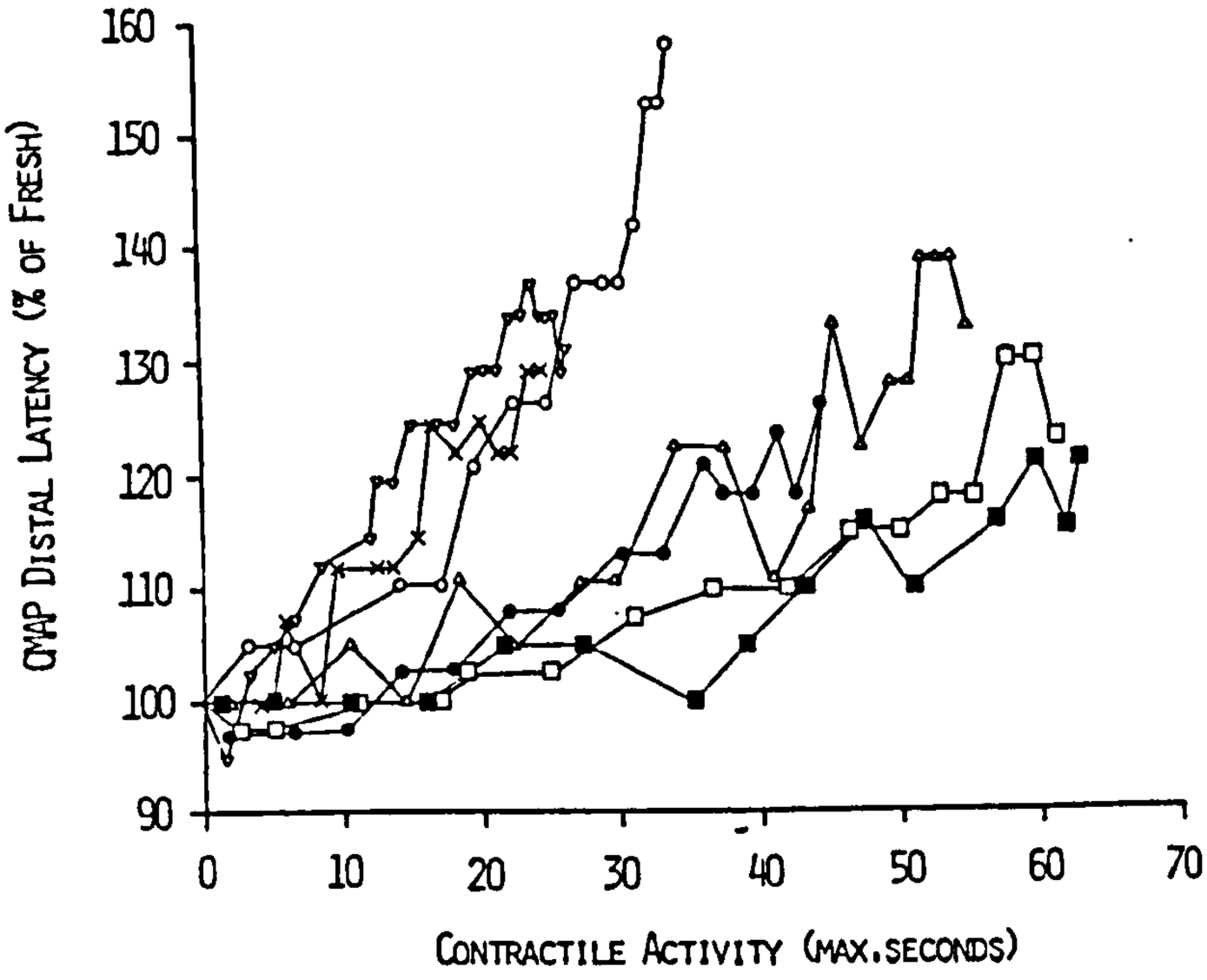
For technical reasons, the changes in action potential shape were only documented in the last subject studied. Proportional increases were observed for both CMAP distal latency and CMAP rise time during fatiguing activity at all frequencies of stimulation. The increase in distal latency was clearly dependent on the number of impulses delivered and was independent of frequency of stimulation (Figure 4.7b), whereas distal latency was frequency dependent with respect to contractile activity performed (Figure 4.7a). Similarly, CMAP rise time increased proportionally with

impulse number, although the relative rates of increase were greater for the high frequencies of stimulation compared to the low stimulation frequencies (Figure 4.8b), indicating some degree of frequency dependence. The frequency dependence of the increase in CMAP rise time was more marked, however, when CMAP rise time was expressed as a function of contractile activity (Figure 4.8a). CMAP rise time peaked and then plateaued or declined after approximately 700 impulses at 80 and 100 Hz and 1200 impulses at 30-60Hz. The plateauing of CMAP rise time was not clearly evident at 15 or 20 Hz, although the experiment had to be terminated early due to discomfort (Figure 4.8b).

The implication of the changes of CMAP rise time on force generation were investigated by expressing force generation as a function of CMAP rise time (Figure 4.9). The arrows indicate the start of the second, more rapid phase of force decline (Figure 4.9a). The decline in force appeared to be closely related to the peaking of CMAP rise time for frequencies of 30-60Hz (Figure 4.9b). At the highest frequencies (80 and 100Hz), however, CMAP rise time peaked before the second phase of force decline (Figure 4.9b), whereas at the lower frequencies of stimulation the reduction in force did not appear to be related to CMAP rise time (Figure 4.9c).

Figure 4.7 Changes in CMAP distal latency in relation to A) contractile activity performed and B) numbers of impulses in one subject (H.D.) during ischaemic stimulated activity. Symbols as follows:(■) 15Hz, (□) 20Hz, (△) 30Hz, (●) 40Hz, (○) 60Hz, (▽) 80Hz and (×) 100Hz. Ischaemia was induced 3 minutes before activity commenced. Stimulated activity consisted of intermittent tetani of 2 seconds, 0.5 seconds rest, for upto a total of 3600 impulses or until discomfort became limiting.

A


CMAP Distal Latency (% of Fresh)
160
150
140
130
120
110
100
90
0
10
20
30
40
50
60
70
Contractile Activity (max.seconds)

B

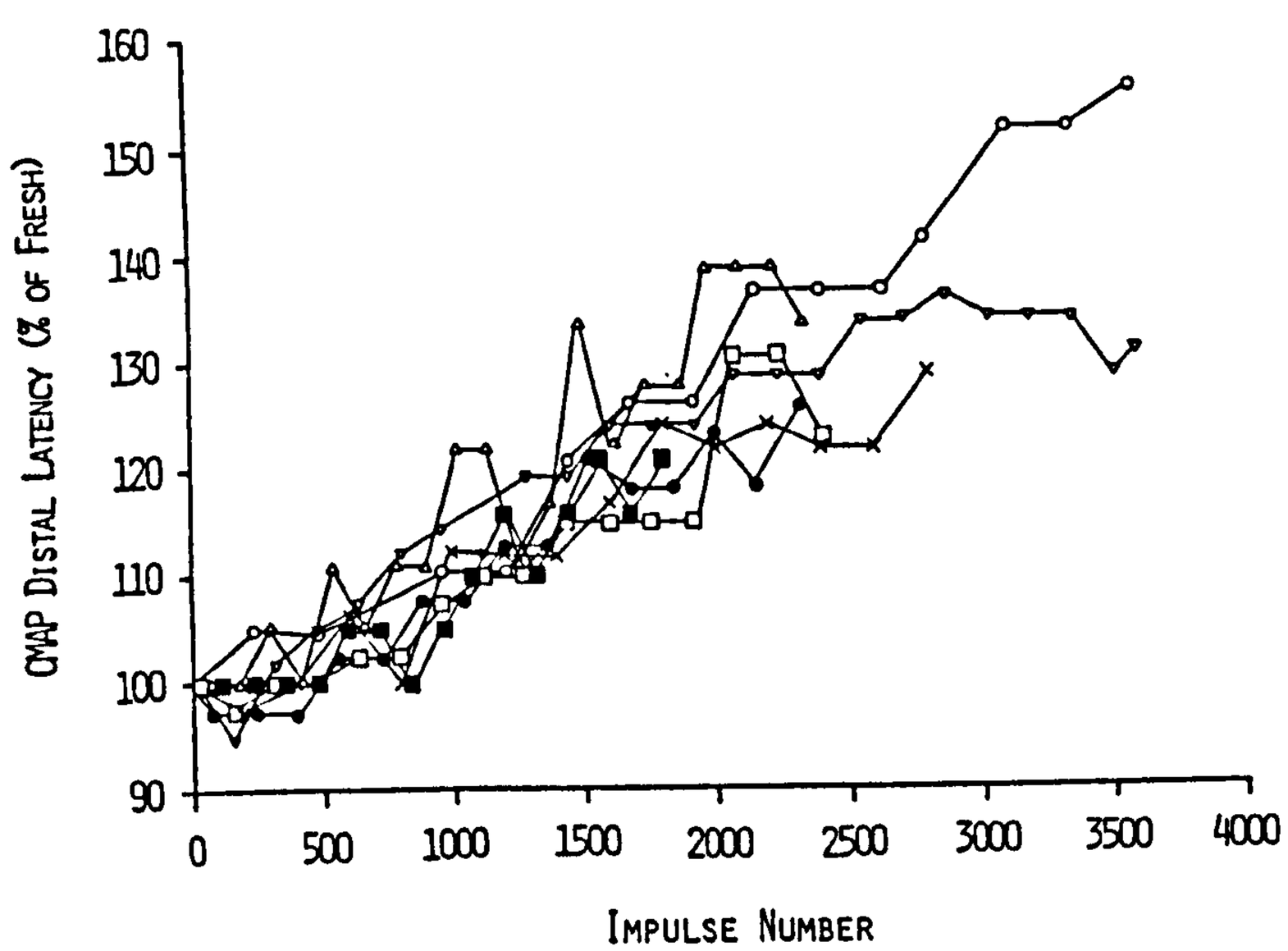
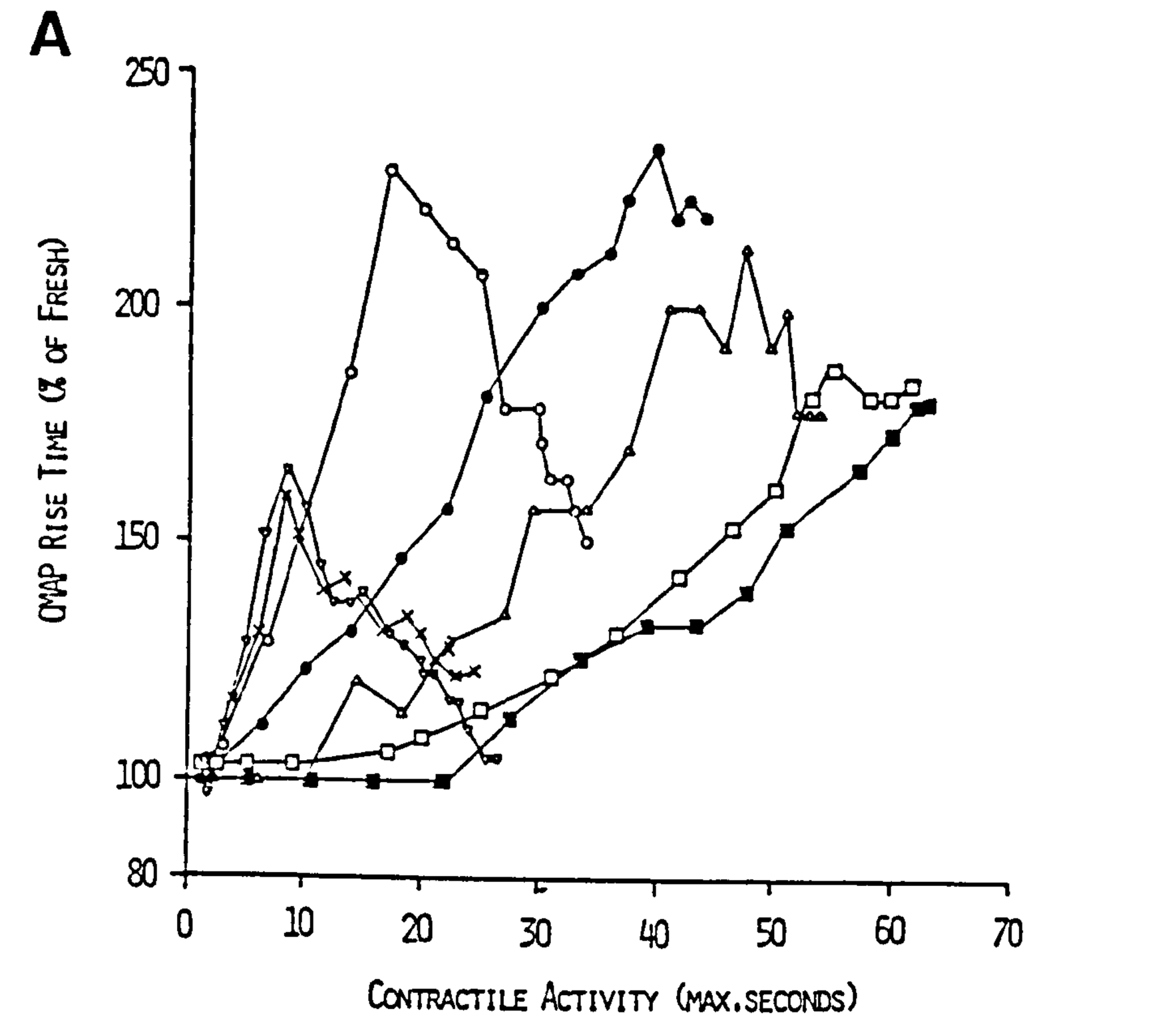

CMAP Distal Latency (% of Fresh)
160
150
140
130
120
110
100
90
0
500
1000
1500
2000
2500
3000
3500
4000
Impulse Number

Figure 4.8 Changes in CMAP rise time in relation to A) contractile activity performed and B) numbers of impulses in one subject (H.D.) during ischaemic stimulated activity. Symbols as for Figure 4.7. Ischaemia was induced 3 minutes before activity commenced. Stimulated activity consisted of intermittent tetani of 2 seconds, 0.5 seconds rest, for upto a total of 3600 impulses or until discomfort became limiting.


A
CMAP Rise Time (% of Fresh)
250
200
150
100
80
0
10
20
30
40
50
60
70
Contractile Activity (max.seconds)

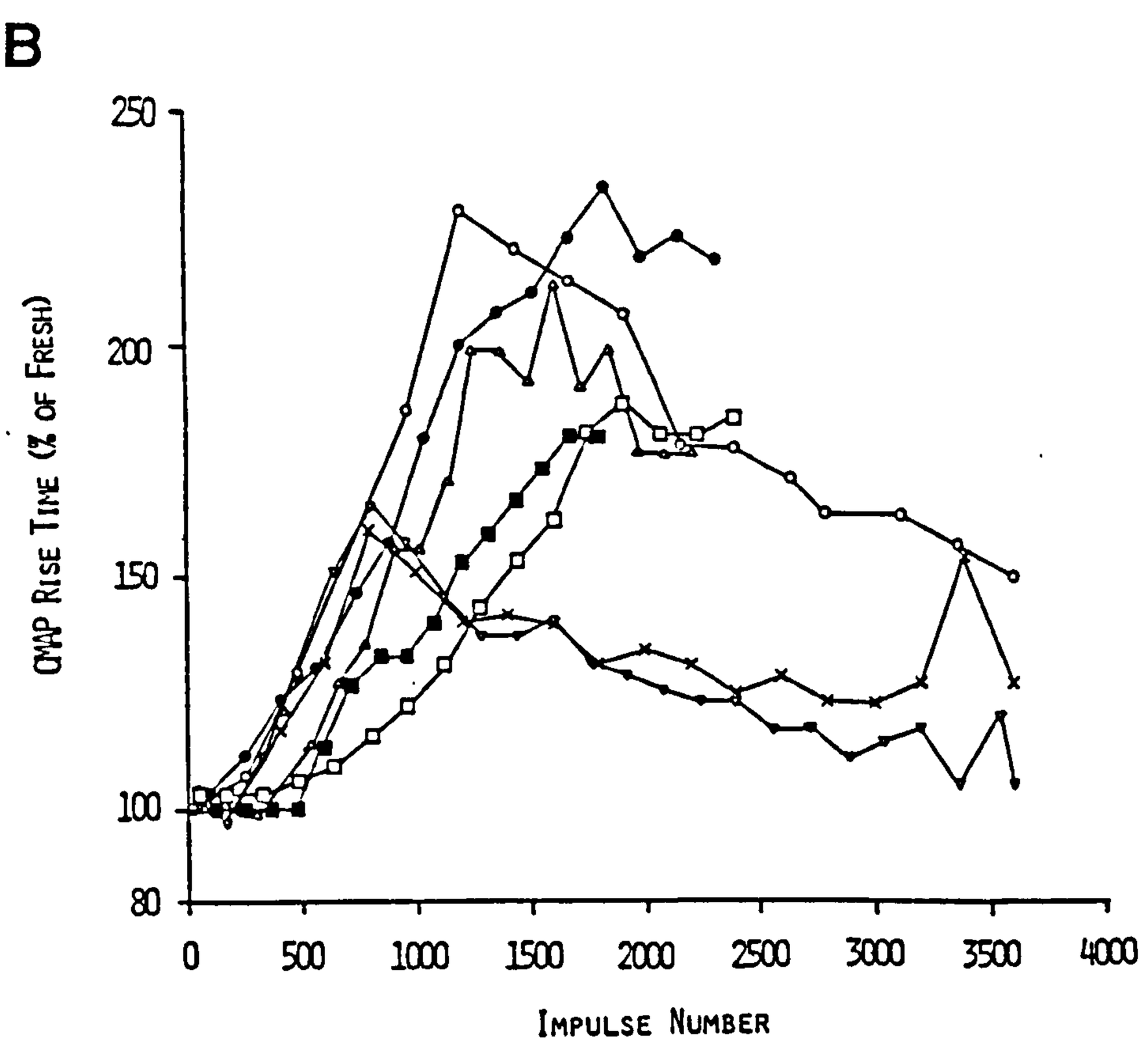
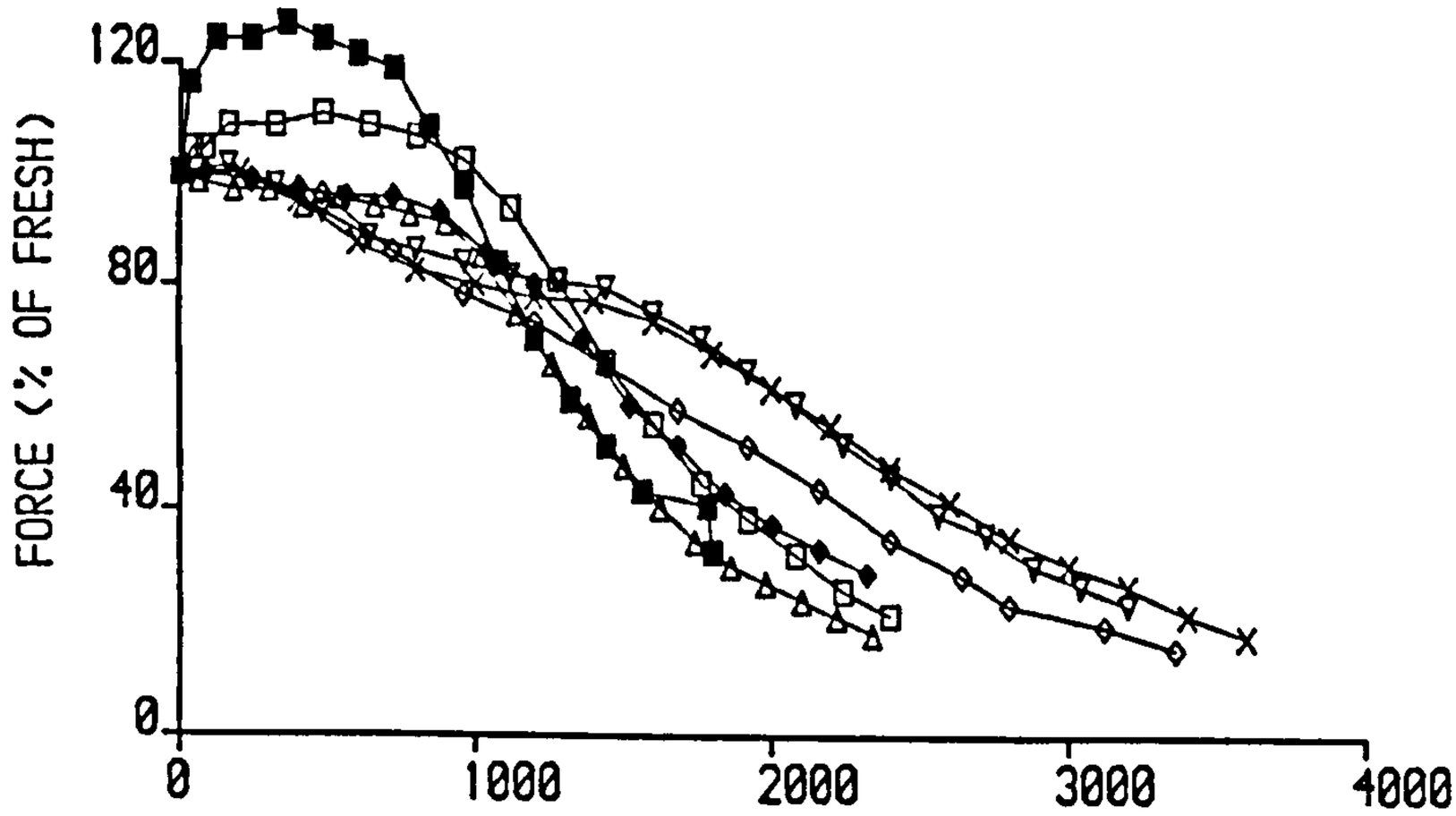

B
CMAP Rise Time (% of Fresh)
250
200
150
100
80
0
500
1000
1500
2000
2500
3000
3500
4000
Impulse Number

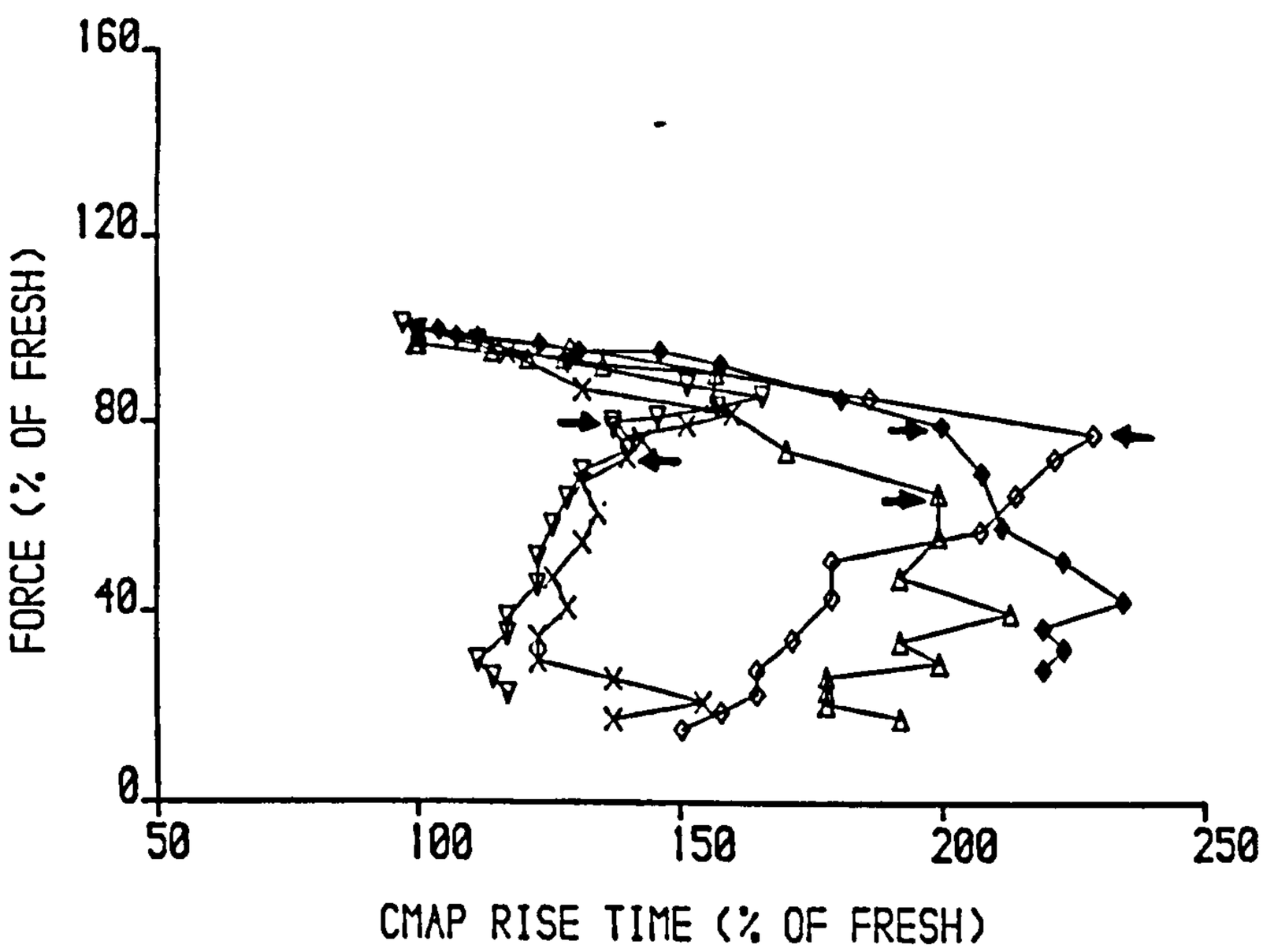
Figure 4.9 Changes in force generation in relation to CMAP rise time at various frequencies in subject HD during stimulated ischaemic fatiguing activity. A) Change in force in relation to impulse numbers at various frequencies. B) Relation of force generation to CMAP rise time for frequencies 30-100Hz. C) Relation of force generation to CMAP rise time at 15 and 20Hz.

Symbols: (■) 15Hz, (□) 20Hz, (△) 30Hz, (◆) 40Hz, (◇) 60Hz, (▽) 80Hz, (×) 100Hz.

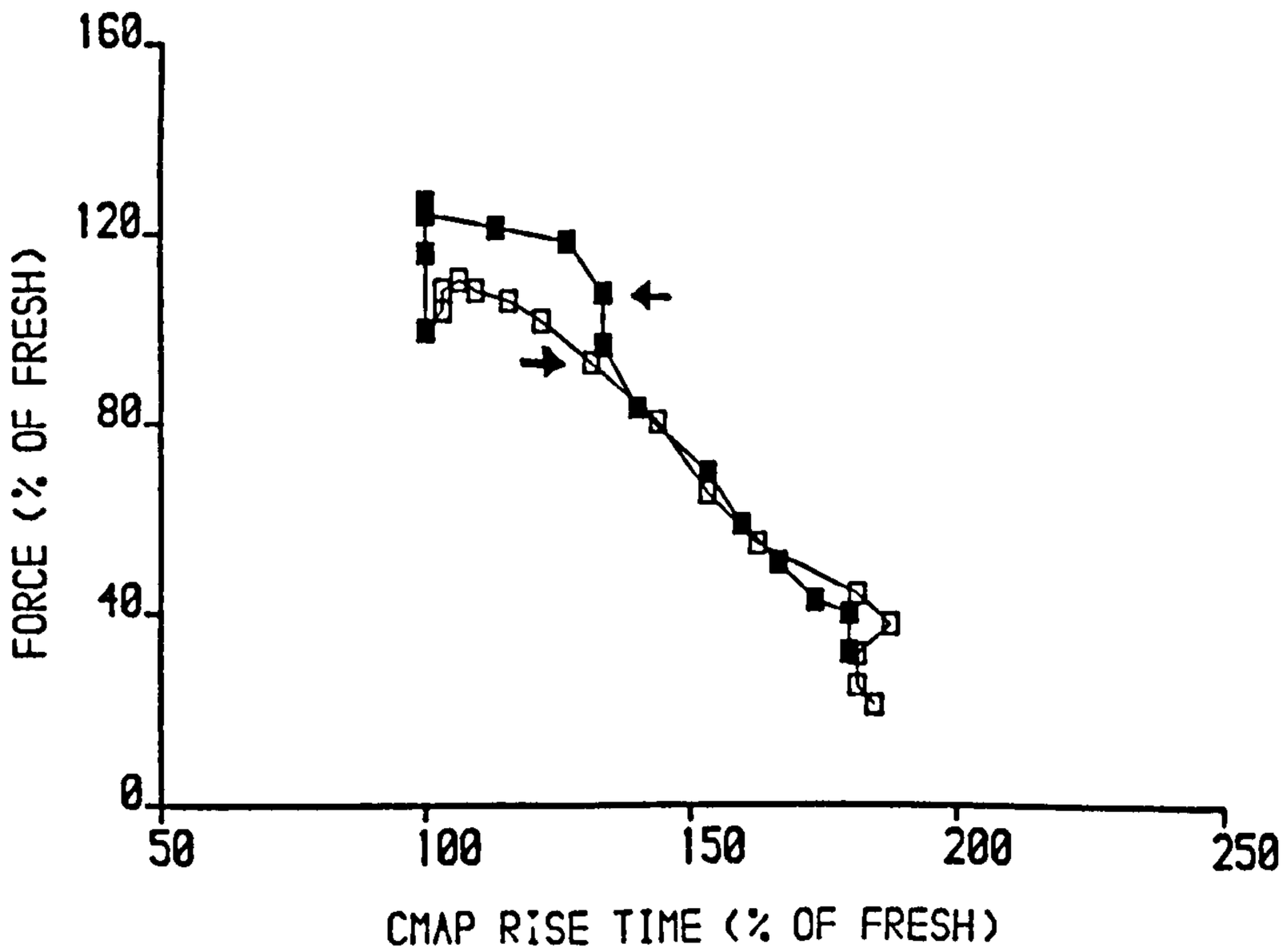
A


120
80
40
0
FORCE (% OF FRESH)
0
1000
2000
3000
4000
IMPULSE NUMBER

B


160
120
80
40
0
FORCE (% OF FRESH)
50
100
150
200
250
CMAP RISE TIME (% OF FRESH)

C


160
120
80
40
0
FORCE (% OF FRESH)
50
100
150
200
250
CMAP RISE TIME (% OF FRESH)

4.4 DISCUSSION

The results of this study suggest that the decline in force is largely due to excitatory factors rather than metabolic factors as proposed by Duchateau & Hainuat (1985) and Garland *et al.*, (1986) since the relationship between force generation and numbers of impulses delivered appears to be largely frequency independent (but see 4.4.2). The linear relationship between MRR and contractile activity supports the suggestion that contractile activity is probably a measure of the metabolic cost of a contraction, but for only up to 30 max.seconds of activity. This finding therefore further supports the suggestion that metabolic factors are not primarily responsible for the observed reduction in force. Furthermore, changes in CMAP amplitude appeared to be similarly independent of frequency, and may indicate that excitation propagation impairment contributes to force failure.

4.4.1 Frequency independence of MRR during stimulated activity

These results support and extend those of Wiles & Edwards (1982a) who investigated the frequency dependence of MRR in relation to activity performed for up to 11 max.seconds in a single individual. These results are therefore consistent with the view that the reduction in MRR reflects the contractile history of those fibres contracting at the time when the measurement is made.

It should be noted that the MRR is not a true measure of the relaxation rate since the latter is reflected by the exponential part of the decay curve of force. Previous studies in man have measured relaxation rate characteristics as the half-time of the exponential phase (Edwards *et al.*, 1972a) following voluntary contractions and the time from the last impulse to 95% or 50% of the plateau tension following stimulated contractions (Edwards *et al.*, 1977a) which correlates with the former measurement. The use of the first differential of force (which is a measure of the maximal rate of change of force), however, has been shown to provide a reliable and sensitive indicator of the changes in relaxation rate slowing, which overcomes some of the difficulties encountered in determining the MRR at the end of a voluntary

contraction (Wiles *et al.*, 1979; Wiles, 1980). It thus offers a convenient practical means of determining relaxation rate changes.

It has been assumed that the reduction in MRR during fatiguing activity is most likely to be dependent on metabolic factors (Wiles & Edwards, 1982a & b). This is supported by the observations of its failure to recover during ischaemic conditions (Wiles & Edwards, 1982b), its dependence on muscle temperature (Wiles & Edwards, 1982a) and a similar Q_{10} to many enzyme catalyzed reactions. The mechanism of MRR slowing is thought to involve a reduction in the rate of Ca^{2+} sequestration as a consequence of altered affinity of ATP for hydrolysis (Dawson *et al.*, 1980) or to changes in the rate of cross-bridge cycling (which is dependent on ATP for detachement) (Edwards *et al.*, 1975). The reduction in MRR is not due to preferential fatigue of type II fibres since MRR may decline in the absence of decrement in force or the action potential amplitude (Wiles, 1980). The energy turnover rate per unit force increases with a decrease in fusion of a tetanus during stimulated contractions as indicated by a greater metabolic heat production at low-frequencies of stimulation (Wiles, 1980), presumably due to the increase in number of cross-bridge reactivations and excess internal work done by the contractile elements against the series elastic components. For the adductor pollicis, oscillation of force and metabolic heat production per unit force are correlated at stimulation frequencies of 18Hz and below (Wiles, 1980). Hence, the rate of slowing of relaxation rate may be greater for a given amount of contractile activity performed during stimulated contractions at low frequency or repeated intermittent contractions. This may account for the discrepancies between various studies in which it has been proposed that fatigue is correlated with metabolic factors e.g., in NMR studies in frog muscle (Dawson *et al.*, 1978; 1980) and in man (Garland *et al.*, 1986) (see below) rather than to electrophysiological factors as suggested by others (Jones *et al.*, 1979; Marsden *et al.*, 1983). Although some degree of oscillation occurs during stimulation at 20Hz in fresh muscle, i.e., fusion is incomplete, from the work of Wiles (1980) it may be assumed that the excess internal work represented by this degree of fusion is

negligable. The demonstration of the frequency independence of MRR when expressed as a function of contractile activity in the present study therefore supports the view that the contractile activity performed by a muscle indirectly reflects the metabolic cost of a contraction for up to 30 max.seconds of activity at stimulation frequencies of 20Hz and above.

4.4.2 Frequency independence of force generation in relation to impulse numbers

It was noted that the decline in force at each stimulation frequency was generally biphasic and to a first approximation appears to be a function of impulse numbers delivered, although it was difficult to determine the precise point at which force declined more rapidly in Figure 4.3a. It could be argued that at low stimulation frequencies (20 and 40Hz), force declines earlier than at high frequencies (60-100Hz) since the rapid decline in force occured after 800 impulses compared to 1200 impulses respectively. However, the degree of variation in force between subjects was greatest at 20 and 40Hz (Table 4.2) and no significant difference was apparent between any of the force curves after 1200 impulses had been delivered. The more rapid decline in force at lower stimulations frequencies may have been due to a failure of complete activation of the contractile apparatus due to less accumulation of Ca^{2+} owing to excitation contraction coupling impairment (MacIntosh & Gardiner, 1987). Alternatively metabolic factors may, in part, contribute to force loss. However, markedly different amounts of contractile activity were performed at each frequency before the rapid phase of force loss was observed, and therefore argues against such a proposition. These results therefore are not identical to those of Marsden *et al.*, (1983), although this may be explained in part by differences in experimental design and presentation of the data. Furthermore, the results for only one individual were presented by Marsden *et al.*, (1983) and thus the variation of force curves for a group of individuals may reveal similar results to those of this study. It is also of interest that the reduction in force during ischaemic activity observed for frequencies between 20-100Hz in chapter 3 (Figure 3.6c-e) occurred

after approximately 1536 impulses, a figure not too dissimilar from that observed in the present study and that of Marsden *et al.*, (1983) over a similar frequency range.

These results constrast with those of Garland *et al.*, (1986), however, who found that similar amounts of contractile activity were performed at the point of fatigue of 15Hz and 30Hz tetani. From the discussion above (section 4.4.1) it would appear that this latter point may be explained by the under estimation of the metabolic requirements of the contraction performed at the lower frequency used by Garland *et al.*, (1986) since it is likely that fusion will be incomplete and hence more energy is utilized by the muscle for internal work against series elastic componants and in cross-bridge reactivations.

Further studies by Marsden *et al.*, (1976), which have been subsequently confirmed by others (Jones *et al.*, 1979), have shown that a maximum voluntary contraction may be mimicked by an electrical tetanus whose frequency is progressively reduced. This force curve, when plotted against numbers of impulses delivered, was similar to that obtained by a tetanus of 60Hz, but only 44% as long in duration (Merton, 1981). Since force failure appears dependent on the numbers of impulses delivered to the muscle and independent of stimulation frequency, it was suggested by Marsden *et al.*, (1976) that slowing of motor unit discharge rates observed during maximal voluntary contractions (Marsden *et al.*, 1971) may serve as a mechanism to prolong a sustained contraction. This is consistent with the observations of a concomitant slowing of relaxation rate (Bigland-Ritchie *et al.*, 1983) which would optimize force generation and offset the rapid decline in force that would otherwise result at high stimulation frequencies. The mechanism of myofibrillar activation failure is not clear, however.

4.4.3 Significance of prolongation of the action potential in relation to changes in excitation and force loss

A similar high-frequency safety-factor to that reported in Chapter 3, part III was apparent, demonstrating its independence to pattern of stimulation. Also in keeping with the observations in chapter 3, the reduction in force at lower frequencies

of stimulation was not directly related to CMAP amplitude. Clearly, the reduction in force cannot simply be explained by the decline in CMAP amplitude alone.

The prolongation of the CMAP during stimulated activity confirms the results of many other studies in which muscle is stimulated (Naess & Storm-Mathisen, 1955; Bigland-Ritchie *et al.*, 1979; Lindstrom *et al.*, 1970). Of interest was the frequency independence of the increase in the relative durations of the action potential, as determined from the distal latency and rise times, the former of which was directly proportional to impulse numbers delivered, although the latter appeared frequency independent in part. These observations are consistent with the view that accumulation of some factor produced per impulse impairs the propagation of the action potential resulting in a decrease in amplitude and conduction velocity which subsequently leads to excitation failure and hence force failure.

Several studies in isolated muscle preparations (Krnjevic & Miledi, 1958; Jones *et al.*, 1979; Bigland-Ritchie *et al.*, 1979; Jeul, 1988) and theoretical calculations (Adrian & Peachey, 1973) suggest that accumulation of K^+ in the extracellular space or the reduction of intracellular Na^+ at high stimulation frequencies leads to a decline in action potential amplitude and slowing of conduction velocity (see chapter 1, section 1.4.3.1). Marked changes of transmembrane Na^+ and K^+ concentrations have also been reported during exhaustive dynamic exercise in man (Sjogaard *et al.*, 1985; Vyskocil *et al.*, 1983) in which the interstitial K^+ measured with intramuscular electrodes has been reported to increase up to 8-9 mM (Vyskocil *et al.*, 1983) and recent studies have shown that isolated mouse extensor digitorum longus muscle fibres incubated with 15mM K^+ are rendered totally inexcitable with a doubling in conduction time at concentrations of 10mM (Jeul, 1988). Clearly, K^+ accumulation appears an attractive proposition to account for the changes observed.

Several studies have indicated that slowing of action potential propagation may be due to metabolic factors. Luttgau (1965) working on isolated frog muscle provided evidence to suggest that a factor associated with contractile activity

influenced CMAP characteristics since iodoacetate/cyanide poisoned muscle could conduct action potentials for longer without a decrement in amplitude compared to that in unpoisoned muscle (up to several thousand impulses at 100Hz compared to a few hundred in unpoisoned muscle). Mortimer *et al.*, (1970) found that slowing of conduction velocity in cat gastrocnemius muscle could be abolished by perfusing muscle with oxygen-free dextran solution. It has been suggested that H^+ ions may influence the action potential as a consequence of their charge effect on the charge associated with ion channels (Bass & Moore, 1973; Blatz, 1984). The results of the present study would not support such a view, however, since the relationship of CMAP latency and rise time were frequency dependent with respect to contractile activity performed. Furthermore, the study of Luttgau (1965) is not supported by those of Jones & Bigland-Ritchie (1986) since stimulated iodoacetate poisoned mouse diaphragm produces similar qualitative changes in the action potential shape when differing concentrations of KCl are added to the bathing medium. This may have been due to the different types of preparation used (single frog fibre preparations compared to strips of mouse diaphragm) and experimental set up in which the external K^+ may have been more diffuse in the preparation of Luttgau (1965). Eventual failure of the action potential was reported in the studies of Luttagau for a fibre stimulated at 77Hz for more than 1200 impulses however. Isolated short toe muscle fibres of *xenopus* which have been fatigued to the extent where force generation is minimal have been shown to produce similar changes in action potential amplitude and duration compared to functional cells during stimulated activity (Lannergren & Westerblad, 1987). This was attributed to the failure of regenerative activity in the T-tubules, since a reduction in action potential amplitude was not seen for de-tubulated fibres. Although the relationship between regenerative activity in the T-tubule and changes in the sarcolemmal membrane is not clear, the influence of altered T-tubular electrolytes on changes in sarcolemmal function may be of significance. Changes in the intracellular pH of mouse soleus and extensor digitorum longus within the physiological range (pH 6.4-7.4) is reported to have minimal effects on conduction

velocity and does not influence excitability (Jeul, 1988) and in man, there appears to be no correlation between recovery of the action potential and venous lactate concentrations from the biceps brachii (Duchateau *et al.*, 1987). These studies therefore suggest that the accumulation of metabolic bi-products of muscle contraction, in particular H^+ does not appear to alter action potential characteristics during stimulated activity.

The frequency independence of CMAP distal latency and rise time suggests that the continous efflux/influx of K^+ and Na^+ exceeds the counteracting action of the Na^+/K^+-ATPase pump, which restores the ionic gradient, over the frequency range investigated. *In vitro* studies suggest skeletal muscle posseses a considerable spare capacity for active Na^+/K^+ transport (Clausen, 1986). However, during activity, K^+ deficiency and anoxia, the loss of K^+ into the venous blood suggests that the function of Na^+/K^+ pump becomes limiting (Clausen, 1986). The results of the present study suggest that the maximum capacity for Na^+/K^+ restoration is exceeded at 15Hz. It has been suggested that the reserve capacity of the Na^+/K^+ pump in human muscle corresponds to a maintained stimulation frequency of 40Hz (Vollestad & Sejersted, 1988). This discrepancy may be accounted for, in part, by a reduction in the rate of Na^+/K^+ exchange during stimulated activity, possibly as a consequence of a reduction in Na^+/K^+-ATPase activity due to inhibition by metabolites or reduced energy supply. This proposition is supported by the greater contractile activity performed at low-stimulation frequencies compared to high and also by the suggestion that ATP turnover rate is reduced during fatigue (Edwards *et al.*, 1975; Dawson *et al.*, 1978). Thus, the changes in CMAP characteristics at low-stimulation frequencies may still be attributed to the accumulation of extracellular K^+ or depletion of intracellular Na^+ as a consequence of altered metabolism.

Other changes in membrane ion conductances may also occur as a result of altered transmembrane K^+ gradients, possibly involving an inactivation of the voltage-dependent Na^+ channel (Jeul, 1988). The uptake of Ca^{2+} into the T-tubules which appears dependent on the numbers of stimuli delivered (Bianchi & Narayan,

1982) may also influence Ca^{2+} sensitive K^+ channels (Pallota, 1985) and may have important implications on excitation-contraction coupling impairement within the T-tubular network. In fact, due to the high surface-to-to volume ratio of the T-tubules, it is likely that alterations in ionic composition may alter to a greater extent (Jones *et al.*, 1979) than that at the sarcolemmal membrane. This may explain the similarity of the decline in force curves observed in the present study which is not reflected by the alterations in CMAP amplitude.

It was noted, however, that although the changes in distal latency and rise time in the present study were in the same direction, they were not identical i.e., some degree of frequency dependence of rise-time was apparent and peaking of rise-time was observed at all but the lowest frequencies. The two measurements permit the separation of the serial and parallel componants of the CMAP; the distal latency encompasses the passage of the evoked potential from the skin surface, along the motor nerve, across the NMJ to the sarcolemmal membrane reflecting the passage of the fastest propagated action potential, whereas the rise time is a measure of the passage of the summated individual action potentials along the sarcolemmal membrane alone. Several factors may influence CMAP shape, including: a) slowing of conduction velocity, b) dispersion of action potentials (due to differences in fibre type properties and NMJ transmission slowing) c) NMJ failure and d) a reduction in excitability of individual action potentials. The contribution of NMJ delay to prolongation of the action potential is likely to be negligable in relation to the changes along the sarcolemmal membrane and is discussed further in the next chapter. Hence, the measurement of distal latency may be assumed to reflect the changes in conduction velocity along the sarcolemmal membrane. The influences of conduction velocity slowing and dispersion cannot be differentiated, however, from measurements of rise time and furthermore, CMAP distal latency may not be linearly related to conduction velocity due to the possible dropping out of cells. It has been suggested that changes in dispersion and conduction velocity may be separated by determining changes in area in relation to CMAP amplitude changes, since

conduction velocity results in broadening of the action potential (and hence an increase in area) without loss of amplitude whereas dispersion would lead to broadening with a reduction in CMAP amplitude and hence no increase in area (Bigland-Ritchie *et al.*, 1979; Duchateau & Hainaut, 1985). It is probable that similar interpretations can be made from measurements of rise time and amplitude. This form of analysis assumes that only conduction velocity and dispersion alter CMAP amplitude whereas (c) and (d) above may also contribute to a reduction in CMAP amplitude. Thus, the interpretation of the CMAP is complex and has to be made with caution.

The apparent frequency dependence of CMAP rise time when expressed as a function of impulse number may therefore have been due to a greater dispersion of individual action potentials at high frequency. This may additionally explain, in part, the reduction of CMAP amplitude at high stimulation frequencies. Alternatively, failure at the NMJ may have occurred resulting in a reduction in CMAP amplitude, although this is probably unlikely initially since force did not decline by a comparable amount at high stimulation frequencies and furthermore, the shape of the action potential would be expected to become 'sharper' i.e., more defined peaks. Direct stimulation of isolated curarized mouse muscle produces a similar decrement of force as in indirectly stimulated preparations (Jones, 1981) further supporting the suggestion that failure at the NMJ is not necessarily involved. A further explanation is that recovery of transmembrane electrolytes may have occured in the period between each impulse in a number of fibres resulting in recovery of the CMAP rise time at low stimulation frequencies which would also result in maintenance of CMAP amplitude. This would be expected to be reflected by some degree of recovery of the distal latency too if electrolyte accumulation is assumed to be responsible for prolongation of the action potential, assuming that the distal latency predominantly reflects changes in conduction velocity of the sarcolemmal membrane. This was not observed however.

The plateauing and subsequent decline of CMAP rise time (Figure 4.2) is most likely to reflect an apparent loss of excitability in a number of fibres. A loss in excitability is most likely to occur in the slowest propagating fibres, thereby explaining the observed increase in CMAP rise time. The dependence of force on CMAP rise time was not directly related at the highest and lowest frequencies investigated however (Figure 4.9) suggesting that CMAP rise time is not a good indicator of the changes in excitation that may lead to force failure.

4.4.4 Limitations of experimental design

Mention of the limitations of the experimental design must be made, since it is not possible to investigate the relative changes and interrelationships of MRR, force and excitation in a single continuous stimulated fatiguing contraction.

In expressing each variable as a percentage of the control tetanus, an error was introduced when each variable was expressed as a function of impulse number. It was not possible to standardise the number of impulses delivered in each tetanus at various frequencies since this could have resulted in prolonged contractions at low-frequencies of stimulation and possibly very brief contractions at high stimulation frequencies. e.g., at 100Hz a 1 second contraction would deliver 100 impulses, whereas at 20Hz this number of impulses would require 5 seconds of tetanic contraction and 10 seconds at 10Hz. Therefore, a 2 second contraction was used as a compromise. This also eliminated possible errors in obtaining the evoked CMAP since skin movements over the muscle may occur as a contraction develops, thereby altering the position of the recording electrode during recording.

A further comment on the frequencies of stimulation used in this study should be made, since these fall on the upper end (plateau portion) of the frequency:force curve (Figure 3.3). Consequently the differences in the contractile activity performed at each frequency was small, thus explaining the apparent frequency independence of MRR when expressed as a function of duration of contraction (Figure 4.2c). It may be argued that these frequencies are non-physiological, since the mean motor-unit discharge rate in the adductor pollicis during sustained maximal activity is of the

order 10-30Hz (Bellemare *et al.*, 1983) except at the onset of a contraction where much higher discharge rates may be achieved. Nevertheless, these frequencies permit investigation of the frequency dependence of fatigue mechanisms over a five-fold range of stimulation frequency, over which it was shown in the previous chapter and the present study, that a biphasic reduction of force does occur, despite differences in CMAP amplitude. Furthermore, the lowest frequency of 20Hz may be regarded as physiological since this frequency falls within the mean motor unit discharge rate recorded from the adductor pollicis (Bellemare *et al.*, 1983).

Ideally, further experiments could be performed in which single sustained contractions are empoyed to fatigue the adductor pollicis. This has the advantage that no recovery of excitatory factors or force may occur as is possible during the brief intervals used to allow relaxation of force, but in such a design it would not be possible to obtain relaxation rate characteristics, or the detailed changes that may occur in action potential characteristics. Furthermore, it would still not be possible to satisfactorally design a paradigm stimulation pattern in which changes in contractile characteristics and excitation could be related to impulse numbers for the reasons given above.

4.5 CONCLUSIONS

This study supports the hypothesis that fatigue may result predominantly from failure of excitation propagation, possibly due to accumulation or depletion of electrolytes or an unknown factor at the sarcolemmal membrane or deeper within the T-tubular network. Although the contractile activity performed over the frequency range studied was linearly related to MRR, thereby supporting the suggestion that contractile activity reflects metabolic cost of a contraction under ischaemic conditions, it was clear that force failure was not dependent on contractile activity performed. The dependence of force on excitatory factors is further evident from the observation of the frequency independence of the serial order effect of prolongation of the distal latency in a preliminary study presented for one subject. It is unlikely that the factor prolonging the action potential is a metabolic by-product of contraction

alone since changes in the CMAP distal latency and rise-time were also frequency dependent with respect to contractile activity performed, although the involvement of metabolic factors at low stimulation frequencies cannot be dismissed. Further studies are indicated to determine whether membrane function is influenced by metabolic factors, or just excitatory events alone. However, the possibility exists that prolongation of the action potential is also due to compartmentalization of accumulation of electrolytes, depleted energy supply for membrane function or to metabolic charge effects which do not depend on the contractile activity performed by the muscle, although there is no evidence to support this. The numbers of stimuli delivered and the pattern in which they are delivered may therefore be of importance in determining the rate at which muscle fatigues.

4.6 SUMMARY

1. Human adductor pollicis was fatigued on separate occasions using intermittent trains of stimuli (2 seconds activity, 0.5 seconds rest) over a range of frequencies of 20-100 Hz during ischaemic conditions to investigate whether excitatory or metabolic factors influence force generation and to determine the frequency dependence of MRR slowing in relation to contractile activity performed as an indicator of metabolic cost of a contraction. In addition, the changes in CMAP characteristics during stimulation at 15-100Hz was investigated in one subject.

2. The relationship between MRR (which is thought to be dependent on metabolic factors) and contractile activity was frequency independent for up to 30 max. seconds of activity. The relation of force generation and excitation to contractile activity (reflecting the metabolic cost of a contraction) was therefore determined.

3. Force decline was biphasic and frequency independent when expressed as a function of impulse number. CMAP amplitude declined in a similar, but not identical, fashion to force, the decline in CMAP amplitude being greatest initially at high stimulation frequencies. A similar 'safety-factor' to that reported in chapter 3, in

which high frequency force was maintained despite a marked decline in CMAP amplitude, was observed.

4. CMAP distal latency, reflecting a slowing of conduction velocity in the fastest propagated action potentials, increased linearly and proportionally with impulse numbers delivered and was frequency independent over the frequency range investigated. CMAP rise time similarly increased, but appeared in part to be frequency dependent, possibly due to phase changes of conduction velocity of different fibre type populations.

5. These results are consistant with the view that accumulation of a factor in relation to each impulse delivered alters the propagation of excitation which may lead to the eventual failure of propagation, particularly within the T-tubules and hence result in a decline in force. Numbers of impulses delivered during stimulated activity may be an important factor in determining the fatiguability (rate of force loss) in a stimulated contraction.

CHAPTER 5: ISCHAEMIC AND NON-OCCLUDED RECOVERY OF THE ACTION POTENTIAL FOLLOWING LOW AND HIGH - FREQUENCY ISCHAEMIC STIMULATED ACTIVITY

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CHAPTER 5: ISCHAEMIC AND NON-OCCLUDED RECOVERY OF THE ACTION POTENTIAL FOLLOWING LOW AND HIGH - FREQUENCY ISCHAEMIC STIMULATED ACTIVITY

5.1 INTRODUCTION

The interrelationship between energy and excitation failure in the development of fatigue may be of considerable importance (Edwards, 1981; Stephens & Taylor, 1972). Several studies indicate that the by-products of metabolism, notably H^+ ions, alter membrane characteristics resulting in a reduction in membrane potential (Jennische, 1982) and prolongation of conduction velocity (Mortimer *et al.*, 1970; Lindstrom *et al.*, 1970; Juel, 1988). Bigland-Ritchie *et al.*, (1979) have disputed the suggestion by Mortimer *et al.*, (1970) that slowing of conduction velocity is due to accumulation of metabolic by-products of contraction since similar changes may be produced by accumulation of K^+ or reduction of Na^+, including a reduction in CMAP amplitude. This view is supported by the results of the previous chapter, in which it was shown that CMAP distal latency was independent of frequency of stimulation for frequencies of 15-100Hz. Furthermore, Bigland-Ritchie *et al.*, (1979) have shown that the reduction in CMAP area that occurs with high frequency stimulation in the adductor pollicis may be reversed by reducing the frequency of stimulation, further supporting the above view. This latter point may be of particular importance in maintaining excitation and hence preserving force generation.

A study was therefore designed to investigate the effect of energy metabolism, as reflected by the contractile activity performed, on excitation to clarify the role of energy metabolism on membrane function during stimulated fatiguing activity. In order to isolate the effects of energy metabolism on membrane function, the *recovery* of the CMAP was investigated during ischaemic conditions following stimulated activity at a high (100 Hz) and low (20 Hz) frequency for identical numbers of impulses, where the stimulated adductor pollicis model may be considered as a 'closed system' preventing the supply of substrates necessary for oxidative metabolism

or removal of by-products of metabolism or electrolytes. In addition, the recovery of twitch force was also monitored to investigate changes in excitation-contraction coupling which is thought to be impaired as a result of failure of membrane impulse propagation (Milner-Brown & Miller, 1986). Only three normal subjects were investigated in this study due to the discomfort induced by the prolonged stimulation and ischaemia.

Patients with the disturbances of energy metabolism offer an alternative approach to the study of muscle function in man, allowing advantage to be taken of paradigm disorders which allow the study of selected mechanisms in isolation. Patients with McArdle's disease (McArdle, 1951) lack the enzyme myophosphorylase and thus have a defect in anaerobic glycogenolysis. For this reason, two patients under the care of Professor RHT Edwards were additionally studied in the hope of further elucidating the role of energy supply in membrane function and fatigue. A similar stimulation procedure to that employed in normal subjects was used, but fatiguing activity consisted of a shorter stimulation period at only 20 Hz in order not to induce contracture formation which may result from excessive activity in these individuals. Thus under ischaemic conditions, energy supply from both oxidative phosphorylation and glycogenolysis, is impaired.

5.2 METHODS

5.2.1 Experimental subjects

Three normal subjects (2 males, 1 female), aged 24-25 and two patients with McArdle's disease (males aged 23 & 44) participated in this study.

5.2.2 Measurement of contractile properties

The adductor pollicis of the left hand was prepared for electrophysiological monitoring and stimulation as described in chapter 2.

5.2.3 Electromyography

Records of single evoked action potentials were selected and digitized under computer control (Apple IIe) and stored on floppy disc (NIC MED 80) for subsequent analysis as described in chapter 4.

5.2.4 Experimental procedure

5.2.4.1 Normal subjects

The changes in action potential characteristics were studied during stimulated fatiguing stimulated activity at 20 and 100Hz and during subsequent ischaemic and non-occluded recovery. Each subject undertook three activity protocols, two at 20Hz and one at 100Hz.

Three control twitches were performed in fresh muscle at 30 second intervals to obtain the CMAP followed by a 2 second control 100Hz tetanus to obtain maximal force generation for the calculation of total contractile activity performed (see analysis). A sphygmomanometer cuff was then inflated above the upper arm and maintained at 220 mmHg. This was followed by three minutes ischaemic rest to reduce oxgen reserves available for oxidative metabolism (see chapter 3, part III).

Fatiguing activity then commenced and consisted of continuous stimulation at 20 and 100 Hz for 2400 impulses to invoke a reduction in CMAP amplitude and a comparable increase in CMAP distal latency (see chapter 4). Twitch stimuli were then introduced at 1 and 5 seconds and at intervals of 10 seconds up to 60 seconds followed by 30 second intervals for up to 4 minutes to follow ischaemic recovery of the action potential. The cuff was then deflated to follow the non-occluded recovery

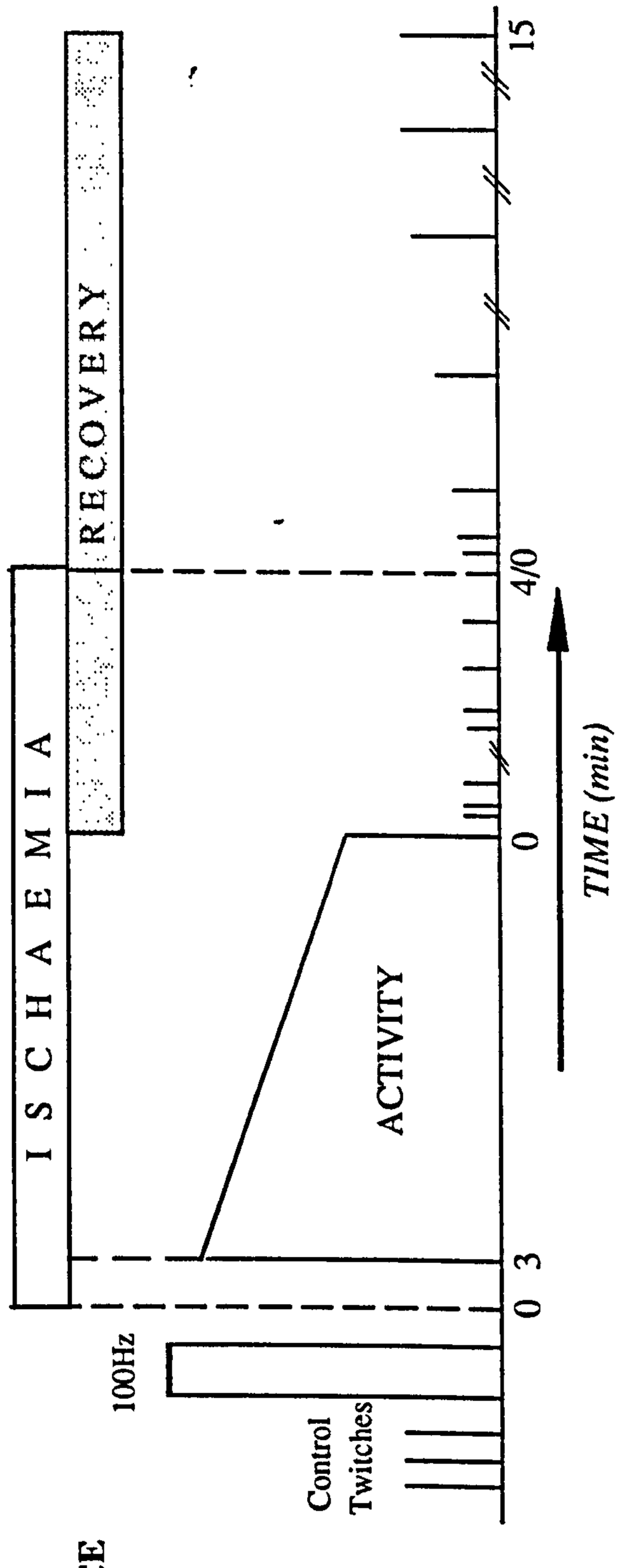


Figure 5.1 Schematic of protocol used to fatigue the adductor pollicis and follow ischaemic/non-occluded recovery of the twitch. The CMAP was digitized and stored on floppy disc. Activity was as follows: 1. Normal subjects; 20Hz x 2400 impulses, 20Hz x 1200 impulses, 100Hz x 2400 impulses. 2. McArdle patients; 20Hz x 1040 impulses. Time is not shown to scale.

of the action potential at 0.5, 1, 2, 3, 5, 10 and 15 minutes. A diagrammatic representation of the stimulation protocol is shown in Figure 5.1.

In order to compare the influence of numbers of stimuli on changes in action potential characteristics and subsequent recovery, the adductor pollicis was stimulated continuously at 20 Hz for 1200 impulses and ischaemic recovery of the action potential documented as above.

These protocols were performed in random order and at least one week apart to ensure full recovery of the adductor pollicis.

5.2.4.2 Patients

One activity procedure was performed on the two McArdle patients. A reduction in CMAP amplitude was induced by stimulation of the adductor pollicis at 20 Hz for 1040 impulses. This produced a similar decrement in CMAP amplitude to that obtained in normal subjects stimulated at 20 Hz for 2400 impulses and is also similar to that previously used by Brandt *et al.*, (1977) and Wiles (1980) for the same muscle, allowing a direct comparison of CMAP characteristics and force generation. Ischaemic recovery of action potential characteristics was followed in the same manner as for normal subjects, but for only up to one minute to prevent possible discomfort and possible development of contracture. The cuff was then deflated and non-occluded recovery was monitored as described for the normal subjects.

5.2.5 Analysis

The evoked CMAP was analysed in the same manner as in chapter 4. CMAP distal latency, rise time and amplitude were measured from the stored data on the computer and expressed as a percentage of that obtained in fresh muscle. Control values were calculated as the mean of the pre-fatigue twitch data. Force data of the twitch was measured from the oscillograph record and expressed as a percentage of twitch force in fresh muscle. The total contractile activity performed was calculated as described in chapter 4 (analysis) and expressed as max.seconds. Figures have been constructed using individual sets of data since statistical analysis was not possible in view of the small number of subjects investigated.

5.3 RESULTS

5.3.1 NORMAL SUBJECTS

5.3.1.1 Effect of repetitive stimulation on CMAP characteristics and force generation

Ischaemic stimulated activity resulted in changes of CMAP characteristics following all contractions. The most notable changes were a decline in CMAP amplitude, which was most marked at the end of 100Hz stimulation and least following 1200 impulses at 20Hz. CMAP distal latency increased most markedly following 2400 impulses at both 20 and 100Hz (Figure 5.2). However, the changes in CMAP rise time were not the same as for those of distal latency: At 100 Hz, CMAP rise time decreased in two subjects and increased by 10% in one subject (Figure 5.3).

The greatest reduction in force occured after stimulation at 20 Hz for 2400 impulses, although this varied between 64 and 91.5%. The reduction in force at 100Hz for the same number of impulses was not as great however. The shorter contraction at 20 Hz resulted only in a modest reduction of force by about 25% for all three subjects. The contractile activity performed for each contraction reflected the changes in force for the given durations of contraction and are shown in Table 5.1. Thus, more contractile activity was performed during activity at 20Hz for 2400 impulses compared to that after 1200 impulses and after 2400 impulses at 100Hz.

5.3.1.2.a Ischaemic recovery of the action potential

CMAP amplitude recovered almost immediately at all frequencies, but was incomplete following stimulated activity at 20Hz for 2400 impulses, and plateaued at 70% of control value for one subject (HG) and 90% for the other two subjects (HD, AL) (Figure 5.2). Recovery of CMAP amplitude was more rapid following activity at 100Hz compared to that at 20Hz for the same number of impulses. Distal latency also appeared to recover rapidly initially at 100 Hz, but this was also incomplete and similarly for 20Hz after 1200 and 2400 impulses. The distal latency then appeared to plateau following all three contractions for the continued duration of the ischaemic period (Figure 5.3). The level at which distal latency plateaued was linearly correlated

to the contractile activity performed (shown in Figure 5.5) (r=0.989) and could be expressed by the equation:

$$\% \text{ distal latency} = 0.241 \times \text{contractile activity} + 99.5$$

In contrast, for subjects HG and AL, the CMAP rise time did not appear to recover following activity at 20Hz for 2400 impulses except for subject HD (Figure 5.3). Even then this was incomplete and remained greater than that observed for the other two subjects. Rise time following 100Hz activity did not change throughout the ischaemic recovery period.

5.3.1.2.b Ischaemic recovery of the twitch

Twitch force was depressed throughout the ischaemic recovery period following all three activity protocols (Figure 5.6). The decline in twitch force was greatest following activity at 20 Hz for 2400 impulses and least following activity at 100 Hz for the same number of impulses. Furthermore, twitch force partially recovered following activity at 100Hz to near initial control values. In two subjects (AL and HG) twitch force declined by only 20 and 27% respectively following activity for 1200 impulses at 20Hz, but then declined in the next 30 seconds of ischaemic rest to 40% of initial control values after which only a slight decline in force occurred.

5.3.1.3.a Non-occluded recovery of the action potential

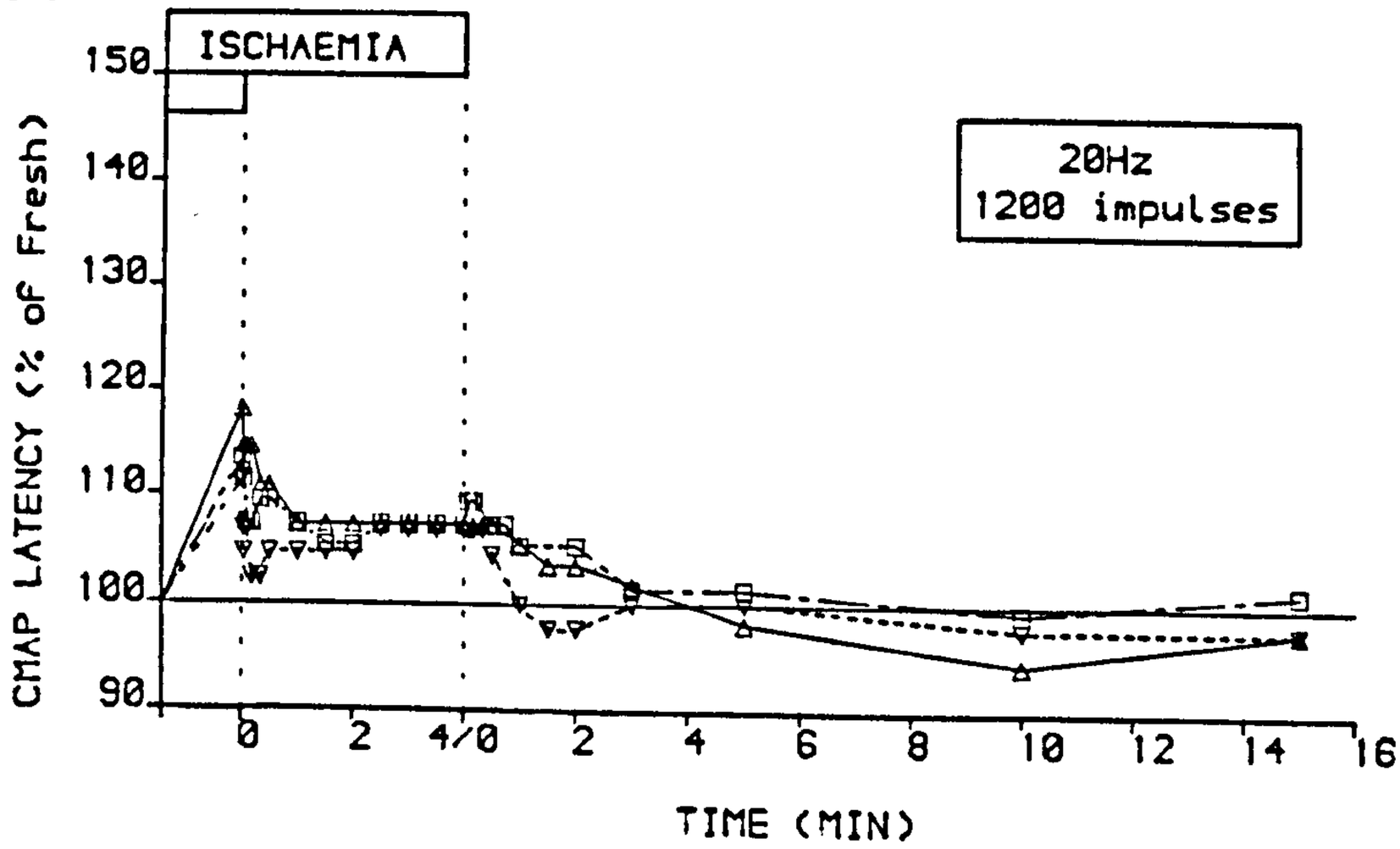
On deflation of the cuff, which permitted reperfusion of the muscle with blood, CMAP amplitude rapidly recovered to near prefatigue values in all cases and in some instances increased beyond initial control values (Figure 5.4). The changes in CMAP distal latency and rise time following the contractions at 20Hz followed a slower time course, recovering to initial values by 2-3 minutes. Thereafter the rise time was noted to become faster than the initial control values by as much as 10-20% (Figure 5.3), which did not recover during the 15 minute period of recovery. The

Table 5.1 Contractile activity (as max.seconds) performed by each subject during fatiguing activity of the adductor pollicis of 20Hz - 1200 impulses, 20Hz - 2400 impulses, 100Hz - 2400 impulses.

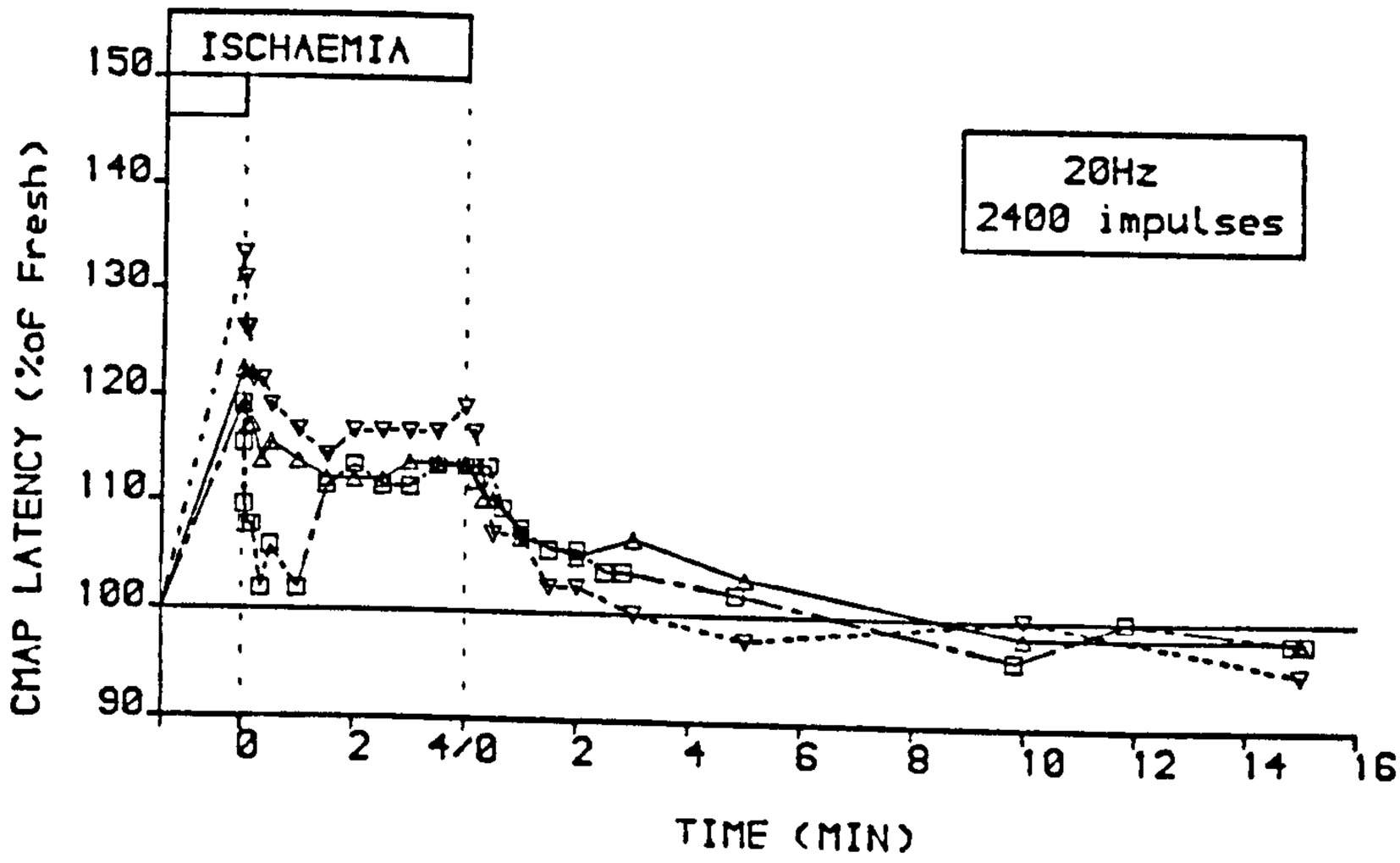
Subject	Contractile activity
20Hz, 1200impulses	
HG	53.2
HD	42.9
AL	38.7
100Hz, 2400 impulses	
HG	17.4
HD	19.1
AL	14.7
20Hz, 2400 impulses	
HG	38.6
HD	81.0
AL	57.1

Figure 5.2 Ischaemic and non-occluded recovery of CMAP distal latency following: A) 1200 impulses at 20Hz, B) 2400 impulses at 20Hz and C) 2400 impulses at 100Hz in three subjects. Following activity the most marked changes in distal latency occurred after 2400 impulses at 20 and 100Hz. However, the greatest degree of ischaemic recovery occurred after activity at 100Hz, although this was not quite complete. Distal latency did not recover completely following activity at all frequencies until circulation was restored. Symbols: (△) AL, (▽) HD, (□) HG.

A


ISCHAEMIA
20Hz
1200 impulses
CMAP LATENCY (% of Fresh)
150
140
130
120
110
100
90
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)

B


ISCHAEMIA
20Hz
2400 impulses
CMAP LATENCY (%of Fresh)
150
140
130
120
110
100
90
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)

C

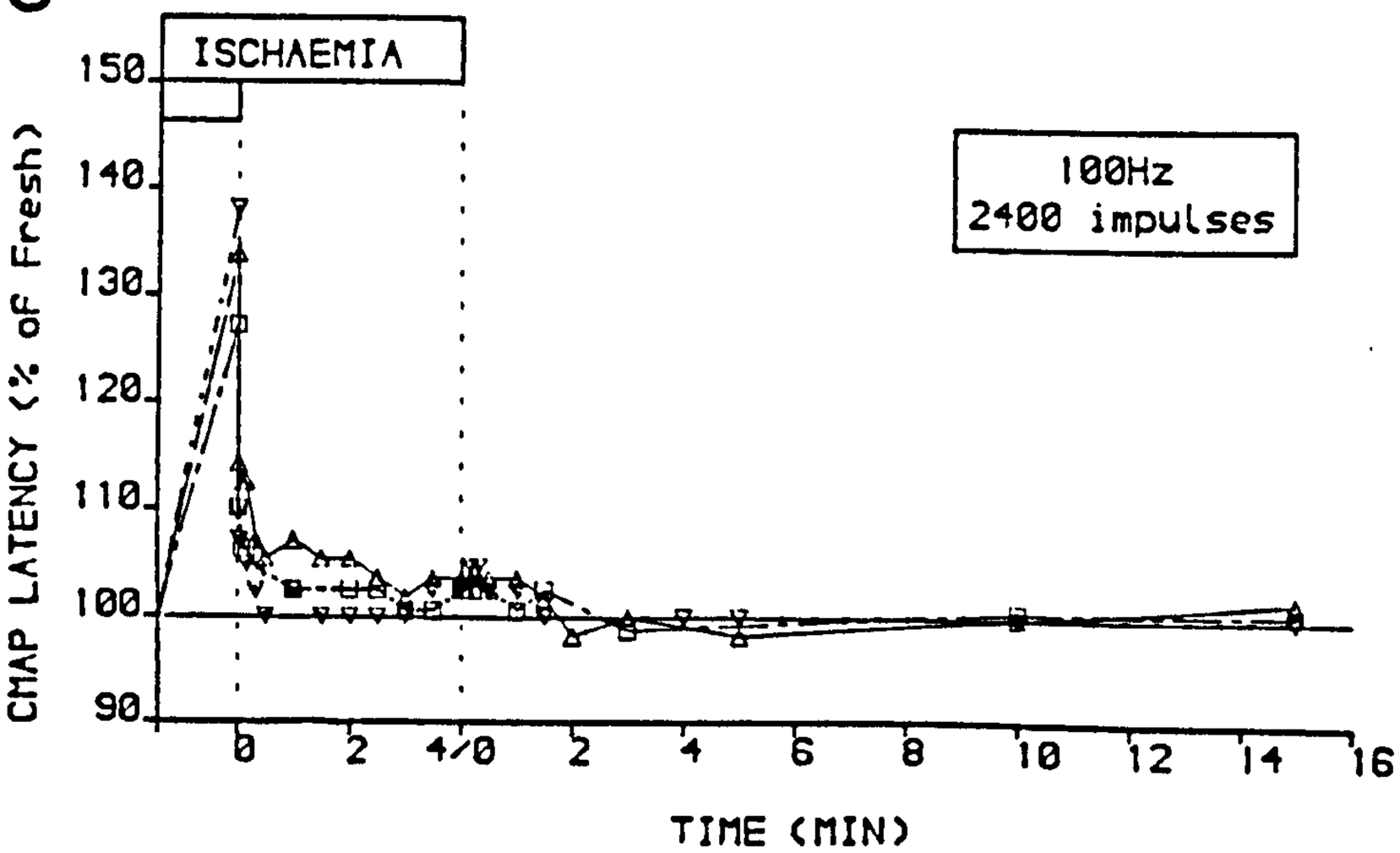
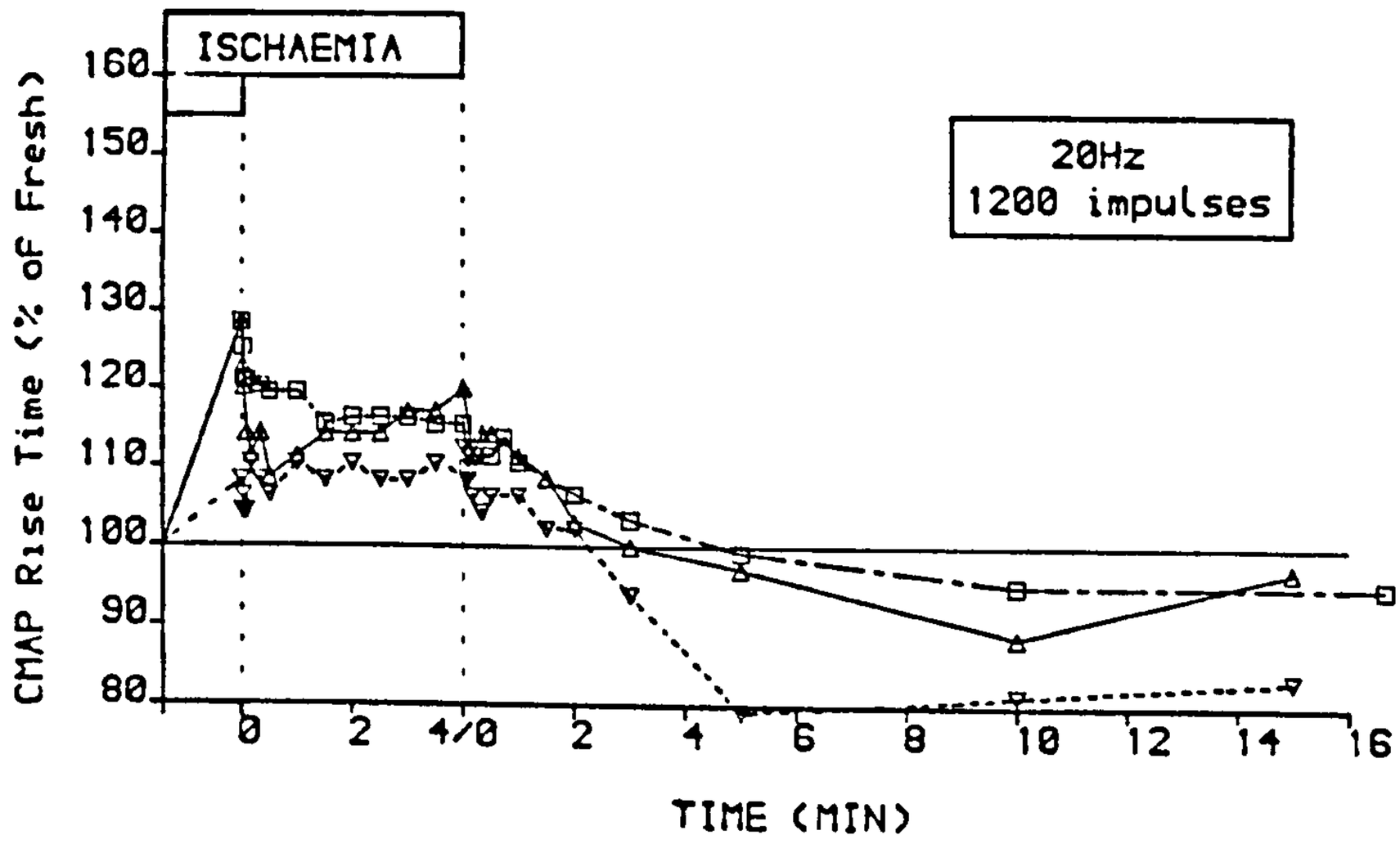
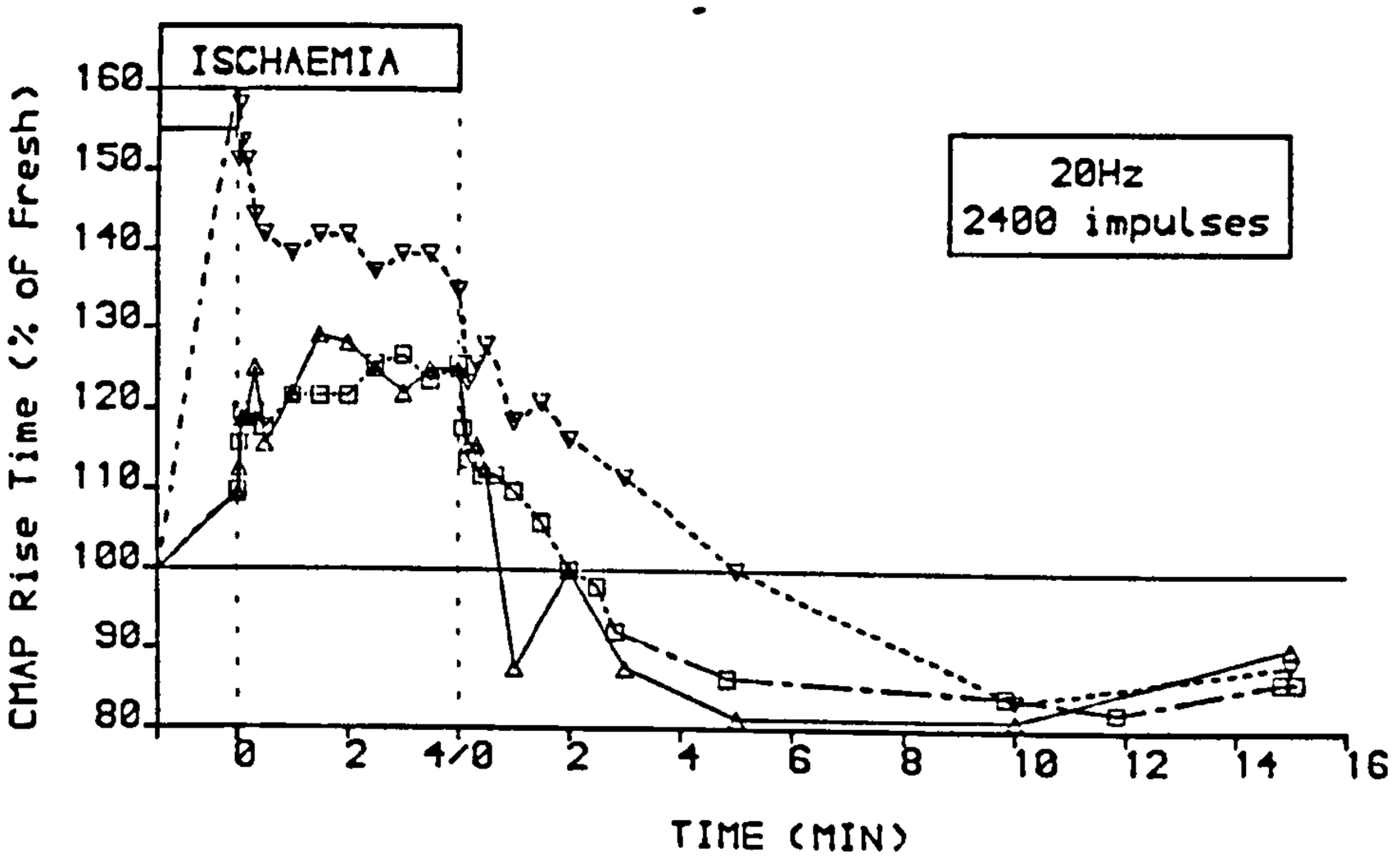

ISCHAEMIA
100Hz
2400 impulses
CMAP LATENCY (% of Fresh)
150
140
130
120
110
100
90
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)

Figure 5.3 Ischaemic and non-occluded recovery of CMAP rise time following: A) 1200 impulses at 20Hz, B) 2400 impulses at 20Hz and C) 2400 impulses at 100Hz in three subjects. The greatest change in CMAP rise time occurred after 2400 impulses at 20Hz. In contrast no increase was noted at 100Hz. CMAP rise time did not recover during ischaemic conditions until circulation was restored, during which CMAP rise time appeared to decrease to values less than initial control. Symbols: (△) AL, (▽) HD, (□) HG.

A


ISCHAEMIA
20Hz
1200 impulses
CMAP Rise Time (% of Fresh)
160
150
140
130
120
110
100
90
80
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)

B


ISCHAEMIA
20Hz
2400 impulses
CMAP Rise Time (% of Fresh)
160
150
140
130
120
110
100
90
80
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)

C

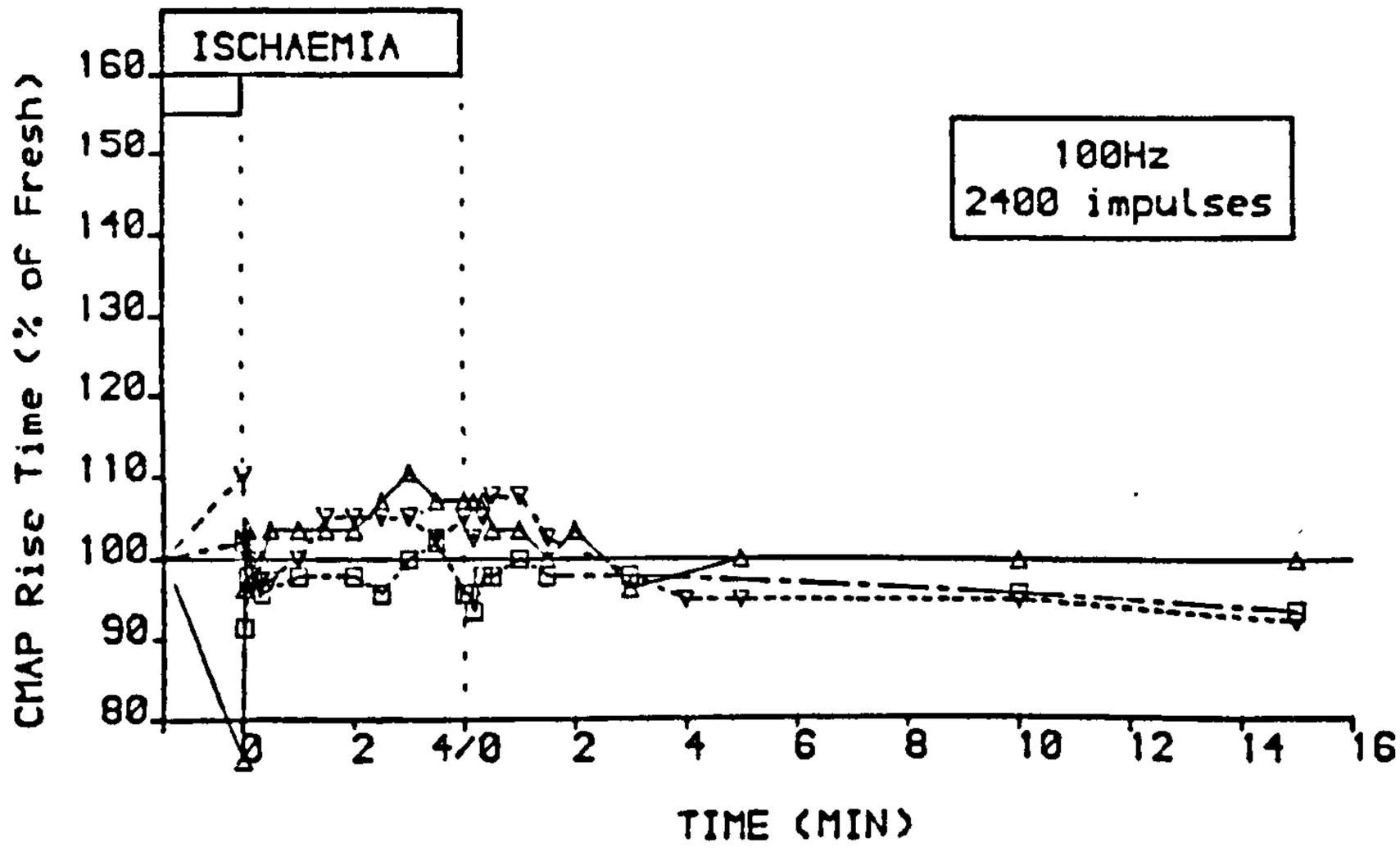

ISCHAEMIA
100Hz
2400 impulses
CMAP Rise Time (% of Fresh)
160
150
140
130
120
110
100
90
80
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)

Figure 5.4 Ischaemic and non-occluded recovery of CMAP amplitude following: A) 1200 impulses at 20Hz, B) 2400 impulses at 20Hz and C) 2400 impulses at 100Hz in three subjects. The reduction in CMAP amplitude was least following 1200 impulses activity at 20Hz and greatest following activity at 100Hz. CMAP amplitude showed near complete recovery during ischaemic conditions after activity in all cases. This was in marked contrast to that seen for CMAP distal latency (Figure 5.2).
Symbols: (△) AL, (▽) HD, (□) HG.

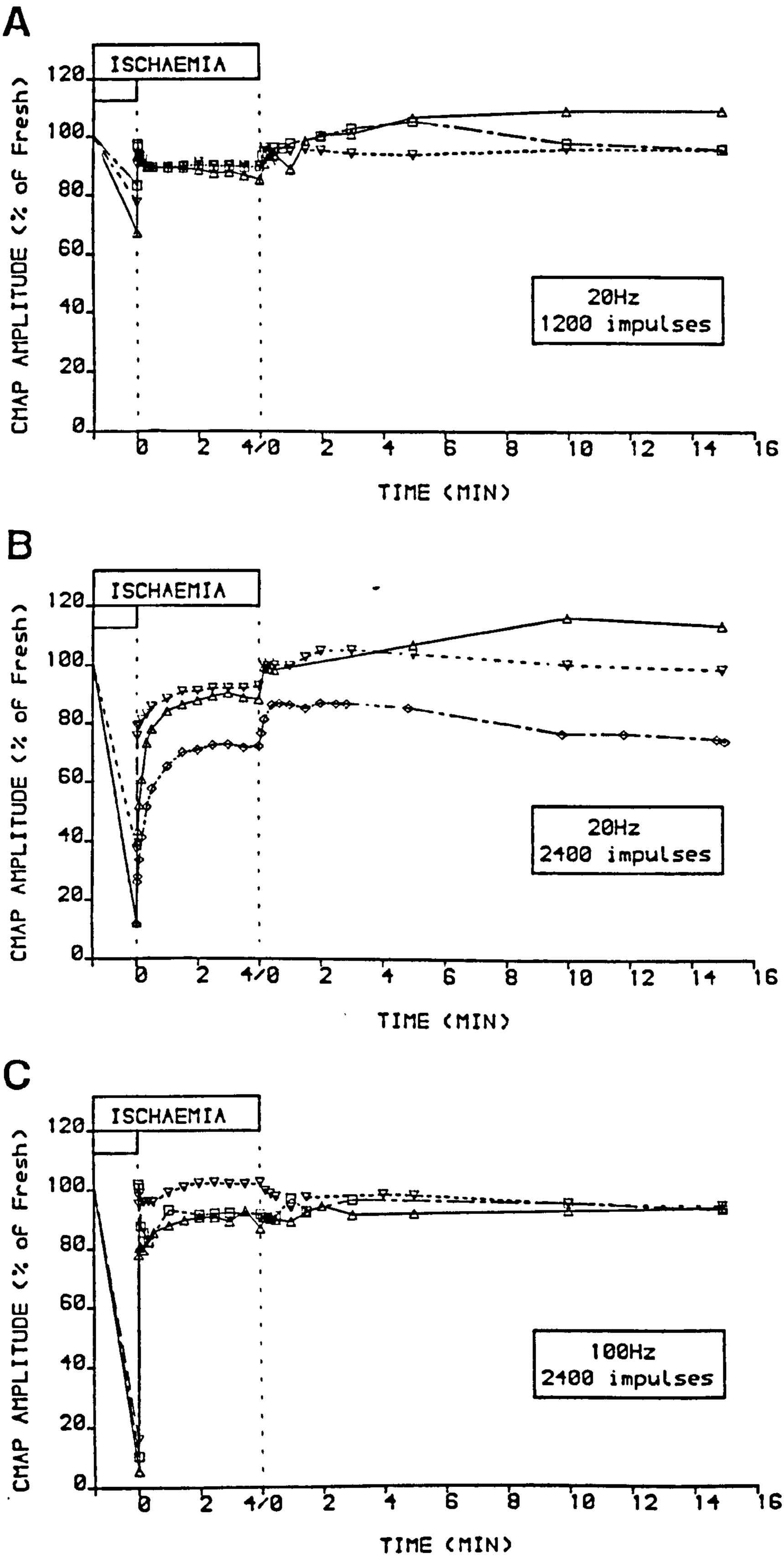

A
ISCHAEMIA
CMAP AMPLITUDE (% of Fresh)
120
100
80
60
40
20
0
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)
20Hz
1200 impulses
B
ISCHAEMIA
CMAP AMPLITUDE (% of Fresh)
120
100
80
60
40
20
0
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)
20Hz
2400 impulses
C
ISCHAEMIA
CMAP AMPLITUDE (% of Fresh)
120
100
80
60
40
20
0
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)
100Hz
2400 impulses

Figure 5.5 Relationship of ischaemic plateau value of CMAP distal latency (as a percentage of control) and contractile activity performed during stimulated activity. The line shown is the best fit (least squares regression) for all the points shown and is described by the equation:

$$\% \text{ distal latency} = 0.241 \times \text{contractile activity} + 99.5 \quad (r = 0.989)$$

Symbols are as follows: (●) AL, (φ) HD, (o) HG.

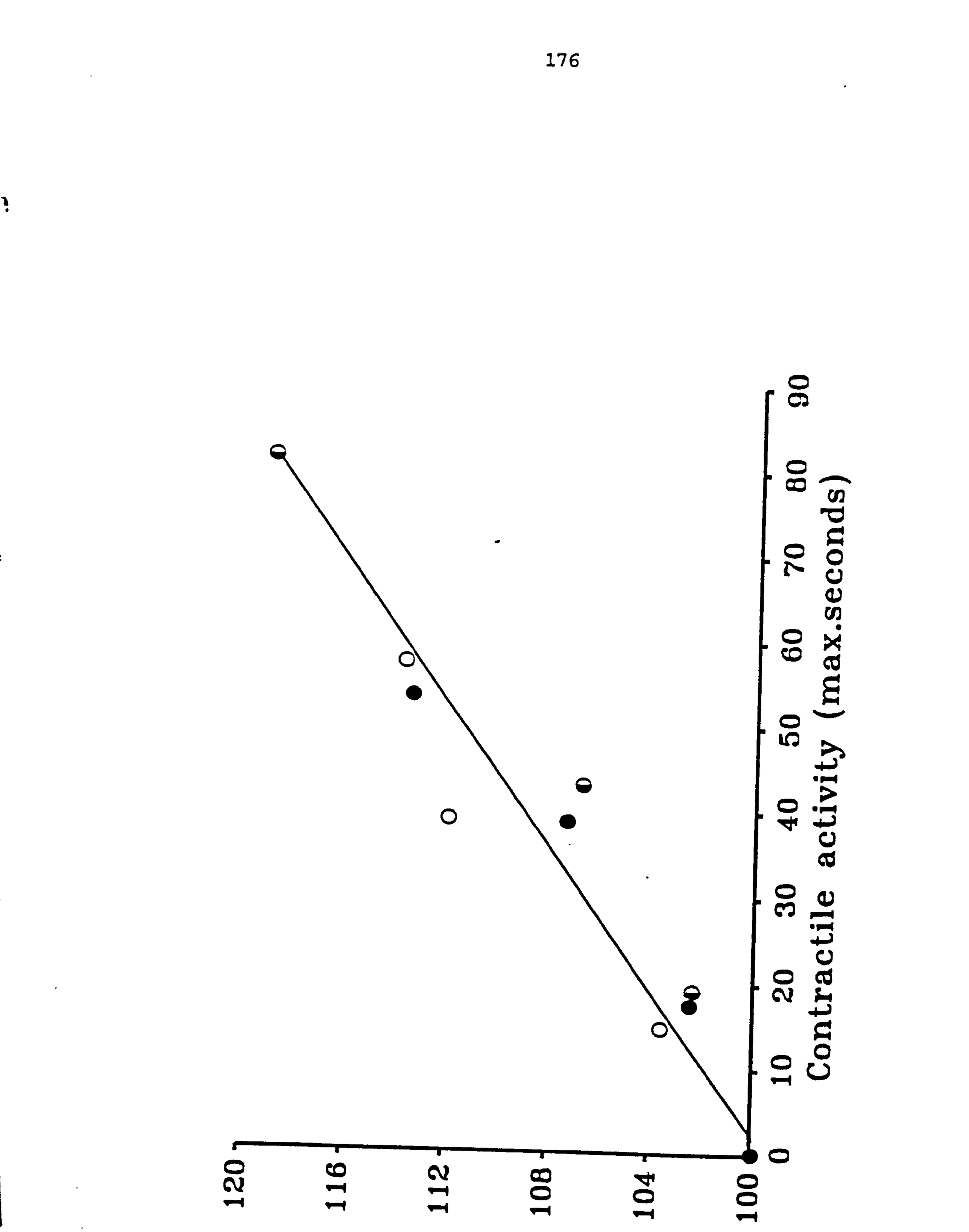
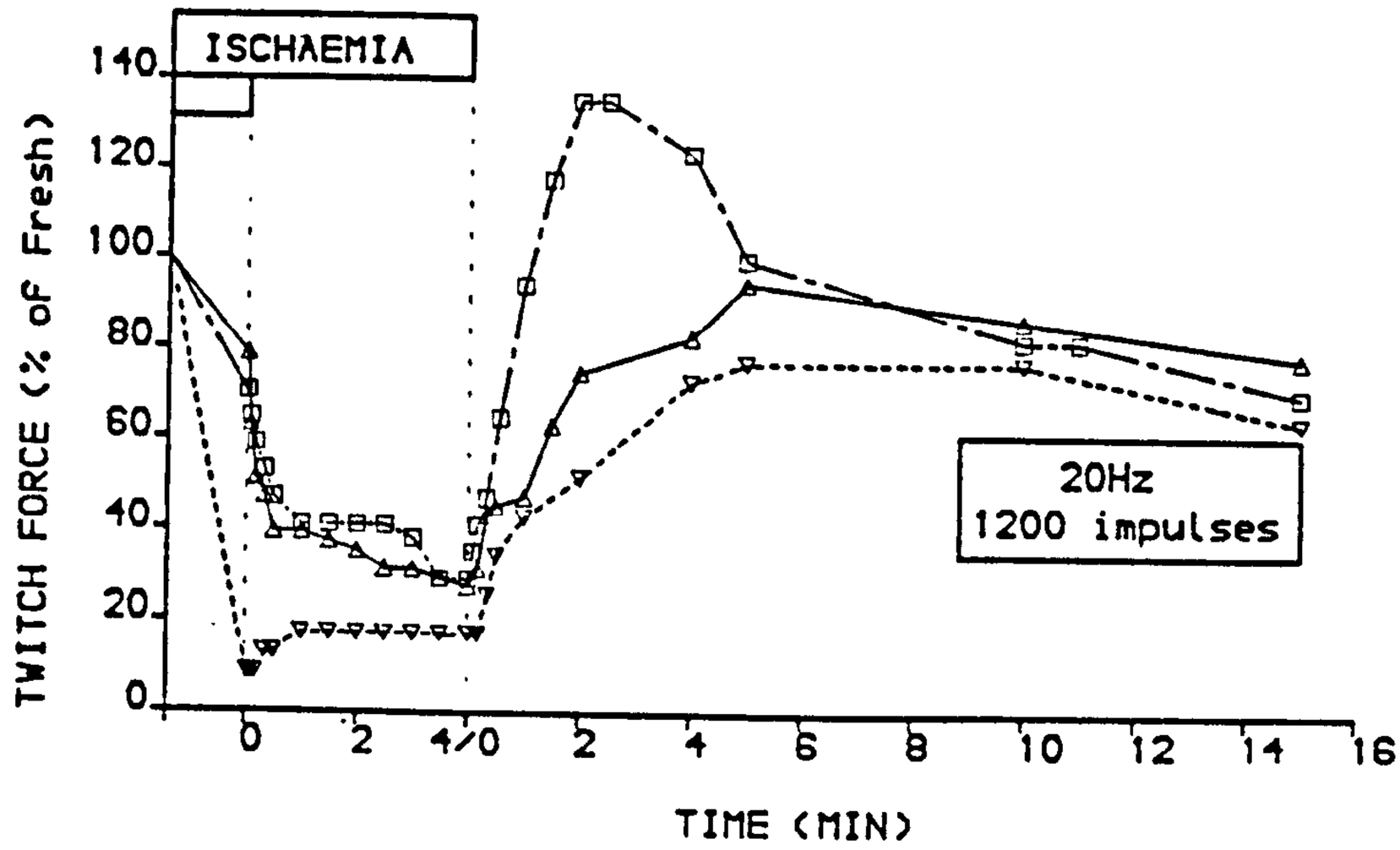

Distal Latency (% of fresh)
120
116
112
108
104
100
Contractile activity (max.seconds)
0
10
20
30
40
50
60
70
80
90

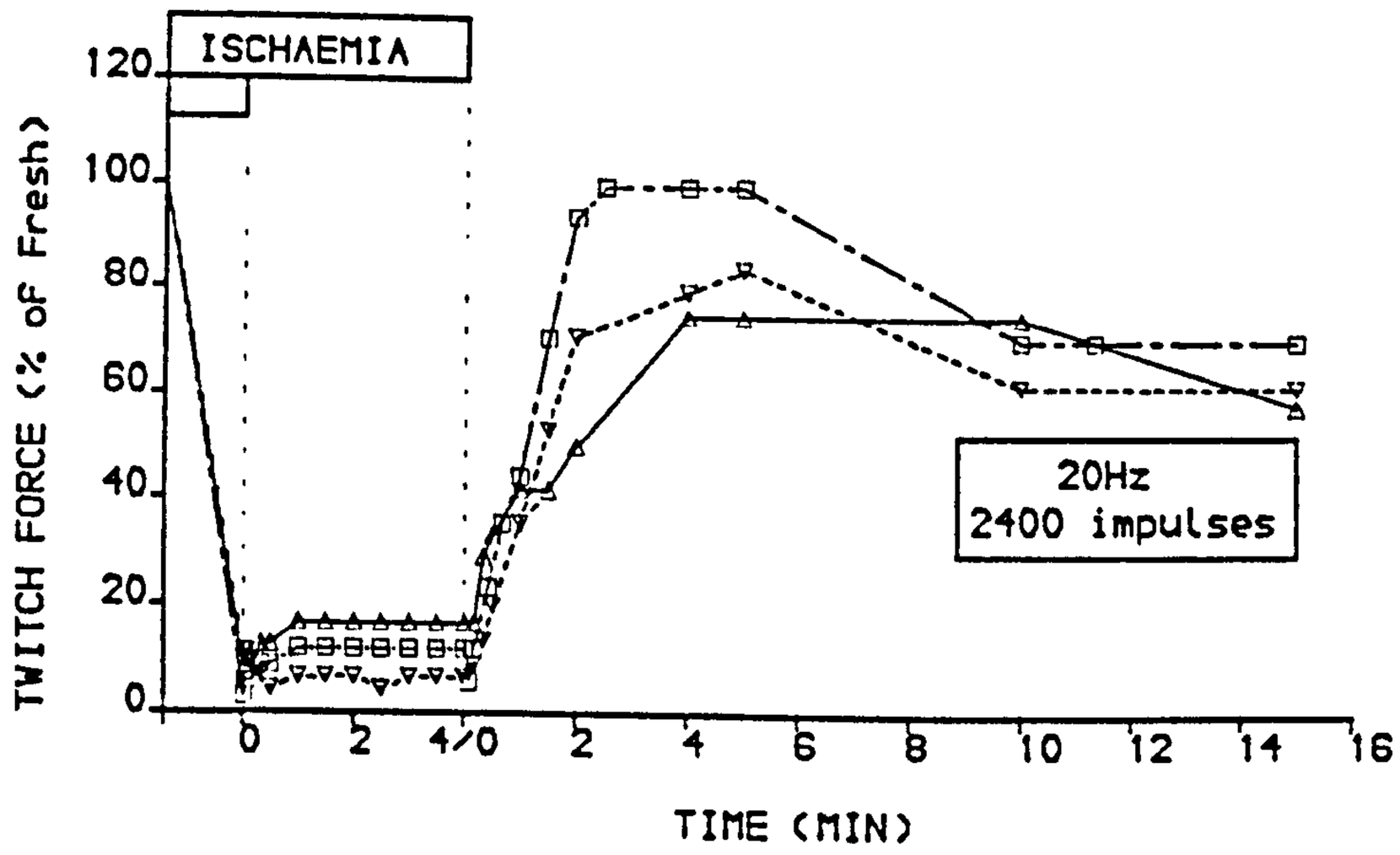
Figure 5.6 Ischaemic and non-occluded recovery of twitch force following: A) 1200 impulses at 20Hz, B) 2400 impulses at 20Hz and C) 2400 impulses at 100Hz in three subjects. Note that little or no recovery of twitch force occurs during conditions of ischaemia until the circulation is restored. The degree of decline of the twitch was least following activity at 100Hz and greatest following activity at 20Hz for 2400 impulses, possibly reflecting the metabolic cost of the contraction.

Symbols: (△) AL, (▽) HD, (□) HG.

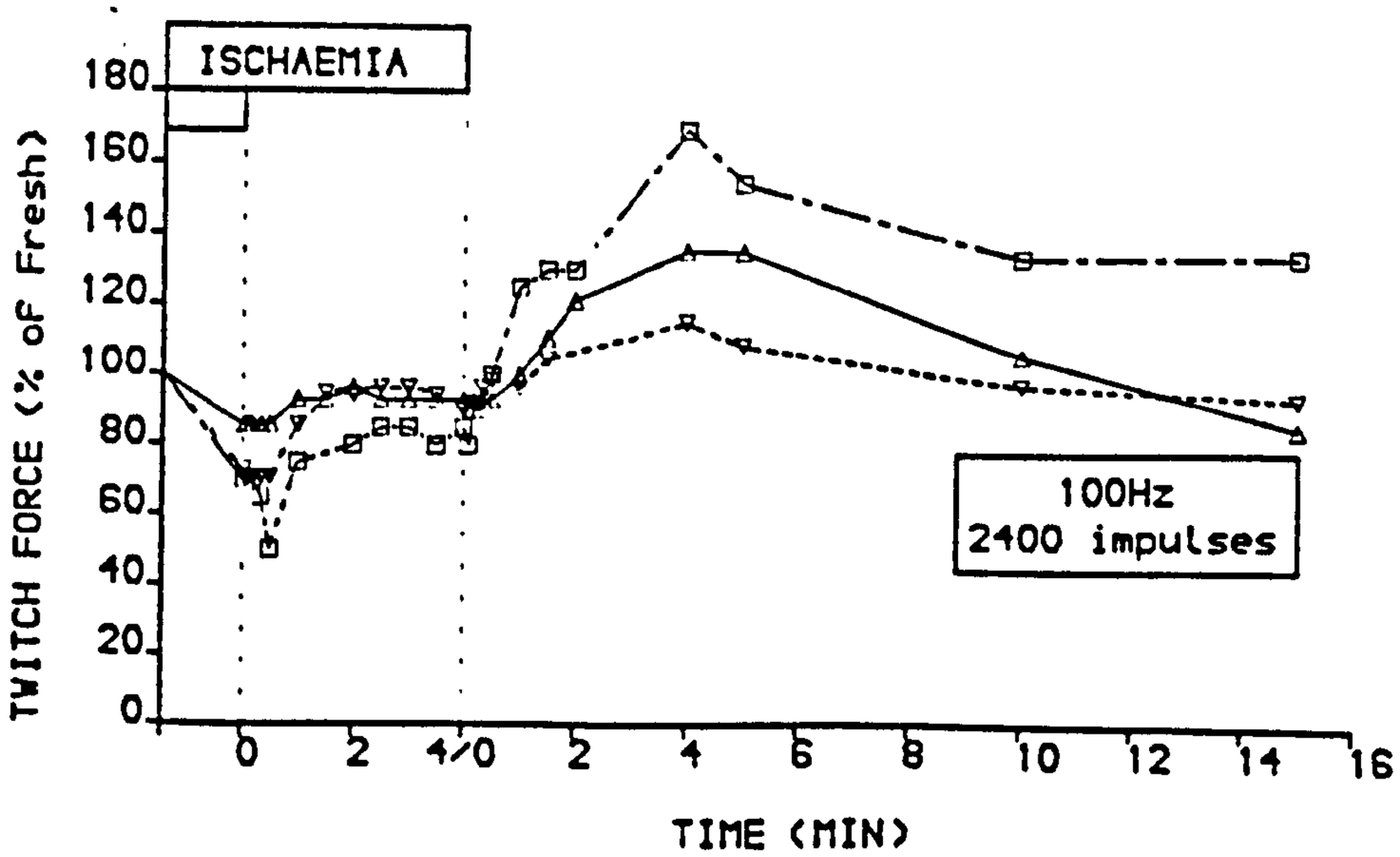
A


ISCHAEMIA
TWITCH FORCE (% of Fresh)
140
120
100
80
60
40
20
0
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)
20Hz
1200 impulses

B


ISCHAEMIA
TWITCH FORCE (% of Fresh)
120
100
80
60
40
20
0
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)
20Hz
2400 impulses

C


ISCHAEMIA
TWITCH FORCE (% of Fresh)
180
160
140
120
100
80
60
40
20
0
0
2
4/0
2
4
6
8
10
12
14
16
TIME (MIN)
100Hz
2400 impulses

non-occluded changes in rise-time following activity at 100Hz was not as marked however.

5.3.1.3.b Non-occluded recovery of twitch force

The recovery of non-occluded twitch force in subjects AL and HD was maximal, but incomplete by 5 minutes following activity at 20 Hz for 1200 and 2400 impulses. However, twitch force in subject HG potentiated to 138% of the initial value by 2 minutes after restoration of the circulation following activity at 20 Hz for 1200 impulses, and fully recovered by 2 minutes after 2400 impulses activity which then declined to 70% of the initial fresh muscle value.

In contrast to the recovery following activity at 20Hz, twitch force potentiated during the non-occluded recovery period following activity at 100 Hz in all subjects (Figure 5.6). This was again greatest for subject HG.

5.3.2PATIENTS

5.3.2.1 Effect of repetitive stimulation on action potential characteristics and force generation

The changes in CMAP characteristics before, after ischaemic recovery and following non-occluded recovery are shown in Figure 5.7 for one McArdle patient along with those of a normal subject who underwent 2400 impulses activity at 20Hz to reduce CMAP amplitude to a similar degree.

After approximately 20 seconds stimulation (400 impulses) CMAP amplitude and force rapidly declined to 50% of control values. By the end of stimulated activity (1040 impulses), the CMAP amplitude had declined to 21.5% and 49.2% of initial fresh control values in the two patients, whereas the distal latency and rise time was similar to pre-fatigue control values (Figure 5.8). Force declined to 22.5 and 34% of initial values, but the baseline had increased by 5.5 and10.9%respectively, indicating that a small degree of contracture may have developed as a result of the fatiguing procedure. The patients did not complain of cramp or pain when asked, however.

5.3.2.2 Ischaemic recovery of the CMAP and twitch force

CMAP amplitude did not recover following the stimulated activity in either patient. It was not possible to measure twitch force since the baseline force had increased and therefore made these measurements unreliable.

5.3.2.3 Non-occluded recovery of the CMAP and twitch force

On return of circulation, CMAP amplitude partially recovered, but in patient AR only 20% recovery occurred and the experiment had to be terminated after 4 minutes due to discomfort, presumably as a result of contracture development. In patient JD, CMAP recovered to 73% of initial by 10 minutes.

Figure 5.7 Tracings of CMAP before, and during ischaemic and non-occluded recovery, following stimulated ischaemic activity at 20Hz for A) 1050 impulses in a patient with McArdle's disease (JD) and B) 2400 impulses in a normal subject. Note that CMAP amplitude did not recover during ischaemic rest in the McArdle patient.

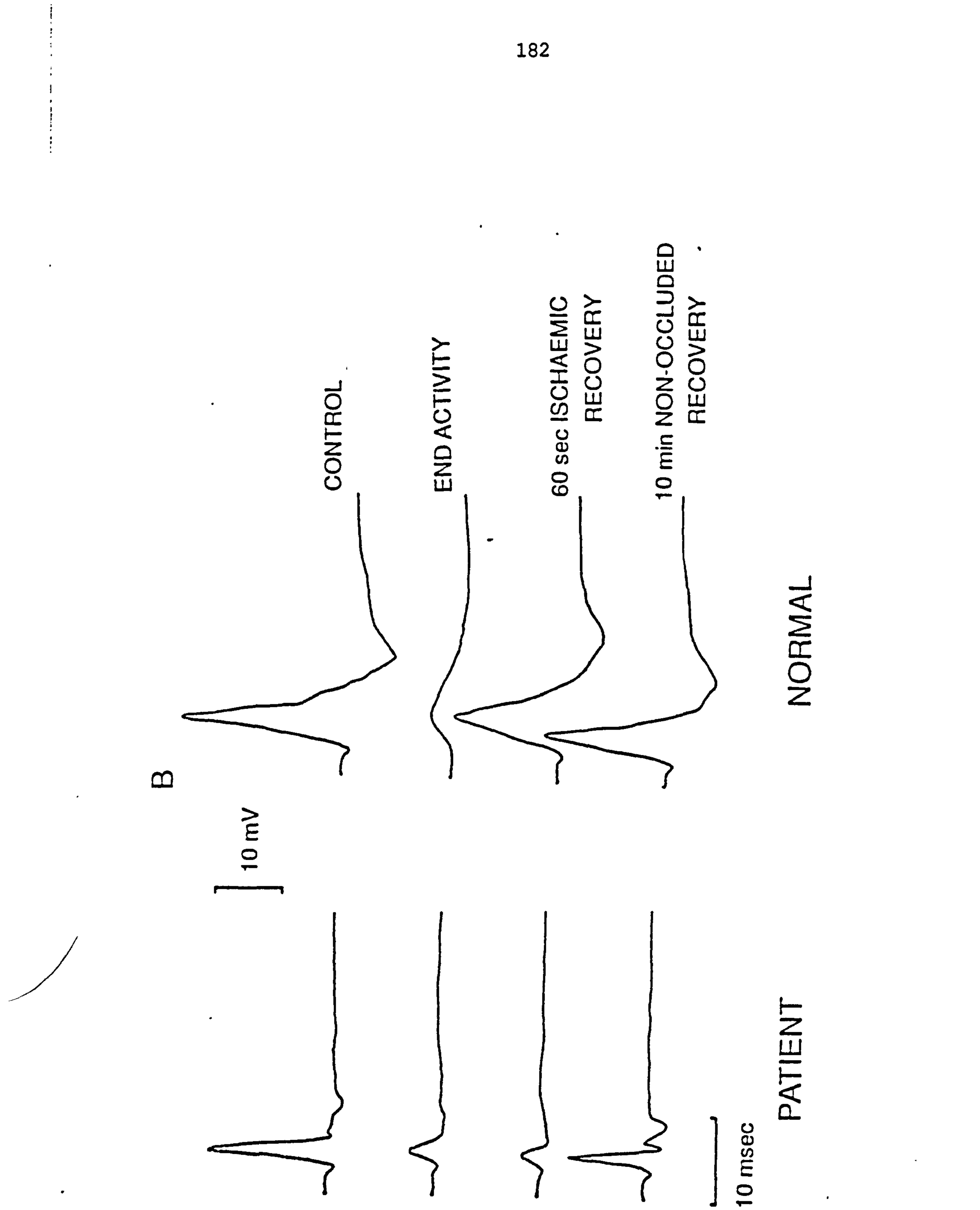
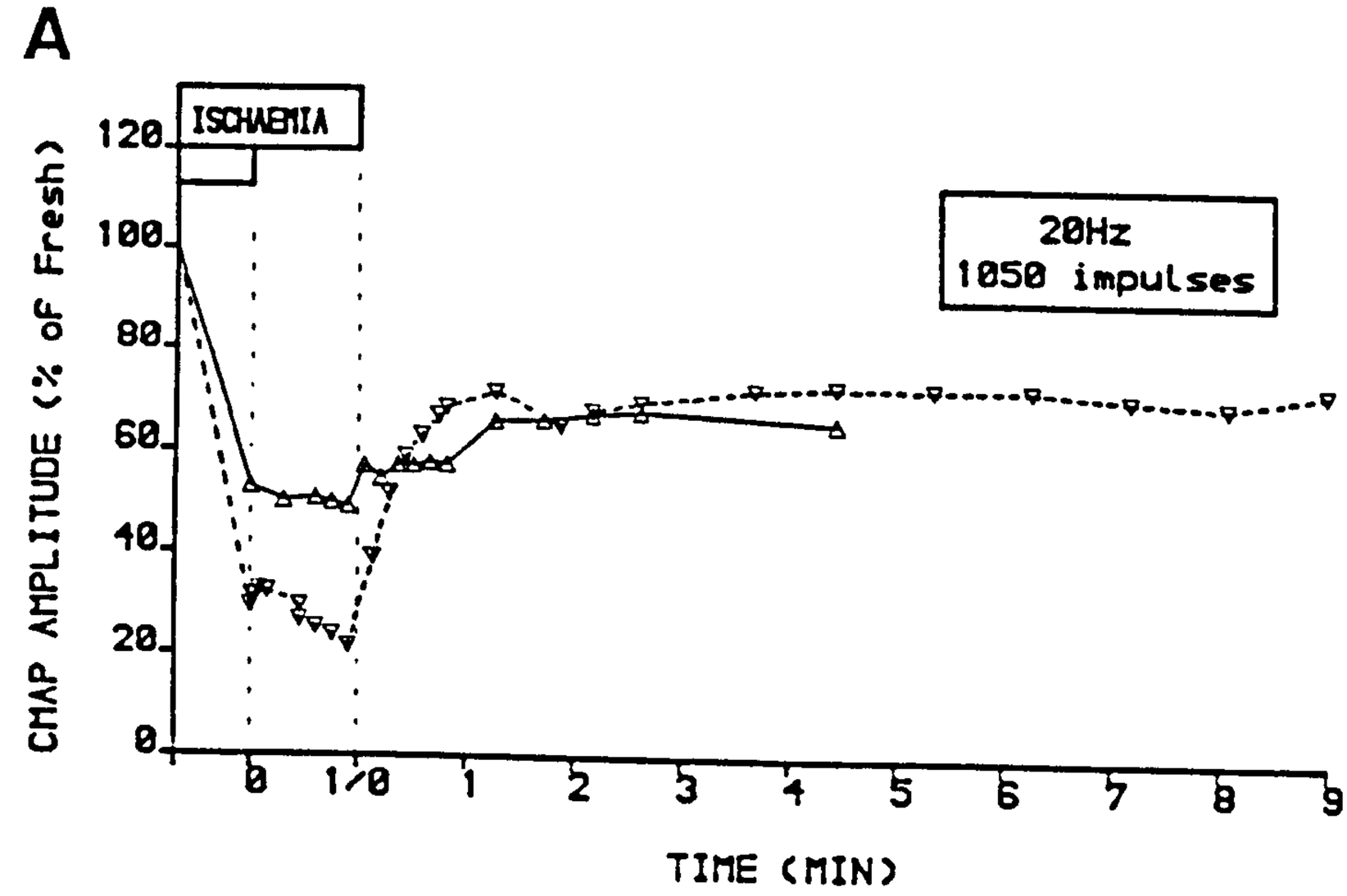
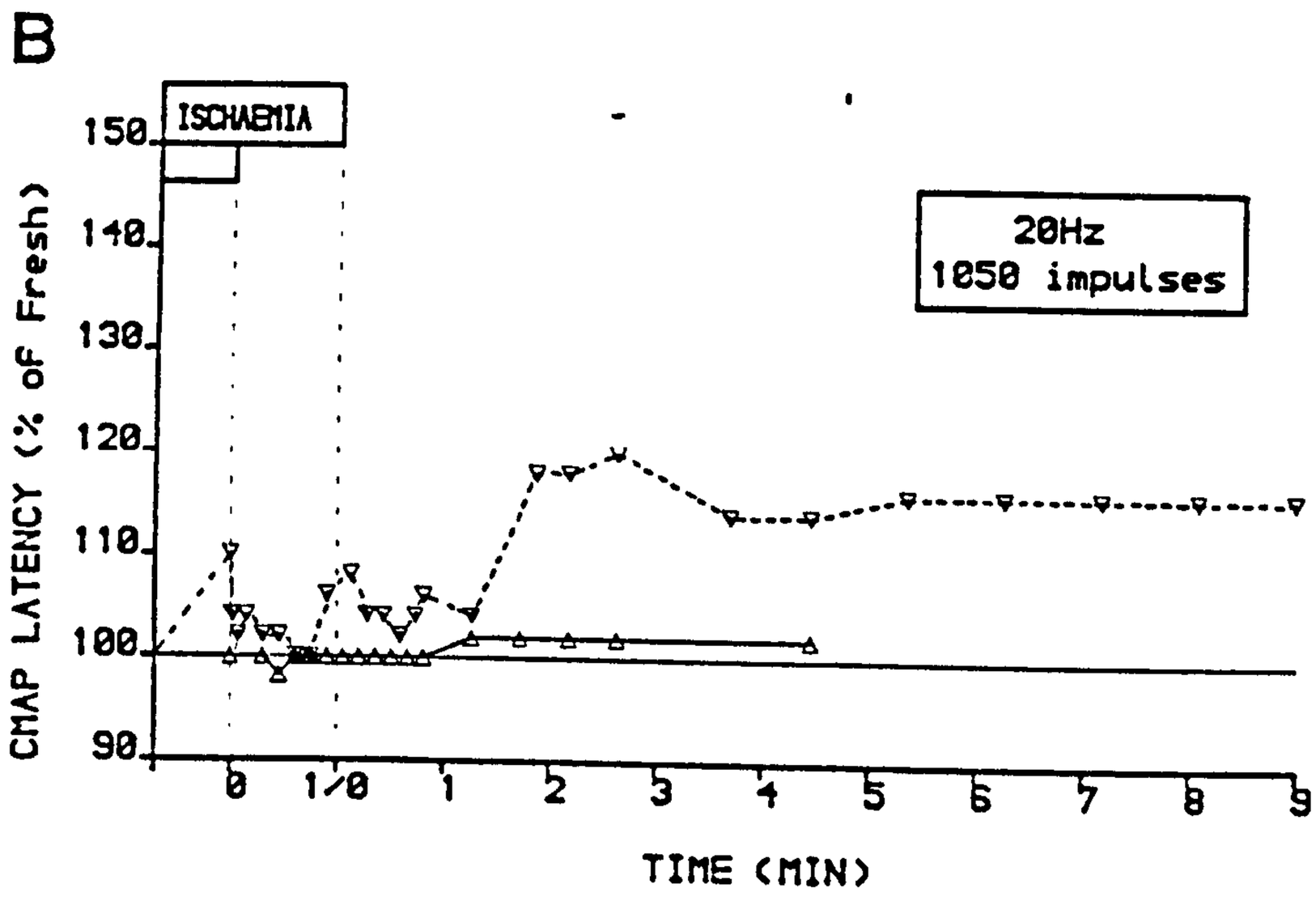
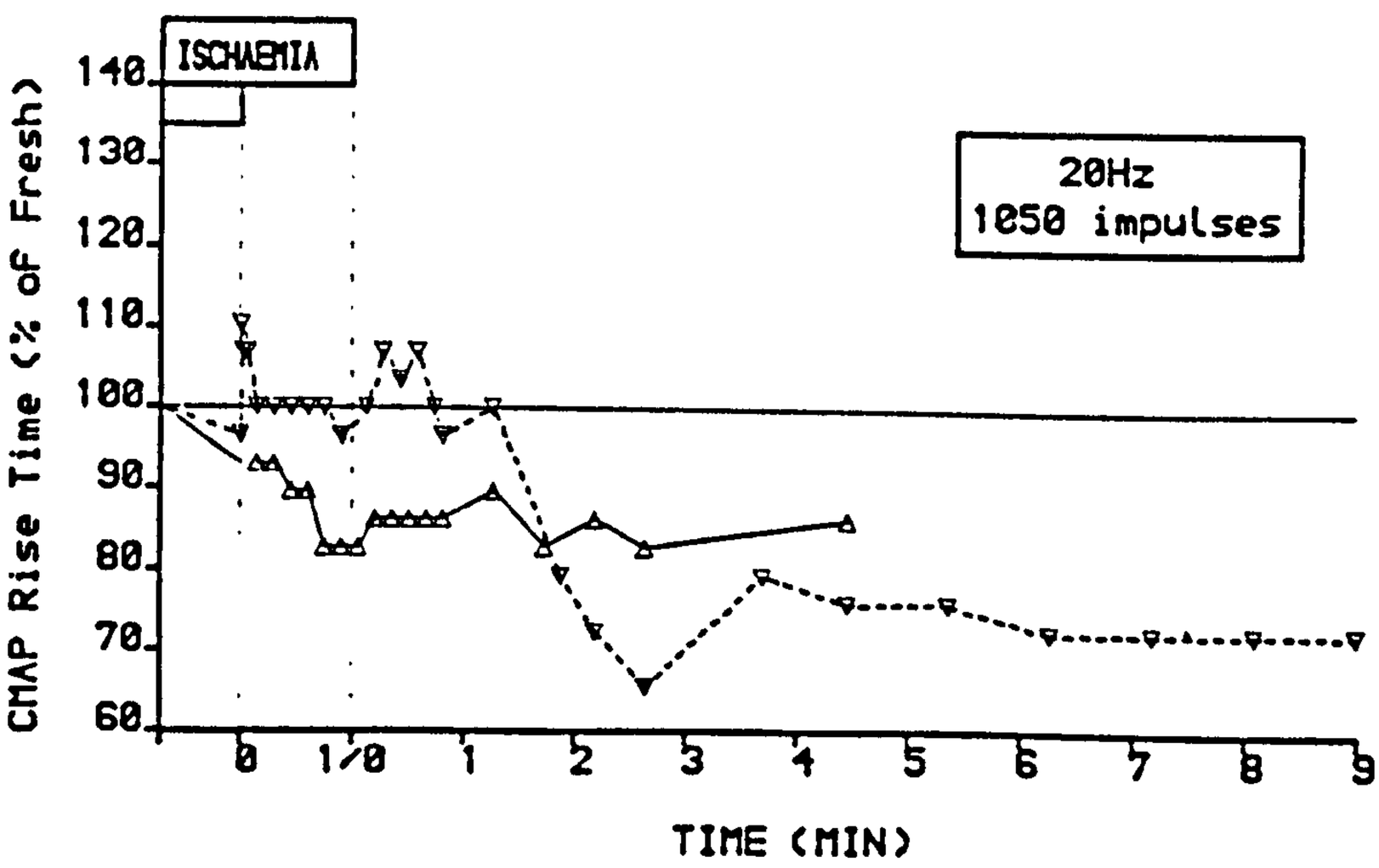

B
CONTROL
END ACTIVITY
60 sec ISCHAEMIC
RECOVERY
10 min NON-OCCLUDED
RECOVERY
NORMAL
10 mV
A
10 msec
PATIENT

Figure 5.8 Ischaemic and non-occluded recovery of A) CMAP amplitude, B) CMAP distal latency and C) CMAP rise time following ischaemic stimulated activity of 1040 impulses at 20Hz in two patients with McArdle's disease. CMAP amplitude did not recover until circulation was restored. This was in marked contrast compared to the normal subjects in which CMAP amplitude recovered almost immediately for a similar decrement in amplitude. Also in contrast to normal subjects, the CMAP distal latency did not increase following the stimulated activity. Symbols (△) AR, (▽) JD.


A
ISCHAEMIA
20Hz
1050 impulses
CMAP AMPLITUDE (% of Fresh)
120
100
80
60
40
20
0
0 1/0 1 2 3 4 5 6 7 8 9
TIME (MIN)


B
ISCHAEMIA
20Hz
1050 impulses
CMAP LATENCY (% of Fresh)
150
140
130
120
110
100
90
0 1/0 1 2 3 4 5 6 7 8 9
TIME (MIN)


C
ISCHAEMIA
20Hz
1050 impulses
CMAP Rise Time (% of Fresh)
140
130
120
110
100
90
80
70
60
0 1/0 1 2 3 4 5 6 7 8 9
TIME (MIN)

5.4 DISCUSSION

The results of this study suggest that two factors may influence excitation in normal subjects; one contributing to the reduction of CMAP amplitude and prolongation of the CMAP which recovers rapidly under ischaemic conditions, and a further factor that is dependent on the contractile history of the muscle causing an increase in CMAP duration and which recovers with a slow time course on return of the circulation only. These observations are consistent with the view that changes in transmembrane electrolyte concentrations may be responsible for the rapid alterations in CMAP shape (Bigland-Ritchie *et al.*, 1979) and additionally that metabolic factors contribute to slowing of impulse propagation.

In contrast, no ischaemic recovery of the CMAP amplitude or changes in CMAP duration were observed in the McArdle patients, implying that the mechanism leading to a loss of excitation diifers to that in normal subjects.

5.4.1 Ischaemic recovery of the action potential in normal subjects

In keeping with the discussion of chapter 4, it would appear that an accumulation (or possibly depletion) of some factor per impulse during stimulated activity is responsible for the initial broadening and reduction in amplitude of the CMAP, which recovers during ischaemic rest, since the most marked changes in CMAP shape occured after 2400 impulses of activity at both 20 and 100Hz. The subsequent ischaemic recovery of the action potential amplitude could not be attributed to recovery of energy supply by oxidative phosphorylation, as illustrated by the failure of recovery of PCr in NMR studies (Edwards *et al.*, 1985), or to removal of accumulated by-products of metabolism by the circulation. The rapidity of recovery of the CMAP amplitude therefore may support the suggestion that accumulation of transmembrane cations is responsible for the reduction in CMAP amplitude and broadening of the action potential, possibly accumulation of extracellular K^+ or depletion of intracellular Na^+ (Jones *et al.*, 1979; Bigland-Ritchie *et al.*, 1979), although it was noted that the rate at which CMAP amplitude was recovered greater following activity at 100Hz compared to that at 20Hz, suggesting a slowing in

the mechanism that restores electrolyte balance. Metabolic factors may be responsible for this, perhaps impairing the function of the ionic membrane pumps, as discussed in more detail in the previous chapter (chapter 4). The appearance of the rapid ischaemic recovery of the CMAP amplitude may alternatively be explained by the recovery of previously inactive cells, possibly owing to propagation failure at the NMJ or at pre-synaptic sites, particularly at the high stimulation frequency (Naess & Storm-Mathisen, 1955; Krnjevic & Miledi, 1958; Thesleff, 1959). However, it has been demonstrated that the reduction in force at high stimulation frequencies produced by direct stimulation in mouse soleus muscle may be similar to that obtained by indirect stimulation of the human adductor pollicis (Jones *et al.*, 1979; Jones, 1981), suggesting that the contribution of NMJ or pre-synaptic failure is probably insignificant. This form of excitation failure cannot be dismissed though, in view of the many reports in the literature mentioned above. The incomplete ischaemic recovery of the CMAP amplitude may have also been due to metabolic factors, particularly H^+ as suggested by studies in cat soleus and gastrocnemius muscle which have been ischaemic for up to 4 hours (Jennische, 1982), but recent studies by Juel (1988) on isolated mouse muscle preparations exposed to altered intracellular and extracellular pH concentrations suggest that for changes in pH within the physiological range, excitability is not reduced.

Of significance, is the partial, but rapid recovery of the CMAP distal latency during ischaemia which was followed by a slower, but complete recovery on restoration of the circulation. By maintaining ischaemia following stimulated activity, it appears that it has been possible to separate two factors contributing to altered prolongation of the CMAP, one involving a fast phase of recovery, suggestive of recovery of transmembrane electrolytes and a slow phase on return of circulation, indicating the involvement of metabolic factors. This latter point is further supported by the finding of a good correlation between the ischaemic plateau value of the distal latency and the contractile activity performed shown in Figure 5.5. It is unlikely that accumulated transmembrane electrolytes may be responsible for the persistance of the

broadening of the action potential since CMAP amplitude appeared nearly completely recovered at a time when it would be expected to be reduced. It may be argued that this is not always the case, since the CMAP amplitude remained reduced during ischaemic recovery in one subject following activity at 20Hz (Figure 5.4B), but even during subsequent non-occluded recovery the CMAP amplitude did not fully recover suggesting that the recording of the evoked potential was poor, possibly due to changes in the recording electrode position or drying of the electrode.

Several workers have suggested that slowing of membrane conduction velocity may be attributed to the increase in H^+ as a consequence of the metabolic activity of the muscle during activity (Mortimer *et al.*, 1970; Lindstrom *et al.*, 1979; Juel, 1988). It is unlikely that the increase in CMAP distal latency is due to the generation of these metabolites by ischaemia alone since the relative period of ischaemia in the present study was considerably shorter than that necessary to produce changes in membrane conduction velocity as shown for cat gastrocnemius muscle (Mortimer *et al.*, 1970). Whether other metabolites generated as a consequence of metabolic activity contribute to slowing of action potential propagation is not yet established.

It may be argued that the prolongation of the distal latency is due to slowing of nerve conduction velocity or to slowed synaptic transmission across the NMJ. It is likely that some slowing of conduction velocity does occur and also slowing of NMJ transmission, but the energy demand in contracting muscle is likely to be greater than that by excitable membranes and hence accumulation of metabolites as a by-product of contraction are more likely to influence the sarcolemmal membrane to a relatively larger degree than at other sites of electrical transmission.

The contribution of dispersion of individual action potentials cannot account for the increased duration of the distal latency since this measurement is likely to reflect only the fastest propagated action potentials and will not be dependent on the parallel summing of the individual action potentials as is the CMAP rise-time. In the present study, CMAP rise-time was prolonged during activity at 20Hz and did not

recover while ischaemia was maintained, supporting the suggestion that sarcolemmal membrane changes probably are of more significance than slowing of NMJ transmission or nerve conduction, but as mentioned in the previous chapter, several factors may influence this measurement. It is of course possible that dropping out of cells may also influence the distal latency. But this cannot be determined from these studies. The observation that CMAP rise time did not increase at all following activity at 100Hz is therefore not surprising, since it is likely that at this frequency, loss of excitation of individual cells occurs and hence masks the prolongation of the action potential. It would be unreasonable to suggest that at this frequency prolongation of the action potential does not occur in view of the many studies that demonstrate slowing of conduction velocity in the literature as a result of alterations in transmembrane cations.

The inference of the increased prolongation of the CMAP and the parallel reduction in twitch force generation is that force development is coupled to impaired propagation of the CMAP. The reduction in force cannot be due to loss of excitation (measured over the muscle) as suggested by Milner-Brown & Miller, (1986), since during conditions of ischaemia, CMAP amplitude recovered rapidly in this study when force did not. Whether the loss of force is dependent on excitation impairment as a result of prolongation of the action potential or whether both are due to similar factors is not clear. Myofibrillar inactivation by H^+ and Pi have received much attention (Donaldson & Hermansen, 1974; Fabiato & Fabiato, 1978; Metzger & Moss, 1987; Brandt *et al.*, 1982; Miller *et al.*, 1988). The non-occluded recovery of the CMAP distal latency and twitch force do appear to follow a similar time course, complete recovery being apparent after 4 minutes at 20Hz (2400 impulses), but difficulties arise in determining twitch force recovery due to several other factors which may alter it, for example, post-tetanic potentiation, which is evident in the present study following activity at 100Hz, and long-term low-frequency fatigue (Edwards *et al.*, 1977b).

5.4.2 McArdle's disease

Previous studies by other workers have shown that these individuals fatigue prematurely during ischaemic supramaximal stimulation at 20Hz (Edwards & Wiles, 1981), which is confirmed in this study, and also over a frequency range of 1 to 100Hz during non-occluded intermittent stimulated contractions (Cooper *et al.*, 1987). This premature fatigue is also associated with a reduction in CMAP amplitude (Figure 5.8; Dyken *et al.*, 1967; Brandt *et al.*, 1977; Cooper *et al.*, 1987). Owing to the metabolic defect in these individuals, it was hoped to gain some further insight as to the possible role of energy metabolism in membrane function.

The failure of recovery of CMAP amplitude during ischaemic conditions following stimulated activity in this stūdy confirms the findings of Wiles (1980) and suggests that the accumulation of some product of excitation or metabolism, or reduction in energy availability may affect the excitation process which may then lead to force failure. This is also supported by the slow recovery of CMAP amplitude on return of circulation. It is also possible that the reduction in CMAP amplitude may be secondary to other factors which influence force production.

It is tempting to suggest that the decline in CMAP amplitude is due to a marked reduction in energy availability in view of the enzymatic defect in these individuals, since a significant quantity of energy supply is probably necessary for ionic pumps for maintenance of membrane potential and also with respect to uptake of Ca^{2+} by the sarcoplasmic reticulum. In this respect, the ischaemic human McArdle muscle model appears similar to the poisoned muscle model described by Luttgau (1965) in which amphibian muscle fibres were poisoned with iodoacetate and cyanide to prevent energy supply by glycolysis and oxidative phosphorylation. However, in contrast to the premature decrement of CMAP amplitude observed in the present study, Luttgau (1965) demonstrated that the action potential could be propagated for hundreds of impulses without much decrement in amplitude, although the stimulation frequency employed was 100Hz compared to 20Hz used in this study. In a further similar study on mouse soleus muscle in which glycolysis was prevented by adding

iodoacetate to the bathing medium (Jones & Bigland-Ritchie, 1986), it was shown that electrical stimulation resulted in a marked decrement in CMAP amplitude and a prolongation of the action potential, consistent with a reduction in conduction velocity. The changes in action potential characteristics in that study were similar to those produced by high-frequency stimulation and increased extracellular KCl added to the medium, suggesting that Na^+/K^+ pump function was reduced or inhibited, resulting in altered transmembrane electrolyte concentrations and hence reduced excitability and slowed conduction velocity. It is of interest, therefore, that **no change** in either the CMAP distal latency or rise-time was apparent in the two McArdle patients following fatiguing activity, which is in stark comparison to that seen in the normal subjects for a similar decline in CMAP amplitude. The decline in CMAP amplitude may therefore have not resulted from a reduction in excitability owing to transmembrane electrolyte accumulation through the failure of ionic pump mechanisms. Alternatively, the broadening of the action potential is masked by the complete failure of individual action potentials. Consequently the CMAP does not appear to broaden, but force rapidly declines in addition to CMAP amplitude. However, a greater than normal K^+ release from active muscle has been measured from some, but not all cases of myophosphorylase deficiency (Lewis & Haller, 1986). This suggests that the failure of the action potential may be due to other factors.

It has been suggested that the accumulation of inorganic phosphate (Pi), produced as a consequence of activity, may contribute to reduced membrane Na^+/K^+-ATPase function in cardiac muscle (Ponce-Hornos *et al.*, 1982) and in red blood cells (Garay & Garrahan, 1975). It is likely therefore that Ca^{2+}-ATPase activity is also inhibited. This may explain the contracture formation that is characteristics of this condition, and which was experienced by the two patients. However, many reports in the literature suggest that Pi may induce fatigue in these individuals as a result of Pi induced myofibrillar inactivation. This is supported by the observation that Pi is generated in greater quantities in these individuals during fatiguing activity (Lewis *et al.*, 1985). More recent evidence points to the Pi^- form that reduces myofibrillar

activation (Wilkie, 1986; Miller *et al.*, 1988), but since H^+ ions are not produced during activity in these patients it is unlikely that the Pi^- concentrations will be produced in excess to that observed in normal individuals. Furthermore, it has been recently been shown that less Pi^- was produced in a patient with McArdle's disease despite greater fatigue compared to normal individuals (Cady *et al.*, 1988). However, the failure to produce more Pi and Pi^- in the McArdle's patient in that study may have been due to the fact that less cells were functional as a result of excitation failure. A reduction in EMG may support this, but this was not reported. Whether Pi influences membrane function, has a direct effect on myofibrillar activation or acts at both sites is not known. The role of Pi in fatigue remains unclear and requires further study.

A greater concentration of ammonia is also produced in McArdle patients undergoing dynamic activity than is observed in normal individuals (Wagenmakers *et al.*, 1987) and also during isometric contractions of the adductor pollicis (Coakley, personal communication). There is evidence to suggest that ammonium ions may reduce the excitability of the membrane of frog sartorius muscle (Heald, 1975) which may contribute to fatigue, although ammonium ions may also initially induce a potentiating effect on force generation (Heald, 1975). This offers an attractive explanation for the marked reduction in excitability of the membrane and may act by exerting a depolarizing effect on the membrane. However, the ammonium concentrations used by Heald (1975) to obtain a reduction in action potential excitability was of the order of 100 times greater than that reported to be released in the venous blood in McArdle's patients (72 mmol compared to 400-500µmol). It is of course not certain what the local concentration of ammonium ions is at the membrane surface, and furthermore comparisons between experiments in different species and types of preparation have to be made with caution. Further studies are necessary to investigate this proposition.

It is also possible that reduced excitability of the membrane may result from increased conductivity of K^+ through ATP-sensitive K^+-channels which may be activated by depletion of ATP (Spruce *et al.*, 1985) and Ca^{2+} sensitive K^+ channels

which may be activated by Ca^{2+} released during activity (Pallotta, 1985). However, it is of interest that ATP levels do not decline even when contracture development occurs (Rowland *et al.*, 1965).

No satisfactory explanation as yet can account for fatigue in McArdle patients. The model employed in this study is consistent with the suggestion that excitatory processes play an important role in fatigue of these individuals since it would appear that excitation failure of individual fibres reduce the CMAP. The mechanism of excitation failure may involve a reduction in Na^+/K^+-ATPase activity due to metabolite accumulation as a result of the failure of glycogenolysis, but an increase in the duration of the action potential would be expected to occur, particularly if electrolyte accumulation/depletion is responsible for the reduction in excitation. These results therefore suggest that excitation failure is probably the result of metabolite build-up, which occurs in concentrations in excess of that in normal individuals, as a consequence of the metabolic myopathy in these individuals. The extrapolation of the mechanisms leading to fatigue in these individuals to normal individuals must therefore be made with caution.

5.5 CONCLUSIONS

The results of this study suggest that two components influence the propagation of the action potential; one which declines rapidly during ischaemic conditions, which is dependent on numbers of stimuli delivered and a further component that is dependent on the contractile history of the muscle and recovers with a slow time course on restoration of the circulation. It is probable that metabolic factors are responsible, in part, for the slowing of the propagation of the action potential and may also reduce the rate at which transmembrane electrolyte balance may be restored by membrane pumps. In contrast, the loss of excitation in McArdle patients appears to be due to loss of individual action potentials, possibly as a result of build-up of metabolites as a consequence of their myopathy which impairs excitation. It is unlikely that the loss in excitation in these individuals is the result of a

reduced membrane capacity for electrolyte exchange and hence the factors that lead to fatigue may differ markedly to those in normal individuals.

5.6 SUMMARY

1. The ischaemic recovery of the action potential was studied in three normal volunteers and two McArdle patients to investigate the dependence of the CMAP on metabolic factors.

2. In normal subjects, CMAP amplitude declined and recovered rapidly following stimulation at 20 and 100Hz for 2400 impulses. CMAP distal latency became prolonged and only partially, but rapidly, recovered at 20Hz and nearly fully recovered at 100Hz. The final plateau value of recovery showed a good correlation to contractile activity performed. On return of circulation CMAP distal latency fully recovered with a slow time course.

3. In contrast to the normal subjects, CMAP distal latency did not increase by the end of activity. CMAP amplitude declined, but did not recover until circulation was restored.

4. These results suggest that two components influence CMAP duration in normal subjects, one that is dependent on impulse numbers and recovers rapidly during conditions of ischaemia, possibly accumulation of electrolytes, and a component that is dependent on the contractile history of the muscle, possibly H+ or Pi. The CMAP amplitude is probably dependent on transmembrane electrolyte concentrations and the rate of restoration of these.

The changes in the CMAP of McArdle patients suggest a loss of individual action potentials, possibly due to impairment of excitability as a consequence of the metabolites generated owing to their myopathy, rather than a direct consequence of failure of supply of energy for membrane processes.

CHAPTER 6: POTENTIATION OF LOW-FREQUENCY TETANI DURING FATIGUING ACTIVITY

CHAPTER 6: POTENTIATION OF LOW-FREQUENCY TETANI DURING FATIGUING ACTIVITY

6.1 INTRODUCTION

Slowing of relaxation as the sole cause of low-frequency force potentiation was questioned in chapter 3. During stimulated activity it appeared that low-frequency force potentiation resulted from a reduction in relaxation rate since low-frequency force increased at a time when relaxation rate decreased. However, contrary to this, during subsequent recovery it was shown that low-frequency force again potentiated, but at a time when relaxation rate was increasing, hence indicating a further factor contributed to low-frequency force potentiation. It was suggested that post-tetanic potentiation of the twitch may contribute to low-frequency potentiation and hence reduce fatiguability.

It would appear from the literature survey in chapter 1 that little work has previously been carried out regarding the degree of post tetanic potentiation of the twitch and low-frequency force production or the role of post-tetanic potentiation of the twitch in resisting fatigue in human muscle. That the degree of post-tetanic twitch potentiation appears unaltered by the degree of fatigue (Alway *et al.*, 1987) further supports the possible involvement of post-tetanic potentiating mechanisms in resisting fatigue.

The present study was therefore undertaken in order to investigate the role and contribution of post-tetanic potentiation of the twitch and MRR reduction in resisting fatigue during intermittent fatiguing stimulated activity of the human adductor pollicis.

6.2 METHODS

6.2.1 Experimental subjects

Six normal subjects (4 male and 2 female), aged 24 - 33 years, participated in this study

Measurement of muscle contractile properties

Isometric force, MRR, integrated force, and the evoked compound muscle action potentials (CMAP) of adductor pollicis were recorded as described under general methods, chapter 2.

Trains of computer-controlled supramaximal stimuli, delivered via the ulnar nerve at the wrist to contract the adductor pollicis were used to fatigue the adductor pollicis and obtain an ascending or descending programmed stimulation electromyogram (PSEM), depending on the pattern of stimulation applied (Figure 6.1a). The ascending frequency pattern consisted of 1, 10, 20, 50, 100 and 1Hz for 1 second each (10Hz for 2 seconds to obtain a plateau force), with a 1 second gap after 10Hz to allow estimation of the MRR as had been employed in chapter 3. The descending frequency pattern consisted of 1, 100, 50, 20, 10 and 1Hz for 1 second each (10Hz for 2 seconds). The rationale for using the descending PSEM was to examine the role of reduction in MRR in low-frequency potentiation in isolation, since under these conditions post-tetanic potentiation of the twitch would be expected to be maximal. On the other hand, post-tetanic potentiation of the twitch was unlikely to be fully evoked during the ascending PSEM, because of the absence of immediately preceding high-frequency tetani. A comparison would therefore shed light on the role of post-tetanic potentiation of the twitch.

6.2.2 Experimental fatigue protocols

In each subject two series of stimulated, fatiguing contractions were undertaken, one using the ascending PSEM and the other using the descending PSEM. The procedure was identical to that used in chapter 3 (part III) for the determination of MRR at different frequencies during ischaemic conditions from which data from five subjects for the ascending PSEM was obtained.

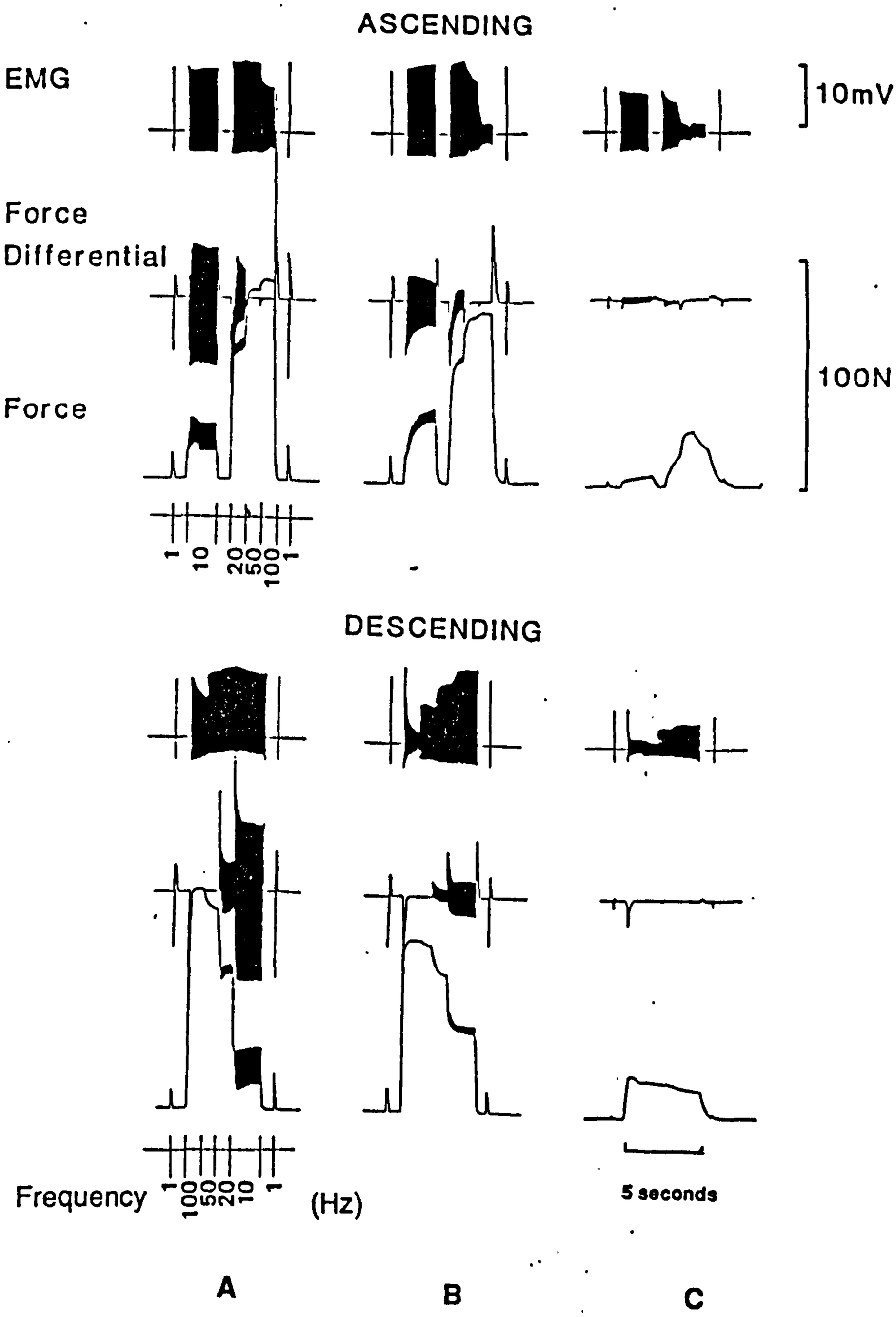


Figure 6.1 Simultaneous oscillographic recordings of compound muscle action potential (CMAP), force and force differential producing the ascending (upper panel) and descending (lower panel) Programmed Stimulation Electro-Myogram (PSEM). A) Fresh muscle; B) seventh contraction C) fifthteenth contraction.

A control ascending/descending PSEM was performed in each subject in unfatigued (fresh) muscle. After a two minute rest period for recovery, a sphygmomanometer cuff was inflated around the upper arm and maintained at 100mmHg above systolic blood pressure to occlude circulation. This was followed by a further 3 minutes ischaemic rest to reduce oxygen reserves available for oxidative metabolism as described in chapter 3. Fatiguing activity followed and consisted of 15 PSEMs at intervals of 5 seconds. The cuff was then deflated and aerobic recovery monitored using the same stimulation pattern at intervals of 0.5, 1, 2, 3, 5, 10 and 15 minutes.

The decay of potentiation of the twitch following an ascending PSEM in fresh muscle was also documented for three-subjects. Twitch stimuli were introduced at intervals for up to 10 minutes during conditions of ischaemia and with the circulation intact on separate occasions.

6.2.3 Analysis

The following measurements were made on each PSEM of the contraction series. For each subject, the force measured at the end of each frequency train was expressed as a percentage of maximum force generated by a 100Hz tetanus in fresh muscle. CMAP amplitude was expressed as a percentage of the equivalent part of the PSEM in fresh muscle, measured at the end of each frequency train.

The ratio of force to excitation amplitude was used to express the 'force generated per impulse of excitation' as described in chapter 3 (part III, methods).

Maximum relaxation rate (MRR) was determined for the twitch and from 10Hz tetani. Maximum contraction rate (MCR) was determined for the twitch only. These were expressed as force change per 10msec (Wiles *et al.*, 1979) and as a percentage of the value obtained in fresh muscle. Paired t-tests were used to compare paired experimental data, a value of $p < 0.5$ being taken as significant. The results reported in the text and used to construct figures are expressed as mean $\pm$ 1 S.D., where n=6.

6.3 RESULTS

6.3.1 Fresh muscle ascending/descending PSEM characteristics

Absolute values of force for each frequency, MRR and MCR characteristics obtained for fresh muscle on the same occasion are shown in Table 6.1. The maximal tetanic force (100Hz) was found to differ significantly between the two stimulation patterns ($p< 0.05$), the ascending PSEM 100Hz tetani being smaller than that obtained in the descending PSEM, indicating that a small degree of fatigue results from the brief pre-100Hz tetani in the ascending PSEM. As expected, the post-tetanic twitch force of the descending PSEM was significantly greater than the pre-tetanic twitch force of the ascending PSEM, and similarly, oscillation of the 10Hz tetani in the former was greater. This was not associated with a significant increase in mean force, however. The increased twitch force of the descending PSEM was accompanied by a significantly faster maximal rate of relaxation compared to the ascending PSEM, although this was not the case for the 10Hz tetani.

6.3.2 Anaerobic activity

Force declined at all frequencies during both stimulation protocols (Figure 6.2, Table 6.2). At stimulation frequencies of 20 to 100Hz, the reduction in force was initially gradual, this being linear and similar up to the 7th contraction, independent of stimulation pattern (Figure 6.2 a-c). Force declined more rapidly thereafter. In contrast to the higher frequencies, 10Hz force showed obvious potentiation, peaking after the 7th contraction (Figure 6.2d). This was greater for the descending PSEM series and was significant ($p<0.001$). Similarly, 20Hz force was significantly greater at the end of fatiguing activity (Table 6.2). The pre-tetanic twitch of the ascending PSEM became fully potentiated by the 2nd contraction, but the decline in twitch force was unexpectedly **greater** for the post-tetanic twitch of the descending PSEM series (Figure 6.2e), contrasting with the changes observed at 10Hz. In addition, the degree of oscillation of force at 10Hz (which reflects the degree of fusion (Bigland-Ritchie *et al.*, 1983a)) was less during the descending than ascending contraction series (Figure 6.3).

Table 6.1 Effect of preceding stimulation frequency on force generation and relaxation in fresh adductor pollicis. Force data in Newtons, maximal relaxation rate and maximal contraction rates as percent change per 10msec. Mean ± 1 S.D. P value obtained from paired t-test.

	Ascending frequency	**Descending frequency**	**p-value of difference**
Twitch Force	8.4±1.9	11.5±3.8	<0.05
Force 10Hz	13.9±6.0	16.5±6.2	NS
Force 20Hz	40.9±6.7	40.3±10.2	NS
Force 50Hz	57.3±11.6	58.0±14.2	NS
Force 100Hz	62.5±12.4	65.2±14.4	<0.05
Max. relaxation rate of twitch	9.3±2.3	11.7±1.8	<0.05
Max. contraction rate of twitch	20.2±1.9	21.6±2.0	NS
Max. relaxation rate 10Hz	9.5±1.4	9.8±1.4	NS

Table 6.2 Comparison of relative changes in force, CMAP amplitude, maximum relaxation rate and changes in F/E ratios, for all frequencies at the end of fatiguing activity. Mean ± 1 S.D., P-values from paired t-test.

Frequency (Hz)	**Ascending frequency**	**Descending frequency**	**p-value of difference**
	Force (% of fresh)		
1	22.9±15.5	0.7(1 subject)	-
10	30.4±16.1	73.2±18.4	0.005
20	22.1±7.9	33.6±8.0	0.05
50	29.8±9.3	25.8±6.9	NS
100	22.2±9.0	23.6±5.8	NS
	CMAP amplitude (% of fresh)		
1	74.9±9.1	58.5±11.7	0.001
10	68.2±11.9	48.8±10.2	0.001
20	54.1±13.5	33.8±8.3	0.005
50	19.5±5.3	18.6±3.9	NS
100	15.3±7.3	16.3±6.0	NS
	F/E ratio		
1	0.22±0.13	0.13(2 subjects)	-
10	0.46±0.25	1.58±0.56	0.005
20	0.42±0.13	0.92±0.28	0.05
50	1.55±0.37	1.32±0.22	NS
100	1.58±0.44	1.59±0.50	NS
	Max. relaxation rate (% of fresh)		
10	16.9±2.7	14.1±4.3	NS

Note that by the 15th contraction the twitch was so small that reliable measurements of maximum relaxation rate could not be made, hence the absences from the table.

However, the low-frequency force differences between the two protocols could not be attributed to differences in MRR since this declined in an identical fashion during both protocols at 10Hz (Figure 6.4a). Changes in twitch relaxation and contraction rates were also independent of stimulation pattern once the initial pre-tetanic twitch of the ascending PSEM had become fully potentiated (Figure 6.4b & c). Maximum twitch contraction rate decreased by only a small extent in contrast to a much greater reduction in maximum relaxation rate.

The decline in CMAP amplitude appeared frequency dependent. Declines at higher frequencies were similar for both stimulation protocols (ie., 50 and 100Hz) (Figure 6.5a,b), but at lower frequencies of stimulation CMAP amplitude declined more during the descending stimulation protocol. This was preceded by a small increase in CMAP amplitude at the lower stimulation frequencies (Figure 6.5c,d). The implication of the changes in CMAP amplitude on the interrelation of force generation and CMAP amplitude is shown in Figure 6.6. The changes in the ratio F/E were similar at high stimulation frequencies, but at low frequencies of stimulation F/E diverged. Hence, F/E increased to >1 at all frequencies except the twitch during the descending frequency protocol, reflecting an improvement in the force generating capacity per action potential amplitude, whereas during ascending stimulated activity, the converse was true at the lower stimulation frequencies (10Hz and 20Hz) reflecting some degree of activation failure. This was also noted for the twitch during both stimulation protocols.

The dependence of force generation on CMAP amplitude is summarized in Figure 6.7 a and b. At high stimulation frequencies (50 & 100Hz) force was well maintained despite a reduction in CMAP amplitude for both ascending and descending PSEMs. However, at lower stimulation frequencies (except twitch), in contrast to the reduction in force at l0 and 20Hz seen during the ascending PSEM, imposing a descending frequency regime of stimulation resulted in a marked preservation of force, despite a decline in CMAP amplitude.

Similar amounts of activity (force x time) resulted during both stimulation protocols (39.0 ± 4.4 max.seconds. and 41.9 ± 3.2 max.seconds ascending and descending protocols respectively). It is likely that the contribution of the improved force generating capacity observed at the lower frequencies of stimulation during the descending series to total activity undergone is masked by the greater activity achieved at the higher stimulation frequencies.

6.3.3 Aerobic recovery

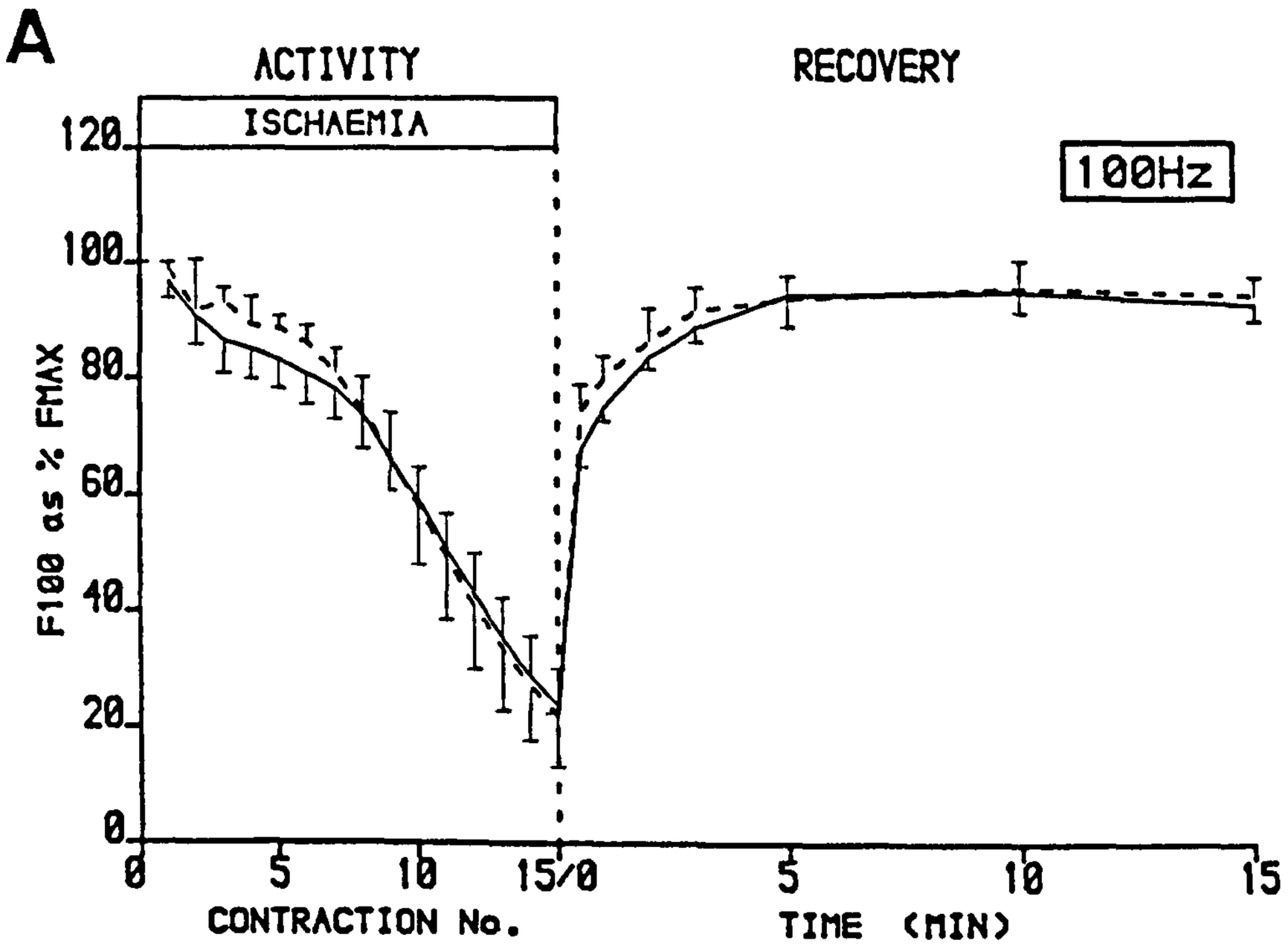
On reperfusing the muscle, force recovered rapidly at high stimulation frequencies towards normal values for both contraction series. Low-frequency force recovered immediately (ie., 30 seconds) following the descending series and 10Hz force potentiated to similar levels seen during activity (Figure 6.2d). In contrast, 10Hz and 20Hz force recovered at a slower rate following the ascending series, peaking after 2 minutes (Figure.6.2c,d). Consequently, the force/MRR relationship was markedly influenced (Figure 6.8). Twitch force recovered at a slower rate than that observed for high frequency tetanic forces, peaking after 2 minutes and then declining to values less than that obtained in fresh muscle (Figure.6.2e).

Recovery of CMAP amplitude appeared dependent on the frequency of stimulation following both stimulation protocols, the rates of recovery being greater at low-frequencies than at high-frequencies (Figure 6.5). The dependence of force on CMAP amplitude during recovery was similar, but opposite, to that seen during fatiguing activity. Recovery of MRR was similar for both series, returning to initial values by 15 minutes (Figure 6.4a).

6.3.4 Decay of post-tetanic twitch potentiation

The decline of the post-PSEM twitch force during ischaemic conditions was initially rapid and was followed by a slower phase, recovery of the twitch being complete by 5-10 minutes (Figure 6.9a). The degree of potentiation was markedly variable between subjects. The decay of twitch potentiation was not due to ischaemic factors reducing twitch force since similar degrees of potentiation and time courses of recovery were observed during non-occluded conditions (Figure 6.9b).

Figure 6.2 Time course of changes of force, as a percentage of maximum force (at 100Hz), during fatiguing activity with circulatory occlusion and during aerobic recovery at: A) 100Hz, B) 50Hz, C) 20Hz, D) 10Hz and E) 1Hz (twitch). The results from the ascending PSEM series appear as dashed lines and those from the descending PSEM series as solid lines. Note that force at 10Hz potentiates more during the descending PSEM series despite a greater decline in twitch force.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ACTIVITY
RECOVERY
ISCHAEMIA
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CONTRACTION No.
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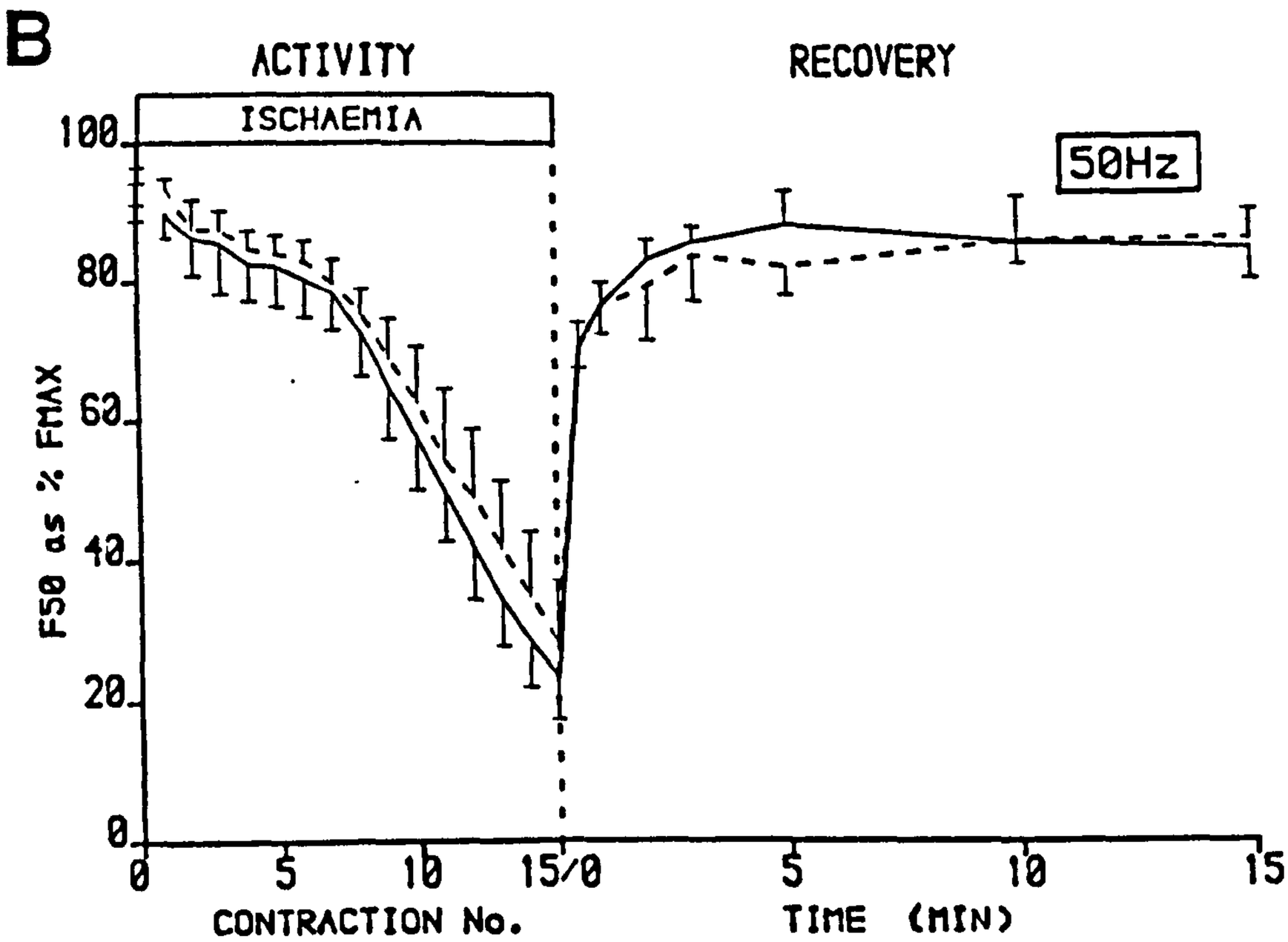
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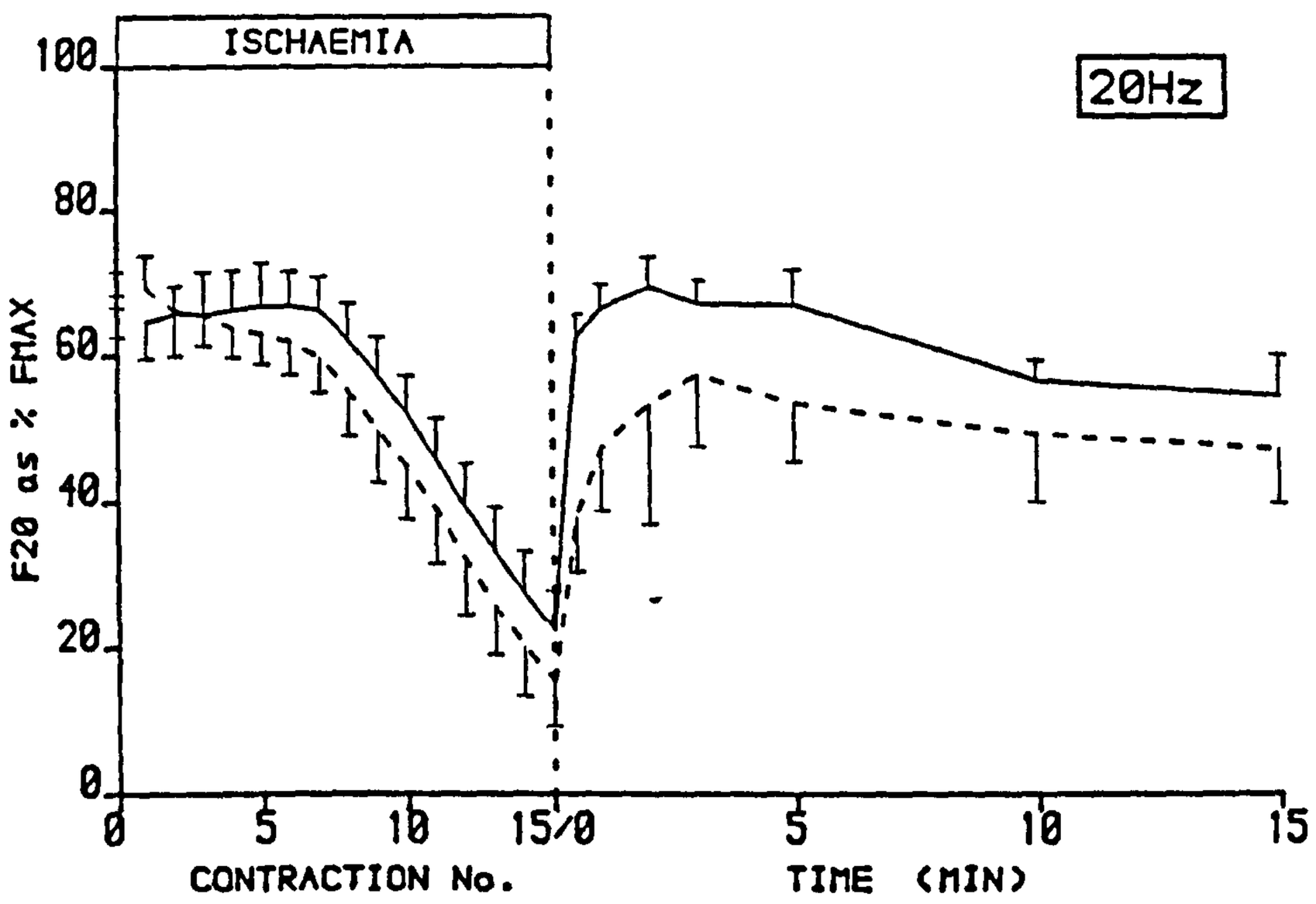
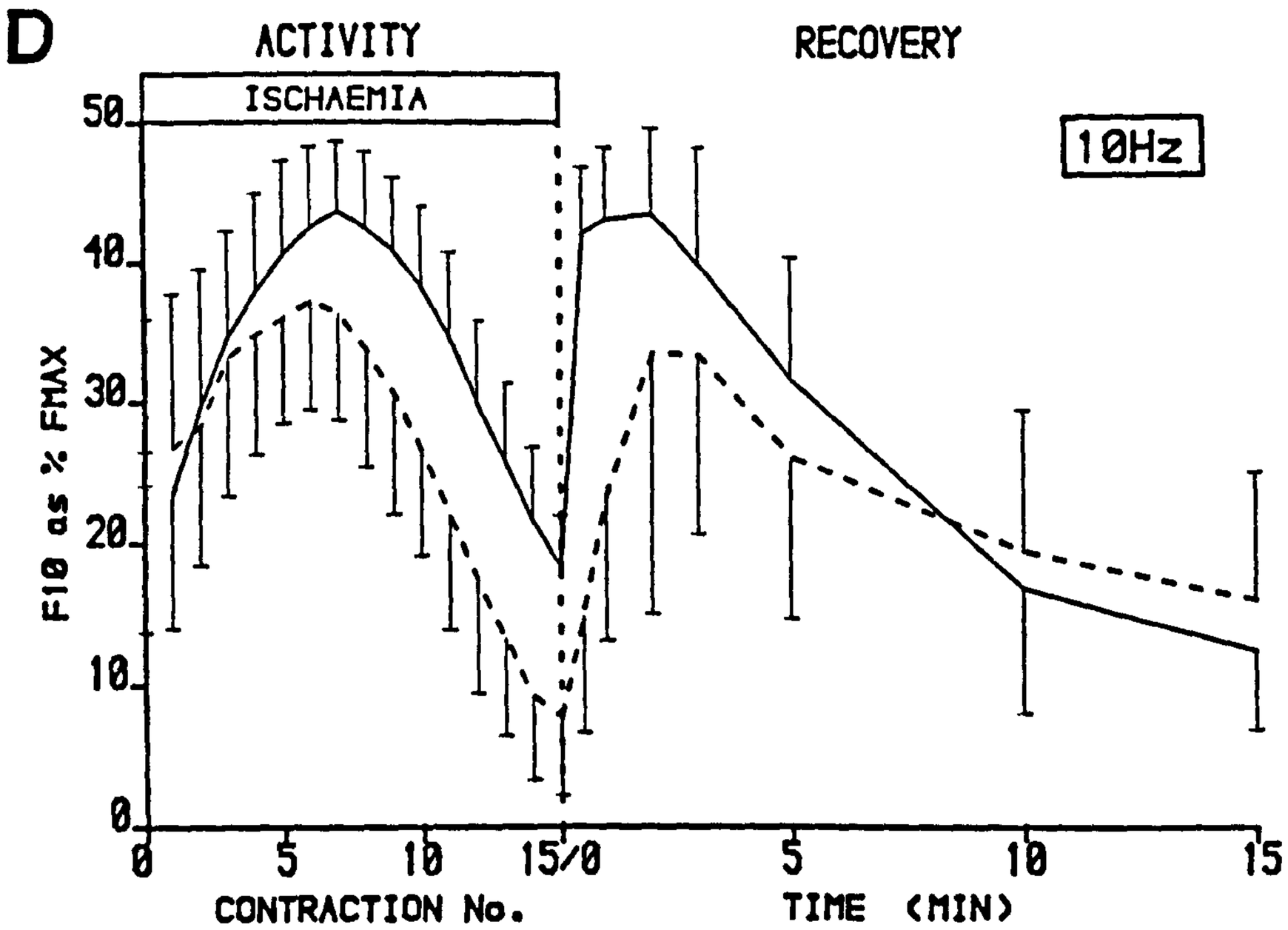
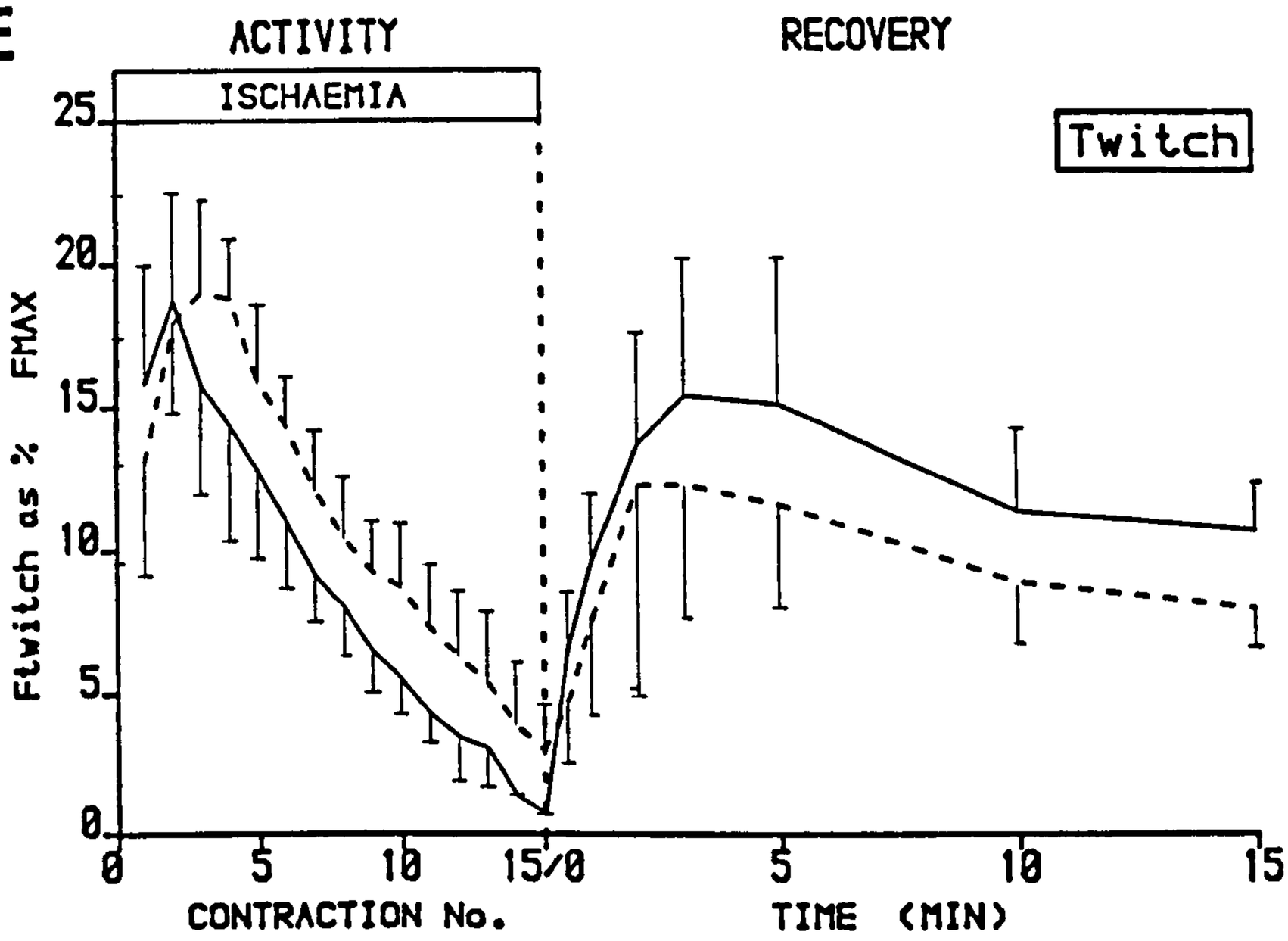

ACTIVITY
RECOVERY
ISCHAEMIA
20Hz
100
80
60
40
20
0
F20 as % FMAX
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

D


ACTIVITY
RECOVERY
ISCHAEMIA
10Hz
50
40
30
20
10
0
F10 as % FMAX
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

E


ACTIVITY
RECOVERY
ISCHAEMIA
Twitch
Ftwitch as % FMAX
25
20
15
10
5
0
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

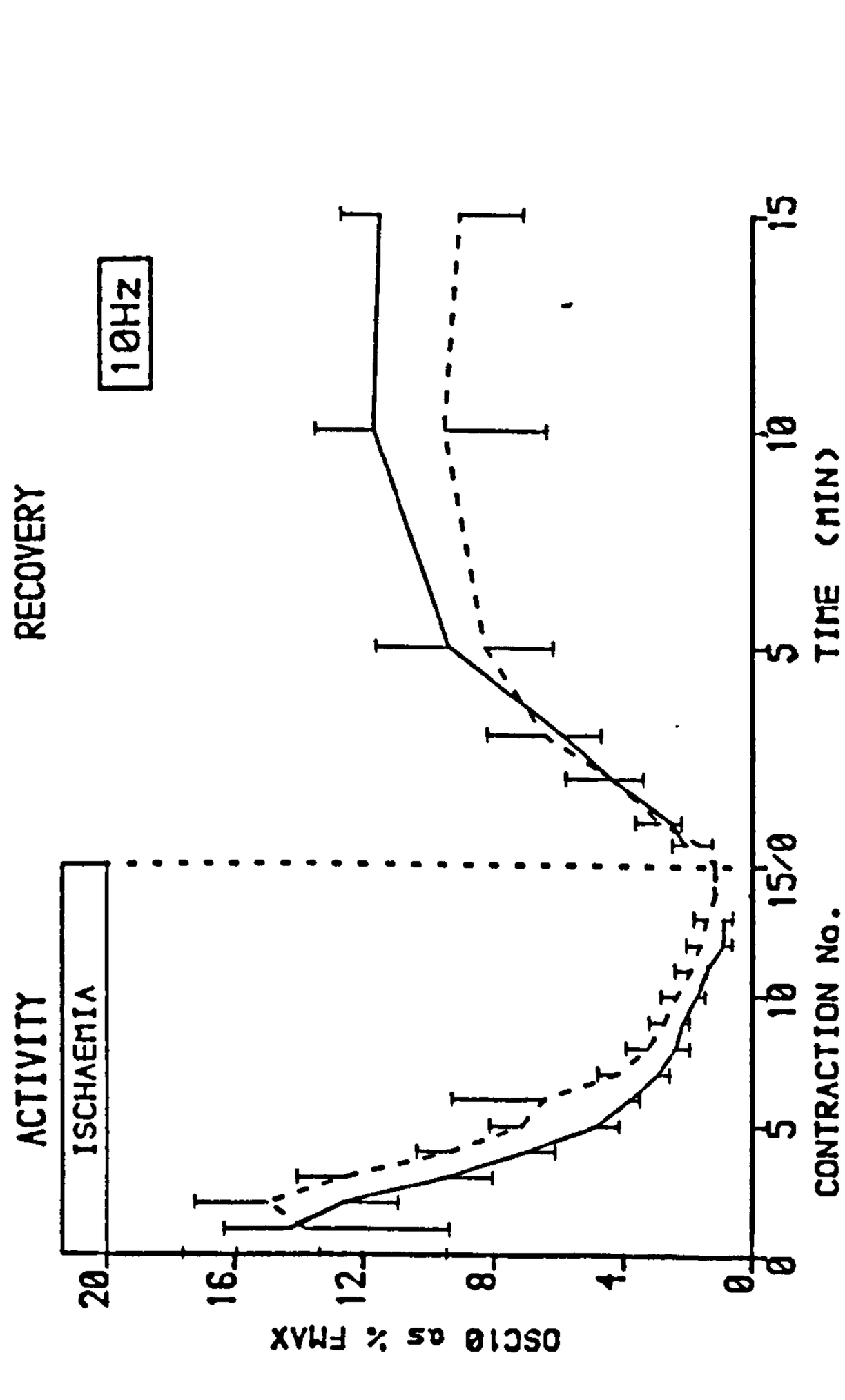


Figure 6.3 Time course of changes in oscillation of force at 10Hz, expressed as a percentage of maximum force, during fatiguing ischaemic activity and aerobic recovery. The results from the ascending PSEM series appear as dashed lines and those from the descending PSEM series as solid lines.

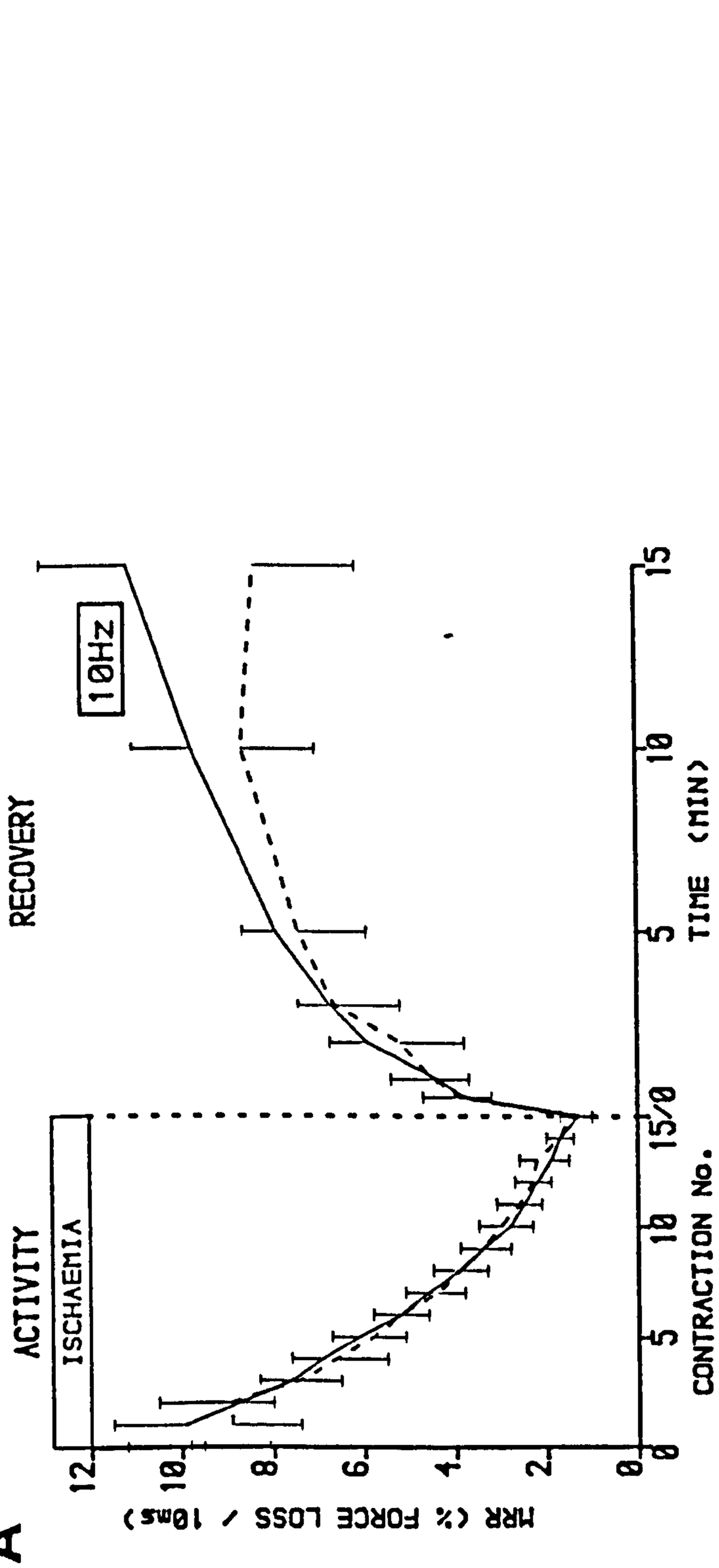
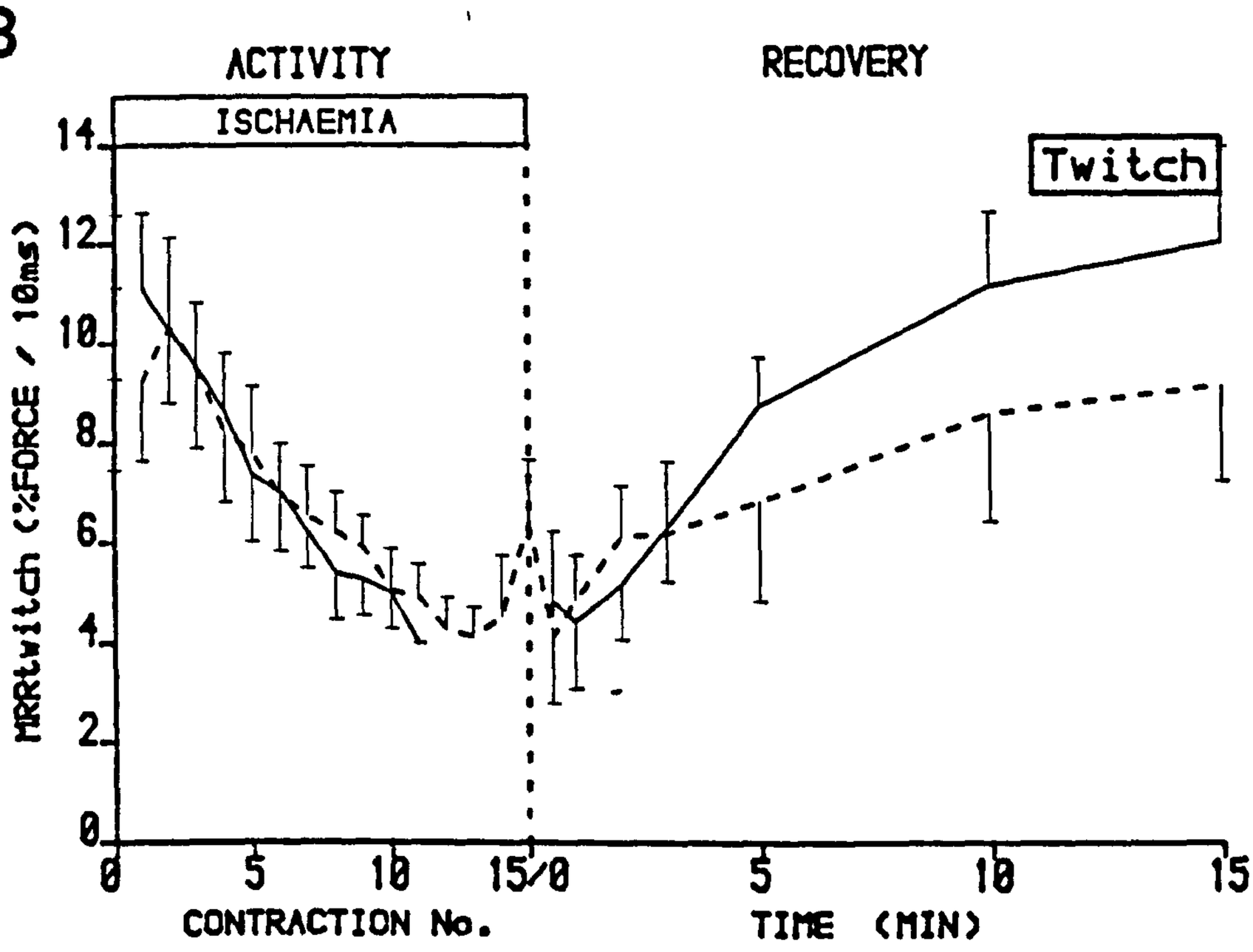


Figure 6.4 Changes in the maximal relaxation rate (MRR, maximum % force loss in 10 ms) after A)10Hz and B)1Hz (twitch). D) Changes in maximal contraction (MCR, maximum % force gain in 10 ms) of the twitch. Symbols as used previously.

B


ACTIVITY
RECOVERY
ISCHAEMIA
Twitch
MRRtwitch (%FORCE / 10ms)
0
2
4
6
8
10
12
14
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

C

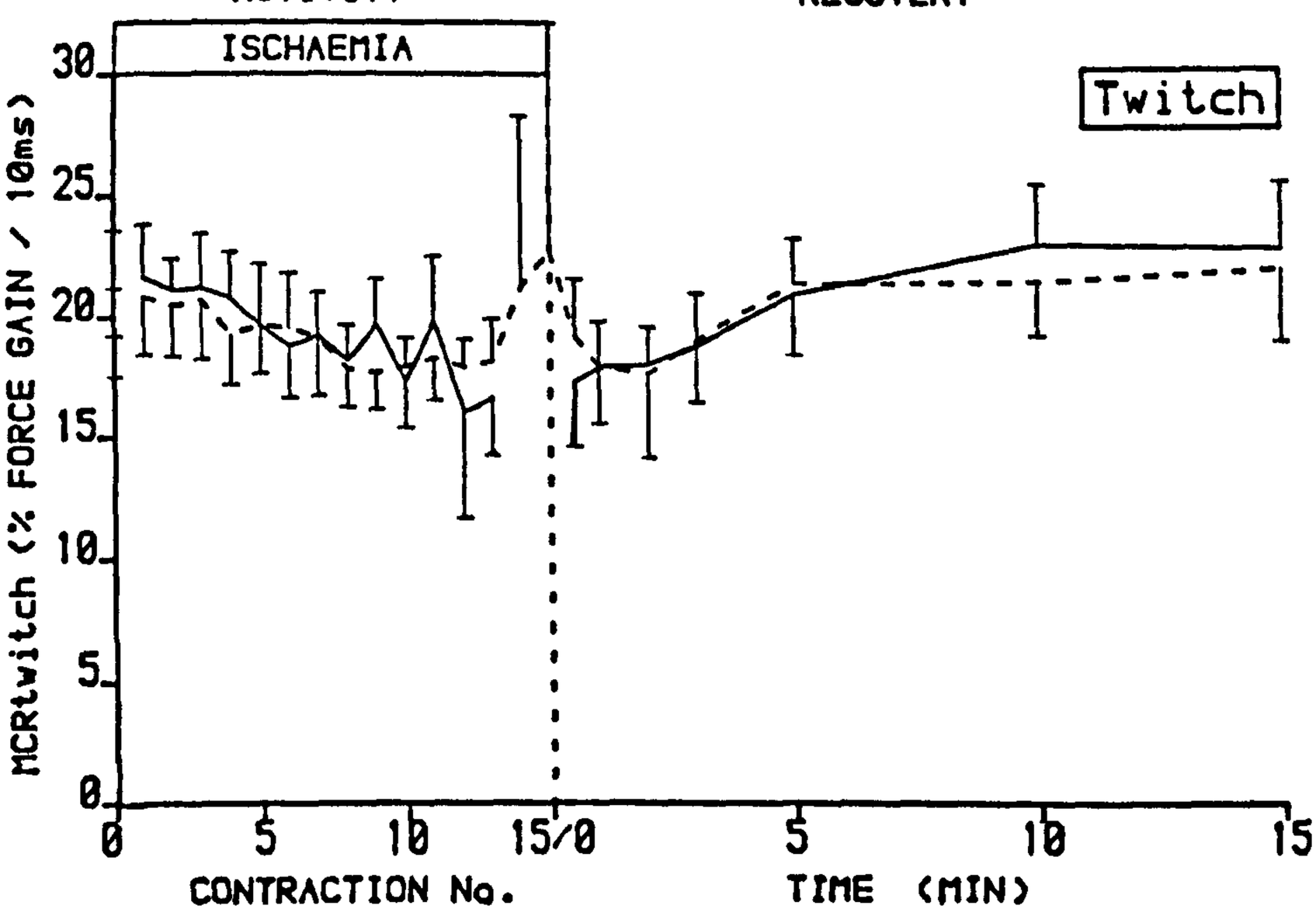
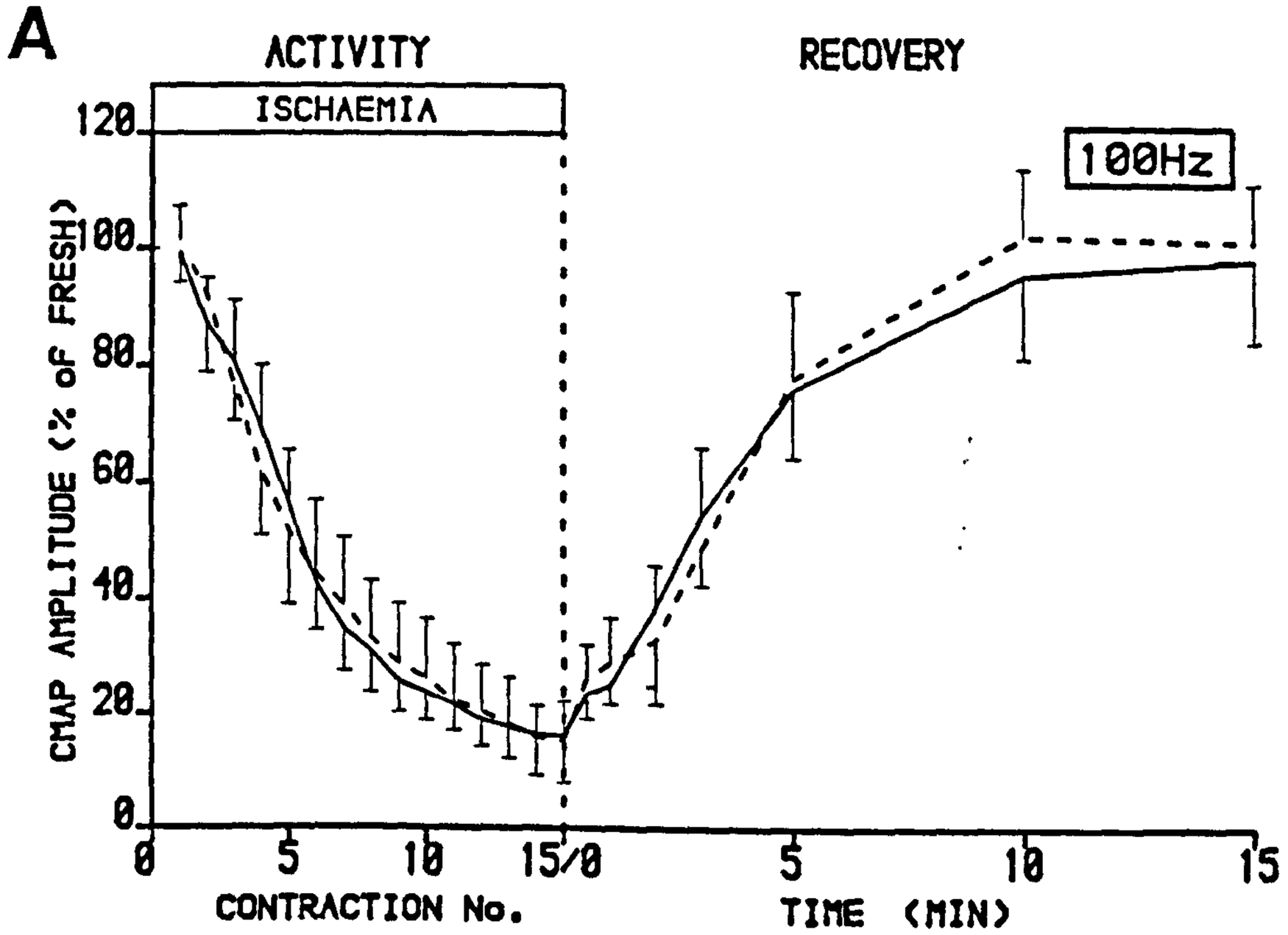
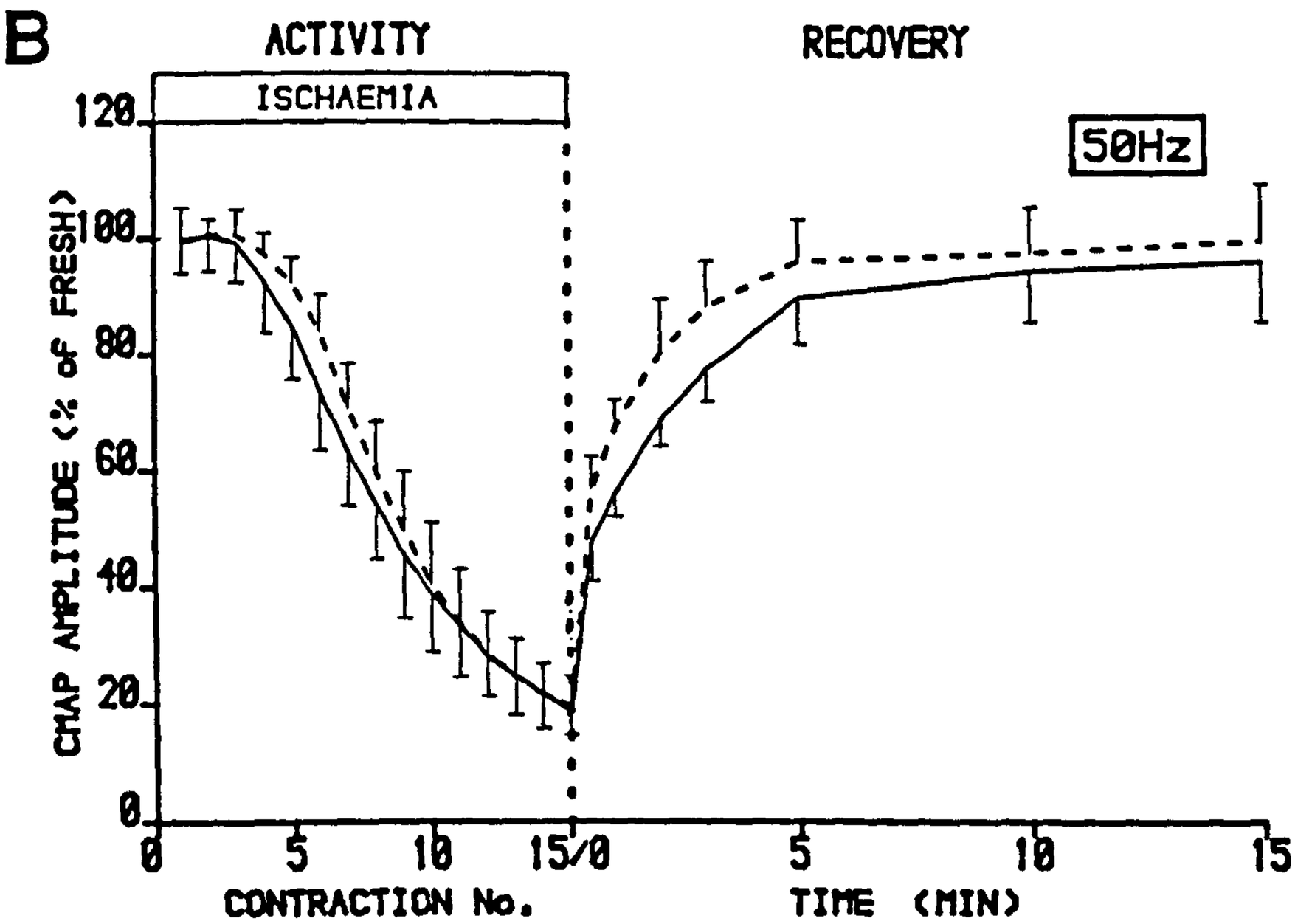
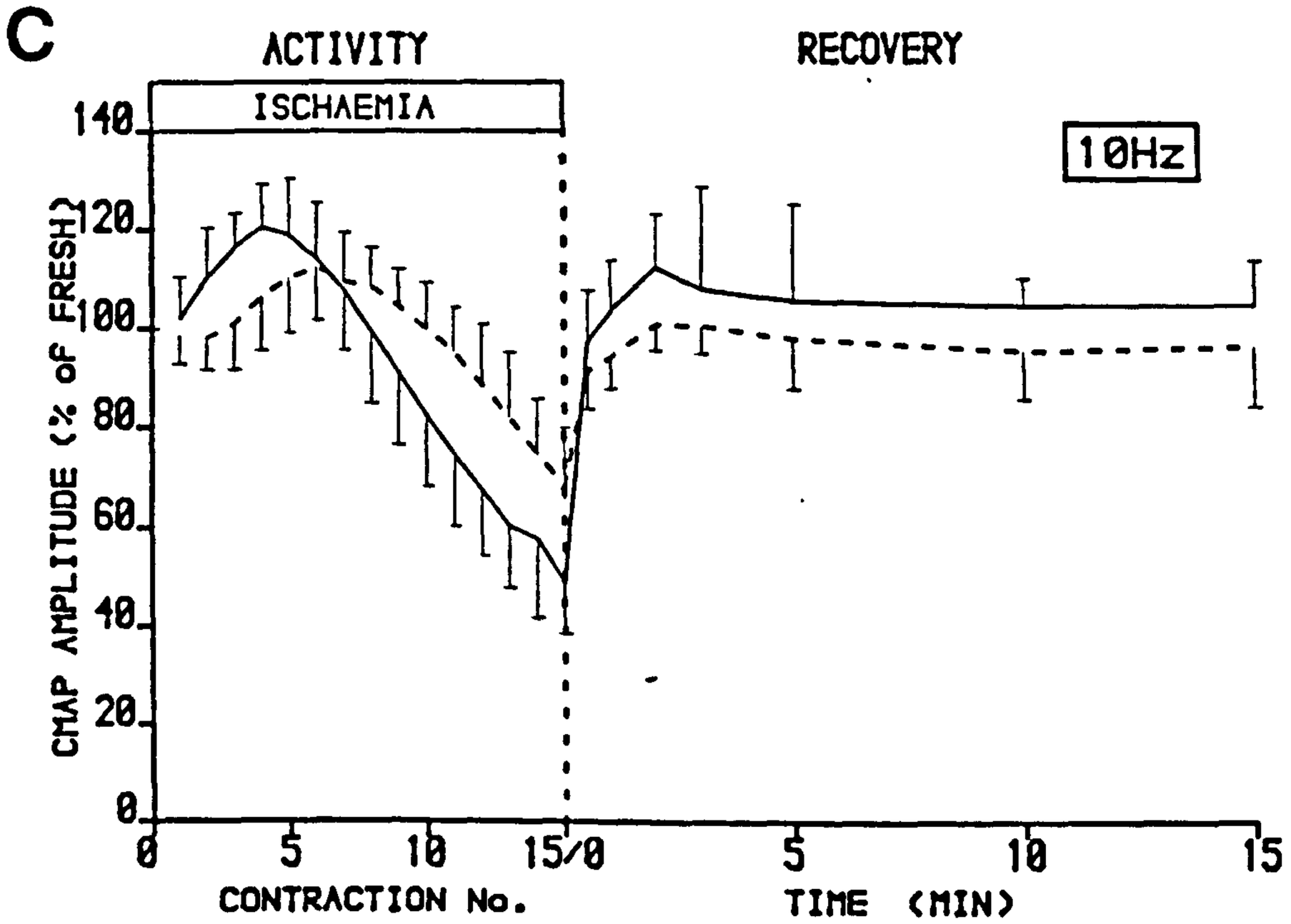
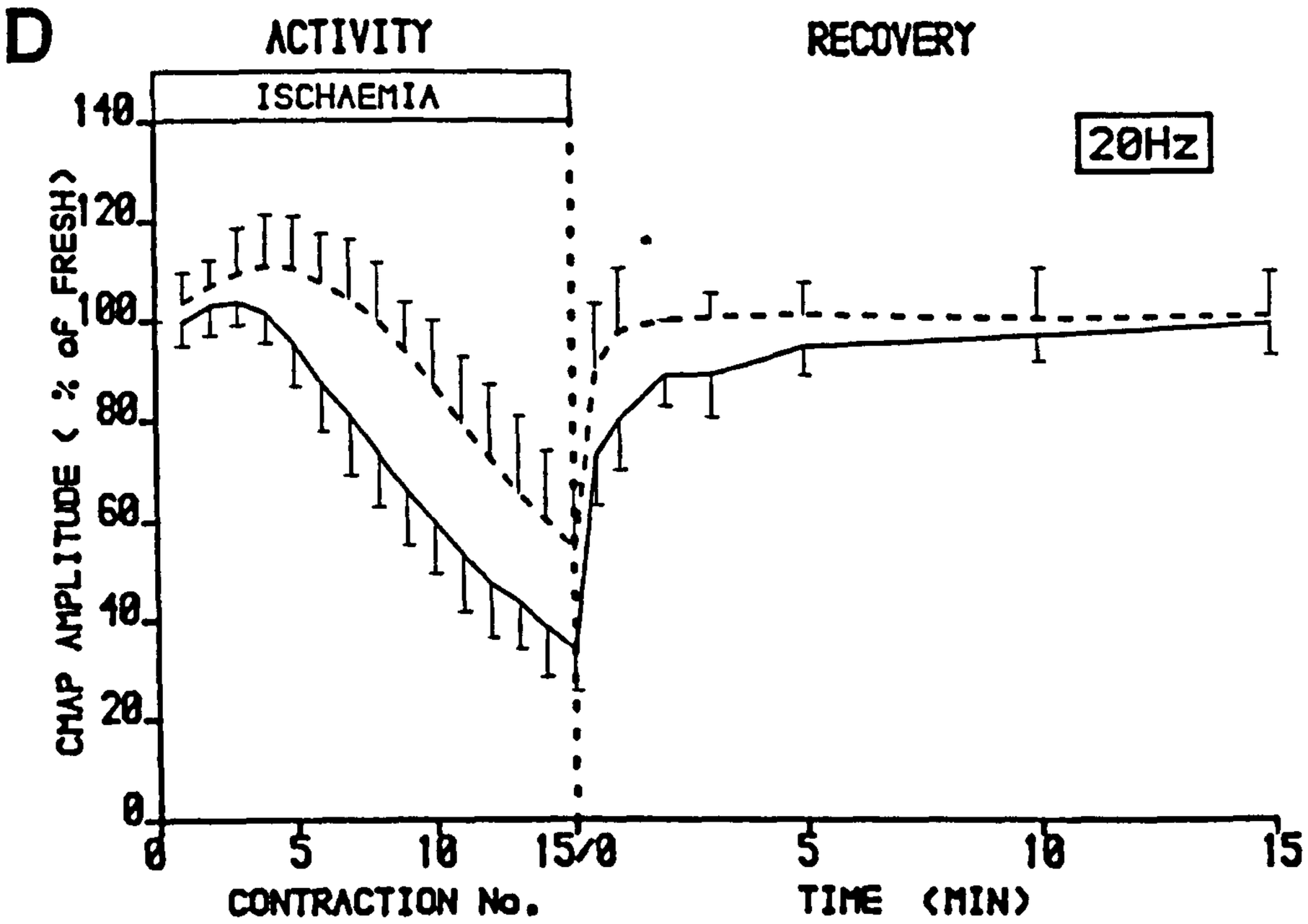

ACTIVITY
RECOVERY
ISCHAEMIA
Twitch
MCRtwitch (% FORCE GAIN / 10ms)
0
5
10
15
20
25
30
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

Figure 6.5 Time course of changes of CMAP amplitude, as a percentage of fresh values, during fatiguing activity with circulatory occlusion and during aerobic recovery at: A) 100Hz, B)50Hz, C)20Hz, D)10Hz and E) 1Hz (twitch). The symbols are as in the previous figure. Note that the CMAP amplitude declines more during the descending PSEM series.


A
ACTIVITY
RECOVERY
ISCHAEMIA
100Hz
120
100
80
60
40
20
0
CMAP AMPLITUDE (% of FRESH)
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)


B
ACTIVITY
RECOVERY
ISCHAEMIA
50Hz
120
100
80
60
40
20
0
CMAP AMPLITUDE (% of FRESH)
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)


C
ACTIVITY
RECOVERY
ISCHAEMIA
10Hz
140
120
100
80
60
40
20
0
CMAP AMPLITUDE (% of FRESH)
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)


D
ACTIVITY
RECOVERY
ISCHAEMIA
20Hz
140
120
100
80
60
40
20
0
CMAP AMPLITUDE (% of FRESH)
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

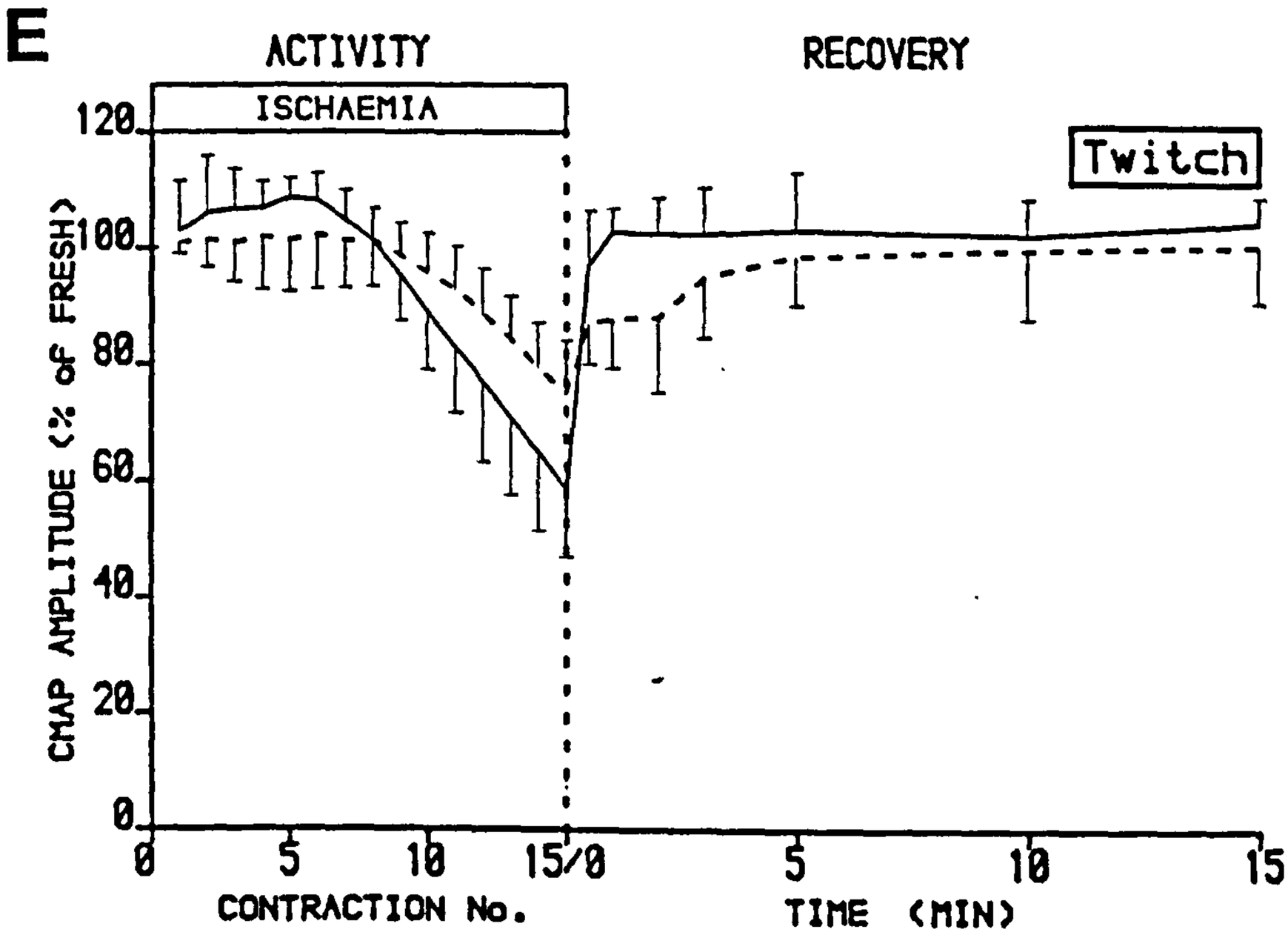
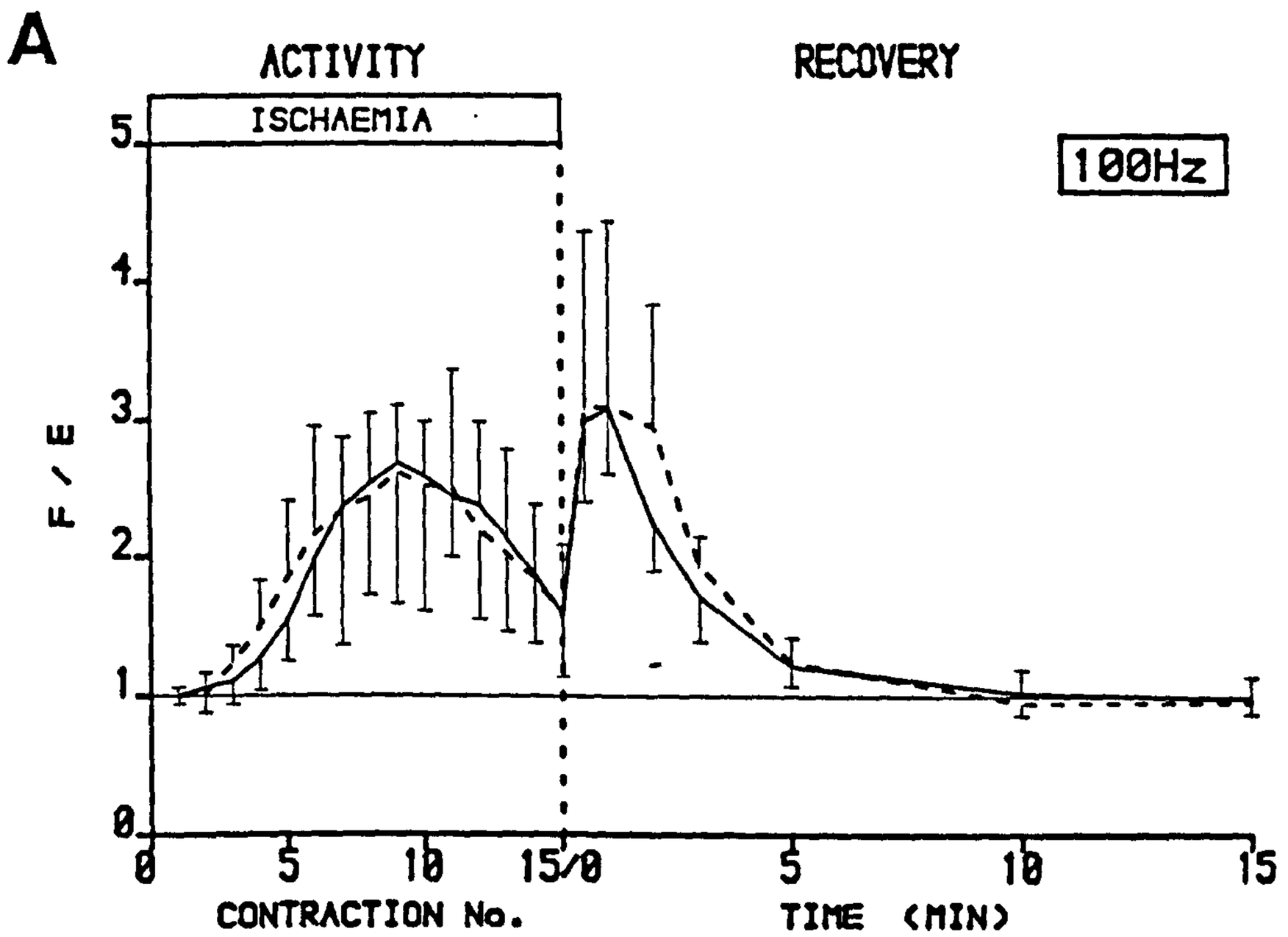
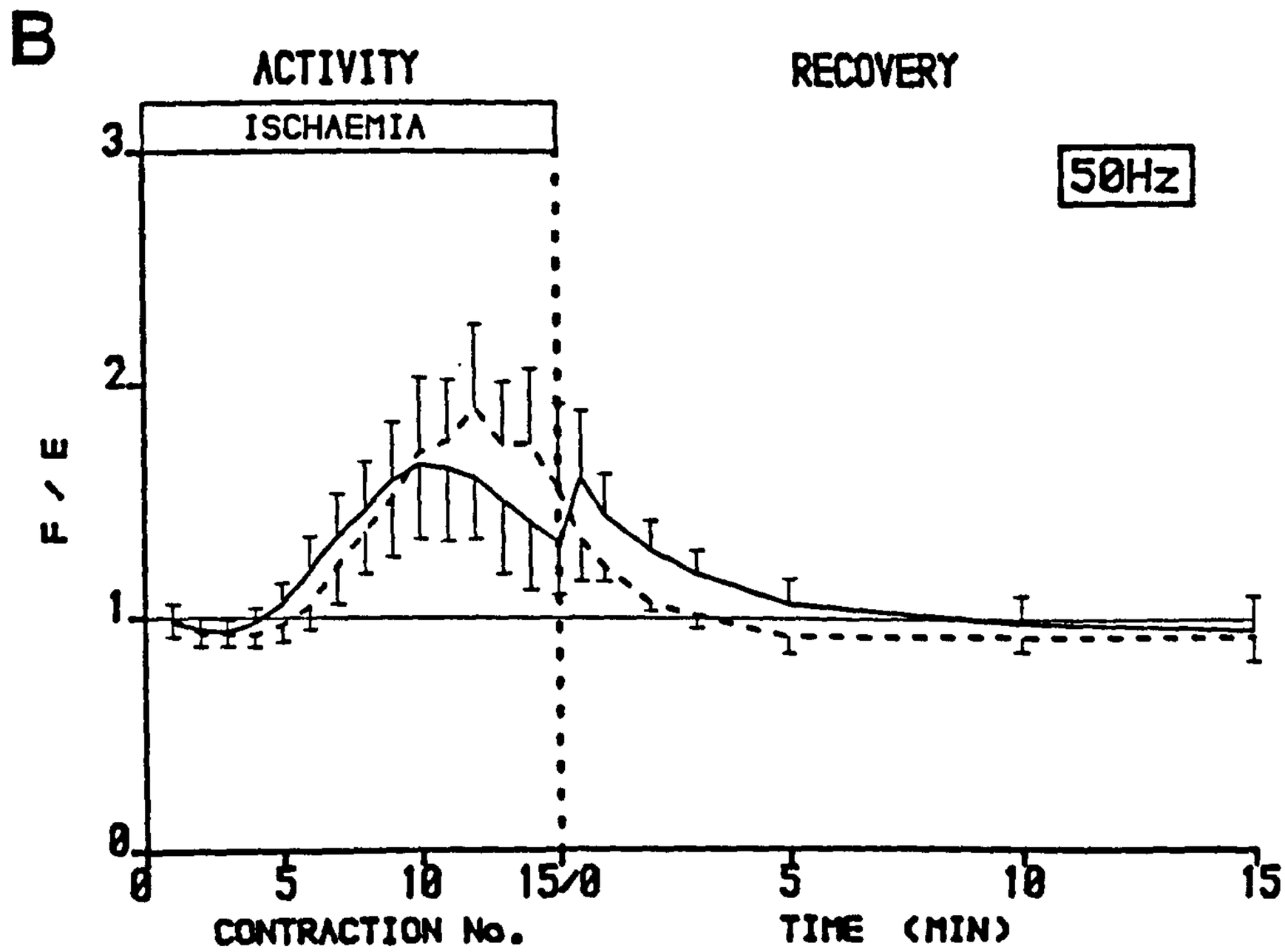
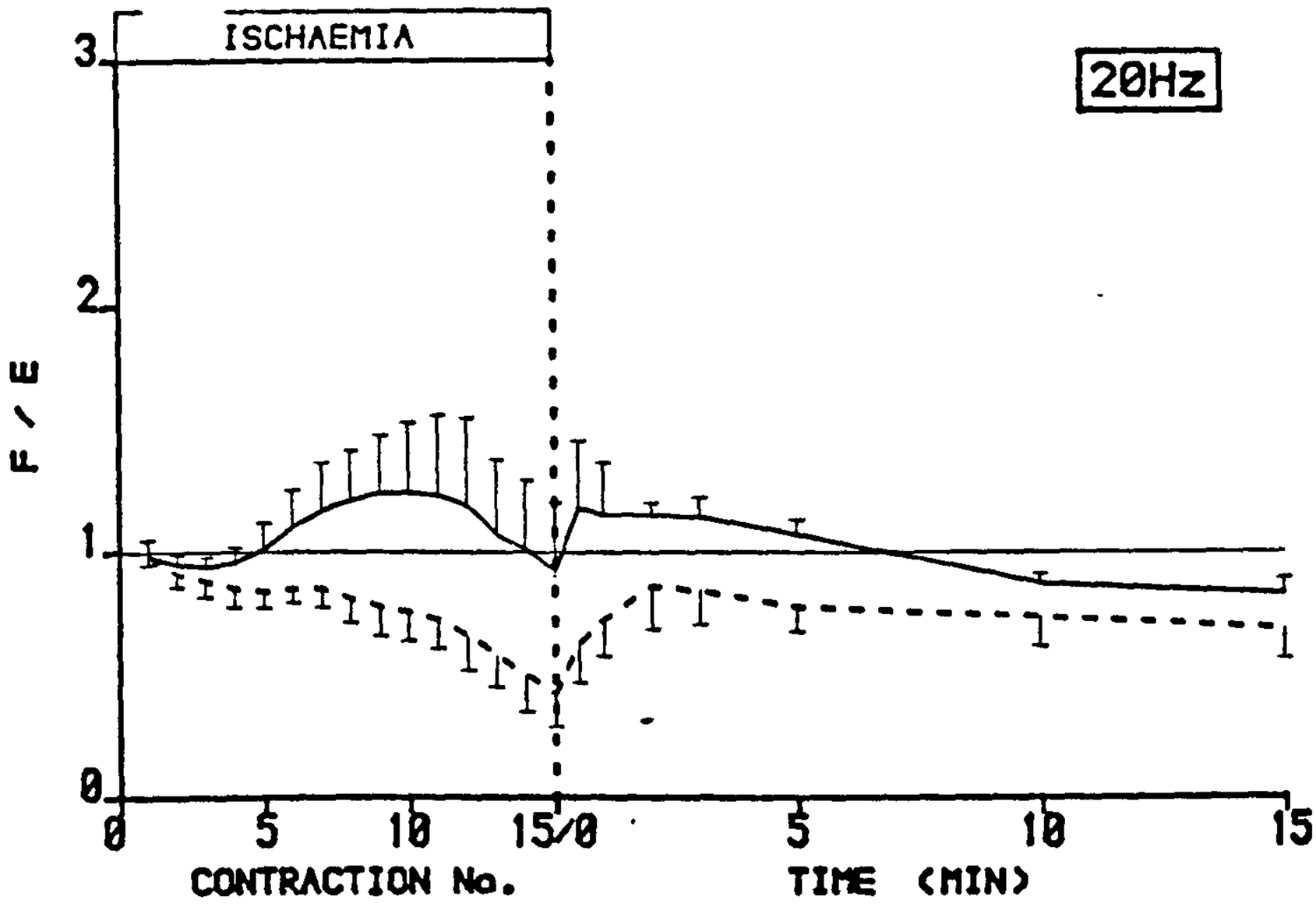

E
ACTIVITY
RECOVERY
ISCHAEMIA
Twitch
120
100
80
60
40
20
0
CMAP AMPLITUDE (% of FRESH)
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

Figure 6.6 Mean F/E ratio changes during fatiguing activity with arterial occlusion and during aerobic recovery at: A)100Hz, B)50Hz, C)20Hz, D)10Hz and E)1Hz (twitch). Symbols as used previously. Note that at 100Hz and 50Hz during activity the changes are similar for both protocols and the ratio remains > 1 because of excitation failure in excess of force failure. At 10Hz during the ascending PSEM series the ratio initially increases to > 1 because of potentiation , before declining to < 1 due to fatigue. When using the descending PSEM series the ratio remains > 1 because potentiation is prolonged. At 1Hz, the ratio declines to < 1 because of force failure in excess of excitation failure.

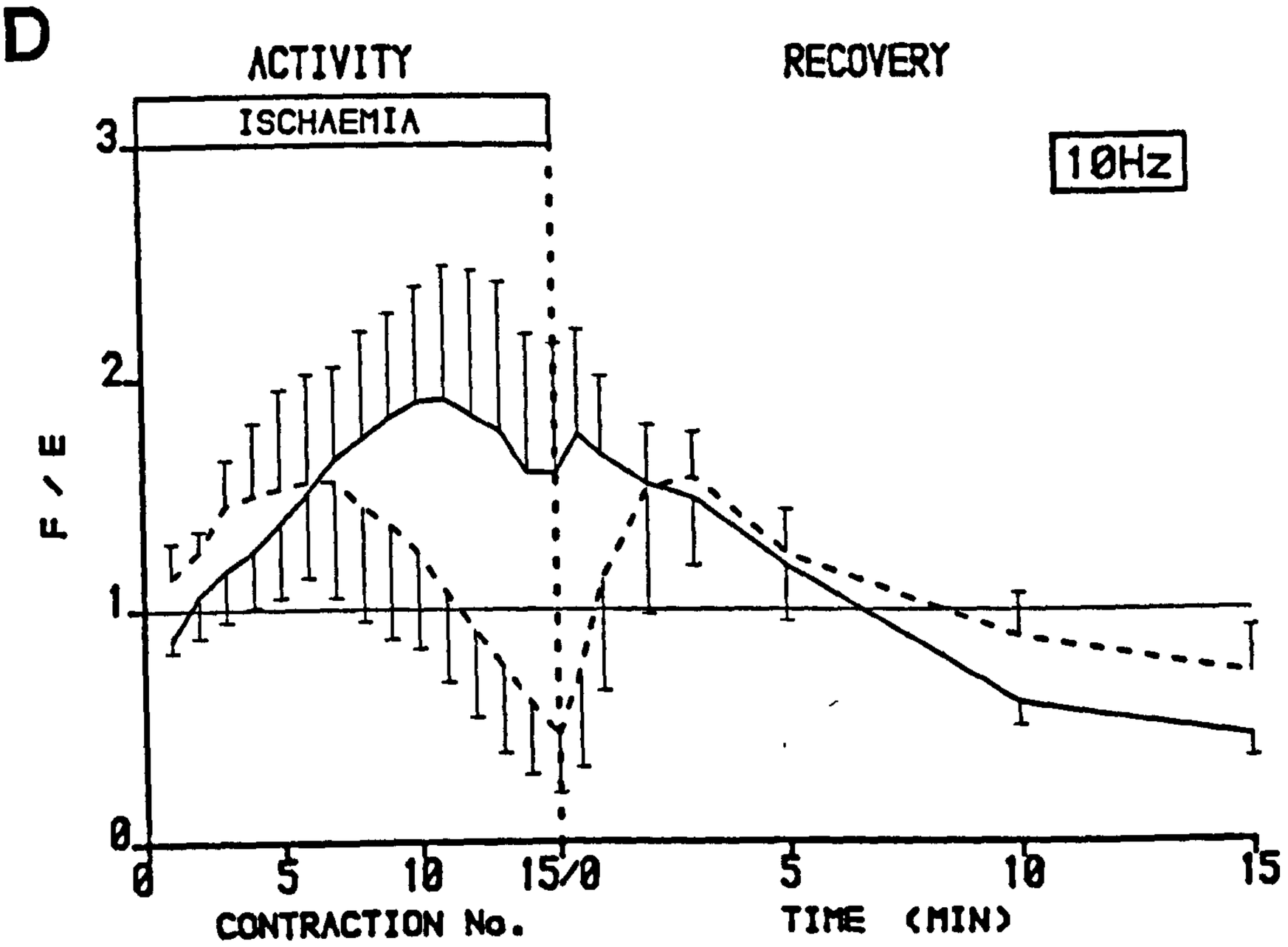

A
ACTIVITY
RECOVERY
ISCHAEMIA
100Hz
F / E
5
4
3
2
1
0
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)


B
ACTIVITY
RECOVERY
ISCHAEMIA
50Hz
F / E
3
2
1
0
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

C


ACTIVITY
RECOVERY
ISCHAEMIA
20Hz
F / E
3
2
1
0
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

D


ACTIVITY
RECOVERY
ISCHAEMIA
10Hz
F / E
3
2
1
0
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

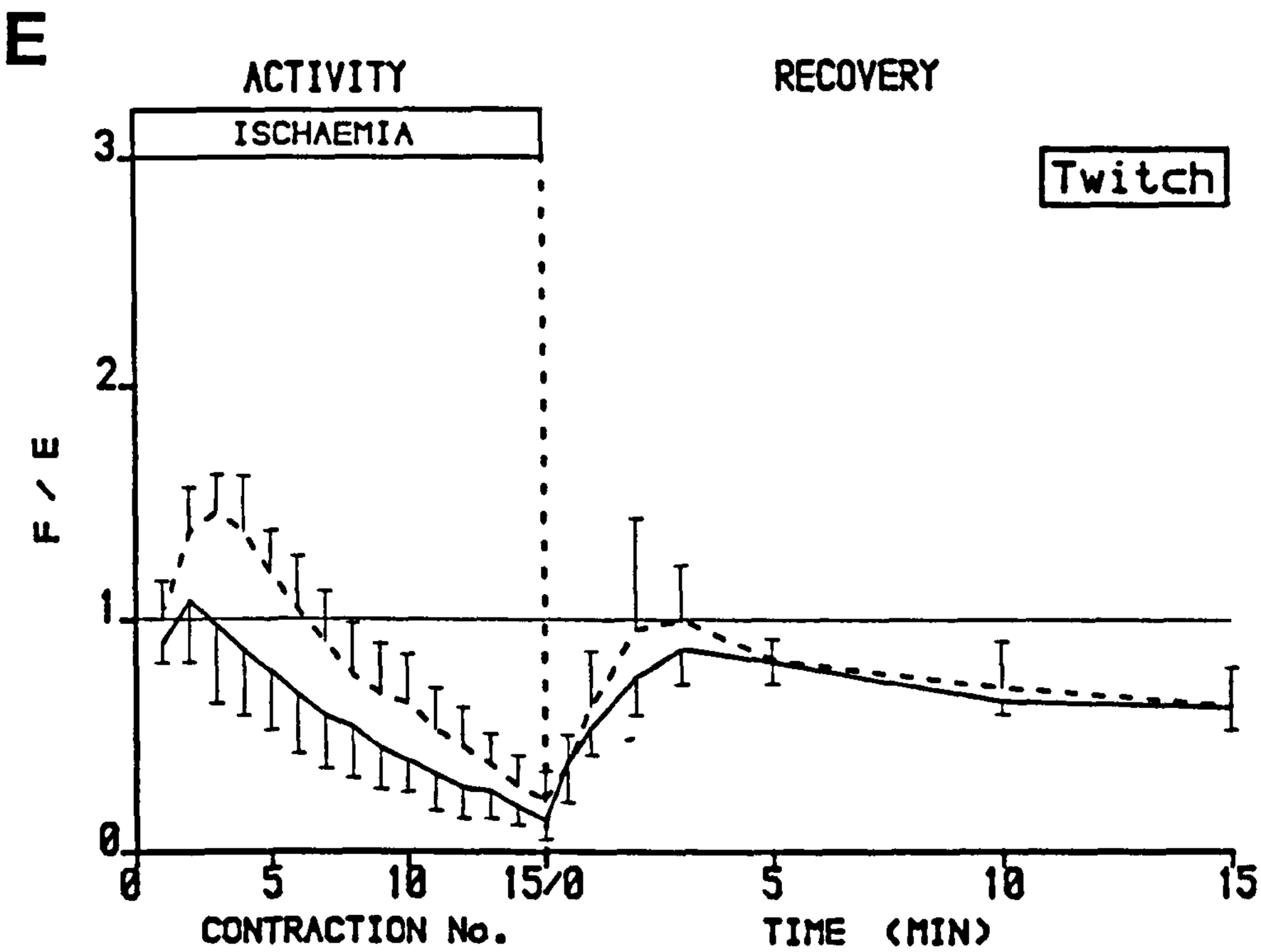
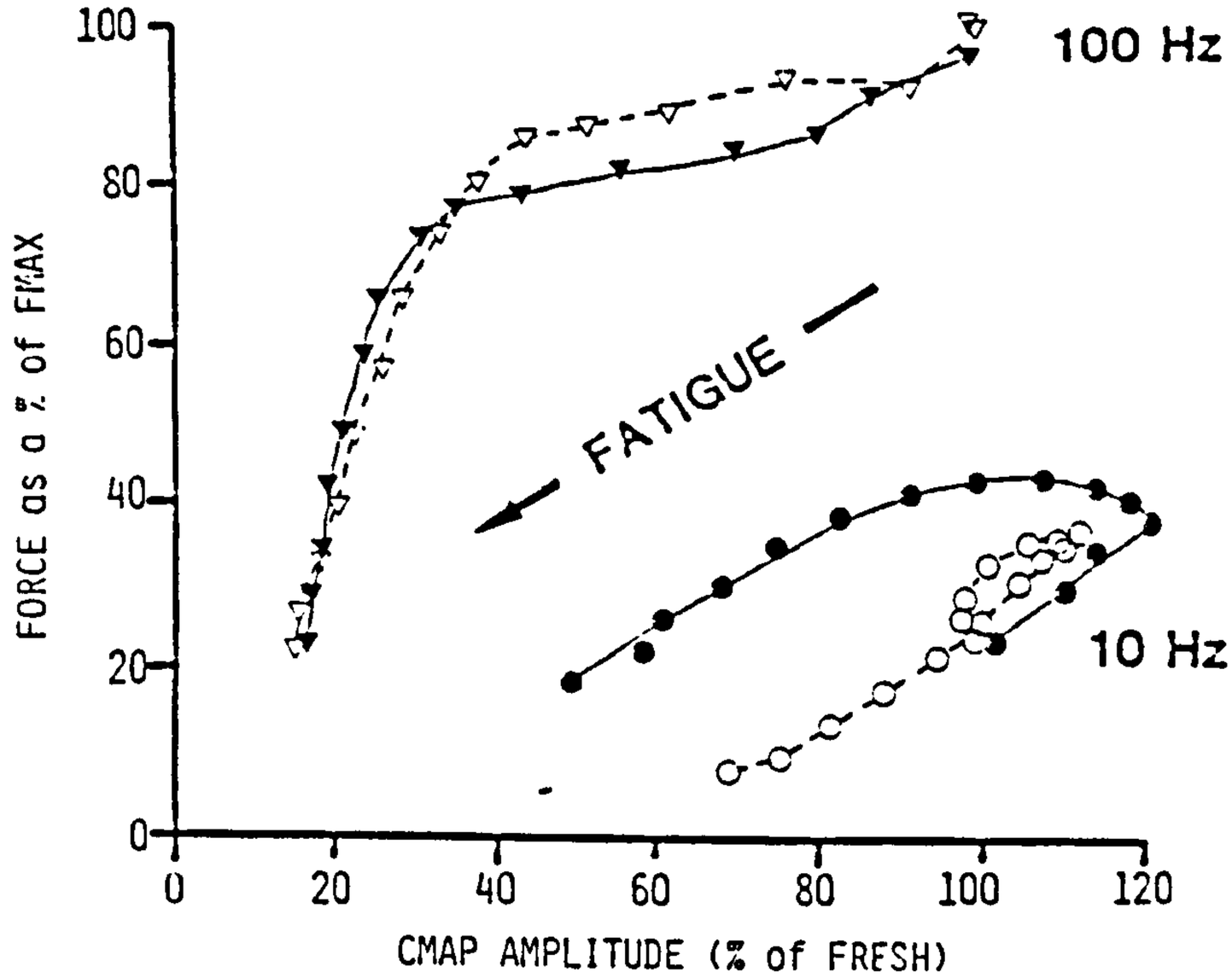

E
ACTIVITY
RECOVERY
ISCHAEMIA
Twitch
3
2
1
0
F / E
0
5
10
15/0
5
10
15
CONTRACTION No.
TIME (MIN)

Figure 6.7 Mean changes in force relative to mean changes in excitation (CMAP amplitude) during fatiguing activity. Open circles and interrupted lines are used to depict ascending PSEM series results and closed symbols and solid lines to depict descending PSEM series results. At high frequency force is initially maintained despite marked early declines in excitation indicating the operation of a 'safety factor'. At low frequency force and excitation are initially potentiated before declining due to fatigue. During the descending PSEM series, force and excitation potentiate to a greater degree and the subsequent declines were clearly less than during the ascending PSEM series. Thus, by preceding low with high-frequency stimulation, low-frequency fatiguability has been reduced. A) 100 and 10Hz, B) 50 and 20Hz.

A


100
80
60
40
20
0
FORCE as a % of FMAX
FATIGUE
100 Hz
10 Hz
0
20
40
60
80
100
120
CMAP AMPLITUDE (% of FRESH)

B

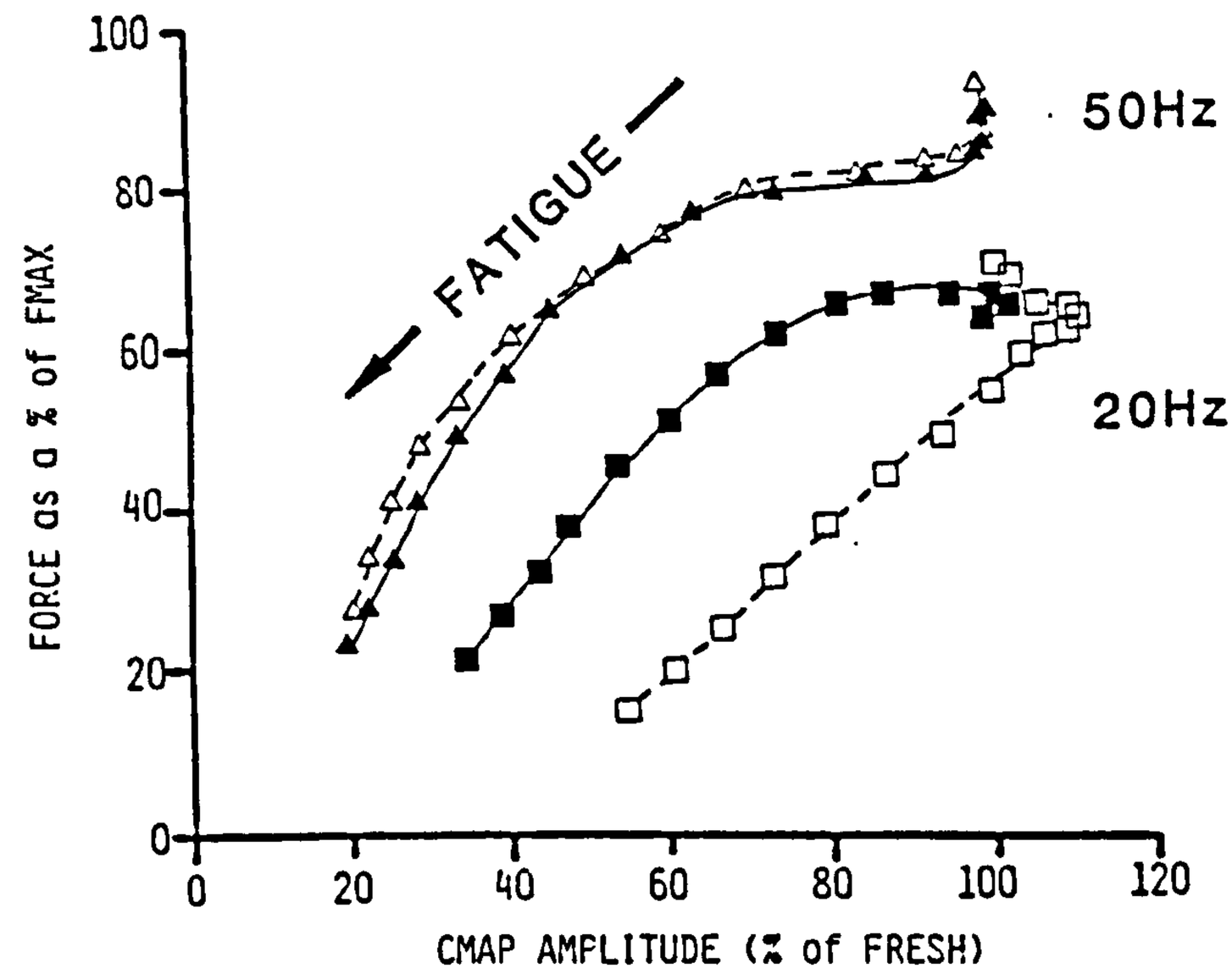
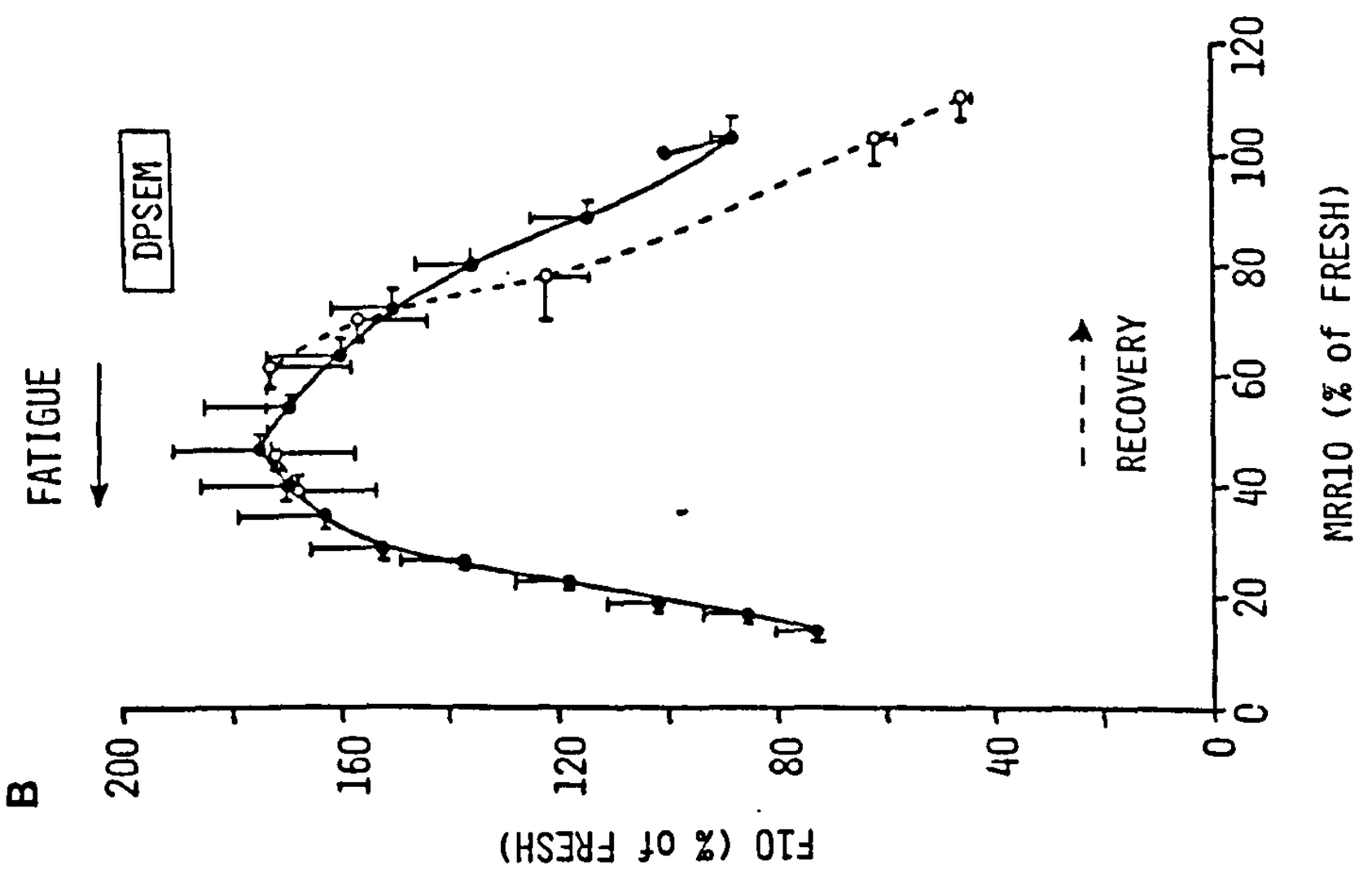

100
80
60
40
20
0
FORCE as a % of FMAX
FATIGUE
50Hz
20Hz
0
20
40
60
80
100
120
CMAP AMPLITUDE (% of FRESH)

Figure 6.8 Changes in mean force as a function of changes in mean MRR at 10Hz during activity (shown as solid lines) and recovery (shown as interrupted lines). A) When using the ascending PSEM, force potentiated during activity to about 170% of fresh values before declining due to fatigue. During recovery force again potentiated but to only about 140% of fresh values. B) When using the descending PSEM potentiation was similar to the ascending PSEM protocol during activity but during recovery potentiation was immediate and of similar degree to that during activity. Mean $\pm$ SEM.


B
FATIGUE
DPSEM
RECOVERY
FIO (% of FRESH)
MRR10 (% of FRESH)
200
160
120
80
40
0
0
20
40
60
80
100
120

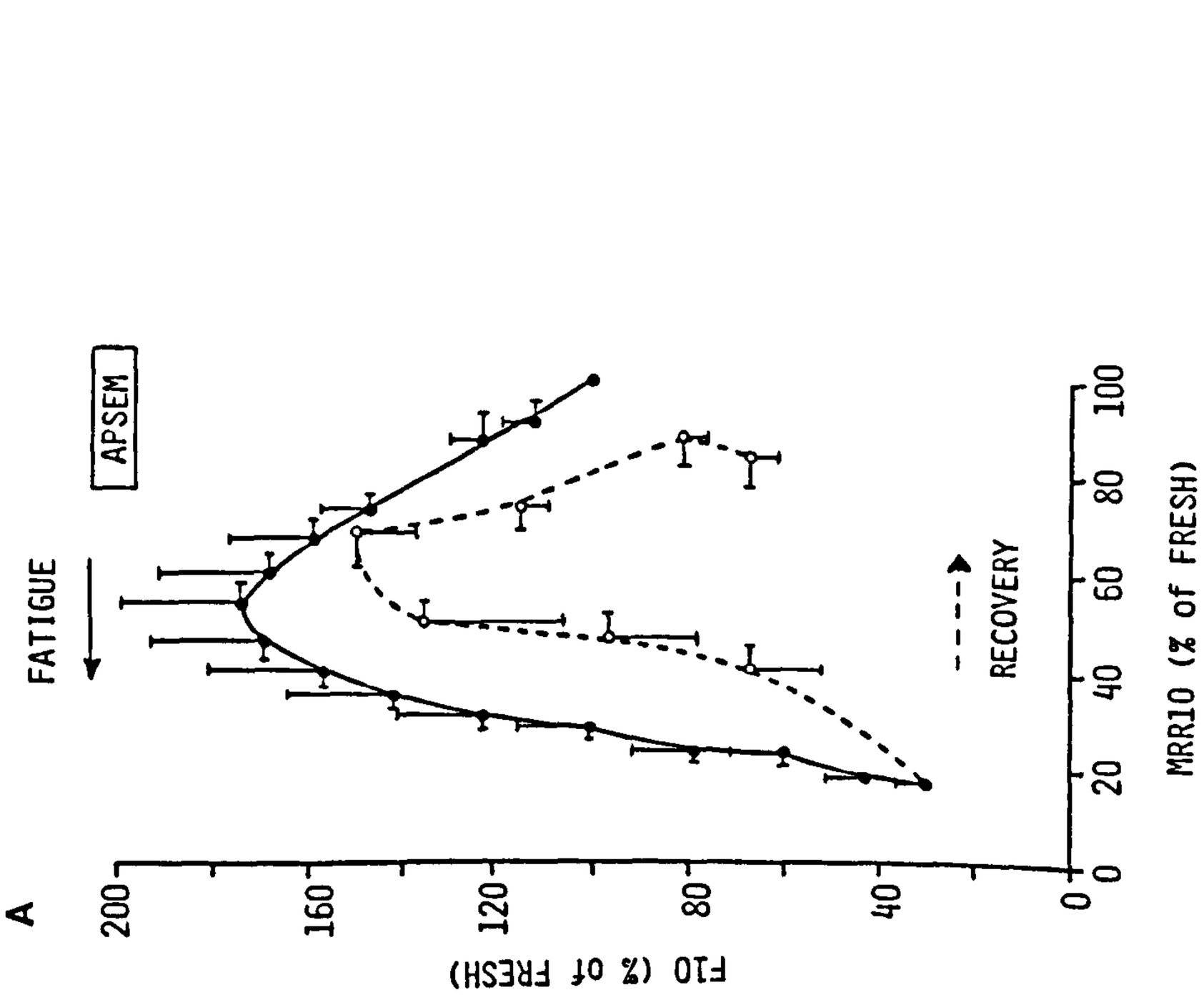
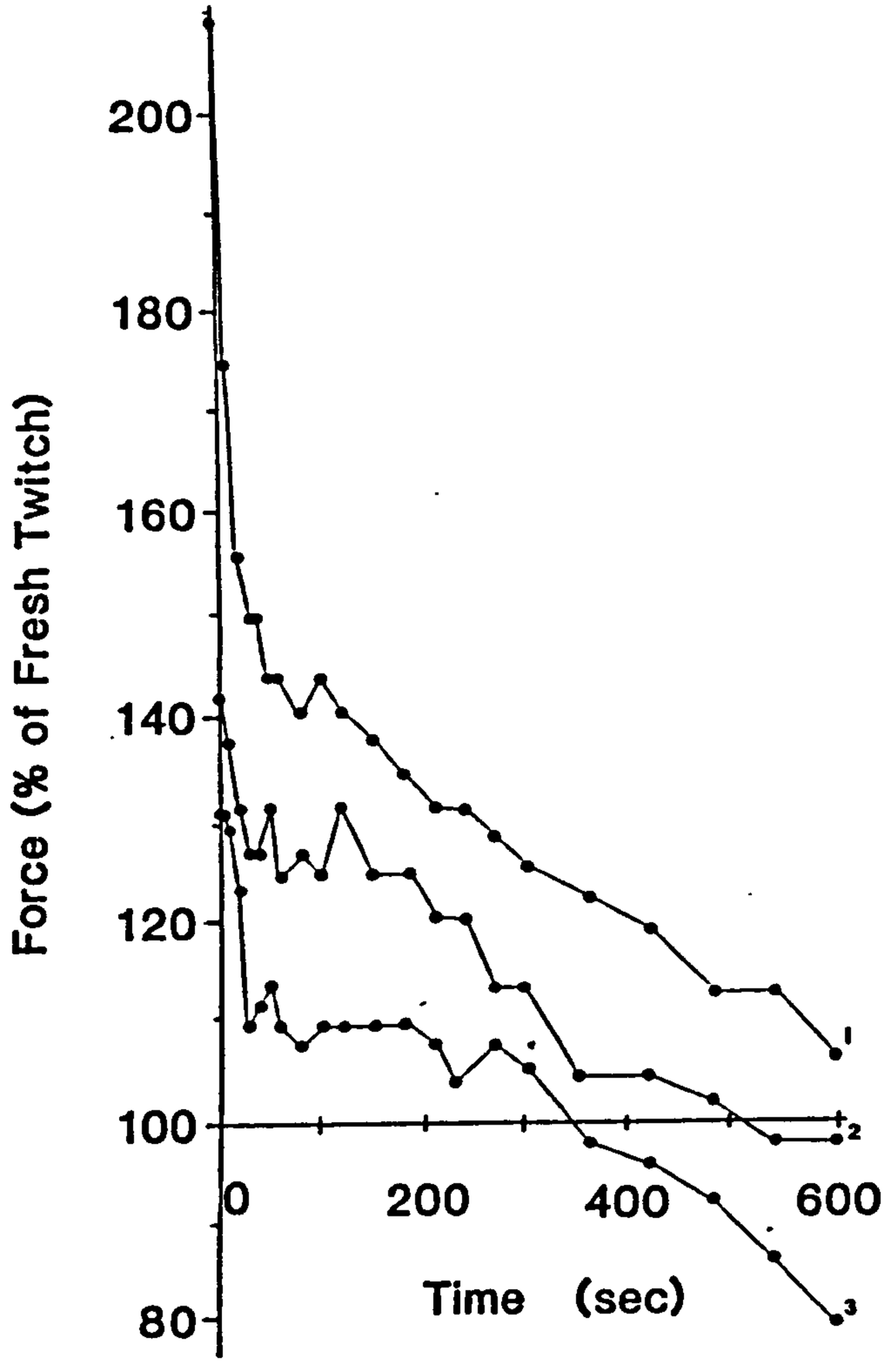
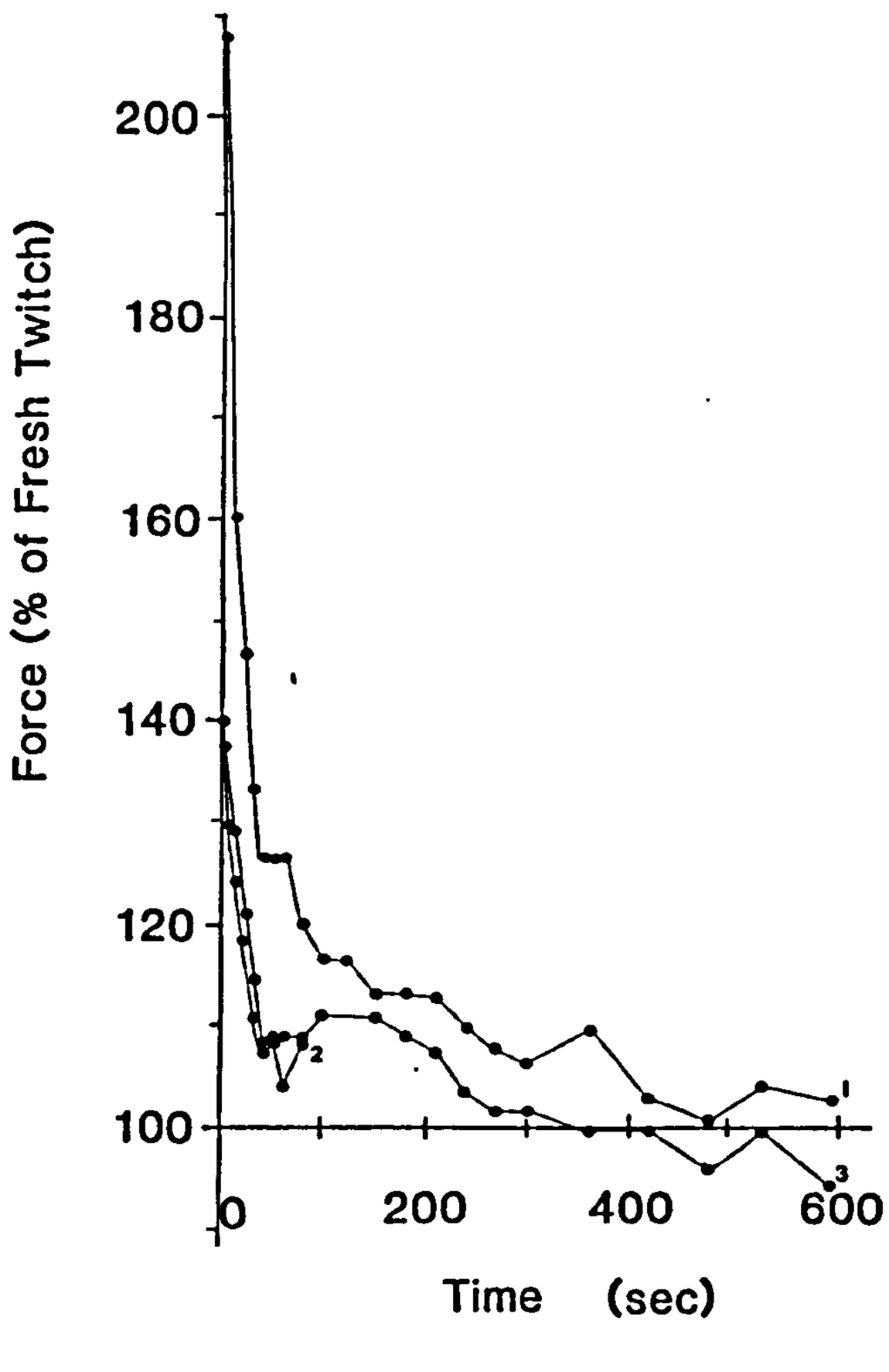

A
FATIGUE
APSEM
RECOVERY
FIO (% of FRESH)
MRR10 (% of FRESH)
200
160
120
80
40
0
0
20
40
60
80
100

Figure 6.9 Post-PSEM potentiation and decay of the twitch during A)circulatory occlusion and B) intact circulation in three subjects. The circulation was occluded three minutes before the PSEM was delivered in (A). Note the initial rapid decline in twitch potentiation followed by a slower phase lasting five-six minutes during both conditions, indicating the decline in twitch force in (A) is probably not due to ischaemia.

A


Force (% of Fresh Twitch)
200
180
160
140
120
100
80
0
200
400
600
Time (sec)
1
2
3

B


Force (% of Fresh Twitch)
200
180
160
140
120
100
0
200
400
600
Time (sec)
1
2
3

6.4 DISCUSSION

The results of this study have clearly demonstrated the beneficial effect on the fatiguability of low-frequency tetani of commencing each stimulated contraction at a high frequency. Full potentiation of the twitch appears to have been achieved, since twitch force did not rise substantially during the descending contraction series. Low-frequency force was observed to potentiate in both contraction series indicating that post-tetanic twitch potentiation could not be responsible for the initial increase in force alone. However, the increase in low-frequency force was **greater** for the descending frequency series and remained so throughout activity, at a time when twitch force became unexpectedly **smaller** than that obtained during the ascending series. Since the MRR declined identically for both contraction series, it appears that a factor over and above twitch force and MRR slowing, is responsible for the greater low-frequency force potentiation seen during the descending PSEM contraction series.

6.4.1 Factors causing low-frequency force potentiation

6.4.1.1 Slowing of MRR

The possible role of the slowing of relaxation rate in potentiating low-frequency force has already been alluded to in chapter 3. It has been suggested that the slowing of relaxation rate, as a consequence of voluntary and stimulated fatiguing activity (Edwards *et al.*, 1972a; Wiles & Edwards, 1982a; Bigland-Ritchie *et al.*, 1983a), may contribute to the reduction of force loss by increasing twitch summation of low-frequency incomplete fused tetani (Bigland-Ritchie *et al.*, 1983a; Jones, 1981). In the present study, this is reflected by the reduction in oscillation (Figure 6.3) observed at 10Hz. The fact that low-frequency force increased during both contraction series in the present study confirms the suggestion that MRR slowing is involved in preventing force loss and indeed increases the F/E ratio to > 1. The degree to which this occurs appears limited, however, as MRR slowing begins to plateau after the seventh PSEM contraction and the F/E ratio declines during the ascending frequency PSEM contraction series.

6.4.1.2 Post-tetanic twitch potentiation

Despite twitch potentiation having been achieved in fresh muscle, low-frequency force generation was not improved by stimulating with a descending frequency pattern of stimuli, probably due to the increase in oscillation of low-frequency force (Table 6.1). This is not surprising, since it is well established that following brief tetanic activity in man, potentiation of the twitch is also characterized by an increase in the peak rate of force development and relaxation of the twitch (present study, Table 6.1; Desmedt & Hainaut, 1968). Continued contractile activity, however, appeared to potentiate low-frequency force generation to a greater degree than when an ascending frequency pattern of stimulation was employed suggesting that potentiation mechanisms continue to act at a time when further slowing of relaxation resisting fatigue is no longer possible.

The simplest explanation to account for the differences in 10Hz force potentiation is that low-frequency post-tetanic potentiating mechanisms may involve alterations in Ca^{2+} kinetics as has been proposed for post-tetanic potentiation of the twitch (Desmedt & Hainaut, 1968; Blinks *et al.*, 1978; MacIntosh & Gardiner, 1987). Work in amphibian muscle using a Ca^{2+} sensitive bioluminescent protein, aequorin, to detect free Ca^{2+} suggests twitch potentiation is not due to release of abnormally large amounts of Ca^{2+} (Blinks et al, 1978). This would probably lead to prolongation of the twitch as is observed with caffeine stimulated twitch contractions (MacIntosh & Gardiner, 1987). In this and other studies (Desmedt & Hainaut, 1968; others), however, post-tetanic twitch potentiation was associated with an increase in maximal contraction rate and, although not seen in the present study, an increase in the rate of slowing which further supports an alternative mechanism for force potentiation.

Such findings have led to the suggestion that post-tetanic potentiation of the twitch results from changes in myofibrillar Ca^{2+} sensitivity. Studies in rabbit skeletal muscle suggest that this may be a result of activity-induced phosphorylation increasing the affinity of myosin for actin (Persechini & Stull, 1987). This is supported by the good correlation between twitch potentiation and myosin light-chain

phosphorylation in rodent fast-twitch muscle (Moore & Stull, 1984). Further evidence to suggest changes in myofibrillar sensitivity to Ca^{2+} may be an important mechanism for post-tetanic force potentiation has been obtained from studies in skinned barnacle fibres in which hysterises of the isometric force-pCa relationship is produced when the concentration of Ca^{2+} in the bathing medium producing a submaximal force is increased and subsequently reduced to the initial concentration (Ridgeway *et al.*, 1983). The authors of this study reported that the hysterises was lost if Ca^{2+} was removed from the bathing medium before returning the Ca^{2+} concentration to initial levels, indicating some dependence of force potentiation on immediate contractile activiation.

An alternative mechanism of post-tetanic twitch potentiation has been proposed in which Ca^{2+} availability is thought to be increased at the site of the myofibrils (Blinks *et al.*, 1978; MacIntosh & Gardiner, 1987). Despite observations that the Ca^{2+} response to a post-tetanic twitch stimulus is reduced (Blinks *et al.*, 1978), it was argued that an increase in activation could still be achieved if Ca^{2+} remained in the sarcoplasm in the vicinity of the myofibrils, but of a mechanically subthreshold amount, or alternatively if Ca^{2+} was taken up or bound by potential 'sinks'; the subsequently released Ca^{2+} becoming available for binding to troponin. More recent studies in amphibian muscle employing a more sensitive aequorin technique than that used by Blinks *et al.*, (1978) support the latter proposition (Cannell, 1986). These have shown that during tetanic activity a Ca^{2+}-binding protein, other than troponin takes up Ca^{2+} in conjunction with the sarcoplasmic reticulum to reduce myofibrillar Ca^{2+} concentration (Cannell, 1986). Such a role has been suggested for parvalbumins (Cannell, 1986) in view of their high affinity for Ca^{2+} (Haiech *et al.*, 1979) and high prevalance in type II fibres (Heizmann *et al.*, 1982). The subsequent release of Ca^{2+} from the Ca^{2+}-binding proteins may last several seconds (Cannell, 1986) during which time it is likely that subsequent twitch stimuli may still be potentiated. A similar mechanism may act in mammalian muscle, although the time course of the decay of potentiation of the twitch following

voluntary or stimulated contractions may be of the order of minutes rather than seconds (present study: Figure 6.9; Close & Hoh, 1968; Desmedt & Hainaut, 1968; Krarup, 1981; Vandervoort *et al.*, 1983).

There is also evidence to suggest that some residual Ca^{2+} remains bound to troponin. The studies of Cannell (1986) have demonstrated that during the relaxation phase following a brief tetanus, myoplasmic Ca^{2+} concentration is still raised at a time when the mechanical response has ceased. This elevated Ca^{2+} is thought to arise from a decrease in the affinity of troponin-C for Ca^{2+} since the detachement of cross-bridges is suggested to lead to a reduction in the binding constant for troponin (Weber & Murray, 1973). More recent studies indicate troponin-C has four binding sites for Ca^{2+} two of which are low affinity sites (Ca^{2+}-binding constant 2×10^7 M^{-1}) and the other two high affinity (Ca^{2+}-binding constant 5×10^5 M^{-1}) (Potter & Gergely, 1974; Leavis *et al.*, 1978). The binding of Ca^{2+} to the low-affinity sites is thought to be important in regulation of actin-myosin interaction (Potter & Gergely, 1974). During relaxation it is probable that the low affinity sites release Ca^{2+} in preference to the high affinity binding sites. Clearly, if a residual amount of Ca^{2+} remains bound to the myofibrils, it is conceivable that a stronger contraction would be produced than if the myofibrils were totally Ca^{2+}-free during subsequent twitch stimuli. Such a mechanism may also have important implications during fatiguing activity where a reduction in the amount of Ca^{2+} released per impulse occurs as a consequence of fatigue (Blinks *et al.*, 1978; MacIntosh & Gardiner, 1987), possibly due to build-up of metabolic by-products (Dawson *et al.*, 1978).

Clearly, the mechanisms suggested for post-tetanic potentiation of the twitch may account, in part, for the present observations of low-frequency force potentiation. Since low-frequency force potentiation occurred at a time when the twitch was declining, however, it may be argued that an alternative mechanism to twitch potentiation is responsible for low-frequency force potentiation. This paradox may similarly be explained by those mechanisms potentiating twitch force. The decline in post-tetanic twitch potentiation has been shown to decline exponentially in

mammalian muscle, including man (Close & Hoh, 1968; Krarup, 1981) and in this study. It appears likely, therefore, that in the time between tetani and twitch during the descending PSEM, the degree of potentiation had already declined considerably. Alternatively, the mechanism potentiating low-frequency force per impulse is short-lived in comparison to that for the twitch.

These effects do not influence high-frequency force generation since activation is already at or near its maximum, as demonstrated by the shape of the frequency:force curve (Merton, 1954; Edwards *et al.*, 1977) which resembles the pCa:force curve (Donaldson & Hermansen, 1978).

6.4.1.3 Other factors influencing low-frequency force potentiation

The proposed mechanisms of post-tetanic twitch potentiation in amphibian and mammalian muscle may explain the results of the present study, although these have necessitated extrapolation from single fibre studies to whole muscle. The events occuring within the ascending/descending PSEM contraction series will therefore reflect the total response of the muscle fibre population, encompassing the inequalities within single fibres and of individual fibre types. Post-tetanic potentiation of the twitch is well documented in type II fibres (e.g., Close & Hoh, 1968) whereas post-tetanic depression has been demonstrated in muscles composed largely of type I fibres, e.g., cat soleus (Buller *et al.*, 1981). It is therefore of interest, that the human adductor pollicis should show a marked degree of twitch potentiation in fresh muscle in view of an 80% occurrence of type I fibres (Round *et al.*, 1984), a proportion similar to that of the human soleus (Johnson *et al.*, 1973), in which post-tetanic depression would be expected to occur. Further studies of low-frequency potentiation in different muscle groups of various fibre type composition is therefore indicated to assess the physiological relevance of potentiation in muscle function and fatigue.

The contribution of passive factors such as series elastic components (Hill *et al.*, 1949) and viscous elastic components to low-frequency force potentiation are likely to be minimal. The series elastic componants of muscle are not known to demonstrate hysteresis properties during contraction and relaxation. Furthermore,

during isometric contractions muscle length is not altered (although under the conditions used in the present study some shortening of muscle length is possible) and therefore the contribution of visco-elastic components on low-frequency force generation is likely to be negligible.

6.4.2 CONCLUSIONS

Although the explanation of low-frequency force potentiation is largely speculative, it is clear from this study, that preceding low-frequency tetani with high frequency stimulation clearly reduces fatiguability of low-frequency force generation and, furthermore, increases the F/E ratio in a similar fashion to that observed at high frequency. It appears that a low-frequency 'safety-factor' functions under the conditions imposed in this study, resembling that observed at high frequency. It is apparent that such a mechanism may only function during prolonged contractile activity where fatigue mechansisms come into play, since in fresh muscle an improvement in force generation at low-stimulation frequencies was not seen.

Other studies are indicated to further investigate the degree and nature of high-frequency stimulation (e.g., frequency and numbers of impulses) on low-frequency force-generation, and also to determine if a similar mechanism acts during maximal voluntary contraction where slowing of relaxation in conjunction with a decline in the motor-unit discharge rate is thought to maintain force and prevent loss of excitation (Bigland-Ritchie, 1981; Marsden *et al.*, 1983).

6.5 SUMMARY

1. The contribution of post-tetanic potentiation of the twitch and MRR slowing to altering the relationship between force generation and excitation was investigated by employing a descending frequency PSEM to potentiate low-frequency force and the twitch.

2. At 50Hz and 100Hz a similar safety-factor was apparent permitting force generation despite a marked reduction in CMAP amplitude as observed in chapter 3.

3. At 10Hz force was initially potentiated, presumably as a result of MRR slowing thereby increasing the F/E ratio to > 1. Potentiation was greater during the descending

frequency PSEM series and the ratio F/E remained > 1, reflecting a further safety-factor at low-frequency. This was not the result of PTP of the twitch force or MRR changes which were identical for the two series.

4. It is hypothesized that the 'extra' potentiation at low-frequency is due to twitch potentiating mechanisms, possibly increased sensitivity of myofibrils to Ca^{2+} or to an increase in myofibrillar Ca^{2+} availability.

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSIONS

The studies described in this thesis have addressed the factors that influence the interrelationship between force generation and excitation during stimulated contractions of the human adductor pollicis. Generally, it has become accepted that if force loss is accompanied by a decline in electrical activity, fatigue is attributed to the failure of excitation, whereas if excitation is not reduced, fatigue is attributed to failure of the contractile system itself. However, during the course of the present studies, it has been shown that for the adductor pollicis, the relationship between force generation and excitation is complex, since endogenous peripheral mechanisms appear to offset the mechanisms leading to fatigue at both high and low frequencies of stimulation, where despite a reduction in excitation, force is maintained or even increased, thereby counteracting the mechanisms leading to fatigue.

The design of the experimental procedures described have employed indirect electrical stimulation of the adductor pollicis under ischaemic or non-occluded conditions. Such a model may be considered as an 'isolated preparation'. This has the advantage of permitting the study of muscle function which does not involve a central nervous component to control of contraction or performance. With this in mind, the interpretation of the investigations undertaken to 'real life' have to be made with caution, for it is only possible to identify how the 'contractile machine' functions under controlled peripheral conditions. For this reason, only the physiological cellular processes that contribute to excitation failure and fatigue resistance during stimulated activity have been discussed in depth in each experimental chapter. Nevertheless, the physiological significance of fatigue resistance mechanisms during voluntary activity may be of importance and further studies are required.

The mechanisms of fatigue resistance described in this thesis may also have important implications in rehabilitation regimes and functional electrical stimulation or wherever fatigue presents a problem, particularly in the design of stimulation

regimes where it may be possible to predict the performance of muscle function during stimulated activity.

This general discussion will therefore attempt to draw together the physiological significance of the processes that may contribute to fatigue and those that may serve to resist fatigue during voluntary and stimulated activity and in addition outline the opportunities that exist for future work.

7.1 Peripheral mechanisms resisting fatigue during stimulated activity

7.1.1 Fatigue resistance mechanisms at high stimulation frequency

Differences in the fatiguability of skeletal muscle at high and low frequencies of stimulation is well established and has been demonstrated in animal preparations (Davies & Davies, 1932; Naess & Storm-Mathisen, 1955; Jones *et al.*, 1979) as well as in human studies (Jones *et al.*, 1979; Naess & Storm-Mathisen, 1955). The development and application of the computer controlled generated programmed package of various frequency trains to fatigue the human adductor pollicis (chapter 3, part III) has additionally shown that at high stimulation frequencies, a safety-factor acts within which excitation (measured as the amplitide of the CMAP) can markedly alter without affect on force whereas at low stimulation frequency, force is potentiated during both ischaemic and non-occluded conditions. These observations suggest that the underlying mechanisms that are thought to contribute to force failure may be offset, thereby resisting fatigue.

The concept of a safety-factor (chapter 3, part III) has been suggested by others (Sandow, 1952; Metzger & Fitts, 1986; Edwards, 1983), but has not previously been demonstrated for high-frequency stimulated activity, although reinterpretation of the data of Marsden *et al.*, (1983) in which the adductor pollicis was fatigued with continuous trains of stimuli at frequencies of 5-200Hz, expressing force as a function of CMAP amplitude, similarly demonstrates the existance of the safety-factor. The mechanism of the high-frequency safety-factor may involve a decline in the CMAP amplitude as a result of 'running-in' of successive impulses, which may result in a reduction in the amount of Ca^{2+} released per impulse. However, the high frequency

of stimulation probably allows sufficient Ca^{2+} to accumulate in the myoplasm to achieve full activation of the contractile apparatus. At lower frequencies insufficient myoplasmic concentrations of Ca^{2+} are probably not achieved to fully activate the contractile apparatus and hence submaximal forces are generated.

From chapter 3, it is not clear why the safety-factor should be lost after eight contractions during ischaemic conditions. Furthermore, force declined in a similar manner at all frequencies. The decline in CMAP amplitude that was observed at 100Hz cannot explain the loss of force since at lower stimulation frequencies of 10-20Hz CMAP amplitude had not significantly altered. Such a mechanism, however, supports the suggestion of Edwards (1983) that the muscle protects itself from the possible utilization of energy reserves which may consequently lead to the development of rigor and hence irrepairable damage.

The results of chapter 4 suggest that the most likely explanation for the loss in the safety-factor and biphasic decline in force which is discussed below is probably impairment of the propagation of the action potential, possibly within the T-tubular network where alterations in ionic concentrations are thought to be most marked (Bezanilla *et al.*, 1972; Bigland-Ritchie *et al.*, 1979). This is supported by the finding that force loss was largely frequency independent when plotted as a function of impulse number, but clearly frequency dependent with respect to contractile activity performed. Since contractile activity performed was suggested to reflect the metabolic cost of a contraction, as shown by the good correlation between MRR and contractile activity in chapter 4, it would appear that metabolic factors do not seem to be directly responsible for force loss over the frequency range investigated. It is not clear as to whether metabolic factors may contribute to force loss at lower stimulation frequencies, however. Accumulation of K^+ in the extracellular space and T-tubules and depletion of intracellular Na^+ has been suggested by several investigators as the factor impairing propagation of the action potential at high frequency (Bigland-Ritchie *et al.*, 1979; Jones 1981). It is therefore of interest that in the study of the change in action potential duration carried out in one subject, the increase in distal

latency appeared to be linearly related to the numbers of impulses delivered, independent of frequency over a range of 15-100Hz and was clearly frequency dependent with respect to contractile activity performed. This supports the above proposition of Bigland-Ritchie*et al.*, (1979) and Jones (1981), which is further supported by studies in isolated single cell preparations (e.g., Juel, 1988) as discussed in chapter 4, but metabolic factors may also contribute to prolongation of the action potential (see below). It is also possible that other factors, possibly involving Ca^{2+}, influence propagation of the action potential in view of the increasing evidence of the role of T-tubules in taking up Ca^{2+} (Bianchi & Narayan, 1982) and the presence of Ca^{2+} activated K^{+} channels (Pallotta, 1985).

The involvement of metabolič factors in altering CMAP characteristics is supported by the results of chapter 5, in which a good correlation between the prolongation of CMAP distal latency and the contractile activity performed was apparent. The increased broadening of the CMAP distal latency cannot be due to accumulation of extracellular K^{+} or depletion of intracellular Na^{+} alone as suggested by Jones *et al.*, (1979), since it occured at a time when CMAP amplitude had nearly recovered to pre-fatigue values (approximately 90% recovery). It is therefore likely that propagation of the action potential may be rapidly impaired even at low stimulation frequencies as a result of accumulation of metabolic factors, possibly H^{+} or Pi, and hence lead to fatigue. Such a concept thus supports the view of Edwards (1983) that metabolic factors may influence excitation as predicted by the 'catastrophe' model which is discussed in chapter 1, section 1.5.

It may be argued that the distal latency, from which the conclusions above are drawn may also reflect changes in the components of neuromuscular junction delay and changes in conduction of the peripheral nerves besides changes in the propagation of the action potential. However, the contribution of the delay at these sites is likely to be relatively small in relation to the changes along the sarcolemmal membrane, considering the marked changes in electrolyte distribution and proportion of metabolites generated by contractile processes. Furthermore, the changes in CMAP

rise-time, itself dependent on the changes in parallel components making up the summated action potential, are of similar proportion, further suggesting that the measurement of distal latency probably reflects events at the sarcolemmal membrane. The contribution of slowing at the afore mentioned sites cannot be ruled out however. Difficulties were experienced in measuring the distal latency in chapter 4 due to the resolution of the sampling rate of the action potential, which may have introduced a sampling error. Furthermore, only one subject was studied, although the results of chapter 5, of the frequency independent increase in distal latency in three subjects, supports the results of chapter 4.

Twitch force appeared to be reduced in relation to the prolongation of the CMAP, suggesting that excitation-contraction coupling impairment may be dependent on the successful propagation of the action potential. However, whether the loss of force is a result of cause or effect of prolongation of the action potential under the conditions used in these studies has as yet to be determined. Further studies are clearly necessary to investigate the role of metabolic factors on CMAP characteristics.

It is of interest that the high-frequency safety-factor is not evident in patients with McArdle's disease, as evidenced in the figures of Cooper *et al.*, (1987) where a parallel decline in force and CMAP amplitude was observed at frequencies of 1 to 100Hz during intermittent non-occluded fatiguing activity. The results of chapter 5 suggest that excitation loss in these individuals may be due to loss of individual cells as a consequence of their myopathy, although it is thought that this may be due to metabolite accumulation rather than to failure of energy supply as a result of impaired glycogenolysis. This observation thus supports the concept that the loss of safety-factor in normal individuals is a result of impairment of excitation.

In summary, it would appear that despite alterations in the action potential at high stimulation frequencies, a safety-factor prevents the loss of maximal force generation. However, the function of this safety-factor may be limited since propagation of the action potential becomes impaired, possibly as a result of

accumulation of electrolytes in the extracellular and T-tubular space and/or the influence of metabolites on membrane properties, thus resulting in a loss of force.

7.1.2 Fatigue resistance mechanisms at low stimulation frequency

Slowing of relaxation rate has generally been thought to assist in maintaining force generation during low frequency stimulated activity (Jones, 1981) and during voluntary activity (Bigland-Ritchie *et al.*, 1983a). However, the studies of chapter 3 and 6 indicate that slowing of relaxation is not the only factor that may contribute to force maintenance, and that post-tetanic potentiation of low-frequency tetani may act over and above MRR. It is not clear whether the mechanisms that contribute to this phenomenon are similar to that of post-tetanic twitch potentiation, which is thought to involve alteration in myofibrillar sensitivity to Ca^{2+} (Moore & Stull, 1984) or an increase in myofibrillar Ca^{2+} availability (Blinks *et al.*, 1978; MacIntosh & Gardiner, 1987), since post-tetanic potentiation of low-frequency tetani was apparent even when twitch force had completely declined. This suggests that the mechanisms of post-tetanic twitch potentiation and post-tetanic low-frequency potentiation may differ, but the involvement of alterations in Ca^{2+} kinetics cannot be ruled out. Further studies of the decay of low-frequency potentiation may be useful in elucidating the mechanism of this phenomenon. In this respect, the influences of briefly altering the duration of rest between potentiated low-frequency tetanic contractions may allow documentation of the decay kinetics of this form of potentiation.

The role of low frequency force potentiation in overcoming long-term low-frequency fatigue was not determined in these studies. It would appear that slowing of MRR cannot overcome this form of fatigue since long-term low-frequency fatigue is apparent even when MRR has recovered. It may be that severe excitation-contraction coupling impairment, resulting in a reduced Ca^{2+} release per impulse (Gardiner *et al.*, 1986) may result in loss of post-tetanic twitch potentiation (Miller *et al.*, 1987). This may be due to the fact that type II fibres are more susceptible to long term low-frequency fatigue as suggested by studies in rats (Kugelberg & Lindegren, 1979).

This would also explain why potentiation of low frequency force was not observed during recovery following non-occluded activity in chapter 3.

It should be pointed out that there appears to be no advantage of post-tetanic potentiation in developing mean force of incompletely fused low-frequency tetani in fresh muscle due to an increase in the contraction and relaxation rates of the unsummated twitch (Desmedt & Hainaut, 1968). Post-tetanic potentiation of low-frequency force may therefore contribute to preventing force loss at a time when a substantial increase in metabolic acidosis and myoplasmic Pi concentration is likely, both of which may alter excitation as well as myofibrillar activity or when excitation-contraction coupling impairment is apparent.

7.2 Functional implications of fatigue resistance during voluntary activity

Despite the arguments against extrapolating results of stimulation studies to those of voluntary activity, there is no reason to suppose that the same mechanisms that resist fatigue may not be operating during voluntary activity. Several studies have attempted to explain the fatigue characteristics of voluntary contractions by simulating the changes of force of maximal voluntary activity with stimulated contractions (Jones *et al.*, 1979; Marsden *et al.*, 1983). Difficulties arise, however, when the differences of motor unit properties are considered, particularly within muscle groups of mixed fibre type composition (Marsden et al. 1983; Bigland-Ritchie, 1984). The following part of this discussion therefore is largely speculative, but may further add to the present understanding of fatigue and perhaps should be considered when analysing fatigue during voluntary activity.

7.2.1 High frequency safety-factor

The physiological significance of the 'safety-factor' at high frequencies is probably limited, for during sustained maximal voluntary contractions, high motor-unit discharge rates may be encountered only briefly at the onset of a contraction during ballistic activity (Desmedt & Godaux, 1977). Furthermore, the reduction in CMAP amplitude during a maximal voluntary contraction is not apparent for at least 60 seconds (Bigland-Ritchie *et al.*, 1982), although this point has been disputed by

others (Stephens & Taylor, 1972; Marsden *et al.*, 1983). The upper limit of motor unit discharge may be of the order of 100-120Hz (Desmedt & Godaux, 1977), and instantaneous motor unit discharge rates as high as 150Hz have been reported for abberant motor units of the adductor pollicis (Marsden *et al.*, 1971). Mean motor unit discharge rates decline rapidly during sustained maximal activity, particularly those with high initial firing rates (Grimby *et al.*, 1981; Stephens & Taylor 1972; De Luca *et al.*, 1982a; Bigland-Ritchie *et al.*, 1982), however. It is thus conceivable that if the high-frequency safety-factor does have a role during voluntary activity, it is only in the brief initial phase of contraction where a loss of excitation may result due to cancelling effects of successive action potentials since repolarization may be incomplete owing to the short interspike period. This may be a distinct disadvantage if fatigue limits motor performance which is dependent on the rate of development of force particularly during dynamic ballistic activity.

7.2.2 Low frequency force potentiation

Generally, fatigue resistance at low stimulation frequencies has been attributed to slowing of relaxation rate (Jones, 1981; Bigland-Ritchie *et al.*, 1983a) in view of its good correlation to the decline in mean motor unit discharge rate (Bigland-Ritchie *et al.*, 1983a). However, it is also possible that potentiation of low-frequency tetani acts over and above slowing of MRR in resisting fatigue as suggested by the results of chapter 6. Although both post-tetanic potentiation of the twitch and low-frequency tetani occured following high-frequency stimulated activity during the present studies, it is not clear as to whether the same mechanisms initiating these processes occur during voluntary activity. Several studies have shown that twitch force may be potentiated by brief maximal contractions (e.g., Vandervoort *et al.*, 1983). So it is therefore possible that low-frequency potentiation occurs during voluntary activity, assuming similar mechanisms are responsible for potentiation of force.

It is also possible that brief bursts of high-frequency discharge rates contribute to post-tetanic potentiation mechanisms by promoting an increase in activation

associated with post-tetanic activity and hence contribute to fatigue resistance. There is evidence to suggest that the discharge rates of high-threshold motor units of the first dorsal interosseus in man may occur in brief bursts of 50 Hz (De Luca *et al.*, 1982b). More recent evidence in the same muscle indicates that this phenomenon may become more pronounced as fatigue ensues (Oldham, 1987). It is thought that these brief bursts of high frequency activity may assist in force generation by promoting fusion of high threshold motor units which have fast contraction times, thereby producing a more fused contraction in addition to low-threshold motor units (De Luca *et al.*, 1982b).

Low-frequency force potentiation has only been investigated in the adductor pollicis in the present studies. It is not known whether low-frequency potentiation occurs in other muscle groups, or if this phenomenon is specific to muscle fibre-type, as is observed for twitch potentiation. The requirement of such a mechanism in type I fibres may be unecessary in view of their high degree of fatigue resistance, long contraction times (30-100msec in the first dorsal interosseus, (Milner-Brown *et al.*, 1973)) and hence lower discharge rates necessary for fusion (approximately 20Hz). However, the adductor pollicis would appear to be an exception to this, in view of the large proportion of type I fibres and unusual contractile properties (Round *et al.*, 1984). The unusual properties of the adductor pollicis have not been explained, and it is possible that other muscles may show a similar paradox.

7.2.3 Functional aspects of fatigue resistance during dynamic activity

Everyday function of skeletal muscle does not involve sustained maximal contractions. Rather, during normal everyday activity it is likely dynamic contractions are performed in the simplest of tasks. The investigation of excitation and force generation cannot easily be applied to dynamic contractions since force is dependent on muscle length and hence testing of fatigue has generally been performed during static contractions. However, during dynamic fatiguing activity it is possible to measure power output of specific muscle groups which, by definition, declines during fatiguing activity (Edwards, 1983). The power output of a muscle is determined by

both force and the velocity of a contraction. Although it has not been possible to study this in the present investigations, it would be of interest to see how the present findings in this thesis may influence dynamic activity. The extrapolation of observations made during isometric stimulated activity to those of voluntary dynamic activity needs to be made with care, however, for the reasons mentioned above.

The contribution of high-frequency fatigue probably depends upon the rate of contraction cycling, and hence on the relative time of activation and rest during which recovery of the processes responsible for this type of fatigue may occur. Rapid dynamic activity may result in repeated bursts of ballistic activity, during which time the high-frequency safety-factor may become important. This will be dependent on which muscle fibre types are recruited and the metabolic demand of the recruited fibres, which, in order to develop a high power output will require a high stimulation rate and a high degree of ATP utilization. Other factors, particularly central factors may also influence power output although again the contribution of this in dynamic contractions is not clearly established.

The contribution of slowing of relaxation to the generation of force during isometric contractions is clearly important, but during dynamic activity its influence to altering power output may not be so simple. Much will depend on the mechanism of slowing of relaxation since if this is a result of reduced cross bridge cycling (Edwards *et al.*, 1975) then a shift of the force/velocity curve would occur resulting in a reduction of power. On the other hand, if slowing of relaxation is due to a reduction in the rate of Ca^{2+} uptake by the sarcoplasmic reticulum, then it is unlikely that the force/velocity relationship is altered and hence power will not be reduced.

It is likely that potentiation of the twitch also occurs during dynamic activity suggesting that post-tetanic potentiation of low-frequency force may contribute to force generation. In fresh muscle post-tetanic potentiation of the isometric twitch is associated with an increase in contraction rate (Desmedt & Hainuat, 1968), but a depression in the maximal velocity of shortening (Kushmerick & Crow, 1983). This too would result in a reduction in power output rather than enhance it. Whether or not

an obligatory decrease in power output occurs during dynamic contractions or whether the mechanism of low-frequency force potentiation acts in a similar fashion to twitch potentiation, has yet to be determined.

7.2.4 Interrelation of fatigue resistance mechanisms during voluntary isometric activity

The reduction in motor unit discharge rate in conjunction with a slowing of relaxation rate described above (section 7.3.1) is suggested to further minimize fatigue during prolonged voluntary effort in man (Bigland-Ritchie *et al.*, 1983a; Marsden *et al.*, 1983). This process has been described as the muscles' 'natural wisdom' (Marsden *et al.*, 1983), and may serve as a mechanism protecting against excitation failure at the neuromuscular junction or at the excitable membranes of the muscle cell (Jones & Edwards, 1986).

A hypothetical model of the interaction of the muscles' 'natural wisdom' and fatigue resistance mechanisms discussed above is shown in Figure 7.1. At the onset of a contraction where high motor unit discharge rates are encountered, optimal force generation is achieved during the time when the high-frequency safety-factor may act to prevent loss of parallel active fibres through excitation failure. As the contraction proceeds, a reduction in motor unit discharge rate occurs with a concommitant slowing of relaxation rate, protecting against further excitation failure. Potentiating mechanisms ensure maximal activation of the contractile apparatus, possibly in an attempt to overcome the inhibition of contractile function due to metabolites produced as a by-product of contraction. Thus optimal force generation is achieved.

7.3 Implications of fatigue resistance in functional electrical stimulation

The technique of stimulating muscle to generate force has long been of interest for therapy regimes and rehabilitation (McNeal, 1977). Functional electrical stimulation serves to assist physiological function of many organs of the body (Table 1.2) through direct and indirect stimulation of muscle and thus necessitates a thorough understanding of neuro-muscular physiological function. The success of FES devices

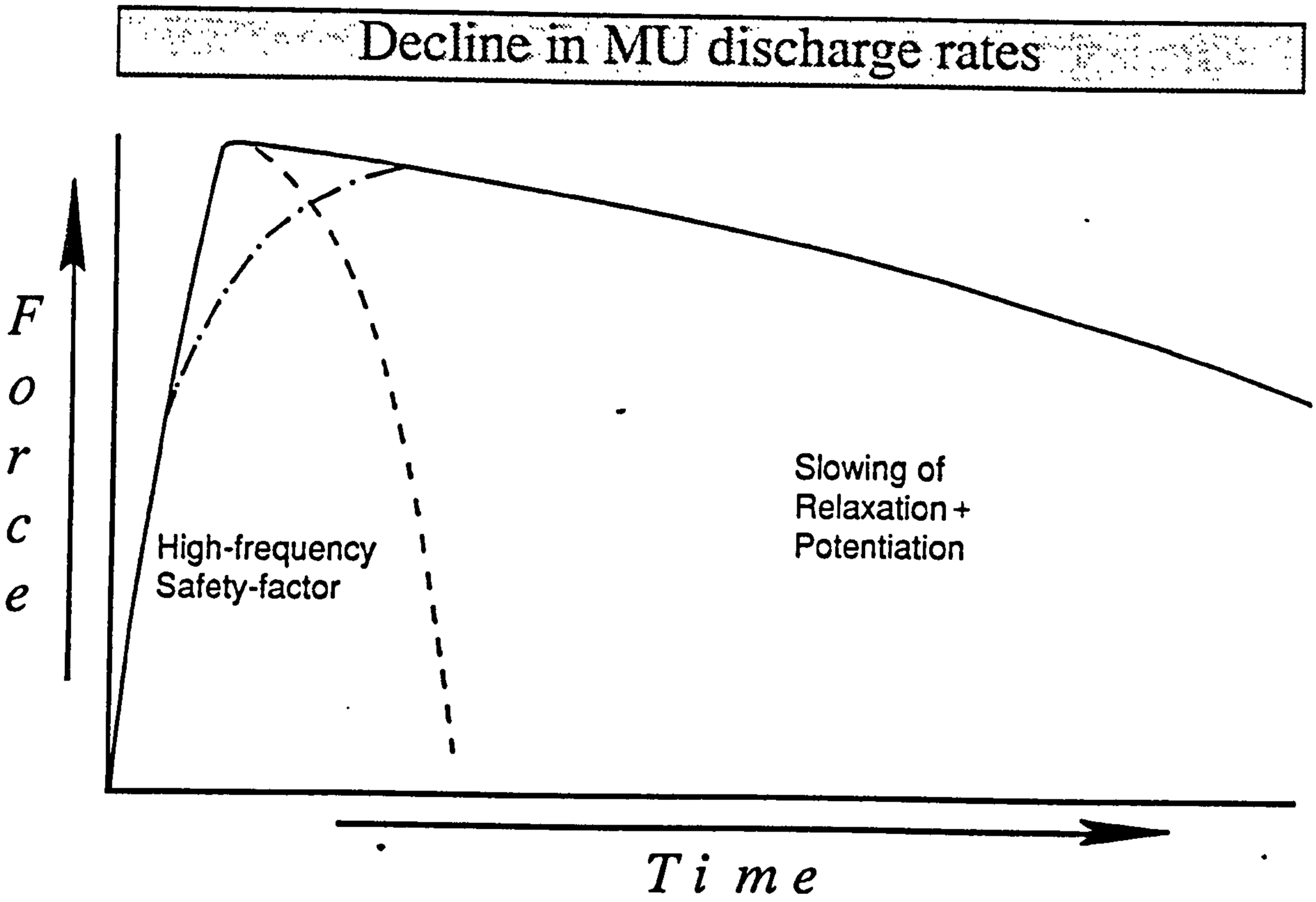


Figure 7.1 Hypothetical model of fatigue resistance mechanisms during sustained maximal voluntary contractions. At the onset of a maximal voluntary contraction the high-frequency safety-factor prevents loss of force generation that may result from excitation failure. As the contraction proceeds, motor unit discharge rates decline. Slowing of relaxation and potentiation of low-frequency force then become increasingly more important to optimize force generation.

depends upon many factors, including fatigue, response variability, influences on peripheral tissue and central organization (Table 7.2).

Problems may arise in assessing the criteria for the appropriate stimulation for the task required e.g., for locomotive activity or finite control of hand muscle for grasping movements, or in diaphragm pacing by phrenic nerve stimulation and in the development of fatigue resistant skeletal muscle for the purposes of assissting cardiac pumping (Acker *et al.*, 1987). In developing stimulation regimes therefore, strength, fatigue resistance, control of the degree of contraction, quality of the contraction (smooth or unfused) and speed of contraction may all be considered of importance.

7.4.1 Prediction of muscle function during FES

The results of the present studies could be utilized in resisting fatigue during stimulated activity, but this will depend on the nature of the stimulation to be applied and the particular task required. The pattern of excitation a muscle receives during voluntary contractions cannot be imitated by any type of artificial nerve stimulation in view of the wide range of fibre-type / discharge rate matching (Bellamere *et al.*, 1983) that is observed within a single muscle. Therefore a more practical approach based on a compromise of the frequency:force characteristics and fatiguability characteristics of the muscle to be stimulated has to be employed. Clearly, the performance of an electrically stimulated contraction will be a function of the frequency of stimulation applied: The attainment of a force will be dependent on the frequency:force characteristics of the muscle to be stimulated as will the changes in force profile with respect to duration of stimulation (Naess & Storm-Mathisen, 1955; Jones *et al.*, 1979; as shown in the present study, chapters 3 and 4). An example of this is shown in Figure 7.2 for the adductor pollicis. The relationship between frequency and 'fatiguability' of the adductor pollicis, expressed as % force loss per second for the first 30 seconds of a stimulated contraction (data from chapter 4, Figure 4.3C), is shown together with the force that may be obtained in fresh muscle for a given frequency of stimulation. This figure also demonstrates that the task/performance characteristics of a muscle is limited by stimulation frequency.

TABLE 7.1 Factors influencing performance of functional electrical stimulation (FES)

1. Effects of FES on musculo-skeletal system:

   i. Effect of stimulation on tissue (e.g., damage)

   ii. Fatigue mechanisms

   iii. Electrical stimulation parameters

   iv. Stimulating electrodes - percutaneous, intramuscular, nerve supply

   v. Alterations in function as a result of stimulation e.g., plasticity changes

2. Effects on central nervous system

3. Practical considerations e.g., packaging, energy supply, patient acceptabililty.

Thus, a task in which maximal force generation is necessary will require a high stimulation frequency, but not without cost to performance, i.e., high frequency stimulation gives the benefit of force, but the penalty of greater fatiguability whereas low stimulation gives the benefit of a prolonged contraction, but only at low force levels. Furthermore, fusion is likely to be incomplete at very low stimulation frequencies which may contribute to some degree of discomfort. Such a model is perhaps too simple since the rate of decline of force is not strictly linear during the course of a prolonged stimulated contraction (see Figure 4.3c), although several 'fatiguability' curves encompassing lower frequencies of stimulation may be obtained for a given duration of contraction.

The results of this thesis may have considerable functional importance since it has been demonstrated that fatigue resistance of a muscle may be altered simply by the way in which it is stimulated. Thus, careful design of stimulation regimes in which fatigue resistant mechanisms are utilized may benefit the production and maintenance of force. The frequency dependence of the contribution of potentiation and safety-factor mechanisms for a given frequency during stimulated activity is shown in Figure 7.3. The percentage contributions of low-frequency force potentiation and the safety-factor have been calculated as the maximum percentage that would be achieved in a contraction for a given frequency of stimulation based on the results from chapters 3 (Figure 3.6) and 6 (Figure 6.2). The contribution of the high-frequency safety-factor becomes less important as stimulation frequency is reduced and its significance is likely to be of importance early on in a contraction as discussed for voluntary contractions above (section 7.2.1). The contribution of the potentiating mechanism has been calculated as a percentage of that at 10Hz at the end of fatiguing activity in chapter 6. Potentiation becomes less important in resisting fatigue as stimulation frequency is increased.

It has not been possible to predict the time course of the changes in the safety-factor or potentiation since the time dependent changes were not investigated for each frequency alone. However, it is likely that during high frequency stimulated activity,

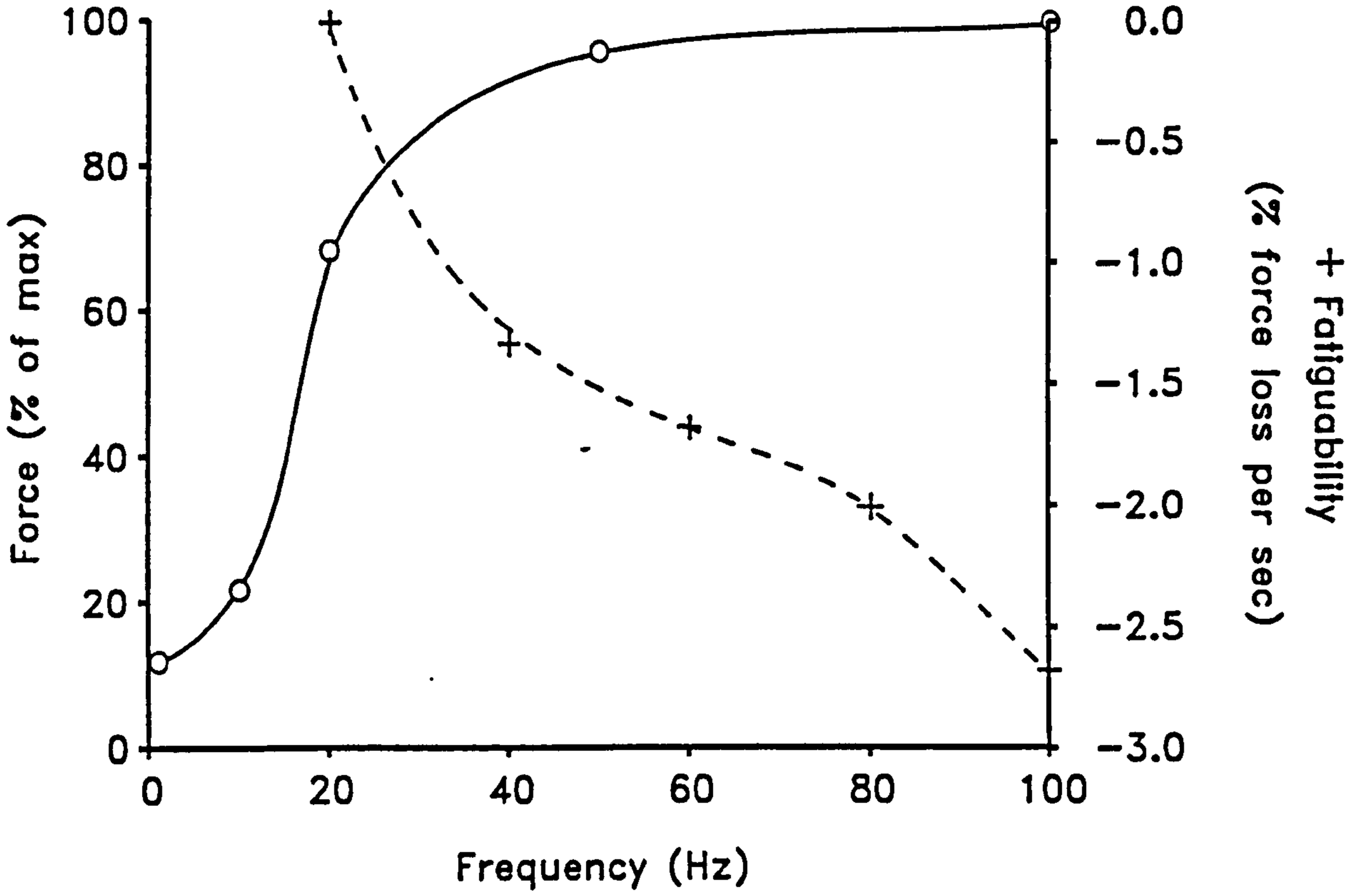


Figure 7.2 Fatiguability (+) (as a percentage of force loss per second) of human adductor pollicis following 30 seconds of stimulated fatiguing activity at force generated over a frequency range of 1-100Hz shown by the frequency:force curve (○). Data from chapter 4 (Figure 4.3c) and chapter 3 (Figure 3.4). Note that the rate of force decline becomes greater as frequency of stimulation is increased, although maximal force generation is achieved only at the higher frequencies.

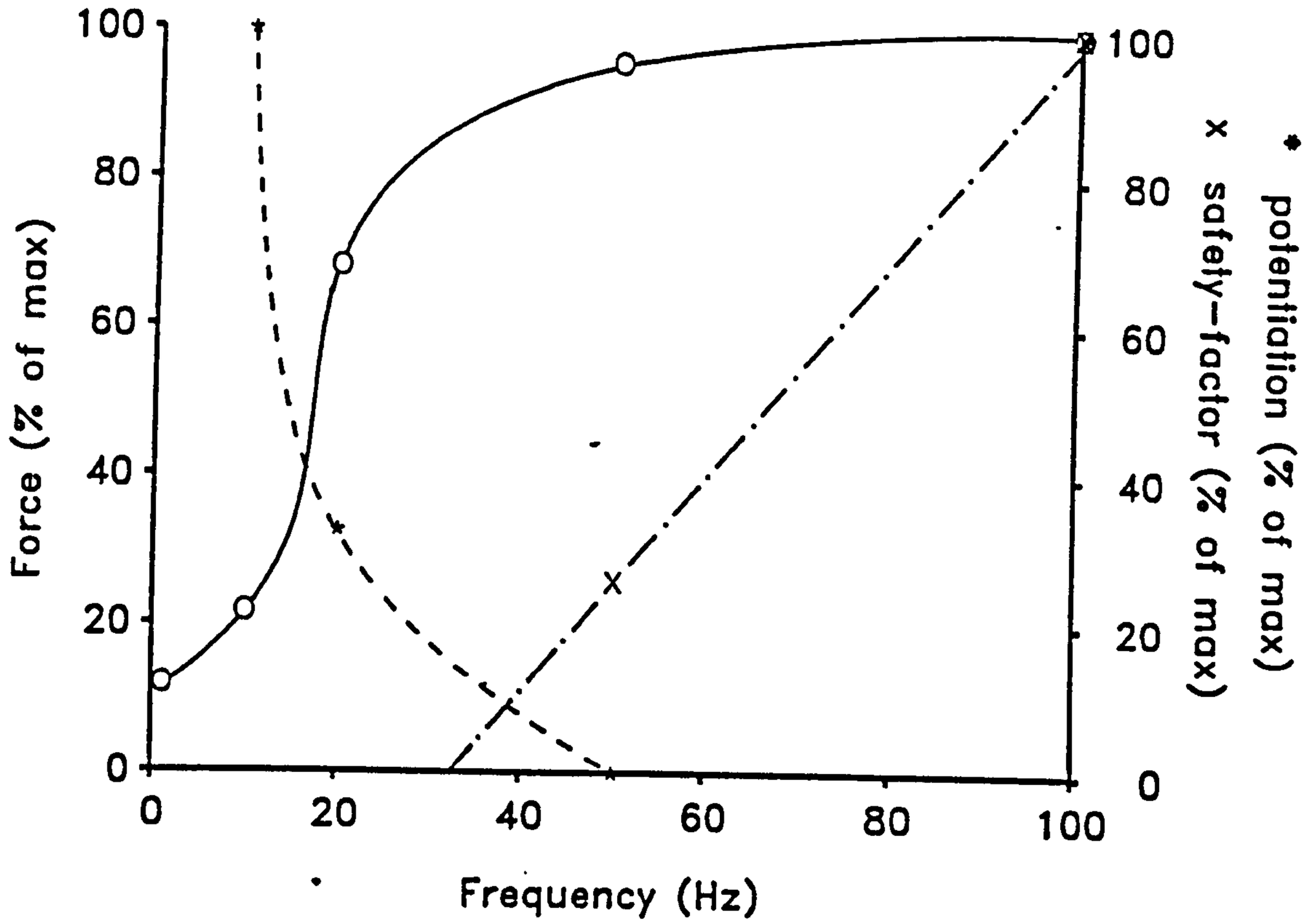


Figure 7.3 Contribution of high-frequency .safety-factor (×) and low-frequency potentiating mechanisms (✳) during stimulated activity in relation to frequency for a given degree of force generation (○) in fresh muscle. The percentage contributions of the safety-factor and potentiation mechanisms have been calculated as the maximum percentage that would be achieved in a contraction for a given frequency of stimulation, as obtained from the results from chapters 3 (Figure 3.6) and 6 (Figure 6.2). The time course of the changes in the safety-factor or potentiation were not investigated for each individual frequency.

the contribution of the safety-factor is short-lived since force rapidly declines. Its role may be of importance during brief intermittent contractions where ballistic type activity may be required. During repeated contractions with ischaemic conditions, action potential fade becomes more evident at high frequency (Bigland-Ritchie *et al.*, 1983a), presumably due to the prolongation of the action potential, and it is therefore likely that the relative contribution of the safety-factor to resisting fatigue declines. In contrast, low-frequency potentiation was evident throughout fatiguing activity studied in chapter 6 and thus may resist fatigue during prolonged activity. Of course the benefit of these mechanisms will depend on the nature of the contraction to be performed and hence further studies are necessary, for example, to determine whether low-frequency potentiation is apparent during non-occluded contractions as well as during ischaemic conditions and what factors may alter the contribution of the safety-factor and potentiating mechanisms to force generation during different forms of stimulation.

Difficulties may arise, however, in designing suitable regimes for stimulation, for brief bursts of high frequency stimulation in order to achieve potentiation of low-frequency force may be undesirable in a functional electrical stimulation protocol and furthermore may lead to premature fatigue, counteracting the benefits of fatigue resistance mechanisms. Pain associated with ischaemic activity may become discomforting, and indeed was experienced by several subjects during the course of the present studies, particularly under ischaemic conditions.

7.5 CONCLUSIONS

Several mechanisms appear to resist fatigue during stimulated activity. At high frequencies a safety-factor appears to act within which force is maintained at a time when CMAP amplitude is declining as a consequence of running in of action potentials. At low stimulation frequencies, slowing of relaxation and low-frequency force potentiation, when immediately preceded by high frequency activity, may act to offset excitation-contraction coupling impairment or enhance force generation in the light of build-up of metabolites that reduce myofibrillar activation. Although the

findings of this study have been discussed in relation to voluntary activity, the significance of these findings to voluntary contractions are made with caution, since the model employed does not represent contractions in 'real life' during which central mechanisms may be of importance. It is during stimulated activity, however, that the results presented in this thesis may be of considerable benefit to clinical medicine and in furthering the development and understanding of muscle function.

APPENDICES

Please note that due to the large numbers of data produced by the experimental procedures described in this thesis, only data where specifically stated in the text appears in the following appendices. Data for individual experiments may be obtained on request from the author.

Appendix 1 Strain gauge calibration

Force (N)	Oscillograph dispacement (mm)
0.0	0.0
4.9	4.0
4.9	4.0
9.8	8.0
24.5	17.5
24.5	18.0
29.4	22.0
29.4	20.5
49.0	38.5
49.0	38.0
73.6	56.0
73.6	55.0
78.5	60.0
78.5	59.5
98.1	74.0
98.1	73.0
122.6	90.0
122.6	88.5

Fylde amplifier gain : X 20

Data obtained in random order.

Regression equation:

Force = 1.35 x deflection - 0.61

r = 0.999.

The strain gauge was calibrated on a regular basis and the values shown are typical for a procedure. Paired values were made throughout each calibration to assess repeatability.

Appendix 2 Force differentiator and integrator calibration

See appendix 3 for details of differentiator calibration.

1. Force Differentiator

Negative ramp slope MRR		Positive ramp slope MCR	
Ramp function ($mm.s^{-1}$)	Differential deflection (mm)	Ramp function ($mm.s^{-1}$)	Differential deflection (mm)
1308.5	140.0	1074.6	108.0
1294.7	140.5	1079.0	107.5
563.2	60.5	460.9	47.5
563.2	60.5	460.9	47.5
686.2	73.0	556.0	57.0
679.0	73.0	538.8	57.5
106.4	9.0	87.7	8.0
89.5	8.0	73.3	8.0
401.0	42.0	336.2	33.0
410.5	42.0	334.8	33.0
229.2	23.5	189.7	19.5
234.0	23.5	189.7	19.5

Regression equations:

1. MRR: ramp slope = 9.99 x deflection - 4.27, r = 0.999

2. MCR: ramp slope = 9.17 x deflection + 14.3, r = 1.000

2. Force Integrator

Force deflection (mm)	Duration (sec)	Force x duration (mm.sec)	Integrator deflection (mm)
29	10	290	8.25
19	5	95	2.5
49	20	980	29
49	18	882	27
39.5	5	197.5	6
34	15	510	15
50	10	500	14.5
60.5	10	605	19
20	30	600	18.5
59.5	5	297.5	9.25
40	15	600	19.5

Regression equation:

Force x time = 0.0305 x deflection - 0.093, r=0.998

Calculation of max.seconds = integrator deflection (mm) / 0.0305 / max.force (mm)

Appendix 3 Protocol for calibration of force differentor

Apparatus: Servomex signal generator (model L.F.141, Sevomex Controls, Ltd)

Procedure:

1. Replace Balance unit (Fylde) with special 'cal' unit + jack plug.

2. Connect jack plug to Servomex. Blue to 'output' (O) and red to 0 volts (X).

3. Set output volts to near zero and multiplier to X0.01 (actual output required < 6mV).

4. Set waveform to triangular.

5. Set waveform output to SWITCHED

6. Swith output voltage to $\pm$.

7. Frequency set to x1 x10 s^{-1}.

8. If waveform ouput is set to 'switched' use 1/2 cycle switch to obtain final output.

9. Set paper record time on oscillograph to 1.0 seconds.

10. Set paper speed to 125mm.s^{-1}, i.e., 25x5mm, time 0.1sec.

11. With 'continous' mode obtain a 'force' deflection of about 10cm by adjusting output voltage.

12. If necessary, use screw driver to set zero of force galvanometer.

13. Differential galvanometer should rest at 1.

14. Start calibration at top, but make random.

15. To record, switch paper drive on and at same time (or briefly after) switch 1/2 cycle swith down and then up.

Appendix 4 Development of computerised stimulator controller

Two menu driven programmable pulse train generator routines, PULG12 and NSTIM, were written for an Apple II microcomputer fitted with a specially constructed pulse generator card. PULG12 generates a single channel 5 volt, 50usec output pulse whereas NSTIM gives the user 8 independent computer controlled outputs. Both programmes allow 20 trains of impulses over a frequency range of 0 to 500Hz, of a duration from 2msec to 255 seconds.

The application of a programmable pulse train generator to the study of muscle function allows rapid alteration and precise control over stimulation patterns, which may be developed based around a simple editing routine and permanently stored on disc and recalled when necessary. The advantage of this to other systems (Zeiderman *et al.*, 1984; Mills, personal communication) which use fixed patterns stored in read only memory (ROM) is obvious, although the switching between different patterns of stimulation may be limited by recall time of the disc system.

Software

Both programmes are written in two parts;

a)the 'front-end' written in Applesoft Basic, allowing menu-driven development of pulse trains and control of execution. NSTIM calls a second BASIC program, NPULG03 for control of the ASSEMBLER pulse controller routine below,

b)the 'pulse controller' routines, written in 6502 mini-assembler.

PULG12 calls a single channel controller assembler routine, ASS2. NPULG03 calls an eight channel controller assembler routine, H1. Listings of PULG12, NSTIM, NPULG03, ASS2 and H1 are given below. Please note that programme comments have only been given in listing ASS2. Further details of programme development and explanation can be obtained from the author.

```
10  REM
20  HIMEM: 8192
30  REM  PROGRAMMABLE STIMULATION UNIT.
40  REM  H. GIBSON DEC.1984
50  HOME
55  VTAB 10
60  PRINT  TAB( 4)"PROGRAMMABLE STIMULATION UNIT."
65  FOR A = 1 TO 2000: NEXT
70  PRINT
80  PRINT "WAIT ...."
90  PRINT "   SPENDING A QUICK PENNY- BACK SOON!"
92  REM  LOAD M/C FOR 6522 PULSE GENERATION
95 D$ =  CHR$ (4): REM  CTRL-D
100  PRINT D$;"BLOAD ASS2 ,D1"
110  DIM STIM(20,3),DR(20),R(20),MULT(20),FRDUR(20),MLL(20),MTLTI(20)
120  HOME
130  VTAB 10
140  PRINT  TAB( 16)"MAIN MENU"
150  PRINT  TAB( 16)"---------"
160  PRINT
170  PRINT  TAB( 17)"1.LOAD"
180  PRINT  TAB( 17)"2.CATALOG"
190  PRINT  TAB( 17)"3.EDIT"
200  PRINT  TAB( 17)"4.RUN"
205  PRINT  TAB( 17)"5.QUIT"
210  GET A$
215 A =  VAL (A$)
220  ON A GOSUB 1000,2000,3000,4000,240
230  GOTO 120
240  END
370  PRINT : INPUT "(C)              ONTINUE/(E)ND ";A$
1000  REM  READ FILES
1010  HOME : VTAB 10
1020  PRINT "LOAD FILE"
1025  PRINT
1030  INPUT "FILE NAME: ";F$
1040  PRINT D$;"OPEN";F$;",D2"
1050  PRINT D$;"READ";F$
1060  INPUT N
```

```
1070  FOR A = 1 TO N
1080  INPUT STIM(A,1)
1085  INPUT STIM(A,2)
1090  NEXT
1100  PRINT D$;"CLOSE";F$
1110  RETURN
2000  PRINT
2010  PRINT D$;"CATALOG,D2"
2020  PRINT : PRINT "PRESS ANY KEY TO CONTINUE"
2030  GET K
2040  RETURN
3000  REM  EDIT FILES MODE.
3005  HOME
3010  VTAB 10    .
3020  PRINT  TAB( 16)"EDIT MENU"
3030  PRINT  TAB( 16)"*********"
3035  PRINT
3040  PRINT  TAB( 13)"1.DELETE FILE"
3050  PRINT  TAB( 13)"2.MODIFY FILE"
3060  PRINT  TAB( 13)"3.NEW FILE"
3070  PRINT  TAB( 13)"4.SAVE FILE"
3080  PRINT  TAB( 13)"5.QUIT"
3090  GET A$
3095 A =  VAL (A$)
3100  ON A GOSUB 3200,3250,3300,3900,120
3110  GOTO 3000
3200  PRINT "DELETE FILE ": PRINT
3210  INPUT "NAME OF FILE TO DELETE: ";F$
3220  PRINT D$;"DELETE";F$;",D2"
3230  PRINT "PRESS ANY KEY TO CONTINUE": GET K
3240  RETURN
3250  GOTO 3410
3300  REM  EDIT FACILITY
3305 N = 0
3310  HOME : VTAB 10
3320  PRINT  TAB( 12)"OPENING NEW FILE"
3325 N = N + 1
3330  PRINT
```

```
3340  PRINT "PULSE TRAIN: ";N: PRINT
3350  INPUT "FREQUENCY (HZ)? ";STIM(N,1)
3360  INPUT "DURATION (SEC)? ";STIM(N,2)
3370  PRINT : INPUT "(C)ONTINUE/(E)ND? ";A$
3380  IF A$ = "C" THEN  GOTO 3310
3390  IF A$ = "E" THEN  GOTO 3410
3400  GOTO 3370
3410  HOME
3420  VTAB 5
3430  PRINT "PULSE TRAIN    FREQUENCY      DURATION"
3440  PRINT "                  HZ          SEC  "
3450  PRINT : FOR A = 1 TO N
3460  PRINT A,STIM(A,1),STIM(A,2)
3470  NEXT
3475  PRINT
3480  INPUT "ALTER PULSE TRAIN (Y/N)? ";A$
3490  IF A$ = "Y" THEN  GOTO 3520
3500  IF A$ = "N" THEN  GOTO 3600
3510  GOTO 3480
3520  INPUT "PULSE TRAIN NO.? ";P
3530  INPUT "DELETE? ";D$
3540  IF D$ = "Y" THEN  GOTO 3560
3550  IF D$ = "N" THEN  GOTO 3570
3555  GOTO 3530
3560 STIM(P,1) = 0:STIM(P,2) = 0: GOTO 3410
3570  INPUT "NEW FREQUENCY: ";STIM(P,1)
3580  INPUT "NEW DURATION: ";STIM(P,2)
3590  GOTO 3410
3600  RETURN
3900  HOME : VTAB 10
3910  PRINT "SAVE FILE": PRINT
3920  INPUT "FILE NAME: ";F$
3930  PRINT D$;"OPEN";F$;",D2"
3940  PRINT D$;"WRITE";F$
3950  PRINT N
3960  FOR A = 1 TO N
3970  PRINT STIM(A,1)
3975  PRINT STIM(A,2)
3980  NEXT
```

```
3990  PRINT D$;"CLOSE";F$
3995  RETURN
4000  REM  POKE DATA
4004 B = 0
4006  FOR A = 1 TO N
4007  IF STIM(A,1) = 0 THEN MLL(A) = STIM(A,2) * 500:STIM(A,1) = 1:STIM(A,2) = 1
: GOTO 4009
4008 MLL(A) = 500 / STIM(A,1)
4009 FRDUR(A) = STIM(A,1) * STIM(A,2)
4010  IF FRDUR(A) > 255 THEN DR(A) =  INT (FRDUR(A) / 255):R(A) = FRDUR(A) - (DR
(A) * 255): GOTO 4012
4011 DR(A) = 0:R(A) = FRDUR(A)
4012  IF MLL(A) > 255 THEN MTLTI(A) =  INT (MLL(A) / 255):MULT(A) =  INT (MLL(A)
 - (MTLTI(A) * 255)): GOTO 4014
4013 MTLTI(A) = 0:MULT(A) =  INT (MLL(A))
4014  NEXT
4015  FOR A = 0 TO (4 * (N - 1)) STEP 4
4016 C = (A / 4) + 1
4020  POKE 6912 + A,DR(C)
4030  POKE 6913 + A,R(C)
4040  POKE 6915 + A,MULT(C)
4041  POKE 6914 + A,MTLTI(C)
4042  REM  PRINT "DR= ";DR(C): PRINT "R= ";R(C): PRINT "MULT= ";MULT(C): PRINT "
MTLTI(C)= ";MTLTI(C): STOP
4043  NEXT
4045  POKE 6656,4 * N
4047  CALL 768
4050  HOME : VTAB 5
4055  PRINT  TAB( 18)"MENU"
4060  PRINT  TAB( 18)"****"
4065  PRINT : PRINT  TAB( 15)"1.SINGLE"
4070  PRINT  TAB( 15)"2.COUNT"
4075  PRINT  TAB( 15)"3.FREE RUN"
4077  PRINT  TAB( 15)"4.RETURN TO"
4078  PRINT  TAB( 15)"  MAIN MENU"
4080  GET Z$
```

```
4083 Z =  VAL (Z$)
4085  ON Z GOTO 4100,4200,4300,4090
4087  GOTO 4050
4090  RETURN
4100  CALL 821
4110  GOTO 4050
4200  HOME : VTAB 10
4210  INPUT "ENTER NUMBER OF CYCLES: ";NC
4215  VTAB 15: PRINT "PRESS ANY KEY TO STOP"
4220  FOR AA = 1 TO NC
4225  VTAB (20): PRINT AA
4230  CALL 821
4240  NEXT
4250  GOTO 4050
4300  REM
4302  HOME : VTAB 15: PRINT "PRESS ANY KEY TO STOP"
4305  CALL 821
4307 XI% =  PEEK ( - 16384): IF XI% > 128 THEN  GOTO 4320
4310  GOTO 4305
4320  GOTO 4050
```

```
20  REM  HIMEM:8192?
30  REM  PROGRAMMABLE STIMULATION UNIT.
40  REM  H.GIBSON MAY 1985
50  HOME
55  VTAB 10
60  PRINT  TAB( 4)"PROGRAMMABLE STIMULATION UNIT."
65  FOR A = 1 TO 2000: NEXT
70  PRINT
95 D$ =  CHR$ (4): REM  CTRL-D
110  DIM STIM(7,19,1),DR(20),R(20),MULT(20),FRDUR(20),MLL(20),MTLTI(20),TT(7)
120  HOME
130  VTAB 10
140  PRINT  TAB( 18)"MENU"
150  PRINT  TAB( 18)"****"
160  PRINT
170  PRINT  TAB( 17)"1.LOAD"
180  PRINT  TAB( 17)"2.CATALOG"
190  PRINT  TAB( 17)"3.EDIT"
200  PRINT  TAB( 17)"4.RUN"
205  PRINT  TAB( 17)"5.QUIT"
210  GET A$
215 A =  VAL (A$)
220  ON A GOSUB 1000,2000,3000,4000,240
230  GOTO 120
240  END
370  PRINT : INPUT "(C)              ONTINUE/(E)ND ";A$
1000  REM  READ FILES
1010  HOME : VTAB 10
1020  PRINT "LOAD FILE"
1025  PRINT
1030  INPUT "FILE NAME:  ";F$
1035  PRINT
1040  PRINT D$;"OPEN";F$;",D2"
1050  PRINT D$;"READ";F$
```

```
1060  FOR CHAN = 0 TO 7
1065  INPUT TT(CH)
1070  FOR TRAIN = 0 TO TT(CH) - 1
1073  FOR INFO = 0 TO 1
1075  INPUT STIM(CHAN,TRAIN,INFO)
1080  NEXT
1085  NEXT
1090  NEXT
1100  PRINT D$;"CLOSE";F$
1110  RETURN
2000  PRINT
2010  PRINT D$;"CATALOG,D2"
2020  PRINT : PRINT "PRESS ANY KEY TO CONTINUE"
2030  GET K
2040  RETURN
3000  REM  EDIT FILES MODE.
3005  HOME
3010  VTAB 10
3020  PRINT  TAB( 16)"EDIT MENU"
3030  PRINT  TAB( 16)"*********"
3035  PRINT
3040  PRINT  TAB( 13)"1.DELETE FILE"
3050  PRINT  TAB( 13)"2.MODIFY FILE"
3060  PRINT  TAB( 13)"3.NEW FILE"
3070  PRINT  TAB( 13)"4.SAVE FILE"
3080  PRINT  TAB( 13)"5.QUIT"
3090  GET A$
3095 A =  VAL (A$)
3100  ON A GOSUB 3200,3250,3300,3900,120
3110  GOTO 3000
3200  PRINT "DELETE FILE ": PRINT
3210  INPUT "NAME OF FILE TO DELETE: ";F$
3220  PRINT D$;"DELETE";F$;",D2"
3230  PRINT "PRESS ANY KEY TO CONTINUE": GET K
3240  RETURN
3250  GOTO 3410
3300  REM  EDIT FACILITY
3310  HOME : PRINT : INPUT "HOW MANY CHANNELS ? ";CAN
3311  FOR CH = 0 TO CAN - 1
3312 N =  - 1
3315 N = N + 1
```

```
3315 N = N + 1
3318  HOME : VTAB (10)
3320  PRINT  TAB( 12)"OPENING NEW FILE"
3330  PRINT
3334  PRINT "CHANNEL: ";CH
3340  PRINT "PULSE TRAIN: ";N + 1: PRINT
3350  INPUT "FREQUENCY (HZ)? ";STIM(CH,N,0)
3360  INPUT "DURATION (SEC)? ";STIM(CH,N,1)
3370  PRINT : INPUT "(C)ONTINUE/(E)ND? ";A$
3380  IF A$ = "C" THEN  GOTO 3315
3390 TT(CH) = N + 1
3400  NEXT
3410  HOME
3420  VTAB 5
3425  FOR CH = 0 TO 7
3427  PRINT "CHANNEL: ";CH
3430  PRINT "PULSE TRAIN    FREQUENCY     DURATION"
3440  PRINT "                 HZ          SEC   "
3450  PRINT : FOR A = 0 TO 19
3460  PRINT A + 1,STIM(CH,A,0),STIM(CH,A,1)
3470  NEXT
3472  PRINT "PRESS ANY KEY FOR NEXT CHANNEL": GET A
3473  NEXT
3475  PRINT
3480  INPUT "ALTER PULSE TRAIN (Y/N)? ";A$
3490  IF A$ = "Y" THEN  GOTO 3515
3500  IF A$ = "N" THEN  GOTO 3600
3510  GOTO 3480
3515  INPUT "CHANNEL NO.? ";CH
3520  INPUT "PULSE TRAIN NO.? ";P
3530  INPUT "DELETE? ";DD$
3540  IF DD$ = "Y" THEN  GOTO 3560
3550  IF DD$ = "N" THEN  GOTO 3570
3555  GOTO 3530
3560 STIM(CH,P - 1,0) = 0:STIM(CH,P - 1,1) = 0: GOTO 3410
3570  INPUT "NEW FREQUENCY: ";STIM(CH,P - 1,0)
3580  INPUT "NEW DURATION: ";STIM(CH,P - 1,1)
3590  GOTO 3410
3600  RETURN
```

```
3900  HOME : VTAB 10
3910  PRINT "SAVE FILE": PRINT
3920  INPUT "FILE NAME: ";F$
3925  PRINT
3930  PRINT D$;"OPEN";F$;",D2"
3940  PRINT D$;"WRITE";F$
3950  FOR CHAN = 0 TO 7
3955  PRINT TT(CH)
3960  FOR TRAIN = 0 TO TT(CH) - 1
3965  FOR INFO = 0 TO 1
3970  PRINT STIM(CHAN,TRAIN,INFO)
3975  NEXT
3976  NEXT
3977  NEXT
3990  PRINT D$;"CLOSE";F$
3995  RETURN

4000  REM  LOAD RUN PROGRAM
4005  PRINT
4010  PRINT  CHR$ (4);"RUN NPULG03,D1"
```

```
10  REM
20  REM  HIMEM:8192?
30  REM  PROGRAMMABLE STIMULATION UNIT.
40  REM  H.GIBSON MAY 1985
50  HOME
60 D$ =  CHR$ (4)
70  REM  LOAD M/C
80  PRINT
90  PRINT D$;"BLOAD H1,D1"
110  DIM STIM(7,19,1),DR(20),R(20),MULT(20),FRDUR(20),MLL(20),MTLTI(20),TT(7)
120  HOME
1000  REM  READ FILES
1010  HOME : VTAB 10
1020  PRINT "LOAD FILE"
1025  PRINT
1030  INPUT "FILE NAME: ";F$
1035  PRINT
1040  PRINT D$;"OPEN";F$;",D2"
1050  PRINT D$;"READ";F$
1060  FOR CHAN = 0 TO 7
1065  INPUT TT(CH)
1070  FOR TRAIN = 0 TO TT(CH) - 1
1073  FOR INFO = 0 TO 1
1075  INPUT STIM(CHAN,TRAIN,INFO)
1080  NEXT
1085  NEXT
1090  NEXT
1100  PRINT D$;"CLOSE";F$
3000  FOR AA = 6656 TO 7296
3010  POKE AA,0
3020  NEXT
4000  REM  POKE DATA
4004 B = 0
4005  FOR CH = 0 TO 7
4006  FOR A = 0 TO TT(CH) - 1
```

```
4007  IF STIM(CH,A,0) = 0 THEN MLL(A) = STIM(CH,A,1) * 200:STIM(CH,A,0) = 1:STIM
(CH,A,1) = 1: GOTO 4009
4008 MLL(A) = 200 / STIM(CH,A,0)
4009 FRDUR(A) = STIM(CH,A,0) * STIM(CH,A,1)
4010  IF FRDUR(A) > 255 THEN DR(A) =  INT (FRDUR(A) / 255):R(A) = FRDUR(A) - (DR
(A) * 255): GOTO 4012
4011 DR(A) = 0:R(A) = FRDUR(A)
4012  IF MLL(A) > 255 THEN MTLTI(A) =  INT (MLL(A) / 255):MULT(A) = MLL(A) - (MT
LTI(A) * 255): GOTO 4014
4013 MTLTI(A) = 0:MULT(A) = MLL(A): IF MULT(A) = 0 THEN MULT(A) = 1
4014  NEXT
4015  FOR A = 0 TO (4 * (TT(CH) - 1)) STEP 4
4016 C = A / 4
4020  POKE 6658 + A + CH * 80,DR(C)
4030  POKE 6659 + A + CH * 80,R(C) - 1
4040  POKE 6657 + A + CH * 80,MULT(C) - 1
4041  POKE 6656 + A + CH * 80,MTLTI(C)
4043  NEXT
4045  POKE 7296 + CH,TT(CH)
4046  NEXT
4047  CALL 544
4050  HOME : VTAB (10)
4055  PRINT  TAB( 18)"MENU"
4060  PRINT  TAB( 18)"****"
4065  PRINT : PRINT  TAB( 15)"1.SINGLE"
4070  PRINT  TAB( 15)"2.COUNT"
4075  PRINT  TAB( 15)"3.FREE RUN"
4076  PRINT  TAB( 15)"4.LOAD NEW FILE"
4077  PRINT  TAB( 15)"5.RETURN TO"
4078  PRINT  TAB( 15)"  MAIN MENU"
4080  GET Z$
4083 Z =  VAL (Z$)
4085  ON Z GOTO 4100,4200,4300,4400,4088
4087  GOTO 4050
4088  PRINT
4090  PRINT  CHR$ (4);"RUN NSTIM,D1"
4095  STOP
```

```
4100  CALL 578
4110  GOTO 4050
4200  HOME : VTAB 10
4210  INPUT "ENTER NUMBER OF CYCLES: ";NC
4215  VTAB 15: PRINT "PRESS ANY KEY TO STOP"
4220  FOR AA = 1 TO NC
4230  CALL 578
4235  VTAB 20: PRINT AA
4240  NEXT
4250  GOTO 4050
4300  REM
4302  HOME : VTAB 15: PRINT "PRESS ANY KEY TO STOP"
4305  CALL 578
4307 XI% =  PEEK ( - 16384): IF XI% > 128 THEN  GOTO 4320
4310  GOTO 4305
4320  GOTO 4050
4400  GOTO 1000
```

ASS2

```
1A00 = TRAIN NUMBER TIMES 4
1A01-1A08 = CHANB
1B00 = MSB FRDUR
1B01 = LSB FRDUR
1B02 = MSB MULT
1B03 = LSB MULT


0300- SETUP.      A9 FF       LDA #$FF
0302-             8D B3 C0    STA $C0B3    ;MAKE OUTPUT A DDRA
0305-             8D B2 C0    STA $C0B2    ;MAKE B OUTPUT DDRB
0308-             8D B0 C0    STA $C0B0    ;SET B SIDE HIGH VIAB
030B-             A9 7F       LDA #$7F     ;i.e.#% 11111111
030D-             8D BE CO    STA $C0BE    ;DISABLE ALL VIA
                                           INTERUPTS IER
0310-             78          SEI          ;SET UP INTERUPTS STATUS
0311-             A9 40       LDA #$40     ;MAKE TIMER 1 IN FREE
                                           RUN MODE
0313-             8D BB C0    STA $C0BB    ;WITH NO O/P ON BIT 7 OF B
                                           SIDE ACR.
0316-             A9 00       LDA #$00     ;(ACR BITS 5, 6 & 7
                                           CONTROL MODES OF
                                           OPERATION OF CLOCKS)
0318-             8D B1 C0    STA $C0B1    ;(VIAA) SET A SIDE LOW
031B-             8D B4 C0    STA $C0B4    ;SET UP TIMER
031E-             A9 14       LDA #$08     ;FOR 2 MSEC
0320-             8D B5 C0    STA $C0B5    ;AS SOON AS T=1H SET
                                           THEN  TIMING STARTS
0323-             EA          NOP
0324- SETPAT.     A9 80       LDA #$80     ;SETPAT SETS THE
                                           PATTERNS
0326-             A0 07       LDY #$07     ;FOR THE VIA BITS 0 TO 7.
0328- SET1.       99 01 1A    STA 41A01,Y        ;STORE IN CHANTB,Y
032B-             4A          LSR          ;SHIFT RIGHT,IN EFFECT
                                           HALVE VALUE
032C-             88          DEY          ;AND STORE IN CHANTB -1
032D-             10 F9       BPL $0328    ;(SET1) BRANCH TO SET1 IF
                                           CARRY IS POSITIVE STILL
                                           TO SET1.
032F-             60          RTS          ;END SETUP AND SETPAT
0330-             EA          NOP
0331-             EA          NOP
0332-             EA          NOP
0333-             EA          NOP
0334-             EA          NOP
0335-             A2 00       LDX #$00     ;X-REG IS USED AS A
                                           POINTER IN DATA
                                           TABLE.START WITH X=0
0337-TESTTRAIN.   A9 00       LDA #$00     ;TEST IF FRDUR <0
0339-TESTTRAIN+1.DD 01 1B     CMP $1B01,X        ;TEST LSB FRDUR
033C-             D0 12       BNE $0350    ;(START) ROUTINE TO
                                           GENERATE PULSES
033E-             DD 00 1B    CMP $1B00,X        ;TEST MSD DURATION
                                                 IF LSB = 0
0342-             DO OD       BNE $0350    ;(START).
0343- INCTRAIN.   E8          INX          ;INCREMENT X REG BY 4
                                           BECAUSE OF PATTERN OF
                                           STORAGE OF DATA
0344-             E8          INX          IN GROUPS OF 4,
0345-             E8          INX          ;SINCE CURRENT TRAIN IS
                                           COMPLETED.
0346-             E8          INX
0347-             EC 00 1A    CPX $1A00    ;COMPARE X WITH TRAIN
                                           NO X 4
034A-             F0 73       BEQ $03BF    ;(END). IF SAME WE HAVE
                                           FINISHED!
034C-             4C 37 03    JMP $0337    ;TESTTRAIN). ELSE START
                                           NEXT TRAIN OF PULSES
034F-             EA          NOP
```

```
0350- START.      BD 00 1B    LDA $1B00,X       ;LOAD ACC WITH MSB
                                                & LSB OF FRDUR : ROUTINE
                                                TO TEST IF FRDUR=-1. IF
                                                NOT CONTINUE ELSE
                                                INCREMENT TRAIN AND
                                                TEST NEXT TRAIN OF
                                                PULSES.
0353-             48          PHA               ;PUSH ONTO STACK
0354-             BD 01 1B    LDA $1B01,X       ;LOAD ACC WITH LSB
                                                FRDUR
0357- DECFRDUR.48             PHA
0358-             68          PLA               ;PULL OFF LSB FRDUR
0359-             A8          TAY               ;AND PUT INTO Y-REG
035A-             C0 00       CPY #$00          ;TEST WITH ZERO
035C-             F0 06       BEQ $0364         ;(TESTMSB) AND IF ZERO
                                                TEST MSB
035E-             88          DEY               ;ELSE DECREMENT LSB
035F-             98          TYA               ;AND PUSH BACK ONTO
                                                STACK
0360-             48          PHA
0361-             4C 73 03    JMP $0373         ;(MULT) DO SAME AS
                                                ABOVE TO MULT
0364- TESTMSB.    68          PLA               ;MSB FRDUR
0365-             A8          TAY
0366-             C0 00       CPY #$00
0368-             F0 D9       BEQ $0343         ;(INCTRAIN) IF FRDUR
                                                FINISHED GO ON TO NEXT
                                                TRAIN
036A-             88          DEY               ;DECREMENT MSB
036B-             98          TYA
036C-             48          PHA               ;AND PUSH ONTO STACK
036D-             A9 FF       LDA #$FF          ;MAKE LSB=255
036F-             48          PHA
0370-             EA          NOP
0371-             EA          NOP
0372-             EA          NOP
0373- MULT.       BD 02 1B    LDA $1B02,X       ;LOAD ACC WITH MSB
                                                MULT
0376-             48          PHA               ;PUSH ONTO STACK
0377-             BD 03 1B    LDA $1B03,X       ;LOAD ACC WITH LSB
                                                MULT
037A-             48          PHA               ;PUSH ONTO STACK
037B- LSBMULT.    68          PLA               ;PULL OFF LSB
037C-             A8          TAY               ;TRANSFER TO Y-REG
037D- CHECK.      C0 00       CPY #$00          ;AND SEE IF ZERO
037F-             F0 15       BEQ $0396         ;(MSBMULT) IF SO TEST
                                                MSB MULT
0381- WAIT.       A9 40       LDA #40           ;ELSE CHECK IF VIA
                                                COUNTER HAS COUNTED
                                                  DOWN
0383- SYNCH.      2C BD C0    BIT $C0BD         ;(IFR)
0386-             F0 FB       BEQ $0383         ;(SYNCH) MODIFICATION TO
                                                ALLOW INTERRUPT BY ANY
                                                KEY
0388- KEYPRESS.   A9 80       LDA #$80
038A-             ED 00 C0    SBC $C000         ;TEST IF APPLE KEY PRESS
                                                REGISTER DONE
038D-             30 31       BMI $03C0         ;(END+1) YES SO GO ON TO
                                                FINISH
038F-             AD B4 C0    LDA $C0B4         ;TIMER CLEAR FLAG
0392-             88          DEY               ;DECREMENT MULT
0393-             4C 7D 03    JMP $037D         ;(CHECK) AND GO THROUGH
                                                CHECH IF ZERO (DONE)
                                                ROUTINE AGAIN
0396- MSBMULT.    68          PLA               ;IF MULT LSB=0 TEST MULT
MSB
0397-             A8          TAY
0398-             C0 00       CPY #$00
```

```
039A-              F0 0A       BEQ $03A6    ;(FLASH) IF MULT IS DONE,
                                            PULSE OUTPUT OF VIA
039C-              88          DEY          ;ELSE DECREMENT
                                            MULTMSB BY ONE
039D-              98          TYA
039E-              48          PHA          ;PUSH ONTO STACK
039F-              A9 FF       LDA #$FF     ;AND MAKE MULT LSB=255
03A1-              48          PHA          ;PUSH ONTO STACK READY
                                            TO BE RETESTED
03A2-              4C 7B 03    JMP $037B    ;(LSBMULT)
03A5-              EA          NOP
03A6- FLASH.       A0 00       LDY #$00
03A8-              B9 01 1A    LDA $1A01,Y        ;LOAD ACC WITH
                                                  CHANB,Y PATTERN
03AB-              8D B1 C0    STA $C0B1    ;(VIAA) PULSE OUT
03AE-              49 FF       EOR #$FF
03B0-              8D B0 C0    STA $COBO    ;(VIAB)
03B3-              A9 00       LDA #$00
03B5-              8D B1 C0    STA $COB1    ;(VIAA)
03B8-              AD B0 C0    LDA $C0B0    ;(VIAB)
03BB-              EA          NOP
03BC-              4C 58 03    JMP $0358    ;(DECFRDUR)
03BF- END.         60          RTS          ;RETURN TO BASIC
03C0- END+1.       68          PLA          ;PUT STACK INTO ORIGINAL
                                            STATUS
03C1-              68          PLA
03C2-              68          PLA
03C3-              60          RTS          ;RETURN TO BASIC
```

```
0364-   8D B0 C0     STA   $C0B0
0367-   A9 00        LDA   £$00
0369-   8D B1 C0     STA   $C0B1
036C-   A9 FF        LDA   £$FF
036E-   EA           NOP
036F-   A9 00        LDA   £$00
0371-   A6 01        LDX   $01
0373-   D5 73        CMP   $73,X
0375-   F0 05        BEQ   $037C
0377-   D6 73        DEC   $73,X
0379-   4C E7 02     JMP   $02E7
037C-   D5 72        CMP   $72,X
037E-   F0 09        BEQ   $0389
0380-   D6 72        DEC   $72,X
0382-   A9 FF        LDA   £$FF
0384-   95 73        STA   $73,X
0386-   4C E7 02     JMP   $02E7
0389-   20 C6 03     JSR   $03C6
038C-   F0 F8        BEQ   $0386
038E-   4C CF 02     JMP   $02CF
0391-   EA -         NOP
0392-   EA           NOP
0393-   20 B5 03     JSR   $03B5
0396-   A6 01        LDX   $01
0398-   B1 61        LDA   ($61),Y
039A-   95 70        STA   $70,X
039C-   E8           INX
039D-   EA           NOP
039E-   C8           INY
039F-   C0 04        CPY   £$04
03A1-   D0 F5        BNE   $0398
03A3-   4C E7 02     JMP   $02E7
03A6-   A9 00        LDA   £$00
03A8-   85 00        STA   $00
03AA-   85 02        STA   $02
03AC-   85 04        STA   $04
03AE-   85 01        STA   $01
03B0-   A9 05        LDA   £$05
03B2-   85 03        STA   $03
03B4-   60           RTS
03B5-   A0 00        LDY   £$00
03B7-   A2 00        LDX   £$00
03B9-   A1 03        LDA   ($03,X)
03BB-   85 61        STA   $61
03BD-   E6 03        INC   $03
03BF-   A1 03        LDA   ($03,X)
03C1-   85 62        STA   $62
03C3-   C6 03        DEC   $03
03C5-   60           RTS
03C6-   A6 00        LDX   $00
03C8-   D6 15        DEC   $15,X
03CA-   A9 00        LDA   £$00
03CC-   D5 15        CMP   $15,X
03CE-   60           RTS
03CF-   A6 00        LDX   $00
03D1-   A9 00        LDA   £$00
03D3-   D5 15        CMP   $15,X
03D5-   60           RTS
```

```
02F3-   E6 01       INC   $01
02F5-   A9 80       LDA   £$80
02F7-   ED 00 C0    SBC   $C000
02FA-   30 19       BMI   $0315
02FC-   A2 08       LDX   £$08
02FE-   A9 00       LDA   £$00
0300-   15 14       ORA   $14,X
0302-   CA          DEX
0303-   D0 FB       BNE   $0300
0305-   C9 00       CMP   £$00
0307-   F0 0C       BEQ   $0315
0309-   A9 08       LDA   £$08
030B-   C5 00       CMP   $00
030D-   F0 29       BEQ   $0338
030F-   4C C7 02    JMP   $02C7
0312-   4C 20 03    JMP   $0320
0315-   A2 00 -     LDX   £$00
0317-   BD 00 1D    LDA   $1D00,X
031A-   95 00       STA   $00,X
031C-   E8          INX
031D-   D0 F8       BNE   $0317
031F-   60          RTS
0320-   A6 01       LDX   $01
0322-   D5 71       CMP   $71,X
0324-   F0 05       BEQ   $032B
0326-   D6 71       DEC   $71,X
0328-   4C E7 02    JMP   $02E7
032B-   D5 70       CMP   $70,X
032D-   F0 1D       BEQ   $034C
032F-   D6 70       DEC   $70,X
0331-   A9 FF       LDA   £$FF
0333-   95 71       STA   $71,X
0335-   4C E7 02    JMP   $02E7
0338-   20 A6 03    JSR   $03A6
033B-   A9 00       LDA   £$00
033D-   85 00       STA   $00
033F-   A9 40       LDA   £$40
0341-   2C BD C0    BIT   $C0BD
0344-   F0 FB       BEQ   $0341
0346-   AD B4 C0    LDA   $C0B4
0349-   4C C7 02    JMP   $02C7
034C-   20 B5 03    JSR   $03B5
034F-   B1 61       LDA   ($61),Y
0351-   A6 01       LDX   $01
0353-   95 70       STA   $70,X
0355-   E8          INX
0356-   C8          INY
0357-   B1 61       LDA   ($61),Y
0359-   95 70       STA   $70,X
035B-   A6 00       LDX   $00
035D-   B5 A0       LDA   $A0,X
035F-   8D B1 C0    STA   $C0B1
0362-   49 FF       EOR   £$FF
```

```
0292-   A9 00       LDA   £$00
0294-   85 60       STA   $60
0296-   20 B5 03    JSR   $03B5
0299-   A2 04       LDX   £$04
029B-   B1 61       LDA   ($61),Y
029D-   85 63       STA   $63
029F-   98          TYA
02A0-   48          PHA
02A1-   A4 60       LDY   $60
02A3-   A5 63       LDA   $63
02A5-   99 70 00    STA   $0070,Y
02A8-   E6 60       INC   $60
02AA-   68          PLA
02AB-   A8          TAY
02AC-   C8          INY
02AD-   CA          DEX
02AE-   D0 EB       BNE   $029B
02B0-   E6 03       INC   $03
02B2-   E6 03       INC   $03
02B4-   A5 60       LDA   $60
02B6-   C9 20       CMP   £$20
02B8-   D0 DC       BNE   $0296
02BA-   20 A6 03    JSR   $03A6
02BD-   A2 08       LDX   £$08
02BF-   BD 7F 1C    LDA   $1C7F,X
02C2-   95 14       STA   $14,X
02C4-   CA          DEX
02C5-   D0 F8       BNE   $02BF
02C7-   20 CF 03    JSR   $03CF
02CA-   D0 54       BNE   $0320
02CC-   4C E7 02    JMP   $02E7
02CF-   18          CLC
02D0-   A2 00       LDX   £$00
02D2-   A1 03       LDA   ($03,X)
02D4-   69 04       ADC   £$04
02D6-   81 03       STA   ($03,X)
02D8-   B0 03       BCS   $02DD
02DA-   4C 93 03    JMP   $0393
02DD-   E6 03       INC   $03
02DF-   A1 03       LDA   ($03,X)
02E1-   69 01       ADC   £$01
02E3-   81 03       STA   ($03,X)
02E5-   C6 03       DEC   $03
02E7-   E6 00       INC   $00
02E9-   E6 03       INC   $03
02EB-   E6 03       INC   $03
02ED-   E6 01       INC   $01
02EF-   E6 01       INC   $01
02F1-   E6 01       INC   $01
```

```
0220-   A9 FF       LDA   £$FF
0222-   8D B3 C0    STA   $C0B3
0225-   8D B2 C0    STA   $C0B2
0228-   8D B0 C0    STA   $C0B0
022B-   A9 74       LDA   £$74
022D-   8D BE C0    STA   $C0BE
0230-   78          SEI
0231-   A9 40       LDA   £$40
0233-   8D BB C0    STA   $C0BB
0236-   A9 D0       LDA   £$D0
0238-   8D B1 C0    STA   $C0B1
023B-   8D B4 C0    STA   $C0B4
023E-   A9 14       LDA   £$14
0240-   8D B5 C0    STA   $C0B5
0243-   60          RTS
0244-   A2 00       LDX   £$00
0246-   B5 00  -    LDA   $00,X
0248-   9D 00 1D    STA   $1D00,X
024B-   E8          INX
024C-   D0 F8       BNE   $0246
024E-   A9 80       LDA   £$80
0250-   A2 07       LDX   £$07
0252-   95 A0       STA   $A0,X
0254-   4A          LSR
0255-   EA          NOP
0256-   CA          DEX
0257-   10 F9       BPL   $0252
0259-   A9 00       LDA   £$00
025B-   85 05       STA   $05
025D-   A9 50       LDA   £$50
025F-   85 07       STA   $07
0261-   A9 A0       LDA   £$A0
0263-   85 09       STA   $09
0265-   A9 F0       LDA   £$F0
0267-   85 0B       STA   $0B
0269-   A9 40       LDA   £$40
026B-   85 0D       STA   $0D
026D-   A9 90       LDA   £$90
026F-   85 0F       STA   $0F
0271-   A9 E0       LDA   £$E0
0273-   85 11       STA   $11
0275-   A9 30       LDA   £$30
0277-   85 13       STA   $13
0279-   A9 1A       LDA   £$1A
027B-   85 06       STA   $06
027D-   85 08       STA   $08
027F-   85 0A       STA   $0A
0281-   85 0C       STA   $0C
0283-   A9 1B       LDA   £$1B
0285-   85 0E       STA   $0E
0287-   85 10       STA   $10
0289-   85 12       STA   $12
028B-   A9 1C       LDA   £$1C
028D-   85 14       STA   $14
028F-   20 A6 03    JSR   $03A6
```

Hardware

The stimulator controller is based around an Apple II microcomputer. Although software development and execution may be carried out on the basic machine, no facilities are provided for direct input/output lines to other devices. Internal expansion slots are provided, however, for the installation of additional circuitary.

To provide the necessary output lines for the pulse generator an interface card was contructed employing a 'versatile interface adapter' (VIA) integrated circuit to provide the signal output lines. The circuit was supplied by Dr G Kidd, dept of physiology. A circuit diagram of the card is shown in Figure A4.1. The signal outputs are buffered to prevent damage by exceeding the devices current limitations and fed to TTL circuitary to provide pulses of a suitable duration for the trigger input of the Devices 3072 stimulator. Transistor drivers increase the final output level to approximately 10 volts, which when biased is reduced to 0 volt.

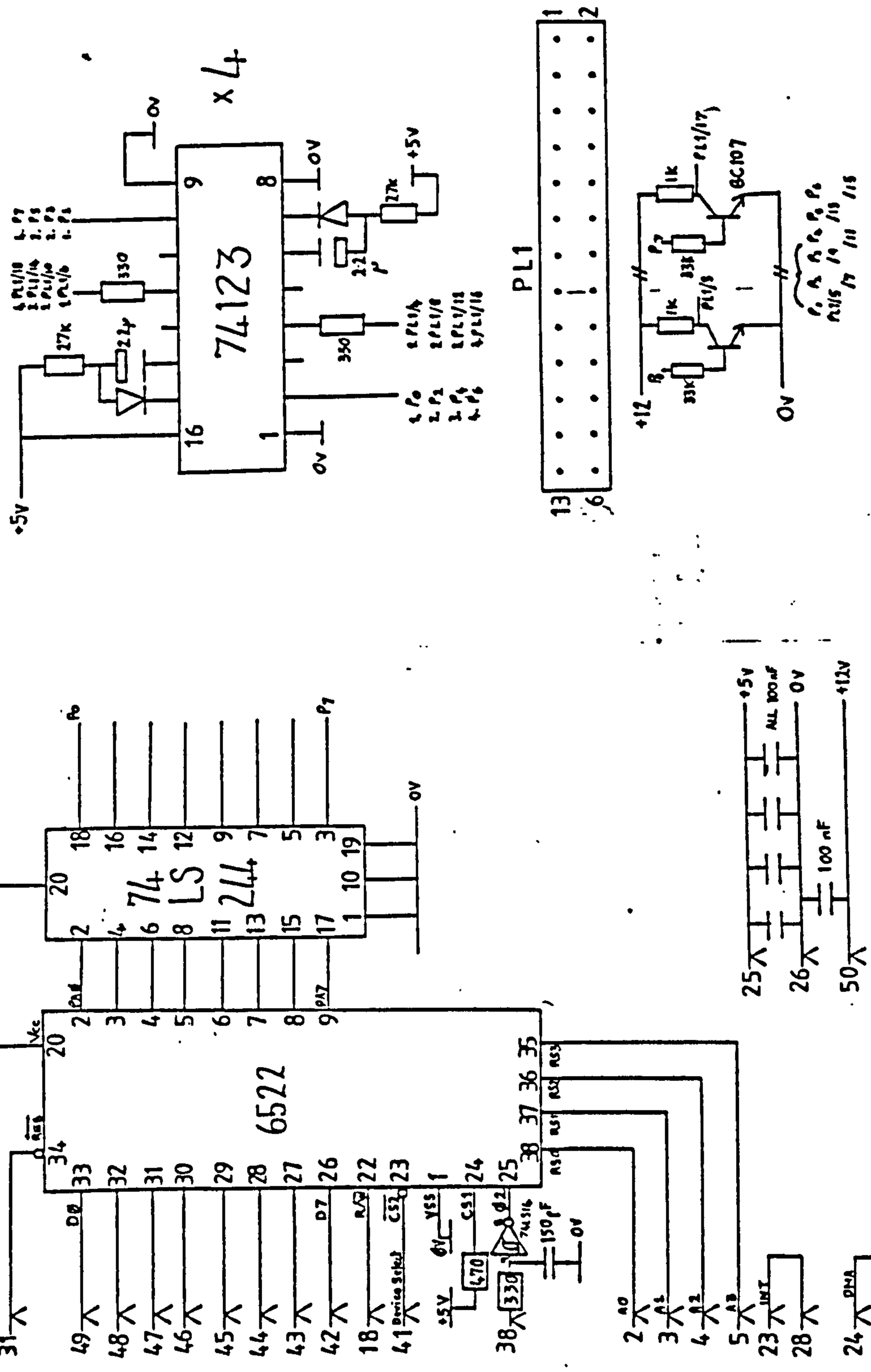


Figure A4.1 Circuit details of Apple II stimulator controller card.

Appendix 5 Individual coefficients of variation for force (expressed as a percentage of maximum) and MRR (% force loss per 10 msec) of 5 second PSEM for repeated inter/intra day measurements. Chapter 3, part II.

Inter-day coefficients of variation (3 measurements per subject)

Subject	MRR	20/50	%F10	%F20	%F50	%tw	%ptw
HG (M)	4.9	5.0	25.0	4.6	0.4	26.3	2.0
RS (F)	4.7	0.9	36.1	1.0	0.1	10.9	17.2
AL (M)	3.2	0.6	11.6	0.5	0.1	8.6	3.1
BC (M)	5.4	2.1	6.0	2.0	0.1	12.6	2.7
MS (F)	1.9	2.0	1.9	1.7	0.3	17.1	4.9
SP (F)	11.4	1.2	5.0	3.2	2.1	5.7	4.2
MJ (M)	6.0	1.3	33.7	2.7	1.4	2.3	*
JC (M)	12.5	1.0	15.2	2.0	1.0	9.6	2.9
MH (M)	2.3	2.6	10.7	4.7	2.2	9.0	1.0

Intra-day coefficient of variation (2 visits)

Subject	MRR	20/50	%F10	%F20	%F50	%tw	%ptw
LB (F)	6.2	1.4	1.1	1.3	0.1	3.2	5.6
SP (F)	4.1	1.1	3.1	1.2	0.9	1.6	4.6
N (F)	2.6	1.9	2.5	2.4	0.5	0.6	6.0
C (F)	2.3	5.7	6.8	6.1	0.5	0.5	2.3
HD (F)	6.2	1.4	10.1	1.3	0.1	1.3	7.2
RS (F)	4.9	1.5	2.2	1.9	0.8	1.3	8.1
S (F)	*	1.1	12.4	1.1	1.1	1.2	9.7
PM (F)	2.4	1.5	15.5	5.3	2.8	0.8	5.3
JW (F)	1.0	3.8	6.9	4.7	1.6	0.2	1.2
MS (F)	7.4	1.3	5.2	1.1	0.7	0.7	2.9
RC (M)	1.8	1.8	3.2	1.2	0.5	1.3	4.8
SS (M)	1.2	0.3	7.1	0.8	1.1	1.0	3.4
HG (M)	1.9	3.8	11.4	3.3	0.5	1.7	9.4
PM (M)	3.8	1.7	18.9	2.1	0.5	1.2	8.9
AL (M)	5.4	0.8	5.2	1.0	0.6	0.4	2.3
JE (M)	1.0	1.4	5.3	1.7	0.3	0.3	2.0
JC (M)	2.4	2.8	18.6	3.6	1.1	2.6	11.8
MH (M)	3.1	0.9	8.4	1.8	1.5	0.7	4.5
MJ (M)	5.9	2.1	4.8	1.8	0.3	2.0	14.6
RB (M)	8.7	0.8	19.5	1.0	1.5	0.7	4.9

Appendix 6 Comparison of frequency force data for 5s PSEM and 8s PSEM. Chapter 3, part II.

5 second PSEM

	%Twitch	%Force 10Hz	%Force 20Hz	%Force 50Hz	%Post twitch	MRR
Subject						
HG	15.0	24.2	66.7	95.0	20.0	11.7
AL	12.6	17.6	63.9	95.8	15.1	12.9
RS	11.3	20.0	71.3	95.0	20.0	8.6
AW	11.0	13.4	63.0	94.5	19.7	12.4
AL	13.5	16.3	71.1	97.1	20.2	11.9
IC	12.1	18.8	69.8	96.6	18.8	10.8
DH	15.5	22.7	72.7	95.5	22.7	13.5
RC	21.9	42.9	76.2	94.3	28.6	11.4
MS	21.3	26.3	76.3	96.3	25.0	11.8
SP	15.5	23.9	71.8	98.6	19.7	9.7

8 second PSEM

HG	15.4	25.6	70.9	94.9	20.5	9.8
AL	14.5	22.2	70.9	96.6	16.2	10.7
RS	*	33.8	76.6	96.1	*	8.8
AW	10.9	16.4	68.0	95.3	19.5	11.2
AL	11.4	21.0	76.2	98.1	20.0	11.0
IC	11.8	25.0	76.4	97.2	*	9.9
DH	19.6	29.4	78.4	98.0	22.5	10.4
RC	23.4	44.9	77.6	93.5	27.1	10.7
MS	22.3	31.6	78.9	96.1	25.0	10.1
SP	19.1	27.9	75.0	100.0	22.1	8.2

Appendix 7 Influence of sex on force (as percentage of maximum) and relaxation characteristics of 5 second PSEM (Chapter 3, part II).

Subject	sex	age	MRR	Twitch	10Hz	20Hz	50Hz	Post twitch	20/50 ratio
LB	F	22	10.8	16.9	27.0	69.7	96.6	18.0	72.1
SP	F	28	11.4	14.3	25.7	68.6	95.7	18.6	71.6
N	F	27	10.6	13.4	27.9	71.2	96.2	18.3	74.0
C	F	33	12.5	13.8	22.3	73.8	95.4	20.8	77.4
HD	F	26	11.8	10.0	13.8	66.3	95.0	18.8	69.7
RS	F	25	8.2	9.6	33.7	72.3	95.2	15.7	75.9
S	F	35	*	9.6	15.2	64.6	92.9	13.6	69.6
PM	F	39	11.5	11.3	20.0	62.5	95.0	13.8	65.8
JW	F	26	11.4	9.3	17.4	62.8	*	15.1	*
MS	F	31	11.4	16.7	25.6	74.4	96.7	23.3	77.0
RC	M	34	12.3	18.3	46.7	78.3	94.2	27.5	83.1
SS	M	28	9.6	16.4	38.3	80.5	99.2	28.9	81.1
HG	M	26	10.9	10.2	16.9	62.5	95.6	20.6	65.4
PM	M	26	12.0	8.9	10.6	65.0	95.9	14.6	67.8
AL	M	25	12.5	11.9	19.3	70.6	97.3	19.3	72.6
JE	M	21	12.4	8.9	9.6	58.5	94.8	14.1	61.7
JC	M	32	11.7	12.0	19.4	64.8	96.3	20.4	67.3
MH	M	30	11.4	10.3	23.3	74.0	97.9	16.4	75.5
MJ	M	36	*	10.4	25	67.4	96.5	*	69.8
RB	M	34	8.7	7.0	15.7	64.4	97.4	13.9	66.1

Appendix 8 Subjects participating in experiments of chapter 3, part III and chapter 6

Chapter 3

1. Frequency dependence of relationship between force and excitation

A. Wagenmakers	(M)	34
H. Gibson	(M)	26
R. Cooper	(M)	34
M. Stokes	(F)	31
M. Hartley	(M)	30
N. Parr	(M)	30
M. Jackson	(M)	36
P. Smith	(M)	29
J. Coakley	(M)	32

2. Frequency dependence of changes in relaxation characteristics

H. Gibson	(M)	26
M. Stokes	(F)	31
J. Coakley	(M)	32
A. Wagenmakers	(M)	34
R. Cooper	(M)	34

Chapter 6

1. Ascending/descending fatigue experiments

Subject	sex	age
R. Cooper	(M)	34
J. Coakley	(M)	32
R. Savage	(F)	25
A. Wagenmakers	(M)	34
M. Stokes	(F)	31
H. Gibson	(M)	26

2. Potentiation of the twitch

A. Wagenmakers	(M)	34
R. Cooper	(M)	34
H. Gibson	(M)	26

Appendix 9 Relation between force, CMAP amplitude and maximal relaxation rate to contractile activity performed

CA - contractile activity performed, F - force, MRR - maximum relaxation rate
Subject: R.Cooper. male. 34

Frequency

20				40				60				80				100			
CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	C	F	CMAP	MRR
0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0
5.7	93.4	109.7	80.6	3.9	85.2	*	88.3	3.7	97.0	93.5	85.8	2.2	96.0	109.1	96.2	2.5	95.1	109.5	100.8
10.6	96.1	112.5	70.8	7.7	91.1	*	73.8	8.8	94.0	87.1	74.2	4.8	92.0	88.6	92.0	5.0	91.1	83.3	90.3
15.5	97.4	111.1	55.1	11.9	92.1	95.7	61.9	12.9	89.0	74.2	67.0	6.7	88.5	77.3	86.0	7.3	84.2	66.7	88.6
22.4	97.4	108.1	39.2	16.5	88.1	85.5	53.6	17.0	86.0	58.1	59.1	9.1	84.0	65 9	82.4	9.4	81.2	61.9	81.4
27.9	92.1	100.0	31.3	20.7	87.1	76.8	45.9	20.6	80.0	46.8	52.5	11.1	81.0	54.5	79.1	11.2	79.2	54.8	77.0
32.8	79.0	83.3	22.2	24.6	79.2	63.8	39.4	24.3	70.0	35.5	46.2	13.5	79.0	50.0	74.7	13.5	75.2	54.7	72.1
36.5	61.8	63.9	18.3	28.1	70.3	53.6	34.1	27.3	54.0	24.2	40.5	15.6	78.0	43 2	70.2	15.4	71.3	45.2	66.6
39.9	47.4	48.6	15.2	31.2	60.4	49.3	30.5	29.9	40.0	20.2	33.6	17.4	74.0	43.2	65.8	17.3	63.4	35.7	61.5
42.2	36.8	37.5	12.6	34.0	49 5	43.5	27.5	31.8	28.0	16.1	27.0	19 2	70.0	29.5	61.1	19.2	52.5	28.6	56 5
44.3	29.0	31.9	10.7	35.8	37.6	37.7	25.5	32.9	20.0	12.9	25.2	21.0	63.0	27.3	57.0	20.8	41.6	19.1	53 0
45.7	22.4	27.8	6.9	37.2	29.7	31.9	21.6	33.6	14.0	12.9	21.0	22.6	54.5	25.0	51.8	21.9	31.7	19.1	45.5
46.8	17.1	25.0	*	38.6	22.8	26.1	21.1	34.4	10.0	12.1	21.0	24.2	47.0	20.5	47.3	22.9	23 8	19.1	42.8
47.4	11.8	20.1	*	39.3	17.8	23.2	18.0	34.6	8.0	12.9	15.8	25.3	39.0	18.2	41.6	23.7	17.8	16.7	38.0
48.0	7.9	18.8	*	40.4	14.8	24.6	16.2	34.8	5.0	12.9		26.3	32.0	18.2	37.4	23.8	14.9	16.7	34.2
49.1	6.6	15.3	*	40.7	11.9	24.6	13.5	35.0	4.0	12.9		27.1	26.0	18.2	34.5	24.6	10.9	16.7	31.1
49.4	2.6	12.5	*	41.1	8.9	26.1	*					27.5	20.5	15.9	29.2	25.0	7.9	16.7	32.1
				41.4	6.9	27.5	*					28.3	16.0	15 9	29 4	25.4	6.9	16.7	30.6
				41.6	5.9	27.5	*					28.7	13.0	15.9	26.3	25.6	5 0	16.7	34.2
				41.7	5.0	29.0	*					28.9	10.0	15.9	25 6				
				41.9	4.0	29.0	*					29.3	8.0	15.9	21.4				
				42.1	3.0	29.0	*					29.7	6.0	15.9	*				
				42.1	2.5	29.0	*					29.5	5.5	15.9	*				

Appendix 9 continued

Subject: H.Gibson. male. 26

Frequency

20				40				60				80				100			
CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	C	F	CMAP	MRR
0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0
6.0	78.6	96.3	99.2	5.3	95.6	87.5	83.6	4.2	93.6	93.7	87.7	2.3	94.3	100.0	92.9	2.2	93.7	105 5	94.1
12.2	82.1	92.6	83.5	10.0	93.8	89.6	75.1	8.5	88.7	91.1	77.0	4.6	92.1	86.7	81.8	4.7	88.7	71.2	83 4
18.0	84.5	64.4	61.8	15.7	92.0	87.5	64.2	12.6	83.9	75.9	69.5	6.7	87.5	74.4	79.7	6.9	84 5	57.5	81.6
24.3	84.5	85.2	47.2	20.0	88.5	78.1	53.1	16.4	79.0	63.3	61.9	8.9	83.8	55.6	77.4	9.1	81.0	57.8	80.2
30.3	75.0	70.4	33.5	24.7	83.2	64.6	45.2	19.7	71.8	46.8	54.1	10.9	80.8	42.2	74.9	11.0	78.2	50.7	75 9
35.1	63.1	51.9	30.5	30.0	76.1	54.2	38.8	22.7	61.3	35.4	45.8	12.9	78.5	34.4	70.8	13.0	73.9	46.6	72.6
39.4	47.6	38.9	26.4	34.3	67.3	45.8	33.9	25.2	48.4	25.3	38.7	14.7	74.7	28.9	66.1	14.9	69.0	38.4	67.1
42.3	33.3	33.3	21.5	38.0	57.5	36.5	28.0	27.3	37.1	20.3	31.0	15.8	69.4	25.6	60.5	16.3	62.0	34.3	61.1
44.0	23.8	25.9	15.1	40.7	47.8	31.3	25.3	29.1	27.4	15.2	28.8	18.0	61.1	23.3	54.5	18.1	55 6	28.8	53.8
45.4	16.7	24.1	16.2	43.7	38.9	29.2	24.1	30.3	21.0	12.7	24.0	19 6	52.1	18.9	47.4	19.3	47.9	23.3	47 2
46.6	13.1	22.2	13.7	45.3	33.6	22.9	21.9	31.2	15.3	10.1	21.1	20.7	44.5	16.7	41.6	20.7	40.8	20.5	42.9
47.4	9.5	18.5	9.4	47.3	28.3	20.8	18.9	31.8	11.3	8.9	19.1	22.0	36.2	13.3	37.5	21.8	33.8	17.8	40.1
48.1	6.0	18.5	*	49.0	23.9	20.8	16.9	32.1	8.1	7.6	17.8	22.7	30.2	12.2	32.7	22.6	28.2	16.4	36.1
48.6	3.6	17.6	*	50.0	21.2	19.8	15.8	32.4	5.6	6.3	*	23.6	24.2	11.1	30.7	23.4	23.9	16.4	35 4
48.7	2.4	16.7	*	51.3	17.7	16.7	11.4	32.6	4.0	5.1	*	24.2	20.4	8.9	25.8	24.0	19.7	16.4	31.5
				52.0	15.9	16.7	10.5					24.7	17.4	7.8	24.9	24 6	16.2	16.4	31.4
				53.3	14.2	14.6						25.0	14.3	7.8	19.4	25.1	13.3	16.4	29.6
				54.0	12.4	12.5						25.6	11.3	7.8	21.8	25.4	11.3	16.4	25.1
				54.7	9.7	11.5						25.9	9.1	7.8	20.5				
				55.2	8.9	11.5						26 2	8.3	8.9	14.9				
				55.7	7.1	11.5						26.3	6.8	8.9	13.6				
				56.0	6.2	9.4						26.5	5.7	8.9	*				
												26.6	4.5	8.9	*				

Appendix 9 continued

Subject: J.Coakley. male. 32

Frequency

20				40				60				80				100			
CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	C	F	CMAP	MRR
0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0
7.2	101.6	102.5	83.4	4.5	98.4	90.1	87.5	4.3	100.0	105.4	85.8	2.4	96.9	100.0	93.4	2.9	97.5	100.0	92.5
14.5	103.3	100.0	70.5	9.0	100.0	94.5	75.9	8.7	100.0	105.4	73.5	4.8	94.9	89.6	90.9	5.9	97.5	82.4	85.2
22.6	103.3	96.2	56.8	13.7	102.2	94.5	64.3	13.0	95.5	97.3	61.2	6.8	92.8	85.1	86.4	7.8	93.8	67.6	80.0
29.8	101.6	96.2	40.6	18.8	101.1	90.1	54.9	17.4	90.9	86.5	54.5	9.2	89.7	77.6	75.9	10.7	90.0	52.9	76.4
37.0	98.4	91.1	32.0	23.5	97.8	81.3	45.4	21.3	85.2	75.7	48.8	11.2	87.6	68.7	76.7	13.2	88.8	44.1	68.4
44.3	90.2	81.0	25.3	27.8	91.3	72.5	38.5	25.2	77.3	62.2	43.5	13.2	86.6	56.7	72.7	15.6	87.5	38.2	65.3
51.1	77.1	73.4	19.8	32.2	84.8	60.4	33.9	27.8	65.9	48.7	37.6	15.6	84.5	46.3	66.3	18.5	85.0	35.3	58.8
55.7	63.9	63.3	17.0	36.1	77.2	52.7	30.0	30.4	54.5	35.2	32.5	17.6	82.5	41.8	62.7	20.5	80.0	35.3	53.6
60.4	55.7	53.2	11.7	40.0	68.5	46.1	27.0	32.8	43.2	32.4	32.8	19.6	79.4	35.8	57.6	22.9	72.5	29.4	50.5
63.8	45.9	45.6	9 5	42.4	58.7	41.8	20.5	34.6	35.2	24.3	22.6	21.6	74.2	34.3	54.6	24.9	63.8	23.5	46.2
67.2	41.0	40.5	9.3	45.3	51.1	37.4	19.0	35.6	27.3	21.6	19.5	23.6	69.1	32.8	47.4	26.3	53.8	22.1	39.9
				47.8	44.6	34.1	14.5	36.7	20.5	21.6	17.3	25.2	61.9	29.9	41.8	28.3	45.0	22.1	31.8
				49.8	39.1	30.8	11.8	37.6	18.2	20.3	14.6	26 8	55.7	25.4	38.7	29.3	37.5	22.1	28 6
				51.8	34.8	28.6	12.0	38.2	13.6	20.3	13.0	28.0	47.4	23.9	35.5	30.7	30.0	22.1	26.8
				53.3	30.4	24.2	12.0	38.5	11.3	20.3	7.8	29 6	43.3	22.4	25.9	31.2	25.0	22.1	21.4
				54.9	26.1	24.2	10.6					30.8	39.2	22.4	22.0	32.2	20.0	22.1	24.6
				56.1	22.8	25.3	8.1					31.6	35.1	20.9	22.1	32 9	17.5	22.1	20.4
				57.3	20.6	24.2	*					32.8	30.9	19.4	19.5	33.2	15.0	22.1	17.9
				58.4	19.6	24.2	*					33 6	27.8	19.4	18.6				
				59.2	16.3	24.2	*												
				60.0	14.7	24.2	*												
				60.8	13.0	24.2	*												
				61.2	12.0	24.2	*												

Appendix 9 continued

Subject: D.Hill. male. 26

Frequency

20				40				60				80				100			
CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	C	F	CMAP	MRR
0 0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0
7.2	96.3	97.1	84.6	5.1	92.2	100.0	87.6	4.4	94 6	94.3	88.5	2.3	96.4	101.4	98.7	2.3	95.8	103.7	96.5
14.4	92.5	94.1	70.9	9.5	93.2	97.5	75.1	8.8	91.0	91.4	78.4	4.2	91.9	93.2	96.5	4.3	89.9	68.5	91.8
21.0	90.6	91.2	55.4	14.5	93.2	95.0	64.4	13.2	87.4	77.1	67.4	6.5	87.5	81.1	93.1	6.5	84 0	48.1	89.3
27.9	90.6	88.2	40.6	19.6	91.3	90.0	52.3	17.3	82.9	60.0	55.2	8.5	83.9	66.2	87.4	8.7	80.7	44.4	84.5
34.7	87.5	76.5	28.2	24.4	89.3	80.0	42.2	21.4	73.9	45.7	46.2	10.2	80.4	54.1	82.3	10.5	75.6	51.9	80.1
41.3	75.0	61.8	20.3	29.1	83.5	65.0	35.0	25.1	58.6	34.3	39.7	12.0	76.8	45.9	77.7	12.4	70.6	50.0	72.9
46.2	58.8	47.1	16.2	33.1	71.8	52.5	30.0	27.1	40.5	27.1	34.4	13.6	73.2	39.2	72.7	13 9	62.2	40.7	65.8
49 8	43.8	41.2	13.0	36.4	60.2	42.5	25.6	28.8	26.1	20.0	29.7	15.6	66.1	36.5	65.9	15.5	51.3	37.0	60.6
53.1	33.8	35.3	8.4	39.3	48.5	35.0	20.6	30.2	16.2	14.3	23.9	16.9	57.1	33.8	59.3	16.7	41.2	31.5	53.4
56.1	27.5	29.4	6.9	42.5	35.9	27.5	19.3	30.8	9.9	11.4	19.6	18.2	47.3	27.0	52.8	17.9	31.9	22.2	45.1
58.0	21.3	26.5	4.5	44.0	25.2	25.0	18.3	31.2	6.3	8 6	12.3	19.5	37.5	21.6	49.5	18.6	23.5	14.8	40.2
				45.1	19.4	20.0	15.8	31.4	3.6	8 6	*	20.5	30.4	17.6	42.5	19.2	16.8	11.1	36.1
				45.8	15.5	15.0	9.9	31.5	3.6	7.1	*	21.5	23.2	15.5	38.2	19 8	12 6	11.1	30.1
								31.5	3.2	7.1	*	21.8	18.8	14.9	34.4	20.3	10.0	11.1	33 8
								31.5	2.3	7.1	*	22.8	14.3	14.9	28 2	20.5	6.7	11.1	33.8
												23.4	10.7	14 9	30.1	20.6	5.0	11.1	*
												23.4	7.1	14.9	33.9	20.8	3 4	11.1	*
												23.7	5.4	13 5	22 6	20 9	2 5	11.1	*
												23 9	3.6	13 5	*				
												24.1	2.7	13 5	*				
												24.1	1.8	12 8	*				
												24.2	1.8	12 8	*				
												24.4	1.4	12.8	*				

Appendix 9 continued

Subject: M.Stokes. female 30

Frequency

20				40				60				80				100			
CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	C	F	CMAP	MRR
				0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0
				4.4	101.3	100.0	82.5	4.9	98.8	101.5	86.9	2.6	94.9	108 9	91.2	2.7	97.7	103.2	96.1
				9.3	98.8	90.2	73.5	9.5	95.1	91.0	74.3	5.2	91.6	92.5	87.8	5.3	90.9	77.4	86.1
				13.9	97.5	80.4	64.2	13.7	90.1	74.6	62.6	7.4	87.2	77.6	79.3	7.8	84.1	62 9	78.1
				18.6	96.3	*	54.8	18.0	85.2	53.7	55.2	9.7	82.7	65.7	76.1	9 8	77.3	51 6	73.2
				22.8	93.8	60.8	45.7	21.9	79.0	37.3	47.6	12.1	78 2	52.2	72.5	12.0	76.1	41.9	68.9
				26.9	88.8	50.9	39.6	25.4	70.4	29.9	43.5	14.1	74.6	41.8	67.5	14 2	75.0	41 9	63.3
				31.2	80.0	45.1	34.3	29.2	60.5	26.9	36.9	15.8	72.6	37.3	62.5	16.2	71.6	38.7	59.3
				34.9	70.0	33.3	29.8	32.2	45.7	22.4	30.9	17.6	70.4	34.3	57.1	18.2	68.2	37.1	53.3
				38.1	62.5	25.5	24.6	34.6	35.8	17.9	26.3	19.7	69.3	32.8	53.6	20.0	62.5	29.0	50.1
				40.9	52.5	22.6	20.9	35.6	25.9	14.9	24.9	21.5	64.8	31.3	50.9	21.6	54.5	22.6	48.1
				42.8	43.8	21.6	20.0	37.1	20.9	14.9	22.4	23.3	61.5	26.9	46.9	23.1	45.5	19.4	44.4
				44.6	37.5	19.6	17.6	38.0	16.7	13.4	17.6	24.2	55.8	22.4	44.3	24.0	38.6	17.7	39.2
				46.0	32.5	16.7	15.2	38.5	12.3	11.9	19.1	26.4	49.2	17.9	41.9	25.6	31.8	12.9	36.5
				47.7	27.5	15.7	13.9	39.3	11.1	11.2	15.9	27.7	42.5	16.4	38.9	26.4	26.1	12.9	34.8
				48.8	23.8	15.7	11.6	39.5	9.9	8.9	*	28.6	35.8	16.4	34.6	27.1	21.6	11.3	28.1
				49.8	20.0	14.7	10.9					29.9	31.3	14.9	32.9	27.6	18.2	11.3	27.8
				50.7	17.5	13.7	*					30.8	26.8	11.9	30.8	28.2	15.9	11.3	25.4
				51.2	15.0	13.7	*					31.6	22.3	11.9	34.6	28.4	12.5	11.3	24.2
				51.8	13.8	11.7	*					32.1	21.2	11.9	26.7				
				52.3	11.9	10.8	*					32.9	17.9	11.9	25.9				
				53.0	10.0	9.8	*					33.4	15.6	11.9	26.4				
				53.5	8.8	9.8	*					33.8	14.5	11.9	21.3				
				53.5	8.8	9.8	*												

Appendix 9 continued

Subject: H.Downey. female. 26

Frequency

20				40				60				80				100			
CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR	CA	F	CMAP	MRR
0.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0					0 0	100.0	100.0	100.0	0.0	100.0	100.0	100.0
2.5	108.5	115.8	79.9	1.7	100.0	98.6	96.8					1.5	103.4	101.4	94.9	1.6	100.0	106 8	90 0
11.0	108.5	118.9	64.5	6.4	98.6	99.2	76.4					3 2	101.1	106.5	87.8	4.1	95.0	88 6	77.2
16.9	110.6	118.1	48.2	10.2	97.1	95.7	61.9					5.1	97.7	82.3	80.6	6.2	87.5	74 0	73.7
19.0	108.5	116.6	36.9	14.1	95.7	94.1	48.3					6.6	93.1	71.7	71.6	8.2	82.5	61.0	68.7
25.3	106.4	109.6	23.5	17.9	95.7	87.3	40.4					8.4	88.5	62.7	72.2	9.5	80.0	52.3	59.7
31.2	102.1	80.8	19.6	22.2	92.9	77.7	30.1					9 9	86.2	55.3	65.5	11.5	77.5	49.7	62.4
36.7	93.6	86.4	16.0	25.6	85.7	67.4	26.3					11.0	83.9	46.9	59.6	13 5	76.9	46.1	56.4
42.2	80.8	74.2	14.4	29.9	80.0	57.9	21.5					12.4	81.6	44.4	58.9	15.4	73.1	41.6	53.2
46.4	65.9	63.9	12.6	32.9	70.0	48.2	21.5					13.9	80.5	38.7	55.2	16.5	67.5	32.1	47.7
50.2	55.3	51.2	12.0	35.9	58.6	41.4	19.3					15.4	79.3	36.2	46.7	18.5	61.3	29 2	38.1
53.2	44.6	37.5	11.2	37.6	51.4	33.8	16.7					16.8	74.7	31.6	47.1	20.2	55.0	20.8	36.4
55.7	38.3	31.9	8.7	39.7	42.8	29.3	15.1					18.3	70.1	25.9	42.3	21.4	47.5	15.6	28.1
58.2	31.9	28.8	*	41.4	37.1	25.0	11.5					19.8	64.4	18 8	37.4	22.2	41.3	13 9	26.9
59.9	25.5	26.0	*	42.7	32.9	22.9	11.5					20.1	58 6	17.4	33.2	23.5	35.0	10.7	25.4
61.6	21.3	22.8	*	44.7	28.6	19.9	7.5					21.2	51.7	17.2	32.2	24.3	30.0	*	22.2
												22.3	45.9	14.4	26.2	25.3	26.3	8.4	16.9
												23.0	39.1	12.2	23.7	25.7	21.3	7.5	15.7
												23.8	35.6	10.6	18.2	26.3	17.5	*	12.7
												24.5	29.9	8 9	21.7				
												25.3	26.4	9.3	21.0				
												25.6	22.9	7.6	*				
												26.0	20.7	7.1	13.4				
												26.6	18.4	6.5	10.1				

Appendix 10 Relation of force, CMAP amplitude and MRR to impulse numbers (chapter 4)

20 Hz

Impulse number	Force			CMAP amplitude			MRR		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
0	100.0	0.0	5	100.0	0.0	5	100.0	0.0	5
160	95.7	11.1	5	104.3	8.4	5	85.5	7.8	5
320	96.5	10.2	5	103.6	11.6	5	72.0	6.9	5
480	97.3	10.3	5	102.2	11.7	5	55.5	4.9	5
640	96.5	9.3	5	98.9	13.3	5	40.9	3.8	5
800	91.9	11.8	5	89.5	16.2	5	29.7	4.0	5
960	81.8	14.9	5	71.8	14.1	5	23.6	4.5	5
1120	67.8	17.9	5	61.9	19.2	5	19.3	4.3	5
1280	53.9	18.7	5	52.1	16.6	5	16.2	3.3	5
1440	43.2	17.2	5	43.2	15.2	5	12.1	2.4	5
1760	34.9	15.5	5	36.4	11.4	5	11.1	3.4	5
1600	28.5	13.6	5	30.9	7.8	5	9.1	3.6	5
1920	21.6	14.9	3	25.2	6.8	3	9.1	0.5	2
2080	16.6	13.6	3	22.5	5.5	3	*	*	*
2240	12.3	11.6	3	20.8	4.5	3	*	*	*
2400	10.1	9.9	3	18.3	4.0	3	*	*	*
2560	2.6	*	1	12.5	*	1	*	*	*

Appendix 10 continued

40 Hz

	Force			CMAP amplitude			MRR		
Impulse number	Mean	SD	N	Mean	SD	N	Mean	SD	N
0	100.0	0.0	5	100.0	0.0	5	100.0	0.0	5
160	92.4	6.4	5	94.4	6.6	4	85.9	2.7	5
320	90.6	8.7	5	93.0	3.7	4	74.7	1.0	5
480	88.0	13.4	5	90.6	6.6	5	63.8	1.1	5
640	83.8	16.5	5	85.9	5.6	4	53.8	1.1	5
800	79.8	19.6	5	72.7	9.4	5	44.9	1.5	5
960	73.2	20.0	5	61.3	8.7	5	38.3	1.9	5
1120	64.7	17.8	5	51.5	6.3	5	33.2	1.9	5
1280	55.6	15.1	5	42.9	8.3	5	28.8	2.0	5
1440	47.1	12.7	5	36.3	8.6	5	25.0	2.7	5
1760	37.1	11.4	5	31.8	7:8	5	22.1	2.6	5
1600	30.3	9.3	5	27.8	6.7	5	20.2	1.6	5
1920	24.5	8.8	5	24.1	6.2	5	17.6	2.6	5
2080	20.3	8.1	5	21.3	6.2	5	14.4	3.4	5
2240	18.9	6.9	4	22.2	5.6	4	14.5	1.9	4
2400	16.4	5.6	4	20.3	4.8	4	12.2	1.0	4
2560	13.9	5.1	4	20.4	5.6	4	10.7	0.3	3
2726	11.7	5.0	4	20.3	7.1	4	8.1	*	1
2880	11.1	4.7	3	19.5	7.5	4	*	*	*
3040	9.5	4.4	3	19.1	8.9	4	*	*	*
3200	8.3	4.0	3	18.9	9.1	4	*	*	*
3360	6.7	3.5	3	18.6	9.4	4	*	*	*
3520	5.8	3.2	3	18.1	10.0	4	*	*	*
3600	5.4	4.8	2	21.0	10.0	3	*	*	*

Appendix 10 continued.

60 Hz

Impulse number	Force			CMAP amplitude			MRR		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
0	100.0	0.0	6	100.0	0.4	6	100.0	0.0	6
240	97.1	2.5	6	98.8	5.6	6	85.3	4.2	6
480	94.2	4.0	6	98.8	9.0	6	73.1	6.1	6
720	89.3	3.8	6	82.7	11.1	6	62.7	7.7	6
960	85.0	3.9	6	66.2	12.4	6	54.7	6.8	6
1200	78.0	4.7	6	51.5	13.4	6	46.7	8.3	6
1400	68.5	7.1	6	39.9	11.6	6	40.6	8.1	6
1680	55.8	10.1	6	31.5	9.5	6	35.4	5.9	6
1920	43.5	11.7	6	24.1	6.0	6	30.8	2.3	6
2160	33.7	12.5	6	19.9	6.9	6	27.9	3.0	6
2400	25.9	12.0	6	15.7	4.8	6	24.0	2.7	6
2640	19.8	10.1	6	13.5	4.6	6	19.5	3.7	6
2800	15.1	8.7	6	12.8	4.7	6	18.0	2.2	5
3120	12.2	7.3	6	11.6	4.8	6	15.5	3.3	5
3360	9.6	6.1	6	11.1	5.1	6	14.5	2.1	2
3600	7.8	5.2	6	10.4	5.5	6	7.8	*	1

Appendix 10 continued

80 Hz

Impulse number	Force			CMAP amplitude			MRR		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
0	100.0	0.0	5	100.0	0.0	5	100.0	0.0	5
160	95.8	1.1	5	103.9	4.7	5	95.9	3.6	5
320	92.5	1.3	5	90.1	2.7	5	90.8	3.9	5
480	88.7	2.3	5	79.1	4.1	5	83.8	7.2	5
640	84.8	2.8	5	66.2	7.8	5	75.5	12.1	5
800	81.6	3.5	5	54.4	9.5	5	70.6	16.3	5
960	79.2	4.5	5	45.8	8.4	5	65.4	17.7	5
1120	76.6	4.9	5	39.0	6.6	5	60.1	17.8	5
1280	72.5	6.3	5	36.3	7.0	5	55.4	17.0	5
1440	67.4	8.7	5	31.1	4.9	5	51.0	15.7	5
1760	60.3	10.7	5	27.8	5.8	5	46.8	15.7	5
1600	53.4	12.7	5	24.6	6.0	5	42.5	14.5	5
1920	46.3	13.1	5	20.7	6.2	5	37.8	13.9	5
2080	39.5	13.3	5	17.8	4.9	5	34.5	12.6	5
2240	33.0	12.0	5	16.9	4.7	5	31.4	11.5	5
2400	28.0	11.7	5	16.2	4.9	5	26.4	10.2	5
2560	23.8	11.4	5	15.2	5.2	5	24.4	10.1	5
2726	19.9	11.1	5	14.3	4.9	5	24.8	10.4	5
2880	16.6	10.0	5	13.7	4.3	5	22.2	9.8	5
3040	14.3	9.9	5	13.7	4.3	5	19.7	8.7	4
3200	12.3	8.8	5	13.6	3.5	5	18.9	7.2	4
3360	10.5	8.3	5	13.5	3.5	5	18.2	8.8	3
3520	9.6	7.7	5	13.5	3.5	5	14.9	6.2	3
3600	2.9	2.3	2	12.8	*	5	8.9	*	1

Appendix 10 continued

100 Hz

Impulse number	Force			CMAP amplitude			MRR		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
0	100.0	0.0	6	100.0	0.0	6	100.0	0.0	6
200	96.6	2.2	6	104.8	3.3	6	95.0	3.7	6
400	92.2	3.4	6	78.6	7.7	6	85.7	5.2	6
600	86.4	3.9	6	62.8	9.0	6	81.9	6.1	6
800	82.1	4.2	6	54.4	6.5	6	77.4	5.8	6
1000	79.7	4.8	6	49.3	5.1	6	71.7	7.5	6
1200	76.6	5.8	6	46.9	6.0	6	68.1	5.0	6
1400	72.7	7.7	6	40.7	4.2	6	62.3	4.7	6
1600	66.3	9.9	6	36.8	2.6	6	57.2	4.2	6
1800	58.6	11.2	6	29.9	1.5	6	52.0	3.2	6
2000	50.2	12.2	6	23.3	3.3	6	46.3	4.9	6
2200	41.7	12.4	6	19.5	2.5	6	41.6	3.4	6
2400	34.3	12.0	6	17.2	3.7	6	36.4	5.5	6
2600	28.2	11.1	6	15.5	3.6	6	32.7	4.7	6
2800	23.3	9.3	6	15.0	4.3	6	31.7	4.4	6
3000	19.0	8.7	6	15.5	4.5	5	28.0	5.2	6
3200	15.6	7.9	6	14.3	5.0	6	26.6	6.2	5
3400	13.1	6.7	6	14.2	5.2	6	24.3	6.3	5
3600	10.6	5.8	6	15.5	4.6	5	22.8	8.1	5

Appendix 11 Mean and SD for force generation and CMAP amplitude, chapter 4.

20Hz			40Hz			60Hz			80Hz		
mean	SD	N	mean	SD	N	mean	SD	N	mean	SD	N
Force											
63.1	3.1	5	86.7	5.8	5	94.1	3.1	6	96.1	2.8	6
60.4	7.7	5	82.7	8.5	5	91.4	4.8	6	92.8	3.3	6
60.9	6.9	5	83.2	7.4	5	88.6	5.7	6	89.7	3.5	6
61.3	6.6	5	83.4	7.9	5	84.0	5.3	6	85.9	3.9	6
60.9	6.2	5	81.2	8.7	5	79.9	5.1	6	82.0	3.8	6
57.5	7.3	5	78.8	8.9	5	73.4	6.0	6	79.1	4.3	6
50.6	7.9	5	73.2	9.1	5	64.5	8.0	6	76.8	5.0	6
41.1	8.5	5	65.4	9.6	5	52.6	10.5	6	74.4	5.6	6
32.1	8.6	5	56.9	10.0	5	41.1	11.5	6	71.0	7.3	6
25.9	8.8	5	48.5	10.7	5	31.8	12.0	6	66.7	9.8	6
20.7	7.8	5	39.2	10.8	5	24.5	11.3	6	60.4	11.7	6
16.9	7.1	5	32.2	10.7	5	18.7	9.6	6	54.1	13.5	6
11.9	6.6	3	26.9	10.2	5	14.2	8.2	6	47.5	14.0	6
8.8	5.9	3	22.7	9.6	5	11.6	6.8	6	41.1	14.1	6
6.7	5.5	3	16.1	11.9	4	9.1	5.8	6	34.9	13.1	6
			18.6	7.6	4	7.4	4.9	6	29.9	12.5	6
			15.7	6.8	4				25.5	11.7	6
			13.6	6.3	4				21.8	11.5	6
			12.0	5.7	4				18.2	10.2	6
			10.7	5.7	4				15.8	9.8	6
			9.1	4.8	4				11.5	8.0	5
			7.7	4.6	4				9.8	7.5	5
			6.8	4.1	4				8.8	6.8	5
			6.9	4.6	3				7.0	7.5	3
CMAP amplitude											
100.0	0.0	5	100.0	0.0	5	100.0	0.0	6	100.0	0.0	5
104.3	8.4	5	94.4	6.6	4	98.9	5.6	6	103.9	4.7	5
103.6	11.6	5	93.0	3.7	4	95.8	9.0	6	90.1	2.7	5
102.2	11.7	5	90.6	6.6	4	82.7	11.2	6	79.1	4.1	5
98.9	13.3	5	86.0	5.6	4	66.2	12.4	6	66.2	7.8	5
89.5	16.2	5	72.7	9.4	5	51.5	13.4	6	54.4	9.5	5
71.7	14.1	5	61.3	8.7	5	40.0	11.6	6	45.8	8.4	5
61.9	19.2	5	51.5	6.3	5	31.5	9.5	6	39.0	6.6	5
15.1	16.6	5	42.9	8.3	5	24.1	6.0	6	36.3	7.0	5
43.2	15.2	5	36.3	8.6	5	19.9	6.9	6	31.0	4.9	5
36.4	11.4	5	31.8	7.8	5	15.7	4.8	6	27.6	5.8	5
30.9	7.8	5	27.8	6.7	5	13.6	4.6	5	24.6	6.0	5
25.2	6.8	3	24.1	6.2	5	12.8	4.7	6	20.7	6.2	5
22.5	5.5	3	21.3	6.2	5	11.6	4.8	6	17.8	4.9	5
20.8	4.5	3	22.2	5.6	4	11.1	5.1	6	16.9	4.7	5
18.3	4.0	3	20.3	4.8	4	10.4	5.5	6	16.2	4.9	5
			20.4	5.6	4				15.2	5.2	5
			20.3	7.1	4				14.3	4.9	5
			19.5	7.5	4				13.7	4.3	5
			19.1	8.9	4				13.7	4.3	5
			18.9	9.1	4				13.6	3.5	5
			18.6	9.4	4				13.5	3.5	5
			18.1	10.0	4				13.5	3.5	5
			21.0	10.0	3						

Please note that data for 100Hz is presented in appendix 10

BIBLIOGRAPHY

Acker A, Hammond RL, Mannion D, Salmons S & Stephenson LW. (1987) Skeletal muscle as the potential power source for a cardiovascular pump: assessment *in vivo*. Science, **236**, 324-327.

Adrian RH & Peachey LD (1973) Reconstruction of the action potential of frog sartorius muscle. Journal of Physiology, **235**, 103-131.

Ahlborg G & Felig P (1982) Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. Journal of Clinical Investigation, **69**, 45-54.

Alesso HP (1978) Reliability analysis of the elememtary catastrophe theory model of muscle contraction. Engineering in medicine, **7**, 21-30.

Alway SE, Hughson RL, Green HJ, Patla AE & Frank JS (1987) Twitch potentiation after fatiguing exercise in man. European Journal of Applied Physiology, **56**, 461-466.

Asmussen E (1934) Untersuchungen uber die mechanische Reaktion der Skelettmuskelfuser. Skand Arch Physiol, **70**, 233-272.

Asmussen E (1979) Muscle Fatigue. Medecine and Science in Sports and Exercercise, **11**, 313-321.

Baldwin KM, Reitman JS, Terjung RL, Winder WW & Holloszy JO (1973) Substrate depletion in different types of muscle and in liver during prolonged running. American Journal of Physiology, **225**, 1034-1050.

Barcroft H & Millen JLE. (1939) The blood flow through muscle during sustained contraction. Journal of Physiology, **97**, 17-31.

Barnes RH, Labadan B, Siyamoglu B & Bradfield RB (1964) Effects of exercise and administration of aspartic acid on blood ammonia in the rat. American Journal of Physiology, **207**, 1242-1246.

Barnes WS (1980) The relationship between maximum isometric strength and intramuscular circulatory occlusion. Ergonomics, **23**, 351-357.

Bass L & Moore MJ (1973) The role of protons in nerve conduction. Progress in Biophysics and Molecular Biology, **27**, 143-151.

Belanger AY & Quinlan J (1982) Muscle function studies in human planter-flexor and dorsi-flexor muscles. Canadian Journal of Neurological Science, **9**, 358-359.

Belanger AY & McComas AJ (1981) Extent of motor unit activation during effort. Journal of Applied Physiology, **51**, 1131-1135.

Bellemare F & Bigland-Ritchie B (1987) Central components of diaphragmatic fatigue assessed by phrenic nerve stimulation. Journal of Applied Physiology, **62**, 1307-1316.

Bergstrom J, Harris RC & Hultman E & Nordesjo LO (1971) Energy rich phosphagens. In Dynamic and static work: Muscle metabolism during exercise, ed. Pernow B & Saltin B, London: Plenum Press.

Bergstrom J, Berniade V, Hultman E & Roch-Norland AE (1967) Relation between glycogen and electrolyte metabolism in human muscle. In Symposium uber Transport und Function intracellularer Elektrolyte, ed. Kruck F, Munchen-Berlin-Wien, Schuren und Schwarzenberg.

Bergstrom, J Hermansen L, Hultman E & Saltin B (1967) Diet, muscle glycogen and physical performance. Acta Physiological Scandinavica, **71**, 140-150.

Bezanilla F, Caputo C, Gonzalez-Serratos H & Venosa RA (1972) Sodium dependance of the inward spread of activation in isolated twitch muscle fibres of the frog. Journal of Physiology, **223**, 507-523.

Bianchi CP & Narayan S (1982) Muscle fatigue and the role of the Transverse Tubules. Science, **215**, 295-296.

Bigland-Ritchie B, Johansson R, Lippold OCJ, Smith S & Woods JJ (1983b) Changes in motoneurone firing rates during sustained maximal voluntary contractions. Journal of Physiology, **340**, 335-346.

Bigland-Ritchie B, Johansson R, Lippold OCJ & Woods (1983a) Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. Journal of Neurophysiology, **50**, 313-324.

Bigland-Ritchie B (1981) EMG and fatigue of human voluntary and stimulated contractions. In Human muscle fatigue: physiological mechanisms, Ciba Foundation Symposium No. 82, ed. Porter R & Whelan J, London: Pitman Medical.

Bigland-Ritchie B, Jones DA & Woods JJ (1979) Excitation frequency and muscle fatigue. Electrical responses during human sustained and stimulated contractions. Experimental Physiology, **64**, 414-427.

Bigland-Ritchie B & Woods JJ (1984) Changes in muscle contractile properties and neural control during human muscular fatigue. Muscle & Nerve, **7**, 691-699.

Bigland-Ritchie B, Jones DA, Hosking GP & Edwards RHT (1978) Central and Peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. Clinical Science and Molecular Medecine, **54**, 609-614.

Bigland-Ritchie B, Kakula CB, Lippold OLJ & Woods JJ (1982) The absence of neuromuscular transmission failure in sustained maximal volantary contractions. Journal of Physiology, **330**, 265-278.

Bigland B & Lippold OCJ (1954) Motor unit activity in the voluntary contraction of human muscle. Journal of Physiology, **125**, 322-335.

Bigland-Ritchie B, Dawson NJ, Johansson R & Lippold OCJ (1985) Reflex control of motor neurone firing rates during fatigue in man. Journal of Physiology, **365**, 23P.

Bigland-Ritchie B & Woods JJ (1976) Integrated electromyogram and oxygen uptake during positive and negative work. Journal of Physiology, **260**, 267-277.

Bigland-Ritchie B, Cafarelli E & Vollestad NK (1986a) Fatigue of submaximal static contractions. Acta Physiological Scandinavica, Suppl. 556, 137-148.

Bigland-Ritchie B, Donovan EF & Roussos CS (1981) Conduction velocity and EMG power spectrum changes in fatigue of sustained maximal efforts. Journal of Applied Physiology, **51**, 1300-1305.

Bigland-Ritchie B (1984) Changes in muscle contractile properties and neural control during human muscular fatigue. Muscle & Nerve, **7**, 691-699.

Bigland-Ritchie BR, Dawson NJ, Johansson RS & Lippold OCJ (1986b) Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. Journal of Physiology, **379**, 451-459.

Blanchard EM, Pan B & Solaro RJ (1984) The effect of acidic pH on the ATPase activity and troponin Ca^{2+} binding of rabbit skeletal myofilaments. Journal of Biological Chemistry, **259**, 3181-3186.

Blatz AL (1984) Asymmetric proton block of inward rectifier K channels in skeletal muscle. Pflugers Arch, **401**, 402-407.

Blinks JR, Rudel R & Taylor SR (1978) Calcium transients in isolated amphibian skeletal muscle fibres; detection with aequorin. Journal of Physiology, **277**, 291-323.

Boobis LH, Williams C & Wootton SA (1982) Human muscle metabolism during brief maximal exercise. Journal of Physiology, **338**, 21-22P.

Botelho SY & Cander L (1953) Post-tetanic Potentiation before and during ischaemia in intact human skeletal muscle. Journal of Applied Physiology, **6**, 221-228.

Brandt PW, Cox RN, Kawai M & Robinson T (1982) Regulation of tension in skinned muscle fibres. Journal of General Physiology, **79**, 997-1016.

Briscoe G (1931) Adequate electrical stimuli for posture and movement. Journal of Physiology, **71**, 292-308.

Brown, GL & von Euler, US (1938) The after-effects of a tetanus on mammalian muscle. Journal of Physiology, **93**, 39-60.

Brown GL & Burns BD (1949) Fatigue and neuromuscular block in mammalian skeletal muscle. Proceedings of the Royal Society of London (Series B), **136**, 182-195.

Bruce SA, Phillips SK & Woledge RC (1986) Isometric force in maximum voluntary contractions related to an estimate of the cross-sectional area of the adductor pollicis in the human subject. Journal of Physiology, **372**, 31P.

Buller AJ, Kean CJC, Ranatunga KW & Smith JM (1981) Post-tetanic depression of twitch tension in the cat soleus muscle. Experimental Neurology, **73**, 78-89.

Cambell DT & Hille B (1976) Kinetic and pharmacological properties of the sodium channel of frog skeletal muscle. Journal of General Physiology, **67**, 309-323.

Cannell, MB (1986) Effect of tetanus duration on the free calcium during the relaxation of frog skeletal muscle fibres. Journal of Physiology, **376**, 203-218.

Clausen T (1986) Regulation of active Na^+-K^+ transport in skeletal muscle. Physiological Reviews, **66**, 542-580.

Close, R & Hoh, JFY (1968) The after-effects of repetitive stimulation on the isometric twitch contraction of rat fast skeletal muscle. Journal of Physiology, **197**, 461-477.

Connolly, R Gough, W & Winegrad, S (1971) Characteristics of isometric twitch of skeletal muscle immediately after a tetanus. A study of the influence of the distribution of calcium within the sarcoplasmic reticulum on the twitch. Journal of Physiology, **57**, 697-709.

Cooke R & Pate E (1985) Inhibition of muscle contraction by the products of ATP hydrolysis: ADP and Phosphate. Biophysical Journal, **47**, 25a.

Cooper RG, Edwards RHT, Gibson H & Stokes MJ. (1988) Human muscle fatigue: frequency dependence of excitation and force generation. Journal of Physiology, **397**, 585-599.

Cooper RG, Edwards RHT & Stokes M (1987) Human muscle fatigue: excitation-contraction coupling failure in McArdle's disease. Journal of Physiology, **394**, 142P.

Davies CTM & White J (1982) Muscle weakness following dynamic excercise in humans. Journal of Applied Physiology, **53**, 236-241.

Davies CTM & White MJ (1981) Muscle weakness following eccentric work in man. Pflugers Archiv, **392**, 168-171.

Davies CTM & Thompson MW (1979) Aerobic performance of female marathon & male ultramarathon athletes. European Journal of Applied Physiology, **41**, 233-245.

Davies H & Davies PA (1932) Fatigue in skeletal muscle in relation to the frequency of stimulation. American Journal of Physiology, **101**, 339-356.

Dawson MJ, Gadian DG & Wilkie DR (1980) Mechanical relaxation rate and metabolism studied in fatiguing muscle by phosphorous nuclear magnetic resonance. Journal of Physiology, **299**, 465-484.

Dawson MJ, Gadian DG & Wilkie DR (1978) Muscular fatigue investigated by phosphorus nuclear magnetic resonance. Nature, **274**, 861-866.

Dawson MJ (1983) Phosphorus metabolism and the control of glycolysis studies by Nuclear Magnetic Resonance. In Biochemistry of exercise, ed. Knuttgen HG, Champaign: Human Kinetics.

De Luca CJ, LeFever RS, McCue MP & Xenakis AP (1982a) Control scheme governing concurrently active human motor units during voluntary contractions. Journal of Physiology, **329**, 129-142.

De Luca CJ (1984) Myoelectrical manifestations of localized muscular fatigue in humans. CRC Critical Reviews of Biomed Engineer, **11**, 251-279.

De Luca CJ, LeFever RS, McCue MP & Xenakis AP (1982b) Behaviour of human motor units in different muscles during linearly varying contractions. Journal of Physiology, **329**, 113-128.

Deleze J, Lazrak A & Rakotonirina VS (1986) Isolation of the tubular action potential in the frog skeletal muscle fibre. Journal of Physiology, **371**, 149P.

Desmedt, JE & Hainuat, K (1968) Kinetics of myofilament activation in potentiated contraction: Staircase phenomenon in Human skeletal muscle. Nature, **217**, 529-532.
Desmedt JE & Godaux E (1977) Ballistic contractions in man: characteristic recruitment pattern of single motor units of the tibialis anterior muscle. Journal of Physiology, **264**, 673-693.

Donaldson SKB & Hermansen L (1978) Differential direct effects of H^+ on Ca^{2+} - activated force of skinned fibers from the soleus, cardiac, adductor magnus muscles of rabbits. Pflugers Archiv, **376**, 55-65.

Duchateau J & Hainuat K (1985) Electrical and mechanical failures during sustained and intermittent contractions in humans. Journal of Applied Physiology, **58**, 942-947.

Duchateau J, Montigny L & Hainaut K (1987) Electro-mechanical failures and lactate production during fatigue. European Journal of Applied Physiology, **56**, 287-291.

Dyken ML, Smith DM & Peake RL (1967) An electromyographic diagnostic screening test in McArdle's disease and a case report. Neurology, **17**, 45-50.

Edwards RHT (1981) Human muscle function and fatigue. In Human Muscle Fatigue: Physiological Mechanisms, Ciba Foundation Symposium 82, ed. Porter R & Whelan J, London: Pitman Medical.

Edwards RHT, Griffiths RD & Cady EB (1985) Topical magnetic resonance for the study of muscle metabolism in human myopathy. Clinical Physiology, **5**, 93-109.

Edwards RHT (1983) Biochemical basis of fatigue in exercise: Catastrophy theory of muscular fatigue. In Biochemistry of Exercise, ed. Knuttgen HG, Vogel JA & Poortmans J, Champaign: Human Kinetics.

Edwards RHT, Hill DK, Jones DA & Merton PA (1977b) Fatigue of long duration in human skeletal muscle after exercise. Journal of Physiology, **272**, 769-778.

Edwards RHT, Young A, Hosking GP & Jones DA (1977a) Human skeletal muscle function: description of tests and normal values. Clinical Science and Molecular Medicine, **52**, 283-290.

Edwards, RHT, Hill, DK & Jones DA (1972b) Effect of fatigue on the time course of relaxation of skeletal muscle in man. Journal of Physiology, **227**, 26-27P.

Edwards RHT & Newham DJ (1984) Force frequency relationship determined by percutaneous stimulation of the quadriceps muscle. Journal of Physiology, **353**, 129P.

Edwards RHT & Wiles CM (1981) Energy exchange in human skeletal muscle. Circulation Research, **48**, 111-117.

Edwards RHT, Mills KR & Newham DJ (1981) Greater low frequency fatigue produced by eccentric than concentric muscle contractions. Journal of Physiology, **317**, 17P.

Edwards RHT, Harris RC, Hultman E, Kaiser L, Koh & Nordesjo LO (1972a) Effect of temperature on muscle energy metabolism and endurance during successive isometric contractions, sustained to fatigue, of the quadriceps in man. Journal of Physiology, **220**, 335-352.

Edwards RHT (1980) Studies of muscular performance in normal and dystrophic subjects. British Medical Bulletin, **36**, 159-164.

Edwards RHT, Hill DK & Jones DA (1975) Heat production and chemical changes during isometric contractions of the human quadriceps muscle. Journal of Physiology, **251**, 303-315.

Edwards RHT, Hill DK & McDonnell M (1972c) Myothermal and intramuscular pressure measurements during isometric contractions of the human quadriceps muscle. Journal of Physiology, **224**, 58P-59P.

Fabiato A & Fabiato F (1978) Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. Journal of Physiology, **276**, 233-255.

Farragher D, Kidd GL & Tallis R (1987) Eutrophic electrical stimulation for Bell's Palsy. Clinical Rehabilitation, **1**, 265-271.

Feng TP (1931) The heat-tension ratio in prolonged tetanic contractions. Proceedings of the Royal Society of London (series b), **108**, 522-537.

Fink R & Luttgau HC (1976) An evaluation of the membrane constants and the potassium conductance in metabolically exhausted muscle fibres. Journal of Physiology, **263**, 215-238.

Fitch S & McComas AJ (1985) Influence of human muscle length on fatigue. Journal of Physiology, **362**, 205-213.

Fuchs F (1979) The relationship between pH and the amount of calcium bound to glycerinated muscle fibres. Biochimica et biophysica acta, **585**, 477-479.

Fulton JF (1925) The inseparability of mechanical and electrical responses in skeletal muscle. American Journal of Physiology, **75**, 261-266.

Gadian DG (1982) Nuclear magnetic resonance and its applications to living systems. In Nuclear magnetic resonance and its application to living systems, ed. Gadian DG, New York: Oxford University Press.

Garay RP & Garrahan PJ (1975) The interaction of adenosinetriphosphate and inorganic phosphate with the sodium pump in red cells. Journal of Physiology, **249**, 51-67.

Garland J, Garner SH & McComas AJ (1986) Relationship between numbers and frequencies of stimuli in human muscle fatigue. Journal of Physiology, **384**, 33P.

Glen WWL, Holcomb WG, Hogan JF, Kaneyuki T & Kim J (1977) Long term stimulation of the phrenic nerve for diaphragm pacing. In Functional Electrical Stimulation. Applications in Neural Prostheses, ed. Hambrecht FT & Reswick JB, New York, Basel: Dekker, Inc.

Gollnick PD, Armstrong RD, Sembrowich WL, Shepherd RE & Saltin B (1973) Glycogen depletion pattern in human skeletal muscle fibres after heavy exercise. Journal of Applied Physiology, **34**, 615-618.

Gollnick PD, Karlsson J, Piehl K & Saltin B (1974) Selective glycogen depletion in skeletal muscle fibres in man following sustained contractions. Journal of Physiology, **241**, 59-67.

Gollnick PD, Armstrong RB, Saubertiv CW, Piehl K & Saltin B (1972) Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. Journal of Applied Physiology, **33**, 312-319.

Grabowski W, Lobsiger EA & Luttgau H (1972) The effect of repetitive stimulation at low frequencies upon the electrical and mechanical activity of single muscle fibres. Pflugers Archiv, **334**, 222-239.

Green HJ (1986) Muscle Power: Fibre type recruitment, metabolism & fatigue. In Human muscle power, ed. Jones ML, McCartney N & McComas AJ (eds), chapter 7. Champaign: Human Kinetics.

Grimby L, Hannerz J & Hedman B (1981) The fatigue and voluntary discharge properties of single motor units in man. Journal of Physiology, **316**, 545-554.

Haiech, J, Derancourt, J, Pechere, JF & Demaille, JG (1979) Magnesium and calcium binding to parvalbumins: Evidence for differences between parvalbumins and an explanation of their relaxing function. Biochemistry, **18**, 2752-2758.

Hanson J & Persson A (1971) Changes in the action potential and contraction of isolated frog muscle after repetitive stimulation. Acta Physiologica Scandinavica, **81**, 340-348.

Harris, RC, Hultman, E, Kaijser, L & Nordesjo, LO (1975) The effect of circulatory occlusion on isometric exercise capacity and energy metabolism of the quadriceps muscle in man. Scandinavian Journal of Clinical and Laboratory Investigation, **35**, 87-95.

Harris RC, Edwards RHT, Hultman E & Nordesjo L-O (1976) The time course of phosphorylcreatine resynthesis during recovery of the quadriceps in man. European Journal of Physiology, **367**, 137-142.

Heald DE (1975) Influence of ammonium ions on mechanical and electrophysiological reponses of skeletal muscle. American Journal of Physiology, **229**, 1174-1179.

Heizmann CW, Berchtold MW & Rowlerson AM (1982) Correlation of parvalbumin concentration with relaxation speed in mammalian muscles. Proceedings of the National Acadamy of Sciences, **79**, 7243-7247.

Hermansen L, Hultman E & Saltin B (1976) Muscle glycogen during prolonged severe exercise. Acta Physiological Scandinavica, **71**, 129-139.

Hibberd MG, Dantzig JA, Trentham DR & Goldman YE (1985) Phosphate release and force generation in skeletal muscle fibres. Science, **228**, 1317-1319.

Hickson RC, Rennie MJ, Conlee RK, Winder WW & Holloszy JO (1977) Effects of increased plasma free fatty acids on glycogen utilization and endurance. Journal of Applied Physiology, **43**, 829-833.

Hill DK, McDonnell MJ & Merton PA (1979) Direct stimulation of the adductor pollicis in man. Journal of Physiology, **300**, 2P-3P.

Hill AV, Long CNH & Lupton H (1924) Muscular exercise lactic acid, and the supply and utilization of oxygen. Parts I-III. Proceedings of the Royal Society of London (Series B), **96**, 438-478.

Hultman E & Sjoholm H (1983) Electromyogram, force and relaxation time during and after continuous stimulation of human skeletal muscle *in situ*. Journal of Physiology, **339**, 33-40.

Hultman H & Spriet LL (1986) Skeletal muscle metabolism, contraction force and glycogen utilization during prolonged electrical stimulation in humans. Journal of Physiology, **374**, 493-501.

Hultman E, Bergstrom J & McLennan A N (1967) Breakdown and resynthesis of adenosine triphophate in connection with muscular work in Man. Scandinavian Journal of Clinical and Laboratory Investigation, **19**, 56-66.

Hultman E & Bergstrom J (1971) Local energy-supplying substrates as limiting factors in different types of leg muscle work in normal Man. In Limiting factors of physical performance, ed. Keul J, Stuttgart: G Theime.

Ikai M, Yabe K, Ishu K (1967) Muskelkraft und muskulare ermudung bein willkurlicher anspannung und elektrischer reizung des muskels. Sportartz und sportmedizin, 197-211.

Jansson E, Dudley GA, Norman B & Tesch PA (1987) ATP and IMP in single human muscle fibres after high intensity exercise. Clinical Physiology, **7**, 337-345.

Jennische E (1982) Relation between membrane potential and lactate in gastrocnemius and soleus muscle of the cat during tourniquet and post ischemic reflow. Pflugers Archiv, **394**, 329-332.

Jeul C (1988) Muscle action potential propagation velocity changes during activity. Muscle & Nerve, **11**, 714-719.

Johnson MA, Polgar J, Weightman D & Appleton D (1973) Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. Journal of the Neurological sciences, **18**, 111-129.

Jones DA, Bigland-Ritchie B & Edwards RHT (1979) Excitation frequency and muscle fatigue: mechanical responses during voluntary and stimulated contractions. Experimental Neurology, **64**, 401-413.

Jones DA (1979) Changes in excitation threshold as a cause of muscular fatigue. Journal of Physiology, **295**, 90P-91P.

Jones DA & Edwards RHT (1986) Muscle strength and metabolism. In Recent achievements in restorative neurology 2: progressive neuromuscular diseases, ed. Dimitrijevic, Kakulas & Vrbova, Ch.11. Basel: Karger.

Jones DA & Bigland-Ritchie B (1986) Electrical and contractile changes in muscle fatigue. In Biochemistry of exercise. International series on sport sciences. Vol 16, ed. Baltin B, Illinois: Human Kinetics.

Katanuma N, Okada M & Nishii Y (1966) Regulation of the urea cycle and TCA cycle by ammonia.. In Advances in enzyme regulation Vol 4, ed. Weaver G, New York: Pergamon Press.

Kawai M, Wolen C & Comaccia T (1985) The effect of phosphate (Pi) on crossbridge kinetics in psoas fibres indicates that the forward cycling rate increases with Pi. Biophysical Journal, **47**, 24a.

Khan RA & Bengtsson B (1985) Fatigue in frog skeletal muscle fibres and effects of methylxanthine derivatives. Acta Physiologica Scandinavica, **124**, 35-41.

Komi PV & Rusko H (1974) Quantitative evaluation of mechanical and electrical changes during fatigue loading of eccentric and concentric work. Scandinavian Journal of Rehabilitation Medicine. Suppl., 3, 121-126.

Krarup, C (1981) Enhaancement and diminution of mechanical tension evoked by staircase and by tetanus in rat muscle. Journal of Physiology, **311**, 355-372.

Krnjevic K & Miledi R (1958) Failure of neuromuscular propagation in rats. Journal of Physiology, **140**, 440-461.

Kugelberg E & Lindegren B (1979) Transmission and contraction fatique of rat motor units in relation to succinate dehydrogenase activity of motor unit fibres. Journal of Physiology, **288**, 285-300.

Kushmerick MJ & Crow MT (1983) Regulation of energetics and mechanics by myosin light chain phosphorylation in fast twitch skeletal muscle. Foundation Proceedings, **42**, 14-20.

Lannergren J & Westerblad H (1987) Action potential fatigue in single skeletal muscle fibres of Xenopus. Acta Physiological Scandinavica, **129**, 311-318.

Leavis PC, Rosenfeld SS, Gergely J, Grabarek Z & Drabikowski W (1978) Proteolytic fragments of Troponin C. The Journal of Biological Chemistry, **253**, 5452-5459.

Lennmarken C, Bergman T, Larsson J & Larsson LE (1985) Skeletal muscle function in man : force, relaxation rate endurance and contaction time - dependence on sex and age. Clinical Physiology, **5**, 243-255.

Lewis SF, Haller RG, Cook JD & Nunnally RL (1985) Muscle fatigue in McArdle's disease studied by 31P-NMR: effect of glucose infusion. Journal of Applied Physiology, **59**, 1991-1994.

Lindstrom L, Magnusseon R & Petersen I (1970) Muscular fatigue and action potential conduction velocity changes studied with frequency analysis of EMG signals. Electromyography and Clinical Neurophysiology, **10**, 341-356.

Lippold OCJ, Redfearn JWT & Vuco J (1960) The electromyography of fatigue. Ergonomics, **3**, 121-131.

Lowenstein JM (1972) Ammonia production in muscle and other tissues: the purine nucleotide cycle. Physiological Reviews, **52**, 382-414.

Luttgau, HC (1965) The effect of metabolic inhibitors on the fatigue of the action potential in single muscle fibres. Journal of Physiology, **178**, 45-67.

MacIntosh, BR & Gardiner, PF (1987) Post tetanic potentiation and skeletal muscle function: Interactions with caffiene. Canadian Journal of Physiological Pharmacology, **65**, 260-268.

MacIntosh BR, Stainsby WN & Gladden LB (1983) Fatigue from incompletely fused tetanic contractions in skeletal muscle *in situ*. Journal of Applied Physiology, **55**, 976-982.

Marsden CD, Meadows JC & Merton PA. (1983) "Muscular wisdom" that minimizes fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. In Motor control mechanisms in health and disease., ed. Desmedt JE, New York: Raven Press.

Marsden CD, Meadows JC & Merton PA (1971) Isolated single motor units in human muscle and their rate of discharge during maximal voluntary effort. Journal of Physiology, **217**, 12P-13P.

McArdle B (1951) Myopathy due to a defect in muscle glycogen breakdown. Clinical Science, **10**, 13-33.

McNeal DR (1977) 2000 years of electrical stimulation. In Functional electrical stimulation. Applications in neural prostheses, ed. Hambrecht FT & Reswick KB, New York, Basel: Dekker, Inc.

Merton PA (1954) Voluntary strength and fatigue. Journal of Physiology, **123**, 553-564.

Merton PA (1981) In Human muscle fatigue: physiological mechanisms, Ciba Foundation Symposium No. 82. eds Porter R & Whelan J. London : Pitman Medical p152.

Metzger JM & Fitts RH (1986) Fatigue from high and low frequency muscle stimulation role of sarcolemma action potentials. Experimental Neurology, **93**, 320-333.

Metzger JM & Moss RL (1987) Greater hydrogen ion-induced depression of tension and velocity in skinned single fibres of rat fast than slow muscles. Journal of Physiology, **393**, 727-742.

Miller RG, Boska MD, Moussavi RS, Carson PJ & Weiner MW (1988) 31P Nuclear Magnetic Resonance studies of high energy phosphates and pH in human muscle fatigue. The Journal of Clinical Investigation, **81**, 1190-1196.

Miller RG, Giannini D, Milner-Brown HS, Layzer RB, Koretsky AP, Hooper D & Weiner MW (1987) Effects of fatiguing exercise on high-energy phosphates, force, and emg: evidence for three phases of recovery. Muscle & Nerve, **10**, 810-821.

Mills KR (1982) Power spectral analysis of electromyogram and compound muscle action potential during muscle fatigue and recovery. Journal of Physiology, **326**, 401-409.

Milner-Brown HS, Stein RB & Yemm R (1983) The orderly recruitment of human motor units during voluntary isometric contractions. Journal of Physiology, **230**, 359-370.

Milner-Brown HS & Miller RG (1986) Muscle membrane excitation and impulse propagation velocity are reduced during muscle fatigue. Muscle & Nerve, **9**, 367-374.

Moore, RL & Stull, JT (1984) Myosin light chain phosphorylation in fast and slow skeletal muscles *in situ*. American Journal of Physiology, **247**, C462-C471.

Mori S & Ishida A (1976) Synchronization of motor units and its simulation in parallel feedback system. Biol. Cybernetics, **21**, 107-111.

Mortimer J, Magusson R & Petersen I (1970) Conduction velocity in ischaemic muscle: effect on EMG frequency spectrum. American Journal of Physiology, **219**, 1324-1329.

Mosso A (1915) Fatigue. In Fatigue. 3rd edition, ed. Mosso A, London: Allen & Unwin.

Moxham J, Morris AJR, Spiro S, Edwards RHT & Green M (1980a) The contractile properties and fatigue of the diaphragm in man. Clinical Science, **58**, 6P.

Moxham J, Wiles CM, Newham D & Edwards RHT (1980b) Sternomastoid muscle function and fatigue in man. Clinical Science, **59**, 463-468.

Muller EA (1935) Die Erholung nach statischer Haltearbeit. Arbeitsphysiologie, **8**, 72.

Naess K & Storm-Mathisen A (1955) Fatigue and sustained tetanic contractions. Acta physiologica Scandinavia, **34**, 351-366.

Newham DJ, McPhail G, Mills KR & Edwards RHT (1983b) Ultrastructural changes after concentric and eccentric contractions of human muscle. Journal of Neurological Science, **61**, 102-122.

Newham DJ, Mills KR, Quigley BM & Edwards (1983a) Pain and fatigue after concentric and eccentric muscle contractions. Clinical Science, **64**, 55-62.

Newsholme EA (1981) The glucose/fatty acid cycle and physical exhaustion. In Human muscle fatigue: physiological mechanisms. Ciba Foundation Symposium No 82, ed. Porter R & Whelan J, London: Pitman medical.

O'Reilly KJ, Warhol MJ, Fielding RA, Frontera WR, Meredith CN & Evans WJ (1987) Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. Journal of Applied Physiology, **63**, 252-256.

Oldham JA (1987) Rehabilitation of skeletal muscle in the arthritic hand. Thesis. University of Liverpool.

Orchardson R (1978) The generation of nerve impulses in mammalian axons by changing the concentrations of the normal constituants of extracellular fluid. Journal of Physiology, **275**, 177-189.

Pallotta BS (1985) Calcium activated potassium channels in rat muscle inactivated from a short duration open state. Journal of Physiology, **363**, 501-516.

Persechini, A & Stull, JT (1984) Phosphorylation kinetics of skeletal muscle myosin and the effect of phosphorylation on actomyosin adenosinetriphosphate activity. Biochemistry, **23**, 4144-4150.

Piehl k (1974) Time course for refilling of glycogen stores in human muscle fibres following excercise induced glycogen depletion. Acta Physiologica Scandinavia, **90**, 297-302.

Piper J, di Prampero PE & Certelli P (1968) Oxygen debt and high-energy phosphate in gastrocnemius muscle of the dog. American Journal of Physiology, **215**, 523-531.

Ponce-Hornos JE & Langer GA (1982) Effects of inorganic phophate on ion exchange, energy state and contraction in mammalian heart. American Journal of Physiology, **242**, H79-H88.

Potter JD & Gergely J (1974) Troponin, tropomyosin and actin interactions in the Ca^{2+}-regulation of muscle contraction. Biochemistry, **13**, 2697-2705.

Rack PMH & Westbury R (1969) The effects of length and stimulus rate on tension in the isometric cat soleus muscle. Journal of Physiology, **204**, 443-460.

Ranatunga KW, Sharpe B & Turnbull B (1987) Contractions of a human skeletal muscle at different temperatures. Journal of Physiology, **390**, 383-395.

Ridgway, EB, Gordon, AM & Martyn, DA (1983) Hysteresis in the force-calcium relation in muscle. Science, **219**, 1075-1077.

Round JM, Jones DA, Chapman SJ, Edwards RHT, Ward PS & Fodden DL (1984) The anatomy and fibre type composition of the Human adductor pollicis in relation to its contractile properties. Journal of Neurological Sciences, **66**, 263-292.

Rowland LP, Araki S & Carmal P (1965) Contracture in McArdle's disease. Archives of Neurology, **13**, 541-544.

Sacks J & Cleland MG (1960) Absence of phosphate interchanges in repetitive muscular contraction. American Journal of Physiology, **198**, 300-302.

Sahlin K (1983) Effects of acidosis on energy metabolism and force generation in skeletal muscle. In Biochemistry of exercise, ed. Knuttgen HG & Vogel H, Champaign: Human Kinetics.

Sahlin K, Harris RC & Hultman E (1975) Creatine kinase equilibrium and lactate content compared with muscle pH in tissue samples obtained after isometric contraction. Biochemical Journal, **152**, 173-180.

Sandow A (1952) Excitation-contraction coupling in muscular response. Yale Journal of Biology and Medicine, **25**, 176-201.

Schwab RS (1953) Motivation and Measurement of fatigue. In Fatigue, ed. Floyd & Wilford, London: Lewis & Co..

Siesjo BK (1982) Lactic acidosis in the brain occurance triggering mechanisms and pathophysiological importance. Ciba foundation symposium 87, 77-88.

Simonson E (1971) Physiology of work capacity and fatigue. Springfield: Charles Treatment.

Sjodin B (1976) Lactate dehydrogenase in human skeletal muscle. Physiologica Scandinavica (Supplement), **436**, 5-32.

Sjogaard G, Adams RP & Saltin B (1985) Water and ion shifts in skeletal muscle of humans with intense dynamic knee extension. American Journal of Physiology, **248**, R190-R196.

Sjoholm H, Sahlin K, Edstrom L & Hultman E (1983) Quantitative estimation of anaerobic and oxidative energy metabolism and contraction characteristics in intact human skeletal muscle in response to electrical stimulation. Clinical Physiology, **3**, 227-239.

Slomic A, Rosenfalk A & Buchthal F (1968) Electrical and mechanical responses of normal and myasthenic muscle with particular reference to the staircase phenomenon. Brain Research, **10**, 1-78.

Spruce AE, Standen NB & Stanfield PR (1985) Voltage-dependant ATP sensitive potassium channels of skeletal muscle membrane. Nature, **316**, 736-738.

Stalberg E (1966) Propagation velocity in human muscle fibres *in situ*. Acta Physiologica Scandinavica Suppl.287.

Stephens JA & Taylor A. (1972) Fatigue of maintained voluntary muscle contraction in man. Journal of Physiology, **220**, 1-18.

Susak Z, Levy M, Mizrahi J & Isakov E (1986) The duration of isometric contraction while using FES with stimuli of different parameters. *Proceedings of 2nd Vienna* International Workshop on Functional Electrostimulation, Vienna (Austria), September 21-24.

Takamori, M, Gutmann, L & Shane, SR (1971) Contractile properties of human skeletal muscle. Archives of Neurology, **25**, 535-546.

Tashiro S (1922) Ammonia production in the nerve fibre during excitation. American Journal of physiology, **60**, 519-543.

Taylor DJ, Styles P, Matthews PM, Arnold DA, Gadian DG, Bore P & adda GK (1986) Energetics of human muscle: exercise - induced ATP depletion. Magnetic Resonance in Medicine, **3**, 44-54.

Thesleff S (1959) Motor end-plate 'desensitization' by repetitive nerve stimuli. Journal of Physiology, **148**, 659-664.

Ui M (1966) A role of phosphofructokinase in pH dependent regulation of glycolysis. Biochimica et Biophysica Acta, **124**, 310-322.

Vandervoort, AA, Quinlan, J & McComas, AJ (1983) Twitch potentiation after voluntary contraction. Experimental Neurology, **81**, 141-152.

Vollestad NK & Blom PCS (1985) Effect of varying exercise intensity on glycogen depletion in human muscle fibres. Acta Physiologica Scandinavica, **125**, 395-405.

Vollestad NK, Vaage O & Hermansen L (1984) Muscle glycogen depletion patterns in type I and subgroups of type II fibres during prolonged severe exercise in man. Acta Physiologica Scandinavica, **122**, 433-441.

Vollestad NK & Sejersted OM (1988) Biochemical correlates of fatigue. European Journal of Applied Physiology, **57**, 336-347.

Vyskocil F, Hnik P, Rehfelat H, Vejsada R & Ujec E (1983) The measurement of K^+_e concentration changes in human muscles during volitional contractions. Pflugers Archiv, **399**, 235-237.

Wagenmakers AJM, Coakley JH & Edwards RHT (1987) Branched-chain amino acid metabolism during exercise in McArdle's disease (myophosphorylase deficiency). In Advances in myochemistry, ed. Benzi G, : John Libby.

Waller AD (1891) The sense of effort: an objective study. Brain, **14**, 179-249.

Waters R (1977) Electrical stimulation of the peroneal and femoral nerves in man. In Functional Electrical Stimulation. Applications in Neural Prostheses, ed. Hambrecht FT & Reswick JB, New York, Basel: Dekker, Inc.

Weber A & Murray JM (1973) Molecular control mechanisms in muscle contraction. Physiological Reviews, **53**, 612-670.

Weichardt W (1904) Uber das Ermudungtoxin und - Autitoxin. Muenchener Medizinische Wochenschrift, **51**, 2121.

Wiles CM & Edwards RHT (1982a) The effect of temperature, ischaemia and contractile activity on the relaxation rate of human muscle. Clinical Physiology, **2**, 485-497.

Wiles CM, Young A, Jones DA & Edwards RHT (1979) Relaxation rate of constituant muscle-fibre types in human quadriceps. Clinical Science, **56**, 47-52.

Wiles CM (1980) The determinants of relaxation rate of human muscle in vivo. PhD Thesis, University of London.

Wiles CM & Edwards RHT (1982b) Metabolic heat production in isometric ischaemic contractions of human adductor pollicis. Clinical Physiology, **2**, 499-512.

Wiles CM, Jones DA & Edwards RHT (1981) Fatigue in human metabolic myopathy. In Human muscle fatigue: physiological mechanisms. Ciba Foundation Symposium No 82, ed. Porter R & Whelan J, London : Pitman Medical.

Wilkie DR, Dawson MJ, Edwards RHT, Gordon RE & Shaw D (1983) 31P NMR studies of resting muscle in normal human subjects. In Cross-bridge mechanisms in muscle contraction. Proceedings of the 2nd international symposium, ed. Pollack G & Sugi H, Seattle.

Wilkie DR (1986) Muscular fatigue: effects of hydrogen ions and inorganic phosphate. Federation Procedings, **45**, 2921-2923.

Woods JJ, Furbish F & Bigland-Ritchie BR (1987) Evidence for a fatigue-induced reflex inhibition of motoneuron firing rates. Journal of Physiology, **58**, 125-137.

Worcel A & Erecinska M (1964) Mechanism of inhibitory action of ammonia on the respiration of rat-liver mitochondria. Biochimica Biophysica Acta, **67**, 27-33.

Zeeman EC (1977) Catastrophe theory. Selected Papers 1972-1977. Addison-Wesley.

Zeiderman MR, Wilson IJ & Price R (1984) An objective test of muscle function using the BBC microcomputer. Journal of Microcomputer Applications, **7**, 309-317.

Sports Medicine 2: 120-132 (1985)
0112-1642/85/0300-0120/$6.50/0

Muscular Exercise and Fatigue

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Summary

The development of muscular fatigue during exercise is a common phenomenon, and several forms depend on the precise type of exercise performed. The causes are still not clearly established, although the involvement of electrical and metabolic factors have been demonstrated. Several techniques which allow for the analysis of muscle function in terms of electrical activation and energy metabolism are (a) a needle biopsy of muscle for histochemical and metabolic studies, (b) magnetic resonance spectroscopy for the non-invasive study of muscle energy metabolism and pH, (c) electromyographic analysis of the electrical characteristics of muscle, and (d) percutaneous electrical stimulation of muscle for the force-frequency and relaxation characteristics of muscle. Endurance training increases the capacity to sustain exercise possibly by altering muscle energy metabolism and contractile properties.

Fatigue is a self-protective mechanism against the damage of contractile machinery of muscle as, for example, with the development of rigor, which occurs if the energy stores are depleted. To illustrate the roles of energy supply and electrical properties in muscle in fatigue, the 'catastrophe theory' used in engineering has been applied. This may explain abrupt changes of function of individual muscle cells, while for the muscle as a whole, fatigue may be manifested as a more gradual loss of force.

Muscle generates force, and failure to maintain force (or power output) during sustained or repeated contractions is termed 'fatigue'. The underlying mechanisms of fatigue in human muscle have evoked much interest for more than a century, and its significance lies not only in the function of 'normal' skeletal muscle, but also in diseased muscle.

The mechanisms underlying fatigue of human skeletal muscle have been discussed under the auspices of the Ciba Foundation. This article reviews some of the causes of fatigue occurring during and after muscular exercise.

1. Voluntary Contraction of Skeletal Muscle

The events leading to a voluntary muscular contraction involve a controlling 'chain of command' (fig. 1) from brain to actomyosin cross-bridge (Edwards, 1981) comprising several links, each of which have been separately analysed in different physiological systems, ranging from whole animal preparations to isolated cells or subcellular fractions. Fatigue may be due to impairment at any one or more of the links in this 'chain'. It is not practical, however, to analyse in detail the relative contributions of different sections of this command chain in man because of the inaccessibility of the tissues or mechanisms involved. Nevertheless, the changes in muscle function associated with fatigue may be identified: loss of force or power output (the defining feature of fatigue), slowing of relaxation, changes in contractile characteristics and alterations in electrical properties, depending on the circumstances of measurement and on how the muscle has been fatigued.

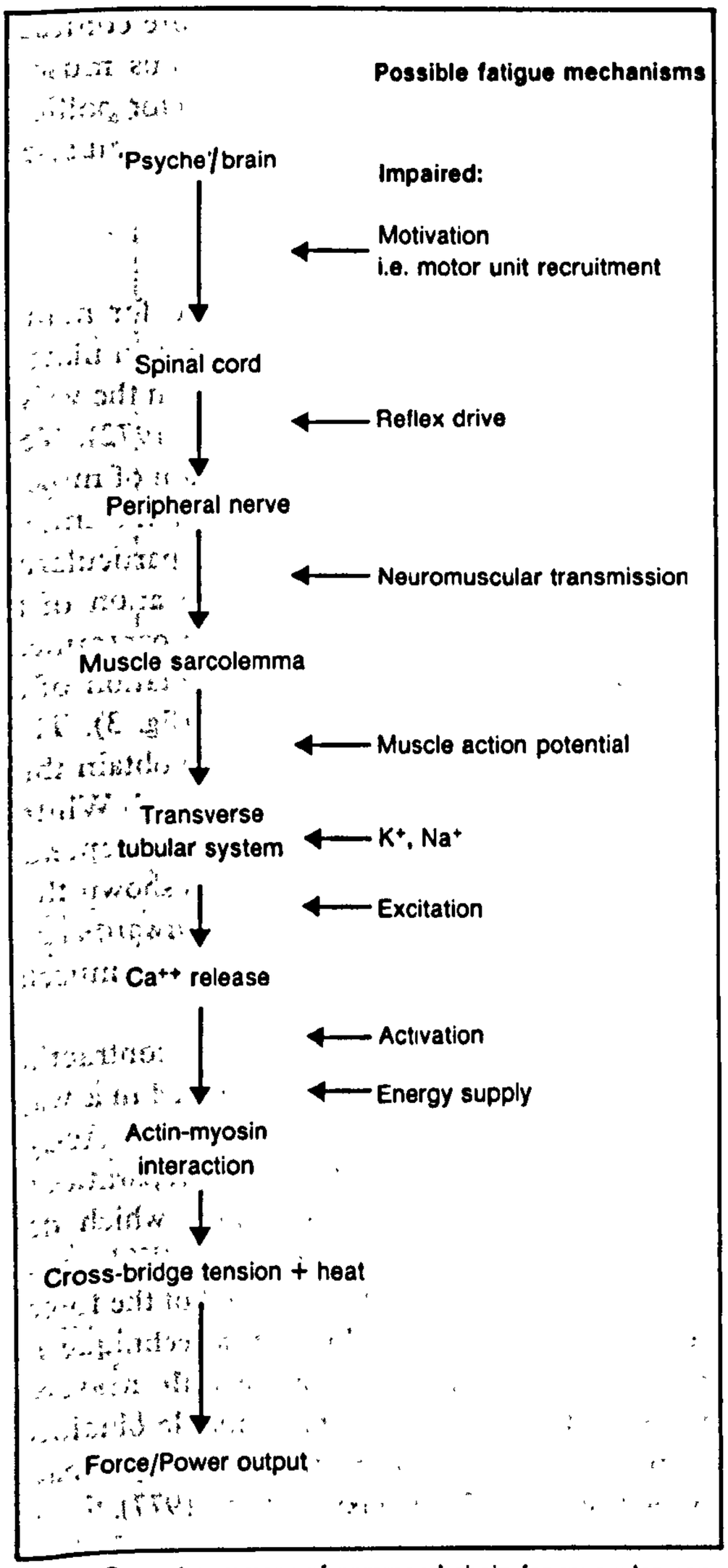

Fig. 1. Current summary of command chain for muscular contraction in man (after Edwards, 1983).

Fatigue may thus involve different processes associated with central nervous command or peripheral mechanisms. These have been broadly classified into 'central' or 'peripheral' (Bigland-Ritchie et al., 1978; Edwards, 1981; see table I). 'Central' causes of fatigue include motivation, impaired transmission down the spinal cord and impaired recruitment of motor neurons. 'Peripheral' causes of fatigue may involve impairment of the function of the peripheral nerves, neuromuscular junction transmission, electrical activity of muscle fibres or the processes of activation within the fibre. The distinction between 'central' and 'peripheral' fatigue is possible by comparing the force generated by a maximal voluntary contraction with that of an electrically stimulated contraction (Bigland-Ritchie et al., 1978; Merton, 1954), or by interpolating twitch stimuli to muscles undergoing voluntary contractions (Belanger and McComas, 1981; Chapman et al., 1984; fig.2). In well-motivated, normal subjects, there is little evidence of central fatigue

Table I. Physiological classification of fatigue (based on Ciba Foundation Symposium No. 82)

Fatigue	Characteristics	Mechanisms
1) Central	Force or heat generated by voluntary effort less than that by electrical stimulation	Failure to sustain recruitment and/or frequency of motor units
2) Peripheral	Same force loss or heat generation with voluntary and stimulated contractions	
a) High frequency	Selective loss of force at high stimulation frequencies	Impaired neuromuscular transmission and/or propagation of muscle action potential
b) Low frequency	Selective loss of force at low stimulation frequencies	Impaired excitation/ contraction coupling

N.B. Only when possible *parallel fatigue*, i.e. dropping out of parallel-acting force producing elements (motor units or individual cells) has been eliminated can contractile fatigue, i.e. failure or force maintenance by the individual elements be recognised.

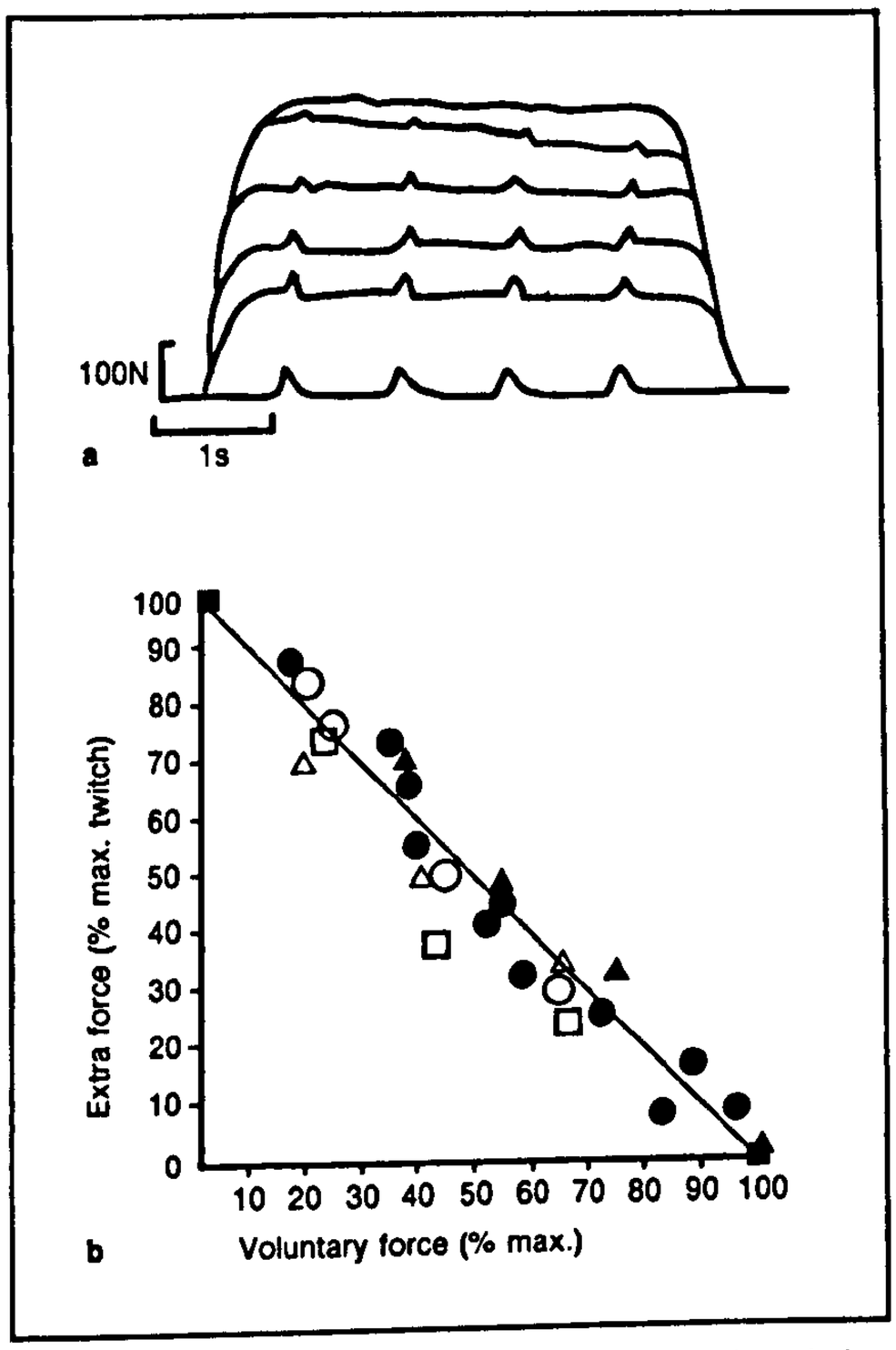

Fig. 2. Extra force generated by interpolated twitches during voluntary contractions at various forces of the quadriceps (after Chapman et al., 1984).

suggesting the decrease in force during a sustained contraction is probably due to peripheral factors. This can be established by the electrical stimulation of a peripheral nerve and by demonstrating failure (independently of motivation or voluntary effort) of muscle contraction force.

2. *Mechanisms of Fatigue in Exercise*

2.1 Investigation of Muscle Fatigue

Two techniques have generally been used to investigate and identify muscle fatigue: the contractile function of muscle in response to electrical stimulation, and secondly, measurement of the surface electromyographic (EMG) power spectrum.

Strain gauges have been used to measure contraction force in the first dorsal interosseous muscle (Stephens and Taylor, 1972), the adductor pollicis (Merton, 1954) and the quadriceps muscles (Edwards et al., 1977a).

2.1.1 Electrical Stimulation

Electrical stimulation has been used for many years to induce muscle fatigue. Merton stimulated the adductor pollicis via the ulnar nerve at the wrist (Merton, 1954; Stephens and Taylor, 1972). Tetanic stimulation of muscle by excitation of motor nerves in large mixed nerve trunks or intramuscular nerve endings is safe and not particularly painful (Edwards et al., 1977a). Application of a series of frequency trains, i.e. a programmed stimulation myogram, allows documentation of a force-frequency curve for the muscle (fig. 3). The validity of percutaneous stimulation to obtain this curve has been questioned (Davies and White, 1982), since it was suggested to be voltage-dependent. However, further investigation has shown this is not the case except at low voltages (Edwards and Newham, 1984) in either fresh or fatigued muscle (Edwards et al., 1984).

Electrical stimulation also allows the contractile properties of human muscle to be studied in a way which is directly comparable to that used in studying the function of isolated muscle preparations. The measurements of relaxation rate, which depends on a number of metabolic factors (Wiles and Edwards, 1982; Wiles et al., 1979) and of the force-frequency curve obtained by such a technique in the intact quadriceps muscle agree with measurements made on strips of human muscle obtained at operation and studied in an isolated organ bath (Moulds et al., 1977; Faulkner et al., 1977).

2.1.2 EMG Studies

The investigation of surface EMG in voluntary and electrically stimulated contractions allows the study of the electrical properties of the muscle. The frequency analysis of muscle action potentials from EMG power spectra has been well studied (Kadefors et al., 1968; Kaiser and Petersen, 1963; Mills, 1982; Moxham et al., 1982). A muscle fatigue

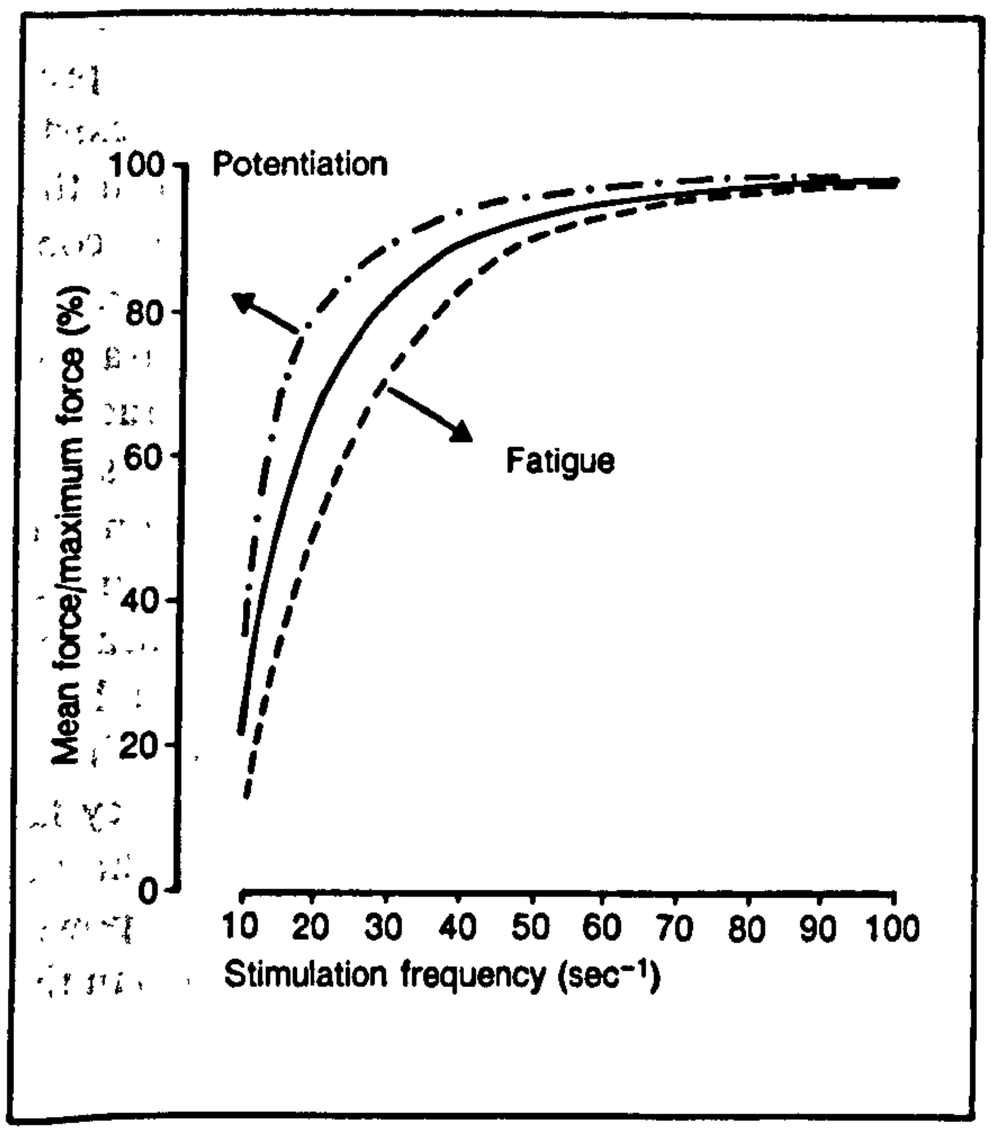

Fig. 3. Changes in the frequency : force curve with fatigue. Note the shift of the curve to the right with a resulting fall in the ratio of force generated by low frequency stimulation to force at high stimulation frequencies, characteristic of low frequency fatigue.

monitor based on detection of myoelectrical signals for observing localised muscular fatigue has been developed by Stulen and De Luca (1982).

2.2 Muscle Fatigue

When defective neural drive (central fatigue) has been eliminated, the failure of a muscle to generate force may be the result of peripheral factors. These may include the electrical properties of muscle, (electromechanical activation) where a failure at the neuromuscular junction or action potential conduction in the fibre surface membrane is the cause of fatigue. Metabolite changes in the muscle cell may also be involved in the development of fatigue, where failure is a consequence of adenosine triphosphate (ATP) depletion or accumulation of H^+ inhibiting calcium-activated myosin ATPase and thereby the function of the contractile machinery (Hermansen, 1981).

2.2.1 High Frequency Fatigue

The first possible site of failure in peripheral fatigue is the neuromuscular junction. The contribution to fatigue in voluntary contractions is probably not as important as first thought (Stephens and Taylor, 1972), since it has been shown recently at least during the first 60 seconds of a maximal voluntary contraction that there is little neuromuscular junction failure (Bigland-Ritchie et al., 1982).

The rapid force loss observed with high frequency electrical pulses is accompanied by a loss of the surface-recorded synchronous action potential (fig. 4), and a change in the EMG power spectrum (Moxham et al., 1982). This cause of fatigue is probably due to a failure of action potentials along the surface membrane of the muscle fibre. It has been suggested that the loss of force and change in electrical properties seen during high frequency stimulation is due to an accumulation of potassium in the T-tubules and interfibre spaces of the muscle (Jones, 1981; Jones et al., 1979). The power spectral shift is not simply due to lactic acid accumulation since it also occurs in patients with myophosphorylase deficiency who are unable to produce lactic acid with ischaemic muscular activity (Mills and Edwards, 1984).

The natural firing frequencies seen in normal voluntary contractions are in the range of 5 to 30Hz. However, the fall in firing frequency during a sustained voluntary contraction (Bigland Ritchie et al., 1983) would seem to minimise the tendency to high frequency fatigue, thus protecting against action potential failure at the peripheral nerve, neuromuscular junction and the muscle fibre membrane itself. Electromechanical failure (high frequency fatigue) occurs more readily in normal muscle when cooled, when there is partial curarisation and in diseases such as myasthenia gravis and myotonia (Edwards, 1980) [fig. 4].

2.2.2 Low Frequency Fatigue

Low frequency fatigue, a specific failure of force generation at low frequencies of stimulation, is thought to represent a failure of activation of the muscle despite adequate excitation (Edwards et al.,

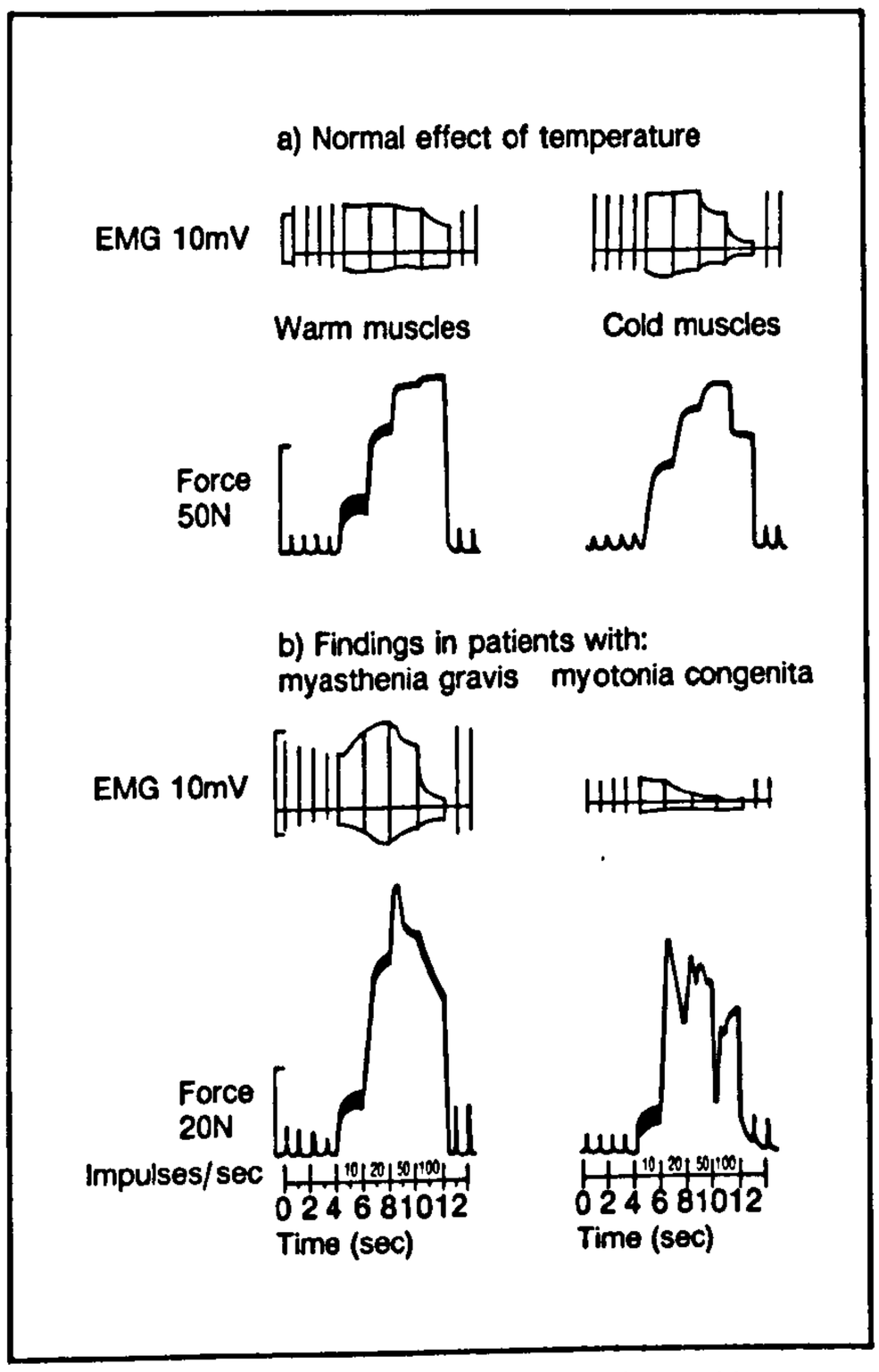

Fig. 4. Programmed stimulation myograms of the adductor pollicis showing evidence of high frequency fatigue in (a) cold muscle (about 25°C) and (b) diseased muscle (after Edwards, 1980).

1977b). The effect of this type of fatigue is to move the steep part of the force-frequency curve to the right (fig. 3). Since it is likely that firing frequency for voluntary contractions in everyday life activities is in the range 5 to 30Hz, this type of fatigue may result in significant force reduction unless a compensatory increase in firing frequency can be achieved or there is concomitant recruitment of further motor units in parallel. In normal subjects this type of fatigue occurs in the quadriceps and adductor pollicis following voluntary dynamic and ischaemic isometric contractions. It is not simply due to lactic acid accumulation as a consequence of muscular activity since it may be demonstrated in individuals with a variety of metabolic defects which influence energy exchanges and lactate production (Wiles et al., 1981). The possibility exists that damage to cellular structures involved in the electromechanical activation processes may contribute to low frequency fatigue since it is more evident in muscles which have been contracted eccentrically, i.e. stretched during contraction (Newham et al., 1983a) [fig. 5]. The latter also show evidence of sarcomere disruption on electron micrographs of needle biopsy samples (Newham et al., 1983b) while muscles which have contracted concentrically, i.e. shorten during contraction, have no damage. It is notable that there is no shift in the EMG power spectrum with low frequency fatigue (Moxham et al., 1982), either during the exercise or during recovery when the EMG power spectrum recovers more rapidly (minutes) than the low frequency fatigue (hours).

2.2.3 Energy Metabolism

The metabolic exchanges with muscular activity are well known (table II): adenosine triphosphate (ATP) is the immediate fuel for muscular contraction, and depending on the intensity and type of exercise, this is regenerated by anaerobic glycolysis or oxidative phosphorylation. Most of what is known of energy supply in exercising human muscle has been learned from needle biopsy studies (Edwards et al., 1980) in normal volunteers. More recently, chemical studies have been made with electrically stimulated contractions (Sjoholm et al., 1983). Resonance spectroscopy (MRS) further allows the non-invasive study of muscles working *in situ* (fig. 6).

The changes in metabolites occurring in active muscle that may cause deterioration in force production include reduced ATP concentrations, increases in lactate, and hence hydrogen ions. These are considered below.

'Nature's Experiments'

1) Lactate accumulation. Inherent disorders of energy metabolism in which there are specific enzyme defects are increasingly being recognised. Such occurrences have provided a further means of in-

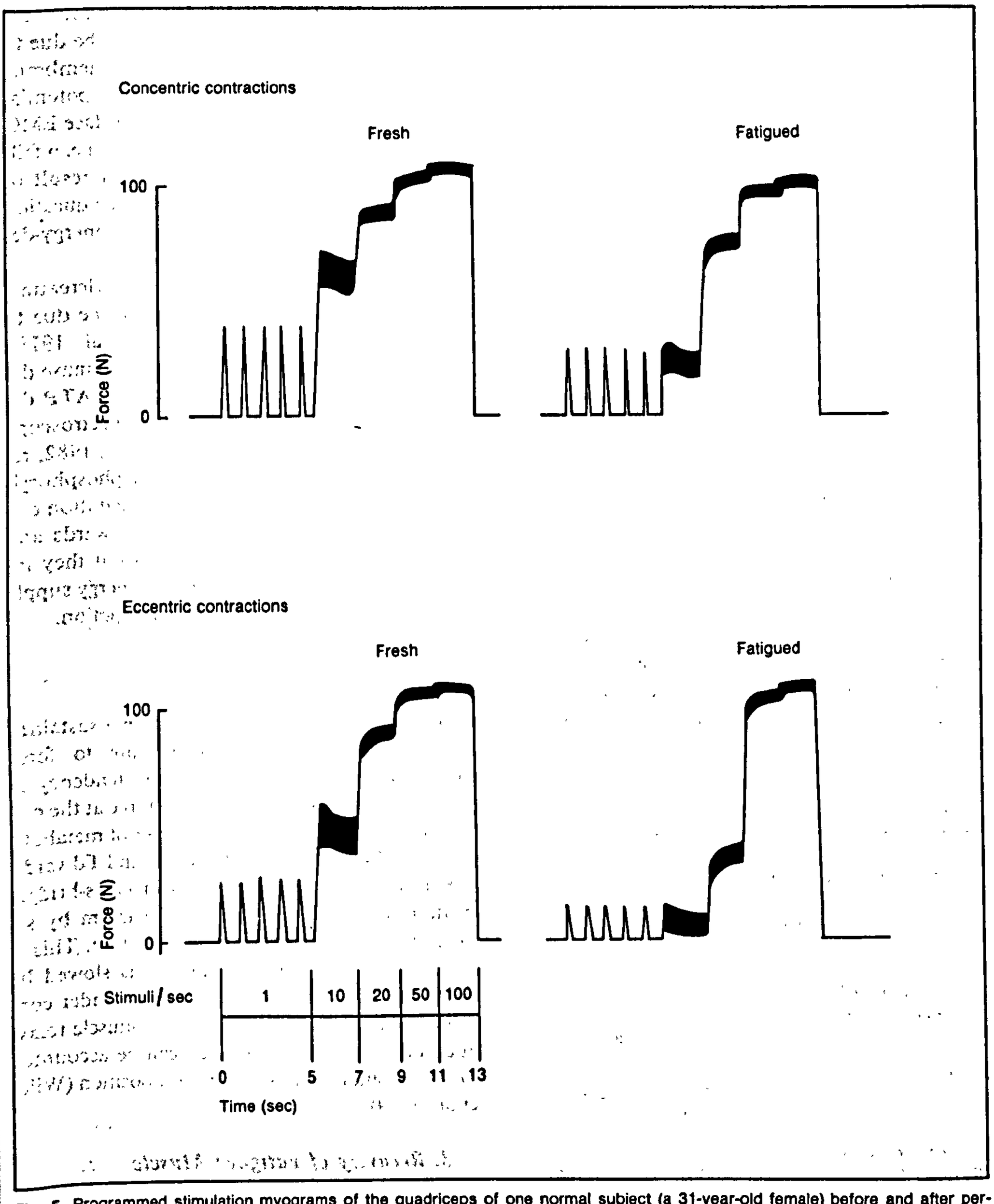

Fig. 5. Programmed stimulation myograms of the quadriceps of one normal subject (a 31-year-old female) before and after performance of similar amounts of work done by either concentric or eccentric contractions. Note greater low frequency fatigue after eccentric contractions (after Newham et al., 1983a).

vestigation of the causes of fatigue in normal human muscle. It may be that 'Nature's experiments' are as important in the study of human muscle fatigue as the discovery that iodoacetate-poisoned muscle can still contract without the formation of lactic acid (Lunsgaard, 1930). The finding that a patient with muscle cramps on exercise did not produce lactic acid on ischaemic exercise (McArdle, 1951) has raised important questions as to the role of lactic acid in the development of fatigue *in vivo*. It might be expected that the accumulation of lactate and hence H^+ ions in muscle would be a cause of fatigue and indeed there is evidence for this (Hermansen, 1981). However, muscle lacking in myophosphorylase, like isolated iodoacetate-poisoned muscles (Edwards et al., 1975), fatigue even more rapidly than normal muscles (Wiles et al., 1981). Similarly, resonance spectroscopy studies in patients with phosphofructokinase deficiency have shown little change in pH with ischaemic exercise but an increase in the position of sugar phosphates (left side of the ^{31}P spectrum, fig. 5) as would be expected from the level of the metabolic block. This was also evident with aerobic exercise. Such studies have shown the presence of phosphorylcreatine and ATP which, if depleted, would result in rigor and damage of the contractile machinery of the muscle. Clearly, lactate is not the cause for the fatigue observed. It is more likely to be due to failure of the excitation of the muscle membrane shown by changes in the evoked action potential (Wiles et al., 1981) and the changes in surface EMG power spectra (Mills and Edwards, 1984), i.e. a failure of electrical activity, rather than a result of metabolic changes. However this begs the question of how far electrical phenomena are energy-dependent.

2) Reduction in ATP and phosphorylcreatine. Although there is no doubt fatigue can be due to a failure of ATP resynthesis (Dawson et al., 1978), in McArdle's disease and phosphofructokinase deficiency, fatigue is not obviously due to ATP depletion as indicated by resonance spectroscopy studies (Ross et al., 1981; Edwards et al., 1982, respectively), but there is depletion of phosphorylcreatine. It is evident that failure of excitation can occur in these glycolytic disorders (Edwards and Wiles, 1981). The cause is uncertain but they indicate the close relationship between energy supply and excitation/activation in muscle function.

Table II. Energy exchange in muscle

ATP is the fuel for contraction

$$H_2O + ATP \xrightarrow[\text{ATPase}]{\text{actomyosin}} ADP + Pi + \text{energy}$$

ATP is regenerated by

1. Creatine kinase (Lohmann reaction)

$$ADP + PCr \rightleftharpoons ATP + Cr \quad (Cr = \text{creatine})$$

2. Glycolysis

$$\underset{\text{(glycogen)}}{1/n\,(C_6H_{10}O_5)} + 3Pi \rightarrow \underset{\text{(lactic acid)}}{2C_3H_6O_3} + 2H_2O + 3ATP$$

3. Oxidative phosphorylation

$$\underset{\text{(pyruvic acid)}}{C_3H_4O_3} + 2\,O_2 + 18ADP + 18Pi \rightarrow 3CO_2 + 20H_2O + 18ATP$$

2.3 Relaxation Rate

The slowing of relaxation rate, as in a sustained isometric contraction, can contribute to force maintenance, thus counteracting the tendency to fatigue (Jones, 1981). Relaxation of force at the end of a contraction depends on a number of metabolic factors (Edwards et al., 1972; Wiles and Edwards, 1982), including the dissociation of cross-bridges made possible by the removal of calcium by sequestration in the sarcoplasmic reticulum. This is further evidenced when the muscle is slowed by cooling (Wiles and Edwards, 1982), or under conditions of hypothyroidism where the muscle relaxation rate is reduced more than can be accounted for by alteration in muscle fibre composition (Wiles et al., 1979).

3. Recovery of Fatigued Muscle

Recovery of fatigued muscle depends on the type of fatigue that develops in the muscle and hence

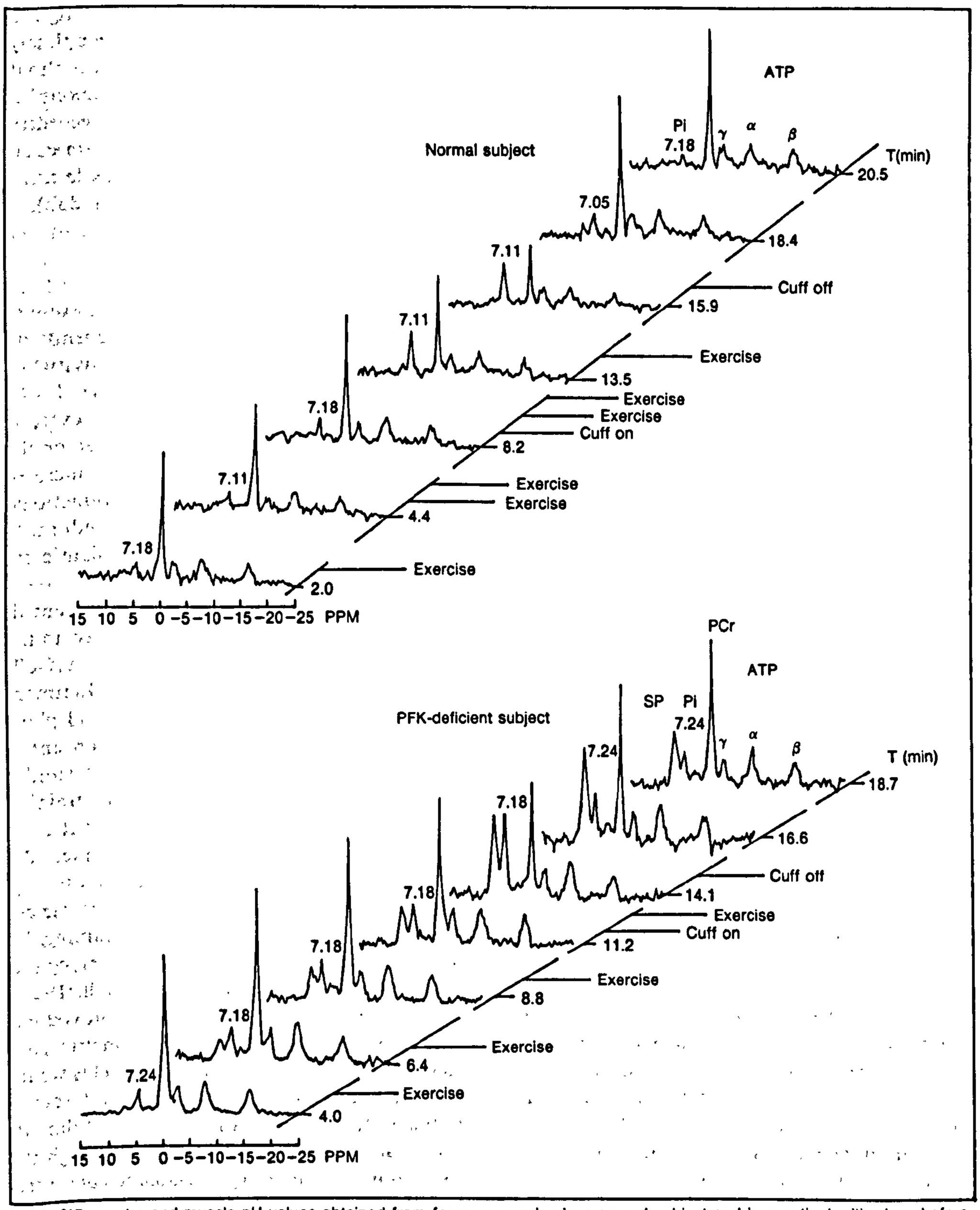

Fig. 6. ^{31}P spectra and muscle pH values obtained from forearm muscles in a normal subject and in a patient with phosphofructokinase deficiency (after Edwards et al., 1982a).

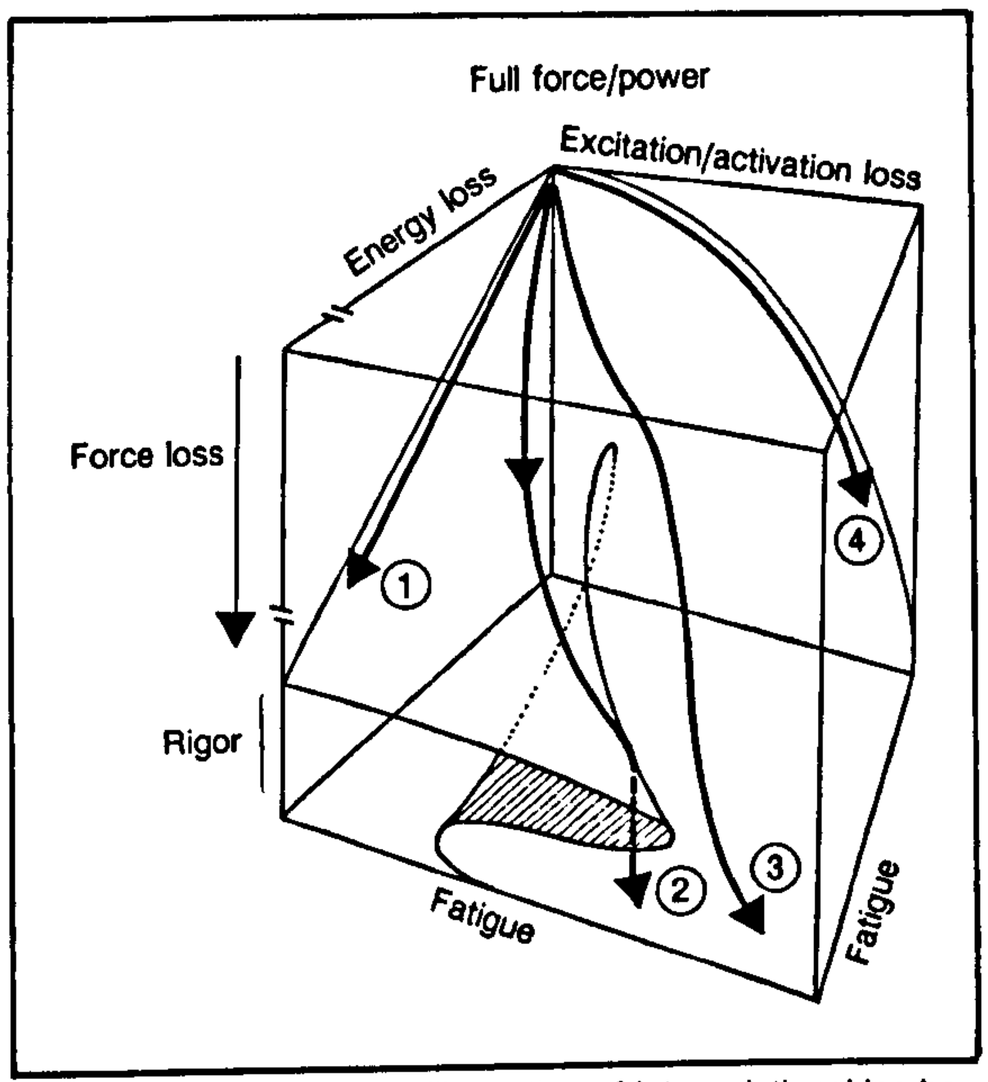

Fig. 7 Catastrophe theory: Illustration of inter-relationships between energy loss and excitation/activation loss in the development of muscular fatigue (force loss). Pathway 1 shows a hypothetical 'pure' loss of energy (under conditions of optimal excitation and activation) with the attendant risk of developing rigor; 4 is pure failure of excitation, e.g. myasthenia gravis; 3 could occur with dynamic exercise while 2 is the 'safety factor' provided by failure of excitation in association with energy loss as seen with isometric contractions and glycolytic disorders (after Edwards, 1983).

on the form that exercise may have taken. In studies on the adductor pollicis where fatigue is induced by electrical stimulation under ischaemic conditions, rapid recovery from high frequency stimulation is apparent following the return of the circulation (Edwards et al., 1977b).

Similarity of the time course of recovery of relaxation rate and mean power frequency of the EMG power spectrum following contraction further supports the hypothesis that there is a close link between electrical and metabolic factors: possibly resynthesis processes in the muscle or degradation or removal of metabolites produced during contraction (Mills, 1982). Further support comes from the fact that neither the slowed relaxation nor the shift of the power spectrum recover with continuing ischaemia, i.e. not until the local circulation is restored.

Conversely, recovery of low frequency fatigue is slow, taking a day or more to recover completely. Dynamic exercise of the quadriceps similarly shows rapid recovery following concentric contractions but slow recovery (a day or more) following eccentric contractions (Edwards et al., 1981; Newham et al., 1983a) where it is likely damage to muscle ultrastructure has occurred (Newham et al., 1983b).

4. Long Term Effects of Exercise

Of interest are the effects of long term exercise on the 'fatiguability' of muscle. This depends on the type of exercise carried out and the activity history of the muscle (McDonagh and Davies, 1984). It is traditional to describe the effects of exercise training on muscle as depending on whether the type of exercise is primarily designed to increase muscle strength (repeated high force contractions) or whether it is intended to increase endurance (prolonged low force contractions of dynamic exercise).

The metabolic changes are well documented. With endurance exercise there is an increase in the activity of mitochondrial enzymes in muscle (Gollnick and Saltin, 1982; Henriksson and Reitman, 1977). Muscle energy metabolites [ATP and phosphorylcreatine (PCr)] are increased in concentration by strength training and somewhat similar changes have been seen with endurance training (Karlsson et al., 1972) with the result that after training, exercise could be continued with less depletion of PCr and less lactate accumulation.

The capacity to sustain endurance dynamic exercise is increased through endurance training, by the result of several cardiovascular, respiratory and metabolic adaptations (Saltin and Rowell, 1980). Strength training can also result in improved endurance of a given (submaximal) isometric contraction (McDonagh and Davies, 1984). This would appear to be due more to the steepness of the curve relating endurance to contraction force (Rohmert, 1960) than to metabolic adaptations, although the observed increase in energy metabolite concentrations (MacDougall et al., 1977) would also be expected to improve endurance of contractions which

depend on anaerobic energy supply processes.

A change in muscle contractile properties by training can reduce fatiguability. Chronic low frequency electrical stimulation is known to improve endurance in animal models, usually as a consequence of an increase in the proportion of slow, fatigue-resistant muscle fibres (Salmons and Henriksson, 1981). Studies carried out in humans using low frequency electrical stimulation [adductor pollicis: 10Hz, 3 hours/day for 6 weeks (Edwards et al., 1982b); tibialis anterior and extensor digitorum longus: 8 to 10Hz, 1h 3-times-daily for 6 weeks (Dubowitz et al., 1982)] have shown the characteristic properties of human muscle can be altered so as to reduce fatiguability. It does this by apparently potentiating the forces generated at low frequencies compared with high, though whether this is a consequence of the alteration in fibre type characteristics, still remains unclear. Long term low frequency stimulation, however, does not appear to increase the maximum voluntary contraction force (Dubowitz et al., 1982).

5. Limiting Factors to Muscle Contraction and Performance

Are there chemical or electrical limits to continuing contractile activity? This has been vigorously debated over the years (Ciba Foundation Symposium, 1981) but the answer may depend on the nature and intensity of the exercise and whether there is an abnormality of muscle metabolism present. Figure 7 illustrates the possible pathways by which fatigue might come about as a result of energy loss, excitation/activation failure or a combination of both. This representation, based on the 'catastrophe theory' model of engineering and mathematical usage (Poston and Stewart, 1978) illustrates 4 hypothetically separate pathways for muscle fatigue while emphasising the way in which they may interact in health to provide a protective mechanism in muscle function (Edwards, 1983). The 'catastrophe theory' has also been applied to muscle contraction relating fibril length, tension state of muscle and calcium ion concentration (Alesso, 1978).

The axes are drawn on the basis of experimental observations of a number of workers. Relating impairment of energy supply to force loss is a linear function (pathway 1; Dawson et al., 1978). This path, if continued indefinitely, could result in the muscle ATP concentration falling to zero and rigor conditions with resulting damage to the contractile mechanism. It is fortunately impossible to drive a healthy human muscle into rigor. It is a possibility that focal rigor may occur when a patient with a glycolytic disorder, such as myophosphorylase or the phosphofructokinase deficiency, exercises to contracture formation.

Excitation frequency has a curvilinear relation to force (Edwards, 1977a) as does the relationship in skinned muscle fibres between ionic calcium concentration and force (Bolitho-Donaldson and Hermansen, 1978). Pathway 4 thus represents pure excitation/activation limitation with no loss of energy. In practice, fatigue may demonstrate a combination of energy loss and some impairment of excitation/activation. In dynamic exercise the brief rhythmic innervation provides protection from the development of high fequency fatigue, thus enabling muscles to utilise a large part of their stored energy. It is possible that in such exercise the vector for fatigue may follow some if not all of pathway 3. In isometric contractions, particularly under ischaemic conditions, the metabolic demand is greater than can be met by energy supply mechanisms and a concomitant impairment of excitation/activation may ensue, thus preventing the muscle going into rigor.

A further separation between energy loss and the development of low frequency fatigue is evident from the greater development of this type of fatigue following eccentric contractions (with a lower metabolic rate) than with concentric contractions (fig. 5). What exactly determines low frequency fatigue is not yet known, but it is possible that tension-related damage is important.

6. Implications for Sports Performance

The concepts of fatigue described here are not claimed to have direct relevance to athletic per-

formance. The body is a complex mechanism and training is a tuning process which improves performance *and* reduces the risk of possible damage caused by unskilled or incoordinated muscular action. Fatigue is likely to be less in such well trained individuals, not only because of adaptations in muscle machinery which favour high performance and endurance, but because this training process improves the conscious or unconscious 'skill' in optimising the function of the muscles in such a way as to minimise the tendency to develop fatigue. It must be realised that special experimental designs and physiological techniques are needed to uncover forms of fatigue (such as low frequency fatigue) which may not be observable with voluntary contractions. Such special experimental conditions used to elucidate mechanisms of human muscle fatigue serve also to show how even optimum performance in a well trained sportsman may be seriously impaired by adverse environmental conditions such as heat and cold, not only because of their effects on general comfort, but specifically because of influences on the cellular mechanisms involved in muscle fatigue.

7. Conclusions

It would appear that there are probably many different types of fatigue, and that each may occur with a particular form of muscular activity. The 'catastrophe theory' of fatigue may validly describe the final common pathway in cellular function leading to impaired performance. It essentially demonstrates that it may be difficult, if not impossible, to recognise the limiting factor when on the edge of a catastrophe cusp, during or at the end of activity. Thus, in aerobic exercise, a marginally deficient rate of ATP supply at one power output with consequent failure of excitation/activation may be adequate and capable of full excitation/activation at an only slightly lower power output. In an isometric contraction, making substantial demands on anaerobic metabolism, the instant when contraction stops and aerobic recovery occurs may allow rapid ATP recovery (and recovery of an excitatory loss) simply because aerobic glycolysis has a considerably greater ATP yield than anaerobic glycolysis. A rapid failure of force may occur with high frequency stimulation with an almost instantaneous improvement in force generation when the frequency is suddenly reduced (Bigland-Ritchie et al., 1979) thus illustrating the 'critical' state of excitation, independent of immediate energy supply.

The cause of fatigue cannot therefore be solely due to electrical or metabolic factors and, furthermore, the relationship between energy supply, excitation/contraction coupling and force production is complicated by the type of exercise carried out. It is, however, possible to investigate further the contribution of electrical and metabolic factors involved in the 'chain of command' (fig. 1) with new and improved techniques and to apply 'catastrophe theory' as a means of describing the events immediately influencing the failure of one of its links causing fatigue.

Acknowledgements

Support from the Wellcome Trust and the Muscular Dystrophy Group of Great Britain is gratefully acknowledged. Henry Gibson is a holder of a University of Liverpool Postgraduate Research Studentship.

References

Alesso, H.P.: Reliability analysis of the elementary catastrophe theory model of muscle contraction. Engineering in Medicine 7: 21-30 (1978).

Belanger, A.V. and McComas, A.J.: Extent of motor unit activation during effort. Journal of Applied Physiology 51: 1131-1135 (1981).

Bigland-Ritchie, B.; Jones, D.A.; Hosking, G.P. and Edwards, R.H.T.: Central and peripheral fatigue in maximum voluntary contractions of human quadriceps muscle. Clinical Science and Molecular Medicine 54: 604-614 (1978).

Bigland-Ritchie, B.; Johansson, R.; Lippold, O.C.J.; Smith, S. and Woods, J.J.: Changes in motorneurone firing rates during sustained maximal voluntary contractions. Journal of Physiology 340: 335-346 (1983).

Bigland-Ritchie, B.; Jones, D.A. and Woods, J.J.: Excitation frequency and muscle fatigue: Electrical responses during human voluntary and stimulated contractions. Experimental Neurology. 64: 414-427 (1979).

Bigland-Ritchie, B.; Kakula, C.B.; Lippold, O.C.J. and Woods, J.J.: The absence of neuromuscular transmission failure in sustained maximal voluntary contractions. Journal of Physiology 330: 265-278 (1982).

Bolitho-Donaldson, S.K. and Hermansen, L.: Differential direct effects of H^+ on Ca^{2+} – activated force of skinned fibres from the soleus, cardiac and adductor magnus muscles of rabbits. Pflugers Archives 376: 55-65 (1978).

Chapman, S.J.: Edwards, R.H.T.; Greig, C. and Rutherford, O.:

Practical application of the twitch interpolation technique for the study of voluntary contraction of the quadriceps muscle in man. Journal of Physiology 353: 3P (1984).
Davies, C.T.M. and White, M.J.: Muscle weakness following dynamic exercise in humans. Journal of Applied Physiology 53: 236-241 (1982).
Dawson, J.J.; Gadian, D.G. and Wilkie, D.R.: Muscular fatigue investigated by phosphorus nuclear magnetic resonance. Nature 274: 861-866 (1978).
Dubowitz, V.; Hyde, S.A.; Scott, O.M. and Vrbova, G.: Effect of long-term electrical stimulation on the fatigue of human muscle. Journal of Physiology 328: 30P-31P (1982).
Edwards, R.H.T.: Studies of muscular performance in normal and dystrophic subjects. British Medical Bulletin 36: 159-164 (1980).
Edwards, R.H.T.: Physiological analysis of skeletal muscle weakness and fatigue. Clinical Science 65: 1-8 (1978).
Edwards, R.H.T.: Human muscle function and fatigue; in Porter and Whelan (Eds) Human Muscle Fatigue: Physiological Mechanisms. Ciba Foundation Symposium No. 82, pp. 1-18 (Pitman Medical, London, 1981).
Edwards, R.H.T.: Biochemical bases of fatigue in exercise performance: Catastrophe theory of muscular fatigue; in Knuttgen et al., (Eds) Biochemistry of Exercise, pp. 3-28 (Human Kinetics, Champaign 1983).
Edwards, R.H.T.; Dawson, M.J.; Wilkie, D.R.; Gordon, R.E. and Shaw, D.: Clinical use of nuclear magnetic resonance in the investigation of myopathy. Lancet 1: 725-731 (1982a).
Edwards, R.H.T.; Efthimiou, J. and Spiro, S.G.: Voltage independence of frequency: force relationships before and after fatiguing dynamic exercise in the sternomastoid muscle. Journal of Physiology 353: 129P (1984).
Edwards, R.H.T.; Jones, D.A. and Newham, D.J.: Low frequency stimulation and changes in human muscle contractile properties. Journal of Physiology 328: 29-30P (1982b).
Edwards, R.H.T.; Hill, D.K. and Jones, D.A.: Effect of fatigue on the time course of relaxation from isometric contraction of skeletal muscle in man. Journal of Physiology 227: 26-27P (1972).
Edwards, R.H.T.; Hill, D.K. and Jones, D.A.: Metabolic changes associated with the slowing of relaxation in fatigued mouse muscle. Journal of Physiology 521: 287-301 (1975).
Edwards, R.H.T.; Hill, D.K.; Jones, D.A. and Merton, P.A.: Fatigue of long duration in human skeletal muscle after exercise. Journal of Physiology 272: 769-778 (1977b).
Edwards, R.H.T.; Mills, K.R. and Newham, D.J.: Greater low frequency fatigue produced by eccentricitation by concentric muscle contraction. Journal of Physiology 317: 17P (1981).
Edwards, R.H.T. and Newham, D.J.: Force-frequency relationship determined by percutaneous stimulation of the quadriceps muscle. Journal of Physiology 353: 128P (1984).
Edwards, R.H.T. and Wiles, C.M. Energy exchange in human skeletal muscle during isometric contraction. Circulation Research 48 (Suppl.): 11-17 (1981).
Edwards, R.H.T.; Young, A.; Hosking, G.P. and Jones, D.A.: Human skeletal muscle function: Description of tests and normal values. Clinical Science and Molecular Medicine 52: 283-290 (1977a).
Edwards, R.H.T.; Young, A. and Wiles, C.M.: Needle biopsy of skeletal muscle in the diagnosis of myopathy and the clinical study of muscle function and repair. New England Journal of Medicine 302: 261-271 (1980).
Faulkner, J.A.; Jones, D.A.; Round, J.M. and Edwards, R.H.T.: Dynamics of energetic processes in human muscle; in Cervetelli and Whipps (Eds) Exercise Bioenergetics and Gas Exchange, pp. 81-90 (Elsevier, North Holland 1980).
Gollnick, P.D. and Saltin, B.: Significance of skeletal muscle oxidative enzyme enhancement with endurance training. Clinical Physiology 2: 1-12 (1982).
Henriksson, J. and Reitman, J.S.: Time course of changes in human skeletal muscle succinate dehydrogenase and cytochrome oxidase activities and maximal oxygen uptake with physical activity and inactivity. Acta Physiologica Scandinavica 99: 91-97 (1977).
Hermansen, L.: Effect of metabolic changes on force generation in skeletal muscle during maximal exercise; in Porter and Whelan (Eds) Human Muscle Fatigue: Physiological Mechanisms. Ciba Foundation Symposium No. 82 pp.75-88 (Pitman Medical, London, 1981).
Jones, D.A.: Muscle fatigue due to changes beyond the neuromuscular junction; in Porter and Whelan (Eds) Human Muscle Fatigue: Physiological Mechanisms, Ciba Foundation Symposium No. 82, pp.178-196 (Pitman Medical, London 1981).
Jones, D.A.; Bigland-Ritchie, B. and Edwards, R.H.T.: Excitation frequency and muscle fatigue: Mechanical responses during voluntary and stimulated contractions. Experimental Neurology 64: 401-413 (1979).
Kadefors, R.; Kaiser, E. and Petersen, I.: Dynamic spectrum analysis of myo-potentials with special reference to muscle fatigue. Electromyography 8: 39-74 (1968).
Kaiser, E. and Petersen, I.: Frequency analysis of muscle action potentials during tetanic contraction. Electromyography 3: 5-17 (1963).
Karlsson, J.; Nordesjo, L.-O.; Jorfeldt, L. and Saltin, B.: Muscle lactate, ATP and CP levels during exercise after physical training in man. Journal of Applied Physiology 33: 119-203 (1972).
Lunsgaard, E.: Untersuchangen uber muskel kontraction ohne milschsaure. Biochemistry Z. 227: 51-59 (1930).
MacDougall, J.D.; Ward, G.R.; Sale, D.G. and Sutton, J.R.: Biochemical adaptation of human skeletal muscle to heavy resistance training and immobilisation. Journal of Applied Physiology 43: 700-703 (1977).
McArdle, B.: Myopathy due to a defect in glycogen breakdown. Clinical Science 10: 13-35 (1951).
McDonagh, M.J.N. and Davies, C.T.M.: Adaptive responses of mammalian skeletal muscle to examination with high loads. European Journal of Applied Physiology 52: 139-155 (1984).
Merton, P.A.: Voluntary strength and fatigue. Journal of Physiology 123: 553-564 (1954).
Mills, K.R.: Power spectral analysis of electromyogram and compound muscle action potential during muscle fatigue and recovery. Journal of Physiology 326: 401-409 (1982).
Mills, K.R. and Edwards, R.H.T.: Muscle fatigue in myophosphorylase deficiency: Power spectral analysis of the electromyogram. Electroencephalography and Clinical Neurophysiology 57: 330-335 (1984).
Moulds, R.F.W.; Young, A.; Jones, D.A. and Edwards, R.H.T.: A study of the contractility, biochemistry and morphology of an isolated preparation of human skeletal muscle. Clinical Science and Molecular Medicine 52: 291-297 (1977).
Moxham, J.; Edwards, R.H.T.; Aubier, M.; De Troyer, G.; Farkas, G.; Macklem, P.T. and Roussos, C.: Changes in EMG power spectrum (high to low ratio) with force fatigue in humans. Journal of Applied Physiology: Respiratory Environmental and Exercise Physiology 53: 1094-1099 (1982).
Newham, D.J.; McPhail, G.; Mills, K.R. and Edwards, R.H.T.: Ultrastructural changes after concentric and eccentric contractions of human muscle. Journal of Neurological Science 61: 109-122 (1983b).
Newham, D.J.; Mills, K.R.; Quigley, B.M. and Edwards, R.H.T.: Pain and fatigue after concentric and eccentric muscle contractions. Clinical Science 64: 55-62 (1983a).
Porter, R. and Whelan, J. (Eds): Ciba Foundation Symposium No. 82: Human Muscle Fatigue: Physiological Mechanisms, pp.1-301 (Pitman Medical, London, 1981).
Poston, T. and Stewart, I.: Catastrophe Theory and its Applications, pp.1-477 (Pitman, London 1978).

Rohmert, W.: Ermittlung von Erholungs pausen fur statische Arbeit des Menschen. Internationale Zeitschrift fur angewandte Physiologie einschliesslich Arbeitsphysiologie 18: 123-164 (1960).

Ross, B.D.; Radda, G.K.; Gadian, D.G.; Rocker, G.; Esiri, M. and Falconer-Smith, J.: Examination of a case of suspected McArdle's Syndrome by ^{31}P nuclear magnetic resonance. New England Journal of Medicine 304: 1338-1342 (1981).

Salmons, S. and Henriksson, J.: The adaptive response of skeletal muscle to increased use. Muscle and Nerve 4: 94-105 (1981).

Saltin, B. and Rowell, L.B.: Functional adaptations to physical activity and inactivity. Federation Proceedings 39: 1506-1513 (1980).

Sjoholm, H.; Sahlin, K.; Edstrom, L. and Hultman, E.: Quantitative estimation of anaerobic and oxidative energy metabolism and contraction characteristics in intact human skeletal muscle in response to electrical stimulation. Clinical Physiology 3: 227-239 (1983).

Stephens, J.A. and Taylor, A.: Fatigue of maintained voluntary contractions in man. Journal of Physiology 220: 1-18 (1972).

Stulen, F.B. and De Luca, C.J.: Muscle fatigue monitor: A noninvasive device for observing localized muscular fatigue. IEEE Transactions of Biomedical Engineering 29: 760-768 (1982).

Wiles, C.M. and Edwards, R.H.T.: Metabolic heat production in isometric ischaemic contractions of human adductor pollicis. Clinical Physiology 2: 499-512 (1982).

Wiles, C.M.; Jones, D.A. and Edwards, R.H.T.: Fatigue in human metabolic myopathy; in Porter and Whelan (Eds) Human Muscle Fatigue: Physiological Mechanisms. Ciba Foundation Symposium No. 82, pp.120-129 (Pitman Medical, London, 1981).

Wiles, C.M.; Young, A.; Jones, D.A. and Edwards, R.H.T.: Muscle relaxation rate, fibre-type composition and energy turnover in hyper- and hypo-thyroid patients. Clinical Science 57: 378-384 (1979).

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[*From the Proceedings of the Physiological Society*, 19–20 *December* 1986
Journal of Physiology, **386**, 113*P*, 1987]

Frequency dependence of excitation and force generation in human adductor pollicis

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In contracting muscle, fatigue may be due to failure of electrical excitation or to contractile failure. Previous studies indicate that force and excitation decrease in parallel (Bigland-Ritchie, Johansson, Lippold, Smith & Woods, 1983; Hultman & Sjoholm, 1983), suggesting that fatigue is a consequence of excitation failure.

The surface compound muscle action potential (c.m.a.p.), maximal relaxation rate (MRR) and force were studied during ischaemic, isometric contractions of adductor pollicis (AP) in 8 healthy males. Contractile properties were examined by programmed stimulation of AP. Supramaximal stimulation was delivered percutaneously via the ulnar nerve at 1, 10, 20, 50 and 100 Hz for 1 s each. The simultaneous recordings produced were termed the programmed stimulation electromyogram ('PSEM' – Fig. 1*a*). The fatiguing activity consisted of 15 PSEMs, 30 s apart.

The results show that fatigue cannot be explained simply by excitation failure, since this relationship differs markedly with frequency. For a 50% force loss during fatiguing activity, c.m.a.p. amplitude declined (mean±S.D.) 77·5±7·0% and 67·0±6·3% at 100 and 50 Hz respectively, but only 9·9±10·5% and 3·1±12·6% at 20 and 10 Hz. Relaxation rate slowed independently of stimulation frequency.

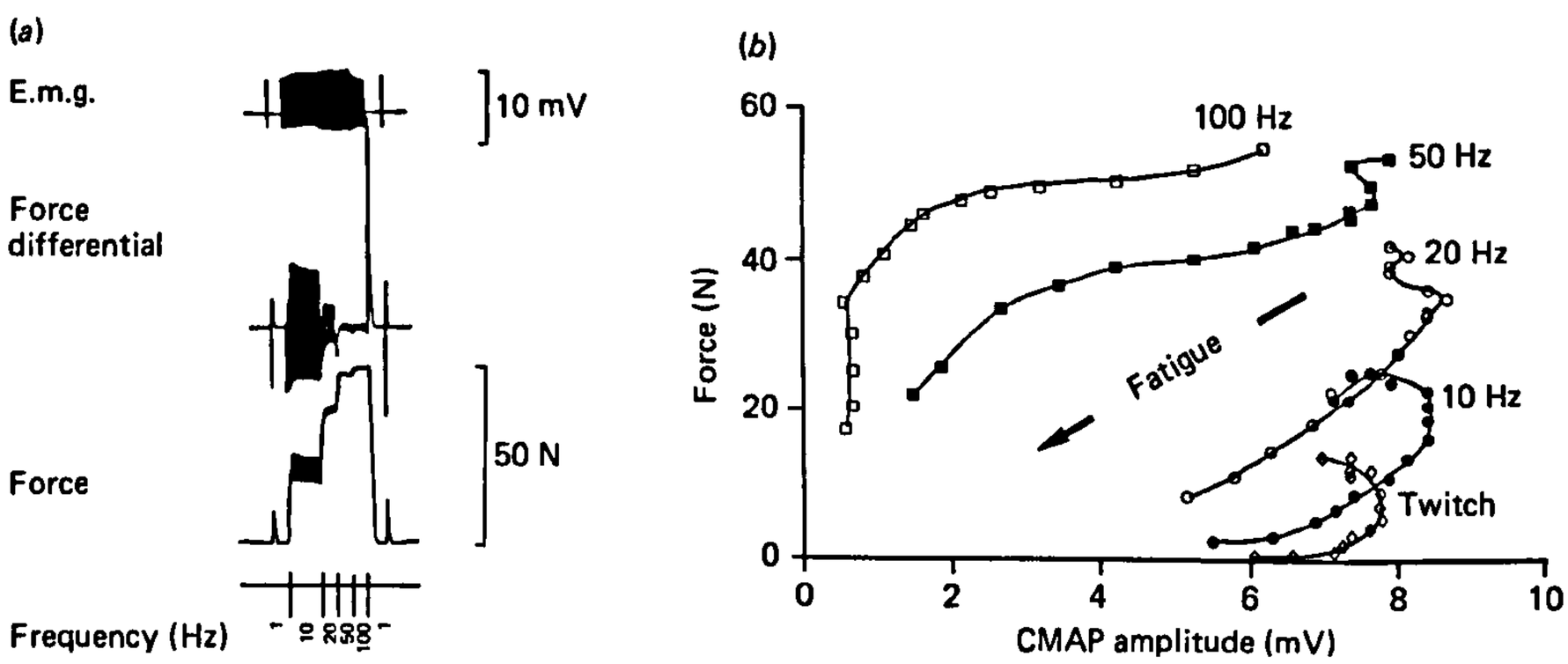

Fig. 1.(*a*). 'PSEM'; (*b*), Absolute values for force and c.m.a.p. for one subject.

Thus at high frequencies force generation is well maintained despite marked loss of excitation, whereas at low frequencies there is marked force loss with little reduction in c.m.a.p. (Figure 1*b*). This seems to indicate that, at high frequencies, a 'safety factor' is operating to maintain force.

Supported by ICI Pharmaceuticals and the Muscular Dystrophy Group of Great Britain.

REFERENCES

Bigland-Ritchie, B., Johansson, R., Lippold, O. C. J., Smith, S. & Woods, J. J. (1983). *J. Physiol.* **340**, 335–346.
Hultman, E. & Sjoholm, H. (1983). *J. Physiol.* **339**, 33–40.

[*From the Proceedings of the Physiological Society*, 27–28 *March* 1987
Journal of Physiology, **390**, 156*P*, 1987]

Human muscle fatigue: effects of ischaemia, perfusion and B-adrenergic blockade

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It is unclear whether increased fatigue experienced during B-blockade (B-b) (Lewis, Jackson & Ramsay, 1984) is peripheral, due to effects on neuromuscular junction and/or contractile apparatus, or due to its effects on the central nervous system. This study examines contractile properties of adductor pollicis (AP) in normal subjects (eight male, one female) both before and during administration of propranolol 80 mg thrice daily (72 hours of therapy was ingested prior to testing).

Supramaximal stimulation was delievered via the ulnar nerve at frequencies of 1, 10, 20, 50 and 100 Hz for 1 s each. Simultaneous recordings of force, maximal relaxation rate (MRR, 10% m s^{-1}) and compound muscle action potential (c.m.a.p.) amplitude were termed the programmed stimulation electromyogram (PSEM). Fatiguing activity consisted of 15 PSEMs 30 s apart with ischaemia and 50 PSEMs 5 s apart without occlusion. B-b was assessed by cycle ergometry (Coltart & Shand, 1970).

B-b was significant at all work rates, e.g. at termination of ergometry, 210–240 W, heart rate (mean ± 1 S.D.) was 130 ± 4/min compared to 202 ± 7/min without B-b (paired t test, $P < 0{\cdot}001$). The MRR in fresh muscle was reduced slightly, $11{\cdot}7 \pm 0{\cdot}6$ compared to $12{\cdot}2 \pm 0{\cdot}8$ without B-b ($P < 0{\cdot}05$). Force, c.m.a.p. amplitude and MRR declined identically during activity with and without B-b. Declines in force and c.m.a.p. were of similar pattern for perfused and ischaemic muscle activity, but of greater degree in the latter (Fig. 1*A* and *B*).

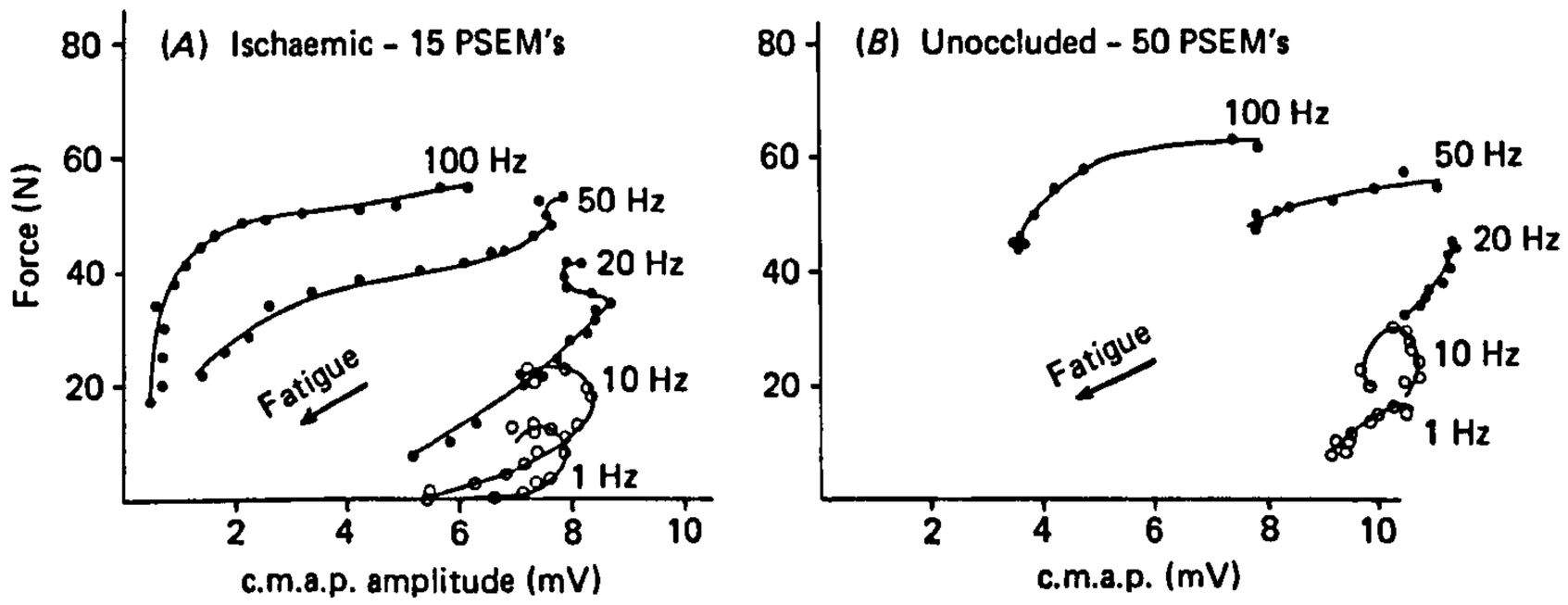

Fig. 1. Force and c.m.a.p. amplitude in one subject: (*A*) ischaemic and (*B*) unoccluded.

The marked reduction in heart rate during B-b was not accompanied by significantly altered skeletal muscle function. Thus increased fatigue in association with B-b is not due to peripheral causes.

Supported by ICI Pharmaceuticals and the Muscular Dystrophy Group of Great Britain.

REFERENCES

Coltart, D. T. & Shand, D. G. (1970). *Br. med. J.* **3**, 731–734.
Lewis, R. V., Jackson, P. R. & Ramsay, L. E. (1984). *Br. J. Clin. Pharmacol.* **18**, 325–330.

Journal of Physiology (1988), **397**, *pp.* 585–599
With 7 text-figures
Printed in Great Britain

HUMAN MUSCLE FATIGUE: FREQUENCY DEPENDENCE OF EXCITATION AND FORCE GENERATION

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(*Received 26 March 1987*)

SUMMARY

1. Human adductor pollicis was fatigued using intermittent trains of programmed stimulation at 1, 10, 20, 50, 100 and 1 Hz, during activity with and without circulatory occlusion, to investigate the relationships between force generation, excitation and maximal relaxation rate (MRR).

2. The relationship between force generation and excitation was markedly dependent on stimulation frequency. Force loss was greatest at low frequencies, with little reduction in excitation, but as frequency increased force was well maintained despite marked loss of excitation.

3. Changes in MRR during activity and recovery were independent of stimulation frequency.

4. Marked increases of force at 1 Hz (pre-tetanic) and 10 Hz occurred, with little reduction in excitation, during activity with and without circulatory occlusion. This may be due to post-tetanic potentiation in addition to slowing of relaxation (MRR).

5. At high frequency a 'safety factor' may thus operate to maintain force, despite obvious loss of excitation, while at low frequencies there may be marked potentiation of force, despite unchanged excitation. These mechanisms could permit resistance to fatigue with muscle function remaining optimal over a range of conditions.

INTRODUCTION

The mechanisms and site of human muscle fatigue remain poorly understood. During isometric contractions, loss of force (fatigue) may be due to failure of electrical excitation or energy supply, as reviewed by Edwards (1981).

Previous studies of human muscle demonstrate that, during both maximal voluntary contractions (Stephens & Taylor, 1972; Komi & Rusko, 1974; Jones, Bigland-Ritchie & Edwards, 1979; Bigland-Ritchie, Johansson, Lippold, Smith & Woods, 1983) and during stimulated fatiguing exercise (Hultman & Sjöholm, 1983; Fitch & McComas, 1985), force and muscle action potential decline in parallel. These studies appear to indicate that force failure is a consequence of excitation failure.

Force and excitation do not always decline simultaneously however. During maximal voluntary contractions (MVC) of human adductor pollicis, continuing until

severe fatigue is induced, superimposed single twitches produced large action potentials but no increases in force (Merton, 1954). Similarly, during intermittent, voluntary, ischaemic activity of human adductor pollicis the force generated by stimulated twitches between contractions declined more than their evoked action potentials (Mills, 1982). During MVCs of the human first dorsal interosseus, force and smooth rectified electromyogram (EMG) initially declined in parallel only to separate later with force declining more rapidly (Stephens & Taylor, 1972). Furthermore, force and action potential may decline in parallel during fatiguing activity but then separate during recovery, with action potential recovering more rapidly than force in both human quadriceps (Hultman & Sjöholm, 1983) and adductor pollicis (Wiles & Edwards, 1982). Following fatiguing activity, force generation at high frequency rapidly returns towards normal but at low frequency may remain depressed for some hours. This 'low-frequency fatigue' may be detected by a reduction in the ratio of forces at 20 and 50 Hz (Edwards, Hill, Jones & Merton, 1977). Since excitation rapidly returns towards normal this phenomenon presumably originates from changes beyond the sarcolemmal membrane. These previous data suggest that force failure is not simply a consequence of excitation failure. There has also been some debate as to whether fatigue is related to the number of stimuli (Marsden, Meadows & Merton, 1983) or is dependent on frequency of excitation (Garland, Garner & McComas, 1986).

The present paper aims to clarify the discrepancies in previous findings by further investigating the interrelationship between excitation and force generation during and after fatiguing activities using standard sequences of stimulated contractions at different frequencies. Preliminary results have been communicated to the Physiological Society (Cooper, Edwards, Gibson & Stokes, 1987*a*, *b*).

METHODS

Experimental subjects

Nine normal subjects (eight male, one female) aged 25–34 years gave their informed consent for participation in this study which was approved by the Local Health Authority Ethics Committee. There was no history of muscle weakness or ingestion of drug therapies in any subject.

Contractile properties of muscle

At the commencement of all experiments, muscle temperature was standardized, by warming the hand and forearm in a water-bath at 45 °C for 10 min, and temperature maintained throughout the experiment with a lamp (Edwards, Young, Hosking & Jones, 1977; Wiles & Edwards, 1982). Stimulated twitches (1 Hz) were used to locate the optimum site and voltage for supramaximal stimulation of the ulnar nerve at the wrist to produce contractions of the adductor pollicis. The stimulator (Devices 3072) was computer driven (Apple II) and delivered trains of stimuli (pulse width 50 μs) in a set pattern of frequencies viz. 1, 10, 20, 50, 100 and 1 Hz for 1 s each (10 Hz for 2 s). The isometric force produced was measured by a strain gauge connected to the proximal phalanx of the thumb via an inextensible band, in an analogous fashion to that of Merton (1954). The force signal was amplified and displayed on an oscillograph (Fig. 1). The relaxation rate was calculated by using a 'force-differentiation' signal. This was generated from the force signal which was electronically differentiated with respect to time and displayed on a second channel on the oscillograph. The differentiator was calibrated against ramps of known slope, produced by a waveform generator (Servomex, LF 141). A differential deflection of 1 cm was equivalent to a ramp slope of 10·02 cm s^{-1}. The maximal relaxation rate (MRR) from a given plateau force was calculated as 10·02 × (force differential deflection/force deflection), see Fig. 1, and gave the maximum percentage of plateau force lost in 10 ms (Wiles, Young, Jones & Edwards, 1979).

Surface electromyography (EMG) was recorded from an electrode over adductor pollicis (Mills, 1982) to measure the compound muscle action potential (CMAP) amplitude. This signal was displayed on a third channel of the oscillograph. Simultaneous recordings of force, force differential and CMAP amplitude were termed the programmed stimulation electromyogram or 'PSEM' (Fig. 1).

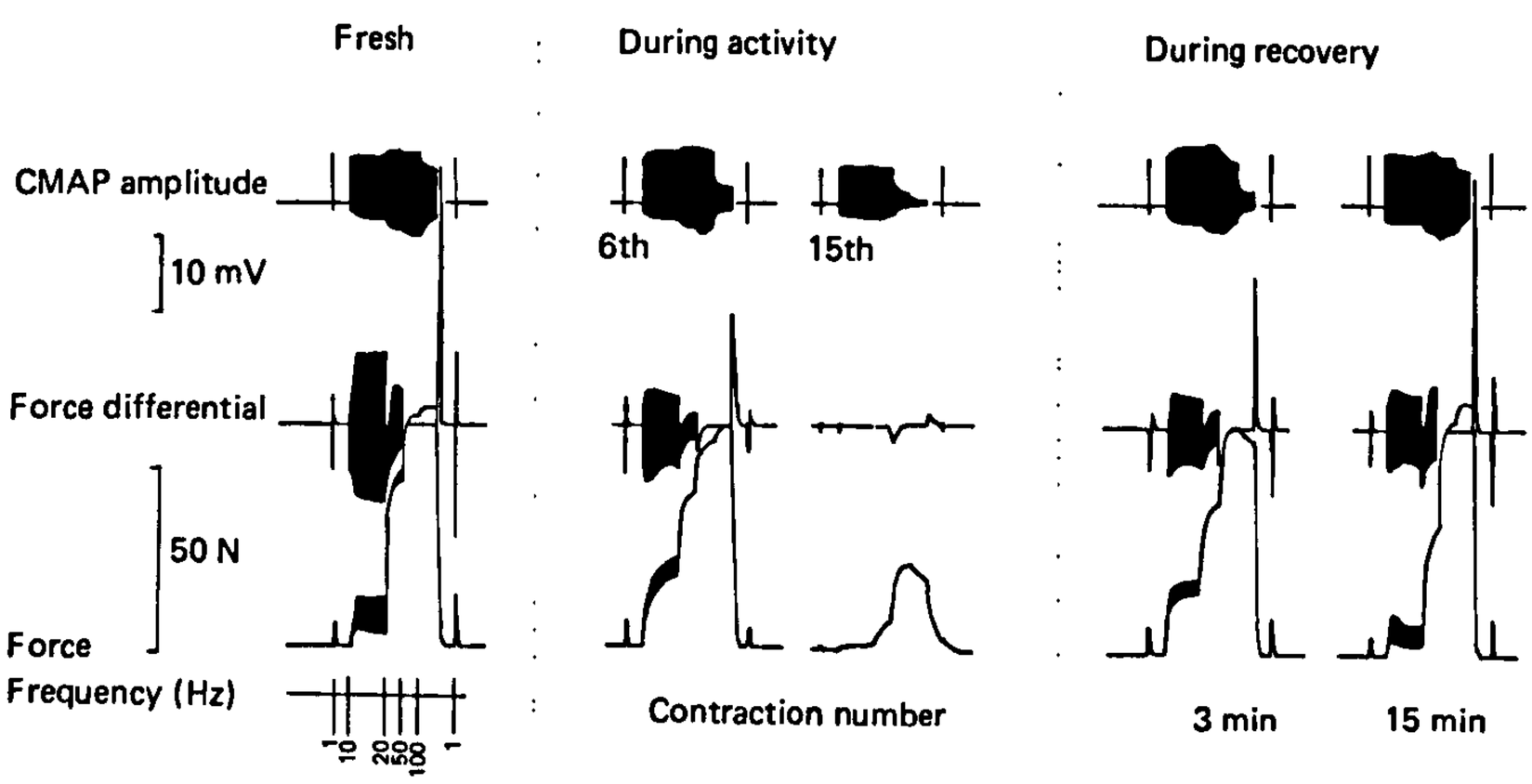

Fig. 1. Simultaneous recordings of compound muscle action potential (CMAP) amplitude, force and force differential, producing the programmed stimulation electromyogram (PSEM). The PSEM from fresh muscle clearly demonstrates post-tetanic potentiation of the twitch. Changes in the PSEM during fatiguing activity and recovery are also demonstrated. (These records are tracings from original recordings.)

Experimental protocols

Activity with arterial occlusion. In each subject, a control PSEM was performed in fresh muscle. A sphygmomanometer cuff on the upper arm was then inflated to 220 mmHg to occlude arterial circulation. A 3 min period of ischaemic rest followed by which time 50% oxygen depletion occurs thus minimizing oxidative metabolism at the commencement of activity (Harris, Hultman, Kaijser & Nordesjo, 1975). Fatiguing activity then commenced and consisted of PSEMs, repeated at intervals of 30 s until fifteen had been completed. The cuff was then deflated and aerobic recovery monitored using the PSEM at intervals of 0·5, 1, 2, 3, 5, 10 and 15 min after the end of activity. Figure 1 demonstrates the typical appearance of the PSEM in fresh muscle and during fatiguing activity and recovery.

Activity without arterial occlusion. A control PSEM was performed in fresh muscle and a 3 min period of rest followed. Fatiguing activity then commenced and consisted of PSEMs, repeated at intervals of 12 s until fifty had been completed. The larger number of PSEMs, and the smaller interval between each, were found necessary to induce force failure. Aerobic recovery was monitored using the PSEM at intervals of 0·5, 1, 2, 3, 5, 10 and 15 min after the end of activity.

These protocols were performed in random order and at least 1 week apart to ensure full recovery of function. The appearances of the PSEM changed in similar fashion to that during activity with circulatory occlusion, but to a lesser degree.

Analysis

On each PSEM the force and CMAP amplitude at each frequency was measured and the MRR from 100 Hz plateau force calculated and expressed as percentages of the equivalent part of the control PSEM. Measurements were made on each PSEM during activity with circulatory occlusion but on every 5th PSEM during activity without occlusion. Frequency: force curves were calculated for fresh muscle and at intervals during activity by expressing all force values as a percentage of that at 100 Hz in fresh muscle. The relationship between force and excitation was assessed using

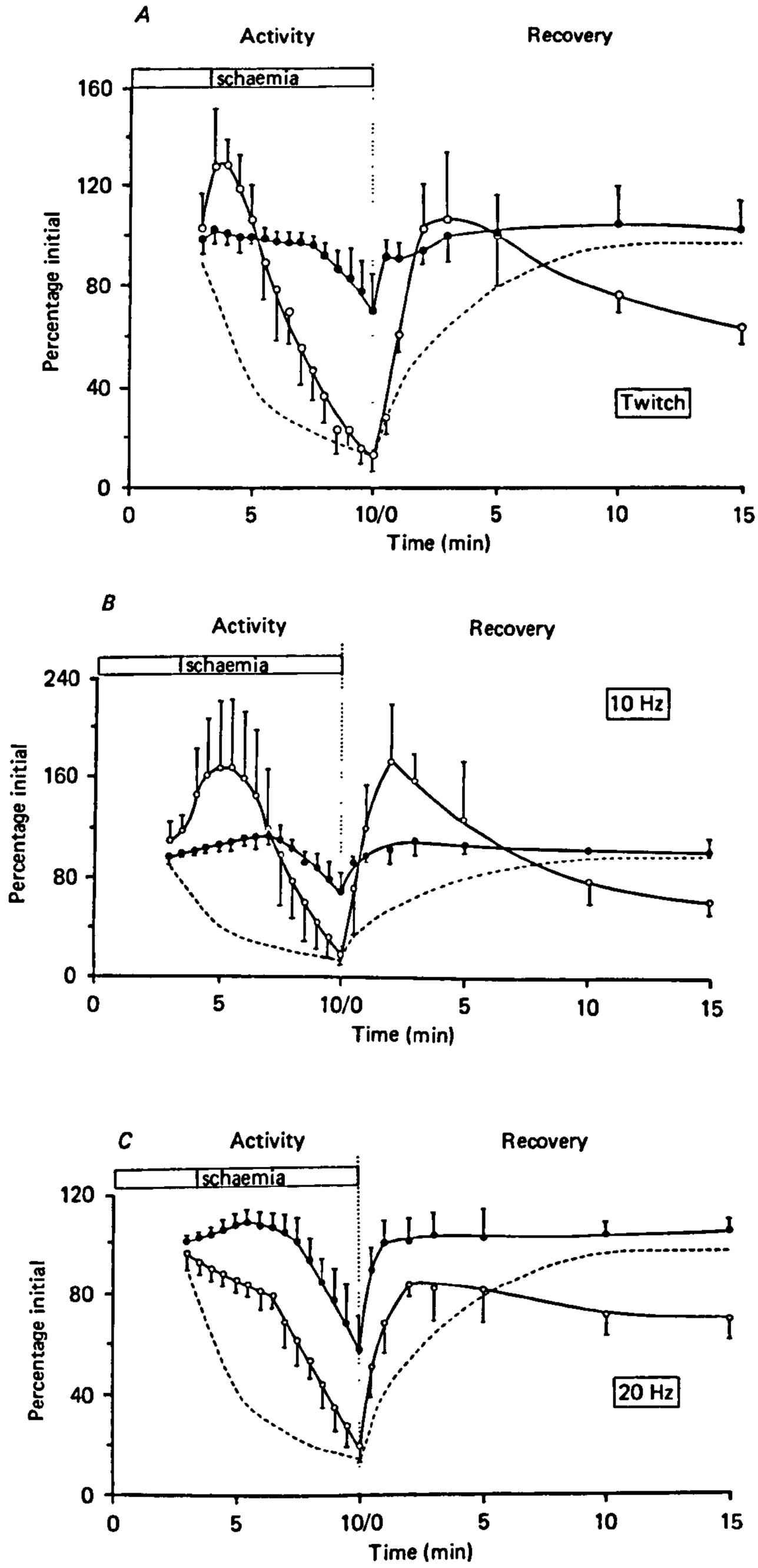

Fig. 2*A–C*. For legend see opposite.

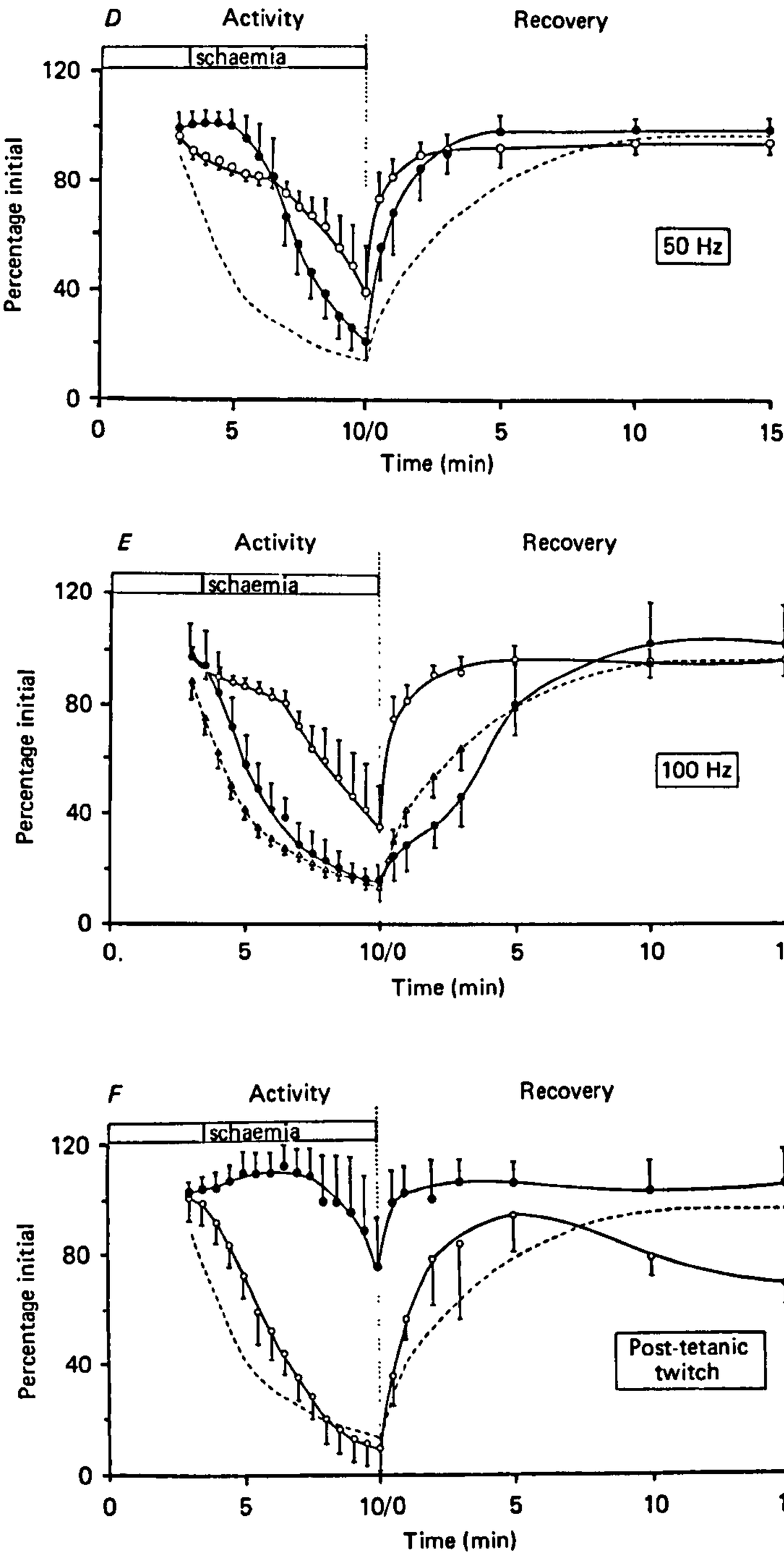

Fig. 2. Time course of changes of force (○) and CMAP amplitude (●) during activity with arterial occlusion (fifteen PSEMs at 30 s intervals) and recovery with intact circulation at various stimulation frequencies. Mean relative changes in MRR at 100 Hz are represented by a dotted line on each range, except at 100 Hz where mean ± 1 S.D. are shown.

the 'force per excitation' (F/E) ratio, calculated for each frequency as follows: force as percentage of force in fresh muscle/excitation (CMAP amplitude) as percentage of that for fresh muscle. This ratio is, by definition, unity (i.e. $F/E = 1$) in fresh muscle. All of the results reported in the text, and those used to construct Figures, are expressed as mean ± 1 S.D., and $n = 9$ unless stated otherwise.

TABLE 1. Force and CMAP amplitude (as percentage of fresh muscle) and F/E ratios at each frequency at the end of activity with and without circulatory occlusion

Activity	Force (%)	CMAP amplitude (%)	F/E ratio
	With arterial occlusion		
Frequency (Hz)			
1	13 ± 4	70 ± 15	0·21 ± 0·05
10	18 ± 7	68 ± 15	0·26 ± 0·17
20	19 ± 7	57 ± 15	0·32 ± 0·09
50	40 ± 16	21 ± 8	1·78 ± 0·48
100	35 ± 17	15 ± 6	2·4 ± 0·96
1	10 ± 5	75 ± 18	0·14 ± 0·05
	Without arterial occlusion		
1	71 ± 15	81 ± 22	0·94 ± 0·24
10	93 ± 31	90 ± 18	1·03 ± 0·36
20	66 ± 7	91 ± 11	0·73 ± 0·11
50	76 ± 5	68 ± 19	1·19 ± 0·34
100	72 ± 7	42 ± 9	1·59 ± 0·44
1	49 ± 9	73 ± 19	0·67 ± 0·16

Mean ± 1 S.D., $n = 9$

RESULTS

Activity with arterial occlusion

During fatiguing activity, force loss occurred at all stimulation frequencies (Fig. 2*A–F*). The degree of force loss clearly depended on stimulation frequency being greater at low than at high frequencies (Table 1). These changes in force, during activity, produced obvious alterations in the frequency: force curve (Fig. 3). The 20/50 Hz ratio fell from 0·70 ± 0·06 in fresh muscle, to 0·35 ± 0·07 at the end of activity.

These declines in force were not associated with equivalent declines in CMAP amplitude (Table 1; Fig. 4*A* and *B*). In contrast to force, CMAP amplitude declined more at high than at low frequencies e.g. for a 50% force loss during activity, CMAP amplitude declined by only 3·1 ± 12·6 and 9·9 ± 10·5% at 10 and 20 Hz respectively, but by 67·0 ± 6·3 and 77·5 ± 7% at 50 and 100 Hz respectively. Changes in the F/E ratio may be caused by changes in excitation and/or force, as shown in Fig. 4*A* and *B*.

The MRR at 100 Hz declined more rapidly than either force or CMAP amplitude, at all frequencies (Fig. 2*A–F*), to 13 ± 5% of its fresh value. The MRR could be measured only for 100 Hz during the PSEM. The time course of slowing and recovery of MRR at 100 Hz, relative to fresh values, is plotted as a dotted line on each panel in Figs 2 and 6 for comparison with force and CMAP amplitude at each frequency. In a separate study of five of the subjects, the PSEM was split by a 0·5 s pause after 10, 20 and 50 Hz during activity to enable the MRR to be calculated at all

frequencies. This demonstrated slight differences in absolute values of MRR at different frequencies but the rates of slowing and recovery were similar at all frequencies (Fig. 5). The frequency dependence of force loss may not therefore be attributed to changes in MRR.

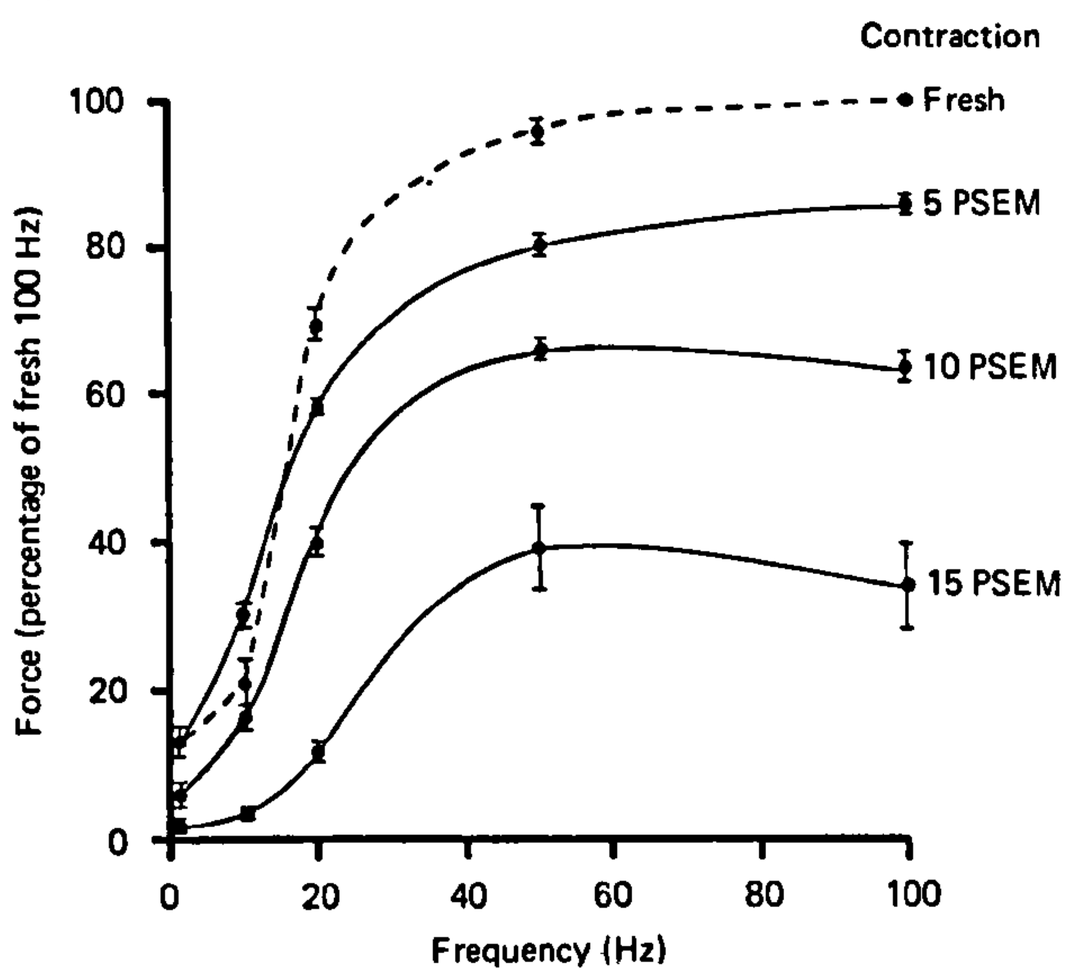

Fig. 3. Frequency:force curves during ischaemic fatiguing activity. Potentiation at low frequency moves curve to left by 5th PSEM; subsequent low-frequency fatigue moves curve to right by 10th PSEM. Progressive high-frequency fatigue moves upper segment of curve progressively downwards.

Aerobic recovery

The immediate effect of reperfusing the muscle was a rapid but incomplete recovery of MRR (Fig. 5) and of force and excitation (Fig. 2*A–F*) at all frequencies. Subsequent rates of recovery of force and CMAP amplitude depended on frequency. The initial rapid recovery of force at high frequency was sustained (Fig. 2*D* and *E*) but there was subsequent low-frequency fatigue (Fig. 2*A–C* and *F*) with F/E ratios < 1 (Fig. 4*A* and *B*). The 20/50 Hz ratio remained reduced, being $0{\cdot}53 \pm 0{\cdot}06$ at 15 min. Excitation at 1, 10, 20 and 50 Hz had returned to normal by 5 min, whereas at 100 Hz there was an initial delay with full recovery only ensuing after 10 min (Fig. 2*E*). The rate of recovery of MRR was slower than that of force or EMG (except for EMG at 100 Hz), with full recovery by 10 min.

Activity without arterial occlusion

During fatiguing activity without arterial occlusion, force loss occurred at all stimulation frequencies except 10 Hz. The declines in force were clearly less than during ischaemic activity but demonstrated a similar frequency dependence (Fig. 6*A–F*). Force was, in general, more severely hampered at low than at high frequencies (see Table 1). The 20/50 Hz ratio fell from $0{\cdot}72 \pm 0{\cdot}05$ in fresh muscle, to $0{\cdot}63 \pm 0{\cdot}07$ at the end of activity.

The declines in force were not associated with equivalent declines in CMAP amplitude which declined more at high than at low frequencies (Table 1; Fig. 6*A–F*).

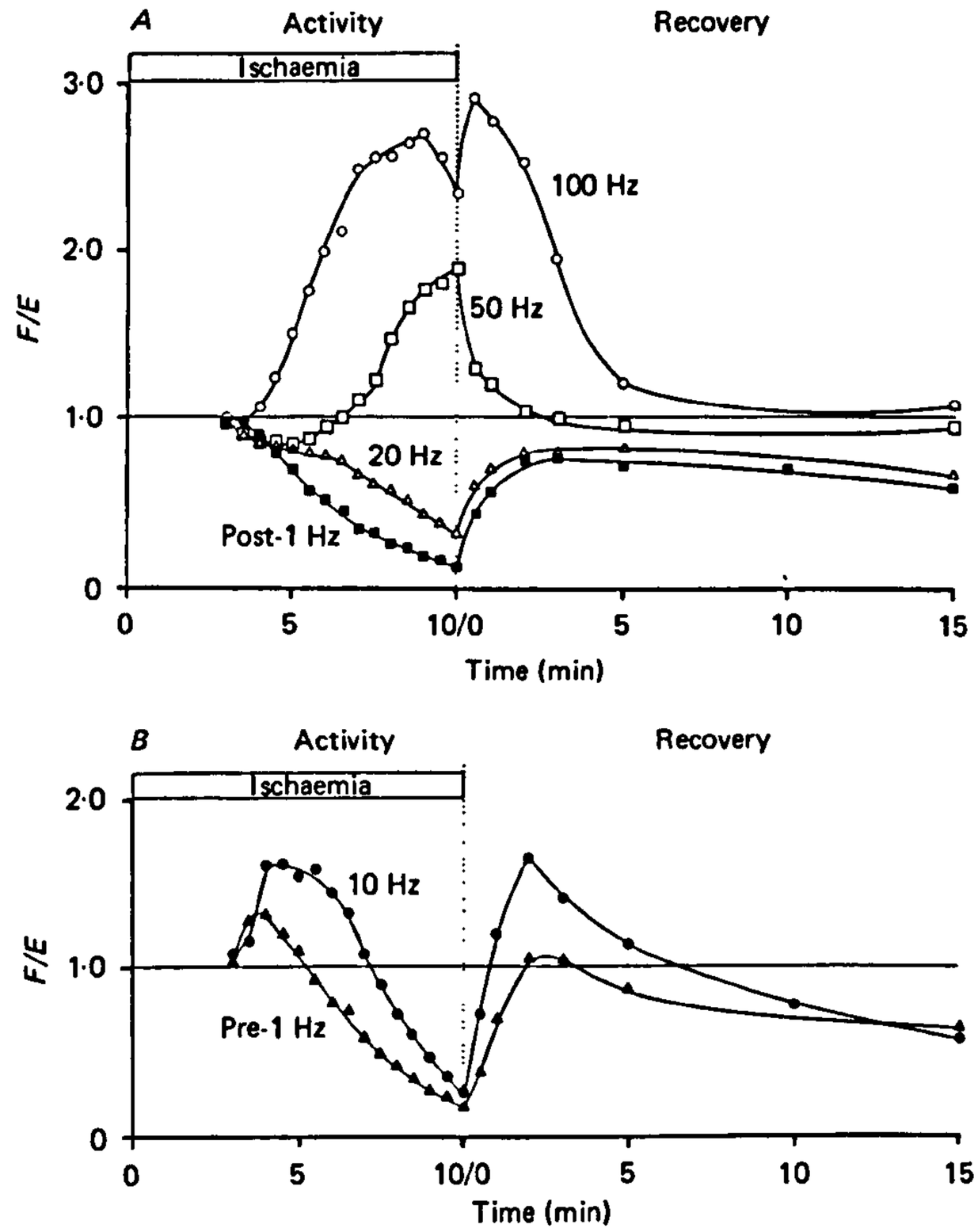

Fig. 4. Mean F/E ratios, during activity with arterial occlusion and recovery with intact circulation. *A*, during activity, the F/E ration is > 1 at high frequency due to excitation failure in excess of force failure but is < 1 at some low frequencies, due to force failure in excess of excitation failure. *B*, during activity at 1 Hz pre-twitch and 10 Hz, however, the F/E ratio is > 1 due to potentiation of force, and then becomes < 1 due to low-frequency fatigue. Similar potentiation occurs during recovery but there is eventual low-frequency fatigue.

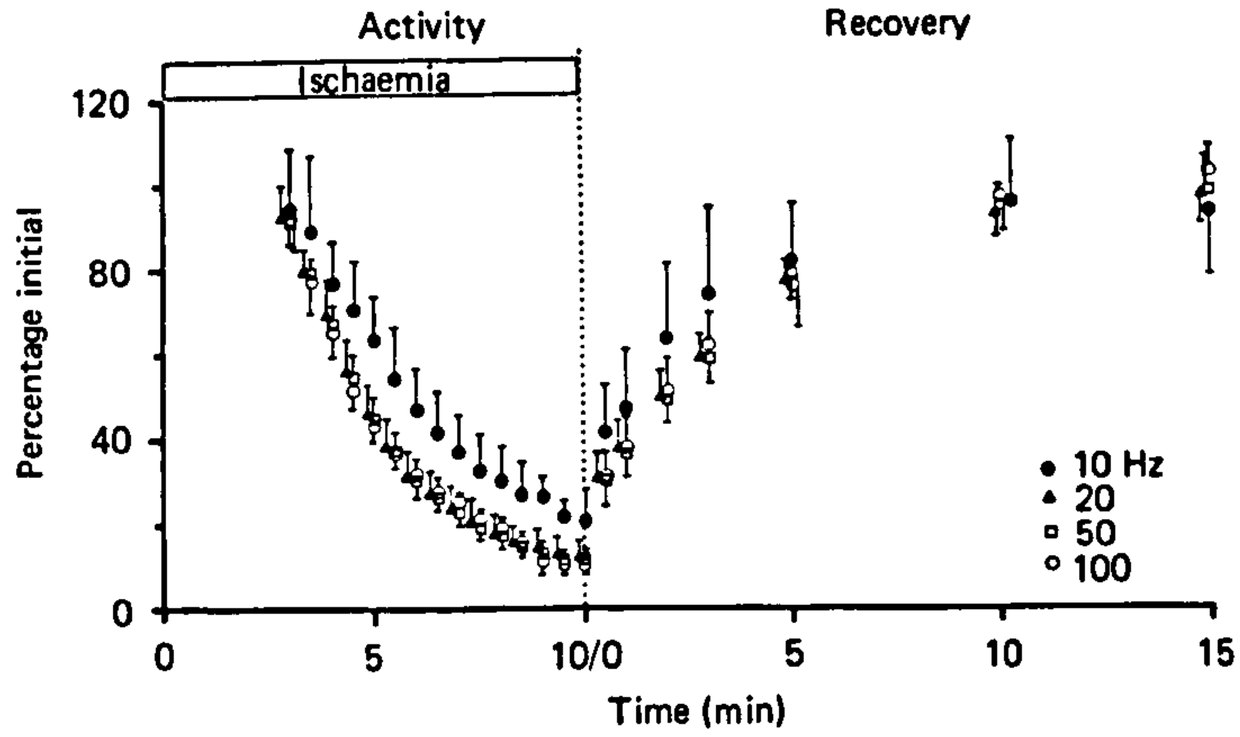

Fig. 5. Declines in MRR at various frequencies: during PSEMs (interrupted after the test frequency) at intervals of 12 s, with arterial occlusion and recovery with intact circulation. This demonstrates that MRR slowing is independent of stimulation frequency. Results mean ± 1 S.D., $n = 5$.

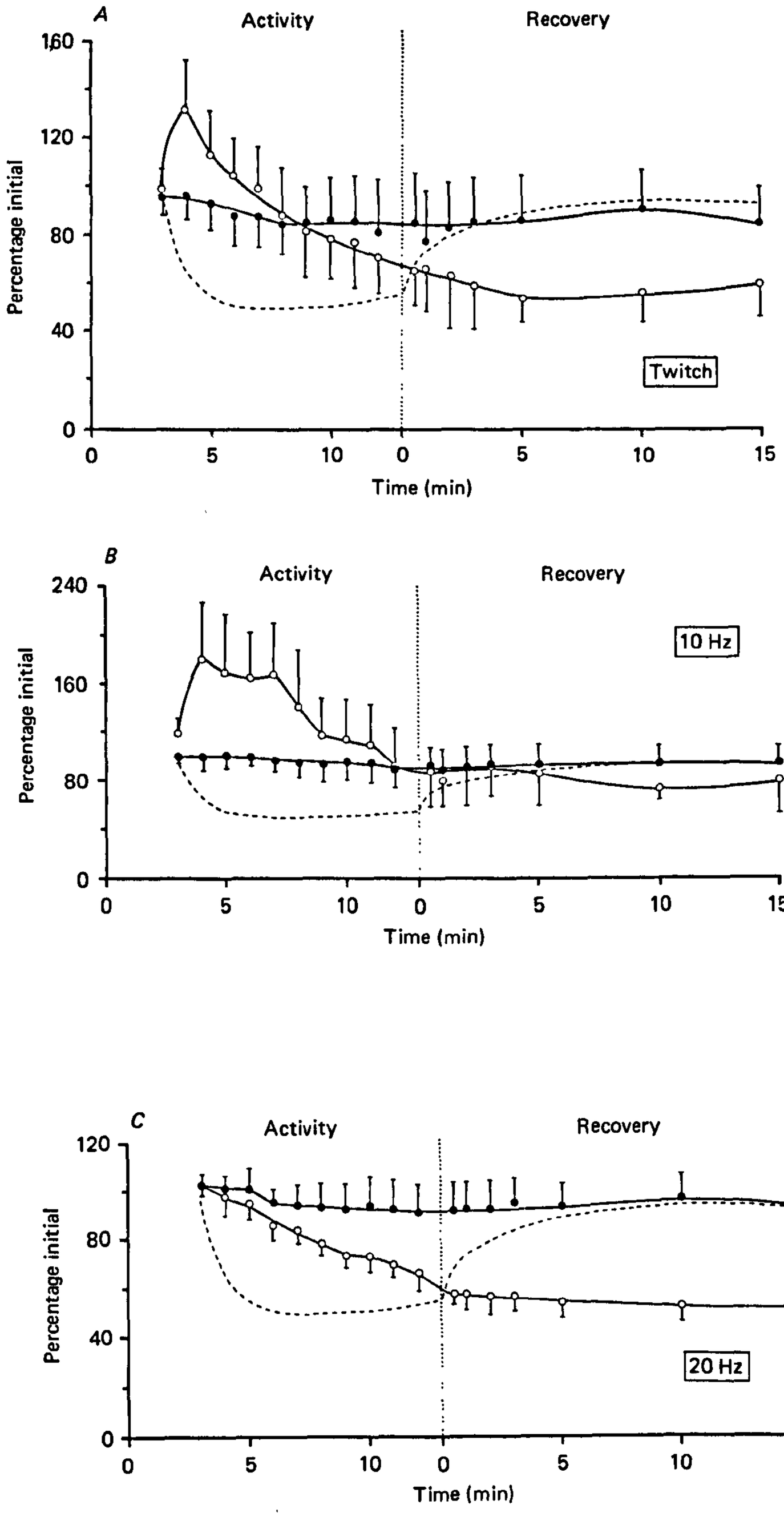

Fig. 6*A–C*. For legend see opposite.

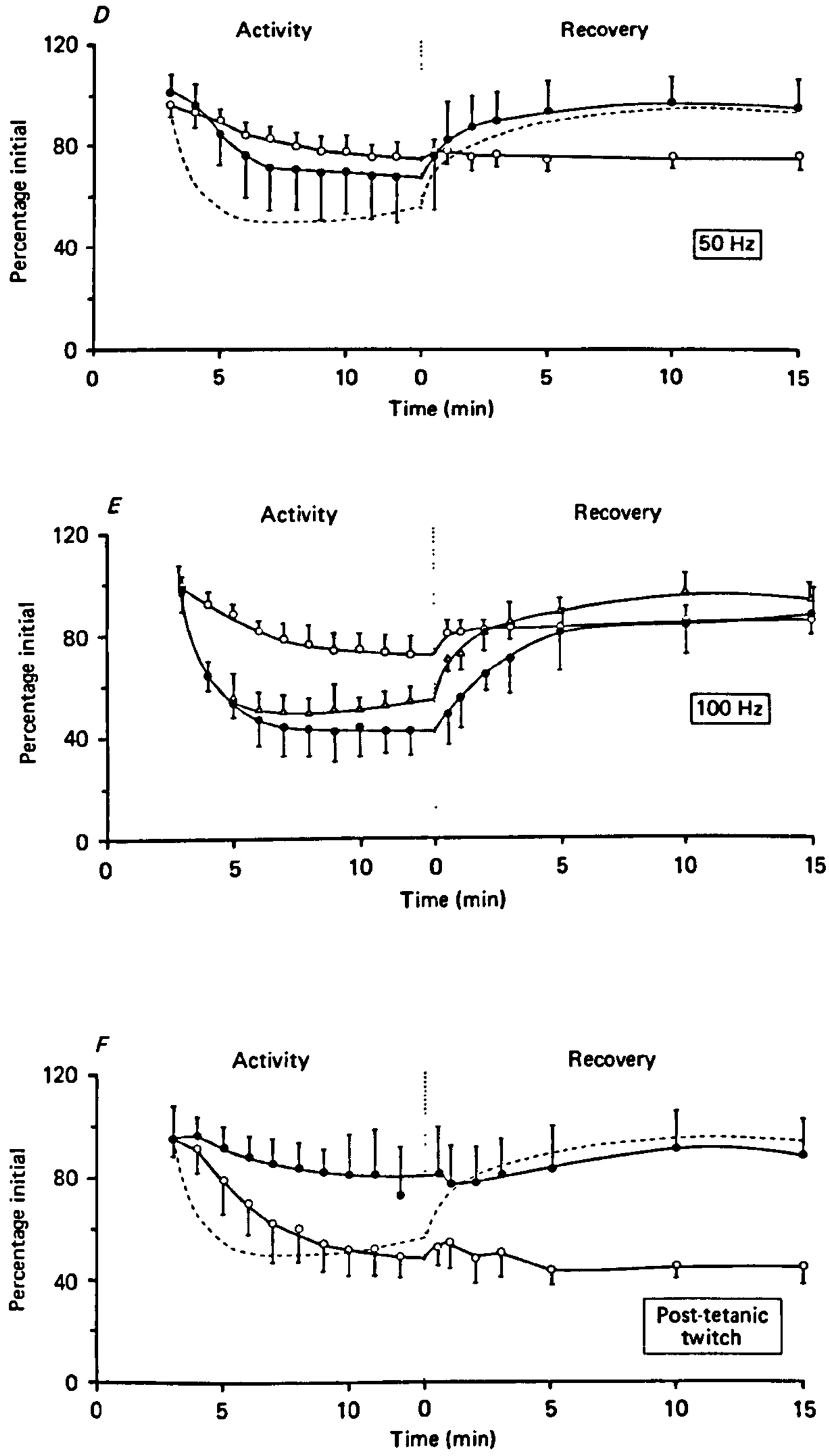

Fig. 6. Time courses of changes of force (○) and CMAP amplitude (●) at various frequencies during activity without circulatory occlusion (fifty PSEMs at intervals of 12 s) and recovery without occlusion. Mean changes in MRR at 100 Hz are represented by a dotted line on each panel, except at 100 Hz where mean ± 1 S.D. are shown. The 1st and every 5th subsequent PSEM were measured.

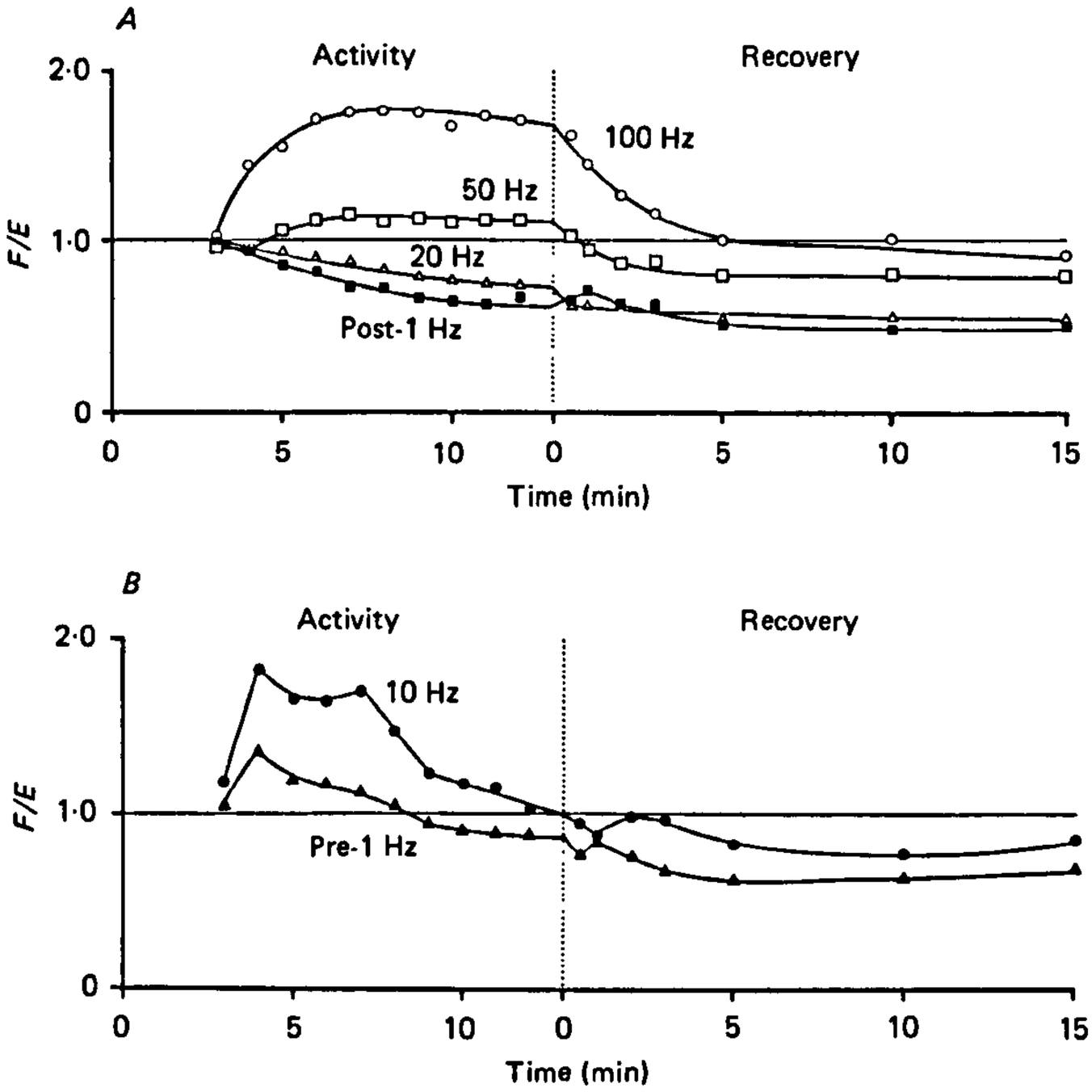

Fig. 7. Mean F/E ratios during activity and recovery without arterial occlusion. *A*, the F/E ratio was > 1 at high frequencies due to reduced excitation, while at some lower frequencies the ratio was < 1 due to excess force failure. *B*, for the 1 Hz pre-tetanic twitch and 10 Hz, the ratio was initially > 1 due to potentiation of force which was not seen during recovery when there was force failure despite normal excitation (low-frequency fatigue).

Frequency:force curves, which are not shown, resembled those seen for ischaemic activity (Fig. 3) but with less high-frequency fatigue. Changes in the F/E ratio are summarized in Fig. 7*A* and *B*. The changes in force, CMAP and the F/E ratio were clearly less than occurred during activity with circulatory occlusion (Table 1).

The MRR at 100 Hz declined more rapidly than either force or CMAP amplitude at all frequencies (Fig. 6*A–F*) to $54 \pm 5\%$.

Aerobic recovery

At low frequencies, force failure persisted at 15 min indicating that low-frequency fatigue had been induced. At high frequencies (50 and 100 Hz) there was rapid initial recovery of force but this was incomplete at 15 min. The 20/50 ratio was reduced to $0{\cdot}50 \pm 0{\cdot}06$ at 15 min and the F/E ratios were < 1 at all frequencies except 100 Hz (7*A* and *B*). Excitation at all frequencies had returned to near normal and MRR was fully recovered within 10 min.

DISCUSSION

Frequency dependence of force and excitation

This study demonstrates that the relationship between force generation and excitation differs markedly according to stimulation frequency and the degree of fatigue or recovery. Changes in force, CMAP amplitude and MRR were in the same direction during activity with ischaemia and with circulation unoccluded but were usually of greater degree with ischaemia. At the end of activity and during recovery obvious reductions in force at low frequencies (1, 10 and 20 Hz) with little loss of excitation ($F/E < 1$) suggests a dissociation between sarcolemmal action potential propagation and eventual myofibrillar force generation. This could result from impairment of sarcoplasmic Ca^{2+} release or myofibrillar Ca^{2+} sensitivity. Fatiguing activity elevates inorganic phosphate (P_i) (Dawson, Gadian & Wilkie, 1980). Since increases in myoplasmic P_i reduce force generation, in the absence of alterations in Ca^{2+} concentration (Brandt, Cox, Kawai & Robinson, 1982), this implies that low-frequency fatigue may be due to P_i-induced myofibrillar Ca^{2+} insensitivity. However, this cannot be the explanation for the obvious persistance of low-frequency fatigue (Edwards *et al.* 1977) since studies of muscle chemistry by ^{31}P-magnetic resonance spectroscopy (Edwards, Griffiths & Cady, 1985) show that P_i has returned to normal values within minutes, not hours, of cessation of activity.

At high frequency (50 and 100 Hz) force was well maintained despite an obvious relative loss of excitation ($F/E > 1$). This implies the existence of a 'safety factor' within which action potential amplitude may decline markedly without effect on force generation. This requires explanation. During continued high-frequency tetanic stimulation, myoplasmic Ca^{2+} concentrations continue to rise despite attainment of plateau force (Blinks, Rüdel & Taylor, 1978). This rise in Ca^{2+} concentration may act to maintain myofibrillar activation despite increasing Ca^{2+} insensitivity as a result of P_i changes. Excitation rates are probably not maintained at the 50–100 Hz level during voluntary contractions so this 'safety factor' may have limited physiological relevance. However, the changes in F/E ratio during an MVC of the first dorsal interosseus (Stephens & Taylor, 1972) were as predicted by the present results, i.e. initially $F/E > 1$ (due to relative excitation failure) but changing to values < 1 (due to relative force failure) as lower firing frequencies become more important.

The discrepancies in previous reports are only apparent, and have arisen because these studies only examined single frequencies, since they all agree with the present findings. Thus at 1 Hz, during fatigue, force declined to a greater degree than CMAP amplitude (Fig. 2*A* and *E*), this being previously observed during maximal voluntary contractions (Merton, 1954) or between intermittent ischaemic contractions (Mills, 1982) of adductor pollicis. Force and excitation declined in parallel (e.g. Figs 2*C* and 6*D*) as previously observed during voluntary contractions by Stephens & Taylor (1972), Komi & Rusko (1974), Jones *et al.* (1979) and Bigland-Ritchie *et al.* (1983) and stimulated contractions at 20 Hz (Hultman & Sjöholm, 1983; Fitch & McComas, 1985). The separation of force and excitation with prolonged activity (e.g. Figs 2*F* and 6*F*) was observed by Stephens & Taylor (1972), and the faster recovery of excitation than force (e.g. Figs 2*C* and 6*C*) was observed

by Wiles & Edwards (1982) and Hultman & Sjöholm (1983). Garland *et al.* (1986) found that fatigue was frequency dependent demonstrating greater reductions in force at 15 Hz than at 30 Hz (after 1200 impulses at each frequency), while the action potential amplitude was only modestly reduced at these low frequencies.

Potentiation of force at low stimulation frequency

In the present study, the relationship between force generation and excitation changed during activity because of potentiation. Thus there was little change in excitation at 10 Hz while the force was initially markedly potentiated (when $F/E > 1$) before declining precipitately (when $F/E < 1$).

Potentiation at low stimulation frequency was previously described, after MVCs of adductor pollicis, and attributed to slowing of MRR causing increased fusion with increased mean force (Bigland-Ritchie, Johannson, Lippold & Woods, 1983; Hultman & Sjöholm, 1983). In the present study, the decline in MRR during ischaemic activity might thus explain the potentiation of force at 10 Hz. However during recovery, when the MRR was *increasing* (Fig. 2*B*), there was again marked potentiation of force at 10 Hz (Fig. 2*B*). Thus, slowing of the MRR as the sole cause of potentiation appears unlikely.

Effect of ischaemia on fatigability

During activity with arterial occlusion, force at all frequencies declined considerably more than without occlusion confirming many previous studies (Merton, 1954; Stephens & Taylor, 1972). Action potential propagation failure may result from impairment of the membrane Na^+–K^+-ATPase, as a consequence of reduced energy supply or accumulation of metabolic waste products, while force failure may result from excitation propagation failure or from impaired Ca^{2+} release or myofibrillar Ca^{2+} sensitivity. It is not possible to drive a normal human muscle into harmful rigor, during stimulated or voluntary contractions, clearly implying that the impairments of excitation and/or myofibrillar activation may act to 'protect' the muscle as previously hypothesized (Edwards, 1983). It is thus of note that, in the present study, force and CMAP amplitude declined precipitately after the 8th or 9th PSEM (Fig. 2).

During activity without occlusion, continued supply of oxygen and substrates and disposal of metabolic products allow effective excitation and activation to proceed for longer. The more severe low-frequency fatigue and a degree of persistent high-frequency fatigue may then represent a metabolic price paid for the greater activity, and may explain the lack of force potentiation at 1 and 10 Hz (Fig. 6*A* and *B*). Since CMAP amplitude rapidly returned to normal, this fatigue was due to changes beyond the sarcolemmal membrane.

Mechanisms of resistance to fatigue

The present study has identified two mechanisms which could permit resistance to fatigue. At high frequency a 'safety factor' appears to maintain force despite marked loss of excitation, while at low frequencies (1 and 10 Hz) there is marked potentiation of force with normal excitation. It is clear that these mechanisms have altered muscle contractile properties, as characterized by the frequency : force relationship (Edwards

et al. 1977) which has previously only been examined before or after activity but is now documented during programmed activity (Fig. 3).

During normal voluntary muscular activity, a wide range of submaximal and occasional maximal contractions are required for various amounts of time. These contractions are generated by a range of frequencies of excitation within the frequency range studied. The mechanisms described may yet contribute to resistance to fatigue although the experimental model used has been unphysiological for the muscle as a whole.

We thank the subjects who took part in this study and gratefully acknowledge support from ICI Pharmaceuticals U.K. and the Muscular Dystrophy Group of Great Britain and Northern Ireland.

REFERENCES

BIGLAND-RITCHIE, B., JOHANSSON, R., LIPPOLD, O. C. J., SMITH, S. & WOODS, J. J. (1983). Changes in motoneurone firing rates during sustained maximal voluntary contractions. *Journal of Physiology* **340**, 335–346.

BIGLAND-RITCHIE, B., JOHANSSON, R., LIPPOLD, O. C. J. & WOODS, J. J. (1983). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *Journal of Neurophysiology* **50**, 313–324.

BLINKS, J. R., RÜDEL, R. & TAYLOR, S. R. (1978). Calcium transients in isolated amphibian skeletal muscle fibres; detection with aequorin. *Journal of Physiology* **277**, 291–323.

BRANDT, P. W., COX, R. N., KAWAI, M. & ROBINSON, T. (1982). Regulation of tension in skinned muscle fibres: Effect of cross-bridge kinetics on apparent Ca^{2+} sensitivity. *Journal of General Physiology* **79**, 997–1016.

COOPER, R. G., EDWARDS, R. H. T., GIBSON, H. & STOKES, M. (1987*a*). Frequency dependence of excitation and force generation of human adductor pollicis. *Journal of Physiology* **386**, 113*P*.

COOPER, R. G., EDWARDS, R. H. T., GIBSON, H. & STOKES, M. (1987*b*). Human muscle fatigue: effects of ischaemia, perfusion, and β-adrenergic blockade. *Journal of Physiology* **390**, 156*P*.

DAWSON, M. J., GADIAN, D. G. & WILKIE, D. R. (1980). Mechanical relaxation rate and metabolism studied in fatiguing muscle by phosphorous nuclear magnetic resonance. *Journal of Physiology* **299**, 465–484.

EDWARDS, R. H. T. (1981). Human muscle function and fatigue. In *Human Muscle Fatigue: Physiological Mechanisms, Ciba Foundation Symposium 82*, pp. 1–18. London: Pitman Medical.

EDWARDS, R. H. T., GRIFFITHS, R. D. & CADY, E. B. (1985). Topical magnetic resonance for the study of muscle metabolism in human myopathy. *Clinical Physiology* **5**, 93–109.

EDWARDS, R. H. T. (1983). Biochemical basis of fatigue in exercise: Catastrophe theory of muscular fatigue. In *Biochemistry of Excercise*, ed. KNUTTGEN, H. G., VOGEL, J. A. & POORTMANS, J., pp. 3–28. Champaign: Human Kinetics.

EDWARDS, R. H. T., HILL, D. K., JONES, D. A. & MERTON, P. A. (1977). Fatigue of long duration in human skeletal muscle after exercise. *Journal of Physiology* **272**, 769–778.

EDWARDS, R. H. T., YOUNG, A., HOSKING, G. P. & JONES, D. A. (1977). Human skeletal muscle function: description of tests and normal values. *Clinical Science and Molecular Medicine* **52**, 283–290.

FITCH, S. & MCCOMAS, A. (1985). Influence of human muscle length on fatigue. *Journal of Physiology* **362**, 205–213.

GARLAND, J., GARNER, S. H. & MCCOMAS, A. J. (1986). Relationship between numbers and frequencies of stimuli in human muscle fatigue. *Journal of Physiology* **384**, 33*P*.

HARRIS, R. C., HULTMAN, E., KAIJSER, L. & NORDESJO, L.-O. (1975). The effect of circulatory occlusion of isometric exercise capacity and energy metabolism of the quadriceps muscle in man. *Scandinavian Journal of Clinical and Laboratory Investigation* **35**, 87–95.

HULTMAN, E. & SJÖHOLM, H. (1983). Electromyogram, force and relaxation time during and after continuous electrical stimulation of human skeletal muscle in *situ*. *Journal of Physiology* **339**, 33–40.

JONES, D. A., BIGLAND-RITCHIE, B. & EDWARDS, R. H. T. (1979). Excitation frequency and muscle fatigue: mechanial responses during voluntary and stimulated contractions. *Experimental Neurology* **64**, 401–413.

KOMI, P. V. & RUSKO, H. (1974). Quantitative evaluation of mechanical and electrical changes during fatigue loading of eccentric and concentric work. *Scandinavian Journal of Rehabilitation Medicine Supplement* **3**, 121–126.

MARSDEN, C. D., MEADOWS, J. C. & MERTON, P. A. (1983). "Muscular Wisdom" that minimises fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. In *Motor Control Mechanisms in Health and Disease*, ed. DESMEDT, J. E., pp. 169–211. New York: Raven Press.

MERTON, P. A. (1954). Voluntary strength and fatigue. *Journal of Physiology* **123**, 553–564.

MILLS, K. R. (1982). Power spectral analysis of electromyogram and compound muscle action potential during muscle fatigue and recovery. *Journal of Physiology* **326**, 401–409.

STEPHENS, J. A. & TAYLOR, A. (1972). Fatigue of maintained voluntary muscle contraction in man. *Journal of Physiology* **220**, 1–18.

WILES, C. M. & EDWARDS, R. H. T. (1982). The effect of temperature, ischaemia and contractile activity on the relaxation rate of human muscle. *Clinical Physiology* **2**, 485–497.

WILES, C. M., YOUNG, A., JONES, D. A. & EDWARDS, R. H. T. (1979). Relaxation rate of constituent muscle-fibre types in human quadriceps. *Clinical Science* **56**, 47–52.

HUMAN MUSCLE FATIGUE: FREQUENCY INDEPENDENCE OF SLOWING OF RELAXATION RATE WITH STIMULATED ISOMETRIC CONTRACTIONS

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The rate of decline of maximum relaxation rate (MRR) during stimulated ischaemic contractions of the adductor pollicis (AP) in one subject appeared dependent on the contractile activity, up to 11 max.seconds, ie., product of force and time divided by the maximal tetanic force in fresh muscle (1). The present study further examines this relationship during stimulated ischaemic activity of AP in five healthy volunteers.

Trains of supramaximal stimulation of 2 seconds duration, separated by 0.5 seconds, were delivered via the ulnar nerve up to a total of 3600 impulses at frequencies of 20, 40, 60, 80 and 100Hz. Isometric force, compound muscle action potential (CMAP) amplitude and MRR were measured simulataneously.

The decline in MRR with contractile activity was linear and of similar degree for all frequencies up to 30 max.seconds and is described by the equation: %MRR = 100 - 2.38 x max.seconds, r=-0.979. The decline thereafter was reduced. When compared with the number of impulses, however, MRR fell more rapidly at low frequencies than high (eg., after 1600 impulses, MRR fell to (mean±SD) 22±3% at 40Hz but only to 58±4% at 100Hz). In contrast, the declines in force and CMAP amplitude were frequency dependent with regard to contractile activity rather than number of impulses.

The fall in MRR appears independent of stimulation pattern. This suggests that metabolic factors influence MRR changes rather than the number or rapidity of impulses delivered.

1. Wiles CM & Edwards RHT. Clinical Physiology. 1982:2:485-497.

153 NO ISCHAEMIC RECOVERY OF THE EVOKED COMPOUND MUSCLE ACTION POTENTIAL IN McARDLE'S DISEASE

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In McArdle's disease (myophosphorylase deficiency), the recovery of the evoked surface compound muscle action potential (CMAP) amplitude of the adductor pollicis (AP) following stimulated activity does not occur anaerobically (Edwards et al., In: Disorders of the Motor unit, Ed, Schotland D., Wiley Med, 1982). The present study examines further the anaerobic recovery of CMAP characteristics following anaerobic activity. Supramaximal stimulation of the AP was delivered via the ulnar nerve at the wrist at 20Hz for 1050 impulses in two patients with McArdle's disease. Ischaemic recovery of CMAP amplitude and conduction velocity (measured from the start of rise of the first negative phase to peak) were studied at intervals for up to one minute and compared to those in three normal subjects following the same stimulation procedure and also after 2400 impulses.

In patients, CMAP amplitude was markedly reduced to 29.4% & 53.0% and did not recover until circulation was restored. Conduction velocity did not change. In normal subjects, CMAP amplitude declined to a similar extent only after the greater activity but recovered almost immediately during ischaemic rest. Conduction velocity was reduced and plateaued at 125%, 135% and 125.5% during ischaemic rest and recovered with a slow time course on return of the circulation.

The decline in CMAP amplitude in McArdle's disease may be due to the greater Pi produced due to impaired glycolisis (N Eng J Med, 1981, 304, 1338). The slowing in conduction velocity in normal subjects may be the result of metabolite influences on membrane function, possibly H^+, rather than of ion distribution across the membrane 'per se'. The excitability of the membrane appears to recover rapidly to near normal levels in ischaemic conditions when conduction velocity does not.

Supported by ICI Pharmaceuticals and the Muscular Dystrophy Group of Great Britain.

Human muscle fatigue: post-tetanic potentiation of low-frequency tetani is not related to altered excitation or slowing of relaxation

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Resistance to fatigue during low-frequency tetani may be due to slowed relaxation (Jones, 1981). The present study examines the contribution of post-tetanic potentiation to this phenomenon by reversing frequencies during stimulated contractions of the adductor pollicis (AP) in 6 normal subjects.

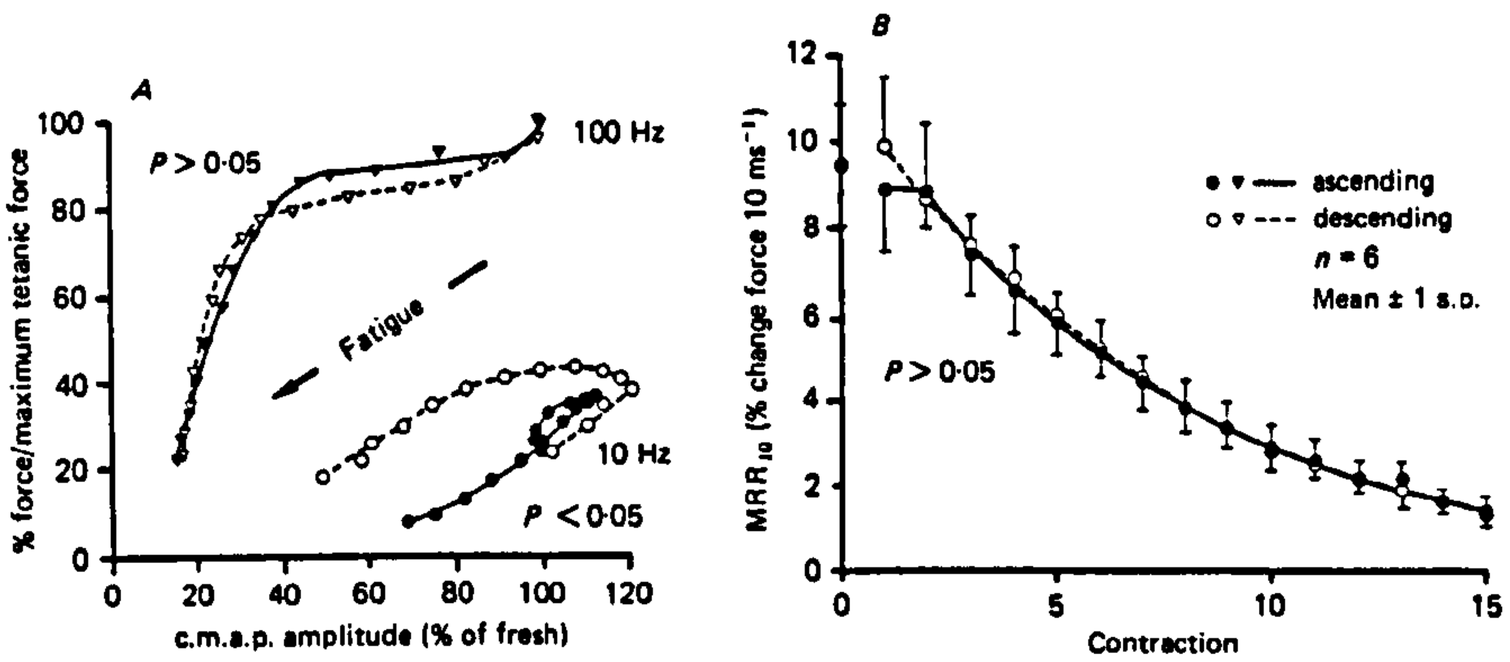

Fig. 1. *A*, mean changes in force and c.m.a.p. amplitude and *B*, MRR 10 during activity.

Fifteen computer-controlled trains of supramaximal stimulation were delivered, via the ulnar nerve, at 5 s intervals in ascending (1, 10, 20, 50, 100 and 1 Hz for 1 s each, with 1 s gap after 10 Hz) and descending (1, 100, 50, 20, 10 and 1 Hz) regimes. These were performed with local circulatory occlusion and one week apart to allow recovery of AP. Isometric force, compound muscle action potential (c.m.a.p.) amplitude and maximum relaxation rate from 10 Hz force (MRR 10) were measured.

At 100 and 50 Hz, changes in force and c.m.a.p. amplitude were similar for both regimes. At low frequencies, force increased initially then declined with activity (Fig. 1*A*). Force at 10 Hz declined to 30 ± 16 (1 s.d.)%, of fresh, at the end of the ascending, but to 73 ± 19% at the end of the descending regime. In contrast, c.m.a.p. amplitude declined less with the ascending than the descending regime: to 68 ± 12% and 49 ± 10% respectively. The decline in MRR was identical for both regimes (Fig. 1*B*).

These results indicate that the increased resistance to fatigue at 10 Hz, if immediately preceded by high-frequency activity, is a form of post-tetanic potentiation, which is independent of changes in MRR or excitation.

Supported by ICI Pharmaceuticals and the Muscular Dystrophy Group of Great Britain.

REFERENCE

Jones, D. A. (1981). In *Human Muscle Fatigue: Physiological Mechanisms* (Ciba Foundation Symposium 82), pp. 178–196. London: Pitman.

Quarterly Journal of Experimental Physiology (1988), 00, 000–000
Printed in Great Britain

MECHANISMS RESISTING FATIGUE IN ISOMETRICALLY CONTRACTING HUMAN SKELETAL MUSCLE

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(MANUSCRIPT RECEIVED 2 JUNE 1988, ACCEPTED 16 JUNE 1988)

SUMMARY

Human adductor pollicis was fatigued during circulatory occlusion by supramaximal stimulation via the ulnar nerve using intermittent trains of stimuli in ascending (1, 10, 20, 50 and 100 Hz) and descending (100, 50, 20, 10 and 1 Hz) frequencies to investigate the contribution of relaxation rate slowing and post-tetanic potentiation (PTP) to fatigue resistance. At 50 and 100 Hz force was initially well maintained despite a marked loss of excitation as indicated by EMG, demonstrating the operation of a high-frequency 'safety factor' which appeared independent of the pattern of stimulation. At 10 Hz, force was initially potentiated before declining during both activity series. Potentiation was greater during the descending frequency series and the rate of decline of force, or fatiguability, was reduced. The 'extra' low-frequency potentiation at 10 Hz was not simply the result of PTP of twitch force, since this declined more during the descending than during the ascending series, nor the result of maximal relaxation rate changes which were identical for both fatiguing series. It is hypothesized that the extra potentiation and reduced fatiguability at low stimulation frequencies, when preceded by high frequency, is the result of increased myofibrillar Ca^{2+} availability and/or sensitivity. These findings may have important practical implications in relation to functional electrical stimulation techniques as used in paraplegia and in other areas of muscle research where fatigue is to be minimized.

INTRODUCTION

During isometric contractions of skeletal muscle, loss of force (fatigue) may result from failure of electrical excitation or energy supply (reviewed by Edwards, 1981). However, little is understood of the peripheral mechanisms that may resist fatigue. Fatigue resistance is of considerable theoretical and practical importance, for example, in relation to development of techniques for functional electrical stimulation in paraplegia (e.g. Susak, Levy, Mizrahi & Isakov, 1987) and in developing cardiac assistance devices employing skeletal muscle (Acker, Hammond, Mannion, Salmons & Stephenson, 1987).

Several studies indicate that slowed relaxation as a consequence of fatiguing activity increases twitch summation and hence potentiates mean force during low-frequency tetani (Edwards, Hill & Jones, 1972; Wiles & Edwards, 1982; Bigland-Ritchie, Johansson, Lippold & Woods, 1983*b*). It was therefore suggested that slowed relaxation could prevent or reduce fatigue (Jones, 1981; Bigland-Ritchie *et al.* 1983*a*). Slowed relaxation appears responsible for the low-frequency force increases observed in other studies (Marsden, Meadows & Merton, 1983; Cooper, Edwards, Gibson & Stokes, 1988). However, slowing of relaxation is unlikely to be the sole cause of low-frequency potentiation, since this occurs

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when the relaxation rate is both declining during fatiguing activity and increasing during subsequent recovery (Cooper *et al.* 1988).

During stimulated isometric contractions 'staircase' potentiation of twitch force occurs during trains at 2–3 Hz (Desmedt & Hainaut, 1968; Krarup, 1981), and following brief tetanic stimulation (Brown & von Euler, 1938; Connolly, Gough & Winegrad, 1971; Takamori, Gutmann & Shane, 1971; Blinks, Rüdel & Taylor, 1978), when it is known as post-tetanic potentiation (PTP). Twitch potentiation also occurs after short (5–10 s) maximal voluntary contractions (Belanger & Quinlan, 1982; Vandervoort, Quinlan & McComas, 1983), presumably as a result of preceding endogenous tetanic activation. Since twitch force as well as relaxation rate affects force generation at low frequency it appears likely that post-tetanic potentiation (PTP) of the twitch could contribute to potentiation of low-frequency tetani.

In the present study we have investigated the role of PTP of the twitch on low-frequency force production during stimulated isometric contractions of the human adductor pollicis. Preliminary results have been communicated to the Physiological Society (Cooper, Edwards, Gibson & Stokes, 1987).

METHODS

Experimental subjects

Six normal subjects (four male, two female), aged 24–33 years, gave their informed consent for participation in this study, which was approved by the Local Health Authority Ethics Committee. There was no history of neuromuscular disorder or drug ingestion in any subject.

Contractile properties of muscle

The experimental apparatus and procedure used were as described previously (Cooper *et al.* 1988). Trains of computer-controlled, supramaximal stimuli (pulse width 50 μs), delivered via the ulnar nerve at the wrist, produced isometric contractions of the adductor pollicis. Simultaneous oscillographic recordings of force, force differential (for calculation of the maximal relaxation rate, MRR; Wiles, Young, Jones & Edwards, 1979) and the evoked surface compound muscle action potential (CMAP) comprised the programmed stimulation electromyogram (PSEM). Stimulation was delivered in either an ascending frequency pattern, consisting of 1, 10, 20, 50 and 100 Hz for 1 s each (10 Hz 2 s) with a 1 s gap after 10 Hz to allow estimation of the MRR, or a descending frequency pattern, consisting of 100, 50, 20, 10 and 1 Hz for 1 s each (10 Hz 2 s) (Fig. 1). The rationale for using the descending PSEM was to examine the role of reduction of MRR in low-frequency potentiation in isolation, since under these conditions PTP of the twitch would be expected to be maximal. On the other hand, PTP of the twitch was unlikely to be fully evoked during the ascending PSEM, because of the absence of immediately preceding high-frequency tetani. A comparison would therefore shed light on the role of PTP of the twitch.

Fatiguing activity protocols

In each subject two series of stimulated, fatiguing contractions were undertaken, one using the ascending PSEM and one using the descending PSEM. At the commencement of each protocol a control PSEM was performed in previously unfatigued (fresh) muscle. A sphygmomanometer cuff was then inflated around the upper arm and maintained 100 mmHg above systolic blood pressure to occlude arterial circulation. A 3 min period of ischaemic rest then followed, by which time approximately 40–50% oxygen depletion could be expected, thus minimizing oxidative metabolism at the commencement of stimulated activity (Harris, Hultman, Kaijser & Nordesjö, 1975). Fatiguing activity then commenced and consisted of repeated ascending or descending PSEMs at intervals of 12 s until fifteen had been completed. The cuff was then deflated and aerobic recovery monitored using the PSEM of intervals of 0·5, 1, 2, 3, 5, 10 and 15 min after the end of activity. The fatiguing procedures were performed in *random* order and at least 1 week apart to ensure full recovery of the adductor pollicis.

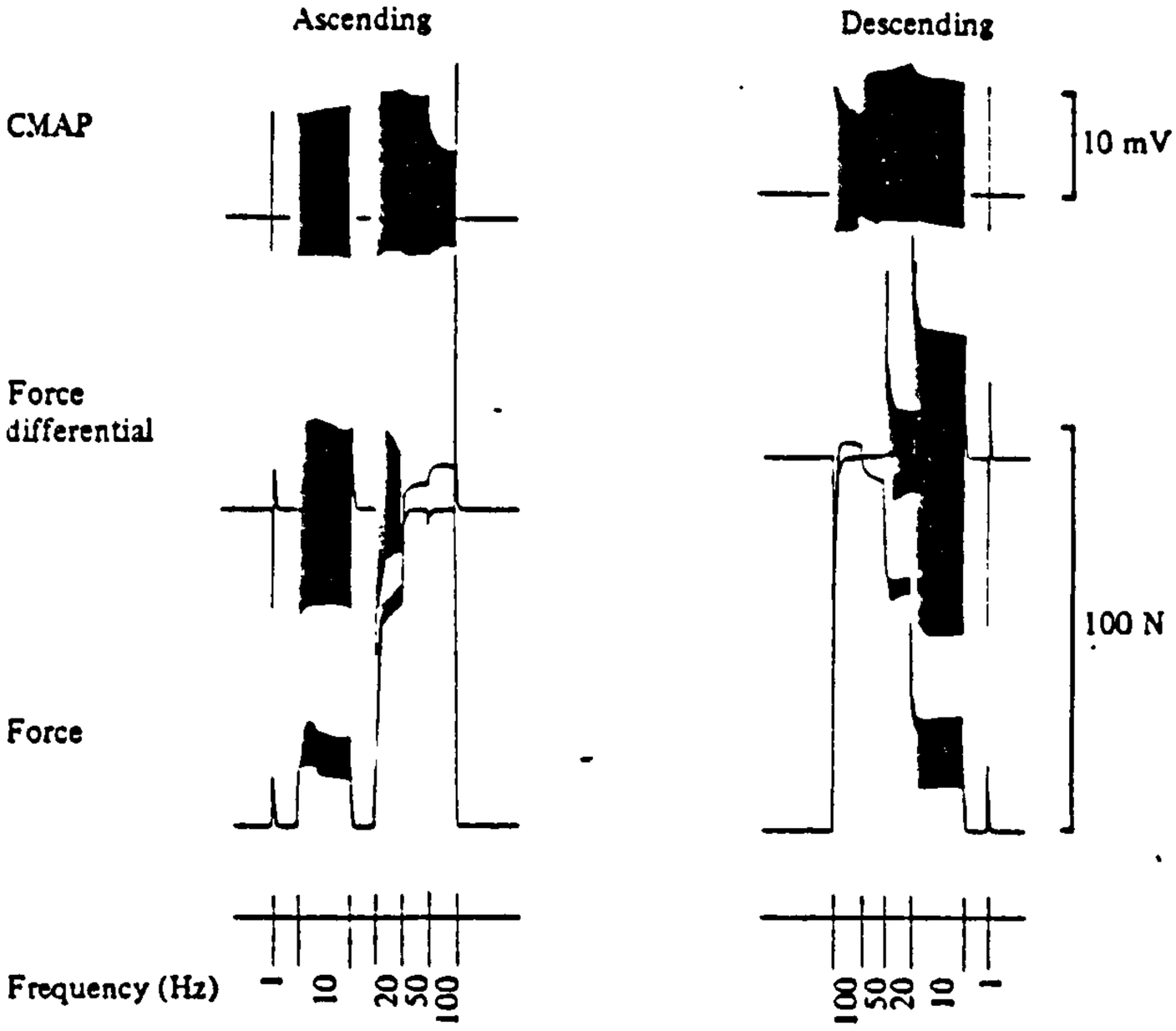

Fig. 1. Simultaneous oscillographic recordings of compound muscle action potential (CMAP), force and force differential producing the programmed stimulation electromyogram (PSEM). The train of stimulation frequencies was delivered in either an ascending or a descending fashion. During the ascending PSEM, a 1 s gap was introduced at the end of the 10 Hz stimulation, to produce a force differential record and allow calculation of MRR.

Analysis

Measurements were made on each PSEM of the contraction series. For each subject the force at each frequency was expressed as a percentage of maximum force generated by fresh muscle, i.e. at 100 Hz. Where oscillation occurred, e.g. 10 Hz, the mean value was used. The CMAP amplitude at each frequency was expressed as a percentage of the equivalent part of the ascending–descending PSEM recorded in fresh muscle. The relationship between force and excitation was assessed using the 'force per excitation' (F/E) ratio (Stephens & Taylor, 1972; Cooper *et al.* 1988). This was calculated for each frequency as: (force as a percentage of that for fresh muscle)/(excitation as a percentage of that for fresh muscle). The MRR at 10 Hz was calculated and expressed as the maximum percentage of force loss in 10 ms (% 10 ms^{-1}; Wiles *et al.* 1979) and also as a percentage of the value obtained from fresh muscle. Statistical analyses, using paired t tests, were made using a Minitab Statistical Package (Minitab Inc.), a value of $P < 0{\cdot}05$ being accepted as significant. The results reported in the text and those used to construct figures are expressed as mean ± S.D., where $n = 6$.

RESULTS

Fresh values

Because of the effects of PTP, twitch force was greater in the descending than in the ascending PSEM; 11·5 ± 3·8 compared to 8·4 ± 1·9 N, $P < 0{\cdot}05$. The force at 100 Hz was also greater in the descending than in the ascending PSEM; 65·2 ± 14·4 compared to 62·5 ± 12·5 N, $P < 0{\cdot}05$, but at other test frequencies forces were not dissimilar. The MRR was similar for both ascending and descending PSEM, 9·8 ± 1·4 and 9·5 ± 1·4 % 10 ms^{-1} respectively.

Table 1. *Relative changes in force, CMAP amplitude and maximum relaxation rate, and changes in F/E ratios, for all frequencies at the end of fatiguing activity*

Frequency (Hz)	Ascending frequency	Descending frequency	*P*-value of difference
	Force (% of fresh values)		
1	22·7 ± 15·5	*	–
10	30·4 ± 16·1	73·2 ± 18·4	0·005
20	22·1 ± 7·9	33·6 ± 8·0	0·05
50	29·8 ± 9·3	25·8 ± 6·9	n.s.
100	22·2 ± 9·0	23·6 ± 5·8	n.s.
	CMAP amplitude (% of fresh values)		
1	74·9 ± 9·1	58·5 ± 11·7	0·001
10	68·2 ± 11·9	48·8 ± 10·2	0·001
20	54·1 ± 13·5	33·8 ± 8·3	0·005
50	19·5 ± 5·3	18·6 ± 3·9	n.s.
100	15·3 ± 7·3	16·3 ± 6·0	n.s.
	F/E_{ratio}		
1	0·22 ± 0·13	*	–
10	0·46 ± 0·25	1·58 ± 0·56	0·005
20	0·42 ± 0·13	0·92 ± 0·28	0·05
50	1·55 ± 0·37	1·32 ± 0·22	n.s.
100	1·58 ± 0·44	1·59 ± 0·50	n.s.
	Maximum relaxation rate (% of fresh value)		
10	16·9 ± 2·7	14·12 ± 4·3	n.s.

Results mean ± 1 S.D., *P* values from paired *t* test. * Note that by the 15th contraction the twitch was so small that reliable measurements of maximum relaxation rate could not be made, hence the absences from the table.

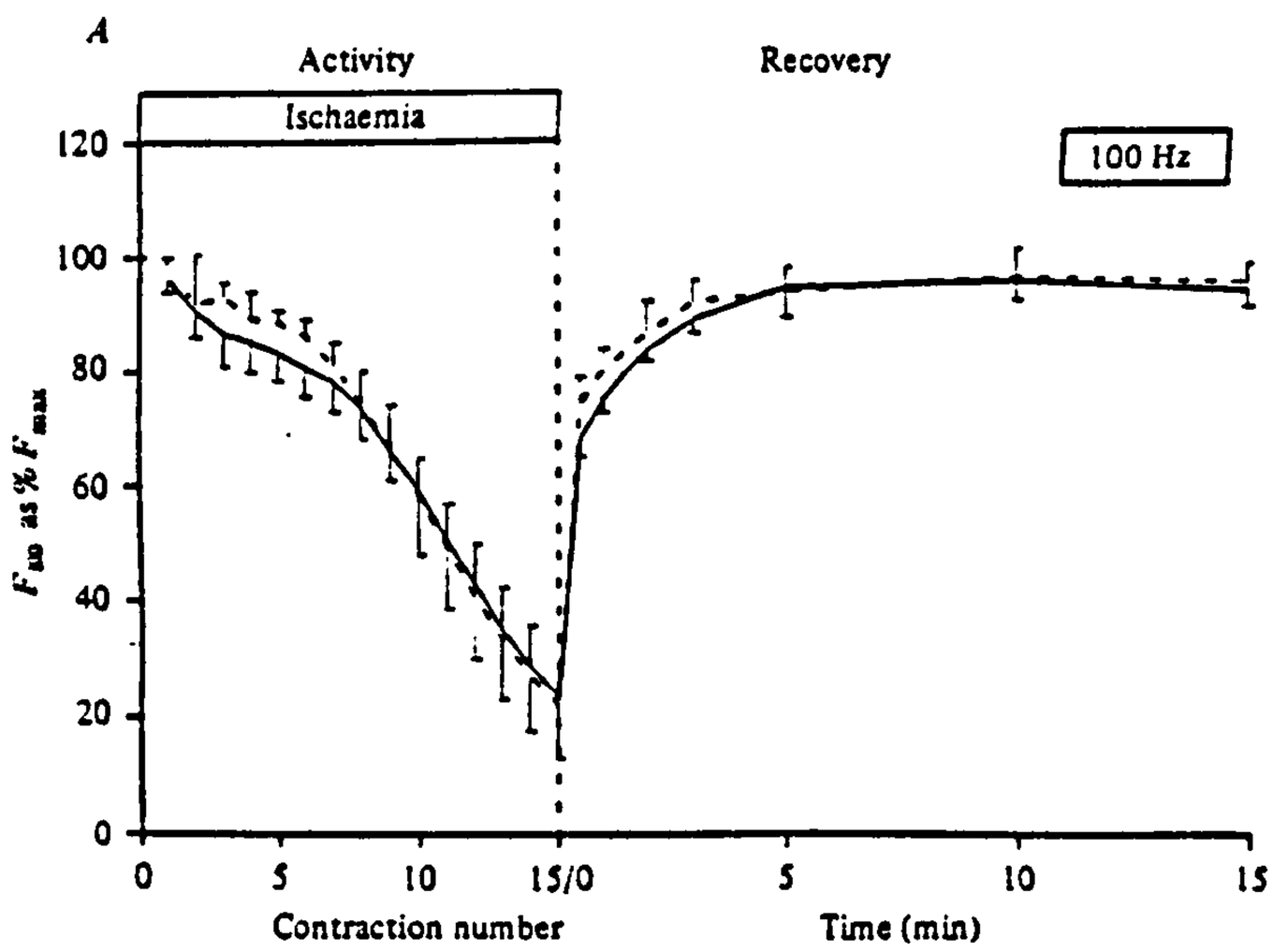

Fig. 2*A*. For legend, see facing page.

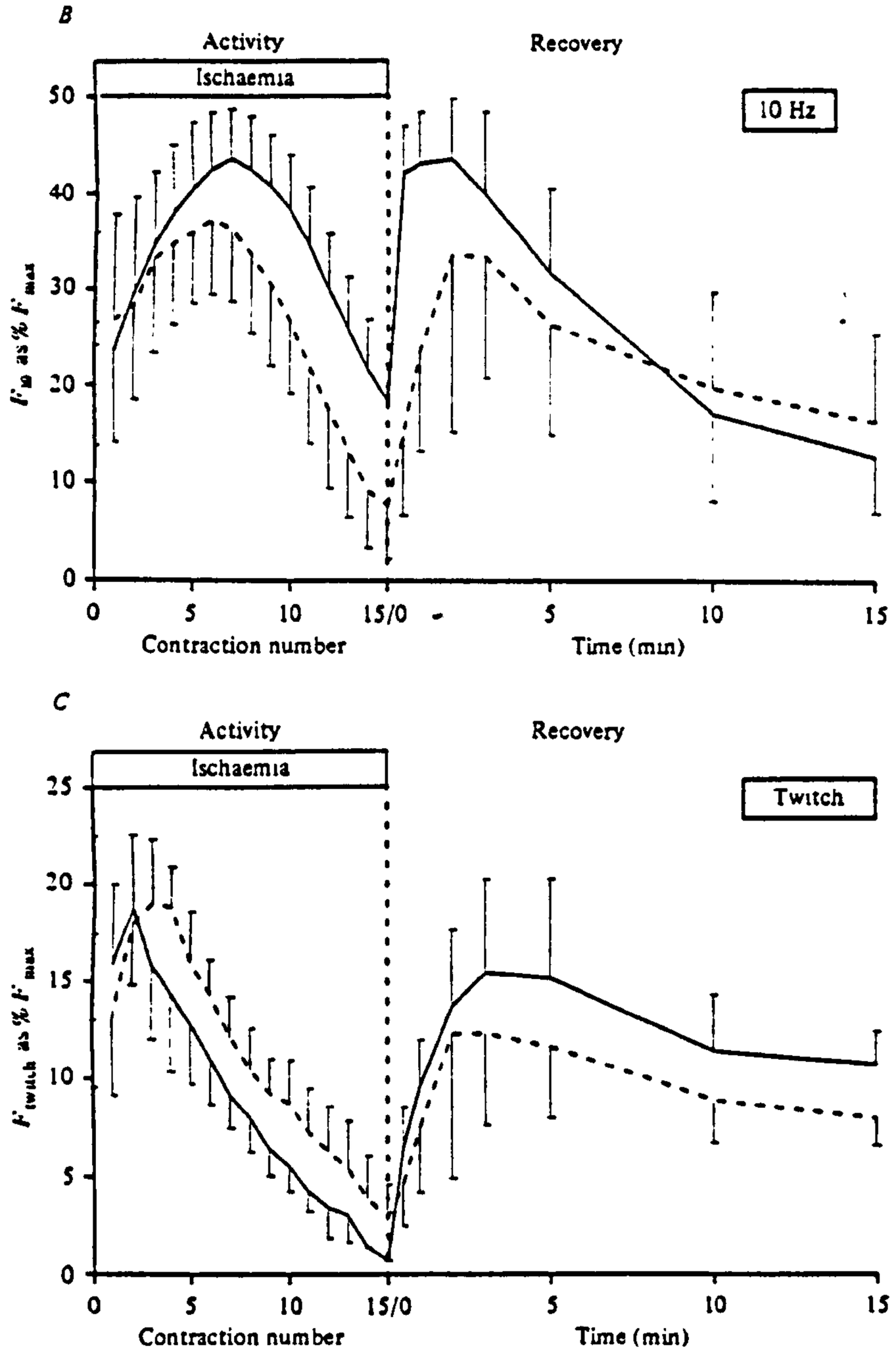

Fig. 2. Time course of changes of force, as a percentage of maximum force (F_{max}) at 100 Hz, during fatiguing activity with circulatory occlusion and during aerobic recovery at: 100 Hz (*A*, F_{100}), 10 Hz (*B*, F_{10}) and 1 Hz (*C*, F_{twitch}). The results from the ascending PSEM series appear as dashed lines and those from the descending PSEM series as continuous lines. Note that force at 10 Hz potentiates more during the descending PSEM series despite a greater decline in twitch force.

Anaerobic activity

During fatiguing activity, force was hampered at all stimulation frequencies (Table 1). The reduction in force at 50 and 100 Hz was similar for ascending and descending contraction series (Fig. 2*A*). At 10 and 20 Hz the force declined *less* during the descending contraction series. In both contraction series there was clear evidence of potentiation at 10 Hz, but this was greater for the descending series (Fig. 2*B*), even though the twitch size declined *more* during this series (Fig. 2*C*).

6

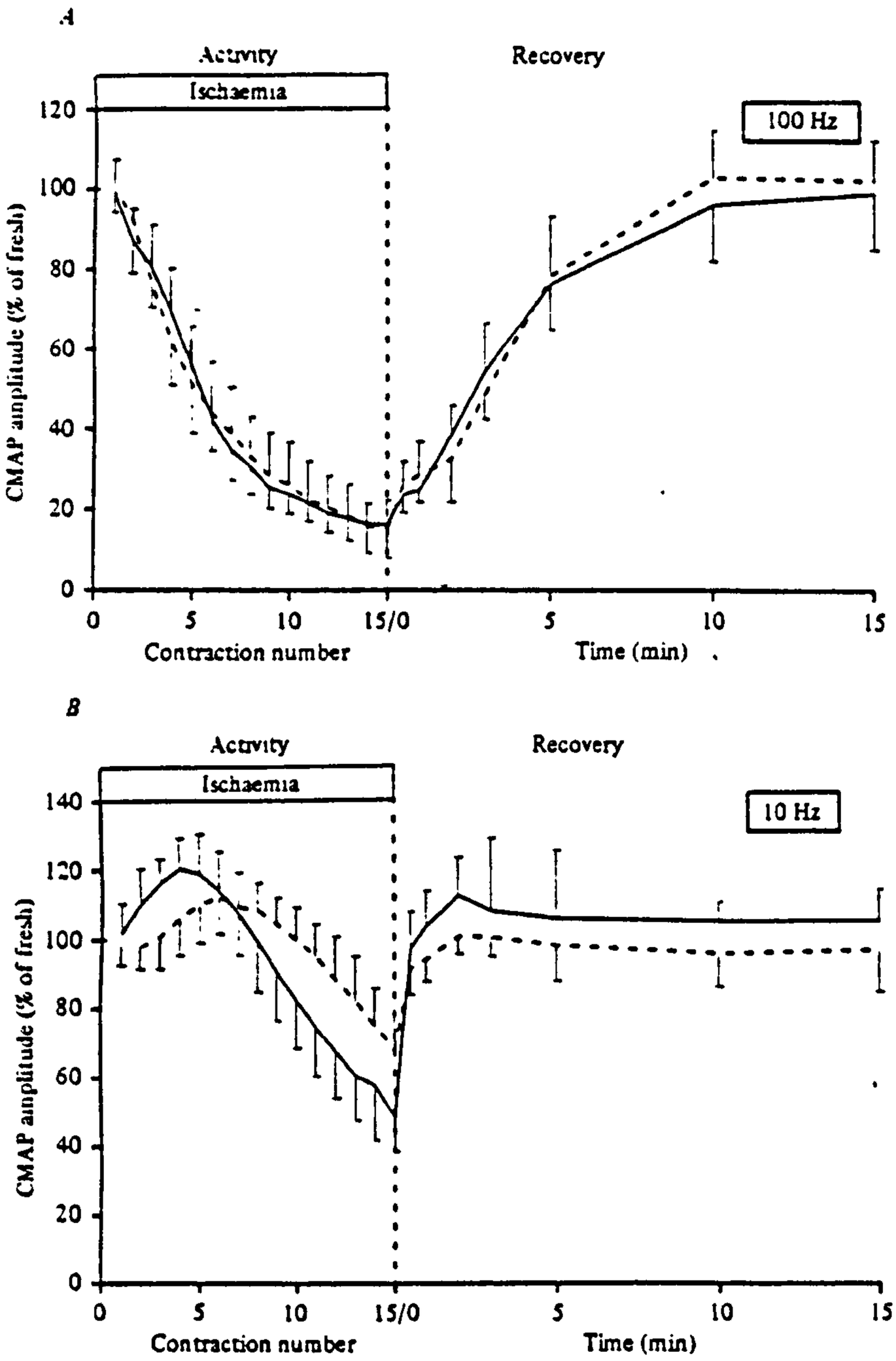

Fig. 3 *A* and *B*. For legend, see facing page.

The changes in excitation were frequency dependent (Table 1). Reduction in CMAP amplitude at 50 and 100 Hz was similar for both series (Fig. 3 *A*) but for the twitch, 10 and 20 Hz, CMAP amplitude declined more during the descending series (Fig. 3 *B* and *C*). There was some potentiation of CMAP at 1, 10 and 20 Hz for both contraction series. These changes in force and excitation altered their interrelationship especially at low frequencies, as evidenced by the *F/E* ratios (Table 1).

The low-frequency force differences between the two contraction series were not attributable to differential changes in MRR since this declined similarly during both series (Figs 4 and 5 *A* and *B*. Table 1).

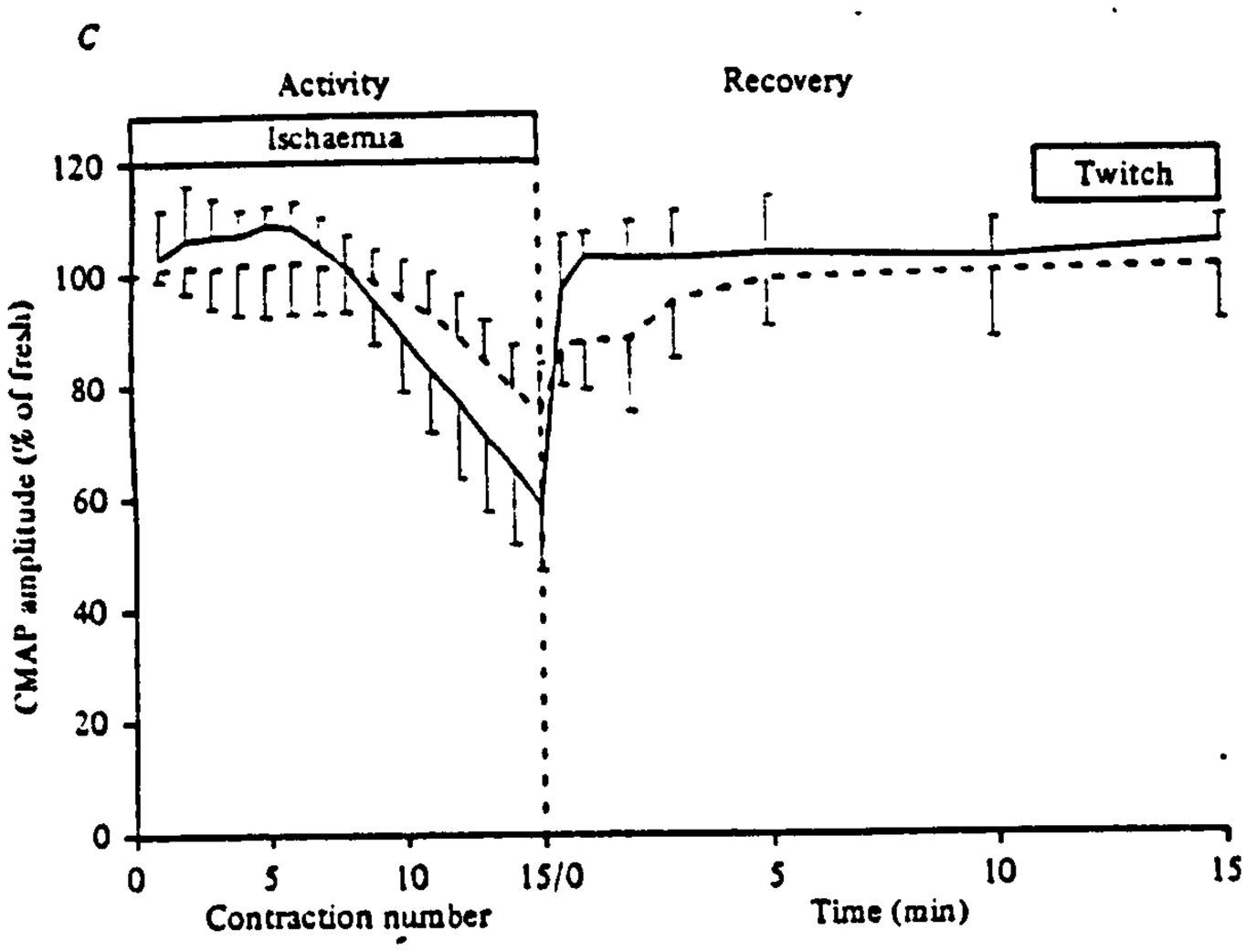

Fig. 3. Time course of changes of CMAP amplitude, as a percentage of fresh values, during fatiguing activity with circulatory occlusion and during aerobic recovery at: *A*, 100 Hz; *B*, 10 Hz; and *C*, 1 Hz (twitch). The symbols are as in the previous figures. Note that the CMAP amplitude declines more during the descending PSEM series.

The relationship between force and CMAP amplitude was similar for both contraction series at high frequencies but there were improvements in fatigue resistance for a given reduction in excitation at lower frequencies during the descending series (Fig. 6*A* and *B*, Table 1).

Aerobic recovery

On reperfusing the muscle there was rapid recovery of force at high frequency for both contraction series (Fig. 2*B*). At 10 Hz there was potentiation of force up to values similar to those seen during activity (Fig. 2*B*). This potentiation reached a maximum at 3–5 min during the ascending PSEM series but was already maximal at 30 s during the descending PSEM series (Fig. 2*B*), with obvious impact on the force–$\dot{M}$RR relationship (Fig. 5*A* and *B*). The CMAP amplitude recovered towards normal values after activity for both series (Fig. 3). The MRR recovered at similar rates for both series (Fig. 4).

DISCUSSION

The results of this study clearly demonstrate the beneficial effect on fatiguability at low frequency of commencing a stimulated contraction at high frequency. Stimulation at various frequencies within the physiological range has, furthermore, given insight into the frequency dependence of fatigue-resistant mechanisms. Full twitch PTP appears to have been achieved by the descending frequency pattern, since twitch force did not rise substantially during this contraction series. The greater force at 10 Hz during the descending series was therefore not unexpected. However, after the second contraction the twitch force in the descending PSEM became smaller than that in the ascending PSEM, despite full PTP, presumably due to fatigue induced by the immediately preceding tetani.

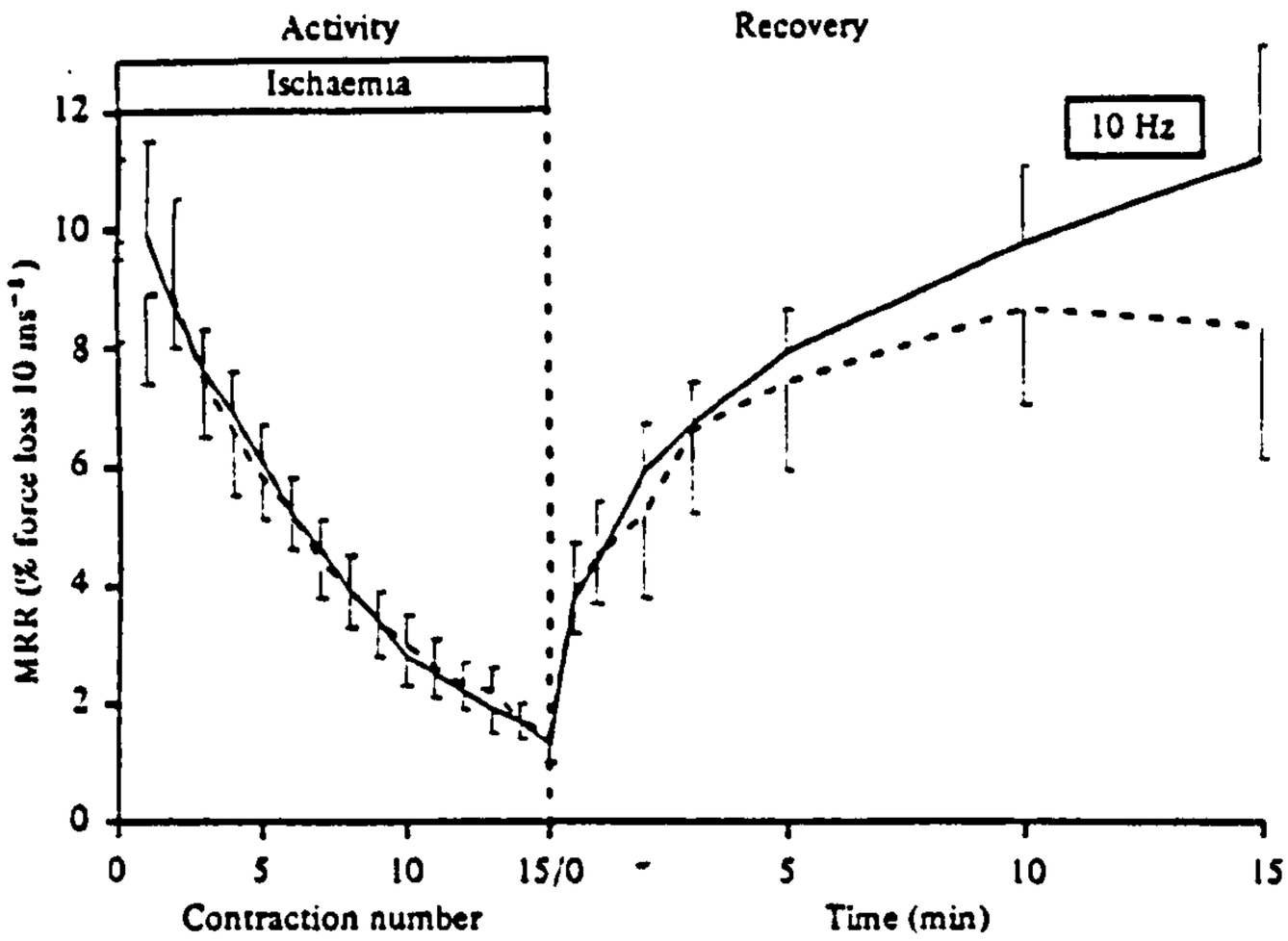

Fig. 4. Changes in the maximal relaxation rate (MRR, maximum percentage force loss in 10 ms) after 10 Hz, symbols as used previously.

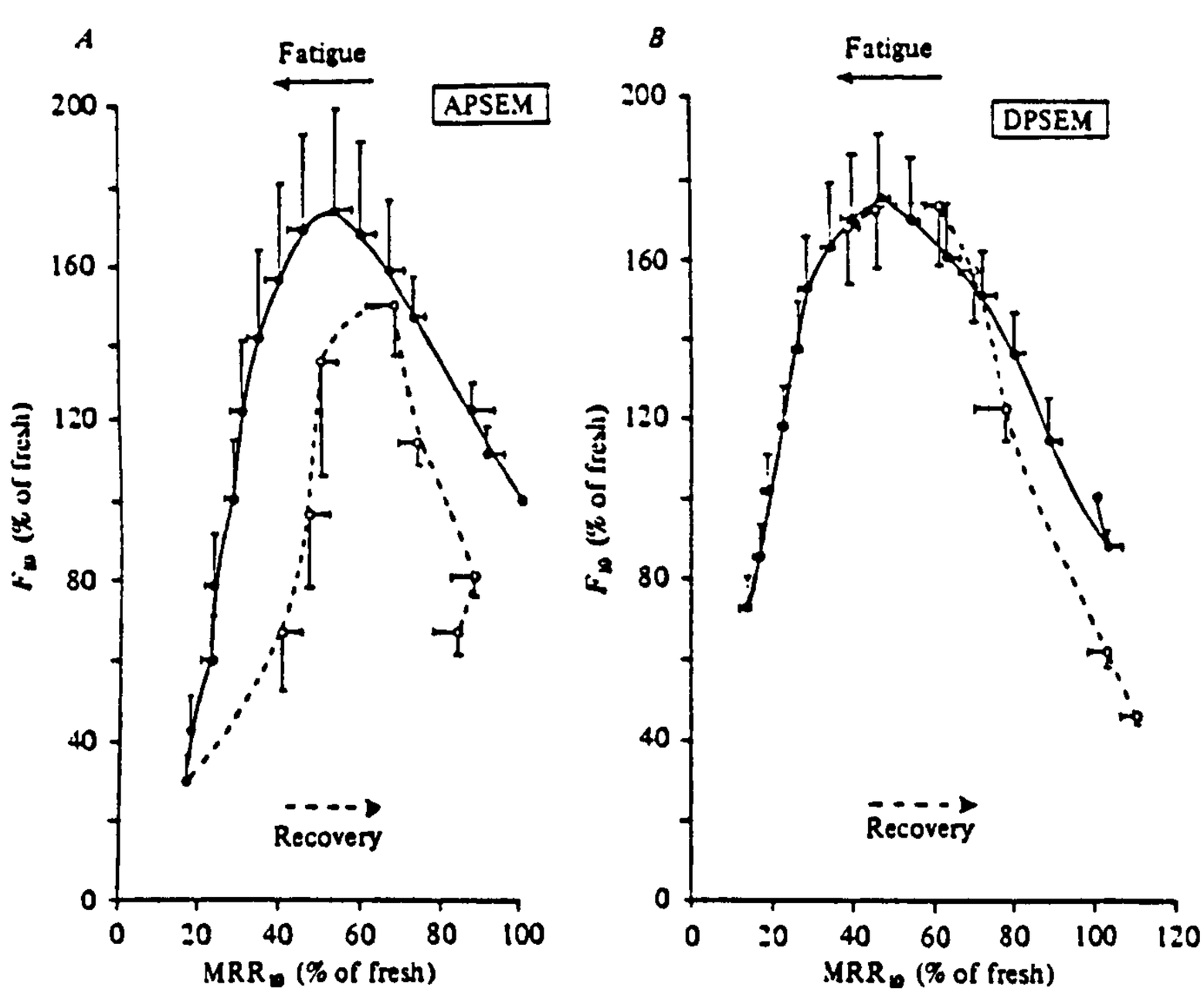

Fig. 5. Changes in mean force (F_{10}) as a function of changes in mean MRR ~~[changes]~~ at 10 Hz (MRR_{10}) during activity (shown as continuous lines) and recovery (shown as interrupted lines). *A*, when using the ascending PSEM (APSEM), force potentiated during activity to about 170% of fresh values before declining due to fatigue. During recovery force again potentiated but to only about 140% of fresh values. *B*, when using the descending PSEM (DPSEM) potentiation was similar to the ascending PSEM protocol during activity but during recovery potentiation was immediate and of similar degree to that during ~~[sensitivity]~~ Mean ± S.E.M.

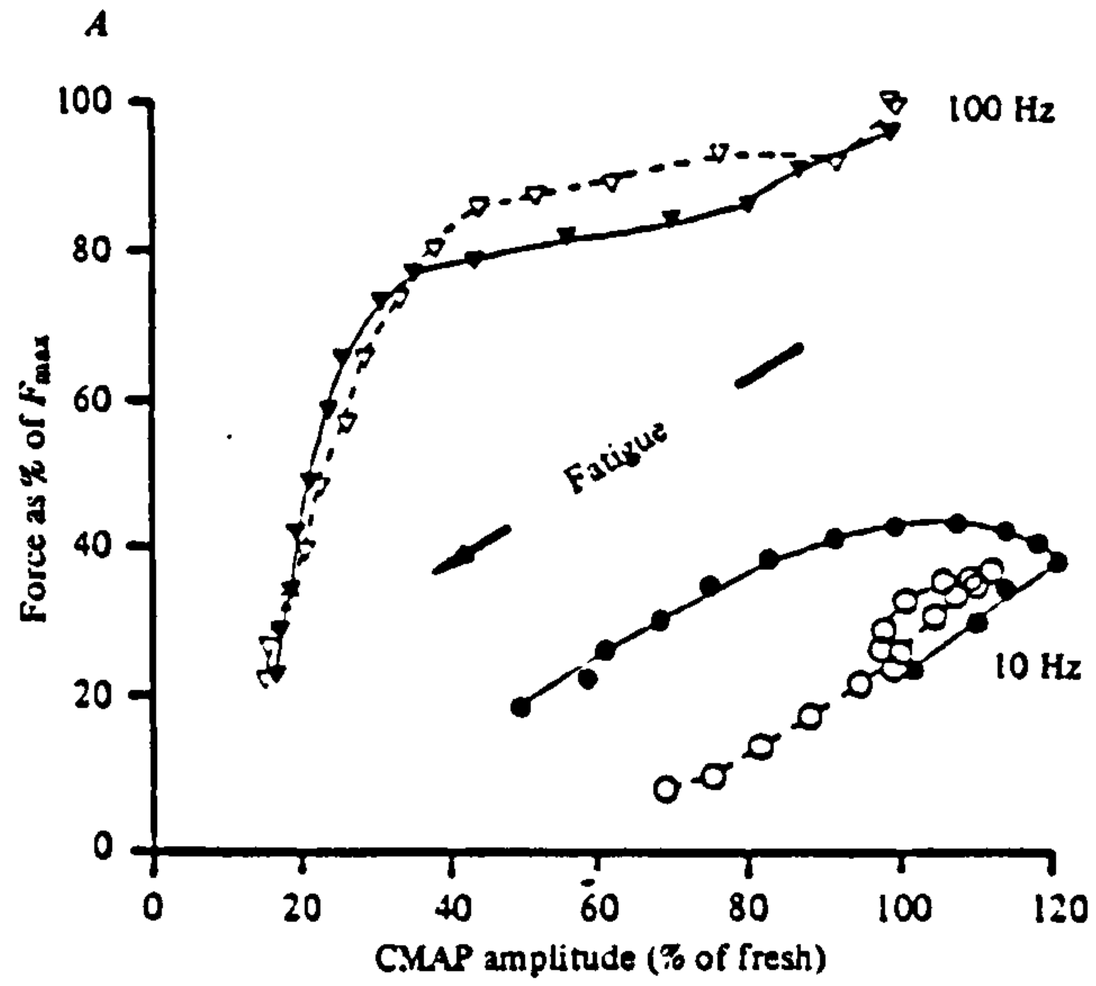

Fig. 6. Mean changes in force relative to mean changes in excitation (CMAP amplitude) during fatiguing activity. ○, ▽ and interrupted lines are used to depict ascending PSEM series results and ●, ▼ and continuous lines to depict descending PSEM series results. At high frequency force is initially maintained despite marked early declines in excitation indicating the operation of a 'safety factor'. At low-frequency force and excitation are initially potentiated before declining due to fatigue. During the descending PSEM series, force and excitation potentiate to a greater degree and the subsequent declines were clearly less than during the ascending PSEM series. Thus, by preceding low- with high-frequency stimulation, low-frequency fatiguability has been reduced. F_{max}, maximum force.

It is clear that MRR slowing is important for low-frequency potentiation, but since the MRR declined identically for both contraction series it appears that a factor, over and above twitch force and MRR slowing, is responsible for the greater low-frequency force potentiation seen during the contraction series using the descending PSEM.

Factors causing 'extra' potentiation and fatigue resistance at low frequency

A possible explanation is that the post-tetanic mechanisms responsible for the potentiation of the twitch also influence low-frequency force production. Post-tetanic twitch potentiation may result from an increase in myofibrillar Ca^{2+} sensitivity since it has been demonstrated in amphibian muscle that Ca^{2+} release into the sarcoplasm is *reduced* despite the increased force (Blinks *et al.* 1978). Studies in rabbit skeletal muscle suggest that this increased sensitivity is the result of activity-induced phosphorylation which increases the affinity of myosin for actin (Persechini & Stull, 1987). This hypothesis is supported by the good correlation between twitch potentiation and myosin light-chain phosphorylation in rodent fast-twitch muscle (Moore & Stull, 1984). Such a mechanism may account for the hysteresis of the force–pCa relationship produced when the same concentration of Ca^{2+} in the solution bathing skinned barnacle fibres is examined immediately before and following a higher Ca^{2+} concentration (Ridgeway, Gordon & Martyn, 1983). This mechanism may also explain the present results since increased myosin phosphorylation resulting from the greater contractile activity immediately preceding each subsequent lower frequency in the descending PSEM could cause increased myofibrillar Ca^{2+} sensitivity.

Increased Ca^{2+} sensitivity is not the only possible explanation for post-tetanic twitch potentiation as there is evidence to suggest increased myofibrillar Ca^{2+} availability. Studies in amphibian muscle indicate that during tetanic activity a Ca^{2+}-binding protein, other than troponin, acts as a Ca^{2+} sink or 'relaxing factor' (Haiech, Derancourt, Pechere & Damaille, 1979) to reduce myofibrillar Ca^{2+} in conjunction with the sarcoplasmic reticulum (Cannell, 1986). Such a role has been suggested for parvalbumins (Cannell, 1986) in view of their high affinity for Ca^{2+} (Haiech *et al.* 1979). During tetanic stimulation, progressive saturation of this protein would result in more of the released Ca^{2+} remaining unbound during subsequent stimuli and hence increase myofibrillar activation (MacIntosh & Gardiner, 1986). This mechanism could also explain the present results since with achievement of plateau force at high frequency in the descending PSEM, continued Ca^{2+} release could saturate non-troponin Ca^{2+}-binding proteins. At the commencement of each lower frequency, the saturation of the Ca^{2+}-binding protein would then be greater than that appropriate for that frequency. The result is that more Ca^{2+} could be available to bind to troponin, producing greater myofibrillar activation.

Clearly, either or both these mechanisms may account for the 'extra' potentiation observed at low frequency. These effects do not influence high-frequency force generation, since activation is already at or near its maximum, as demonstrated by the relationship between frequency and force (Merton, 1954; Edwards, Young, Hosking & Jones, 1977). The paradoxical observation that low-frequency force is potentiated at a time when twitch force is reduced requires explanation. The decay of post-tetanic potentiation in mammalian muscle, including man, has been shown to last for up to several minutes (Close & Hoh, 1968; Desmedt & Hainaut, 1968; Krarup, 1981; Vandervoort *et al.* 1983). However, the decline is initially very rapid then followed by a slower phase (Close & Hoh, 1968; Krarup, 1981; Vandervoort *et al.* 1983). It appears likely, therefore, that in the time between tetani and twitch during the descending PSEM the degree of potentiation had already declined considerably. Alternatively, the mechanism potentiating low-frequency force is short lived in comparison to that for the twitch.

Factors resisting fatigue at high frequency

At high frequency force is maintained despite severe excitation failure indicating the

operation of a 'safety factor' (Cooper *et al.* 1988). The present study indicates its independence of pattern of stimulation frequency. The decline in action potential amplitude cannot be attributed to neuromuscular junction failure since force would also then be expected to decline. Furthermore, excitation failure cannot be explained by failure of propagation of every second or third action potential, as observed in single cell preparations (Lüttgau, 1965), since this would not reduce the overall CMAP amplitude. Prolongation of the action potential, causing successive potentials to run into and cancel each other, is a possible explanation (Marsden *et al.* 1983). However, it appears likely that at high stimulation frequencies, myoplasmic Ca^{2+} levels increase sufficiently to sustain activation of the contractile apparatus despite excitation failure (Cooper *et al.* 1988).

During voluntary contractions, motor unit discharge tends to commence at high frequencies before declining to low frequencies (Bigland-Ritchie, Jones & Woods, 1979; Bigland-Ritchie, 1981; Bigland-Ritchie, Johansson, Lippold, Smith & Woods, 1983*a*; Marsden *et al.* 1983). The presently discussed mechanisms for resisting fatigue may therefore be the basis of physiological strategies for optimizing force production. A practical application is the development of stimulation regimes for 'functional electrical stimulation', in which brief high-frequency activity may be utilized to advantage at the commencement of low-frequency trains of stimuli. Indeed, the physiological significance of these findings may be applied to any area of skeletal muscle research in which fatigue resistance is of importance.

We thank the subjects who took part in this study and gratefully acknowledge support from ICI Pharmaceuticals U.K., and the Muscular Dystrophy Group of Great Britain and Northern Ireland.

REFERENCES

ACKER, A., HAMMOND, R. L., MANNION, J. D., SALMONS, S. & STEPHENSON, L. W. (1987). Skeletal muscle as the potential power source for a cardiovascular pump: assessment in vivo. *Science* **236**, 324–327.

BELANGER, A. Y. & QUINLAN, J. (1982). Muscle function studies in human plantar-flexor and dorsi-flexor muscles. *Canadian Journal of Neurological Science* **9**, 358–359.

BIGLAND-RITCHIE, B. (1981). EMG and fatigue of human voluntary and stimulated contractions. In *Human Muscle Fatigue: Physiological Mechanisms, Ciba Foundation Symposium No. 82*, ed. PORTER, [illegible] & WHELAN, [illegible], pp. 130–156. London: Pitman Medical.

BIGLAND-RITCHIE, B., JOHANSSON, R., LIPPOLD, O. C. J., SMITH, S. & WOODS, J. J. (1983*a*). Changes in motoneurone firing rates during sustained maximal voluntary contractions. *Journal of Physiology* **340**, 335–346.

BIGLAND-RITCHIE, B., JOHANSSON, R., LIPPOLD, O. C. J. & WOODS, J. J. (1983*b*). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *Journal of Neurophysiology* **50**, 313–324.

BIGLAND-RITCHIE, B., JONES, D. A. & WOODS, J. J. (1979). Excitation frequency and muscle fatigue. Electrical responses during human voluntary and stimulated contractions. *Experimental Neurology* **64**, 414–427.

BLINKS, J. R., RÜDEL, R. & TAYLOR, S. R. (1978). Calcium transients in isolated amphibian skeletal muscle fibres: detection with aequorin. *Journal of Physiology* **277**, 291–323.

BROWN, G. L. & VON EULER, U. S. (1938). The after-affects of a tetanus on mammalian muscle. *Journal of Physiology* **93**, 39–60.

CANNELL, M. B. (1986). Effect of tetanus duration on the free calcium during the relaxation of frog skeletal muscle fibres. *Journal of Physiology* **376**, 203–218.

CLOSE, R. & HOH, J. F. Y. (1968). The after-effects of repetitive stimulation on the isometric twitch contraction of rat fast skeletal muscle. *Journal of Physiology* **197**, 461–477.

CONNOLLY, R., GOUGH, W. & WINEGRAD, S. (1971). Characteristics of isometric twitch of skeletal muscle immediately after a tetanus. A study of the influence of the distribution of calcium within the sarcoplasmic reticulum on the twitch. *Journal of General Physiology* **57**, 697–709.

COOPER, R. G., EDWARDS, R. H. T., GIBSON, H. & STOKES, M. (1987). Human muscle fatigue: post-tetanic potentiation of low-frequency tetani is not related to altered excitation or slowing of relaxation. *Journal of Physiology* **394**, 141*P*.

COOPER, R. G., EDWARDS, R. H. T., GIBSON, H. & STOKES, M. (1988). Human muscle fatigue: frequency dependence of excitation and force generation. *Journal of Physiology* **397**, 585–599.

DESMEDT, J. E. & HAINAUT, K. (1968). Kinetics of myofilament activation in potentiated contraction: Staircase phenomenon in human skeletal muscle. *Nature* **217**, 529–532.

EDWARDS, R. H. T. (1981). Human muscle function and fatigue. In *Human Muscle Fatigue: Physiological Mechanisms, Ciba Foundation Symposium No. 82*, ed. PORTER, [illegible] & WHELAN, [illegible], pp. 1–18. London: Pitman Medical.

EDWARDS, R. H. T., HILL, D. K. & JONES, D. A. (1972). Effect of fatigue on the time course of relaxation of skeletal muscle in man. *Journal of Physiology* **227**, 26–27*P*.

EDWARDS, R. H. T., YOUNG, A., HOSKING, G. P. & JONES, D. A. (1977). Human skeletal muscle function: description of tests and normal values. *Clinical Science and Molecular Medicine* **52**, 283–290.

HAIECH, J., DERANCOURT, J., PECHERE, J. F. & DEMAILLE, J. G. (1979). Magnesium and calcium binding to parvalbumins: Evidence for differences between parvalbumins and an explanation of their relaxing function. *Biochemistry* **18**, 2752–2758.

HARRIS, R. C., HULTMAN, E., KAIJSER, L. & NORDESJÖ, L. O. (1975). The effect of circulatory occlusion on isometric exercise capacity and energy metabolism of the quadriceps muscle in man. *Scandinavian Journal of Clinical and Laboratory Investigation* **35**, 87–95.

JONES, D. A. (1981). Muscle fatigue due to changes beyond the neuromuscular junction. In *Human Muscle Fatigue: Physiological Mechanisms, Ciba Foundation Symposium No. 82*, ed. PORTER [illegible] & WHELAN, [illegible], pp. 178–196. London: Pitman Medical.

KRARUP, C. (1981). Enhancement and diminution of mechanical tension evoked by staircase and by tetanus in rat muscle. *Journal of Physiology* **311**, 355–372.

LÜTTGAU, H. C. (1965). The effect of metabolic inhibitors on the fatigue of the action potential in single muscle fibres. *Journal of Physiology* **178**, 45–67.

MACINTOSH, B. R. & GARDINER, P. F. (1987). Post-tetanic potentiation and skeletal muscle function: Interactions with caffeine. *Canadian Journal of Physiological Pharmacology* **65**, 260–268.

MARSDEN, C. D., MEADOWS, J. C. & MERTON, P. A. (1983). 'Muscular wisdom' that minimizes fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. In *Motor Control Mechanisms in Health and Disease*, ed. DESMEDT, J. E., pp. 169–211. New York: Raven Press.

MERTON, P. A. (1954). Voluntary strength and fatigue. *Journal of Physiology* **123**, 553–564.

MOORE, R. L. & STULL, J. T. (1984). Myosin light chain phosphorylation in fast and slow skeletal muscles in situ. *American Journal of Physiology* **247**, C462–471.

PERSECHINI, A. & STULL, J. T. (1984). Phosphorylation kinetics of skeletal muscle myosin and the effect of phosphorylation on actomyosin adenosinetriphosphate activity. *Biochemistry* **23**, 4144–4150.

RIDGWAY, E. B., GORDON, A. M. & MARTYN, D. A. (1983). Hysteresis in the force–calcium relation in muscle. *Science* **219**, 1075–1077.

STEPHENS, J. A. & TAYLOR, A. (1972). Fatigue of maintained voluntary muscle contraction in man. *Journal of Physiology* **220** 1–18.

SUSAK, Z., LEVY, M., MIZRAHI, J. & ISAKOV, E. (1986). The duration of isometric contraction while using FES with stimuli of different parameters. Proceedings of 2nd Vienna International Workshop on Functional Electrostimulation, Vienna (Austria), September 21–24.

TAKAMORI, M., GUTMANN, L. & SHANE, S. R. (1971). Contractile properties of human skeletal muscle. *Archives of Neurology* **25**, 535–546.

VANDERVOORT, A. A., QUINLAN, J. & MCCOMAS, A. J. (1983). Twitch potentiation after voluntary contraction. *Experimental Neurology* **81**, 141–152.

WILES, C. M. & EDWARDS, R. H. T. (1982). The effect of temperature, ischaemia and contractile activity on the relaxation rate of human muscle. *Clinical Science* **2**, 485–497.

WILES, C. M., YOUNG, A., JONES, D. A. & EDWARDS, R. H. T. (1979). Relaxation rate of constituent muscle fibre-type in human quadriceps. *Clinical Science* **56**, 47–52.