EXERCISE INDUCED PAIN AND DAMAGE

IN HUMAN SKELETAL MUSCLE

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ABSTRACT.

Two types of dynamic muscle contractions have been compared to investigate their effects on muscle pain and damage in normal subjects. Damage was assessed by studying changes in isometric force generation, muscle enzyme loss, morphological changes and the patterns of radioisotope uptake.

Delayed onset muscle pain occurred after eccentric but not concentric contractions and was associated with appreciable muscle damage.

A technique was developed to quantitate the intensity and distribution of tenderness in superficial muscles. It was reproducible and showed the bellies as well as attachments to be affected. No discomfort was experienced for approximately eight hours after exercise, and was maximal 1-2 days later.

Dissimilar time courses emerged for the various indicators of damage; impairments of force generation were greatest immediately after exercise and had largely recovered 24 hours later. Small localised areas of morphological damage were seen immediately after exercise, and were more extensive 2-3 days later.

Some subjects had an unexpectedly large and delayed rise

in plasma muscle enzyme levels which increased for 3-6 days. This slow time course paralled the muscle uptake of radioisotope and both imply damaged muscle membranes. The use of radioisotopic techniques showed that increased uptake did not occur in all painful muscles, and thus membrane damage is not the pain stimulus. Morphological changes best approximated, but did not exactly follow, the time course of pain and tenderness.

Both pain and enzyme efflux respond rapidly to training, in the absence of isometric strength changes. The intensity of exercise and the amount of lengthening seemed to be the critical factors in causing damage.

It is concluded that the pain and damage are initiated by high mechanical tensions, but the algesic stimulus remains unclear. These results question the use of intensive eccentric contractions in training and rehabilitiation programmes.

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ABBREVIATIONS AND DEFINITIONS.

Pain	Spontaneously perceived sensation of discomfort.
Tenderness	Sensation of discomfort elicited by pressure.
Eccentric Contraction (negative work)	A contraction during which the active muscle is lengthened.
Concentric Contraction (positive work)	A contraction during which the active muscle shortens.
MVC	Maximal voluntary contraction.
EMG	Electromyogram.
IEMG	Integrated electromyogram.
N	Newtons (9.81N=1kg force).
CK	Creatine kinase.
IU/l	International Units/litre.
Hr	Hour.
Min	Minute.
S	Second.
SD	Standard deviation.
SEM	Standard error of the mean.
n	Number.

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PAPERS BOUND IN.

1. Edwards, R.H.T., Mills, K.R. & Newham, D.J. (1981)
   Greater low frequency fatigue produced by eccentric than concentric contractions.
   Journal of Physiology, 317:17P.

2. Edwards, R.H.T., Mills, K.R. & Newham, D.J. (1981)
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   Journal of Physiology, 317:1P-2P.

3. Newham, D.J., Mills, K.R., Quigley, B.M. & Edwards, R.H.T. (1983)
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   Force, contraction frequency and energy metabolism as determinants of ischaemic muscle pain.
   Pain, 14:149-164.

ETHICAL CONSIDERATIONS.

All procedures have been approved for both normal healthy subjects and patients by the Committee on the Ethics of Clinical Investigations at University College Hospital and The Faculty of Clinical Sciences at University College London.

The normal subjects were volunteers from the staff of University College Hospital and The Faculty of Clinical Sciences, University College London. As such they were familiar with the techniques used and their implications. Informed consent was obtained from the subjects prior to each study.

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INTRODUCTION.

One of the inevitable consequences of working muscles to their limit is pain and damage. There are different types of muscle pain which occur in different situations and a study of the nature and mechanisms of muscle pain and damage is important for understanding both the mechanisms limiting athletic performance and those underlying muscle damage in disease.

There are two types of muscle pain associated with the performance of exercise; ischaemic pain and that which occurs with a delayed onset. These are separate from injuries such as actual ruptures of muscles, tendons and ligaments.

Ischaemic muscle pain occurs during exercise and increases in intensity while the activity continues and the circulation is impaired. Once the exercise is stopped and the circulation restored this type of pain, in normal subjects, disappears within seconds and leaves no residual effects. It has been shown that ischaemic pain is proportional to the energy cost of the exercise and also the contraction frequency (Lewis 1942, Park & Rodbard 1962, Rodbard & Pragay 1968, Mills et al 1982). In normal subjects fatigue occurs and the reduction in force offsets marked damage such as that which occurs in patients with glycolytic defects and in isolated muscle preperations where the muscles can work to the point of contracture (Jones et al 1983).

The second type of exercise related muscle pain is that which occurs with a delayed onset. It is not present during the exercise itself, nor for a matter of hours afterwards. It is usually most intense between twenty-four and forty-eight hours after exercise and may persist for up to one week. It is this type of muscle pain, and the associated muscle damage which is the subject of this thesis.

1.1. PAIN.

Pain is a sensation that is common to nearly all individuals and experience leads us to associate it with physical damage. Pain is felt when a noxious stimulus is applied to the body or pathological tissue damage occurs and when the stimulus is removed the pain goes away. There are obvious anomalies such as chronic neuralgia and phantom limb pain but these are seen to represent aberations in the mechanism rather than a fundamental change in the concept of pain.

The word pain is derived from the Greek for penalty which clearly illustrates the association between sensation and emotion. In earlier times it was seen as emotional rather than physical with Plato and Aristotle describing it as a "passion of the soul" and Marshall (1889) arguing that pain was an emotion and not a sensation.

In view of the emotional and subjective nature of pain it is not suprising that measurement and quantitation have always presented difficulties. In 1952 Hardy, Wolff and Goodell introduced a heat/pain dolorimeter and measured pain 'threshold' and 'reaction'. They were critised by Beecher (1959) who argued that no satisfactory technique for pain threshold measurement had been, or could be, developed and that it was impossible to distinguish between pain sensations

and reactions. Beecher made a valuable distinction between pathological pain and experimentally induced pain, but despite this initial rejection of pain measurements he later contributed to the development of a well accepted technique for measuring the threshold for ischaemic pain (Smith et al 1966). While there are many pitfalls some measurements must be made to enable the investigation of pain mechanisms, but great attention must be paid to the reliability and reproducibility of the methods used.

The work described in this thesis was undertaken with the aim of furthering the knowledge about the type of exercise most associated with delayed onset muscle pain and what forms of damage, if any, it is associated with. It is thought that this will expand the existing knowledge about the mechanisms of muscle damage, and will be relevant to the clinical investigation of patients with disease related muscle damage, and also that found in normal subjects which is exercise related. This will facilitate the design of training programmes for rehabilitation and sports.

1.2. DELAYED ONSET MUSCLE PAIN.

Heavy or unaccustomed exercise is often followed by a type of muscle pain that does not appear until hours after the exercise and may persist for days (Hough 1902, Llewellyn and Jones 1915, Hill 1951 and Taverner 1954).

Asmussen (1953) was the first to associate eccentric rather than concentric contractions with the development of muscle pain. The difference between these two types of dymanic contraction is that the active muscle shortens during a concentric contraction, while it is lengthened during an eccentric contraction. Since then other workers have reported post-exercise pain as occurring only after eccentric contractions (Asmussen 1956, Brendstrup 1962, deVries 1966, Komi and Buskirk 1972, Davies and Barnes 1972) Talag 1973, Rasch 1974, Cobb et al 1975, Abraham 1977 and Mills et al 1981). There has been, however, one report of delayed onset muscle pain after concentric contractions by McGlynn et al (1979).

Activity involving eccentric contractions is also known as negative work, and concentric contractions as positive work. In both cases there are semantic problems with the terminology as exception may be taken to the word "contraction" being used when the active muscle is infact lengthening. The term "negative work" is also open to misunderstandings, but rather than further complicate the

situation by introducing further nomenclature, the existing terminology is used in this work.

Many workers have beleived that delayed onset muscle pain is the result of damage, but there are various ideas about which tissues are damaged. Hough suggested that mechanical damage and rupture of the muscle fibres was responsible for delayed onset muscle pain. Asmussen and also Staton (1952) thought traumatised connective tissue was the cause. This view was supported by Hill (1951) who believed that microscopic mechanical damage was distributed throughout the muscle. Komi and Buskirk (1972) concluded that an overstretching of the elastic elements (i.e. non contractile elements) occured during eccentric contractions and caused the pain. Buchthal and Clemmsen (1940) thought that oedema was responsible for the pain as did Brendstrup (1962). A vicious circle of exercise, ischaemia, pain and increased relex activity leading to further ischaemia was advocated by de Vries (1966).

Clearly there is a concensus that delayed onset muscle pain is associated with some type of trauma and damage, but the tissue primarily affected as well as the underlying cause of the pain is not known. It is particularly interesting that while this type of pain is clearly exercise induced, and is thought to be the result of some form of damage, it is not apparent until hous after the exercise.

1.3. CONCENTRIC AND ECCENTRIC MUSCLE CONTRACTIONS.

There are clear differences between these two types of dynamic muscle contractions in both metabolic and mechanical terms. Both are regularly used in everyday activities and are used in training programmes.

1.3.1. Metabolic differences.

The subjective impression that it is easier to lower a weight than to raise it, although the work done is the same, was initially confirmed experimentally by Chauveau (1896) who measured oxygen uptake during various types of positive work (concentric contractions) and negative work (eccentric contractions). Approximately twice as much oxygen was used to do a given amount of positive work than negative work. More sophisticated techniques were used by Abbott, Bigland and Ritchie (1952) who found the metabolic cost of positive work to be 2.4-5.2 times higher than for negative work, the difference between the two increasing with contraction velocity.

In trying to account for the discrepancy these authors considered the possibility that the work absorbed during stretching of an active muscle, which does not reappear as either thermal or mechanical energy (Hill 1938, Abbott et al 1951), might have been used to stop or reverse the energy utilised to provide chemical processes, however they felt

that the observed difference was too large to be explained in these terms. It has subsequently been shown that this reversal does not occur (Gillis & Marechal 1974).

Asmussen (1953) agreed that the mechanical cost of negative work decreased as velocity increased and reported that the ratio of the energy cost of positive:negative work was 6-12.5.

Abbott and Bigland (1953) measured oxygen consumption during eccentric contractions using a motor driven cycle ergometer with variations of force and speed. When the rate of negative work was increased at a constant speed the rate of oxygen consumption increased rapidly, but when work rate was increased and force kept constant by alteration of pedalling speed the oxygen consumption remained relatively constant. They explained these findings in terms of the force:velocity relationship of active muscle during stretch whereby fewer fibres are employed to generate a given tension as the speed of lengthening increases.

Hill (1965) was of the opinion that in studies such as these the movements should be identical for any comparison to be made. Cycle ergometer studies fulfil this criterion but those using an inclined treadmill where the subjects walk forwards when going both up and down hill, do not. However Margaria (1968) reported the efficiency of positive work to be 25% and that of negative work to be 120% during inclined treadmill walking despite the dissimilarity of the movements. Nagle et al (1965) determined the cost of negative work to be

35% of positive and similar values of 28% and 27% were reported by other workers (Kamon 1970, Gupta et al 1973, Knuttgen et al 1971, Bonde-Petersen et al 1972, Davies and Barnes 1972 and Bigland-Ritchie and Woods 1973) using laddermill, step test, cycle ergometer and treadmill studies.

1.3.2. MECHANICAL DIFFERENCES.

The mechanical conditions during the two types of contraction have also been studied and found to be very different. The relationship between length and active tension was studied by Blix (1895), Asmussen (1936) and Buchthal (1942) who all found in the various muscle preparations used that the tension produced by a muscle which was stretched during activity was greater than when it was allowed to shorten.

The force:velocity curve for muscle described by Fenn and Marsh (1935) and Hill (1938) established that as the velocity of shortening increased the tension a muscle can generate decreased in a hyperbolic manner. This relationship between force and velocity was extended to eccentric contractions by Katz (1939) who found that at shortening velocities (eccentric contractions) the force developed is greater than that at numerically identical positive velocities. As the velocity of stretch increases the tension rises to a plateau at about 1.8 Po (Abbott et al 1952, Komi 1969 & 1973).

Komi & Rusko (1974) reported that repetetive maximal contractions when performed eccentrically resulted in a greater force loss than when performed concentrically, while the electrical activity showed the same pattern for the two types of contraction. They thought the greater fatigue

occuring during eccentric contractions was of mechanical origin and due to the extreme loading of the elastic components during the high tensions developed.

Asmussen (1953) reported that integrated electromyogram (IEMG) was larger when a muscle was shortening against a given weight than when it was lengthening under the same weight. He concluded that this was electromyographic confirmation of his view that the oxygen consumption of a muscle is proportional to the number of active fibres and independent of the tension produced. Support for this theory was provided by Bigland & Lippold (1954), Basmajian (1967) and Bouisset (1973) and Bigland-Ritchie & Woods (1976).

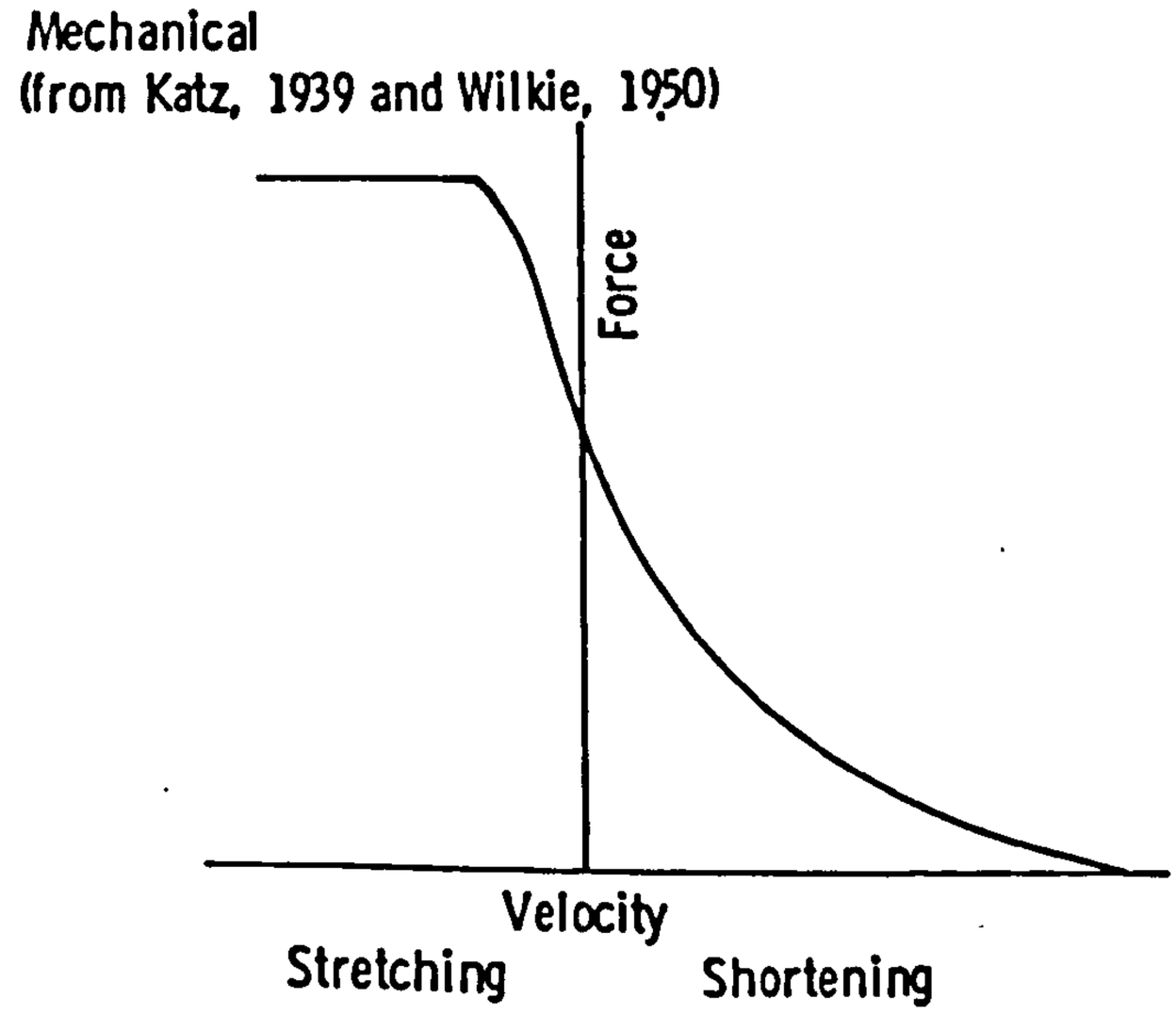
Komi (1973) was unable to find any clear difference in the IEMG:tension relationship with either type of contraction or at any speed of contraction when muscle tension was expressed as a percentage of the maximum voluntary contraction force. Komi & Rusko (1974) studied the electrical changes during fatiguing maximal contractions performed under eccentric and concentric conditions. They found that the IEMG declined continuously during the period and the pattern was similar for both types of contraction.

Bigland-Ritchie & Woods (1976) measured the relationship between IEMG, oxygen consumption and mean torque at various pedalling speeds on a cycle ergometer. The ratio of positive:negative work at 50 revs/min was 1.96 for the IEMG and 6.34 for the oxygen consumption, confirming the earlier results. It is of interest that when using untrained subjects

they found the IEMG to be similar for positive and negative work and the oxygen consumption during negative work to be greater than trained subjects while the oxygen consumption/kg/min during positive work was similar to trained subjects. Therefore the calculated reduction in the rate of oxygen consumption per unit fibre activity was less marked in the untrained subjects.

It has therefore been clearly demonstrated that under eccentric conditions muscle fibres generate greater tension than under concentric conditions. To produce a given force during lengthening fewer fibres are activated in the muscle, and each one generates a higher tension than it is capable of under conditions of active shortening. The force generated by a crossbridge is proportional to the degree of stretch in the series elastic component. During isometric and concentric contractions this is limited to the distance that the myosin head can rotate during the interaction with actin. During stretching the elastic component can be extended further and therefore greater tension generated.

The difference between the two types of contraction is greater in terms of metabolic cost than electrical activity and this is due to the reduced ATP utilisation per unit force which occurs in eccentric conditions (Infante et al 1964, Curtin & Davies 1973, Marechal et al 1974) (Fig. 1).



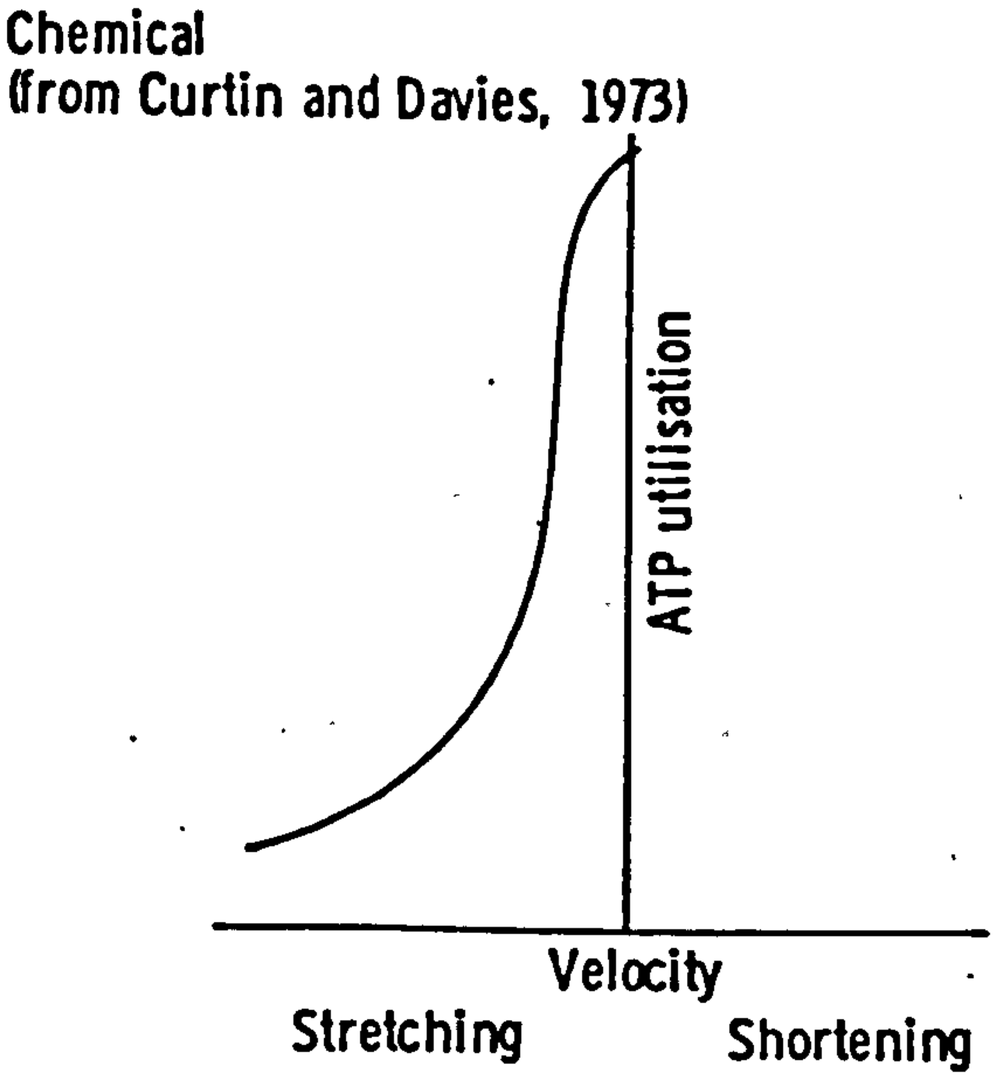


Fig. 1. Mechanical and chemical differences between concentric and eccentric contractions. Under eccentric (stretching) conditions a muscle is capable of generating greater tension than when concentric (shortening) contractions are used but the ATP utilisation is less.

1.4. MUSCLE NOCICEPTORS.

Clearly a knowledge regarding the type of stimuli that result in the sensation of pain is an advantage in understanding the mechanisms responsible. Mammalian sensory neurons are classified by their size and conduction velocity and whether or not they are myelinated. At present both numerical and alphabetical classifications are in common use. The A group of sensory neurones are the largest, have myelinated axons and are subdivided into alpha, beta gamma and delta types. A-alpha neurones relay impulses from primary muscle spindle endings (Group Ia) and golgi tendon organs (Group Ib). Sensory neurones subserving secondary muscle spindle endings, touch and pressure receptors are termed A-beta or Group II fibres. Pain, temperature and some touch receptors relay impulses in A-delta or Group III fibres. The small unmyelinated C or Group IV neurons conduct impulses from pain receptors.

Stimulation of an intensity sufficient to activate thin myelinated (Group III or A-delta) and non-myelinated (GroupIV or C) fibres of cutaneous nerves evokes painful sensations in humans and animals (Pattle and Weddell 1948, Collins, Nulsen and Randt 1960). Stimulation of group II afferents alone has never been shown to cause pain. By using graded stimulus intensities and differential blocking of myelinated or non-myelinated fibres it was established by Torebjork and Hallin (1973) that activation of the Group III and IV fibres

produced the so called fast and slow pain respectively. Fast pain was described as short lasting and well localised with a pin-prick quality, whereas slow pain was of relatively long duration, poorly localised and of a dull, burning quality.

The work that has been done on pain receptors and their sensory nerves is mostly confined to skin and viscera. Little work has been done on muscle nociceptors, mainly because there are appreciable technical difficulties in gaining access to the fibre endings is difficult since most of them are within the muscle bulk. Group III and IV afferent fibres and the freely branching, unencapsulated endings of these fibres have been found throughout skeletal muscle and have been reported as having particularly dense projections in the regions of tendons, aponeuroses and fascial sheaths (Stacey 1969). Sensitivity to mechanical, chemical and thermal stimuli has been demonstrated in these receptors (Paintal 1960, Iggo 1961, Pomeranz et al 1968, Kumazawa and Mizumura 1977, Mense and Schmidt 1977, Mense 1977 a&b). Most of the receptors are polymodal and others have been found to be either slowly or rapidly adapting to mechanical stimuli. Chemosensitive nociceptors have been stimulated by bradykinin, 5-hydroxytryptamine (serotonin), histamine, potassium, hypertonic saline and hydrogen ions.

Some Group IV fibres are stimulated by very strong contractions and will fire more if the contracting muscle becomes ischaemic (Kniffki et al 1981). Group III fibres are not excited by ischaemia (Zimmerman 1976). However some Group

III units show reliable responses to local pressure, stretch and strong contractions (Paintal 1960, Kniffki et al 1981, Ellaway et al 1982).

1.5. ULTRASTRUCTURAL CHANGES ASSOCIATED WITH EXERCISE AND MUSCLE DAMAGE.

Morphological indications of muscle damage can be detected using both light and electron microscopy. There is little data about the morphological damage induced by exercise in normal subjects, in either the presence or absence of muscle pain. Gollnick et al (1969) reported that after prolonged exhaustive exercise (mainly concentric) the muscle ultrastructure was unchanged although some swelling of the mitochondria was seen. Muscle pain was not mentioned, and so presumably was not a significant factor. It has been reported by Friden and coworkers (1981, 1983 a) & b) that heavy eccentric muscle work resulted both in pain and morphological evidence of damage in normal subjects. They performed muscle biopsies before and then two and seven days after exercise. They found the most severe morphological damage in the samples taken two days after exercise. One week later the muscles had virtually recovered.

Hecht et al (1975) found fibre necrosis in rat skeletal muscle after very strenuous dynamic exercise and van Linge (1962) reported signs of both degeneration and regeneration after similar studies. Salminen et al (1981 & 1982), also Vihko and coworkers (1978 a) & b), 1979) reported "lethal and sublethal injuries" in several studies of rat skeletal muscle after severe prolonged exercise. The latter group concluded that the increases in lysosomal activity they

consistently found was an autophagic response, probably reflecting the degeneration of surviving but damaged fibres. Armstrong et al (1983) compared uphill, level and downhill running in rats and found that ultrastructural damage was only seen after eccentric (downhill) running, and that Type I fibres were predominantly affected. However, Friden et al reported that in untrained human subjects the damage was mainly found in Type II fibres.

The morphological features of Z-line streaming and disruption of the internal architecture are taken to be non-specific indicators of damage. They are found in a variety of pathological conditions including Duchenne muscular dystrophy (Milhorat et al 1966, Fardeau 1969), malignant hyperpyrexia (Harriman et al 1977), motor neurone disease (Alfifi & Aleu 1966) and various myopathies (Rewcastle & Humphrey 1965, Engel 1966, Gonatas et al 1969, Dubowitz & Roy 1970, Armstrong et al 1971, Engel et al 1971). In addition such findings have occasionally been seen in the skeletal muscle of normal subjects (Fischman et al 1973 & Meltzer et al 1976) and have also been seen in this laboratory in the muscles of athletes (unpublished).

As Z-line streaming and myofibrillar disruption seem to be associated with both exercise and muscle damage it seemed logical to investigate in this work the association between morphological damage and delayed onset muscle pain.

1.6. MUSCLE ENZYME EFFLUXES ASSOCIATED WITH EXERCISE AND DAMAGE.

The presence of raised levels of muscle enzymes in serum or plasma is taken as a biochemical indication of some form of muscle damage and is known to occur in pathological conditions and also after exercise in normal subjects.

Muscle enzyme efflux occurs in a wide range of myopathic conditions (Pennington 1981) as do myocardial enzymes after infarction (Hearse 1979). In the latter case the time course of the efflux of the enzyme creatine kinase (CK) has been well studied and found to reach peak values 24 hours after infarction. Furthermore the magnitude of the enzyme release is proportional to, and indicative of, the size of the infarct.

Exercise is also known to raise the circulating levels of muscle enzymes in normal healthy human subjects and animals. Most workers have assumed that peak levels occur 24 hours after the event, as with myocardial infarction, and have not measured the activity for longer than this. The time course has been studied by a number of workers (Thomson et al 1975, Kamen et al 1977, Shumate et al 1979 and Brooke et al 1979) and all reported peak values 24 hours after exercise - which consisted mainly of concentric work. In view of the prolonged period for which post exercise muscle pain is experienced it was deci ded in the work reported here to compare the time courses of the enzyme release with that of

other indicators of muscle damage and also muscle pain.

It has consistently been reported that greater muscle enzyme effluxes occur after exercise in untrained than trained subjects (Fowler et al 1968, Ahlborg & Brohult 1967, Misner et al 1973, Maxwell & Bloor 1981). Klausen & Knuttgen (1971) reported similar findings and hypothesised that the training adaptation was due to be muscles reutilising the energy liberated during negative work. However Bonde-Petersen & Knuttgen (1970) later reported that during an eccentric training period there was no evidence of enzymatic adaptation, and also that the training effect took place without any changes in strength.

In addition to agreeing that greater enzyme effluxes occured after exercise with untrained individuals Fowler et al (1968) reported that greater responses occured in female subjects. This is in contrast to the results of Griffiths (1966), Shumate et al (1979) and Thomson & Smith (1980) who all found significantly higher values in men. This led to speculation that oestrogens might play a protective role against large enzyme effluxes in women by stabilising the muscle cell membrane. Smith et al (1979) measured the resting levels of CK in women over a wide range of ages and found them to be similar for prepubertal and postmenopausal females, lower in menstruating women and lowest in pregnant women. They found similar changes at all stages of the menstrual cycle, and no evidence of a diurnal variation.

While there is general agreement that exercise causes

skeletal muscle enzyme efflux in normal subjects the mechanism is not clear. Berg & Haralambie (1978) concluded that with exercise lasting up to five hours the CK rise was proportional to exercise duration. Hagberg et al (1982) were in general agreement with this but pointed out that the type of exercise used affected the magnitude of the CK release. They found that weight lifting exercise caused higher levels than cycling for a similar period, and commented that the lifting exercise caused delayed onset muscle pain, while the cycling exercise did not. Clarkson et al (1982) compared the enzyme efflux after sets of ten maximal isometric contractions with either five or twenty second rest periods between the sets. They found that the longer rest period resulted in the maintainance of higher tension as well as higher CK levels and concluded that intensity and not duration was primarily responsible for the efflux. Fowler et al (1968) were of the opinion that both duration and intensity were important, but that duration was the more so. Burke et al (1982) agreed that a combination of both factors was important and proposed that raised CK levels did not necessarily indicate muscle damage.

Total circulating levels of CK incorporate three isoenzymes. CK MM comes from skeletal muscle, BB from brain tissue (Eppenberger et al 1964) and the third hybrid form - CK MB, from cardiac muscle (Jacobus 1975). It is obviously a possibility that heavy and prolonged exercise may cause the release of CK MB from stressed cardiac muscle. This has been

investigated by Kamen et al (1977), Bornheimer & Lau (1981) and Apple et al (1983) who all found that even after such strenuous exercise as marathon running and exercising at high work rates to exhaustion, the increased circulating CK was of skeletal and not cardiac origin.

While the factors causing muscle enzyme release from the damaged heart have been extensively investigated using isolated preperations, there have been relatively few similar studies on skeletal muscle. What studies there have been (Zierley 1956 & 1957), Dawson 1966, Suarez-Kuntz et al 1981, Jones et al 1983) indicate that metabolic depletion either directly or indirectly causes or initiates changes which allow the enzyme efflux. These studies, in conjunction with the existing knowledge that exercise and muscle damage cause an efflux of muscle enzymes, suggested that the measurement of circulating enzyme levels would be useful in understanding more about the involvement of muscle damage in delayed onset muscle pain.

CHAPTER 2. PAIN AND FATIGUE AFTER CONCENTRIC AND ECCENTRIC CONTRACTIONS.

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2. PAIN AND FATIGUE AFTER CONCENTRIC AND ECCENTRIC CONTRACTIONS.

2.1. Introduction.

In the light of the available literature on delayed onset muscle pain and damage, as discussed in the previous chapter, preliminary studies were carried out to establish whether or not this type of muscle pain does indeed occur after eccentric rather than concentric muscle work. Furthermore, there seemed to be a need to make a direct comparison of these two types of muscle work and determine their effect on muscle fatigue as well as muscle pain. Fatigue is a non-specific term, and in this case was investigated by measuring the changes in the maximal voluntary contraction (MVC) force, the frequency:force relationship, and the electromyogram (EMG).

A step test was used as it allowed the direct comparison of concentric and eccentric exercise of the quadriceps in terms of pain development and fatigue. It was a very useful model for these studies as equal amounts of total work were performed by each quadriceps by means of either positive or negative work in a single study.

The quadriceps muscles have the advantage that by using simple equipment they may be contracted either concentrically or eccentrically, as indeed they often do in everyday life. As the sole muscle group responsible for the movement of knee

extension their contraction force can be accurately measured from either voluntary or electrically stimulated contractions. Furthermore, being superficial it is possible to record the EMG from them and changes in the EMG signal are useful when studying fatigue.

Delayed onset muscle pain and soreness is accompanied by tenderness (discomfort elicited by pressure). It was necessary to quantitate an aspect of discomfort and this was done by the development of a new technique in which the force required to elicit tenderness was measured at predetermined sites over the surface of the muscle. Previous workers have used simple visual-analogue or verbal rating scales, but for the purposed of this work more detailed information was required about the distribution and time course of muscle symptoms. Thus there was a need for a technique which would enable this information to be accquired.

2.2. METHODS.

In these initial studies four normal subjects performed step tests. Three of the subjects exercised for 15 and 20 minutes on separate occasions while the fourth exercised for 20 minutes only. At least two weeks elapsed between tests on an individual subject, and in the second test the quadriceps which had performed eccentric contractions in the first test were used to perform concentric contractions and vica versa. No significant differences were found between the results obtained with the two exercise durations and so the results have been combined.

Three of the subjects were male and one female, the age range was 31-45 years (mean 36.2 years).

2.2.1. Force recording from the quadriceps. (Fig. 2)

The subject was seated in a testing chair (Edwards et al 1977a) based on that described by Tornvall (1963). The knee was flexed to a right angle and an inextensible strap passed round the ankle and horizontally back to the strain gauge. The pelvis was fixed by a seat belt. Force was recorded by a Merlab (Type SF 96-U) strain gauge. The output of a balanced bridge circuit was amplified and recorded on a U.V. oscillograph (S.E. Labs. 3000 D/L onto Kodak direct print paper (T. 1095) through a galvanometer (S.E. Labs B450) with a frequency response of 450Hz.

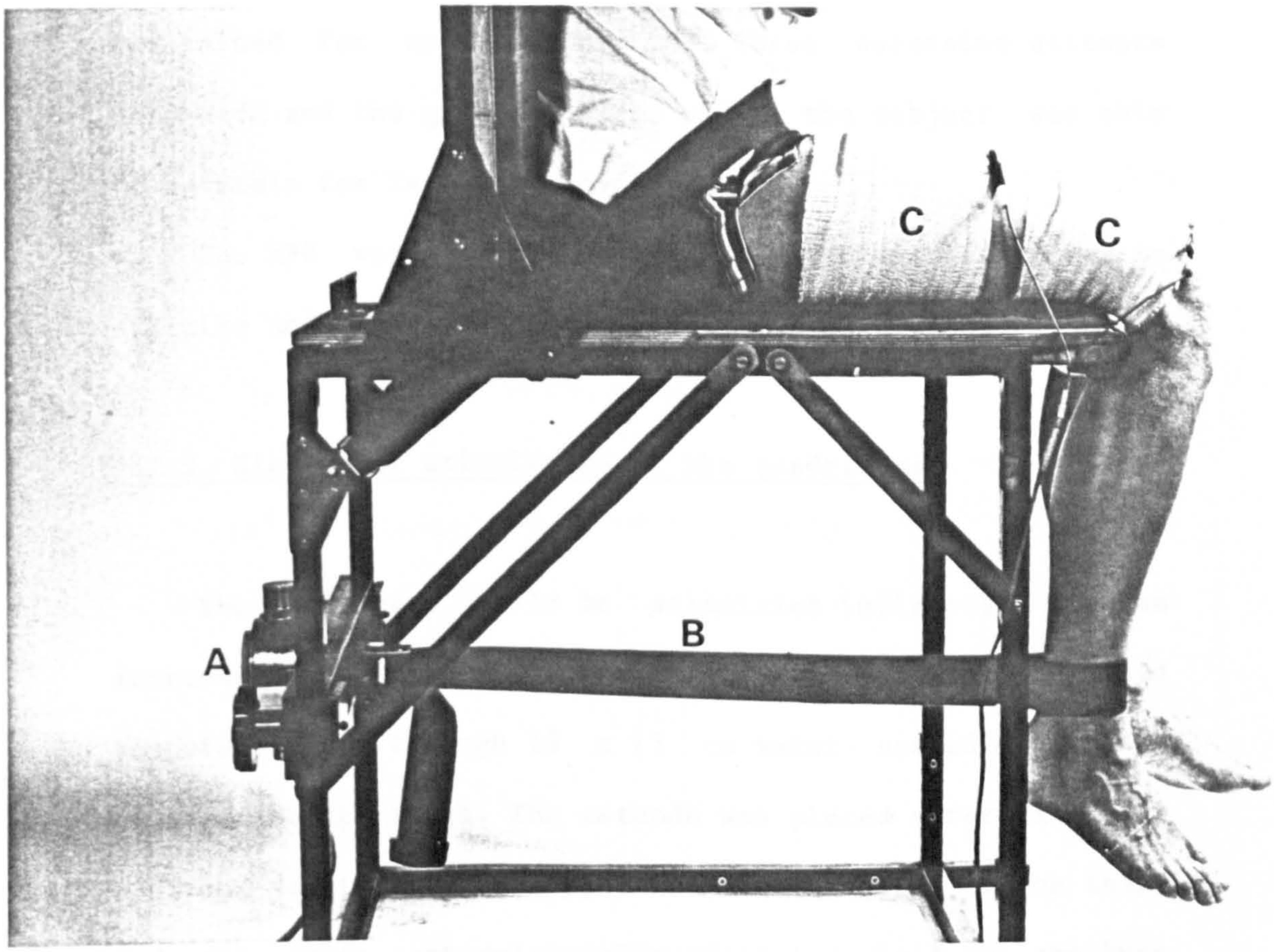

Fig. 2. Apparatus used for force recording and electrical stimulation of the quadriceps. A) Strain gauge, B) strap to ankle, C) Percutaneous stimulating electrodes.

The strain gauge was regularly calibrated using known weights and gave a linear response over the range used.

To determine the maximal voluntary contraction (MVC) force the subjects were asked to make a maximal effort to extend the knee of the appropiate leg. The contractions were maintained for approximately 3s. Three sucessive attempts were made and the greatest force which the subject was able to maintain for 2s was taken as the MVC.

The MVC was measured bilaterally immediately prior to exercise and then at predetermined times afterwards.

2.2.2. Electrical stimulation of the quadriceps.

The quadriceps could be stimulated indirectly via the intramuscular nerves by electrical stimulation passed percutaneously through 13 x 13 cm water soaked electrodes bandaged to the thigh. The cathode was placed anterolaterally over the junction of the upper and middle thirds of the thigh and the anode anterolaterally just proximal to the knee joint. Unidirectional 50us square wave pulses were delivered by a Devices (type 3072) constant voltage stimulator.

The impulse frequency and duration was programmed on a Devices Digitimer (D4030) which was set to deliver impulses at 1 Hz for 5s then 10,20,50 and 100Hz for 2s each. Excitation occurs through the intramuscular nerves or their terminals as very high voltages (approximately 1000v) are required to stimulate the muscle directly (Hill et al 1979).

Although it is not possible to stimulate the whole muscle it is possible to stimulate a portion of the muscle supramaximally. It has been found that if voltages are used such that a force greater than 10% of the MVC are generated at high frequencies of stimulation then the frequency:force characteristics of the muscle are essentially independent of force and the amount of muscle stimulated (Edwards & Newham 1984). This is an important practical consideration when using the quadriceps as inaccurate force:frequency relationships may be obtained if too small a portion of the muscle is activated (Davies & White 1981 & 1982). In practice 200-300 Volts were used to achieve 20-40% of the MVC force.

The frequency:force relationship was determined bilaterally immediately before and at intervals after exercise.

2.2.3. Step tests.

Subjects performed a step test in such a way that the quadriceps of one leg contracted concentrically (stepping up) while the contralateral muscle contracted eccentrically (stepping down). A step height of 46cm was used.

Stepping frequency was set by an electronic metronome (Taktell) which was provided an audible signal at one second intervals. Each stepping phase lasted 1s. One complete cycle consisted of four phases - 1. stepping up, 2. changing distribution of body weight, 3. stepping down and 4. as 2.

Thus a stepping frequency of 15 cycles/min was acheived.

2.2.4. Electromyography.

In two subjects areas over rectus femoris, vastus medialis and lateralis on each leg were prepared by abrasion and alcohol swabs to lower the skin resistance to less than 5 Kohm. These areas were indelibly marked so that identical sites would be used on subsequent testing. Silver/silver chloride cup electrodes were filled with electrode jelly and taped in place. Uni-polar recordings were made from these sites and amplified with reference to an electrode placed over the lower lumbar spine in the mid-line.

Signals were amplified (S.E. Labs type 4901) and band pass filtered between 0.2 and 10K Hz then displayed on a U.V. oscillograph and recorded on light sensitive paper. The 6 raw signals were integrated over 300ms periods and similarly displayed. The equipment was assembled by the Medical Physics Department at University College Hospital and used with the permission of Professor J.P. Moss (U.C.H. Dental School).

The EMG from all three sites was recorded from both legs before and at 5 min intervals throughout the exercise and afterwards at intervals for up to 24 hrs. Recordings were made during three consecutive stepping cycles.

In addition recordings were made from the same sites on the muscles before and after exercise during a submaximal knee extension maintained for 3s. A 3Kg weight was attached

to the foot.

2.2.5. Measurement of knee angle.

To study the relationship between electrical activity in the quadriceps and the angle of the knee, electronic goniometers were used (made by the Medical Physics Department at U.C.H.) A rotary potentiometer with a linear response was mounted as the pivot between two perspex arms. The goniometers were placed laterally on each leg with the potentiometers sited over the centre of rotation of the knee joint and the perspex arms taped in place along the surface marking of the femur and fibula. A signal proportional to the knee angle was displayed on the U.V. oscillograph with the EMG and simultaneously recorded.

2.2.6. Measurement of tenderness.

The severity and distribution of muscle tenderness (the sensation of discomfort elicited by pressure)` was measured over the surface of the muscle. A polythene sheet was marked with a grid of intercepts 2cm apart, the intercepts serving as test sites. The sheet was wrapped around the thigh and the skin marked to ensure identical positioning in subsequent tests. One of two interchangeable pieces of equipment were used to measure the force required to elicit tenderness. In one case the pressure was applied through a flat ended

plastic probe 2cm diameter which forms the head of an electronic force transducer (Penny and Giles Transducer Ltd). The maximal force measurement was displayed on a liquid crystal display which forms part of the unit. The display was reset to zero after testing at each site. In the second case a probe of the same dimensions attached to a strain gauge, the amplified force signal from which was displayed on a U.V. recorder.

At each site a gradually increasing force was applied up to a maximum of 40N. The subject was asked to indicate verbally when tenderness threshold was reached whereupon the probe was immediately withdrawn. If tenderness had not been reached at a force of 40N it was considered not to be present at that site. The upper limit of 40N was arbitrarily decided upon as at forces much greater than this discomfort was experienced in unexercised muscle which were not subjectively painful or tender. Sites were tested in a set order. In this way a record was obtained of the degree of tenderness over the surface of the muscle (Fig. 3).

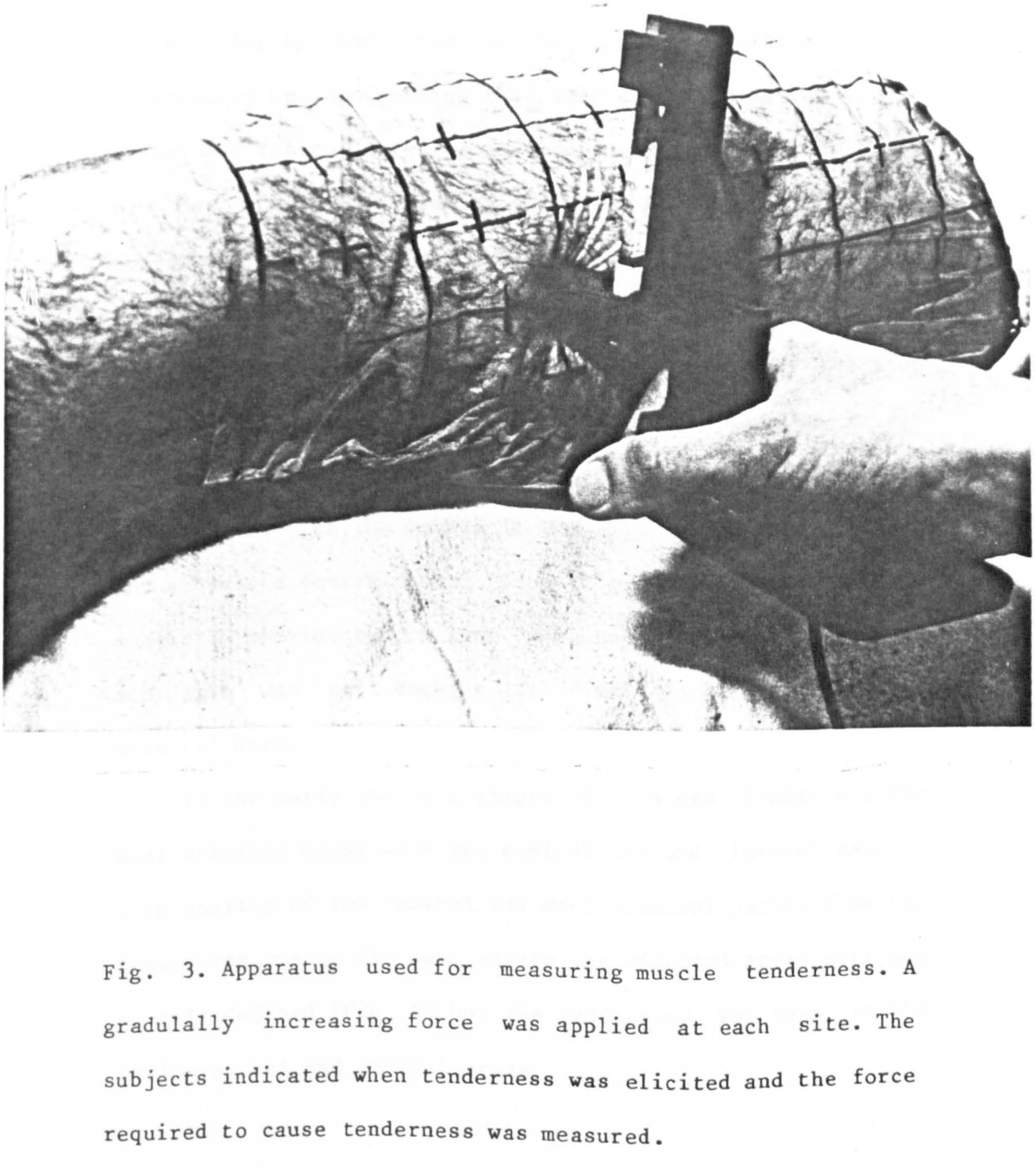

Fig. 3. Apparatus used for measuring muscle tenderness. A gradulally increasing force was applied at each site. The subjects indicated when tenderness was elicited and the force required to cause tenderness was measured.

2.3. RESULTS.

2.3.1. Distribution of muscle pain and tenderness.

During the step test all subjects reported that initially the sense of effort was greater in the leg which was stepping up but later in the test more difficulty was experienced in controlling the step down. While subjective effort and fatigue were reported during stepping actual pain was not. Once pain developed it was only present in the quadriceps of the leg which had stepped down, the contralateral calf and ipsilateral gluteal muscles - all muscles which had contracted eccentrically during the step test. No discomfort was reported in any other muscles by any of the subjects. The most painful procedures were isometric contractions with the muscle in the fully shortened position and eccentric contractions. Isometric contractions with the muscle in mid-length position were not particularly painful and pain was not thought to limit force generation as measured here.

In the early and late stages of pain and tenderness the most affected areas were the mediodistal and lateral aspects with sparing of the central and medioproximal parts. When the tenderness was at its most severe the affected areas were not so well defined (Fig. 4) but the tenderness was less in the medioproximal and central areas.

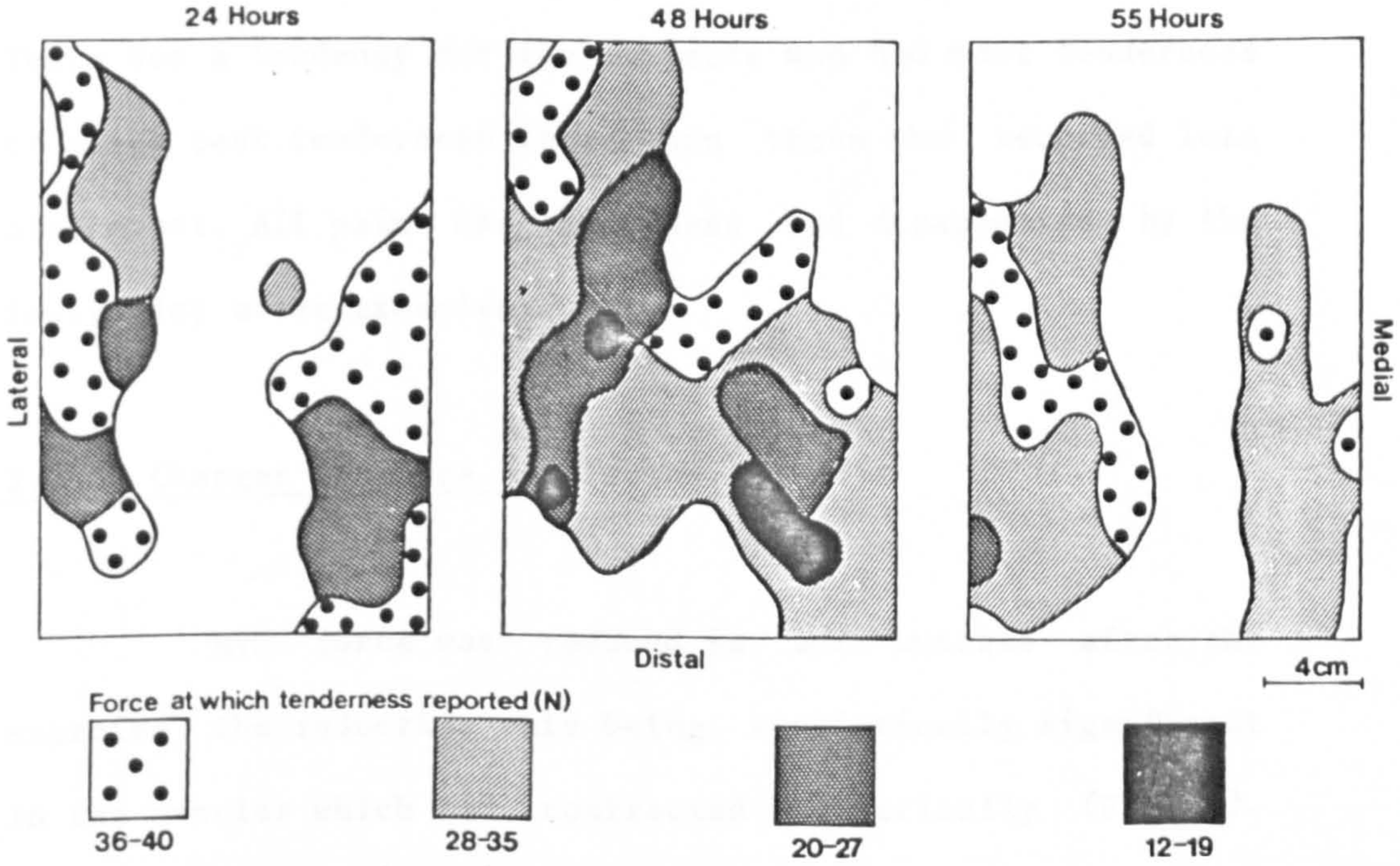

Fig. 4. Distribution and severity of tenderness in the right quadriceps after eccentric contractions. Note relative sparing of the central area of the thigh overlying rectus femoris. Subject: DN, female, 31 yrs.

2.3.2. Time course of pain and tenderness.

The onset of pain and soreness occured between 8 and 10 hours after exercise in all subjects. Tenderness was not measured until 18 hours after exercise and was found to reach peak intensity at between 24 and 48 hours after exercise. There was a tendency for the subjects who had most tenderness to reach peak tenderness later than those who recorded less discomfort. All pain and tenderness had disappeared by the fourth day after exercise.

2.3.3. Changes in force generation.

MVC force was reduced in both muscles after the exercise, the reduction only being statistically significant in the muscles which had contracted eccentrically (Fig. 5). When pre-exercise MVC was compared with that at 2 and 10 minutes after exercise the difference was highly significant ($p<0.001$). Voluntary force recovered over 24 hours. (Appendix i).

As a result of stepping the frequency:force characteristics of both muscles were altered (Appendix ii), greater changes occuring in the muscles which had contracted eccentrically (Figs. 5 & 6). Force generation at the higher frequencies (50 & 100Hz) was relatively preserved while forces generated by low frequency stimulation (1, 10, & 20Hz)

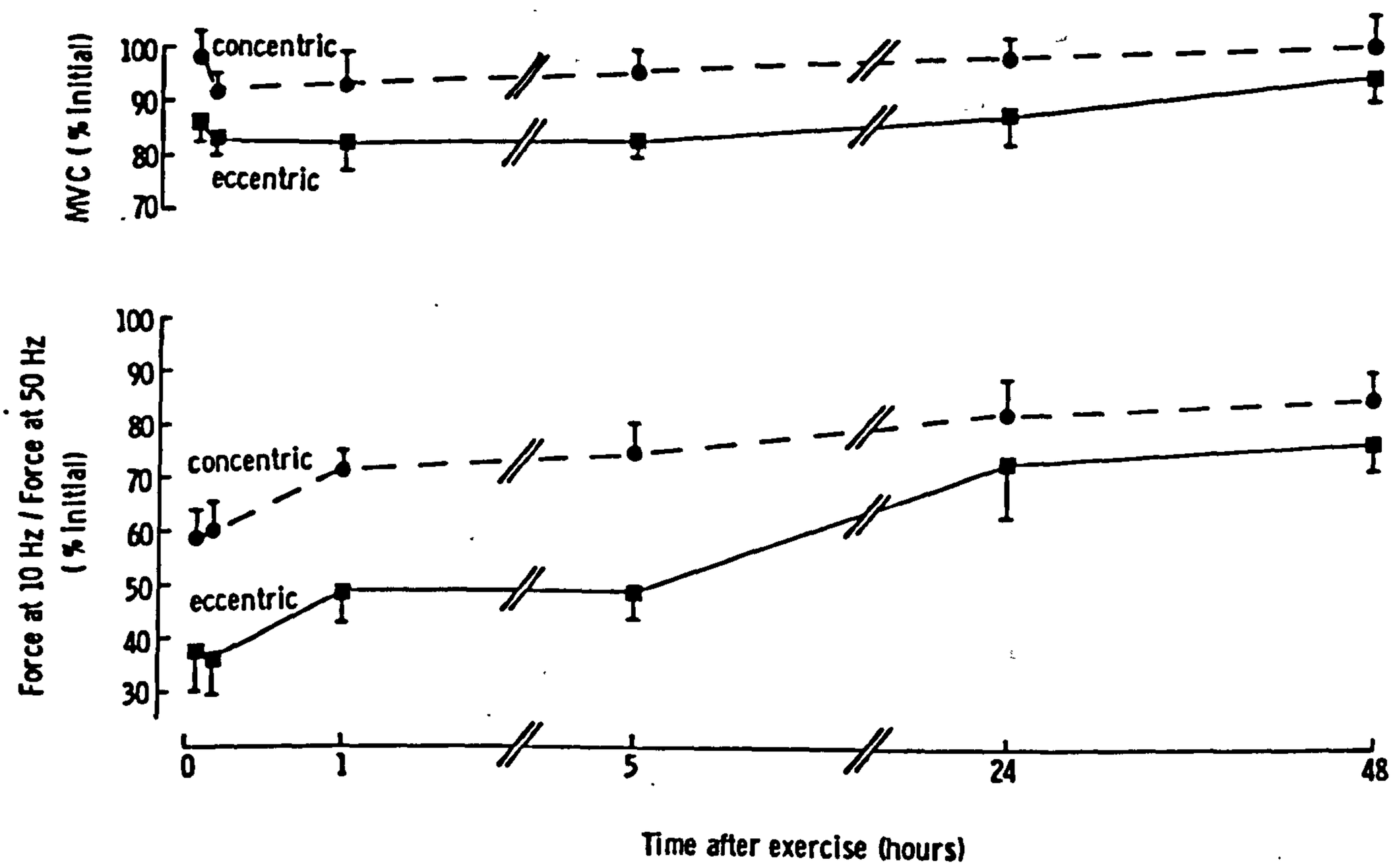

Fig. 5. Changes in MVC and contractile properties of the quadriceps after stepping (Mean ± SEM, n=7). Note greater changes caused by eccentric contractions and the long time course of recovery.

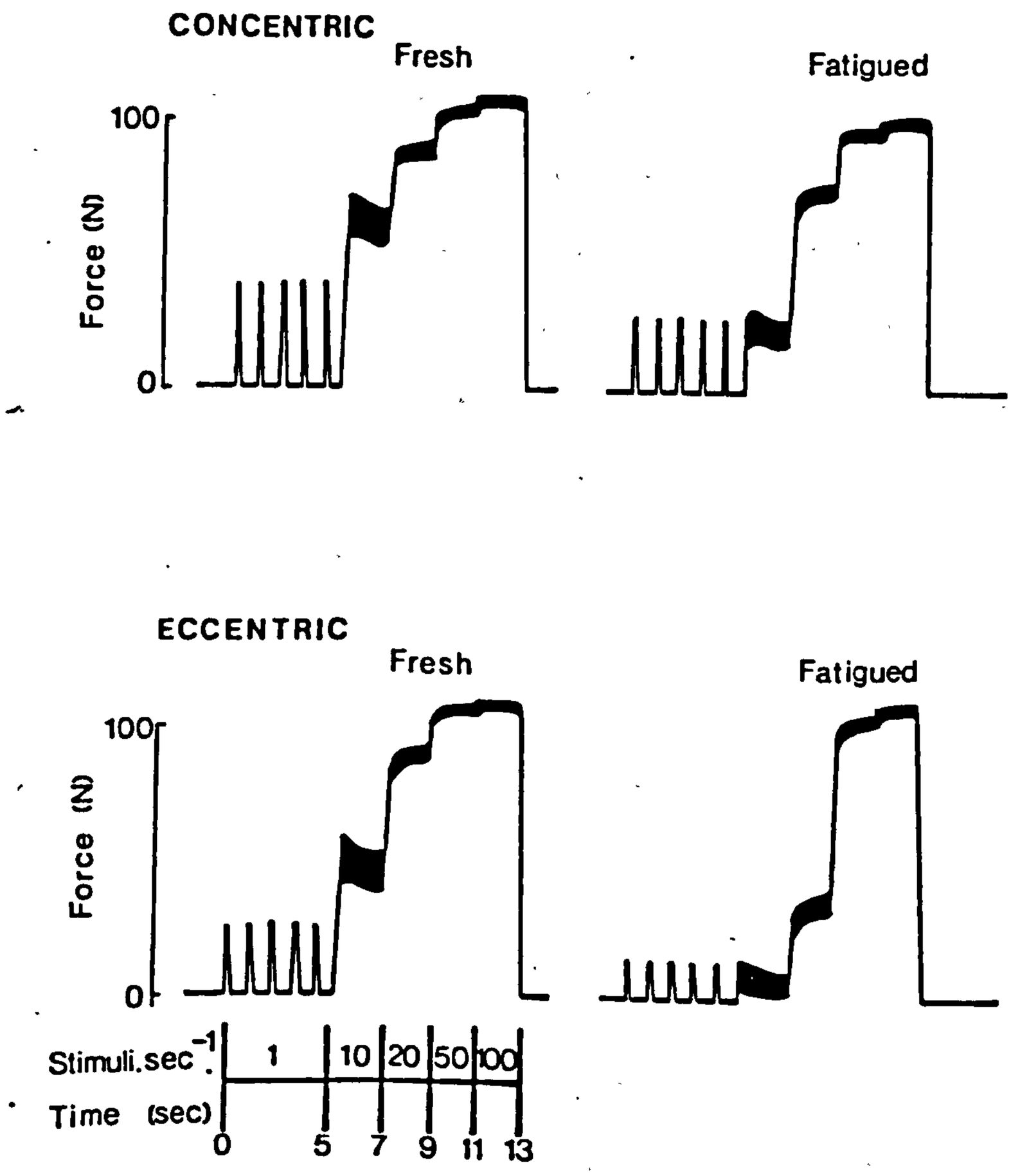

Fig. 6. Contractile properties of the quadriceps before and 10 minutes after stepping for 20 minutes. Note greater change in the fatigued muscle after eccentric contractions. Subject: DN, female, 31yrs.

was reduced. The force produced by stimulation at 10Hz expressed as a percentage of that produced by 50Hz stimulation (10/50%) has been taken as an index of low frequency fatigue. The 10/50% was significantly reduced in both legs in the immediate post-exercise period ($p<0.001$). The difference between the two muscles was significant at 2 minutes ($p<0.05$), 10 minutes ($p<0.05$) after exercise and most highly significant 5 hours after exercise ($p<0.02$) as the muscle which had contracted concentrically showed some degree of recovery which did not take place in the contralateral muscle. Twenty-four and forty-eight hours after exercise there was no significant difference between the two muscles, although when compared to pre-exercise values neither muscle had fully recovered.

2.3.4. Changes in electrical activity.

On two subjects surface EMG recordings were made from areas over rectus femoris and the vasti medialis and lateralis bilaterally and symmetrically whilst stepping before, during and after the actual step test. Recording were also made while the subject performed a dynamic knee extension with a 3Kg weight attached to the foot. Full extension was maintained for approximately 2s. This procedure was carried out before and at intervals after exercise. Similar results were obtained from both subjects.

The area of the integrated EMG (IEMG) was approximately equal from all three sites on each leg, being slightly smaller at sites on the eccentrically contracting muscle only in the first few minutes of the step test. The simultaneous recording of joint angle and IEMG during stepping showed in the concentrically contracting muscle the main burst of electrical activity occuring during the stepping up phase with a smaller burst as the opposite muscle lowered the body weight to the ground to be supported by the former (Fig. 7).

The eccentrically contracting muscle showed two main peaks of activity in each cycle which were approximately similar in amplitude to each other, and shorter in duration than the main peak in the concentrically contracting muscle. One peak occured during the eccentric contraction itself and the other at the time when the leg was taking part of the body weight after the opposite muscle had raised the body up onto the step.

Recordings made during the first minute and then at 5 minute intervals showed a progressive increase in the IEMG area of the eccentrically contracting muscle whilst that of the concentrically contracting muscle remained relatively unchanged (Fig. 8). Using the mean of 3 consecutive steps (Appendix iii) it was found in the concentrically contracting muscle that when the values at the end of the 20 minute stepping period were compared to those obtained at the start of stepping, only the electrical activity recorded from rectus femoris had increased significantly during the

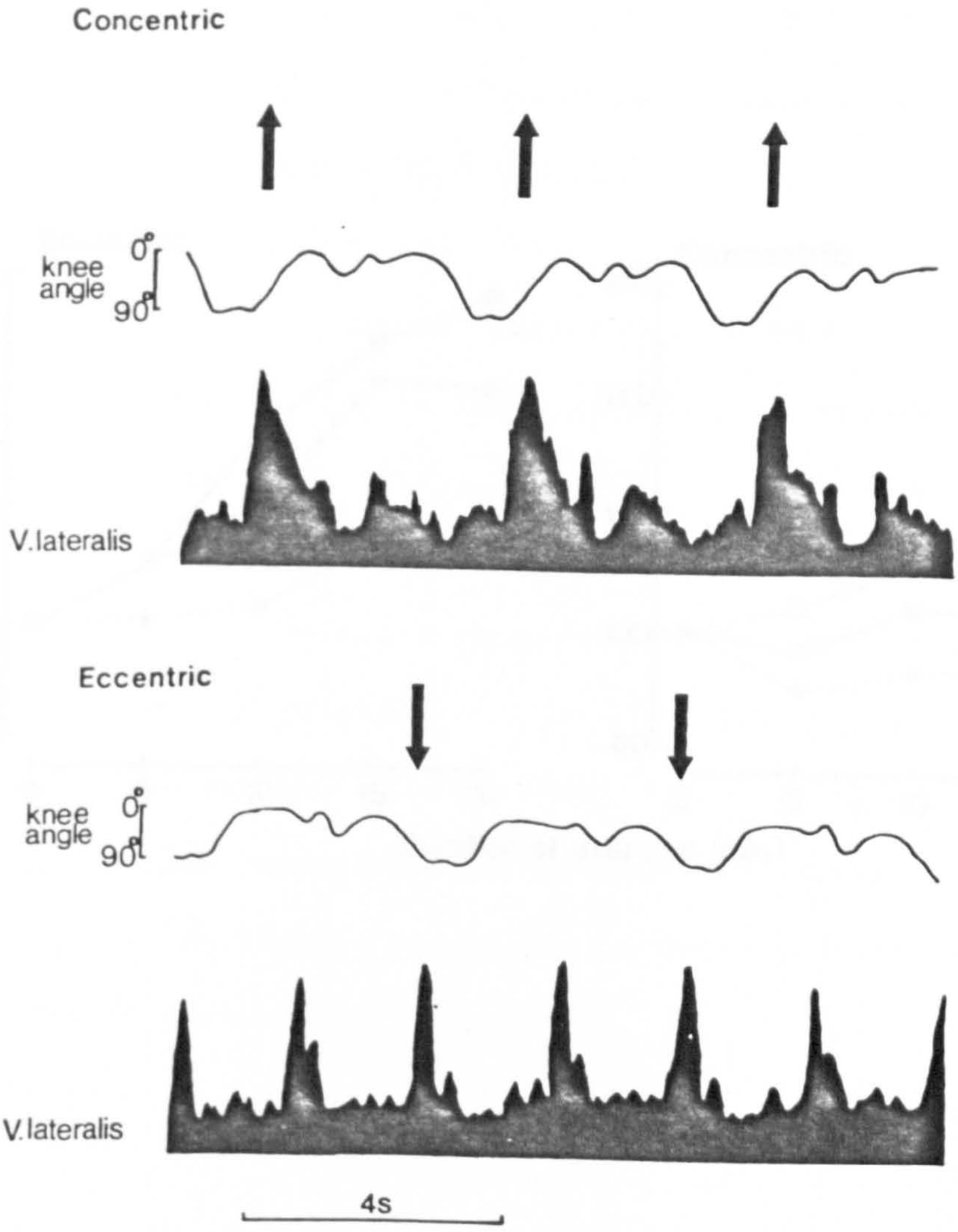

Fig. 7. Knee angle and EMG from vastus lateralis recorded simultaneously during stepping up (↑) and stepping down (↓). Subject: BQ, male, 45 yrs.

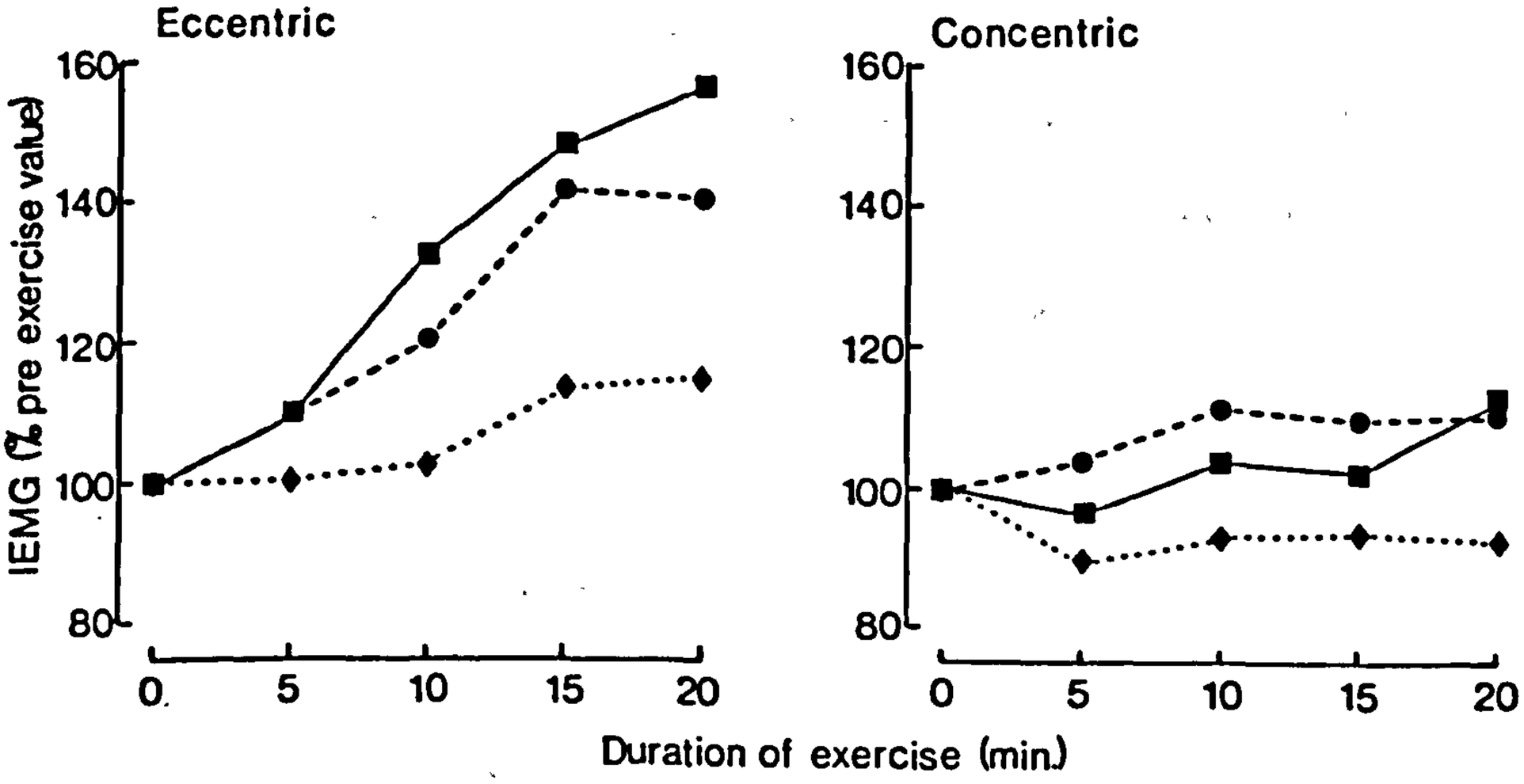

Fig. 8. Changes in electrical activity of the quadriceps during concentric and eccentric contractions. The IEMG area was measured during a 20 minute step test in which the muscles contracted either concentrically or eccentrically. Note progressive increase during eccentric but not concentric activity. Each point is the mean of 3 consecutive contractions. ■ rectus femoris, ● vastus medialis, ◆ vastus lateralis. Subject: BQ, male, 45 yrs.

stepping period to 113% of the initial value ($p<0.05$). In the eccentrically contracting muscle significant increases were seen from all 3 sites, in rectus femoris to 157% ($p<0.001$) in vastus medialis, to 151% ($p<0.005$) and in vastus lateralis to 115% ($p<0.05$) of the initial values. Full recovery had taken place 24 hrs later (Fig. 9).

As a result of the stepping the IEMG area during 2s of submaximal knee extension when compared to values obtained before exercise was unchanged in the muscle which had contracted concentrically. The areas were increased significantly in the early post-exercise period in the muscle which had contracted eccentrically (Fig. 10) with recovery to pre exercise values occuring over 24 hours (Appendix iv).

At no time throughout the testing period was there any evidence of spontaneous electrical activity or inhibiition of activity in painful areas of muscle.

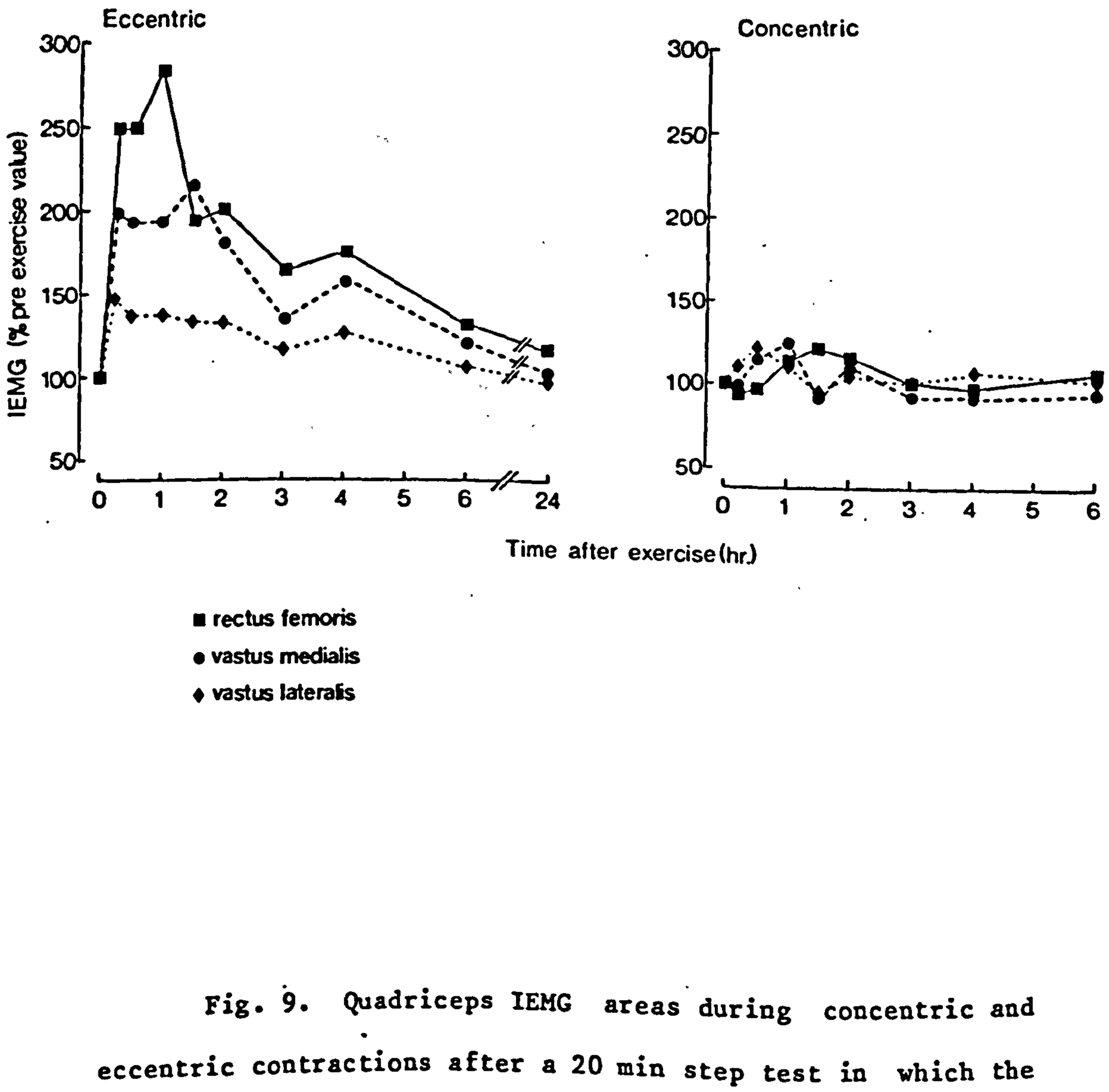

Fig. 9. Quadriceps IEMG areas during concentric and eccentric contractions after a 20 min step test in which the muscles had performed the same type of contraction. Subject: BQ, male, 45 yrs.

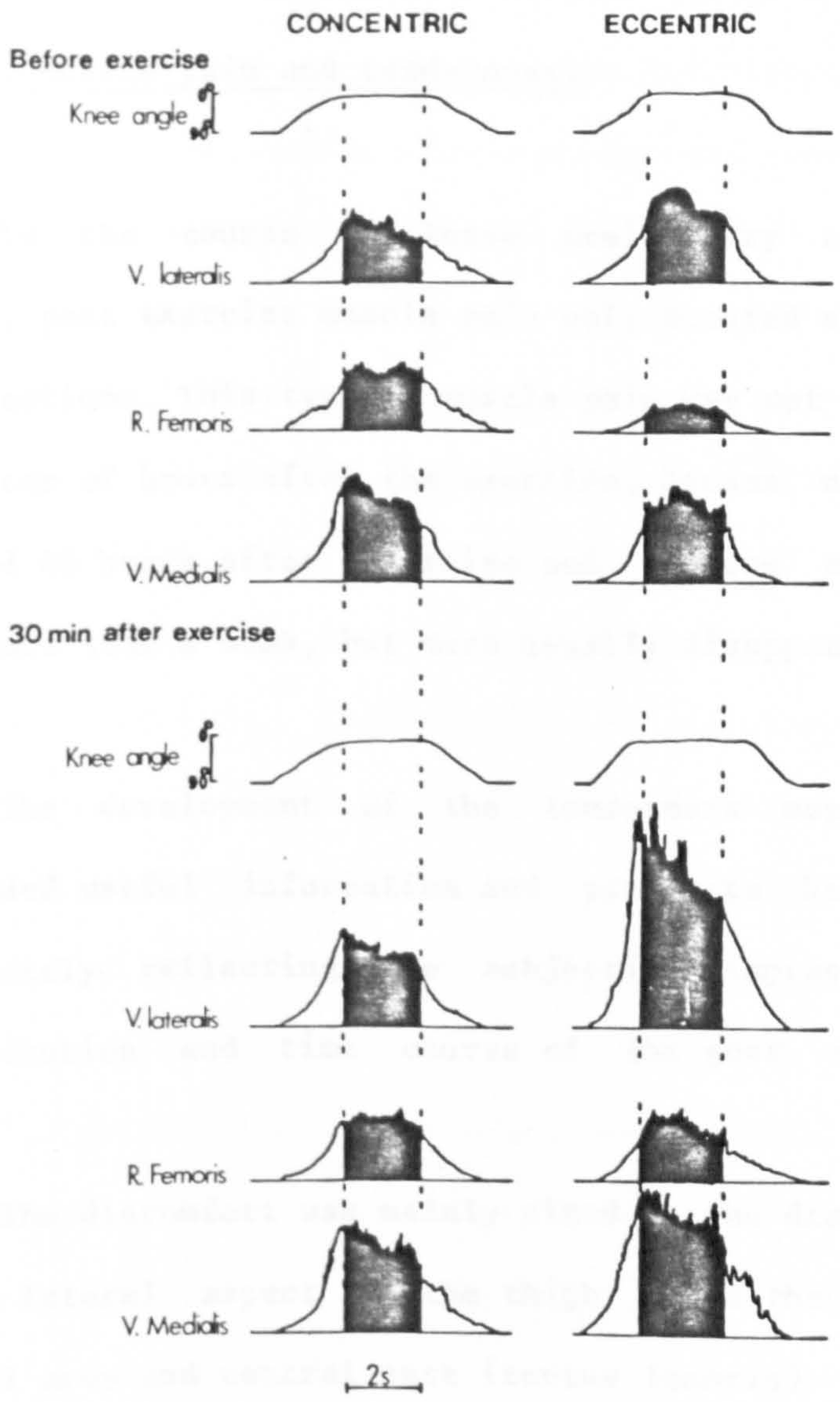

Fig. 10. Quadriceps IEMG areas during concentric and eccentric contractions after a 20 min step test. Each point is mean of 3 consecutive contractions. rectus femoris, vastus medialis, vastus lateralis. Subject: BQ, male, 45yrs.

2.4. DISCUSSION.

2.4.1. Muscle pain and tenderness.

In the course of these preliminary studies delayed onset, post exercise muscle pain only occured after eccentric contractions. This type of muscle pain was not apparent until a matter of hours after the exercise, became maximal between 24 and 48 hours after exercise and in some cases persisted for more than a week, but more usually disappeared after 3-4 days.

The development of the tenderness mapping technique provided useful information and proved to be reproducible, accurately reflecting the subjective impressions of the distribution and time course of the post exercise muscle pain.

The discomfort was mainly sited in the distal-medial and whole lateral aspect of the thigh while the more proximal medial area and central part (rectus femoris) was relatively unaffected.

The most painful areas may correspond to the musculo-tendinous junctions. These results do not discount the hypotheses that either muscle fibres themselves or connective tissues are damaged during exercise and are responsible for the pain. However if either of these tissues are the cause for the pain it is rather suprising that a number of hours pass between the exercise and any awareness of pain or

discomfort. An alternative explanation might be indicated by the EMG studies. The largest increase in the EMG was found in rectus femoris, and this progressive recruitment may indicate greater fatigue and/or failure in the other quadriceps muscles i.e. vastus medialis and lateralis.

While there are many reports that delayed onset muscle pain occurs after eccentric rather than concentric exercise (Asmussen 1952 & 1956, Davies & Barnes 1972, Talag 1973, Abraham 1977, Komi & Viitasalo 1977, McGlynn et al 1979, Davies & White 1981, Friden, Sjostrom & Ekblom 1981 & 1983, Schwane et al 1983, Friden 1983) little attention had been paid to the distribution of symptoms throughout the muscle. Komi & Rusko (1974) and Komi & Buskirk (1972) reported that this type of muscle pain was mainly felt by their subjects in the distal attachments of the elbow flexors. However Friden (1983) reported that after negative work of the quadriceps (performed on an adapted motor driven cycle ergometer) soreness was reported as being evenly distributed throughout the muscles. This might be due to the fact that the exercise itself was different and thus different recruitment patterns may have been used. Furthermore Friden's subjects were performing maximal contractions while the subjects who stepped were performing submaximal eccentric contractions for most or all of the time, and this may also imply different recruitment patterns.

None of the subjects reported delayed onset muscle pain in the muscles which had contracted concentrically. However a

sensation of discomfort was usually experienced in the concentrically contracting muscles at some point actually during the exercise. Once present, this gradually increased in severity as long as the exercise continued although it never reached troublesome proportions. Once the exercise stopped this pain disappeared very rapidly, within seconds, and left no residual effects. This type of pain was never reported in the eccentrically contracting muscles. Both by its nature and its time course, as well as the contraction mode of the affected muscles, this pain was felt to be ischaemic in origin. This is in agreement with the results of other workers that ischaemic pain is proportional to the energy requirements of the work done (McArdle & Verel 1955, Park & Rodbard 1962, Rodbard & Pragay 1968, Mills et al 1981).

The time courses of post exercise muscle pain and the reduction in force generation were different; the latter was recovering before the onset of pain and was virtually completely recovered when pain and tenderness were maximal.

All the subjects noted tremor and instability in the quadriceps which had contracted eccentrically. This was most obvious at the end of exercise and had largely recovered an hour later.

2.4.2. Force generation.

The MVC was reduced in the muscles which had contracted eccentrically but there was no significant force decrement in the muscles which had worked concentrically.

Significant low frequency fatigue was seen bilaterally after exercise, but was greater in the eccentrically worked muscles. Similar results have been reported by Davies & White (1981). The alteration in the contractile properties took longer to recover (approximately 48 hr) than did the MVC decrement (approximately 24 hr). The changes in force generation were greatest in the immediate post exercise period and had largely recovered at the time of maximal pain and tenderness. This indicates that changes in force generation i.e. fatigue and delayed onset muscle pain are not simply and do not share a common mechanism It is possible that the fatigue, either directly or indirectly, predisposes the muscle to some form of damage or tissue changes which inturn result in muscle pain.

An alteration in the contractile properties of human muscle as a result of repetitive, fatiguing contractions has been described in the quadriceps and adductor pollicis by Edwards et al (1977) and also in the diaphragm (Moxham et al 1980a) and sternomastoid (Moxham et al 1980b). The force generated by low frequency stimulation is reduced while the force generated by higher stimulation frequencies (>50Hz) is

preserved. The term 'low frequency fatigue' has been given to this phenomenon which is not associated with depletion of high energy phosphate at a time when the force decrement is evident nor to failure of electrical excitation (Wiles et al 1981, Jones 1981).

2.4.3. Electromyography.

The EMG was recorded from two subjects and the same pattern was seen in both. The only significant increase in the IEMG area per unit force during stepping was seen in the muscles which were contracting eccentrically. Recovery to base line values occured over 24 hours. Komi & Viitasalo (1977) also reported that eccentric contractions caused a greater increase of EMG per unit force than concentric contractions.

It is interesting that the greatest changes in electrical activation were seen in rectus femoris, which was the muscle least affected by post-exercise pain and tenderness. This indicates that muscle fatigue (as demonstrated by an increase in excitation) per se and this type of pain are not directly related, also muscle pain has not been a feature of other work in which low frequency fatigue has been produced by contractions other than eccentric. These results could be interpreted as meaning that the progressive recruitment of rectus femoris during exercise . indicated a progressive fatigue of vastus lateralis and

medialis, which were the muscles most affected by delayed onset pain and tenderness.

The overall progressive increase seen in electrical activity during stepping in the eccentrically contracting muscle is compatible with the oxygen consumption studies reported by Davies and Barnes (1972) where a steady state was not reached during the performance of negative work in contrast to positive work.

Spontaneous electrical activity was not detected at any time during testing when the muscle was painful, arguing against the spasm theory of muscle pain (deVries 1966) and in agreement with the results of McGlynn et al (1979) and Abraham (1977). During the period of muscle pain and tenderness the relative contributions of the three muscles did not significantly alter compared to the pre exercise state, and so there was no evidence of changes in recruitment patterns caused by an inhibition of painful areas of muscle.

The time course of the changes in the EMG and both voluntary and stimulated force generation followed similar time courses, but the magnitude of the changes was not identical. The EMG increase of all three muscles together (during the submaximal knee extension) was greater than the decrement of both MVC and 10/50% (Fig. 11). It was very noticable that functional impairment of the quadriceps was much more obvious when weight bearing on an extended knee, rather than when the knee was flexed or semi-flexed. Therefore the discrepancy between the EMG and force changes

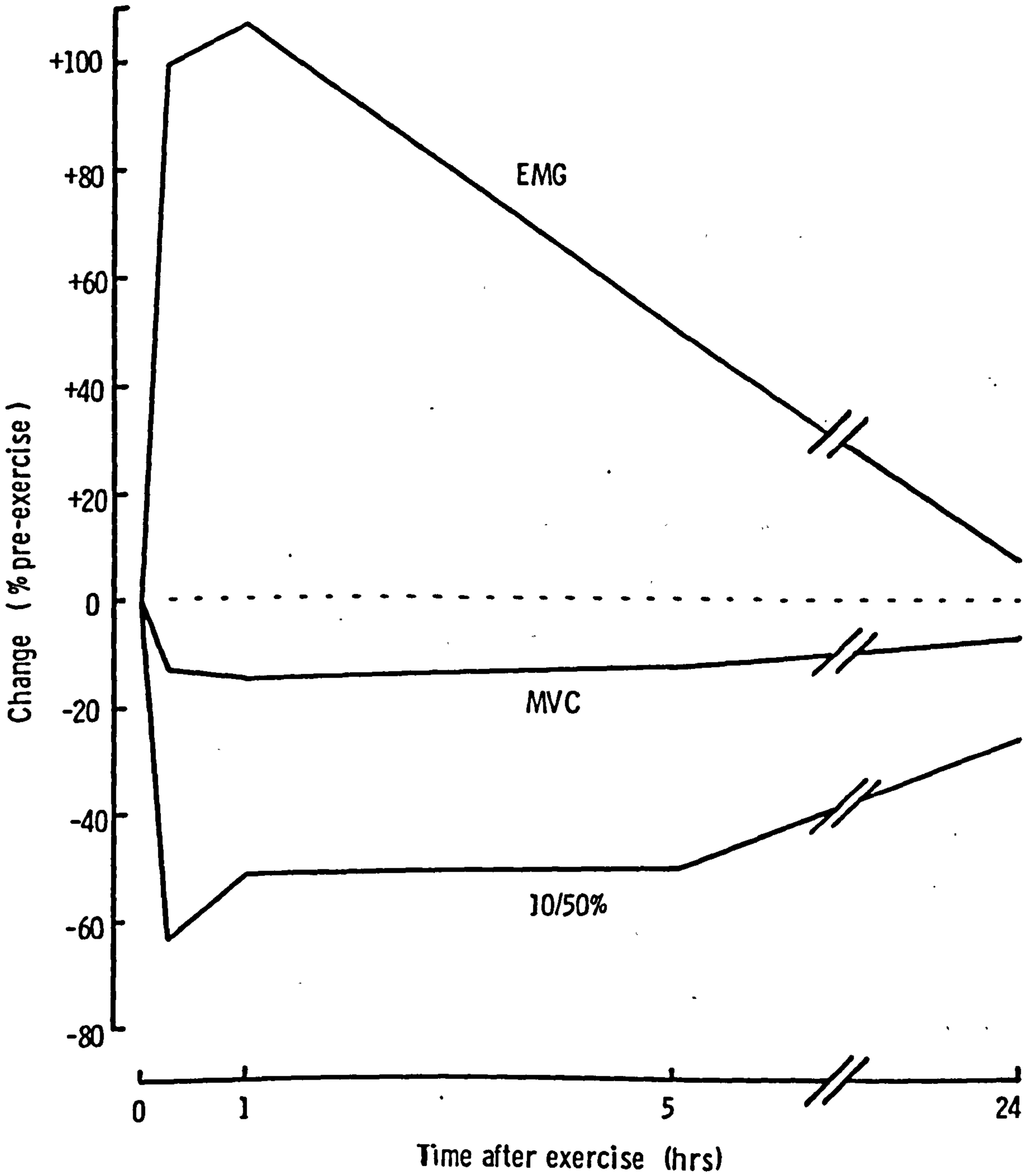

Fig. 11. Changes in the quadriceps EMG and force generation after eccentric contractions. The area of the EMG signal (integrated over 100ms periods) was measured during a submaximal knee extension held for 2s. Isometric force was measured with the knee at 90°.

might be due to their being measured at different joint angles.

These results strongly indicated that eccentric contractions induce delayed onset muscle pain and comparable work performed by concentric contractions do not. The eccentric contractions also caused greater fatigue in terms of force generation and electrical excitation.

In the light of the proven differences between the two types of contraction in their energy demands, and ability to generate tension, it seems unlikely that the pain and fatigue that result from eccentric contractions are caused by the metabolic requirements of the exercise but are probably related to the mechanical stresses in the muscle.

It was thought worthwhile to confirm these results on a larger group of subjects in whom the step height and exercise duration were standardised. Also to measure the circulating levels of a muscle enzyme - creatine kinase (CK) to give more information about the time course of damage after such exercise.

CHAPTER 3. RESPONSE TO ECCENTRIC CONTRACTIONS.

3.1. INTRODUCTION

The study described in the previous chapter had strongly indicated that eccentric contractions are more associated with delayed onset muscle pain and cause a greater impairment in force generation than when a similar amount of work is performed using concentric contractions. However these preliminary results needed to be substantiated on a larger number of subjects using a more standardised exercise test.

In the previous study the use of a fixed height step meant that the amount of stretch imposed on the eccentrically working quadriceps was different for each subject. Relatively more stretch was imposed on those subjects with shorter legs and vice versa and it is possible that this may affect the results.

To get an indication of muscle damage the activity of a muscle enzyme - creatine kinase (CK) was measured in plasma.

3.2. METHODS

3.2.1. Step tests.

The stepping frequency was the same as used in Chapter 2.2.3. (page 41) i.e. 15 cycles/min. The duration was 20 min for those subjects other than the three in the second study in this chapter who stepped to exhaustion. A standardised height step was used for all subjects. This was 110% lower leg length (lateral knee joint line to the ground).

3.2.2. Measurement of tenderness.

This was done in the same way as described in Chapter 2..2.6. (page 43).

3.2.3. Measurements of muscle force.

Voluntary and stimulated force was measured as described in Chapter 2.2.1. (page 38).

3.2.4. Plasma CK activity.

Approximately 10 ml of blood was withdrawn from an antecubital vein before and at intervals after exercise. After seperation by centrifugation at 3000 rpm for 20 min, the plasma CK activity was measured by the Boehinger Mannheim

activated method. This was done in The Department of Chemical Pathology at University College Hospital.

3.3. Results.

3.3.1. Response of 10 normal subjects to a standardised step test.

Ten normal healthy adults acted as subjects. The mean age of the group was 25 yrs (range 21 - 36) years. Six of the subjects were female (mean age 27, range 21 - 36 years) and 4 were male (mean age 23, range 22 - 26 years). One subject was only able to exercise for 15 minutes due to an inability to control the descent from the step. The remaining subjects completed the 20 minute exercise period.

3.3.1. i) Pain and tenderness.

Muscle pain and tenderness occured only in muscles which had contracted eccentrically with the exception of one subject who reported minimal pain during exercise in the quadriceps which had contracted concentrically and measureable tenderness was found in the muscle at 24 hours after exercise only, and was sited over the belly of rectus femoris. Otherwise the time course and distribution of pain and tenderness were as in the preliminary studies reported in the previous chapter.

Peak subjective pain coincided with maximal recorded tenderness and occured at either 24 or 48 hours. The subject who had the highest CK had pain and tenderness for the

longest time (8 days) but as far as could be judged neither was appreciably more severe than in some of the other subjects who had high CK values. The subject who seemed to have the least amount of discomfort had one of the larger CK rises.

3.3.1. ii) Force generation.

MVC force was reduced after the exercise with no significant difference between the two legs (Appendix v). Ten minutes after exercise there was a similar fall in both muscles to 79% of the pre-exercise level. Recovery of voluntary force was slow, occuring over 2 days.

The force generated by stimulation at 10 Hz expressed as a percentage of the force generated by 50Hz stimulation (10/50%) has been taken as an index of low frequency fatigue. Marked low frequency fatigue was seen in the quadriceps which had contracted eccentrically while the contralateral was not significantly affected by the exercise (Fig. 12 & appendix vi). In the muscles which had contracted eccentrically the 10/50% fell to a mean of 55.% of the pre-exercise value ($p<0.001$). Four hours after exercise the decrement showed some recovery being 72% of the initial value ($p<0.001$), at 24 hours after exercise the 10/50% was still reduced at 81% ($p<0.001$) of the initial value. Full recovery had occured 48 hours after exercise, with no significant difference between

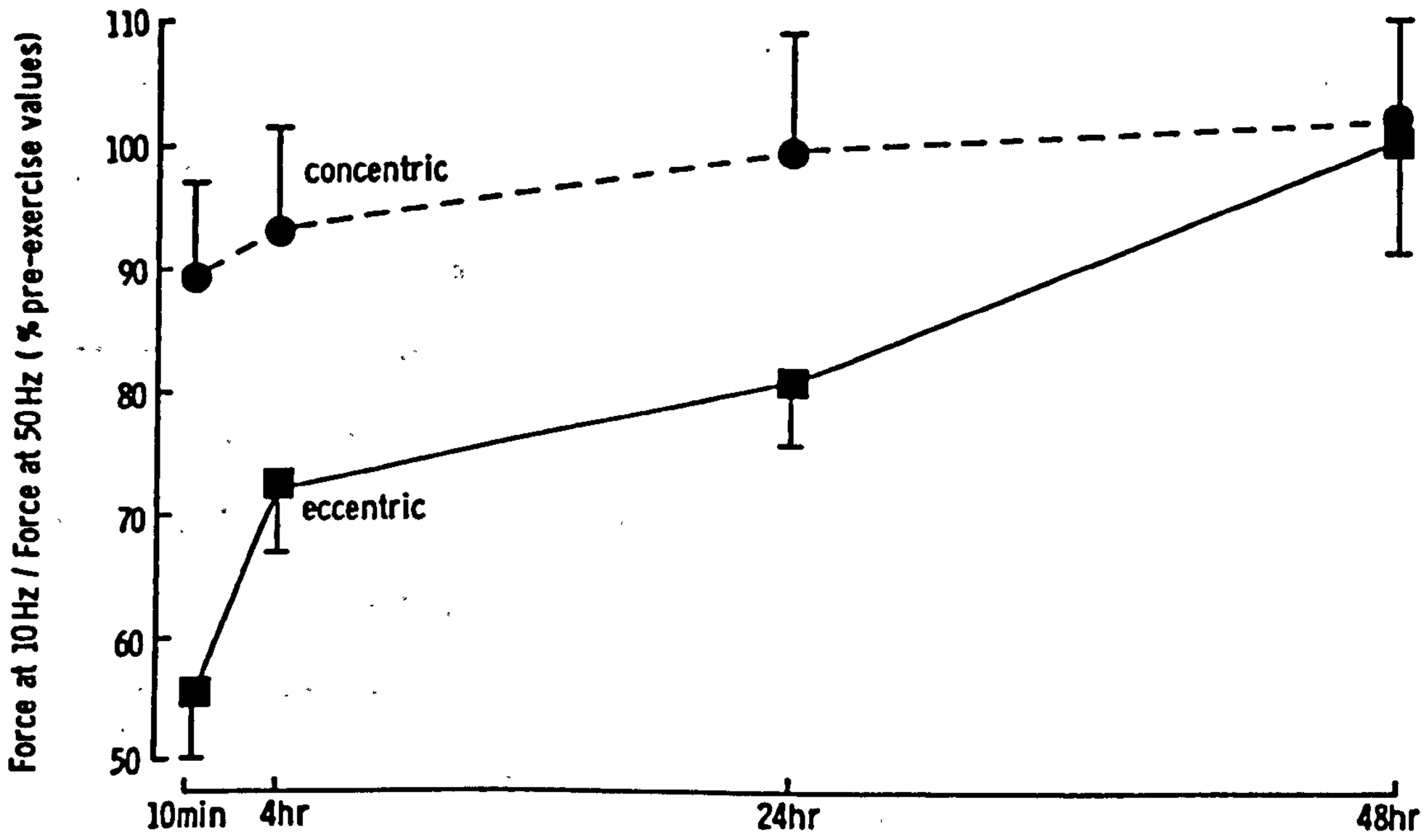

Fig. 12. Recovery of low frequency fatigue after a 20 min standarised step test on 10 normal subjects. Note greater changes in relationship between force generation at high (50Hz) and low (10Hz) frequencies of stimulation in the muscles which had contracted eccentrically. Mean ± SEM.

either the two muscles or pre-exercise values.

The magnitude of the low frequency fatigue in any one subject did not correlate in any obvious way with the MVC:body weight ratio.

3.3.1. iii) Creatine kinase activity.

Thirteen normal adults were the subjects for this study. Inaddition to the 10 of the previous study one female (aged 55 yrs) and two males (aged 25 and 27 yrs) performed the step test but did not have force measurements.

All subjects showed a small CK rise immediately after the exercise and the activity continued to rise during the next 24 hours reaching two or three times the initial levels. Thereafter, in seven of the subjects plasma CK returned to pre-exercise values within the next 24 hours. The remaining five subjects showed an unexpected delayed rise in plasma CK activity which was one to two orders of magnitude greater than the rise seen after 24 hours (Fig 13 & appendix vii). This much greater rise did not begin until the second or third day after exercise, reached a peak between 4 and 5 days and returned to normal between 7 and 9 days after exercise.

Of the five subjects showing this large delayed rise four were female and their ages spanned the whole range of the sample. Changes in muscle force, tenderness and plasma CK in two subjects are shown in Fig 14. Despite similar changes in force and tenderness, the different pattern of CK

efflux is evident. Comparing those subjects who showed the large delayed response with those who did not, there was no difference in the height/weight or quadriceps muscle strenghth/body weight ratios. The height/weight ratio for the subjects who did not show the large delayed CFK rise ranged from 1.9-3.6 cm/kg (2SD about the mean) and for those showing a delayed response the ratio was 2.34-2.98 cm/kg. The quadriceps force/weight ratio for the group not showing the delayed response was 0.63-0.99 N/kg and for those with a delayed response was 0.58-0.94 N/kg. The subjects were therefore exercising at very similar absolute work rates and making similar demands on their quadriceps. Of the subjects who showed the longer delayed response, only one could be described as unfit, the others all participated in regular exercise. In this respect the group did not differ from the subjects who did not have the large delayed enzyme efflux.

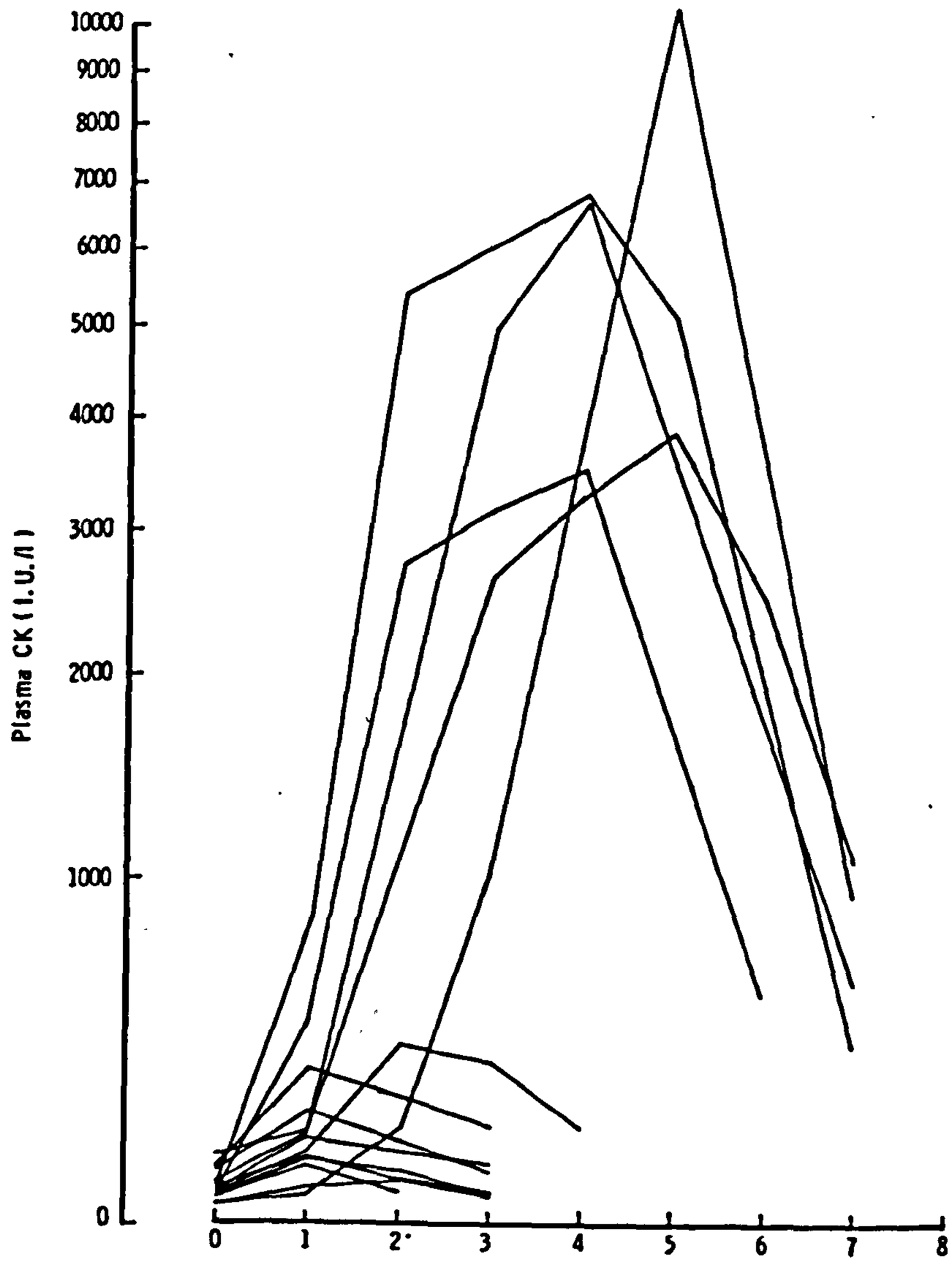

Fig 13. Plasma creatine kinase changes after a standardised step test. Note the two groups of response: those showing smaller increases peaked at 1-2 days after exercise while the larger responses peaked at 4-5 days. n=13.

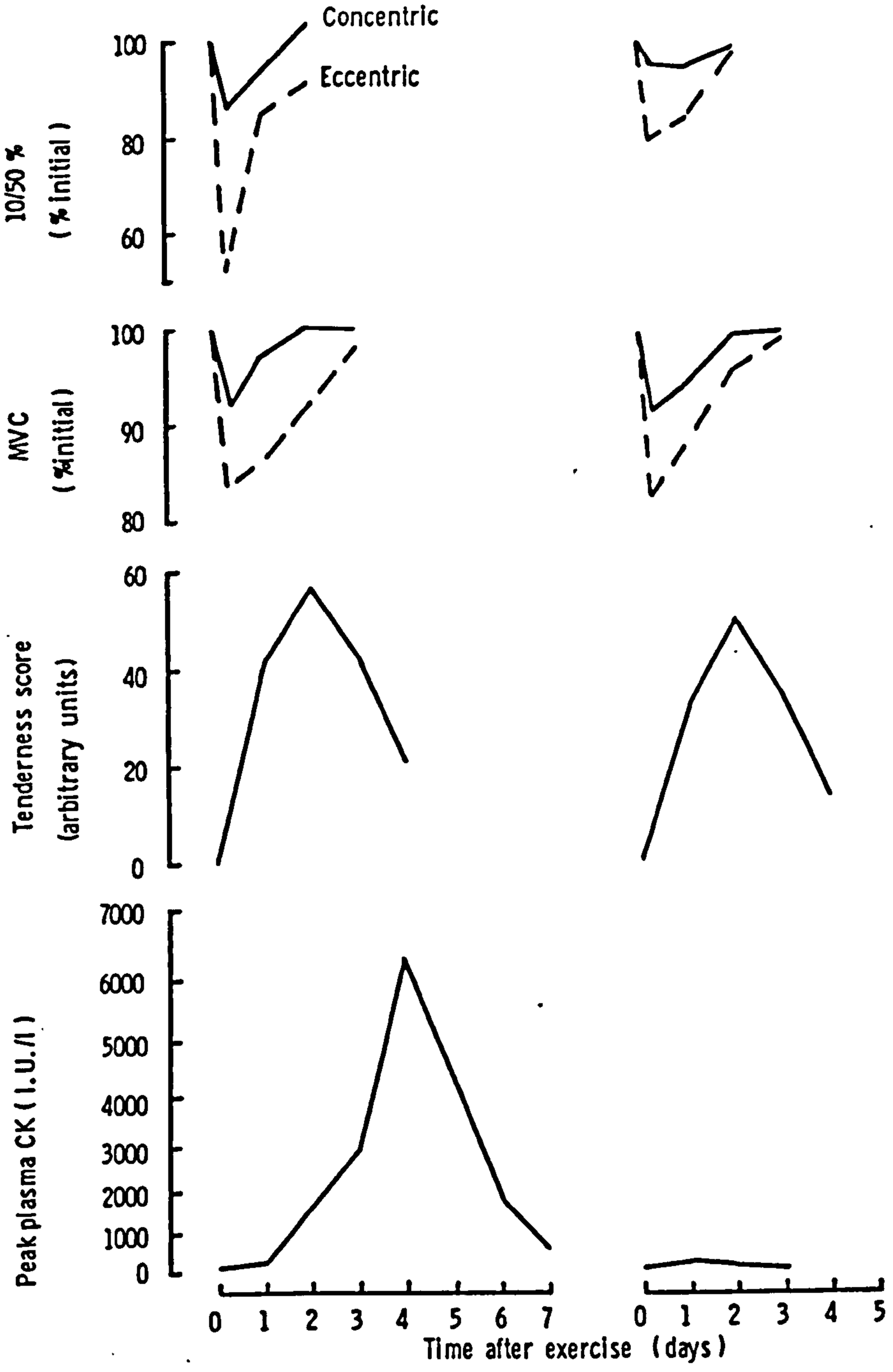

Fig. 14. The relationship between changes in force, tenderness and plasma CK in two subjects after a standardised step test. Note similar changes in force and tenderness despite very different CK results.

Voluntary force was measured in both legs. Tenderness was only experienced after eccentric contractions and the scores are the sum of measured tenderness at all tested sites over the quadriceps muscle.

3.3.2. The effects of prolonged stepping.

It was noted that in every instance where very high CK values had been recorded the subjects were experiencing difficulty in controlling the eccentric contraction by the end of the test. Also most of them had been female. This raised the possibility of whether the large CK effluxes are a sex related phenomena, and also to what extent exercise duration plays a part.

To investigate this 3 healthy male subjects (mean age 27 yrs, range 25 - 30) performed the standardised step test but continued to exhaustion. Of the three subjects in this group, two were fatigued after 50 and 60 minutes and were limited by the failure of the eccentrically working quadriceps to control the descent from the step. The third subject was stopped after two hours although he could have continued, but in common with the other two subjects, he found himself unable to straighten against gravity the leg that had been working eccentrically. No trouble was experienced straightening the leg that had been working concentrically.

3.3.2. i) Pain and tenderness.

All three subjects developed pain and tenderness only in the muscles which had worked eccentrically. The time course and distribution was as in the previous studies. The subject

who stepped for the shortest time had the most tenderness, while the other two had very similar levels.

3.3.2. ii) Creatine kinase activity.

All three subjects showed the large delayed elevation in plasma CK levels after exercise (Fig 15 & appendix viii). The highest value of 34,500 IU/I was seen in the subject who had stepped for the shortest time (50 minutes) and occured on the fifth day after exercise. Peak values occurred 4 days after exercise in the other two subjects, being 1,698 IU/I in the subject who stepped for 1 hour and 2,643 IU/I in the subject who was stopped after 2 hours.

3.3.2. iii) Force generation. (Appendix ix)

Immediately after eccentric work the quadriceps MVC was reduced to a mean of 73.0% of the initial value (range 63.2-78.2). After concentric work the MVC was reduced to 83.0& (range 74.4-90.8) This shows only a slightly greater force loss than that seen in the 10 subjects after stepping for 20 min, where the mean values were 78.8% and 79.8% of the initial force respectively. However the 10/50% was decreased more in both quadriceps and especially in those thaat had worked eccentrically. This was 38.9% (range 36.6-41.3) of the initial value after eccentric contractions and 64.9% (range 50.9-89.9) in the muscles which had contracted

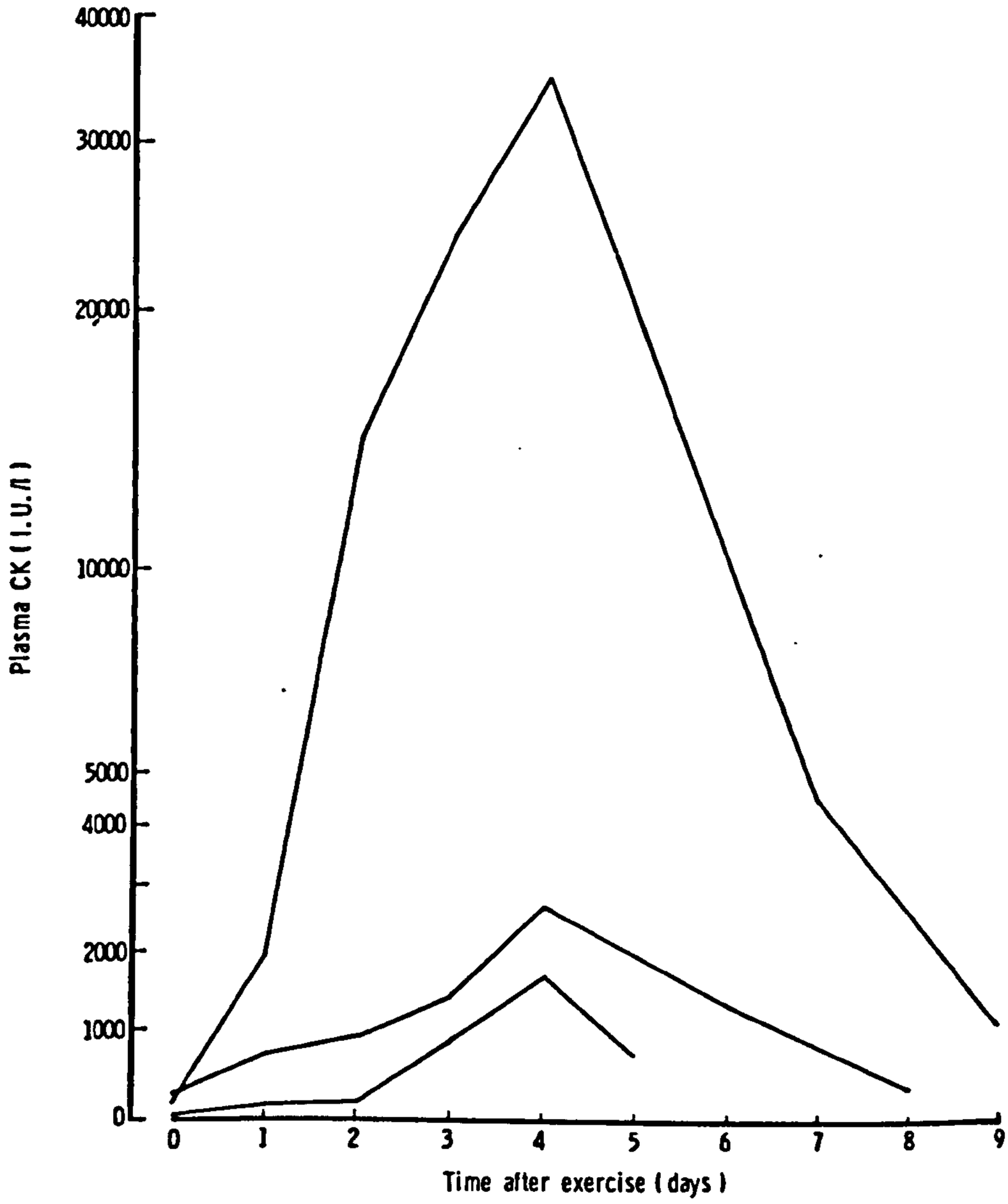

Fig. 15. Plasma creatine kinase in three normal male subjects after prolonged stepping. The subject who had the largest Ck efflux stepped for the shortest time (50 minutes) while the other two stepped for 60min and 2 hours.

concentrically. For the 10 subjects who stepped for 20 min. these figures were 55.0% and 89.5% respectively.

3.4. DISCUSSION.

3.4.1. Muscle pain and tenderness.

These results confirm the preliminary ones of the previous chapter, in that eccentric contractions are responsible for delayed onset muscle pain. In the course of the stepping studies a total of 16 normal subjects performed concentric and eccentric contractions of the quadriceps and delayed onset, post exercise muscle pain only occured after eccentric contractions. This type of muscle pain was not apparent until a matter of hours after the exercise, maximal discomfort occured between 24 and 48 hours after exercise and in some cases persisted for more than a week, but more usually disappeared after 4 days.

In marked contrast, concentric contractions although they were often uncomfortable during the exercise did not, except in a single subject, cause any discomfort once the exercise stopped. In this individual the sudden onset of discomfort was reported in the rectus femoris of the concentrically contracting quadricep and this increased with exercise duration. When the exercise stopped the pain reduced in intensity, but was still present and was exacerbated by activity. Twenty four hours later this muscle was slightly tender, but was not the next day. It would seem that in this case there was an injury of the 'pulled muscle' type.

The discomfort experienced during exercise in the

concentrically contracting muscle was of a burning nature, and to those familiar with the sensation it seemed to be similar to ischaemic pain. As ischaemic pain is proportional to the metabolic cost of the work (McArdle & Verel 1955, Park & Rodbard 1962, Rodbard & Pragay 1968, Mills et al 1982) this emphasises that the energy requirement is greater in concentric contractions. This type of discomfort/pain was not experienced during eccentric contractions, but it was those muscles which contracted eccentrically which subsequently developed delayed onset muscle pain.

The development of the tenderness mapping technique provided useful information and proved to be reliable and reproducible, accurately reflecting the subjective impressions of the distribution and time course of the post exercise muscle pain.

In the quadriceps after stepping the discomfort was mainly sited in the distal-medial and whole lateral aspect of the thigh while the more proximal medial area and central part (rectus femoris) was relatively unaffected.

Initially it was thought that the most painful areas corresponded to those of the musculo-tendinous junctions, but a careful examination of the anatomy of the quadriceps muscle group in dissection studies revealed that there are musculo-tendinous junctions along virtually the whole muscle surface, and that the most painful areas corresponded better to those where the greatest angles occur between the muscle fibres and the line of pull of the tendon. Vulnerability to mechanically

induced damage may well be proportional to the angle of pennation (Gans 1983). These areas are also reported as having the highest density of muscle nociceptors (Stacey 1969, Kumazawa & Mizumura 1977) and this may, at least in part account for the distribution.

The most discomfort seemed to be experienced by those who were unable to fully control the descent of the body weight from the step during the eccentric contraction by the end of the exercise period. It is conceivable that in this situation where the active fibres are generating high tensions and then the sarcomeres are being forcibly pulled apart, the most mechanical damage is likely to occur. From the force:velocity relationship of skeletal muscle (Hill 1938, Abbott et al 1952, Komi 1969 & 1973) it is known that the faster an active muscle is stretched the greater is the force generated.

When there is an inability to control the eccentric contraction, the descent of the body suddenly increases and therefore so does the velocity of lengthening of the quadriceps and thus the tension and so, presumably, the risk of mechanical damage is greater. In this context it is interesting that rectus femoris was the quadricep muscle least affected by pain and tenderness as it is the only one of that group to pass over the hip joint as well as the knee. This means that it has relatively less stretch imposed upon it than the other quadriceps muscles as the lengthening

caused during the eccentric phase of stepping (while the knee flexes) is countered to some extent by the simultaneous shortening that occurs as the hip flexes.

The time courses of post exercise muscle pain and the reduction in force generation were different; the latter was recovering before the onset of pain and was virtually completely recovered when pain and tenderness were maximal. Peak CK values coincided with peak soreness in some subjects, but in those with the large delayed CK rise the circulating ezyzme levels were still increasing at a time when pain and tenderness were decreasing. Furthermore, one subject who seemed to have minimal pain and tenderness had one of the higher CK rises.

The different time courses seen for the various factors examined indicates that there is unlikely to be one common underlying mechanism for them all.

An interesting but poorly defined phenomenon was the tremor and feeling of instability in the leg where the quadriceps had contracted eccentrically. This was most marked during weight bearing on a semi flexed knees and made going down stairs difficult. It was most marked at the end of exercise, it persisted for approximately three hours and had gone before the onset of pain. The presence of this phemomenon corresponds to the time when the MVC was at its lowest and changes in both the frequency:force relationship and IEMG area per unit force were most marked. Therefore it may have been caused by the low and inappropiate forces which

would have been generated by the relatively low normal physiological firing frequency (Clamann 1970, Milner-Brown et al 1973, Bigland Ritchie et al 1982).

These workers report this to be in the range of 10-30 Hz for all but the briefest contractions. These frequencies are in the 'low frequency' group of the stimulation frequencies used here.They are also on the steep part of the frequency:force curve where small increases in frequency result in a large increase in force. The functional significance of low frequency fatigue has not been established but theorectically it would seem that the greatest impairments in force generation occur at the frequencies which are used physiologically.

Another possible explanation for the tremor is that whatever changes occur in the extrafusal muscle fibres could also occur in the intrafusal fibres so the muscle spindles may become either damaged or have their excitability altered. The involvement of the intrafusal fibres could be determined by studying the response to a rapid stretch.

The fact that post exercise muscle pain occurs only after eccentric contractions conclusively demonstrates that this type of pain is not determined by metabolic factors since these are greater during concentric contractions. Furthermore, the time course is incompatible with the pain being related to the energy expenditure of the exercise. Thus the conclusion is that this type of pain is initially caused by the high tensions developed during eccentric exercise, and

is associated with muscle damage.

3.4.2. FORCE GENERATION.

The changes in the post exercise MVCs of the subjects who performed step tests showed great individual variability. In the initial group of 4 normal subjects using the same absolute height step the MVC was significantly more reduced in the muscles which had contracted eccentrically, but in subjects who used the same relative height step there was no significant difference in the strength changes between the two legs. In the first study the step height was less than 110% of the lower leg length for 3 of the 4 subjects and so the discrepancy is not accounted for by differences in muscle length. The subjects in both groups were performing the test for the first time so training effects cannot have been relevant.

The data shows that while concentric work never induced a greater MVC decrement, and some of the subjects had a greater decrement after eccentric work, but for the whole group there was no statistically significant difference. It is interesting that in the preliminary studies using four subjects and a fixed height step the MVC of the eccentrically contracting muscles was reduced more than that of the concentrically contracting ones. However in the group of 10 subjects using the same relative height step no significant difference was found between the MVC the two muscles.

The height of the fixed step (46 cm) was less than 110% of the lower leg length for three of the four subjects. Examination of the data shows that in the study using the same relative height step the MVC of the eccentrically worked muscles was reduced to approximately the same degree as in the same relative height step study but the concentrically worked muscles lost more force than in the former study (note that in this study the height was less than 110% of the lower leg length for three of the subjects). This may well indicate a component of voluntary force fatigue induced by the greater demands, both metabolic and mechanical, made on the concentrically contracting muscles with the higher step.

In view of the long standing interest in eccentric exercise it is perhaps suprising that there is very little in the literature about its short term effects on force generation. What work there has been in this field has been mainly carried out by Komi and his coworkers. The effect on tension and electrical activity of performing 40 MVCs was studied on the forearm flexors (Komi & Rusko 1974) and the quadriceps (Komi & Viitasalo 1977). In both cases it was found that eccentric exercise caused a much greater force loss (50%) than concentric (20%) while the decline in total electrical activity was very similar for both types of work. The concluusion is that the fatigue occurs within the muscle itself and it not the reflection of altered neural activation.

Davies & White (1981) seem to be the only other group to

have compared MVC force after matched concentric and eccentric exercise. Their subjects also performed step tests, but for the much longer time of one hour, and they measured the calf MVC. They found that the muscles which had worked eccentrically showed a significantly greater force loss in the post exercise period than those which had worked concentrically.

In the stepping studies carried out by Davies & White and those in this thesis there are obvious differences, the first being that they studied different muscles. Furthermore the exercise duration used by Davies and White was three times longer. It would be expected that the subjects who stepped for one hour would be more fatigued than those who only exercised for 20 minutes. The more fatigued subjects would be working at a relative intensity which became progressively greater and the effects of fatigue would be compounded. This conclusion is supported by the results reported here in which three subjects stepped to exhaustion - all of them had greater force loss in the muscles which had worked eccentrically.

Previous studies of muscle performance after eccentric exercise have reported that the reduction in muscle strength is greatest when the muscle pain is maximal (Asmussen 1956, Komi & Rusko 1974, Komi & Viitasalo 1977, Friden 1983) and this is in contrast to the results presented here. The major difference between the results of other workers and these is that they measured dynamic eccentric force on the same

equipment that was used to induce the pain. In the studies reported here maximal isometric force was recorded, and it was a consistant finding that when there is post exercise muscle pain, isometric contractions do not exacerbate the discomfort but dymanic contractions and especially eccentric ones, definitely do. The inference is that the strength reduction reported by other workers is not a true indication of the muscles ability to generate force, but simply a reflection of pain which was the limiting factor in the generation of force.

Another factor could be the joint position i.e. muscle length at which force is measured. Subjectively the impaired function was much more marked with the knee extended or only slightly flexed than when it was flexed at a right angle.

There was no obvious correlation between the decrement of the MVC and low frequency fatigue, post-exercise pain or any of the other parameters measured, nor was the greatest force decrement seen in those with the lowest body weight: MVC ratio.

An alteration in the contractile properties of human muscle as a result of repetitive, fatiguing contractions has been described in the quadriceps and adductor pollicis by Edwards et al (1977) and also in the diaphragm (Moxham et al 1980a) and sternomastoid (Moxham et al 1980b). The force generated by low frequency stimulation is reduced while the force generated by higher stimulation frequencies (>50Hz) is relatively preserved. The term 'low frequency fatigue' has

been given to this phenomenon which is not due to depletion of high energy phosphate nor to failure of neural drive.

The degree of low frequency fatigue in the quadriceps which had contracted eccentrically was greater than in the muscle which had contracted concentrically in all normal subjects who stepped on a 46cm step or one which was 110% of the lower leg length.

While the actual mechanism of low frequency fatigue is uncertain, it was thought to be proportional to the amount of work done by the muscle (Wiles et al 1981) however these results make that seem very unlikely. It has recently been suggested that the defect may either be the quantity of calcium released from the sarcoplasmic reticulum in response to a single action potential or to a change in the affinity of the troponin binding site for calcium (Jones 1981) but there is no direct evidence for this. Both these defects would reduce the twitch force but high stimulation frequencies would produce relatively normal force when the interior of the fibre was flooded with calcium.

Changes in the compliance of the elbow flexors after fatiguing voluntary contractions has been described by Vigreux et al (1980) whereby fatigued muscle is more compliant than fresh muscle in studies where electromyographic fatigue was not a factor. If this is so it is possible to imagine a situation where the high tensions generated throughout the muscle during eccentric contractions would increase the compliance of the series elastic component

of muscle to a greater extent than would occur in a concentrically contracting muscle and this could lead to a reduction in force generation which would presumably be greater a low stimulation frequencies thanatthe higher ones. No indication is given of the time course for recovery of the changes in compliance and so its bearing on the present results can only be speculated upon. It could be tested by determining the speed of changes in length of a stretched muscle in response to a rapid release.

3.4.3. PLASMA CREATINE KINASE CHANGES.

Increases in blood levels of CK and other muscle enzymes are known to occur after exercise in normal subjects and also after myocardial infarction and they are taken to indicate muscle damage. In the latter case peak levels occur 24 hours after the pathological event (Hearse 1979) and it seems to have been largely assumed that a similar time course occurs after exercise in normal subjects.

Most groups who have studied enzyme effluxes from skeletal muscle after exercise measured circulating levels only in the immediate exercise period (Fowler et al 1968, Griffiths 1966, Misner 1973). Those who have studied the time course reported peak values 24 hours or less after exercise (Brooke et al 1977 & 1979, Shumate et al 1979).

In the studies reported here a number of subjects showed the same time course as reported by the other workers.

However another response was shown by a number of subjects who had plasma CK rises which were characteristically delayed, taking 3-5 days to reach peak levels, and the magnitude of the efflux was very great. There was considerable variation between the subjects in the actual plasma CK levels which was not obviously related to sex, body composition or general fitness. Despite this the subjects clearly fell into the 'responder' or 'non-responder' groups in terms of their CK changes after exercise.

The extent of the delayed CK rise found in these studies was generally much greater than has previously been reported for other forms of exercise, even thought this has often been of longer duration and greater intensity. Brooke et al (1979) recorded peak values of 1600 IU/l 10-20 hours after cycling for two hours at 50% maximal Oxygen uptake. Griffiths (1966) reported a 20 fold increase after a 53 mile walk. Although large, these increases are still at the lower end of the range of CK rises seen in these studies after only moderate intensity exercise peformed for 20 minutes.

It has been reported that higher enzyme effluxes occur after exercise in untrained subjects by Fowler et al (1968) who also found higher values in women. The latter is in contrast with the the findings of Griffiths (1966) Shumate et al (1979) and Thomson & Smith (1980) who all found markedly higher values in male subjects and hypothesised that oestrogens might play a protective role against large enzyme effluxes by stabilising the muscle membrane. In the studies

reported here women seemed just as likely as men to develop the large delayed CK response. Thus it may be that the results of the other groups reflect a sampling artefact - as individuals seem to be 'responders' or 'non-responders'. Alternatively they could have been habituated to the exercise, as training has been reported to reduce to enxyme efflux after exercise.

Furthermore, if oestrogens did act to stabilise the muscle membrane, then presumably different responses would be seen at different stages of the menstrual cycle, but there was no indication of this.

The prolonged stepping study suggests that given sufficient stress all subjects may show the large delayed CK response. However the stress is not simply exercise duration as the subject who stepped for the shortest time had the largest CK efflux.

Apart from differences in training and sex, CK efflux was thought to be proportional to the intensity (Apple 1981) and especially duration of exercise. In the results reported here very large changes in plasma CK consistently occurred after short exercise periods.

Whatever the mechanism of the CK efflux, this study indicates that it is the eccentric component of the exercise which is responsible for the initial damage. There have been no reports of similar enzyme releases, and the exercise used by other workers has been either cycle ergometry or running on level treadmills. Both these forms of exercise consist

mainly of concentric contractions. However using the stepping exercise, which involves equal amounts of concentric and eccentric contractions, it clearly is not possible to determine which type of contraction causes the enzyme release.

CHAPTER 4. PLASMA CREATINE KINASE CHANGES AFTER CONCENTRIC CONCENTRIC AND ECCENTRIC CONTRACTIONS.

4.1. INTRODUCTION.

In the work carried out so far a considerable amount of enzymic muscle damage had been caused in a number of subjects both by the stepping studies. Particularly striking was both the magnitude and the time course of the largest effluxes. These were appreciably greater and slower than has been described by other workers even after such strenuous and prolonged exercise such as marathon running. Furthermore peak levels have frequently been reported as occuring 24 hours after exercise rather than 3-5 days later which was the case in many of the subjects.

In view of the fatiguing and pain inducing characteristics of eccentric contractions, the most likely explanation for these results compared to those of other workers seemed to be that they investigated enzyme release after exercise such as cycle ergometry - virtually all concentric contractions, or running on level treadmills - mainly concentric contractions. Clearly it is not possible to determine which muscle is responsible for the enzyme efflux using the stepping model.

The aim of the studies reported in this chapter was to establish whether the large delayed CK response was caused by the concentric or eccentric contractions.

Preliminary studies were carried out which were designed to answer this point, but proved unsatisfactory. Initially it was planned to use an escalator, with subjects running down

an escalator which was going up for the eccentric exercise, and up a down going escalator for the concentric. The exercise duration (20 minutes) and stepping frequency (48-50 steps/min) were the same in both tests. However this experimental model did not produce either muscle pain or a CK response in either of the two subjects, and it was thought that the step height (19.5 cm) did not impose an adequate stretch on the eccentrically contracting muscle, reinforcing the contention that a critical amount of lengthening is necessary to induce pain and the enzyme efflux.

Inclined treadmill studies were then used, in initial studies the subjects walked forward on both gradients and tenderness was expected in the quadriceps as in previous studies. This did not occur, neither did a significant plasma CK rise, and it was thought that the explanation for this might be the same as for the escalator study.

The exercise model tried next was to have the subjects walking uphill forwards and downhill backwards. It was hoped that by this method the calf muscles would be sufficiently stretched during their eccentric phase to provoke muscle pain and enzyme efflux. This method has the advantage of satisfying the criteria set by Hill (1965) who emphasised the importance of using identical movements when comparing concentric and eccentric contractions.

Circulating CK may be from either brain tissue, cardiac or skeletal muscle as discussed in the introduction. In order to be certain that the plasma CK measured waas from skeletal

muscle, isoemzymes were measured in samples known to have high total CK values.

4.2. METHODS.

4.2.1. Treadmill studies.

In these studies a motor driven treadmill (P.K. Morgan Ltd.) was inclined at a slope of 13^{o} (23.5%). It has been shown that when the gradient exceeds 6^{o} (10%) uphill walking consists almost entirely of concentric exercise and downhill walking eccentric exercise (Margaria 1938). A speed of 3km/hr was used. For safety reasons the subjects wore a harness which was attached to a trip switch that cut the motor in event of a fall. In addition the subjects had access to a switch which would stop the motor.

Five healthy normal subjects acted as subjects (mean age 30 yrs, range 23-39 yrs). Three were female (aged 23, 25 and 34 yrs) and two were male (aged 32 and 39 yrs). All were physically active and participated in a variety of sports, but none were involved in a regular training programme.

Each subject, on separate occasions, walked up or down an inclined treadmill for one hour.

To minimise possible training effects a minimum of five weeks elapsed between the two tests on each subject. Three subjects performed the downhill test first and the other two performed the uphill test first. Heart rate was monitored throught each test.

4.2.2. CK activity.

Venous blood samples were taken before exercise and at 24 hr intervals afterwards. These were processed in exactly the same way, and the plasma CK activity measured as described in Chapter 3.2.4. (page 68)

4.2.3. Heart rate measurement.

Throughout the duration of both exercise periods the subjects electrocardiogram (ECG) was recorded from three chest electrodes and displayed on an ECG monitor (Sanborn, U.S.A.). The heart rate was measured before and at 10 min intervals during exercise.

4.2.4. CK isoenzyme measurement.

Throughout the course of the studies reported here samples of both serum and plasma from each subject had been stored at -20^{o}C for any subsequent analyses.

To measure the CK isoenzymes, 18 samples of serum known to have very high total CK values were thawed at room temperature. They were from studies using stepping, downhill treadmill walking and eccentric contractions of the elbow flexors.

CK isoenzymes were separated by electrophoresis using Argarose gel (Corning, U.S.A.). Isoenzyme activity was

visualised and quantitated with a scanning densitometer (Corning, U.S.A.) using a flourescent substrate.

Using this technique CK-MB isoenzyme can be detected down to 3.5% of the total CK activity.

This assay was carried out in The Charles Dent Metabolic Laboratory, University College Hospital.

4.3. RESULTS.

4.3.1. Treadmill studies.

All the subjects were able to complete the one hour exercise test. In contrast to walking uphill, the downhill walking felt virtually effortless. Despite this, it was very obvious that considerable muscle fatigue had occured especially in the calf muscles, as immediately after exercise the subjects had difficulty in standing on their toes - a movement which was accompanied by marked tremor as in the quadriceps and elbow flexors after eccentric exercise. Uphill walking, as expected, required much more effort, but at the end of the exercise period none of the subjects had any difficulty in standing on their toes and no tremor was apparent. The subjective impressions of the severity of the exercise is in agreement with the heart rates recorded during exercise. Throughout the exercise period all subjects had higher heart rates when walking uphill than when walking down (Table 1).

After the downhill walking all subjects reported muscle pain and tenderness in the muscles which had worked eccentrically i.e. calves and gluteii. As previously described this did not develop until several hours after the exercise and was maximal between one and two days later. There was considerable variation between the subjects in the

Table 1. Heart rates recorded during inclined treadmill walking for one hour.

	Subject 1		Subject 2		Subject 3		Subject 4		Subject 5	
Sex	F		M,		F		M		F	
Age	25		32		23		39		34	
Ht (cm)	164		166		163		178		180	
Wt (kg)	56		65		55		70		67	
Heart rate (Beats/min)	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
0	94	96	66	80	74	76	60	63	68	65
10 min	158	94	135	122	134	96	130	71	133	98
20 min	160	101	147	118	150	93	138	72	136	95
30 min	165	131	143	113	145	97	135	75	134	102
40 min	156	126	151	117	140	95	145	81	136	96
50 min	155	136	154	120	140	102	143	80	135	98
60 min	157	137	142	113	143	105	149	93	132	106

severity of the pain, which ranged from mild through to severe with appreciable impariment of function. No pain or tenderness developed in any of the subjects after the period of uphill walking.

Both types of exercise resulted in an elevation of plasma CK levels (Appendix x). Overall, after uphill walking only slight increases occured (>200 IU/1) with peak values 24 hours after exercise, while after downhill walking much greater levels were measured (up to nearly 15000 IU/1) and peak values occured between four and seven days after exercise (Table 2). Uphill walking caused slight plasma CK increases which were not more than double the pre exercise values. Four of the five subjects had peak values at 24 hours and thereafter the CK returned to base line values. One subject showed a suggestion of a biphasic response, the second peak of 216 IU/1 occuring on the fourth day after exercise (Fig. 16).

A very different CK response was seen after downhill walking (Fig. 17). Four of the five subjects showed the large delayed rise, previously only seen after stepping exercise. In these subjects the CK level increased by several orders of magnitude (the highest value being nearly 15000 IU/1), and

Table 2. Peak plasma CK values after walking either up or down an inclined treadmill for one hour.

	No. 1		No. 2		No. 3		No. 4		No. 5	
	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
Peak CK (IU/l)	59	14746	216	11305	71	702	67	125	127	6546
Days after exercise	1	7	4	7	1	5	1	1	1	4

Subject numbers correspond to those used in Table 1.

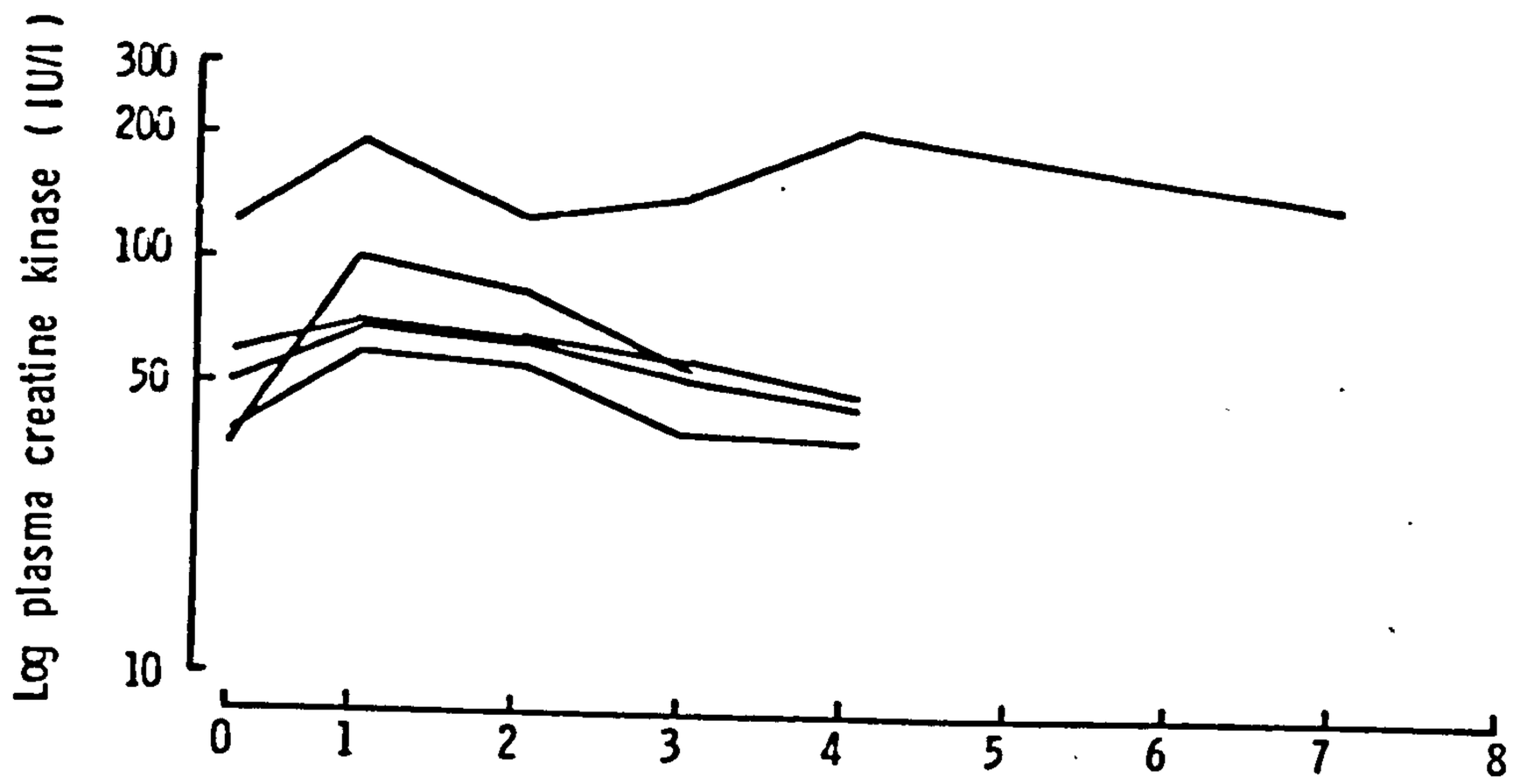

Fig.16 Log plasma CK for five normal subjects after walking up an inclined treadmill for one hour.

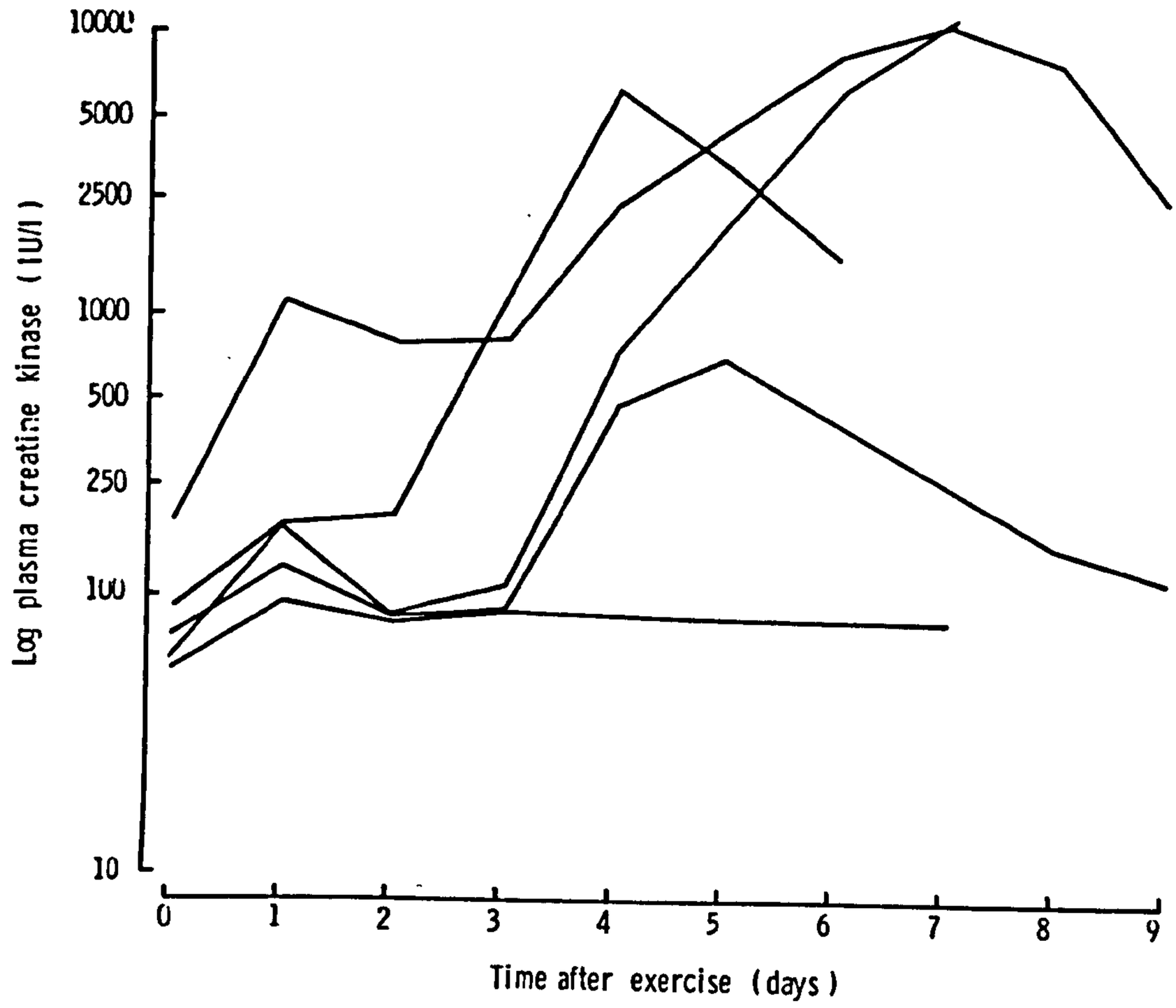

Fig. 17. Log plasma CK in five normal subjects after walking down an inclined treadmill for one hour.

and seven days after exercise. Of these four subjects, three clearly showed a biphasic response with an early peak similar to that after uphill walking, followed by a progressive increase over the next few days to reach peak values. One subject who had the large delayed CK rise, but did not show a clear biphasic response, did have a slowing in the rate at which the CK was rising on the second day after exercise and so it is possible that if blood samples had been taken more frequently a biphasic response may have been seen.

The remaining subject did not show the large delayed response, the highest CK of 125 IU/1 was 24 hours after exercise. He had previously shown the large delayed response after stepping, and also had performed the uphill test first in addition to other treadmill studies some weeks previously, and so might have been habituated to eccentric work.

4.3.2. CK ISOENZYMES.

The 18 samples of sera analysed for CK-MB (cardiac) isoenzyme had total CK activities of 394-12268 IU/1 (mean 3292). In none of them was the CK MB isoenzyme detectable.

4.4. DISCUSSION.

This study clearly showed that eccentric contractions are responsible for the large delayed CK rise. This is compatible with the other results reported here which demonstrate that a comparable amount of work performed by eccentric contractions will cause greater damage in terms of pain and fatigue than concentric contractions.

It would seem that the high forces generated during eccentric contractions cause damage to either the contractile elements or to the membrane systems in the muscle. This may lead first to the release of algesic substances causing the pain and tenderness and then initiate a process leading to the release of large quantities of enzyme. In this case the underlying mechanism cannot have been caused by the metabolic cost of the exercise and hence depletion, as then it would have occurred to a greater extent after concentric contractions.

The factors causing enzyme release from the damagedheart have been intensively investigated using isolated preperations, but there have been few similar studies on skeletal muscle (Zierler 1656 & 1957, Dawson 1966, Suarez-Kurtz & Eastwood 1981, Jones et al 1983). These studies have, in general, demonstrated that metabolic depletion either directly causes, or initiates, changes which allow enzyme efflux. This energy dependance is in contrast to the charcteristics of the large delayed CK rise described here.

The work with isolated prep rations has also shown that physical damage can give rise to enzyme release, but this process is very rapid occurring within minutes (Jones et al 1983/4) and is therefore not compatible with the delay of several days reported here.

In isolated preperations, the energy-dependant enzyme release with the rapdily rapid time course may well be analagous to the early rise in CK seen during the 24 hours after exercise which is reported in this work and in that of many other investigators studying human subjects.

Schmidt & Schmidt (1969) hypothesised that metabolic depletion increased cell membrane permeability and allowed the enzyme efflux. Larger effluxes have been reported after high than low force contractions (King et al 1976, Clarkson et al 1982), but depending upon how the contractions were performed this could implicate either metabolic or mechanical factors and from the published work it is not possible to determine which is the most important. In view of the known differences between the two types of contraction used in this study the clear implication is that the large delayed rise is not determined by metabolic factors - if this were so then the response would have occurred after the uphill (concentric) exercise.

The biphasic response of the efflux seen in a number of subjects is interesting. This has only been reported before in a group of rats performing eccentric exercise by running down an inclined treadmill (Armstrong et al 1983). Another

group of rats in the same study ran on a level treadmill and showed only the smaller and earlier peak. Schwane et al (1983) investigated the effect on normal human subjects of intermittant level and downhill running at a speed which corresponded to 80% maximal oxygen uptake (determined during level running). CK was elevated only after downhill running, but neither the delayed or biphasic response occurred. The differences in the inclination of the treadmill between that study and those reported here add support to the theory that an appreciable amount of lengthening is required to cause appreciable damage and the large enzyme efflux. Further support is given by the negative results of the escalator study and initial treadmill studies reported in this work.

The training effects of exercise on enzyme release described by Hunter & Critz (1971, Magazanck et al (1974) and Siegal et al (1980) in addition to the above factors mean that a critical level of exercise and damage is necessary to cause the large delayed rise, and this will be different for each individual. It may be that all those who have the large delayed rise did infact have the biphasic response, but that the time course is not identical for all individuals. If this is so then the fixed sampling times used in these studies may have caused the biphasic response to have been missed.

The magnitude of the plasm CK seen after cycling or running on level ground is normally no more than a fourfold increase. These levels of 200-300 IU/l are in contrast to the levels found in skeletal muscle diseases such as

polymyositis and Duchenne muscular dystrophy, where the values range form 1,000-10,000 IU/1. These are however very similar to the large delayed rises seen after eccentric contractions and this raises the possibility that the mechanism involved in this form of enzyme release may be more akin to the processes occurring in diseased muscle than are the changes after running or cycling.

The CK was of skeletal muscle origin (CK MM), as the cardiac (CK MB) isoenzyme was not detectable on the samples known to have very high total CK levels. This is in agreement with the results of Kamen et al (1977), Bornheimer & Lau (1981) and Apple et al (1983). They found that the increased circulating CK levels in healthy subjects who had performed such strenuous exercise as running to exhaustion and marathon races was also of skeletal muscle origin.

There are a number of unexplained features of the large delayed CK rise, such as the reason for the delay itself, and the greater sensitivity of some subjects. Another puzzling aspect is that the magnitude of the large delayed CK rise would seem to indicate substantial membrane damage but at the time when peak values are recorded, force generation has largely recovered, and this argues against significant membrane damage. Never the less, it appears to be a phenomenon that warrants further investigation and could be valuable in exploring the mechanisms of skeletal muscle damage and repair in man.

CHAPTER 5. THE EFFECT OF TRAINING, DURATION AND INTENSITY.

5.1. INTRODUCTION

It is well known to anyone who has taken any reasonably vigorous exercise that habituation to a particular type and intensity of exercise protects the muscle in some way against delayed onset muscle pain.

It has often been reported in the literature that both delayed onset muscle pain (Komi & Buskirk 1972, Davies & Barnes 1972, Friden et al 1983) and enzyme effluxes (Fowler 1968, Misner et al 1973, Maxwell & Bloor 1981) only occur after unaccustomed exercise. If this is the case then it is important to establish the time course of any training effects, not only to avoid the effects of training in subjects performing more than one study, but also for any intervention studies which necesitate the subjects performing exercise twice; once when taking the agent being tested and again with a placebo.

It is not clear from the literature whether duration or intensity of the exercise are the most important determinants of delayed onset muscle pain and any associated damage, and this warrants further investigation.

Initially one subject performed a period of regular stepping. Subsequently eccentric contractions of the elbow flexors were performed to study the training effects. This was done in order that the exercise might be restricted, as far as is possible, to only one muscle group and involve predominantly only one type of contraction. Also pain and

tenderness in the elbow flexors of the non-dominant arm is much less of a functional problem for the subjects than pain in large muscle groups of the legs occurs after stepping.

5.2. METHODS.

5.2.1. Step tests.

To examine the effects on force generation, pain and plasma CK levels one normal subject (female 55 yrs) carried out the standardised step test (as described in Chapter 3.2.1. Page 68) at regular intervals for 10 weeks. For the first seven weeks the test was performed once weekly and thereafter twice weekly with the exception of the ninth week when it was done only once. Throughout this period the the quadriceps of one leg performed concentric contractions while the other one performed eccentric contractions.

To assess the specificity of training effects a further test was carried out in the eleventh week in which the contraction pattern was reversed i.e. the muscle which had contracted eccentrically in the training period contracted concentrically and vica versa.

MVC, frequency:force relationship (Chapter 2.2.1. Page 38) and plasma CK (Chapter 3.2.4. Page 68) were measured immediately before exercise and then at 24 hr intervals. Force generation was also measured 10 min after exercise. Tenderness was measured at 24 hr intervals as described in Chpater 2.2.6. (Page 43).

5.2.2. Eccentric contractions of the elbow flexors.

These studies were carried out on the non-dominant arm

of the subjects unless otherwise stated. They sat on a high chair and the upper arm was supported at an angle of 90^{o} to the trunk by a shelf (Fig. 18). The wrist was encircled by felt padding to which was attached an inextensible cord. The This cord passed forward and around a single pulley which was attached to a wall mounted strain gauge. The chair was prevented from moving forward by floor blocks. The cord then passed back underneath the chair and was held by the operator who exerted the extending force which was resisted by the forearm flexor muscles of the subject.

The signal from the strain gauge was amplified and displayed on a UV oscilloscope (Micro Movements). At the start of each test the maximal force that could be generated during an eccentric contraction of the forearm flexors was measured. To do this the subjects attempted to maintain an elbow flexion of 90^{o} while an extending force was applied by the operator.

A visual target was displayed on the UV recorder which was 50% of the maximal eccentric force. To measure the eccentric MVC the subjects were asked to exert as much eccentric force as possible. For the 50% MVC contractions they were asked to maintain the target force throughout the range of movement from 90^{o} flexion to full extention. Each resisted eccentric contraction lasted 3s. The extended elbow was brought back to 90^{o} flexion by an unresisted concentric contraction. Two seconds elapsed between each contraction. This was continued for 20 min. When fatigue resulted in the

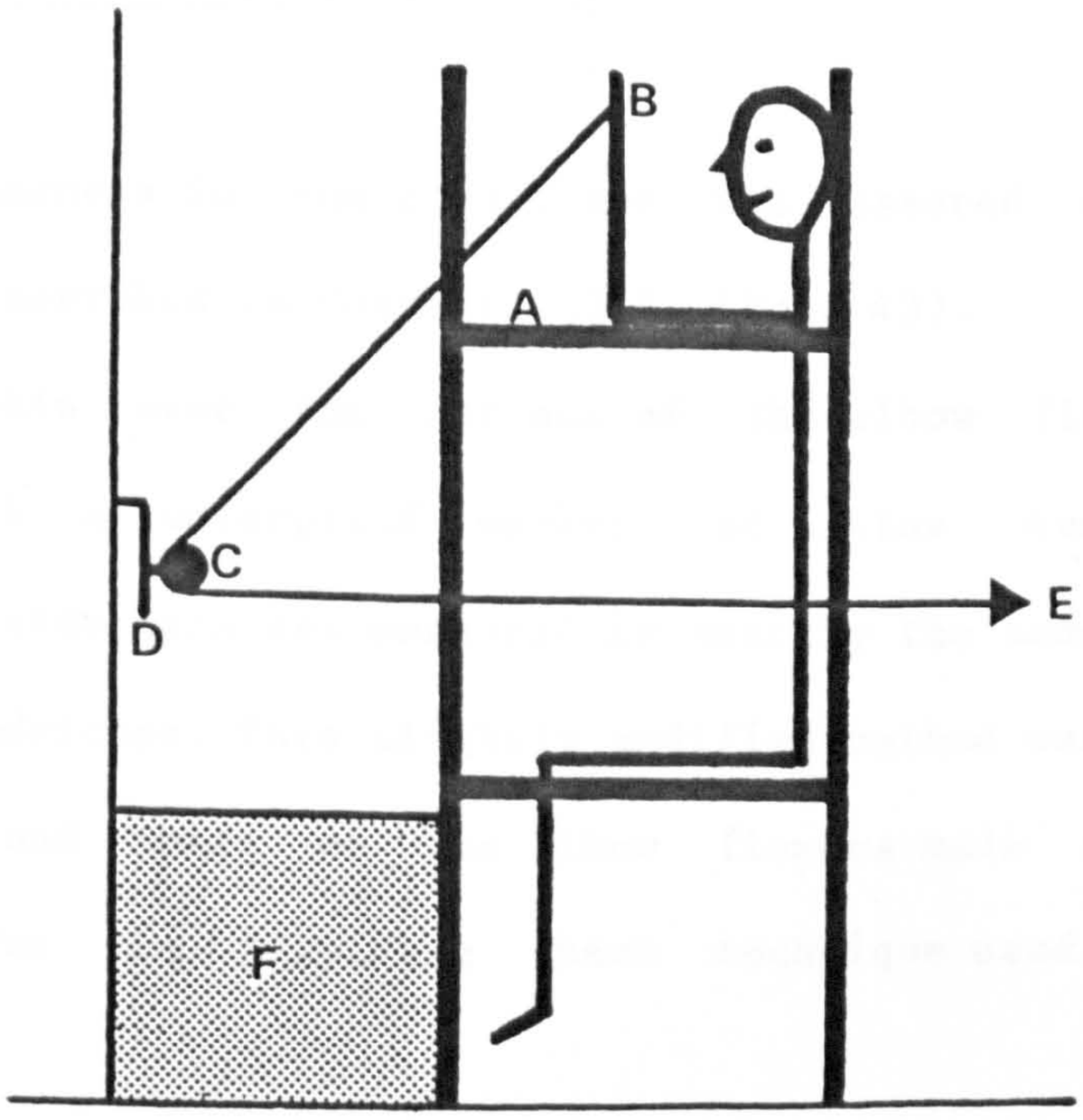

Fig. 18. Apparatus used for eccentric contractions of the elbow flexors. The subject sat on a high chair with the upper arm supported at 90° to the trunk by an adjustable shelf (A). The elbow was flexed to 90° and a rope attached to the wrist (B) passed anteriorly round a pulley (C) which was attached to a wall mounted strain gauge (D). The other end was held by an operator (E) who exerted an extention force which the subject attempted to prevent. Forward movement of the chair was prevented by a block (F).

subjects being unable to reach the target force they were encouraged to get as close to it as possible.

5.2.3. Measurements of tenderness.

Tenderness in the quadriceps was measured using the technique described in Chapter 2.2.6. (Page 43).

The skin over the surface of the elbow flexors was marked with a waterproof marker at sites 4cm apart. Otherwise tenderness was measured in exactly the same way as for the quadriceps. This slightly modified method was used as the size and shape of the elbow flexors made them less suitable for the polythene sheet technique used for the quadriceps.

5.2.4. Tenderness scores.

Using the mapping technique, the figures obtained were inversely proportional to the intensity at each site i.e. the greater the tenderness the less force was required to cause it. To obtain an overall tenderness score for the whole muscle the force required to elicit tenderness at each site was subtracted from the maximal force that was used (40N). These values for all tested sites were summed. Sites where tenderness had not been elicited were given a value of zero.

5.2.5. Plasma CK activity.

Venous blood was withdrawn and the plasma CK measured as described in Chapter 3.2.4. (Page 68).

5.3. RESULTS.

5.3.1. Training effects of regular stepping.

(Appendix xi)

i) Muscle pain and tenderness.

As with other step tests no pain was reported or tenderness elicited at any stage in the muscle which had contracted concentrically during the exercise. Appreciable pain and tenderness occurred in the muscles which had contracted eccentrically during the first two tests, but were markedly reduced in the third week and absent after the fifth week. During the ninth and tenth weeks no pain or tenderness occured. When the contraction pattern was reversed in the eleventh week the quadriceps which contracted eccentrically (concentrically during the training period) developed marked pain and tenderness (Fig. 19) the contralateral muscle being completely free from any discomfort. Tenderness was maximal between 24 and 48 hours after stepping and the distribution was as in all previous studies.

ii) Force generation.

During the course of the 10 week period no change was seen in the fresh or fatigued MVC of either muscle. The degree of low frequency fatigue produced by stepping was also

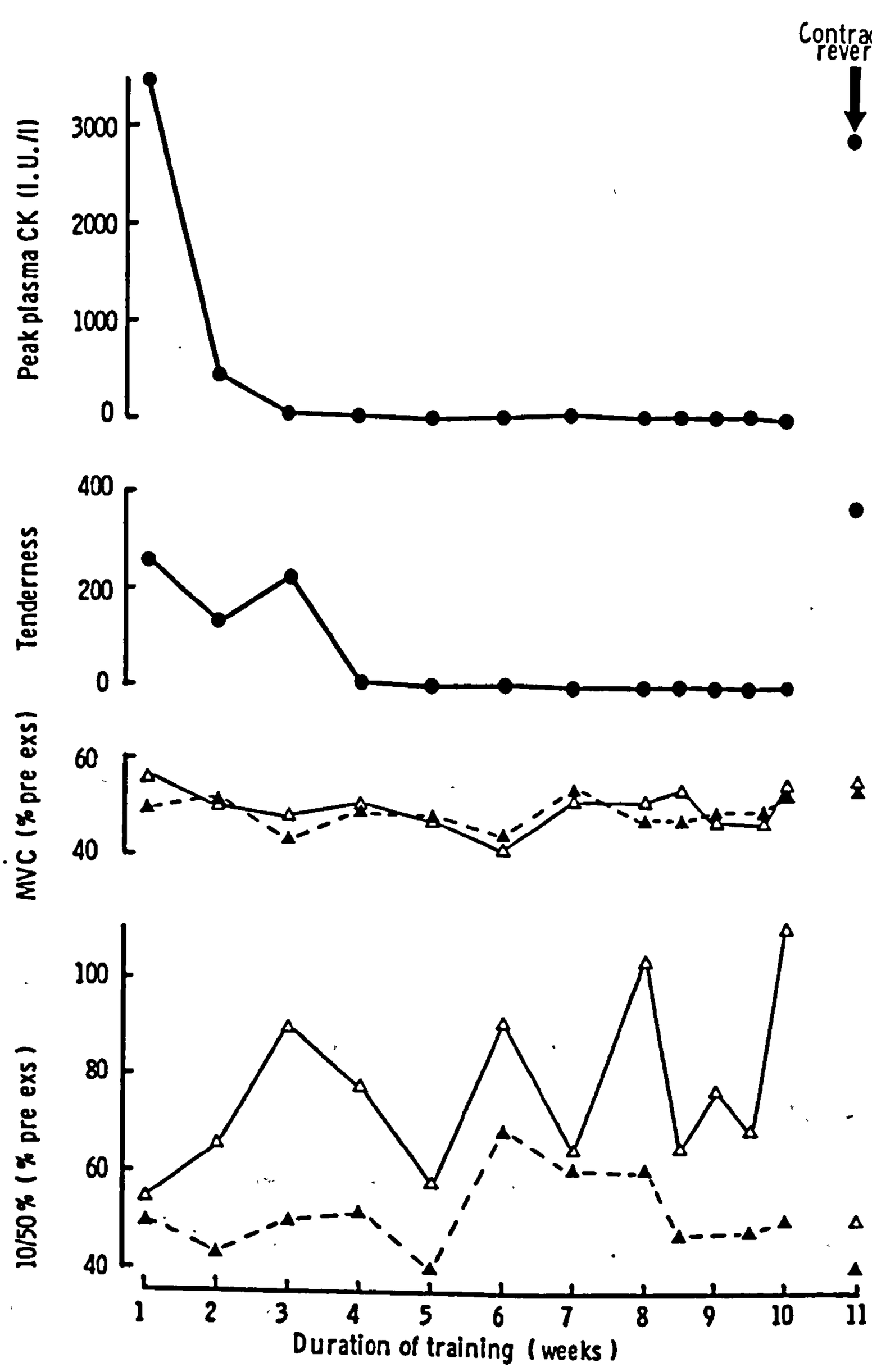

Fig. 19. Effect of training with weekly step tests on tenderness after eccentric contractions, bilateral voluntary force generation and plasma CK. After 10 weeks of training with eccentric (▲) or concentric (△) contractions the stepping pattern was reversed. Note no training effect on either the pre-exercise strength nor the amount of low frequency fatigue caused by the exercise and effects of reversal of the contraction pattern.

unchanged.

iii) Creatine kinase activity.

The first step test resulted in a considerable elevation of the CK level. Pre-exercise the plasma CK was 53 I.U./1 (normal range 10-120 I.U./1), rising to 510 I.U./1 24 hours after exercise, 2,725 I.U./1 at two days and 3,175 I.U./1 at three days and reaching the highest value of 3,500 I.U./1 four days after exercise. The second week the test caused a smaller peak rise of 400 I.U./1 which occured 24 hours after exercise. From the third week onwards the CK values appeared to be unaffected by the exercise.

Reversal of the stepping pattern induced a large rise of CK. From a pre-exercise value of 34 I.U./1 the CK rose to 20424 hours after exercise, 1,458 I.U./1 at 48 hours, 1,731 I.U./1 at three days and 2,870 I.U./1 at 4 days after exercise. The peak value occuring at four days after exercise reflected the same time course as in the initial week of the training period, while the smaller increases seen in sucessive weeks were maximal at 24 hours after exercise.

iv) Specificity of training.

The effects of training were seen in the extent of CK efflux and pain but there were no strength changes or decrease in the alteration of the contractile properties

caused by the stepping. By the fourth week the training changes were mainly completed.

When the contraction pattern was reversed at the end of the 10 week period there was a marked response in terms of both CK activity and pain production that were comparable to those seen in the first week of the training period.

5.3.2. Training effects of eccentric contractions of the elbow flexors.

(Appendix xii)

Two subjects performed the exercise once a week and both showed very similar responses (Fig. 20). The first exercise period resulted in a large CK efflux as well as marked pain and tenderness. By the third week there was no plasma CK response to the exercise. One of the subjects was completely free of pain and tenderness, the other felt no pain or soreness, but was suprised to find that there was some tenderness present, although this was minimal.

Both the subjects then performed the exercise using the contralateral arm which had not performed any regular eccentric work. Both of them had very high CK values, but only in one of them was there significant pain and tenderness.

They then performed the exercise at two, four and twelve week intervals. After two weeks there was a CK response, and

measurable tenderness, but still substantially lower than the first time the exercise was performed. After four weeks a slightly greater response again occurred but it was not until a twelve week period had elapsed that a response similar to that after the first exercise bout was seen to. After a six week period the second subject had a moderate CK rise, which was approximately half that of the first ocassion but no pain or tenderness (Fig. 20).

Another subject trained once every two weeks, and a fourth every four weeks. Both showed a marked training effect (Fig. 21) and in the former subject a four week de-training period still produced a smaller response than on the first ocassion.

In all cases the training effect took place without any changes in muscle force (Table 3). This held both for the eccentric MVC immediately prior to exersise, and the amount of force loss i.e. fatigue, which occured during the exercise. Most of the subjects were unable to reach the target force after 5 min, and none of them were able to do so after 10 min. For all the subjects on all tests the force expressed as a percentage of the pre exercise MVC (mean and SD, n=21) was 40.2% (9.4) at 5 min, 26.3% (8.7) at 10 min, 20.0% (7.3) at 15 min and 17.3% (6.0) at 20 min. Thus the greatest force loss occured in the first half of the exercise period.

The tenderness scores followed the same pattern as the

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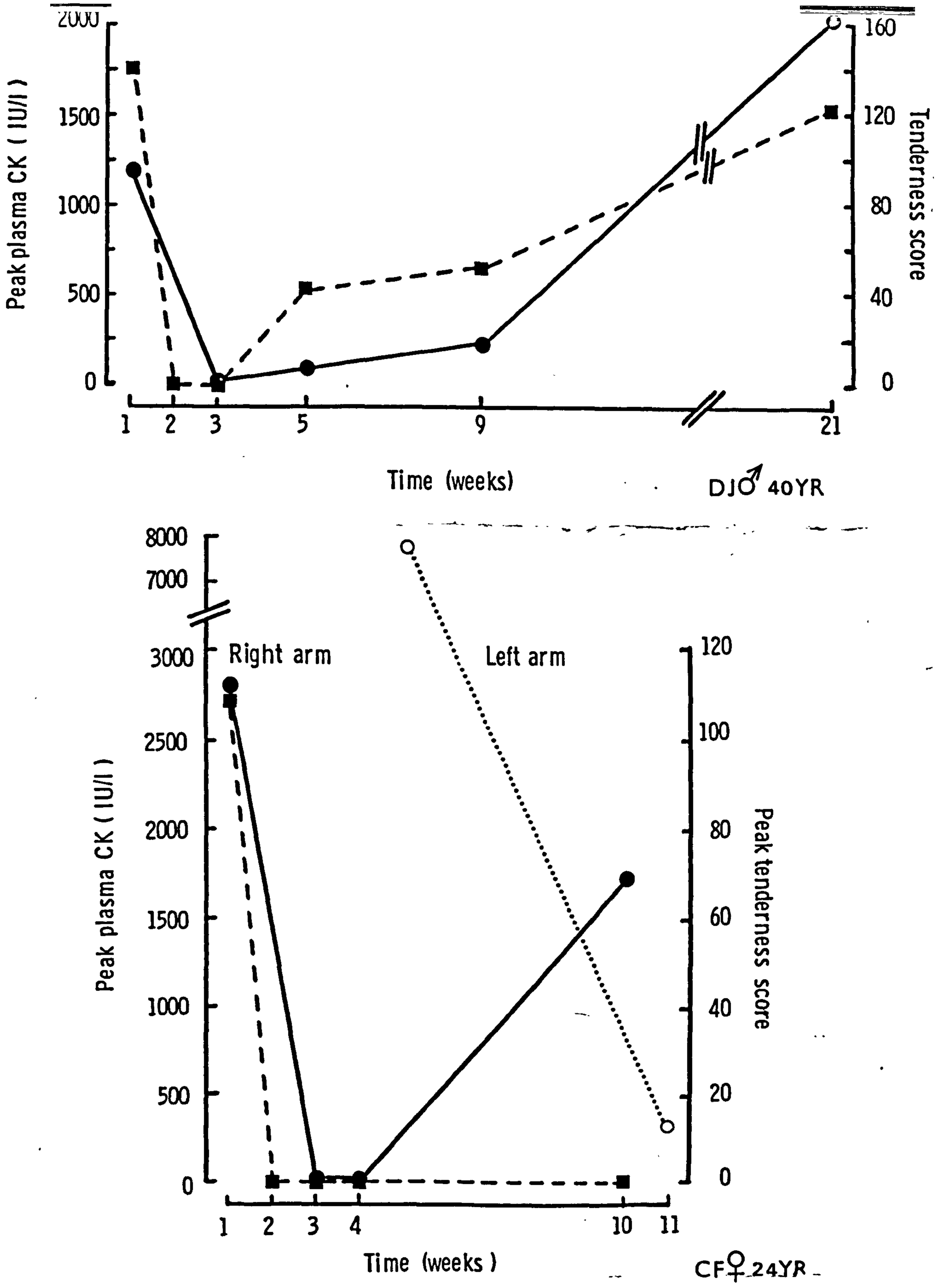
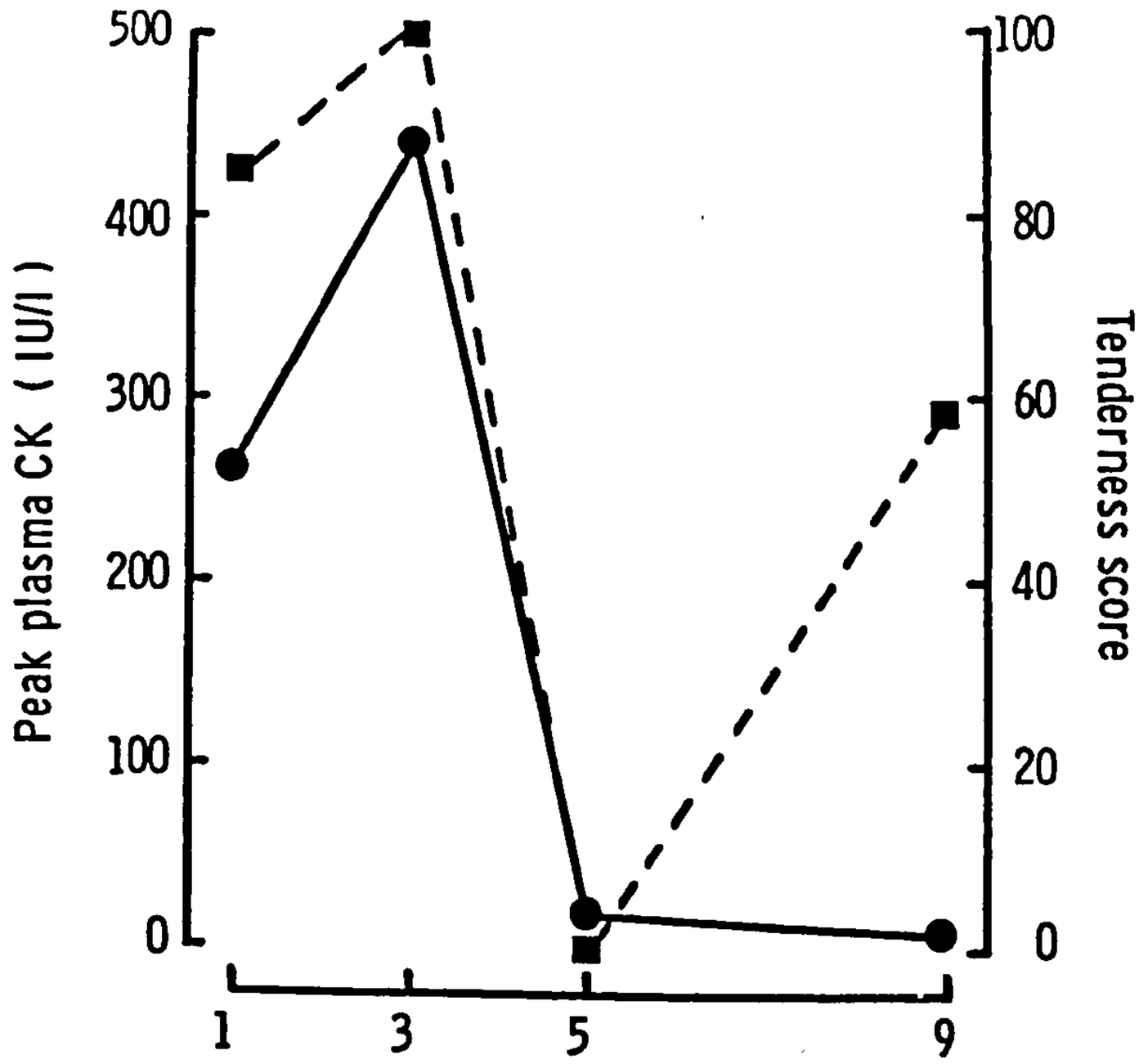


Fig. 20. Peak plasma CK and tenderness after training with weekly eccentric contractions of the elbow flexors, followed by detraining. ●——● CK, ■- - - -■ Tenderness.



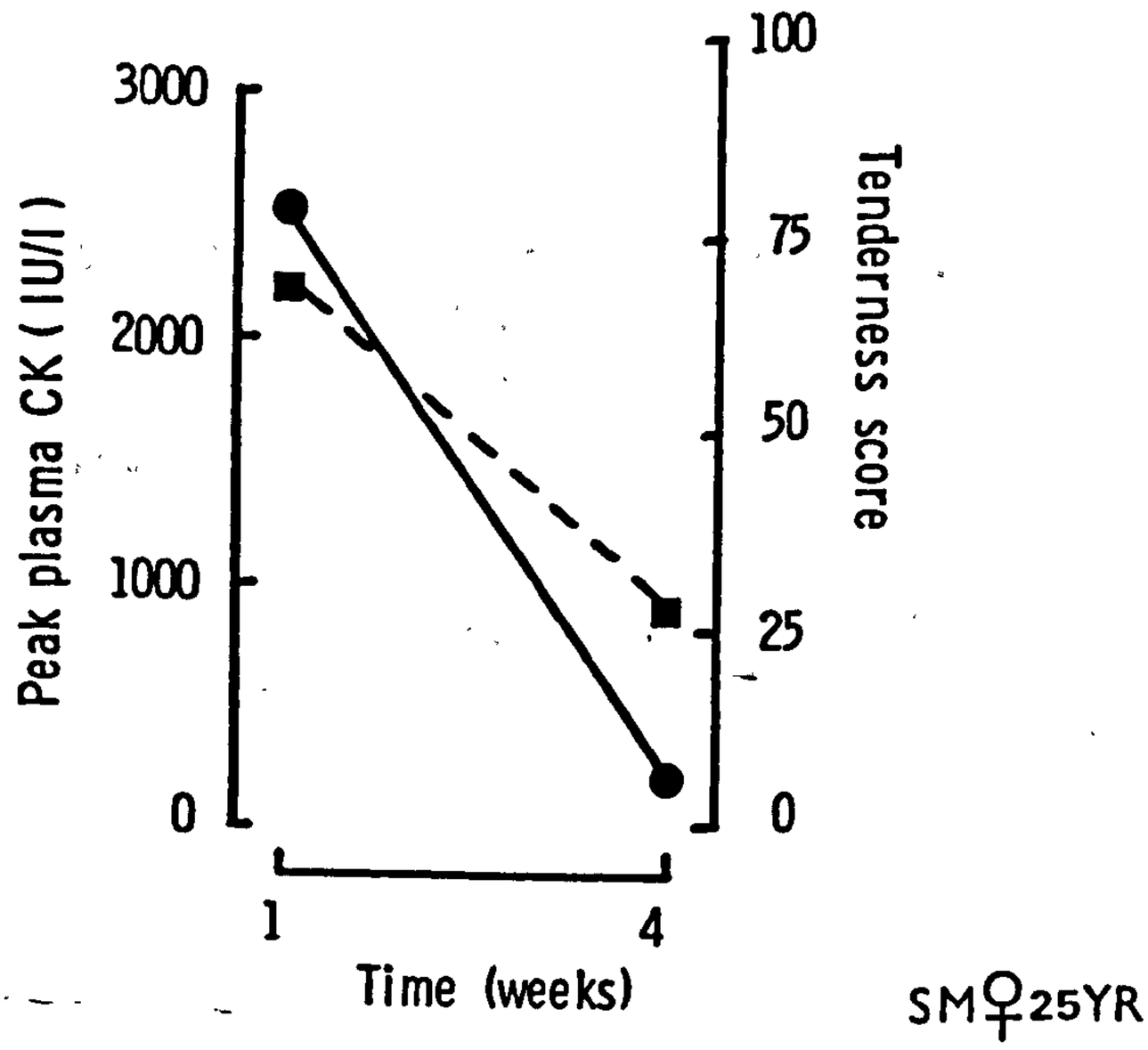


Fig. 21. Peak CK and tenderness after eccentric training of the elbow flexors at two and four week intervals.

●——● CK, ■- - - ■ Tenderness.

Table 3. Force generated by eccentric contractions of the elbow flexors during training.

	MVC (N)	Force (% MVC) during exercise 5 min	10 min	15 min	20 min
Once/week					
C.F.					
Week	1	629.7	37.2	16.9	10.1	8.5
	2	601.7	27.9	25.6	24.4	20.9
	3	503.5	27.7	15.6	17.3	17.3
D.J.						
Week	1	1105.4	52.5	20.5	14.3	14.6
	2	1020.0	24.5	17.6	15.7	12.7
	3	1105.4	48.7	31.6	17.7	16.5
Once every 2 weeks						
O.R.						
Week	1	385.4	52.6	36.3	34.5	29.0
	3	411.2	51.0	25.5	17.0	13.6
	5	503.7	34.7	16.6	16.6	17.2
Once every 4 weeks						
S.M.						
Week	1	561.2	43.0	28.5	20.2	15.8
	5	503.7	44.4	22.2	18.0	12.5

subjective discomfort, and without exception the highest scores coincided with the most painful subjective day. As happened after both stepping and treadmill exercise, with the individuals who demonstrated the large delayed CK rise, pain and tenderness reached highest values before the peak CK values and in many cases all discomfort had completely dissapeared at the time of the peak CK levels.

3.3.3. The effect of duration and intensity on CK and muscle tenderness.

Two normal subjects who volunteered to be subjects for the previous training study showed no response in either CK changes or the development of pain and tenderness to the twenty minute eccentric exercise of the elbow flexors. These two individuals seemed to be ideal subjects in whom to investigate the effects of exercise intensity and duration on both CK and muscle pain.

The area under the force record for the initial period of exercise was measured by planimetry. Subsequently Subject No. 1 was asked to perform the exercise as in the first study, but to continue for double the duration i.e. 40 min. For this subject the area under the force trace was 11.0 cm^2, and on the second 21.0 cm^2. Subject No. 2 performed eccentric maximal contractions. To counteract the effects of fatigue and maintain high force generation three contractions were performed and then the subject rested for five minutes.

In this way a total exercise time of just under five minutes was carried out. For this subject the area under the force trace was 14.9 cm^2 for the first exercise test (i.e. 20 min. with a target of 50% MVC) and slightly less on the second test being 11.5 cm^2.

Subject No. 1, despite having exercised for double the time, only had a moderate CK rise - peak level 356 IU/1, after the second test and had no pain or measurable tenderness. A very different response was seen in Subject No. 2. Although this subject had exercised for a much shorter period, and the total work done was less than on the first occasion, the test using maximal eccentric force produced a much greater response. The large delayed CK rise occured, with peak values of 2,043 IU/1 on the fifth day after exercise. Despite this the subject was not aware of any subjective pain and only complained of "stiffness", and had no measurable tenderness (Fig. 22 & Appendix xiii).

There were no obvious differences between these two subjects who did not respond to the standard 20 min test and those who did. The fresh eccentric MVC of these two subjects was within the range of those who did respond, as was the target force and the amount of fatigue that occured during the exercise. Neither of these subjects was involved in a regular exercise or physical training programme.

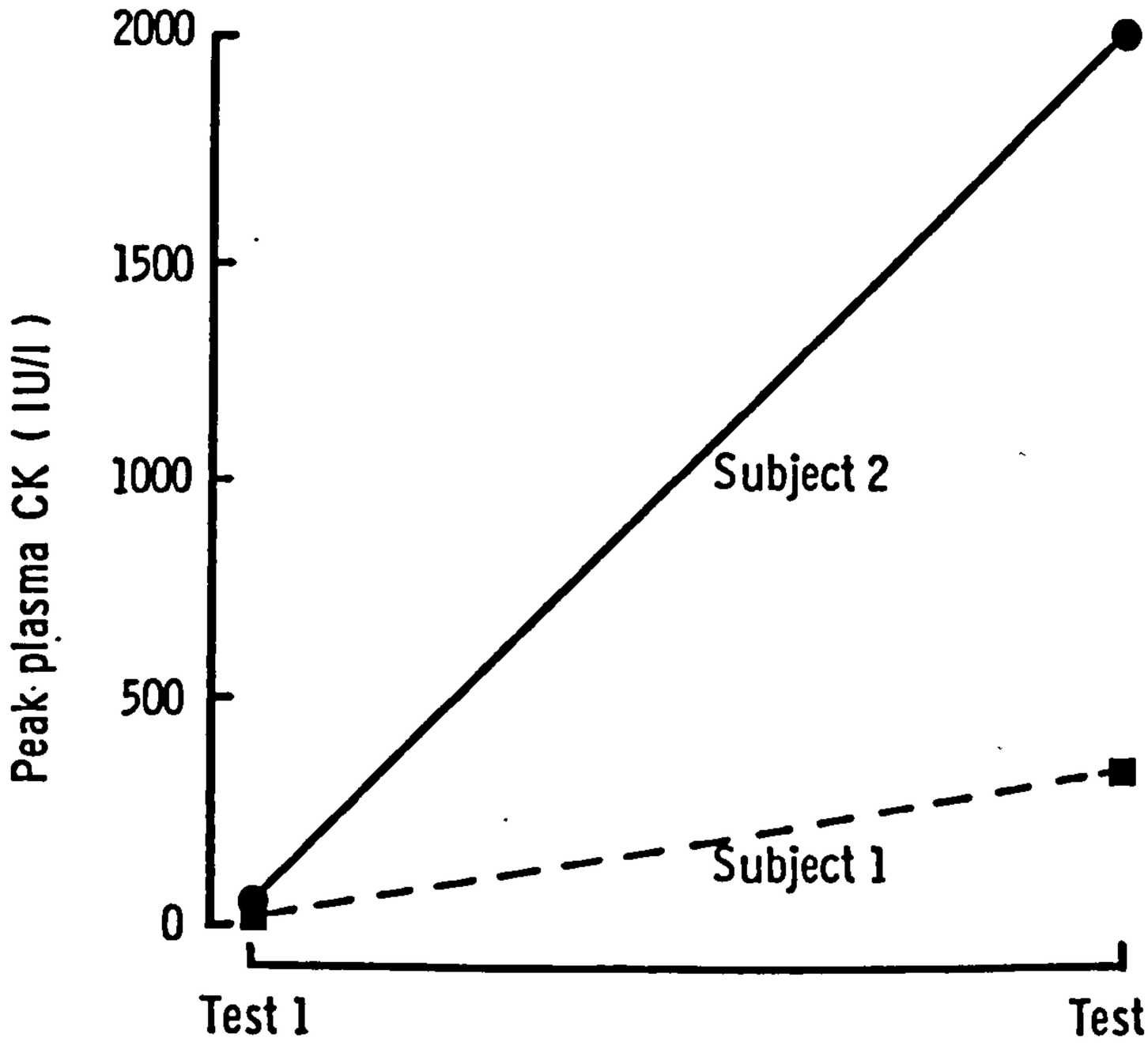


Fig.22. CK changes after eccentric contractions of the elbow flexors with changes in intensity and duration in two normal subjects.

Test 1 was the standardised 20 min test, to which both subjects failed to respond. In Test 2, Subject 1 used the same relative target force (50% MVC) but the duration was doubled. Subject 2 performed intermittant maximal eccentric contractions for a total time of less than 5 min.

5.4. DISCUSSION.

After a period of regular stepping one subject was able to reach a level of training such that no pain or tenderness was caused by the exercise. However the training effect was very specific and when the stepping pattern was reversed, so that the muscles which had contracted concentrically throughout the training period were made to work eccentrically, marked pain and tenderness occured. An indication of the specificity of training effects was also seen in subjects who only performed one step test, as those who performed regular training programmes which consisted of weight training tended to report greater pain and tenderness after stepping than those who were accustomed to performing stretching exercises.

Tenderness mapping of the elbow flexors showed that the bellies were most tender and the subjective impression of the individuals concerned was in agreement with this. The time course of the pain was the same as in the quadriceps and calves. The studies reported in this thesis appear to be the only work where the distribution has been specifically investigated and it was consistently found that pain was felt and tenderness recorded in the bellies as well as the attachments of the elbow flexors and quadriceps. In view of the clear demonstration of very marked biochemical damage it does not seem suprising that some discomfort is experienced in the muscle itself. Indeed, in this case pain would seem to

be fulfilling its classical role as an indicator of damage.

The study of training in the elbow flexors substantiated both the marked training effect, the specificity of training and the absence of strength gain during the training period. In these studies it was found that a 6 week interval between exercise still gave marked protection, but no benefit was seen after a 12 week interval. Bonde-Petersen & Knuttgen (1970) studied habituation to eccentric contractions and also found that the enzyme efflux and delayed onset muscle pain were eliminated with training without any increase in strength. Komi (1983) reported similar results when he investigated the morphological changes with repeated eccentric contractions. Once training had occured and delayed onset muscle pain no longer occurred he found that the morphological damage seen after the initial exercise was not present. He hypothesised the the Z-line was the weak link in the sarcomere and the source of mechanical damage and suggested that in response to training this was thickened and strengthened. Reasonable though this may be there is no evidence to support the suggestion.

As there seems little doubt that no strength changes accompany the training effect, it may be that the non-contractile elements are in someway strengthened, but the exact tissue which adapts and the mechanism of training warrant further investigation.

There was marked individual variation in the degree of discomfort experienced by the subjects. It seems clear that habituation to this form of exercise eliminates delayed onset muscle pain, but in addition to this two subjects proved more resistant to this type of muscle pain. In some of these subjects the large delayed CK rise occured, but they consistently reported "stiffness" rather than actual pain and did not have measurable tenderness. As far as could be determined these subjects were not accustomed to eccentric exercise and so it is unlikely that they would count as trained subjects. This is supported by the fact that training studies the reduction in muscle pain that occured with training was matched by a reduction in the peak CK levels after exercise. It might be that these subjects have particularly high pain thresholds and that what they were experiencing would have been described as pain and tenderness by others, but such psychophysical aspects are beyond the range of this thesis. Another possible explanation is that this pain resistant group of subjects was made up of individuals who were particularly supple and hence have muscles and tendons that were longer than found in the majority of people. If this were true then it may be that during the eccentric exercise their muscle were not lengthened to the critical point at which damage occurs. This remains as speculation, but after an eccentric training study in which the subjects abolished both pain and morphological damage in the absence of any strength changes, Friden (1983)

suggested that the mechanism was by lengthening of the muscles by the addition of sarcomeres. Such an adaptation has been demonstrated in cat muscle after immobilisation in the lengthened position (Goldspink et al 1974).

It was indicated that intensity rather than duration caused a greater enzyme efflux. Similar findings have been reported by King et al (1976) and Clarkson et al (1982). Also in the prolonged stepping study the largest efflux was found in the subject who exercised for the shortest time, which would argue against exercise duration being the critical factor.

The large delayed CK rises showed the same time course as has previously been described in this work. Those who have the large (>1000 IU/l) delayed CK rise do not reach peak values for 3-5 days after exercise and this is hard to account for. It was first seen after stepping, and it was thought that it may be a reflection of the time taken for the enzyme to reach the blood stream from such large muscles. However similar time courses were found after eccentric contractions of both the calves and the elbow flexors, which are much smaller muscles (Fig. 23). This is in the presence of different absolute values thaat are found in different subjects. The absolute and relative changes are shown in Table 4. They indicate that the time course is not the result of some critical rate limiting clearance factor in the clearance being exceeded.

Another possibility is that the circulating levels are a

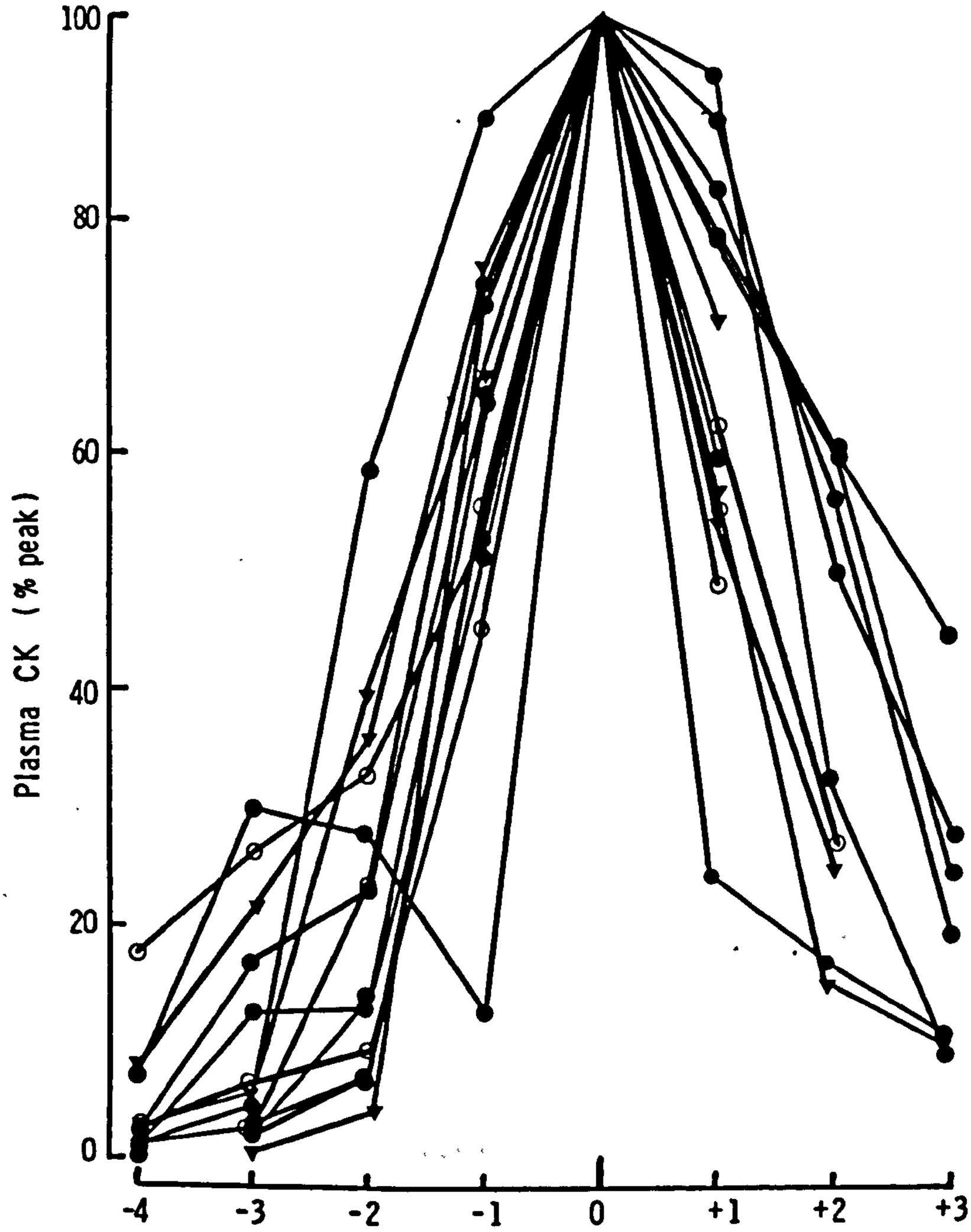


Fig. 23. Time course of the large delayed CK response aafter eccentric contractions in three muscle groups.

● Quadriceps, ▼ Calves, ○ Elbow flexors.

Table. 4. Daily changes in plasma CK in normal subjects with and without the large delayed rise.

SMALL RAPID RESPONSE.

Subject	CK (IU/1)	ΔCK (IU/1)	ΔCK (%)	Subject	CK (IU/1)	ΔCK (IU/1)	ΔCK (%)
CG	62			DN	36		
	129	+67	+108.1		127	+91	+25.3
	117	-12	-9.3		89	-38	-29.9
	61	-56	-47.9		55	-34	-38.2
RH	68			SC	61		
	135	+67	+49.6		71	+10	+16.4
	97	+41	+30.4		68	-3	-4.2
	90	-7	-7.2		53	-15	-22.0
MT	61			DJ	71		
	120	+59	+96.7		125	+54	+76.1
	73	-47	-39.2		87	-38	-30.4
					87	0	0.0

LARGE DELAYED RESPONSE.

KG	154			DJ	75		
	1995	+1841	+1195.4		160	+85	+113.3
	8450	+6455	+323.5		1177	+1017	+635.6
	1440	-7010	-82.9		2111	+934	+79.3
	23700	+22260	+1545.8		1638	-473	-22.4
	3450	-20250	-85.4		1268	-370	-22.6
	2021	-1330	-38.5		946	-322	-25.4
SC	122			SM	30		
	519	+397	+325.4		42	+12	+40.0
	464	-55	-10.6		429	+387	+921.4
	210	-254	-54.7		556	+127	+29.6
	1679	+1469	+699.5		1610	+1054	+189.6
	1346	-333	-19.8		2490	+880	+54.6
	940	-406	-30.2		2229	-261	-10.5
	320	-620	-69.9		1245	-984	-44.1
					642	-603	-48.4

reflection of both breakdown and repair, rather than being specific to damage. One would instinctively expect repair processes to be underway by the third day after injury.

The physical condition of the subjects in terms of cardiovascular fitness and strength did not seem to offer any protection against pain and CK efflux, and specific eccentric training seems to offer the only protection. However some apparently untrained individuals do appear to have greater than usual resistance to the after effects.

The training effects took place after very short and relatively infrequent exercise periods, and last for at least six weeks. Equally striking is that they take place without any increases in strength or in force fatigue during the exercise periods. This is in agreement with the results of Bonde-Petersen et al (1972) and Bonde-Petersen & Knuttgen (1970). It implies that the adaptation is in the length of the muscle, its membrane or non-contractile elements, and not in the fibre cross- sectional area.

CHAPTER 6. ULTRASTRUCTURAL CHANGES AFTER CONCENTRIC AND ECCENTRIC CONTRACTIONS.

Page No

6.1. INTRODUCTION.

The pain and CK efflux consistently found after eccentric work indicate considerable muscle damage. Direct evidence of this can be determined by examining the ultrastructural changes in the affected muscles.

As discussed in the introduction, disruption of the internal architecture of a muscle is taken to represent non-specific damage. Changes of this nature have been described after very vigorous eccentric work of the calf muscles by Friden (1983). Similar, but less marked, changes have also been seen in the muscles of athletes and normal subjects (Meltzer et al 1976) but a comparison of the morphological effects of concentric and eccentric contractions has not been carried out.

Friden performed biopsies before exercise, then two and seven days later. The considerable damage seen two days after exercise had virtually recovered after one week. There is no information about the nature and extent of morphological damage immediately after exercise. This study was carried out to accquire such information. The step test was used as it allows the direct comparison of the effects of the two types of contraction.

6.2. METHODS.

Four normal subjects (one female, aged 22 yrs and three male, aged 24, 27 and 45 yrs) acted as subjects.

The standardised 20 min step test was carried out by the subjects (Chapter 3.2.1. Page 68).

6.2.1. Needle muscle biopsies.

Muscle samples were taken bilaterally from the lateral quadriceps using the needle biopsy technique (Edwards, Young & Wiles 1980).

Three of the subjects had biopsies just prior to exercise. All four had biopsies taken within 15 min of the end of exercise and again between 24 and 48 hrs later when the muscles that had worked eccentrically were painful.

6.2.2. Histology and electron microscopy.

The biopsy samples were fixed in phosphate-buffered glutaraldehyde, post fixed in osmium tetroxide and embedded in Araldite.

For histology, semi-thin sections (0.75 um) were stained with 0.25% toluidine blue. For electron microscopy ultra-thin sections (60-90 nm) were stained with uranyl acetate and lead citrate and examined in a Phillips E.M. 200 electron microscope.

Morphological changes were quantitated by carefully examining the toluidine blue sections. A range of 30-90 (mean 47) individual fibres from each biopsy were studied, and the number of fibres with myofibrillar disruption counted. Areas of disruption affecting one or two adjacent myofibrils and one or two adjacent sarcomeres were designated 'focal'.

Disruption affecting more than two adjacent sarcomeres and more than two adjacent myofibrils were designated 'extensive'. If a fibre did not contain any areas of this size, but contained more than ten focal areas the damage was also designated as extensive.

In fibres which contained more than one extensive area the damage was termed 'very extensive'.

6.2.3. Histochemistry.

The orientated specimens were mounted on cork disks, frozen in isopentane (cooled in liquid Nitrogen) and stored in liquid Nitrogen.

Sections were cut at 10 microns thickness and serial sections were stained with haematoxylin and eosin, a modified trichrome stain for general morphology, a reductase st ain for oxidative activity and with a modified ATPase stain at pH 9.4 (Round, Matthews & Jones 1981) for fibre type classification.

Further sections were stained with oil red O for fat and by a modified Van Gieson stain for connective tissue. An

enzyme stain for acid phosphatase activity was also carried out (for all stains see Dubowitz & Brook 1973).

6.3. RESULTS.

No morphological abnormalities were seen in any of the biopsy samples taken either before exercise or after concentric work. Samples from the muscles which had contracted eccentrically in all four subjects showed morphological changes both in the immediate post exercise period and 1-2 days afterwards. These changes were more marked than in the samples taken immediately after exercise (Fig. 24. & appendix xiv).

Light microscopy showed that immediately after eccentric contractions a mean of 16% of the total fibres counted showed focal changes, a similar number showed extensive changes and 8% had very extensive changes while 58% of the fibres appeared normal.

In the samples taken a mean of 30 hrs after eccentric exercise 6% showed focal changes, extensive and very extensive changes were seen in 23% and 28% of the fibres respectively with 45% of the total fibres counted appeared normal (Fig. 25).

Electronmicroscopy revealed that immediately after exercise many sarcomeres had undergone disruption. In these sarcomeres the myofilaments were disorganised and the Z-line material was often seen to be 'streaming' across the sarcomere. The damage was often focal, affecting only one or two adjacent sarcomeres (Fig. 26a) and one or two adjacent myofibrils, but more widespread areas of damage were seen

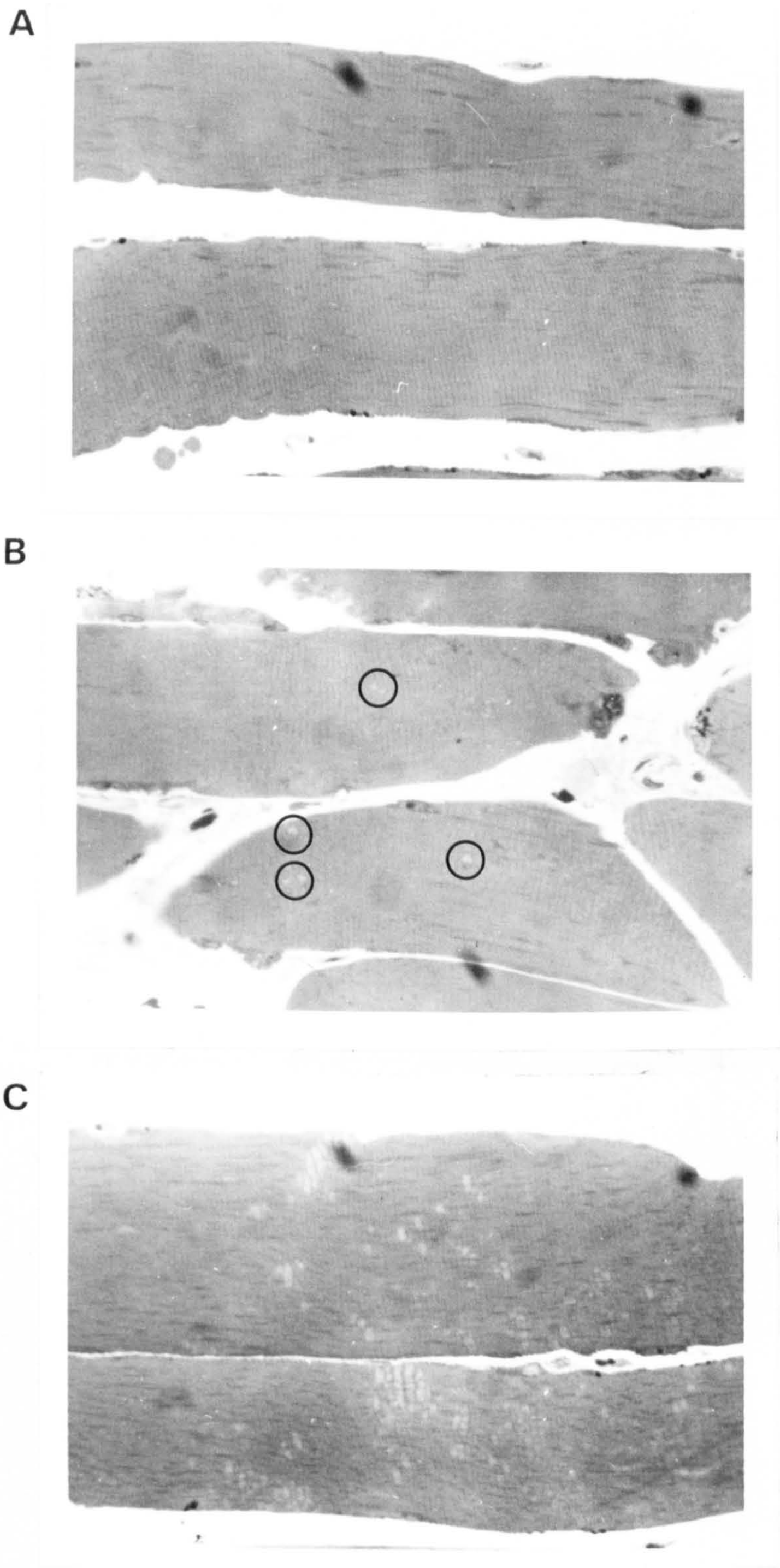


Fig. 24. Typical histological appe arance of muscle biopsy samples a) before, b) immediately after and c) 30 hrs after eccentric contractions. Toluidine blue sections, original magnification x 1000. Circles indicate areas of damage. No changes were seen after concentric contractions.

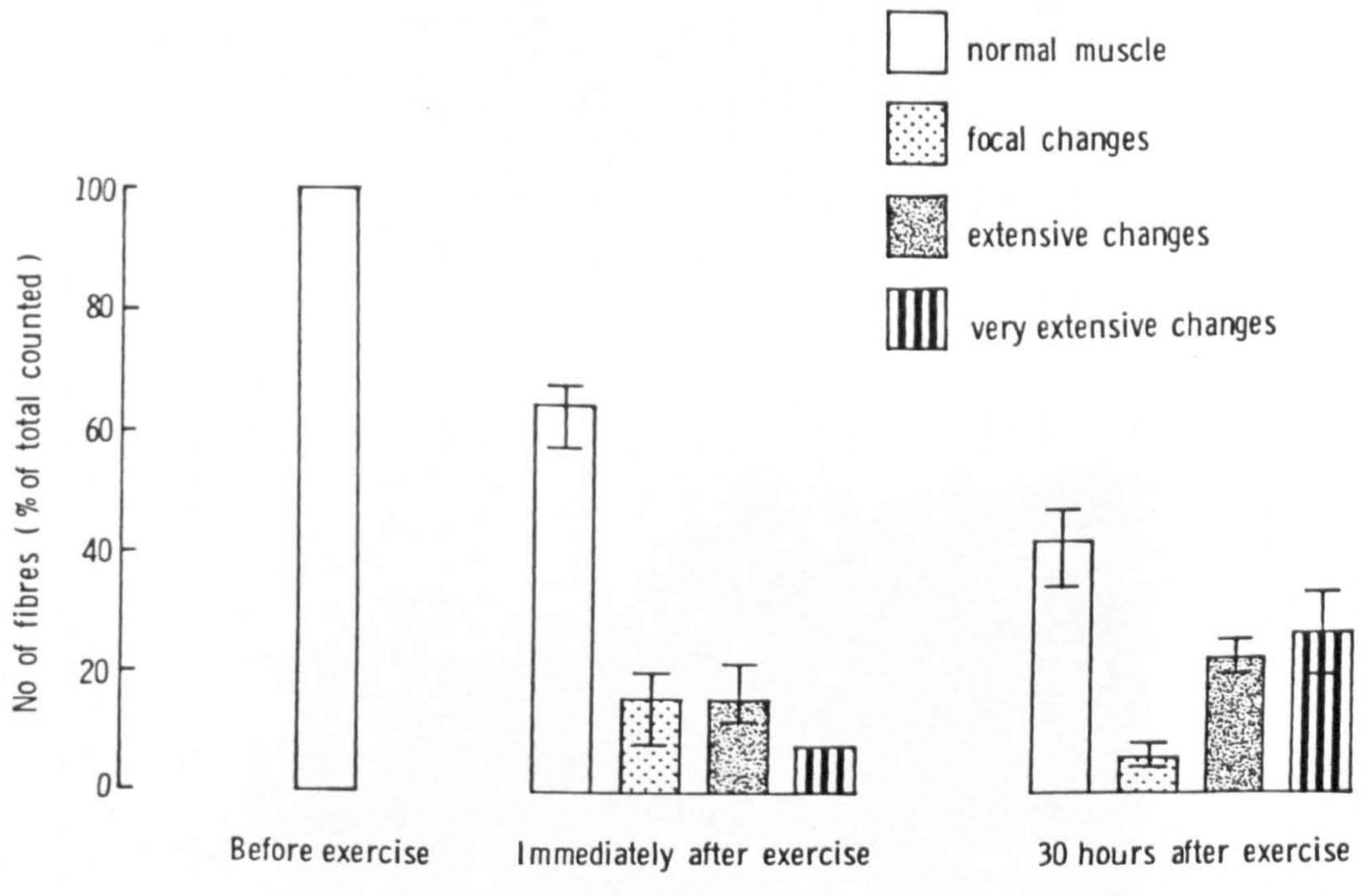
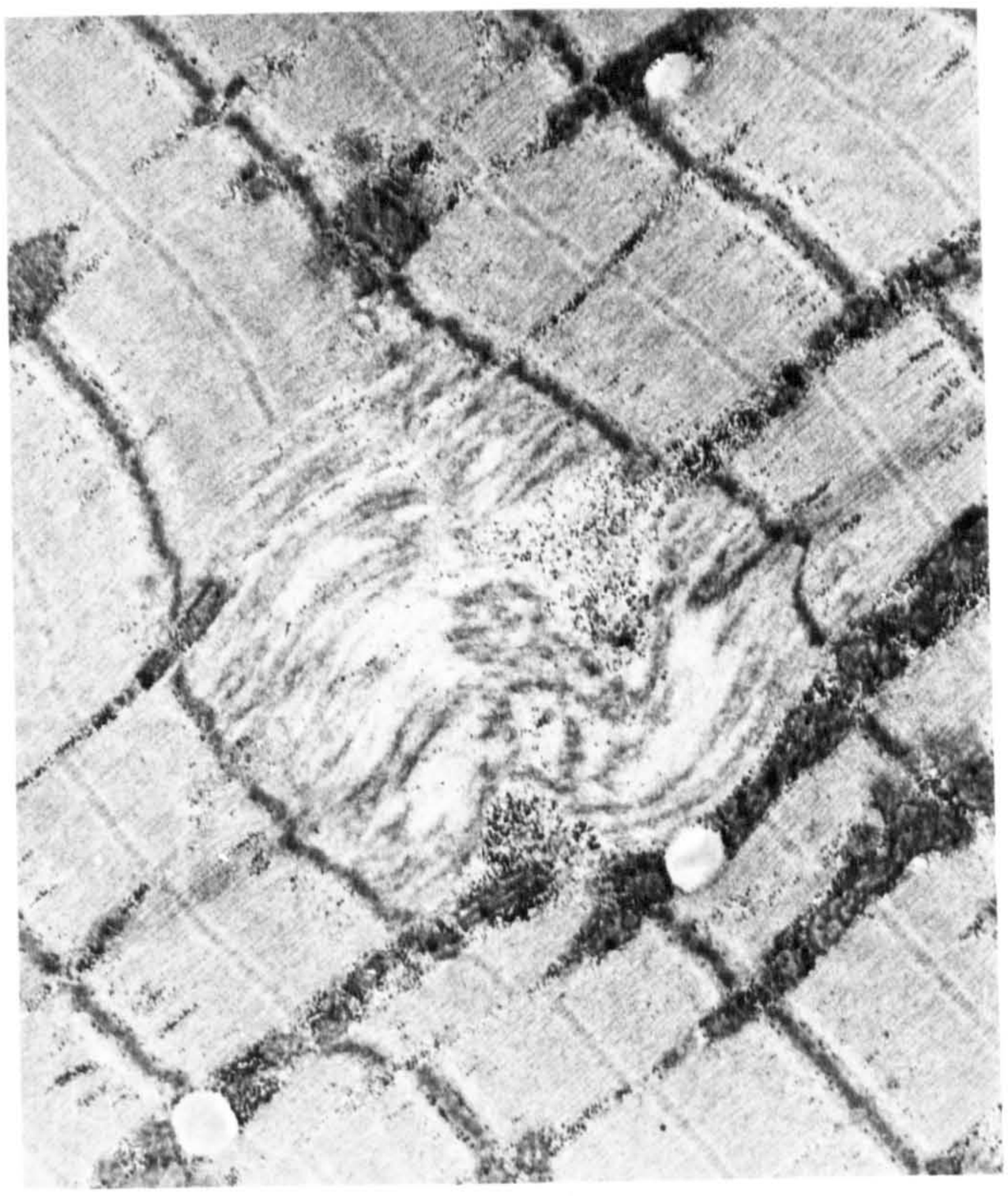


Fig. 25. Quantitation of histological changes in four normal subjects after eccentric contractions (mean and range). Immediately after exercise very extensive changes were only seen in one sample. No changes were seen after concentric contractions.

A



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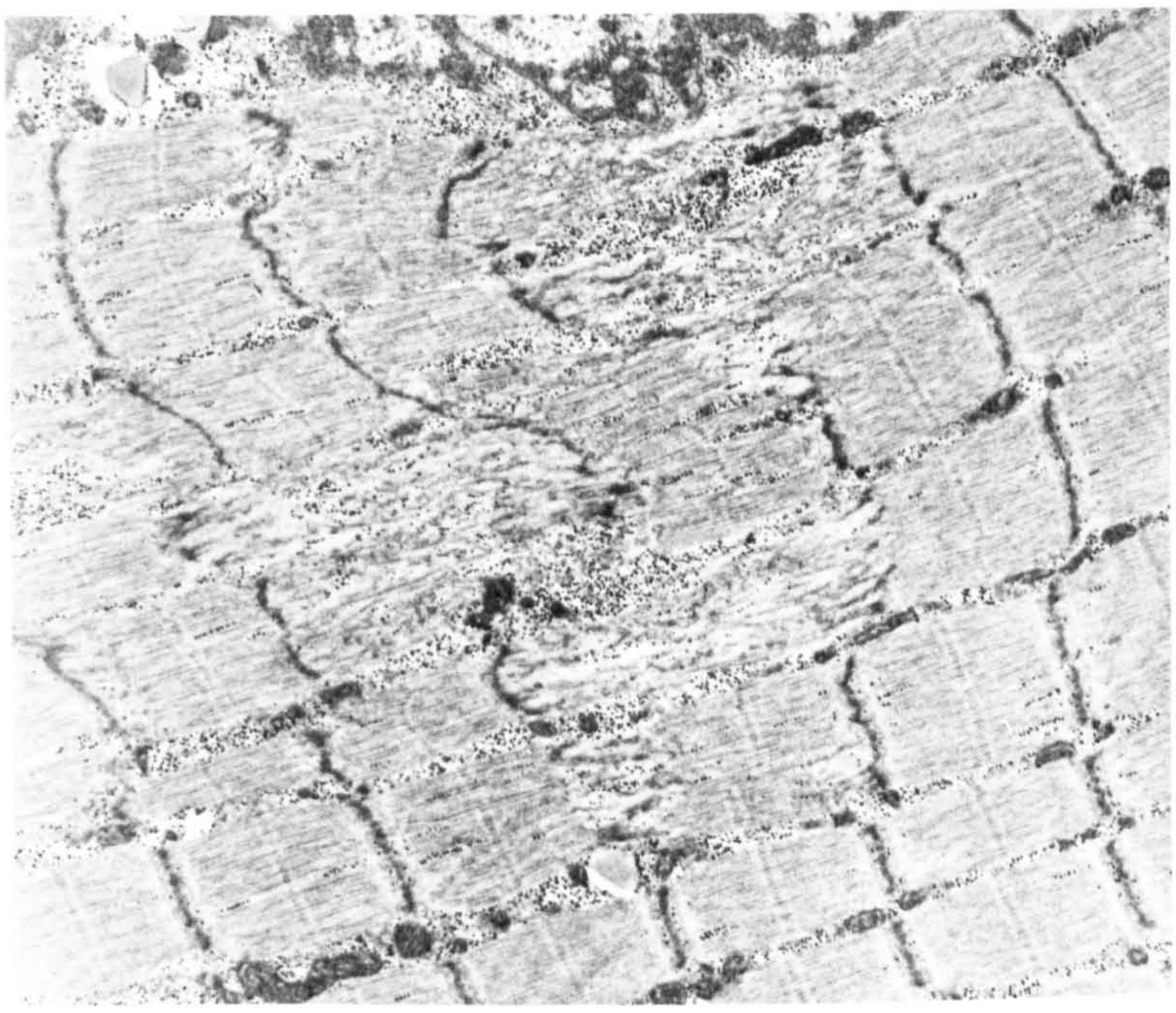

Fig. 26. a) Focal area of disruption affecting only one sarcomere and two adjacent myofilaments. b) Extensive area of sarcomere disruption. Original magnification x 19,000.

(Fig. 26b).

Very occasionally only part of a single sarcomere was affected (Fig. 27). In the larger areas there was sometimes complete distruption of the architecture, leaving the myofilaments randomly orientated and the organelles displaced from their usual position. Loss of Z-lines was also seen (Fig. 28). Sarcomeres adjacent to these areas were usually undamaged but ocassionally showed mild damage in the form of Z-line streaming over part of the sarcomere (Fig. 29) or an apparent widening of the distance between thick and thin myofilaments in the A-band.

In the biopsies taken a mean of 30 hrs after ecentric work the damage was essentially the same as that in the immediate post exercise period but had developed to involve more sarcomeres. Fewer focal areas and more widespread areas of damage were observed.

No abnormalities or changes were seen on any of the sections stained histochemically.

As previously reported pain and tenderness developed only in those muscle which had contracted eccentrically. The time course and distribution of the pain and tenderness was as in the other stepping studies.

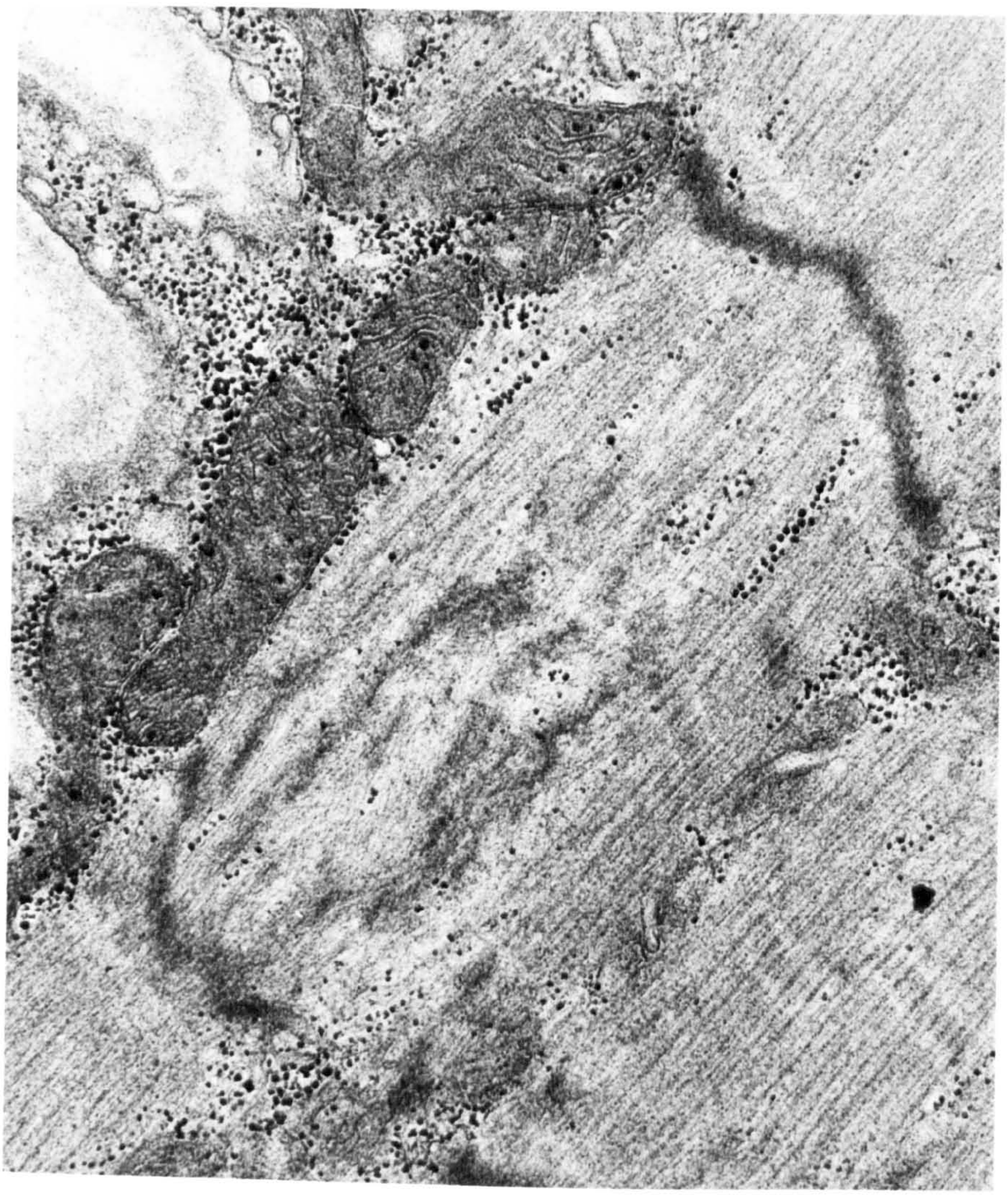

Fig. 27. Focal area of disruption affecting only part of a sarcomere, although both Z-lines appear slightly distorted. Original magnification x 56,000.

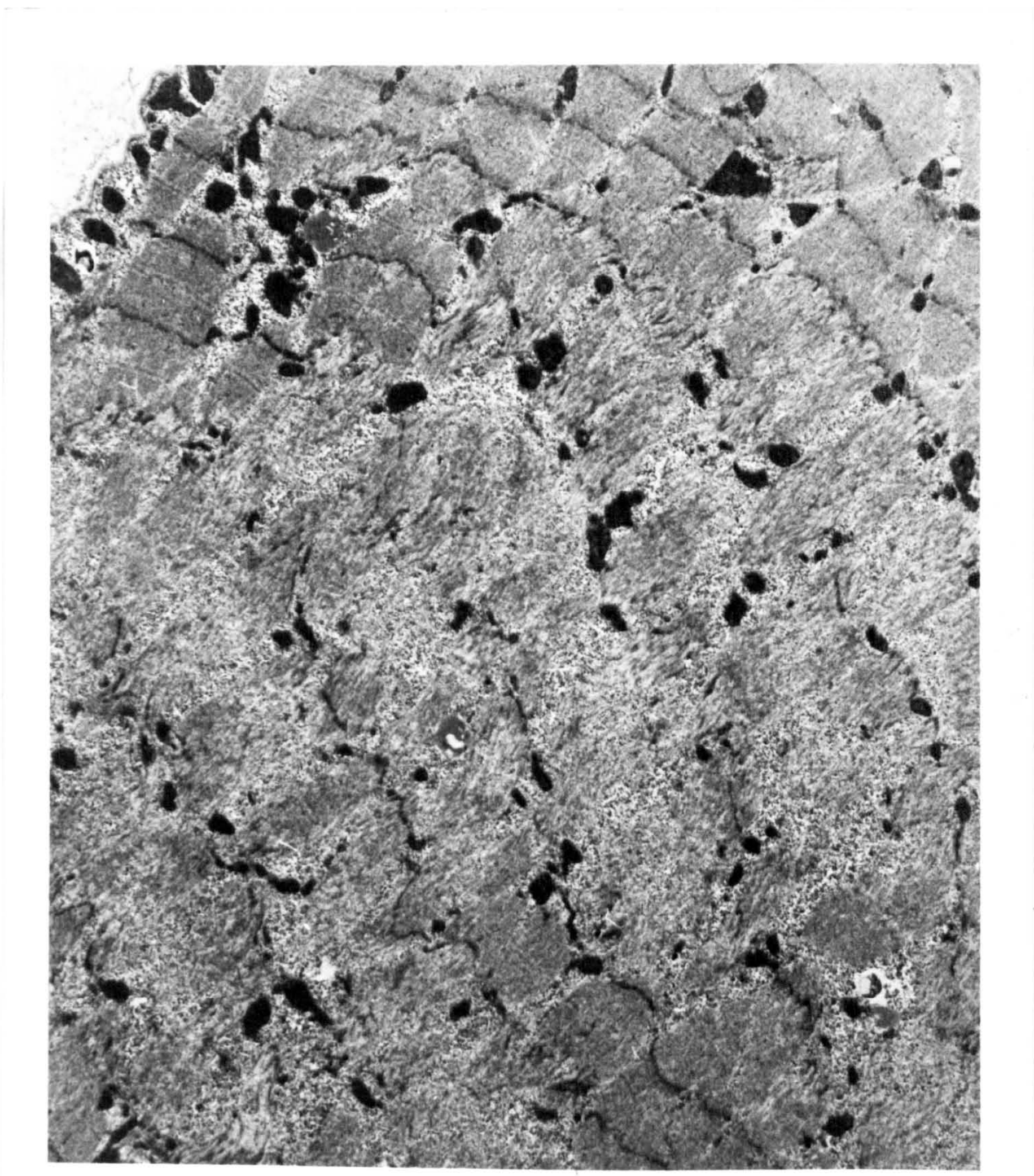

Fig. 28. Very extensive area of architectural disruption. The myofilaments are in dissaray and the Z-lines are distorted or absent. Organelles are displaced. Original magnification x 9,000.

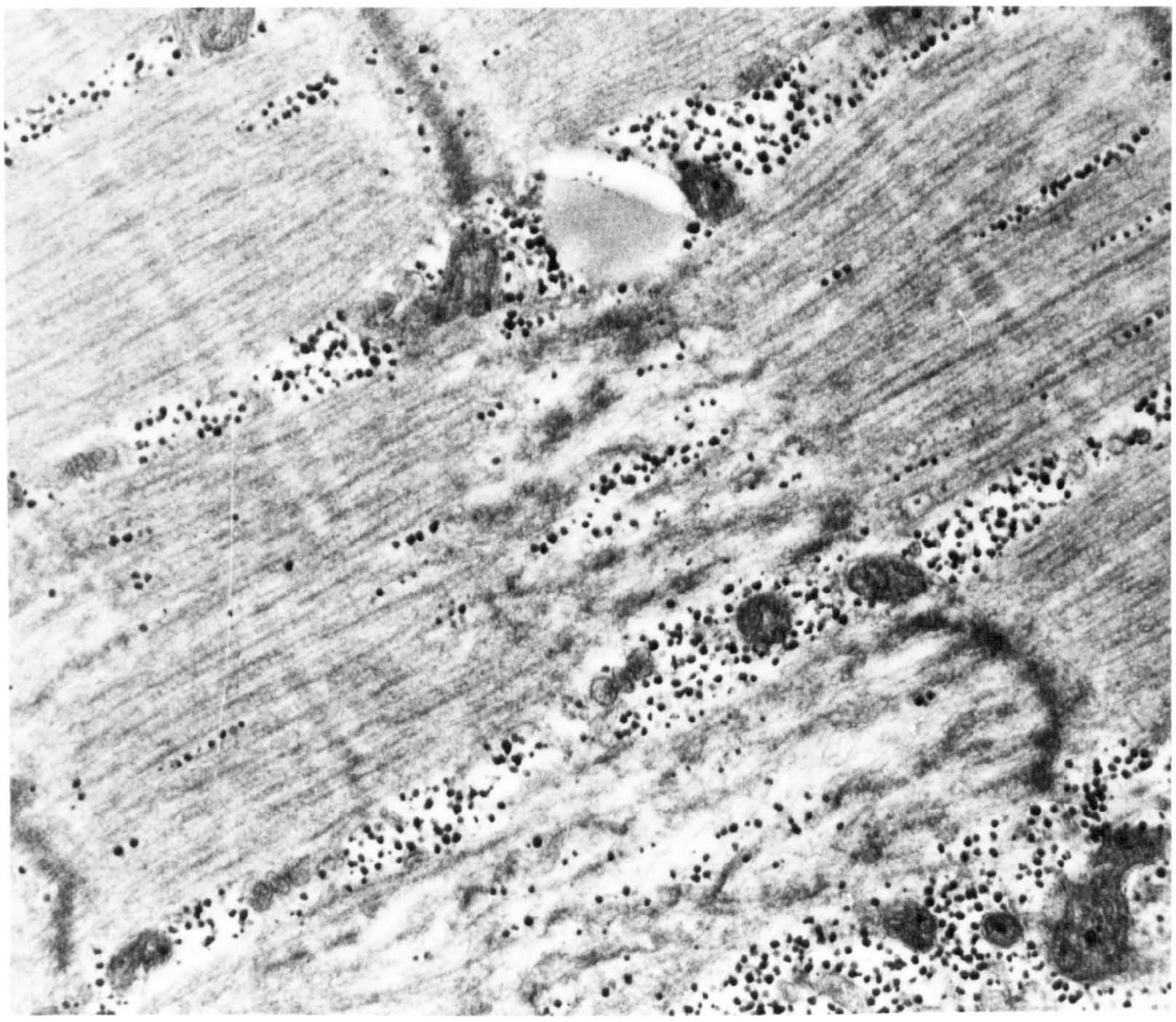

Fig. 29. Sarcomeres adjacent to a large area of disruption. The Z-line between two adjacent sarcomeres is disrupted but the sarcomeres are only partially disrupted. Original magnification x 56,000.

6.4. DISCUSSION.

Needle muscle biopsy samples taken after the performance of the same total work by means of either concentric or eccentric muscle contractions revealed morphological changes indicative of damage only in the muscles which had contracted eccentrically. These changes were only seen with histological preparations and on electron microscopy. They were present immediately after the exercise but were more extensive in the samples taken a mean of 30 hours later. Immediately after eccentric contractions localised areas of Z-line streaming were mainly seen while in the samples taken 30 hours after exercise there were more extensive areas of damage where the internal architecture was completely disrupted.

Changes of this nature are taken to be a non-specific indicator of damage, they are a common finding in many neuromuscular diseases (as reviewed by Cullen & Mastaglia 1982), and have also been reported as occuring in normal subjects by Fischman et al (1973) and Meltzer et al (1976). In these studies no such abnormalities were seen in any of the samples taken before exercise nor after exercise in muscles which had contracted concentrically. As the changes reported here were confined to the muscles which had contracted eccentrically they may be taken to be the consequence of this type of contraction. Friden and coworkers (1981 & 1983 a) & b) reported similar changes after exhausting eccentric contractions, but the morphological

consequences of performing the same total amount of work by either concentric or eccentric contractions had not previously been investigated.

After a series of morphological studies on the effect of eccentric exercise Friden and coworkers (1981 & 1983 a & b) concluded that the Z-line was the site of the initial mechanically induced damage, as the most common early finding was broadening, streaming and disruption at this site. This is in agreement wiith the findings reported here and seems to be a likely explanation for the morphological findings.

In support of this theory is the finding by Friden et al that the morphological damage was seen more in Type II than Type I fibres, the ratio being 2.8:1 in samples taken both immediately after exercise and 3 days later. Despite this they were unable to find any clear evidence for the preferential recruitment of Type II fibres using histochemical staining techniques. An explanation for this might be that as the Type I fibres have broader Z-lines (Prince et al 1981) thay have mechanically stronger connections to the contractile units and are better able to withstand stress.

Whether mechanical overload is the primary or secondary cause of the Z-line damage still remains unclear. A metabolic process occuring during eccentric exercise might cause the release of excessive levels of intracellular calcium which has been shown to be damaging (Jones et al 1984, Sewry & Dubowitz 1984) and may cause a weakness of the Z-line

structure. If this is the case then the calcium activated Z-line protease, soluble alpha-actinin (Fox et al 1975, Beaulaton & Lockshin 1977) may also play a role. However, Friden et al carried out a qualitative analysis of the sarcoplasmic reticulum and could find no swelling or other evidence of damage, making it seem unlikely that this is the primary mechanism of the damage. Furthermore it is hard to imagine a metabolic process which would occur more during eccentric, compared to concentric, muscle activity. This does not exclude the possibility of some metabolically induced damage, which indeed seems quite likely, but suggests that it may be secondary to an initial form of damage which is mechanically induced.

It is particularly interesting that both the extent of the internal disruption and the area involved in the damage was greater in the samples taken 1-2 days after exercise than in the immediate post exercise period as was also found by Friden and collegues. It may be that during stepping some fibres may not be unequivocally disrupted, but so stressed that they are unable to withstand the tensions involved in subsequent normal activity - which will include eccentric contractions, and so the damage is compounded. However it seems unlikely that the increased damage with time is solely due to mechanical factors. While the initial damage appears to be mechanically induced it is possible that it in turn predisposes to a secondary destructive process which is chemically mediated. Vihko et al (1978, 1979) have shown that

the activities of certain acid hydrolases are increased 5-7 days following heavy exercise in mice. The increase was associated with fibre degeneration and necrosis with a marked inflammatory response. There exists in mouse skeketal muscle a calcium activated protease enzyme which causes specific removal of Z-lines (Busch et al 1972) and is thought to be involved in myofibrillary protein turnover (Reveille et al 1976). It is not known whether such an enzyme exists in man, but if it does it is possible that an increase in intracellular calcium during subsequent exercise may activate the enzyme to increase the damage.

Another possible explanation is that lysosomal enzymes are liberated by the damaged structures and so initiate degredation of the myofibrillar material.

In view of the widespread myofibrillar damage seen after eccentric exercise it is rather suprising that isometric force generation has largely recovered at a time when morphological changes seem to be greatest i.e. approximately two days after exercise. This may be accounted for by the system of parallel longitudinal filaments connecting the periphery of successive Z-lines which has been described by Wang & Ramirez-Mitchell (1983). The existence of such a cytoskeletal system, which has also been described by Porter & Tucker (1981) suggests that sarcomeres may be able to transfer tension in the presence of myofibrillar damage.

Evidence of architectural damage was present immediately after exercise when no pain or tenderness was detectable. The

biopsy samples taken at the time of maximal pain and tenderness showed more extensive and profound changes. This is in agreement with Friden (1983) who appears to be the only other worker to study the morphological changes of eccentric exercise. Thus it would seem that morphological damage per se is not the cause of the pain and tenderness. However it is clear that of all the parameters studied the time course of the morphological changes is the most similar to that of the muscle pain. This indicates either a critical level of damage needs to occur for the threshold of the muscle nociceptors to be exceeded, if indeed they are responsive to such damage. Alternatively the damage causes further changes which in turn are the algesic stimulus.

The ultrastructural changes suggest the following sequence; Initially the high tensions exerted during eccentric contractions result in focal sarcomere damage as the A and I bands are pulled apart, causing the Z-line changes seen immediately after exercise and subsequently normal activity, through mechanical and perhaps chemical mechanisms, causes this damage to progres to the total disruption seen 1-2 days after exercise. There is also the possibility that the morphological picture seen 1-2 days after exercise is reflecting regeneration as well as degeneration. This sequence assumes that the initial changes are visible to the techniques used here, and while there is no direct proof of this, the changes seen in Fig. 24 and the data on the numbers of affected fibres is consistent with

this. However, it is notable that in three of the four subjects a larger percentage of fibres show changes in the samples taken 1-2 days after exercise, and in one subject changes were only seen at this time and not immediately after exercise, and furthermore electron microscopy revealed changes affecting only part of a sarcomere. Changes of this nature would not be visible with a light microscope.

The extent of the morphological abnormalities reported here in normal subjects is surprising, particularly in view of the fact that the exercise was neither exhaustive nor of long duration. If similar stresses are imposed upon myopathic muscle it is interesting to speculate about the ability of the muscle to recover. Many myopathic patients are prescribed strengthening exercises and it may be that a significant involvement of eccentric contractions in these exercises may be of doubtful benefit, if not actually harmful.

These results have not fully answered the question of whether the morphological damage causes the CK release from muscle. One of the subjects had a relatively small CK efflux (peak plasma CK of 400 IU/l) yet the morphological changes were quantitatively and qualitatively similar to another subject who had a peak plasma CK of 4900 IU/l. When eccentric exercise induced muscle damage was studied in rats by Armstrong et al (1983) many macrophages were seen. In this study no such changes were seen, nor were any changes seen with histochemical preperations. Three main possibilities arise; firstly that the CK release is not caused by the

morphological damage, and secondly that the mechanism is different in man and animals. The other possibility is that the mechanism is the same in both species and that either biopsies have been taken from the wrong muscle (i.e. the CK is released from some other muscle) or that the right muscles have been biopsied, but at such times that the main changes have been missed.

Therefore it is important to establish the time course and localisation of damage after eccentric exercise.

CHAPTER 7. RADIOISOTOPIC, ENZYMIC AND UNTRASTRUCTURAL EVIDENCE OF MUSCLE DAMAGE.

Page No

7.1. INTRODUCTION.

The uptake of radio-isotope labelled complexes (usually ^{99m}Technetium pyrophosphate (^{99m}Tc-PYP) by damaged muscles has been demonstrated in the last few years. It has been reported in muscle diseases; polymyositis (Spies et al 1975, Messina et al 1978, Bellina et al 1978), McArdles Symdrome (Swift & Brown 1978) and muscular dystrophy (McLean 1977, Giraldi et al 1979, Bulke & Baert 1982). Similar findings have been reported after strenuous exercise which also caused muscle pain (Lentle 1978, Matin et al 1982) the increased uptake being localised to the painful muscles. Uptake does not occur in normal healthy muscle but in rat muscle has been induced by crushing injuries (Robinson & Battaglia 1975). Vita & Harris (1981) injected a myotoxic snake venom into rat muscle and reported that increased uptake occurred with degeneration and inflammation but not in normal, regenerating or denervated muscle.

The results described so far in this work have clearly indicated that eccentric muscle contractions cause pain, fatigue, enzyme release and morphological changes. However there are still some important questions unanswered. It is not clear whether the myofibrillar damage seen with electronmicroscopy causes the enzyme efflux and suprisingly little evidence of damage has been seen on the histochemically stained transverse sections of biopsy samples of the quadriceps, despite very high plasma CK levels.

Stepping exercise involves large muscle groups other than the quadriceps in eccentric work (gluteal and calf muscles) and it is not known what damage occurs in these muscles and to what extent they contribute to the circulating CK levels.

While eccentric contractions have been confined to only one muscle group in the treadmill studies and those of the elbow flexors, these muscles have not been biopsied and so the extent of ultrastructural damage and it time course compared to the CK efflux is not known.

In view of the purported specificity of increased uptake of ^{99m}Tc-PYP to damaged muscles a serial study of muscle isotope uptake, muscle pain, ultrastructural changes and plasma CK was carried out. The aim being to determine the time course and localisation of muscle damage after exercise.

7.2. METHODS.

7.2.1. Exercise tests.

.Initially two normal subjects performed step tests using the standardised height and frequency as described in Chapter 3.2.1. (Page 68).

The first subject (DN, female, 34 yrs) exercised for 1 hour and the second (PB, male, 32 yrs) was stopped after 40 min by an inability to control the eccentric contraction.

Two subjects performed bilateral eccentric contractions of the calf muscles by walking backwards down an inclined treadmill. The slope and inclination was as in Chapter 4.2.1. (Page 99). The first subject (DJ, male, 40 yrs) exercised for 1.5 hrs and the second (DN, female, 34 yrs) for 2 hrs.

7.2.2. Tenderness measurements.

Tenderness was measured over the quadriceps in the subjects who stepped and over the calf muscles in the subjects who used the treadmill. This was carried out as described in Chapter 3.2.2. (Page 68). Tenderness scores were measured as in Chapter 5.2.4. (Page 120)

7.2.3. Needle muscle biopsies.

The two subjects who stepped had bilateral needle biopsies of the vastus lateralis taken three days after exercise. Biopsies were taken only from the muscle which had contracted eccentrically five and ten days after exercise.

One of the subjects who walked down the treadmill (DJ) had unilateral calf biopsies at the same times. The other subject (DN) who used the treadmill had unilateral calf biopsies taken 4, 7, 12 and 20 days after exercise. The biopsy proceedure, processing and evaluation of the samples was as described in Chapter 6.2.1., 6.2.2. & 6.2.3. (Pages 142 & 143)

7.2.4. Plasma CK activity.

Venous blood samples were taken immediately before exercise and then at 24 hour intervals. The plasma was separated and CK activity determined as in Chapter 3.2.4. (page 68).

7.2.5. ^{99m} Tc-PYP uptake.

1.5 milli Curies (mCi) of ^{99m} Technetium labelled pyrophosphate (^{99m}Tc-PYP) (Pyrolite, New England Nuclear Ltd) in a volume of 0.1ml was injected into an antecubital vein. The normal patient dose is approximately 15 mCi and the reduced dose (one tenth) used in this study enabled the subjects to receive up to 10 injections without exceeding the radiation dose normally received in a single study. Approximately three hours later the subject was positioned infront of a Siemens 37 ZLC gamma camera and an image of the appropiate muscle regions was recorded to 200K counts.

Simultaneously digital images were obtained by

collecting the data in 64 x 64 matrices. The data was stored for subsequent analysis of the regions of interest.

For quantitation the regions of interest were outlined and the uptake per unit area computed. The required muscles were outlined together with the patella which is bone not covered by muscle. The uptake was expressed as the muscle:bone ratio. In subjects with normal healthy muscle and bone this ratio is <0.5.

7.3. RESULTS.

7.3.1. Stepping studies.

Very similar results were obtained from both subjects (appendix xv). The first (DN) developed pain and tenderness in the same musles which had been affected in the previous stepping studies i.e. those which had contracted eccentrically - the quadriceps, ipsilateral buttock muscles and contralateral calves. The most discomfort was experienced on the second and third days after exercise. The subject demonstrated the large delayed CK response, the peak plasma value of 3991 IU/l occurring on the fourth day.

The uptake of ^{99m}Tc-PYP in both quadriceps was similar both to each other and to that of normal muscle (muscle:bone <0.5) on all the days when scans were carried out - the first to fifth inclusive and also eighth day after exercise. However on the second day an area of increased uptake was seen on the painful thigh and the muscle:bone uptake ratio was 0.93. This area of increaased uptake was posterior and medial to the femur and using posterior view scans it could be seen that this was an adductor muscle. The amount of uptake in this muscle increased until the fourth day, the muscle:bone uptake being 1.23 on the third day after exercise. The uptake incresed until the fourth day when the ratio was 1.82.

Quadriceps muscle biopsies taken on the third, fifth and

tenth days after exercise revealed no abnormalities when transverse sections stained histochemicaly were examined under a light microscope. However when longitudinal sections, stained with toluidine blue, were examined considerable evidence of damage was seen. Quantitation of the changes revealed that three days after exercise only 27% of the fibres appeared normal, 11% had focal damage, 12% extensive and 50% very extensive changes.

By the fifth day the damage was less obvious and 66% of the fibres appeared normal, 13% had focal changes and 11% and 10% had extensive and very extensive changes respectively. Further recovery had taken place by the tenth day with 90% of the fibres appearing normal, 7% showing focal changes, 2% and 1% showing extensive and very extensive changes.

The second subject (PB) showed very similar results (Fig. 30). Maximal quadriceps tenderness occurred on the second and third days, and the peak plasma CK of 35725 IU/l was on the fifth day . No increased uptake of ^{99m}Tc-PYP was detected in either quadriceps, but as with the other subject was seen in the adductor muscle (Fig. 31). The muscle:bone uptake was 0.58, 0.65, 0.94, 1.45 and 1.87 on the first to fifth days and 1.08 on the eigth day. Posterior views of the pelvis also showed increased uptake in a hip girdle muscle which followed the same time course (Fig. 32). Despite the lack of increased muscle uptake of isotope there was considerable damage seen in the longitudinaly cut biopsy sections stained with toluidine blue. This was greatest in

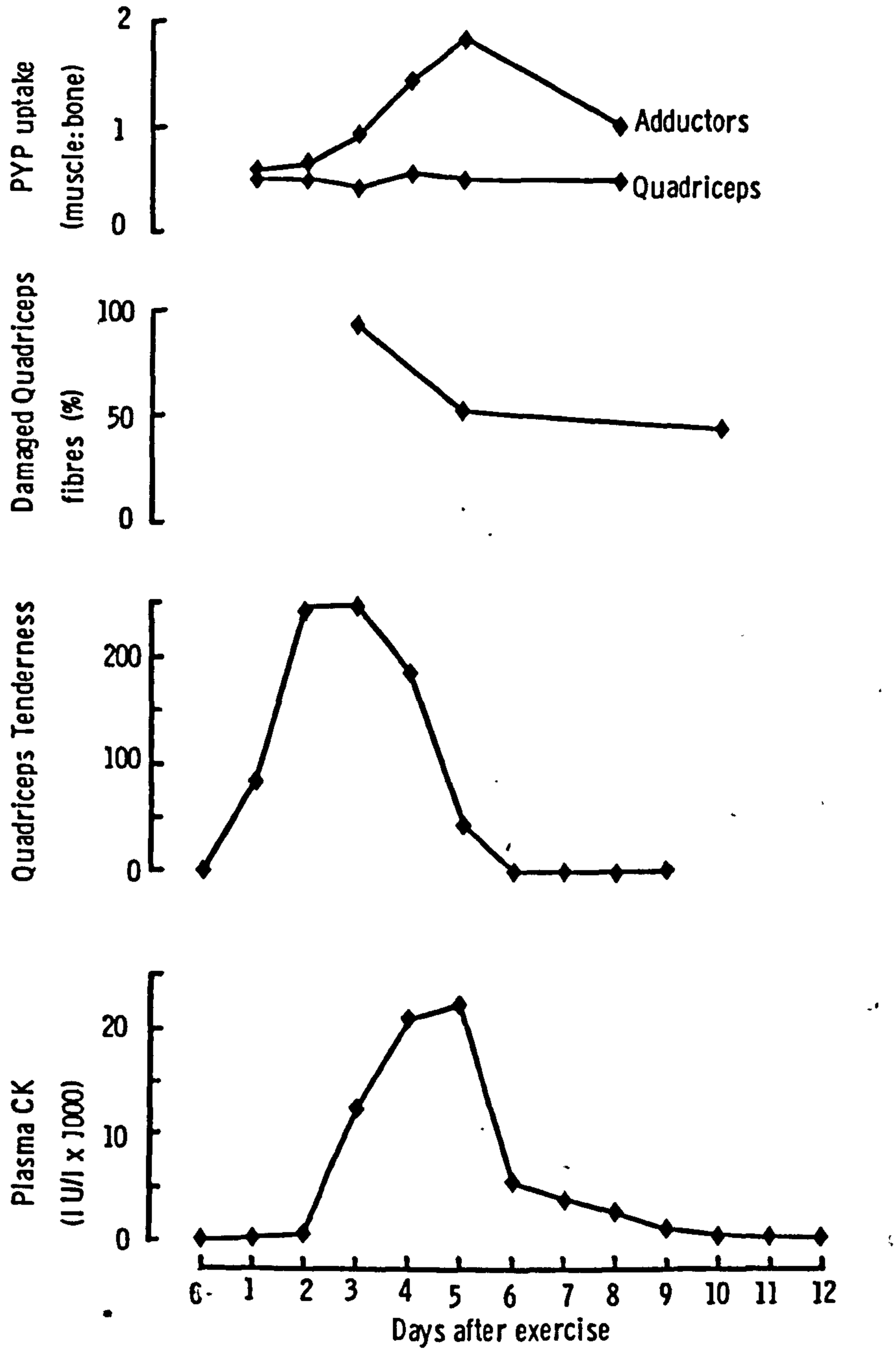

Fig. 30. The time course of muscle tenderness and damage after stepping. Tenderness developed only in the quadriceps which had contracted eccentrically and the biopsy data is from these muscles. Subject PB, male, 31 yrs.

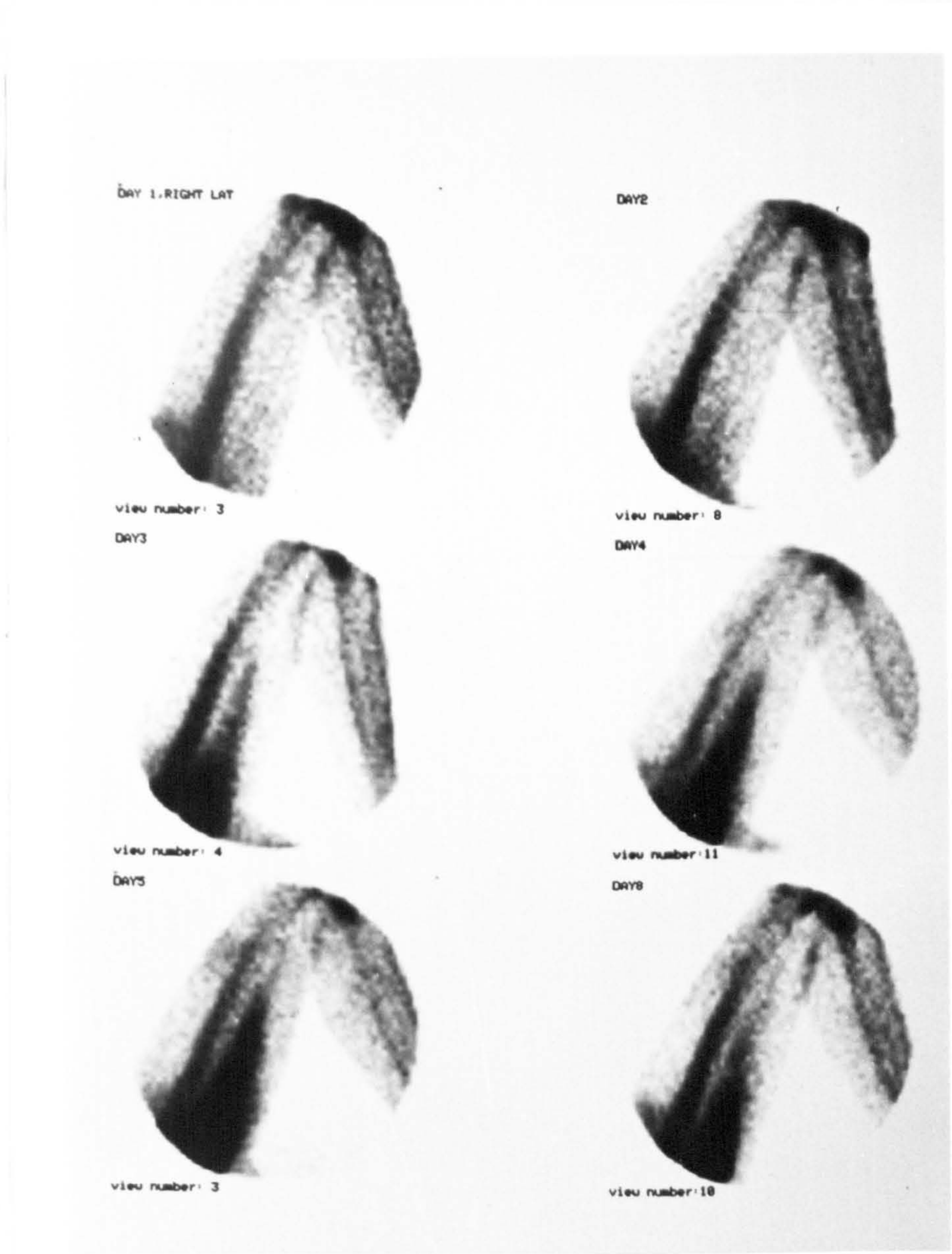

Fig. 31. Lateral views of the thigh showing ^{99m}Tc-PYP uptake after eccentric work. Subject PB, male, 31 yrs.

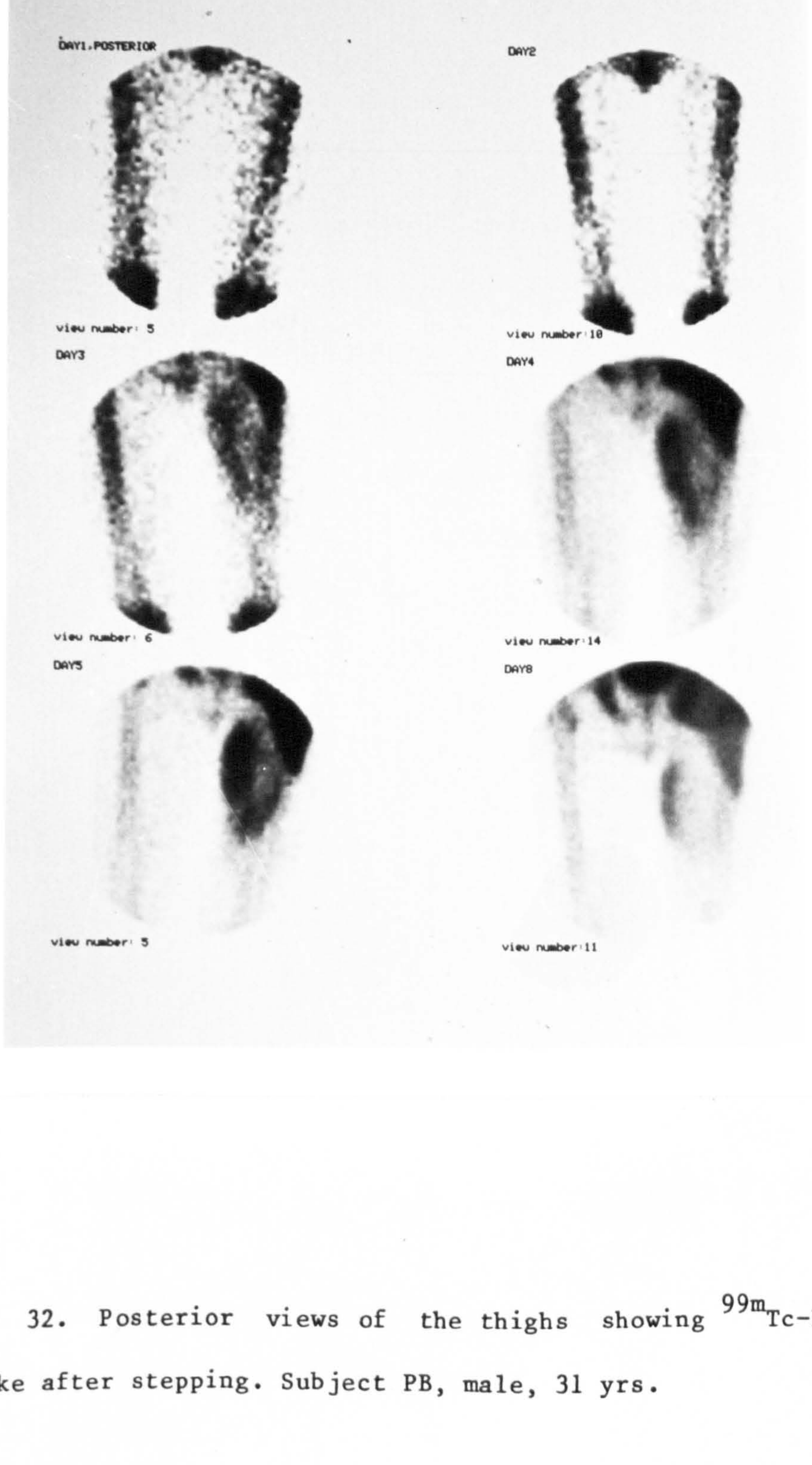

Fig. 32. Posterior views of the thighs showing ^{99m}Tc-PYP uptake after stepping. Subject PB, male, 31 yrs.

the samples taken three days after exercise when only 9% of the fibres appeared normal, focal changes were seen in 2%, extensive in 9% aand very extensive in 80%.

Five days after exercise the proportion of normal looking fibres had increased to 41% while 10% had focal changes, 26% extensive and 23% very extensive changes. By 10 days after exercise 60% of the fibres looked normal, focal changes were seen in 12%, extensive and very extensive changes on 16% and 12% respectively. The combined results for both subjects are shown in Fig. 33. As in other studies, no abnormalities were seen in the muscles which had contracted concentrically, nor in any of the biopsy specimens prepared with histochemical staining techniques.

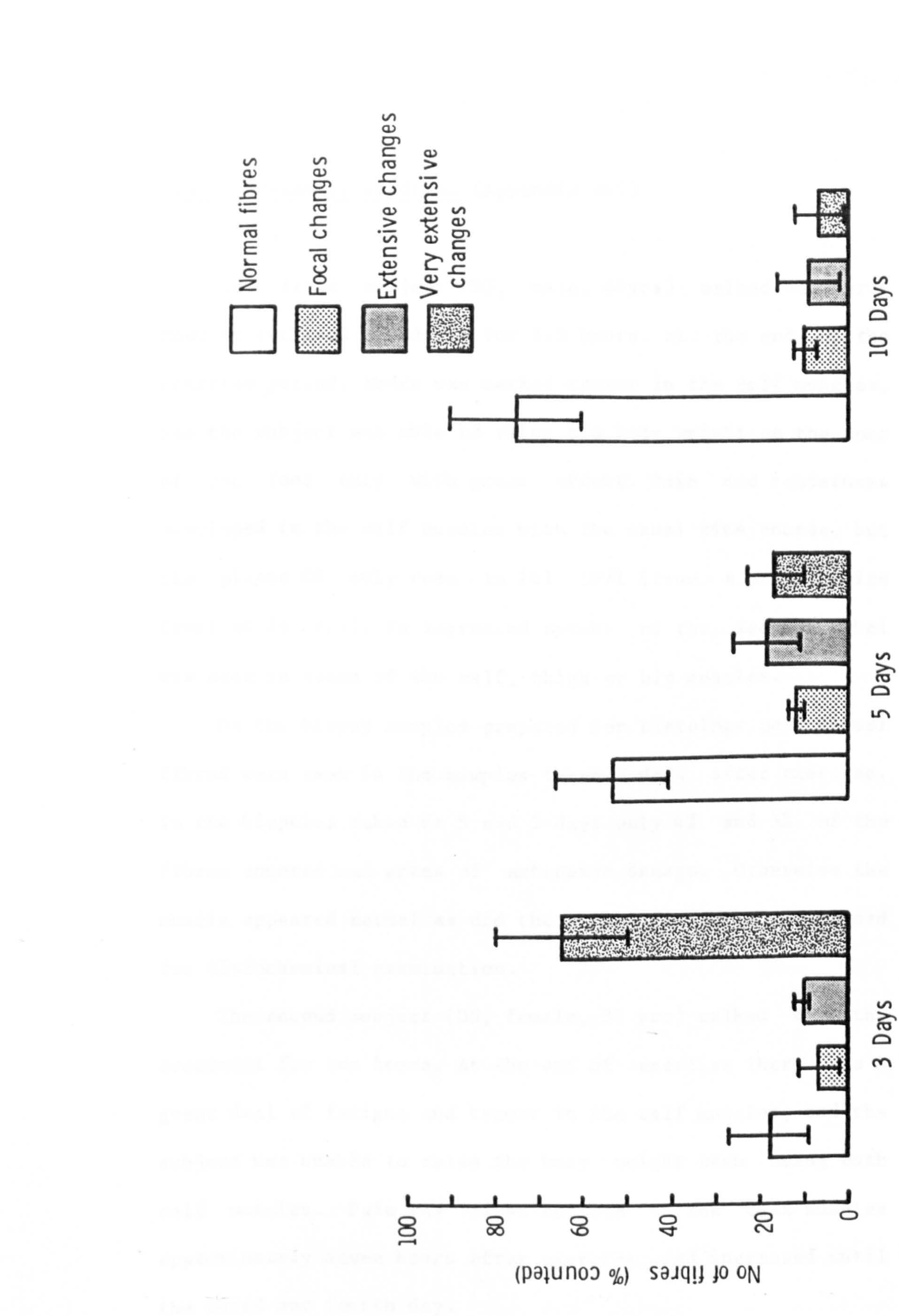

Fig. 33. Quantitation of ultrastructural changes in the quadriceps after eccentric contractions. Mean and range, n=2.

7.3.2. Treadmill studies. (Appendix xvi)

The first subject (DJ, male, 40yrs) walked backwards down an inclined treadmill for 1.5 hours. At the end of the exercise period, there was marked tremor in the calf muscles, and the subject was able to raise his body weight on the toes of one foot only with great effort. Pain and tenderness developed in the calf muscles with the usual time course, but the plasma CK only rose to 141 IU/1 (from a pre-exercise level of 79 IU/1). No increased uptake of the isotope label was seen in scans of the calf, thigh or hip muscles.

On the biopsy samples prepared for histology only normal fibres were seen in the samples taken 3 days after exercise. In the biopsies taken at 5 and 7 days only 4% and 3% of the fibres counted had areas of extensive damage. Otherwise the muscle appeared normal as did the transverse sections stained for histochemical examination.

The second subject (DN, female, 34 yrs) walked down the treadmill for two hours. At the end of exercise there was a great deal of fatigue and tremor in the calf muscles, and the subject was unable to raise the body weight even using both calf muscles. Pain was first noticed in the calf muscles approximately seven hours after exercise, and increased until the third and fourth day.

The plasma CK increased after exercise and the peak values of 77103 IU/1 was measured on the sixth day. Isotope scans were carried out on the third to seventh days

inclusive, and also on the twelth day. Increased uptake was seen in both calf muscles on all occasions. The greatest muscle uptake was on the sixth day after exercise when the muscle:bone uptake was 5.9. Twelve days after exercise the ratio was virtually normal being 0.55 (Figs 34 & 35).

Muscle biopsies were taken from the right calf on the fourth, seventh, twelth and twentieth days after exercise. Histochemical staining techniques revealed minimal changes in the biopsy taken four days after exercise. The fibres were of an even size and the normal chequer board pattern of the fibre types was seen. The fibres were slightly rounded and one area was infiltrated with leucocytes and there was a slight generalised increase in cellularity.

In the samples taken 7 days after exercise there were marked differences in fibre size with rounding of the fibres and increased intercellular spaces. Many cells were were infiltrated with lymphocytes and macrophages. Many macrophages were seen in the intercellular spaces. The type II fibres appeared more severely affected than the type I fibres.

The biopsy taken 14 days after exercise showed a massive infiltration of the intercellular spaces with macrophages. There were many small basophilic fibres, which contained lymphocytes, macrophages and some internal nuclei. These appeared to be type II fibres.

Twenty days after exercise there were still marked differences in fibre size, the smaller cells being basophilic

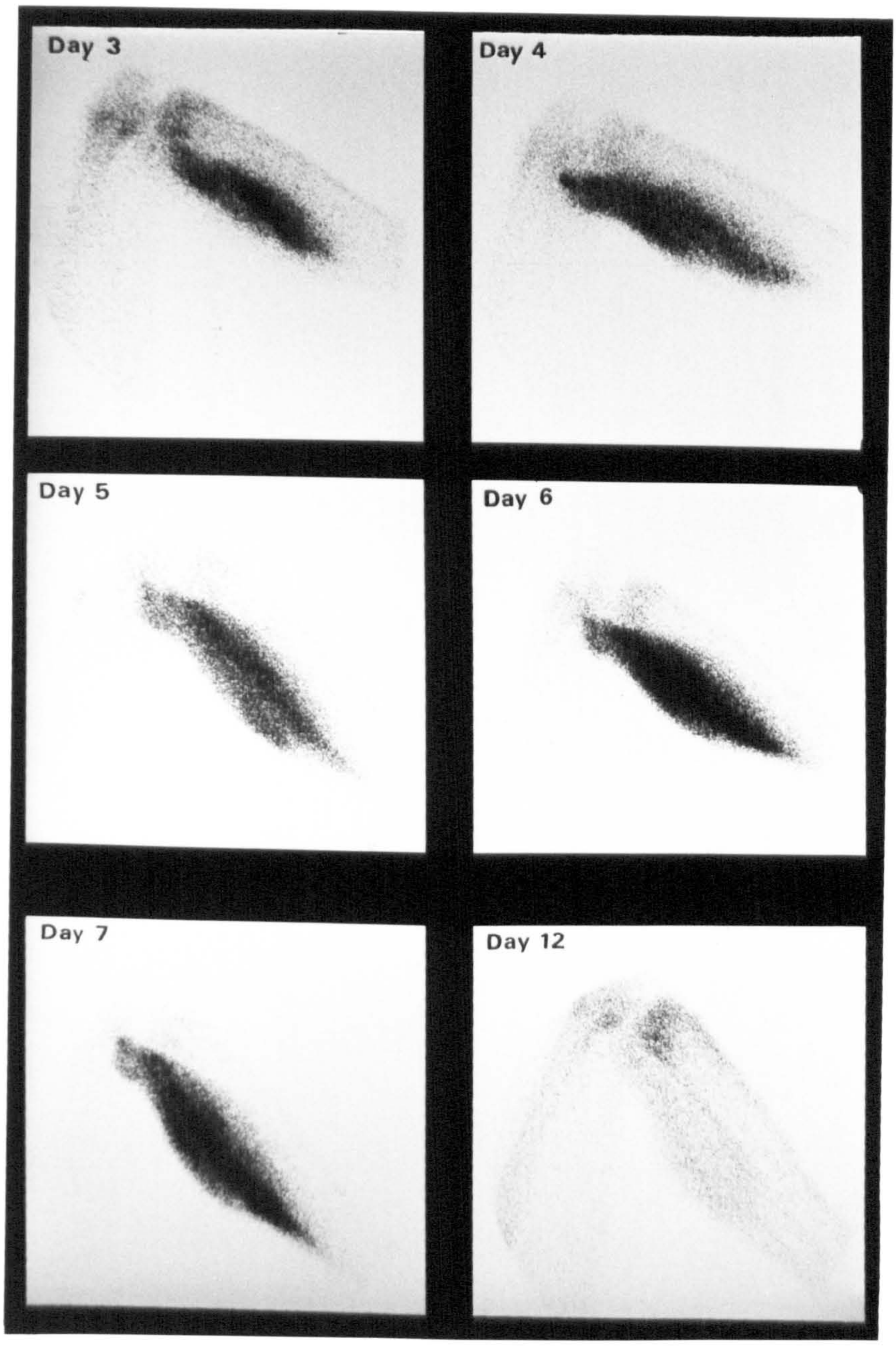

Fig. 34. Lateral views of the right calf showing ^{99m}Tc-PYP uptake after eccentric work. Note increase in area of increased uptake between days 3 & 4. Subject DN, female, 34 yrs.

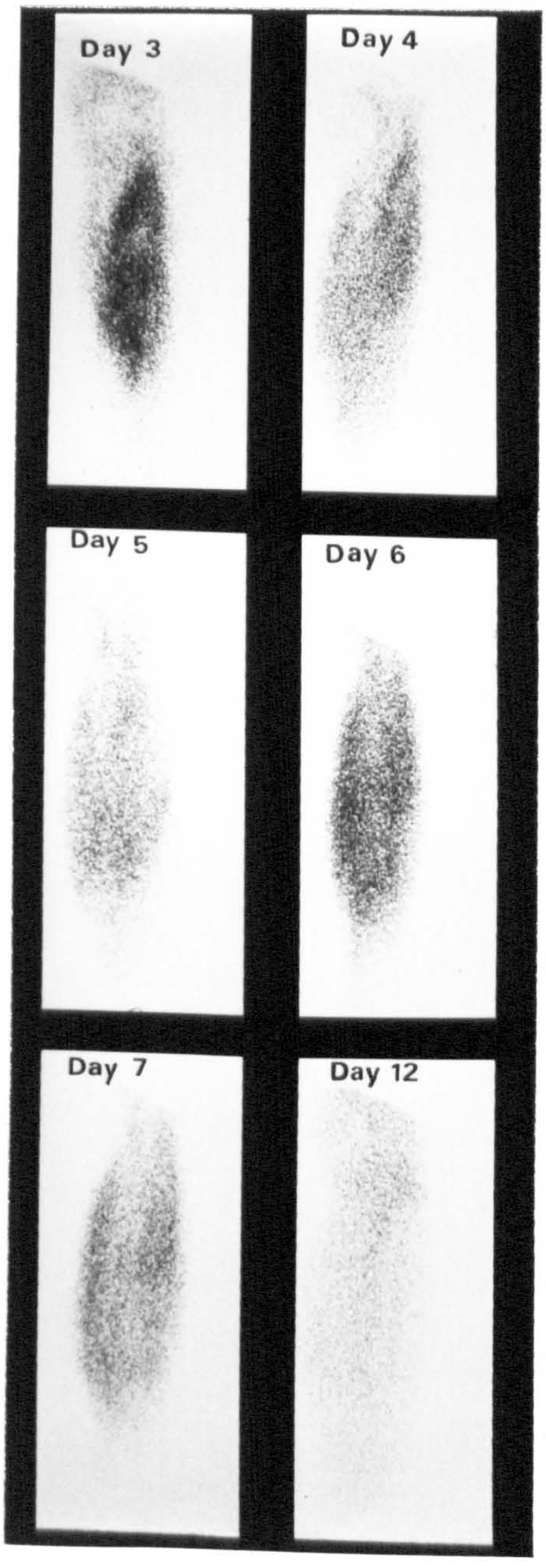

Fig. 35. Posterior views of the right calf showing ^{99m}Tc-PYP uptake after eccentric work. The density of one view cannot be compared with another, but when muscle:bone uptake for each view was calculated the highest ratio was on day 6. Subject DN, female, 34 yrs.

and containing internal nuclei. Increased cellularity and a considerable amount of acid phosphatase activity was seen in the smaller fibres and the interfibre spaces, but was much reduced from the interfibre amount of the previous biopsy. The type II fibres showed considerable recovery in size compared to the previous biopsy, but there was no fibre type grouping or evidence of increased fat or fibrosis.

Typical examples of the appearance of the sections stained with haematoxylin and eosin are shown in Fig. 36 and those stained with ATPase in Fig. 37.

The composite results for this subject are shown in Fig. 38. In agreement with previous studies the pain was maximal before the peak plasma CK and peak muscle uptake of isotope which occurred on the same day. The greatest population of macrophages was not seen until the twelth day after exercise.

Examination of the biopsy samples under an electron microscope showed abnormal Z-lines in virtually every fibre in the samples taken on day 4. Some fibres had lost their thin filaments and the overall picture was one of degeneration. On day 7 most of the fibres had necrotic changes and those that did not had disrupted Z-lines. Degeneration was more marked than in the previous biopsy. The biopsy taken on day 14 showed the marked difference in fibre size that was seen with the histochemical preperations. Disrupted Z-lines were seen in the fibres which were relatively intact. There were numerous macrophages and phagocytosis. The structural damage was worse than in the two

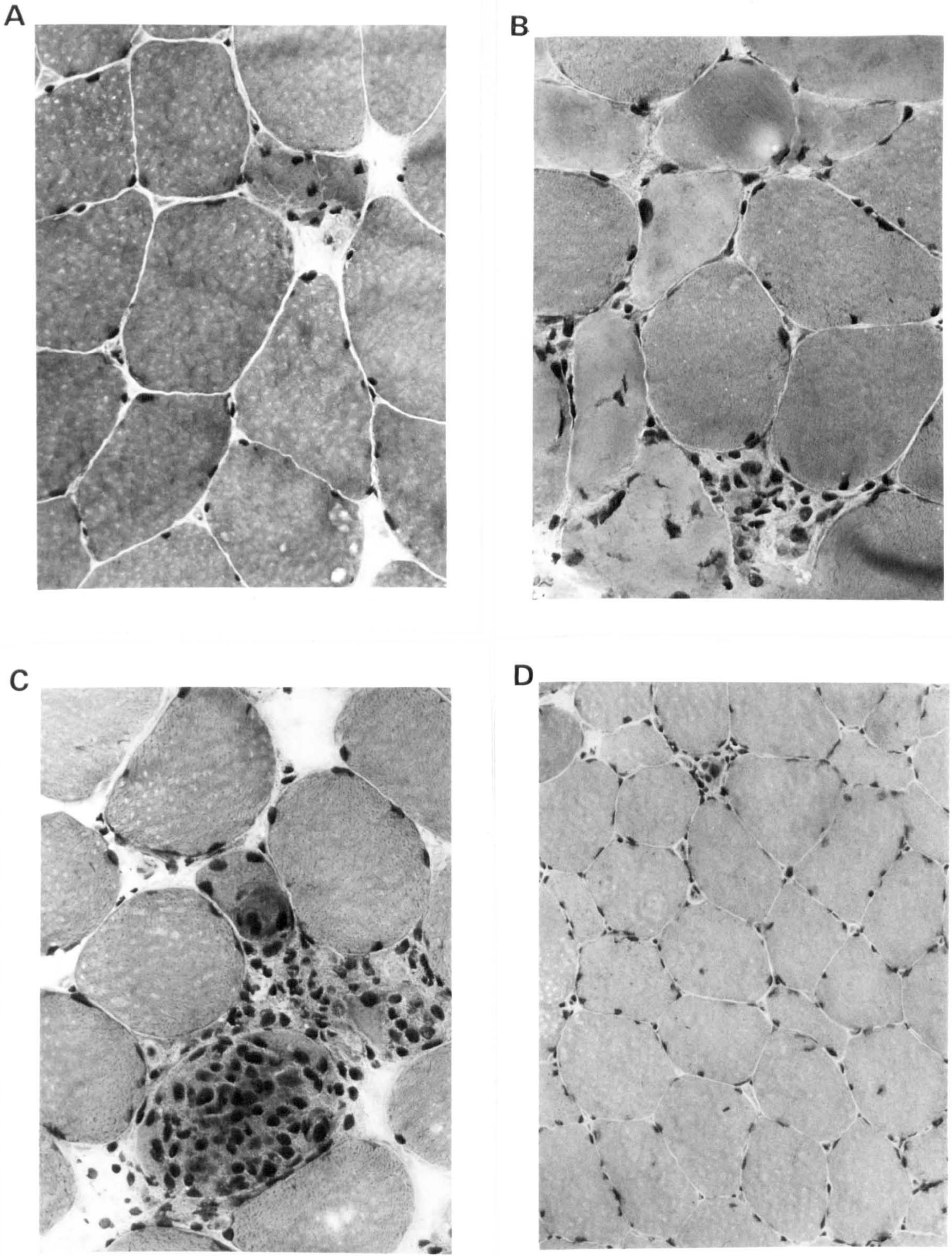

Fig. 36. Transverse sections from biopsy samples of the calf taken a) 4, b) 7, c) 12 and d) 20 days after eccentric exercise. Haematoxylin and eosin stain. Original magnification x 200. Subject DN, female, 34 yrs.

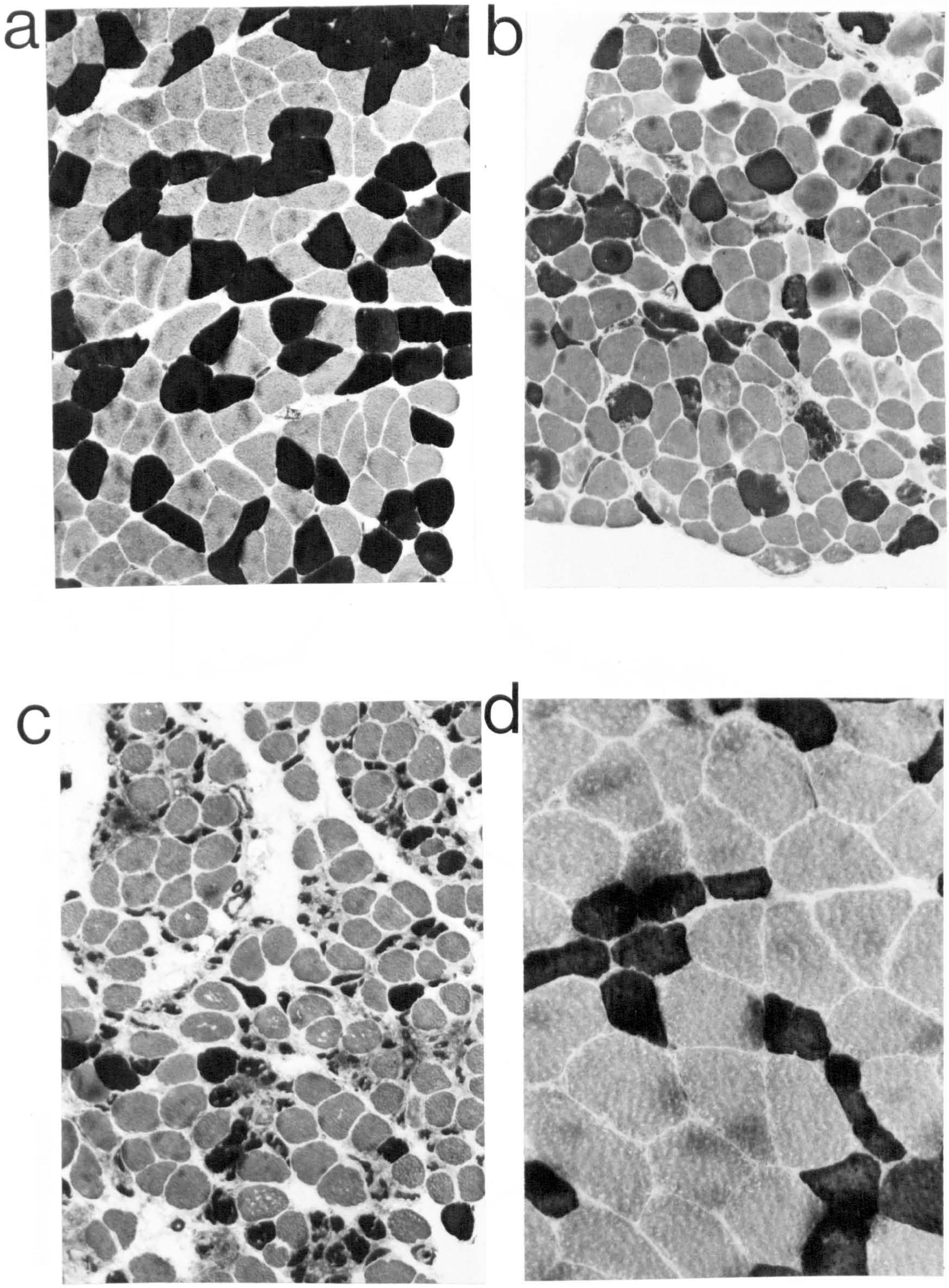

Fig. 37. Transverse sections from biopsy samples taken a) 4, b) 7, c) 12 and d) 20 days after exercise. Note greater size changes in the darker staining fibres (Type II). ATPase, pH 9.4. Original magnification x 100. DN, female, 34 yrs.

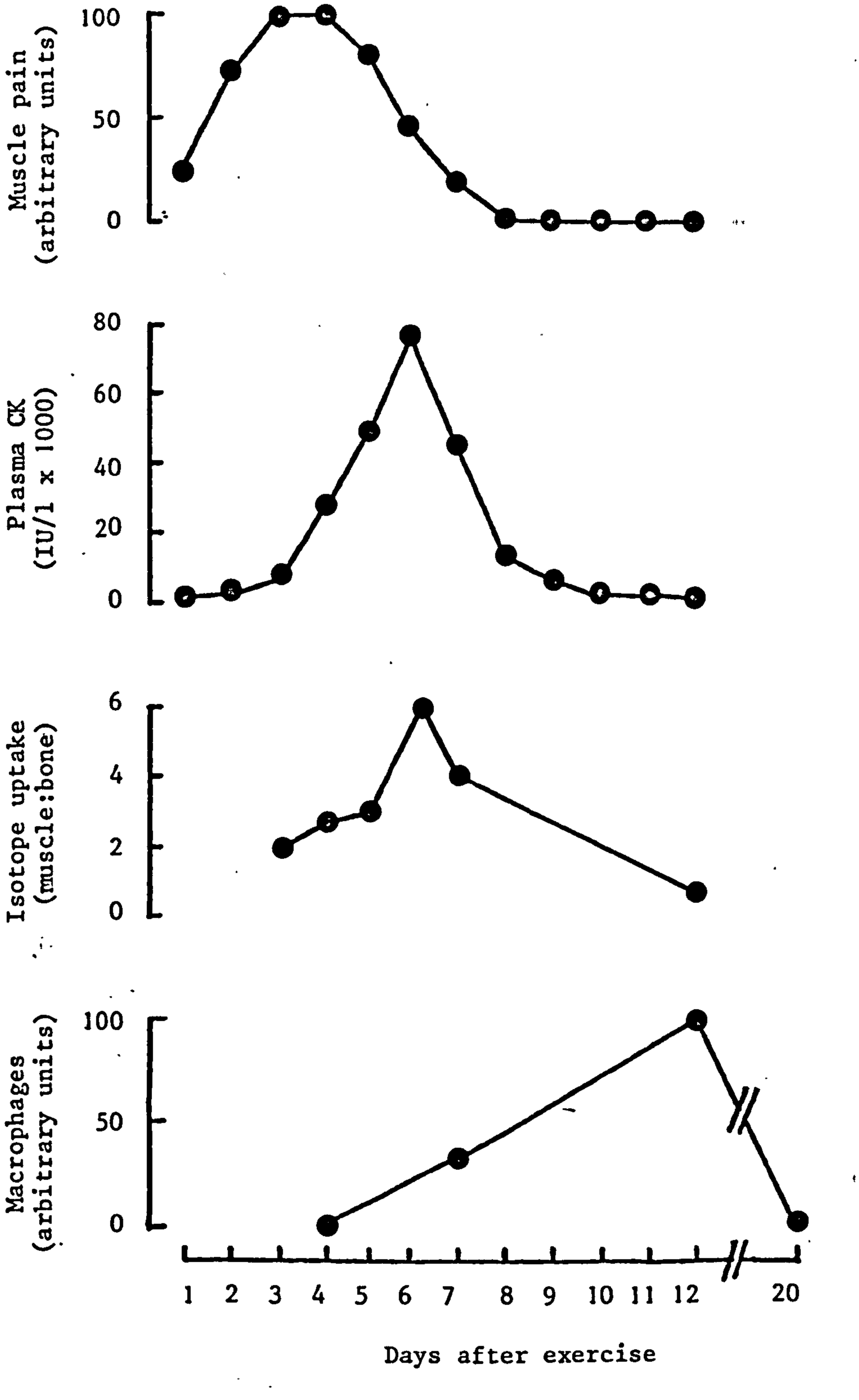

Fig. 38. Time courses of pain, plasma CK, isotope uptake and macrophage infiltration in the calves after eccentric work. DN, female, 34 yrs.

previous biopsies, but with satellite cells and ribosomes the overall picture was one of regeneration. In the last biopsy (day 20) the muscle was almost back to normal, although some Z-lines were disrupted. No macrophages were seen, but internal nuclei and chains of peripheral nuclei, indicating regeneration, were seen.

7.4. DISCUSSION.

These studies fulfilled their original aim and demonstrated both the time course of damage and which muscles were involved. They led to the use of an exercise protocol in which the pain and damage was limited to only one muscle group which it was possible to biopsy.

The stepping studies also provided the rather unexpected result that the quadriceps, which subjectively were as painful as the other affected muscles i.e. ipsilateral buttock muscles and contralateral calves, did not appear damaged on the isotope scans while the other painful muscles did. This strongly suggests that at least a large proportion of the circulating CK originated from muscles other than the quadriceps. It also accounts for lack of damage and absence of macrophages in the histochemically stained biopsy samples from the quadriceps after stepping. This was suprising in view of the fact that many macrophages have been seen in rat muscles damaged by eccentric contractions (Armstrong et al 1983), and one would certainly expect any significant damage to be followed by an invasion of macrophages. It would seem that in this study, as well as that reported in the previous chapter, that the lack of any changes seen with routine histochemical stains of biopsy samples - which was difficult to correlate with the very high plasma CK levels, was due to the fact that the muscles from which the biopsies were taken were not the most damaged ones.

Despite the hip and adductor muscles being painful the tenderness measuring technique was not sucessful. This is presumably because the hip girdle muscles are deep to other muscles and the adductors are covered by a relatively thick layer of subcutaneous fat, even in slender people. Therefore the tenderness mapping technique would appear to be applicable only to superficial muscles.

The treadmill studies had the desired effect of limiting pain and damage to one muscle group - the calves, which have the advantage that they may be biopsied. The first subject experienced pain and tendernes, but did not have the large delayed CK efflux nor an increased uptake of isotope. Neither was any real damage seen on biopsy samples examined with both light and electron microscopes. This provides further evidence that the pain and tenderness are not caused by myofibrillar damage and that they do not share a common mechanism with that which causes the enzyme efflux.

The second subject had marked pain and demonstrated evidence of damage on the isotope scans and this was only in the calf muscles which demonstrated that only the calf muscles were affected. It may reasonably be assumed that the plasma CK originated from these muscles. The time course of the ultrastructural damage was again different to that of the CK efflux, and so the enzyme efflux cannot have been caused by myofibrillar damage. However the peak plasma CK always occurred on the same day as the greatest muscle uptake of isotope. This would suggest that in this case membrane damage

is responsible for both the efflux of enzyme and influx of isotope. Furthermore the long time taken to reach peak CK levels does actually seem to be a true indication of the time course of damage (to the membrane) and not caused by slow clearance from either the muscle or circulation as previously speculated.

Macrophages were seen in the samples taken 7 days after exercise, where the picture was one of degeneration. Many more were seen in the next biopsy and also signs of regeneration were present. Therefore most macrophages were present at a time when the plasma CK was falling and so cannot be contributing significantly to the enzyme leakage.

Ultrastructurally the Z-lines appeared disrupted on most of the fibres in the first biopsy. In the samples from the second biopsy these changes were seen more in the larger, less affected fibres. This raises the possibility that the Z-line changes may be of a regenerative nature rather than reflecting degeneration as previously assumed.

The type II fibres appeared to be the most affected, as reported by Friden (1983). Using a stain for glycogen he also found that there was no indication of preferential type II fibre recruitment. He proposed that these fibres had the narrower and therefore weaker Z-lines and so were more susceptible to mechanical damage. It is known that animal skeletal muscle shows such differences in Z-line thickness between fibre types and it also appears that they are found in human muscle (Payne et al 1975, Prince et al 1981,

Sjostrom et al 1982).

The isotope scans provided useful information about the distribution of damage in the affected muscles. In the earlier scans increased uptake was seen in the bellies of the muscles and later extended towards the attachments. Both soleus and gastrocnemius appeared to be equally affected.

While the similar time courses for enzyme efflux and isotope uptake clearly indicate membrane permeability to relatively large molecules, a gamma count or camera scan does not indicate whether the label is located within the muscle fibre, the inter-fibre space or invading cells. This was investigated by Vita & Harris (1981) who identified labelled material in the free cells (mainly invading phagocytic cells), supernatant - consisting of both interstitial fluid and cytosol and the mitochondria of damaged muscle. Therefore bothoedema fluid and invading cells, as well as the muscle fibres themselves, can take up labelled material. This may well account for the initially suprising results of Kula et al (1976), later confirmed by Messina et al (1978) and Bellina et al (1978). They found that patients with Duchenne muscular dystrophy had high circulating CK levels but accumulated virtually no soft tissue isotope. Conversely patients with polymyositis with low circulating CK levels had high muscle uptake of isotope. The mechanism by which labelled material is taken up by muscle and soft tissue is not known, but appears to be more marked in inflammatory than non-inflammatory myopathic conditions. However in the

treadmill study macrophages and the increased area of interfibre space was most marked on the biopsy samples taken twenty days after exercise, but the soft tissue isotope uptake was greatest on the sixth day when there was less evidence of inflammation.

There is no direct evidence for any ultrastructural recovery while the plasma CK is increasing, but immediately after exercise considerable ultrastructural damage has repeatedly been seen while there is no real change in the plasma CK. Therefore ultrastructural damage does not appear to be synonymous with enzyme efflux, and appears to take place without the membrane necessarily being damaged. It would seem likely that a critical amount of myofibrillar damage can occur and be repaired without the membrane being damaged. However once this critical level of damage is exceeded the cell, including the membrane looses its integrity and breaks down. This causes the CK efflux and invasion of macrophages to ingest the disrupted cell.

However it is not clear what muscle specific agents do activate macrophages. If this was known then more could be learned about primary muscle diseases such as polymyositis. Neither is it known what acts as the algesic stimulus, which clearly preceeds all the indications of damage which have been studied in this work. In addition to the lack of any obvious correlation between pain and damage, muscle fatigue does not have any obvious bearing on pain and tenderness. Impairments of force generation are greatest before the onset

of pain and have largely recovered at the time of maximal discomfort.

Despite these unresolved questions, this study explained some apparent inconsistencies that had been revealed in the previous studies. When the muscles which are biopsied are those which are severely damaged invading marophages are seen and the time course of the enzyme efflux is the same as that of damage and inflammation as indicated by the uptake of isotope. Very different amounts of damage are found in muscles which subjectively seem to be equally painful. In this context it is notable that in patients with polymyositis who have similar morphological changes and circulating levels of CK, some report marked muscle pain and tenderness while others experience little or no discomfort.

CHAPTER 8. GENERAL DISCUSSION AND CONCLUSIONS.

Page No

CHAPTER 8. GENERAL DISCUSSION AND CONCLUSIONS.

In this work the nature and cause of delayed onset muscle pain and damage has been studied and it has been shown that they are associated with eccentric rather than concentric contractions. The different aspects of the work and the way in which it has evolved are discussed in each chapter. There have been some unexpected and possibly confusing turns in the story such as the experiments described in Chapter 7, which showed that the most severely damaged muscle after stepping was not the quadriceps as had been thought, but an adductor and hip girdle muscle. This general discussion will attempt to draw together the data gathered from a number of muscles which have undergone varying degrees of damage. In doing so attention will be drawn to the gaps in the evidence as well as to the opportunities that exist for further work.

8.1. Muscle pain and tenderness.

Pain, measured as tenderness in this work, has always accompanied the damage caused by eccentric contractions. However there are indications that the underlying mechanisms leading to damage and to pain may differ.

Evidence of muscle damage has ranged from the very mild - (with no or only the small and rapid CK release and no ultrastructural changes, to the very severe - with

destruction of half the muscle fibres, yet the pain experienced has varied far less. Even when there has been little evidence of damage (e.g. subject DJ, Chapter 7) the pain was considerable. So it would seem that whatever is the stimulus for severe muscle damage, it is not necessary for tenderness to develop. There is also a suggestion from the training studies that damage and pain may change with slightly different time courses (Chapter 6).

The nature of the algesic agent remains unknown. All that can be said with any confidence is that it is unlikely to be the various metabolites tht might be liberated by working muscle such as Pi, ADP, K etc., since the concentric work does not have any long lasting effects.

The time course of the pain is particularly interesting, being much faster than the major damage to the muscle that results in enzyme release. It does have some similarity to the ultrastructural damage seen on electron microscopy, but there is little evidence to suggest that this is other than coincidental.

It was consistantly reported by the subjects that the painful muscles felt swollen and 'stiff'. This may be related to increases in intramuscular pressure, and it would be worthwhile to mesure these directly. If the pain is related to changes in pressure, then the nociceptors may be either in the muscle itself or in the fascia which is reputed to have a greater density of sensory nerve endings than muscle (Stacey 1969).

The cause of pain may also be examined by intervention studies using specific antagonistic agents such prostaglandin inhibitors and anti-inflammatory agents.

The technique used to measure tenderness appeared to give an accurate represention of both the time course and distribution of pain. However it seems to be applicable to only superficial muscles. This technique has clinical applications as changes in pain are often a useful indication of the state of injury and disease which at present are not quantified.

8.2. Force generation.

The stepping studies gave conflicting results of the effects of the damage on muscle voluntary force generation of the quadriceps (Chapters 2 and 3). However the isotope scanning technique showed that the quadriceps are not the most damaged muscles after stepping. When the eccentric contractions were mainly limited to the elbow flexors there was a considerable loss of voluntary force during the exercise period, and also severely impaired force generation was apparent after eccentric contractions of the calf muscles.

The time course of recovery of MVC force and low frequency fatigue are still uncertain. The quadriceps were the only muscles in which they have been studied in this work, and it has subsequently been shown that they are not

the most appropiate muscle to study. Certainly the biopsy data from the calf muscles after downhill treadmill walking indicates considerable impairments of force generation twelve days after exercise as the type II fibres were dramatically reduced in size. It remains to be seen whether the force generation changes follow the morphological changes.

Other factors which may well affect force generation are the muscle pain itself.or agents in the muscle which may cause reflex inhibition, also the length of the muscle during the force measurements. It was noted throughout that eccentric contractions were particularly uncomfortable. Furthermore the length of the muscle seemed to affect both the discomfort experienced during contractions, and the functional impairments. These factors warrant further investigation.

8.3. Enzyme efflux.

A relatively small and rapid enzyme efflux is well documented in normal subjects after exercise. In this work it was found that eccentric contractions are associated with a very large and delayed enzyme efflux in some individuals and this reached pathological levels. The delay itself is apparently not due to diffusion of the enzyme from the tissue nor to inactivation rates and removal from the circulation. This is shown by the results reported here and also by unpublished work of my own that shows that after major

orthopaedic surgery and needle muscle biopsy the peak levels of circulating muscle enzymes occur twenty-four hours after the event.

Plasma CK was greatest on the same day as the greatest muscle uptake of isotope. Both the enzyme efflux and isotope uptake indicate damaged muscle membranes. Earlier in this work it was speculated that macrophages, which are reported to be found in similarly damaged muscle (Armstrong et al 1983), might be responsible for the membrane damage - by being stimulated to attack muscle fibres and clear up damage. However in the final study it was seen that this was not the case as peak plasma CK levels occurred days before the greatest population of macrophages in the muscle.

8.4. Morphological and ultrastructural changes.

In the quadriceps studies there were discrepancies between the incidence of ultrastructural changes and damage as indicated by the plasma CK release - one of the four subjects had the large delayed CK rise but all had similar ultrastructural changes. The isotope scans largely resolved this anomaly by showing that the most damaged muscles after stepping i.e. presumably those releasing the CK, were infact not the quadriceps as first thought.

From the quantitation of the ultrastructural damage in the quadriceps it seems that the changes (sarcomere disruption and Z-line streaming) are greatest three days

after eccentric exercise. It could be that in the most severely damaged muscles (the calves after downhill treadmill walking and the adductors and hip muscles after stepping, as opposed to the quadriceps) the ultrastructural damage does not recover, but contines to progress leading to cell death after about five days and the large delayed CK rise. However, preliminary examination of the calf muscle biopsies, which were severely damaged, as evidenced by the CK release and isotope uptake, suggests that this may not be the case and the ultrastructural changes as described in the quadriceps represent a regeneration and repair phase.

8.5. Time course of events.

Immediately after eccentric work the only obvious changes are the impairments of both voluntary and stimulated force generation. In the quadriceps, which are not the most severely damaged muscles after stepping, but do show considerable ultrastructural changes and are painful, the low frequency fatigue had largely recovered forty-eight hours later. In the last experiment where the calves were damaged, force was not measured but the biopsy data indicates that there must be considerable force loss twelve days after exercise as the type II fibres were very atrophied and the loss of contractile material could have been up to 50%. Whether force generation made further recovery by the third day - when the muscle fibres seemed fairly normal, and then

subsequently declined with the size of the type II fibres is not known. The study of the longer term changes in force generation would be interesting and useful.

The pain is maximal between twenty-four and forty-eight hours after exercise. Depending on whether the individual has the small rapid or the large delayed CK response, peak CK values are found either before or after the time of maximal pain and tenderness. Ultrastructural changes are present immediately after exercise, when pain is not, and are greatest approximately three days after exercise - after maximal pain. They are similar in individuals who show different CK responses and experience different amounts of discomfort. Thus it is very unlikely that the ultrastructural changes are the algesic stimulus.

In those subjects with the large delayed CK response, muscle enzyme efflux peaks at the same time as the greatest muscle uptake of isotope, and both imply damaged membranes. It was previously speculated that macrophage invasion (to clear up the damaged cells) might be responsible for the time course of the efflux, but this cannot be so as the greatest numbers of macrophages were seen after peak plasma CK levels and muscle isotope uptake. It seems that the role of the macrophages is to clear up already damaged tissue prior to, and perhaps simultaneously with, regeneration.

The time courses are schematically shown in Fig. 39., where the differences between the information accquired from results and speculation are indentified.

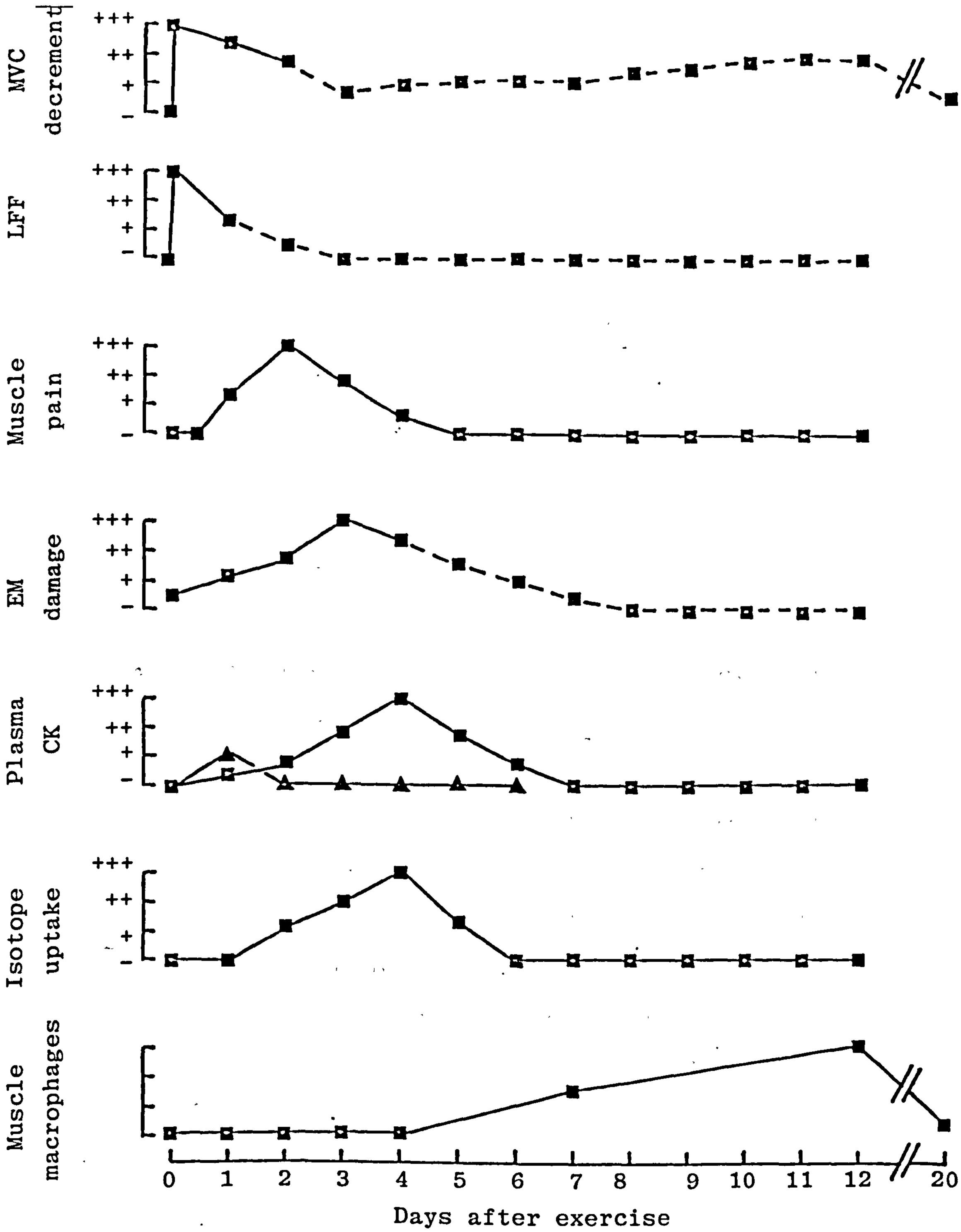

Fig. 39. The time courses of pain and damage after eccecntric exercise. Subjects had either the small rapid CK response or the large delayed one which was accompanied by increased muscle isotope uptake. Where the time course is speculative dashed lines are used.

8.6. Factors affecting pain and damage.

It was striking that training with eccentric contractions gave a very rapid and long lasting protection aginst pain and CK release. The effects were specific to the trained muscle and occurred without any changes in either the absolute strength of the muscle or the fatigue caused by the training regime (Chapter 5).

In view of the specificity of the training effects, humoral factors can be eliminated as causing the adaptation. The nature of the adaptation remains unknown, but there are a number of possible mechanisms whose relevence could be tested.

High tensions are generated by stretching active muscle fibres. In performing eccentric contractions the muscle may adapt by one of two ways when working at submaximal forces. The first is to use few motor units to generate the required force or secondly to use a greater number of motor units. In the first instance the force generated by individual fibres will be higher. It has been suggested that the high tension per active fibre (first option) is responsible for causing damage. One effect of training could therefore be to change the pattern of motor unit recruitment to the second option so that the tension per active fibre is less, and so less likely to cause damage.

If, as has been suggested (Friden 1983) the initial injury is to the Z-lines, they may be strengthened during

training and made better able to withstand the stresses imposed upon them. Any such changes could be detected by measuring the width of the Z-lines seen by electron microscopy.

It could be that the connective tissue is strengthened in response to the training stimulus. The amount of connective tissue breakdown could be determined by measuring the excretion of Hydroxyproline in urine as this has been shown to be a specific breakdown product of connective tissue (Kivirikko 1970). Measurements of the resistance to passive stretch may also give an indication of connective tissue changes.

8.7. Conclusions.

It has been shown that eccentric contractions cause major changes - pain and damage in human skeletal muscle. In normal healthy subjects these changes are of a transitory nature and respond rapidly to training and thus do not seem to have any long lasting effects.

There is evidence that high forces and a degree of stretch are potent stimuli for muscle growth and hypertrophy, and as such the damage caused by eccentric work may be such a stimulus. Conversely, the damage caused by eccentric contractions in normal subjects has often been comparable to that seen in patients with muscle diseases and so might be harmful, particularly when applied to those with an existing

disease and/or weakness.

To find the balance between too much and too little damage would be a most valuable contribution both for athletic training purposes and also clinically for patients undergoing rehabilitation programmes.

Appendix i. MVC force in the quadriceps of 4 normal subjects after stepping on a 46cm step.

The muscles had contracted either concentrically (con) or eccentrically (ecc) in the exercise period.

MVC force is expressed as a percentage of the pre exercise values in each individual.

	Time after exercise											
	2 min		10 min		1 hr		5 hr		24 hr		48 hr	
Subject	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc
KM i)	115.3	103.9	86.2	89.9	115.5	95.1	98.1	83.3	103.4	98.3	112.0	98.7
ii)	84.0	90.3	87.4	90.5	83.3	81.3	104.6	84.5	102.3	85.4	94.7	103.2
BQ i)	101.3	77.1	104.5	93.7	99.6	85.2	100.6	81.5	92.1	73.5	111.4	90.3
ii)	95.3	90.7	84.2	88.1	78.6	85.8	81.8	85.3	89.9	92.4	85.1	93.7
DN i)	107.3	86.8	92.8	83.5	102.6	94.0	99.1	90.8	104.9	99.4	119.0	109.8
ii)	97.2	77.8	96.1	75.9	95.5	76.1	86.2	70.9	98.0	74.8	89.4	77.6
DJ	95.1	76.3	90.3	67.3	76.2	57.3	-	-	-	-	-	-
n	7	7	7	7	7	7	6	6	6	6	6	6
Mean	99.2	86.1	92.1	82.7	93.1	82.1	95.1	82.7	98.4	87.3	101.9	95.5
SD	10.1	10.0	7.1	8.4	14.3	12.8	9.0	6.6	6.2	11.3	14.0	11.2
SEM	3.8	3.8	2.7	3.2	5.4	4.8	3.6	2.7	2.5	4.6	5.7	4.6
Difference between Con & Ecc	$p<0.05$		$p<0.05$		$p<0.05$		$p<0.02$		NS		NS	

Appendix ii. Frequency:force relationship in the quadriceps in 4 normal subjects after stepping on a 46cm step.

The muscles had contracted either concentrically (Con) or eccentrically (ecc) during the exercise period. They were percutaneously stimulated at 10 & 50 Hz. The force generated by 10Hz has been expressed as a percentage of the force generated at 50 Hz (10/50%).
The 10/50% after exercise is expressed as a percentage of the pre exercise values.

		Time after exercise											
		2 min		10 min		1 hr		5 hr		24 hr		48 hr	
Subject		Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc
KM	i)	68.6	41.0	67.1	48.0	84.4	51.0	87.3	59.8	98.8	92.8	99.1.	87.5
	ii)	75.0	58.1	66.8	61.5	65.8	39.0	57.3	51.0	76.8	82.0	75.6	74.1
BQ	i)	80.1	36.1	57.4	29.2	78.9	31.2	77.3	27.9	78.0	40.3	109.2	63.3
	ii)	50.1	64.6	55.5	55.9	79.0	70.4	84.2	64.8	86.5	83.9	84.4	80.6
DN	i)	62.5	23.5	65.9	21.3	60.6	28.6	78.6	44.8	98.9	75.9	91.2	85.2
	ii)	39.2	14.6	30.5	14.0	65.1	24.6	64.8	40.2	67.9	69.9	70.1	65.5
DJ	i)	65.9	22.7	76.8	26.1	67.8	24.7	-	-	-	-	-	-
n		7	7	7	7	7	7	6	6	6	6	6	6
Mean		49.0	37.2	60.0	36.6	71.7	49.0	74.9	48.1	84.5	74.1	88.3	76.0
SD		14.2	18.7	14.8	18.4	9.0	16.9	11.6	13.4	12.6	18.3	14.6	10.1
SEM		5.4	7.1	5.6	7.0	3.4	6.4	4.7	5.5	5.1	7.5	6.0	4.1
Difference between Con & Ecc		NS		$p<0.02$		$p<0.01$		$p<0.01$		NS		NS	

Appendix iii.

EMG activity in the quadriceps during a 20 minute step test.

The muscles worked either cconcentrically (con) or eccentrically (ecc). Recordings were made from three consecutive steps. The area of the integrated EMG (IEMG) signal was measured by plenimetry. Subject BQ.

VL=vastus lateralis, RF=rectus femoris, VM=vastus medialis.

IEMG areas (mm^2) during one stepping phase.

		Concentric			Eccentric		
Time		VL	RF	VM	VL	RF	VM
0	i)	153.7	168.9	182.8	136.6	113.0	109.6
	ii)	171.7	192.7	175.3	134.9	120.5	116.2
	iii)	141.2	173.9	203.6	132.7	125.5	116.8
mean		155.5	178.5	187.2	134.7	119.7	114.2
5 min	i)	141.8	199.3	207.0	132.5	135.8	130.0
	ii)	126.3	152.9	195.1	149.3	123.9	120.5
	iii)	134.8	168.7	185.3	126.0	134.7	132.8
	mean	134.3	173.6	195.8	135.0	131.5	127.7
10 min	i)	142.8	209.2	251.0	139.0	162.8	131.7
	ii)	134.5	198.3	190.1	136.2	169.4	140.0
	iii)	149.5	218.3	220.2	130.3	146.9	144.9
	mean	142.3	208.6	220.4	135.1	159.7	138.9
15 min	i)	207.0	197.5	207.0	145.8	158.1	166.3
	ii)	210.5	190.3	210.5	167.8	176.9	176.8
	iii)	202.1	159.0	202.3	151.9	184.4	145.3
	mean	206.5	182.3	206.6	155.2	173.1	162.8
20 min	i)	153.3	220.8	207.5	137.8	172.5	137.5
	ii)	127.3	198.5	194.8	170.5	187.7	159.0
	iii)	137.8	188.5	231.4	156.5	199.8	157.8
	mean	139.5	202.6	211.2	154.9	186.7	151.4

Mean IEMG area (% of value at start of exs.

5 min	86.4	97.2	104.6	100.2	109.9	111.8
10 min	91.5	116.9	117.7	100.3	133.4	121.6
15 min	132.9	102.1	110.4	115.2	144.6	142.6
20 min	89.7	113.5	122.8	115.0	156.0	132.6

Appendix iv.
EMG activity inthe quadriceps during a submaximal knee extension after a 20 min step test.

Knee extension was maintained for 2 sec.
During the step test the quadriceps had contracted either concentrically or eccentrically. IEMG area was determined as in appendix iii. Subject: BQ.

VL=vastus lateralis, RF=rectus femoris, VM=vastus medialis

		EMG areas (mm^2)					
		Concentric			Eccentric		
Time		VL	RF	VM	VL	RF	VM
Pre exs	i)	189.1	183.5	225.7	233.2	128.2	203.4
	ii)	155.4	168.7	219.2	259.2	136.2	250.8
	iii)	184.6	193.1	257.2	264.8	177.5	241.4
	mean	176.4	181.8	234.0	252.4	147.3	231.9
15 min	i)	199.1	175.0	238.5	329.5	277.7	418.5
post exs	ii)	191.7	173.2	239.6	398.7	404.2	495.4
	iii)	204.9	188.3	233.4	395.7	430.2	488.4
	mean	198.6	178.8	237.2	374.6	370.7	467.4
30 min	i)	232.6	189.0	286.0	333.0	332.7	413.8
post exs	ii)	218.9	170.3	271.4	369.3	395.5	495.2
	iii)	194.1	181.5	256.0	357.6	383.5	457.3
	mean	215.2	180.3	271.1	353.3	370.6	455.4
60 min	i)	209.0	204.7	309.2	337.0	398.4	453.6
post exs	ii)	203.1	231.1	302.3	388.4	444.3	471.1
	iii)	176.0	204.4	279.4	341.1	422.7	433.0
	mean	196.0	213.4	297.0	355.5	421.8	452.6
90 min	i)	177.3	243.1	205.1	333.6	299.2	417.2
post exs	ii)	167.5	223.2	195.3	340.5	325.2	428.1
	iii)	157.9	201.1	187.6	362.5	340.5	487.5
	mean	167.6	222.5	196.0	345.5	321.6	444.3
2 hr	i)	186.5	200.0	278.2	343.6	305.2	403.1
post exs	ii)	203.7	201.8	272.2	331.1	343.2	471.2
	iii)	183.7	227.7	301.4	317.3	295.0	395.8
	mean	191.3	209.8	283.9	330.7	314.5	423.4
3 hr	i)	196.6	207.6	234.9	262.7	193.5	222.7
post exs	ii)	172.6	180.2	195.4	272.0	224.5	315.7
	iii)	175.4	169.3	206.8	326.5	313.0	348.5
	mean	181.5	185.7	212.4	287.1	243.7	295.6
4 hr	i)	195.6	190.5	217.7	304.2	250.0	343.4
post exs	ii)	193.4	179.1	229.5	342.1	257.7	360.7
	iii)	185.8	174.4	223.6	332.2	277.5	389.8
	mean	191.6	181.3	223.6	326.2	261.7	364.6

Time		VL	RF	VM	VL	RF	VM
6 hr	i)	199.3	201.2	229.9	263.0	154.7	266.6
post exs	ii)	171.8	186.5	226.6	285.0	225.5	317.1
	iii)	164.6	187.3	217.3	264.5	201.2	217.3
	mean	178.6	191.7	224.6	270.8	193.8	285.0
24 hr	i)	155.8	165.4	226.2	256.4	187.6	252.0
post exs	ii)	146.6	192.9	253.5	229.0	160.2	219.5
	iii)	143.3	158.2	210.3	266.2	187.0	243.0
	mean	148.6	172.2	230.0	250.5	178.2	238.2

Time	Mean IEMG area (% pre exs)					
15 min	112.6	98.3	101.4	148.1	251.7	201.5
30 min	122.0	99.2	115.8	139.9	251.6	196.4
60 min	111.1	117.4	126.9	140.8	286.3	195.2
90 min	95.0	122.4	85.8	136.9	218.3	191.6
2 hr	108.4	115.4	121.3	131.0	213.5	182.6
3 hr	102.9	102.1	90.8	113.7	165.4	127.5
4 hr	108.6	99.7	95.6	129.2	177.7	157.2
6 hr	101.2	105.4	96.0	107.3	131.6	122.9
24 hr	84.2	94.7	98.3	99.2	120.9	102.7

Appendix v. Quadriceps MVC before and after a standardised 20 min step test in 10 normal subjects.

The quadriceps had worked either concentrically (con) or eccentrically (ecc).

	MVC (N) (pre exs)		MVC after exs (% pre exs)									
			10 min		4 hr		24 hr		30 hr		48 hr	
Subject	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc
RS	587.7	549.1	102.2	85.9	89.1	84.4	92.7	100.0	102.1	107.8	103.7	110.9
MR	405.9	381.3	90.9	87.1	78.8	85.0	90.9	71.1	-	-	84.7	54.8
RH	418.0	406.0	55.9	50.0	88.2	87.9	85.4	90.9	-	-	67.7	69.7
TD	541.2	510.5	61.3	72.3	90.9	84.2	79.4	71.1	-	-	81.8	79.5
CG	362.5	313.2	83.4	87.7	93.5	82.2	98.2	87.0	98.2	87.0	91.1	84.2
LM	738.0	689.0	80.0	83.9	66.7	82.1	82.5	83.4	-	-	76.7	71.4
BS	719.5	615.0	64.9	88.0	40.4	102.0	88.2	91.0	-	72.0	70.1	76.0
MT	615.0	590.0	85.5	79.1	88.0	79.1	80.0	60.5	92.0	81.3	80.0	72.9
JA	344.4	307.5	92.9	84.0	101.8	98.1	92.9	104.0	98.2	94.0	94.7	90.0
DD	514.8	471.9	80.8	70.0	75.8	70.0	76.7	70.0	76.7	61.8	75.0	70.9
n	10	10	10	10	10	10	10	10	5	6	10	10
mean			79.8	78.8	81.3	85.5	86.5	83.4	93.4	84.0	82.5	78.0
SD			14.8	11.9	17.4	9.1	7.4	14.4	10.1	16.2	11.3	14.9
SEM			4.7	3.8	5.5	2.9	2.5	4.5	4.5	6.6	3.6	4.7
Difference between Con & Ecc			NS		NS		NS		NS		NS	

NS=p>0.05

Appendix vi. Stimulated force by the quadriceps before and after a standardised 20 min step test in 10 normal subjects.

The quadriceps had contracted either concentrically (con) or eccentrically (ecc).
The force generated by stimulation at 10 Hz (expressed as a percentage of that at 50 Hz) has been used as an index of the frequency:force relationship.

	10/50% (pre exs)		10 min		10/50% (% pre exs) 4 hr		24 hr		48 hr	
Subject	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc
MR	44.2	56.9	136.4	53.6	71.6	52.7	107.1	85.4	142.0	-
RH	50.3	40.1	128.8	80.5	93.4	83.5	94.0	83.2	68.0	125.0
TD	36.4	44.5	78.6	43.4	150.5	64.3	99.7	90.0	141.8	139.3
CG	49.1	50.4	70.6	33.8	59.7	71.4	55.0	72.6	84.1	64.8
LM	43.4	49.1	87.3	79.0	112.2	59.9	121.9	81.5	80.8	88.8
BS	47.3	53.3	84.1	53.3	86.9	112.4	152.8	116.7	126.8	112.6
MT	44.2	56.1	90.9	39.3	100.0	63.2	92.6	64.3	99.4	83.9
JA	68.6	72.4	66.2	41.7	88.2	72.2	88.2	69.4	95.6	101.2
RS	51.0	49.3	66.7	77.5	64.7	75.5	64.7	75.5	94.1	118.4
DD	50.7	64.8	85.6	48.4	107.2	64.1	123.8	67.2	92.0	71.9
n			10	10	10	10	10	10	10	9
mean			89.5	55.0	93.4	71.9	100.0	80.6	102.5	100.6
SD			22.3	17.6	26.6	16.6	28.6	15.2	25.7	25.2
SEM			7.7	5.6	8.4	5.3	9.1	4.8	8.1	8.4
difference between Con & Ecc			$p<0.005$		$p<0.05$		NS		NS	

Appendix vii.

Plasma CK in 13 normal subjects after a standardised 20 min step test.

Plasma CK (IU/l.

Subject	Sex	Pre exs	After exercise.						
			hrs		Days				
			4	24	2	3	4	5	6
CG	F	62	126	129	117	61	-	-	-
BS	M	183	203	437	417	380	291	-	-
RH	F	68	99	135	97	90	-	-	-
TD	F	220	278	281	170	108	-	-	-
LM	M	117	256	360	296	-	220	-	-
MR	F	72	109	142	1594	4950	6700	-	1800
RS	M	70	143	189	169	160	-	-	-
MT	M	61	90	120	73	-	-	-	-
JA	F	38	64	73	90	71	-	-	-
CS	F	51	-	800	5400	-	6800	5243	-
JR	F	53	-	510	2725	3175	3500	-	565
DJ	M	82	-	170	167	1140	2660	-	3840
DN	F	43	-	68	200	1026	842	10600	890

Appendix viii.

Plasma CK in 3 normal male subjects after prolonged stepping.

Subject	Exs duration	Plasma CK (IU/1)						
		Pre exs	24hr	48 hr	3d	4d	5d	6d
KG	50 min	154	1995	8450	14400	23700	3450	2120
PE	60 min	36	107	150	778	1698	940	-
PB	2 hr	465	696	876	1390	2643	1284	-

Subjects KG and PE stepped to exhaustion (50 & 60 min) while PB exercised for 2 hr and could easily have continued.

Appendix ix. Force changes in the quadriceps after prolonged stepping.

	10 min		24 hr		48 hr		72 hr	
	Con	Ecc	Con	Ecc	Con	Ecc	Con	Ecc
MVC force (% pre exs)								
K.G.	74.44	77.95	82.96	70.0	83.36	55.1	84.8	57.1
P.B.	90.8	63.17	88.3	69.0	99.0	70.2	108.9	86.6
P.E.	83.9	78.2	102.3	83.1	99.8	87.0	-	-
Mean	83.0	73.0	91.2	74.0	94.1	71.8	96.8	71.9
10/50% (% pre exs)								
K.G.	53.8	41.3	101.6	85.9	-	-	-	-
P.B.	89.9	36.6	104.3	75.5	108.2	91.44	-	-
P.E.	50.9	38.9	86.6	70.0	-	-	-	-
Mean	64.9	38.9	97.5	77.1	-	-	-	-

Subject K.G. stepped to exhaustion (50 & 60 min) PB exercised for 2 hr and could have continued.

Appendix x. Plasma CK in 5 normal subjects after walking for 1 hour on an inclined treadmill.

	Plasma CK (IU/1)							
	Pre exs	Days after exercise						
	1	2	3	4	5	6	7	8
Subject								
Downhill walking.								
1 (SM)	59	175	87	110	711	-	6480	14746	-
2 (DC)	220	1090	770	820	2477	-	8476	11305	8104
3 (SC)	55	96	84	88	483	702	149	117	-
4 (DJ)	71	125	87	91	87	85	82	-	-
5 (DN)	92	172	196	-	6546	3491	1613	-	-
Uphill walking.								
1	38	59	57	40	48	-	-	-	-
2	124	192	127	148	216	143	-	-	-
3	61	71	68	53	-	55	-	-	-
4	50	67	64	65	-	-	-	-	-
5	36	127	89	55	50	-	-	-	-

Appendix xi. Muscle force, tenderness and plasma CK during a period of regular stepping.

Subject: JR

	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7	
	Con L	Ecc R	Con L	Ecc R	Con L	Ecc R	Con L	Ecc R	Con L	Ecc R	Con L	Ecc R	Con L	Ecc R
MVC														
Pre exs (N)	557.2	495.7	504.3	510.4	480.9	436.6	504.3	500.6	477.9	487.7	423.4	454.3	529.8	533.8
10 post exs (% pre)	61.8	72.7	98.7	68.6	90.8	62.8	91.2	77.5	87.8	70.2	91.8	75.5	98.8	92.2
10/50% 10 min post exs (% pre)	53.8	49.6	66.3	42.9	89.9	49.7	77.5	51.4	54.1	39.1	91.0	69.0	65.4	63.4
20/50% 10 min post exs (% pre)	84.1	62.7	94.1	68.6	83.1	51.4	86.5	69.5	96.5	61.1	100.8	87.5	93.8	90.2
Peak Tenderness	0	261	0	135	0	251	0	24	0	0	0	55	0	52
Peak plasma CK (IU/1)	3500		*		58		49		39		44		59	

* CK levels still falling from previous week.

	Week 8(i)		(ii)		Week 9		Week 10(i)		(ii)		*Week 11 *	
	Con L	Ecc R	Con L	Ecc R	Con L	Ecc R	Con L	Ecc R	Con L	Ecc R	Ecc L	Con R
MVC pre exs (N)	514.1	487.1	547.3	468.6	481.3	498.9	483.0	493.2	565.8	536.3	543.6	559.6
10 min post exs (% pre)	81.1	85.3	94.6	104.7	92.4	78.1	102.1	99.2	95.6	96.5	59.0	91.9
10/50% 10 min post exs (% pre)	104.5	61.5	65.1	46.8	77.1	85.5	68.9	48.8	112.9	49.8	40.5	50.0
20/50% 10 min post exs (% pre)	94.1	86.2	92.9	77.3	88.1	94.7	88.1	83.9	87.7	86.0	71.0	84.1
Peak Tenderness	0	0	0	7	0	0	0	0	0	0	374	0
Peak plasma CK (IU/1)	35		39		41		41		42		2870	

NB Change of contraction pattern in Week 11.

Appendix xii. The time course of training effects of eccentric exercise of the elbow flexors.

Subjects training once a week.

	Initial MVC (N)	Target (%MVC)	Force (% MVC) 5 min	10 min	15 min	20 min	Tenderness scores 24hr	48 hr	72 hr	4 day	Plasma CK (IU/1) Pre	24 hr	48hr	72 hr	4day	5 day	6day
CS (Female 24 yrs)																	
Week 1	629.7	47.4	37.2	16.9	10.1	8.5	58	106	111	79	39	119	161	1809	2870	2234	1797
2	601.7	50.0	27.9	25.6	24.4	20.9	0	0	0	0	1597	1201	614	300	170	-	52
3	503.5	47.3	27.7	15.6	17.3	17.3	9	0	0	0	52	53	47	59	52	-	38
Cross over (Using contralateral muscle)																	
	433.0	51.7	27.6	24.1	17.2	12.1	0	0	0	0	52	317	434	4580	7748	-	698
6 Weeks detraining																	
	542.2	47.6	26.7	22.7	13.0	12.7	0	0	0	0	47	106	1286	1785	1061	-	88
DJ (Male 40 yrs)																	
Week 1	1105.4	53.6	52.5	20.5	14.3	14.6	144	143	188	58	134	194	1313	458	986	921	461
2	1020.0	49.0	24.5	17.6	15.7	12.7	0	0	0	0	277	184	145	105	96	65	-
3	1105.4	48.7	48.7	31.6	17.7	16.5	0	0	0	0	96	106	113	81	-	-	-
Cross over	1063.5	47.4	47.4	28.9	19.7	14.5	90	85	149	61	74	1565	1572	7700	12268	-	7712
Detraining																	
2 weeks	996.0	50.4	50.4	43.4	36.5	27.8	46	2	0	0	62	82	79	-	137	75	75
4 "	895.5	48.4	48.4	37.5	28.9	28.1	50	54	34	0	81	104	173	282	-	-	96
12 "	1021.5	48.0	41.0	24.0	19.2	15.7	123	123	92	0	75	160	1177	2111	1638	1260	946

The non-dominant arm ws used for the training studies and the dominant one for the cross over studies.

Appendix xii Cont'd.

Subjects training once every two weeks.

	Initial MVC (N)	Target (%MVC)	Force (% MVC) 5 min	10 min	15 min	20 min	Tenderness scores 24hr	48 hr	72 hr	4 day	Plasma CK (IU/l) Pre	24 hr	48hr	72 hr	4day	5 day	6day
OR (Female 24 yrs)																	
Week 1	385.4	52.6	52.6	36.3	34.5	29.0	49	85	23	0	70	90	134	333	318	186	-
2	411.2	51.8	51.8	25.5	17.0	13.6	59	114	69	0	64	69	345	322	509	230	227
3	503.7	48.3	34.7	16.6	16.6	17.2	0	0	0	0	207	127	96	84	76	-	-
After 4 weeks detraining																	
	522.5	48.2	48.2	47.7	33.7	28.4	49	59	54	0	58	65	61	53	64	72	74
Training every four weeks																	
SM (Female 25 yrs)																	
Week 1	561.0	47.5	43.0	28.5	20.2	15.8	47	68	56	10	30	42	556	1610	2490	2290	1254
2	503.7	45.8	44.4	22.2	18.0	12.5	43	35	30	9	31	38	242	605	395	-	-

Appendix xiii. The effect of duration and intensity on plasma CK and muscle tenderness.

	Initial MVC (N)	Target (%MVC)	Force (% MVC) 5 min	10 min	15 min	20 min	Tenderness scores 24hr	48 hr	72 hr	4 day	Pre	Plasma CK (IU/1) 24 hr	48hr	72 hr	4day	5 day	6day
Subject 1 (MT, female 23 yrs)																	
Test 1	426.8	50.8	37.7	23.6	16.4	17.8	0	0	0	0	77	70	60	55	49	54	-
Test 2	518.4	48.6	37.0	22.6	15.1	14.4	0	0	0	0	45	158	401	389	329	124	-
			25 min	30 min	35 min	40 min											
			13.0	12.2	10.3	10.9											
Subject 2 (MS, female 30 yrs)																	
Test 1	937.5	50.7	35.8	20.1	14.2	13.4	0	0	0	0	94	130	133	89	81	-	-
Test 2	867.6	100.0	MVC at end 42.0% initial				0	0	0	0	128	130	147	416	1332	2171	1563

For both subjects Test 1 was the 20 min eccentric exercise of the elbow flexors with a target of 50% eccentric MVC.

In Test 2 Subject 1 performed the exercise with the traget force halved (i.e. 25% MVC) and the duration doubled (i.e. 40 min).

Subject 2 performed 3 MVC's with a 5 min rest between each set for a total exercise time of 5 min.

The area under the force record (cm^2):

Subject 1 - Test 1 = 11.0 and Test 2 = 21.0

Subject 2 - Test 1 = 14.9 and Test 2 = 11.5

Appendix xiv.

Quantitation of morphhological damage in needle biopsy samples from the quadriceps after a standardised 20 min step test in 4 normal subjects.

The damage has been designated as focal, extensive and very extensive. For classification see text.

	No fibres counted	Normal No	Normal %	Focal No	Focal %	Extensive No	Extensive %	Very Extensive No	Very Extensive %
BQ Con									
Pre exs		Fibrofatty tissue only in sample							
End exs	42	42	100.0	0	0	0	0	0	0
+30 hr	63	63	100.0	0	0	0	0	0	0
Ecc									
Pre exs	90	90	100.0	0	0	0	0	0	0
End exs	41	28	68.3	8	19.5	5	2.2	0	0
+30 hr	65	23	35.4	3	4.6	17	26.2	22	33.8
LM Con									
Pre exs	32	32	100.0	0	0	0	0	0	0
End exs	65	65	100.0	0	0	0	0	0	0
+30 hr	35	35	100.0	0	0	0	0	0	0
Ecc									
Pre exs	56	56	100.0	0	0	0	0	0	0
End exs	51	35	68.6	4	7.8	8	15.6	4	7.8
+30 hr	60	29	48.3	5	8.3	14	23.3	12	20.0
MR Con									
End exs	32	32	100.0	0	0	0	0	0	0
+30 hr	58	58	100.0	0	0	0	0	0	0
Ecc									
End exs	43	25	58.1	9	20.9	9	20.9	0	0
+30 hr	40	18	45.0	2	5.0	8	20.0	12	30.0
BS Con									
Pre exs	28	28	100.0	0	0	0	0	0	0
End exs	43	43	100.0	0	0	0	0	0	0
+30 hr	33	33	100.0	0	0	0	0	0	0
Ecc									
Pre exs	46	46	100.0	0	0	0	0	0	0
End exs	39	39	100.0	0	0	0	0	0	0
+30 hr	15	9	60.0	0	0	4	26.7	2	13.3

Appendix xv. Plasma CK, muscle isotope uptake, quadriceps tenderness and ultrastructural changes after stepping.

	Days after exercise.								
	0	1	2	3	4	5	6	7	8
1. Plasma CK (IU/1)									
DN	40	113	550	2046	3991	3877	1324	380	244
PB	93	249	1230	19937	33581	35725	8640	6236	3826
	9	10	11	12					
DN	124	96	63	-					
PB	1738	753	308	310					
2. ^{99m}Tc-PYP uptake (muscle:bone)									
a) Quadriceps									
DN	-	0.58	0.59	0.48	0.50	0.48	-	-	0.5
PB	-	0.52	0.51	0.43	0.57	0.51	-	-	0.5
b) Adductors									
DN	-	0.66	0.93	1.23	1.48	1.82	-	-	0.5
PB	-	0.58	0.65	0.94	1.45	1.87	-	-	1.1
3. Quadriceps tenderness scores									
PB	0	86	278	250	189	46	0	0	-

4. Quadriceps ultrastructural damage

	Total no fibres	Normal n	Normal %	Focal n	Focal %	Extensive n	Extensive %	V. Extensive n	V. Extensive %
DN									
+3 days									
Con	100	97	97	2	2	1	1	0	0
Ecc	73	21	27	8	11	9	12	35	50
+5 days	75	50	66	10	13	8	11	7	10
+10 "	78	70	90	5	7	2	2	1	1
PB									
+3 days									
Con	37	35	92	2	8	0	0	0	0
Ecc	48	4	9	1	2	4	9	39	80
+5 days	100	41	41	10	10	26	26	23	23
+10 "	89	54	60	11	12	14	16	10	12

On the third day after exercise both quadriceps muscles were biopsied. Subsequently biopsies were only taken from the muscle which had contracted eccentrically

Appendix xvi. Plasma CK, muscle tenderness, isotope uptake, and ultrastructural changes in the calf muscles after eccentric contractions.

1. Plasma CK (IU/l)

Days after exercise.

	0	1	2	3	4	5	6	7	8
DJ	79	127	83	72	141	121	-	-	-
DN	35	304	1967	5548	30324	51020	77103	44226	12033

	9	10	11	12	13	14	15	16
DN	6867	4221	1638	636	411	341	227	145

2. ^{99m}Tc-PYP uptake (muscle:bone)

Days after exercise.

	1	2	3	4	5	6	7	12
DJ	-	-	0.4	0.5	-	-	-	-
DN	-	-	1.8	2.7	2.8	6.0	4.1	0.6

3. Calf tenderness scores

DJ	126	235	288	87	0	0	0	0

4. Quadriceps ultrastructural damage

	Total no fibres	Normal n	Normal %	Focal n	Focal %	Extensive n	Extensive %	V. Extensive n	V. Extensive %
DJ									
+3 days	47	47	100	0	0	0	0	0	0
+5 days	47	45	96	0	0	2	4	0	0
+7 days	32	31	97	0	0	1	3	0	0

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Greater low frequency fatigue produced by eccentric than concentric muscle contractions

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Low frequency fatigue has been described following static and dynamic muscle contractions (Edwards *et al.*, 1977), but the cause of this is not clear. To explore this further we have compared the low frequency fatigue produced by concentric contractions (positive work) and eccentric contractions (negative work). O_2 consumption during eccentric contractions is some four to six times less than during concentric contractions (Bigland-Ritchie & Woods, 1973). Low frequency fatigue is defined as impaired force generation at low frequencies of stimulation with relative preservation of force from high frequency stimulation.

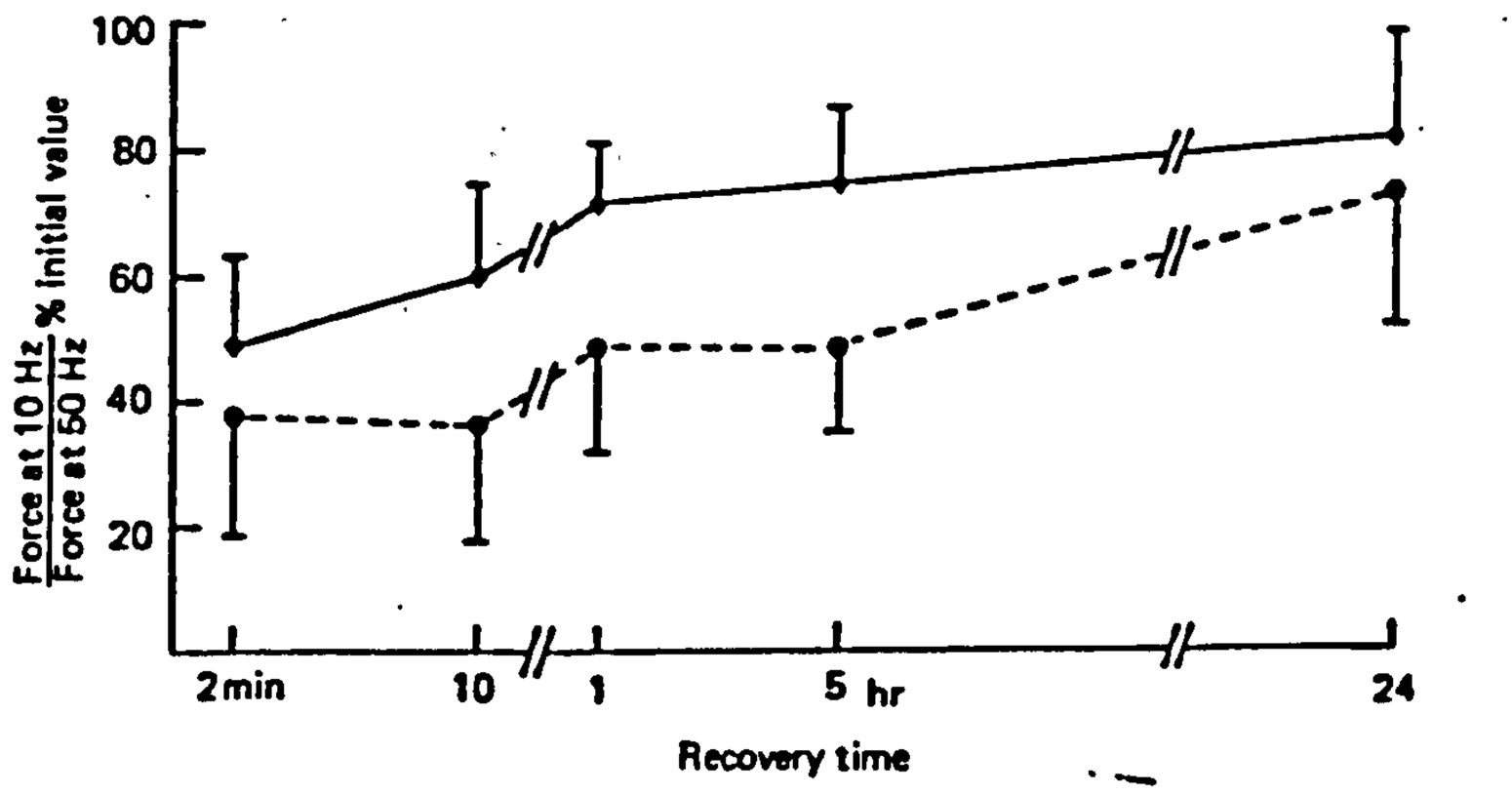

Fig. 1. Recovery of low frequency fatigue following concentric (◆) and eccentric (●) contractions (mean ±S.D., $n = 7$).

In seven studies on three normal subjects, quadriceps contractility was tested by percutaneous stimulation at 1, 10, 20, 50 and 100 Hz, before and after 15 min of stepping. One muscle contracted concentrically throughout (raising the body through 103·5 m) whilst the contralateral muscle contracted eccentrically. Following exercise the ratio of force generated by stimulation at 10 Hz to that at 50 Hz fell when compared to pre-exercise values. This was more marked in the muscle which had been contracting eccentrically (Fig. 1).

These results suggest that low frequency fatigue is a consequence of some injury or mechanical disturbance of the muscle.

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Measurement of severity and distribution of experimental muscle tenderness

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Muscle soreness is common after unaccustomed exercise, being particularly associated with eccentric contractions (Komi & Buskirk, 1972). Affected muscles are also tender on palpation. Four normal subjects performed a 15-min period of stepping

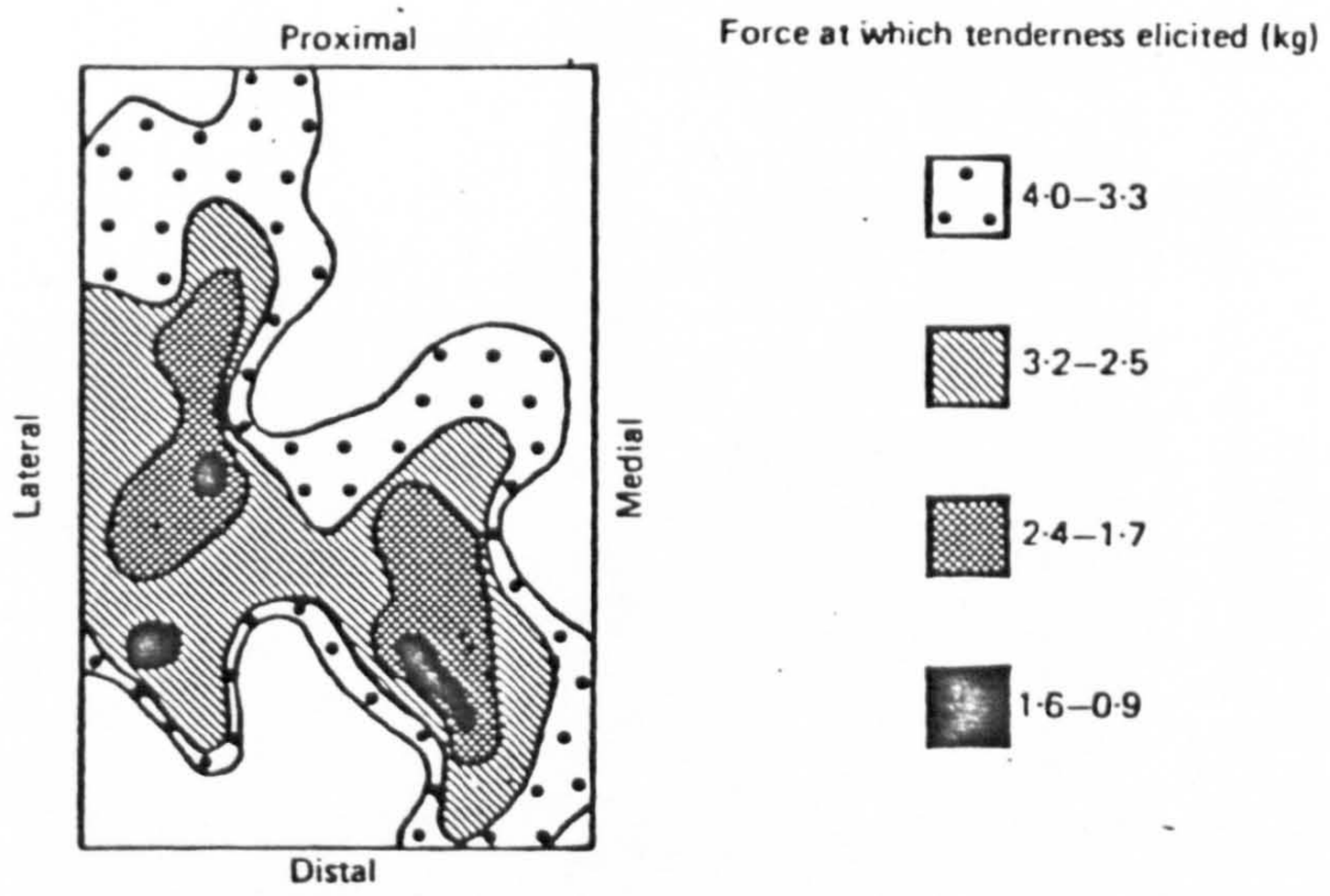

Fig. 1. Typical distribution of tenderness at peak intensity in the right quadriceps of one subject.

(46 cm step) so that the quadriceps muscle of one leg contracted eccentrically throughout. This produced soreness and tenderness, the latter being quantitated by a round-ended wooden probe (2 cm diam.) fixed to a strain gauge. A polythene sheet marked with 4 cm squares enclosed the affected thigh while gradually increasing force was applied to all parts of the muscle in turn. The subject reported the force at which tenderness was first elicited. The exerted force, displayed on a U.V. oscillograph (out of sight of the subject), was increased to a maximum of 4 kg, and if tenderness was not reported at this force it was considered absent. No tenderness was found on the day of exercise; it was first recorded 24 hr later and was maximal at 48 hr. The heads of vastus medialis and lateralis were most tender, with sparing of their proximal regions and of rectus femoris (Fig. 1).

The time course of tenderness and the fact that the opposite muscle which contracted concentrically did not become sore or tender, indicate that the metabolic

[P.T.O.

cost of the exercise is not the cause. The distribution, close to musculotendinous junctions, suggests that mechanical injury may be responsible (Asmussen, 1956).

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Pain and fatigue after concentric and eccentric muscle contractions

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Summary

1. Normal subjects performed a step test in which the quadriceps of one leg contracted concentrically while the contralateral muscle contracted eccentrically.

2. Maximal voluntary force and the force:frequency relationship were altered bilaterally as a result of the exercise, the changes being greater in the muscle which had contracted eccentrically. Recovery occurred over 24 h.

3. Electromyographic studies using three sites on each muscle showed an increase in electrical activation during the exercise only in the muscle which was contracting eccentrically. Recovery followed a time course similar to that of the contractile properties.

4. Pain and tenderness developed only in the muscle which had contracted eccentrically. Pain was first noted approximately 8 h after exercise and was maximal at approximately 48 h after exercise, at which time force generation and electrical activation had returned to pre-exercise values.

5. Eccentric contractions cause more profound changes in some aspects of muscle function than concentric contractions. These changes cannot be explained in simple metabolic terms, and it is suggested that they are the result of mechanical trauma caused by the high tension generated in relatively few active fibres during eccentric contractions.

Key words: eccentric contractions, low-frequency fatigue, muscle pain.

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Abbreviations: EMG, electromyograph; IEMG, integrated electromyograph.

Introduction

Muscle pain occurring 24–48 h after unaccustomed exercise is a phenomenon familiar to most individuals, but the mechanisms responsible for its production are uncertain. Asmussen [1] first indicated that eccentric contractions (those in which the muscle is lengthened during contraction) are particularly associated with pain and soreness. In the intervening period many workers have found this to be an interesting model as it is well established that both the metabolic cost [2–5] and the electrical activity required to produce a given tension [6–9] are less under eccentric conditions than concentric. Komi [10], however, reported the relationship between the integrated electromyograph (IEMG) and percentage of maximum force to be similar whichever type of contraction was used. The fact remains that eccentric rather than concentric contractions predispose to delayed onset, post-exercise pain that is not accounted for in terms of metabolism.

The 'torn tissue' hypothesis of Hough [11] suggested that pain resulted from structural damage in the muscle; de Vries [12] proposed tonic spasms in localized motor units and both Komi [13] and Asmussen [1] put forward overstretching of the connective tissue elements as the cause of pain.

Repetitive, fatiguing isometric and dynamic contractions have been shown to produce specific, long-lasting alterations in contractile properties of muscle such that the force:frequency curve is shifted to the right and electrical

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stimulation at low frequency (1–20 Hz) results in decreased force generation when compared with the fresh muscle, but the force generated by high-frequency stimulation is relatively preserved [14]. This type of fatigue, termed 'low-frequency fatigue', has been demonstrated in the quadriceps, adductor pollicis, diaphragm [15] and sternomastoid [16]. Although the underlying mechanism is not clear, it was assumed that the amount of low-frequency fatigue produced in a muscle was related to the work done by that muscle. Recent work [17] has revealed that eccentric contractions caused greater low-frequency fatigue than concentric.

In this study, normal subjects have performed a step test in which the quadriceps muscle of one leg worked concentrically and the contralateral muscle worked eccentrically. The effects of these two types of contraction on the IEMG, voluntary force and contractile properties of the muscle have been investigated. The degree and distribution of tenderness over the surface of the muscle has been measured [18] in an attempt to define the painful tissue.

Methods

Subjects

Four healthy, normal subjects performed the experiments, three males and one female, the age range being 31–45 years (mean 36·25 years).

Step test

Subjects performed a step test for 15 or 20 min, using a 46 cm step. The stepping pattern was designed so that the quadriceps of one leg contracted concentrically (stepping up) throughout the test, while the contralateral muscle contracted eccentrically (stepping down). A rate of 15 cycles/min was used and an electronic metronome provided audible timing clicks, so that each stepping phase lasted 1 s. During the exercise period a total height of 103·5 m was ascended. Subjects were encouraged to fully control each eccentric contraction and as far as possible to maintain a constant stepping rate.

Force measurements

The force produced by electrically stimulated and maximal voluntary isometric contractions was measured by using previously described techniques [19].

The force:frequency characteristics of the muscle were monitored by percutaneous stimulation at 1 Hz (for 5 s) and 10, 20, 50 and 100 Hz (for 2 s) by using square wave pulses of 50 μs.

Maximal voluntary force measurements and electrical stimulation of the quadriceps muscles of both legs were carried out before exercise, then 2, 10 and 30 min and 1, 5, 24 and 48 h after exercise.

Electromyography

Areas over rectus femoris, vasti medialis and lateralis on each leg were prepared by abrasion and alcohol swabs to lower the skin resistance to less than 5 kohm. These areas were marked so that identical sites would be used on subsequent testing. Silver/silver chloride cup electrodes were filled with electrode jelly and taped in place. Unipolar recordings were made from these sites and amplified with reference to an electrode placed over the lower lumbar spine in the midline. Signals were amplified (S.E. Labs, type 4901) and band pass filtered between 0·2 Hz and 10 kHz, and displayed on a u.v. oscillograph and recorded on light-sensitive paper. The six raw signals were integrated over 300 ms periods and similarly displayed. Recordings were made from these sites at intervals during stepping.

In order to investigate any changes in activation patterns as a result of the exercise, electrical activity of the three muscles on each leg was recorded during active, submaximal contractions during knee extension from 90° to full extension, which was held for 2 s, with a 3 kg weight attached to the foot. These recordings were made before and at intervals after exercise.

To study the relationship between muscular activity and joint angle during stepping and the submaximal knee extension tests, electronic goniometers were used. A rotary potentiometer with a linear response was mounted as the pivot between two long Perspex arms. The goniometers were placed laterally on each leg with the potentiometer sited over the fulcrum of the knee joint and the Perspex arms taped in place along the femur and fibula. A signal, proportional to the knee angle, was displayed on the u.v. oscilloscope with the EMG and simultaneously recorded. Rate of knee extension was kept as constant as possible during the test by displaying to the subject a signal proportional to angular velocity.

Measurement of severity and distribution of muscle tenderness

A polythene sheet marked with a grid of intercepts 2 cm apart, to be used as test sites, was

wrapped around the thigh, the skin of which was marked to ensure constant positioning in subsequent tests. A round-ended, wooden probe (2 cm diameter) was attached to a strain gauge and the amplified force signal was displayed on a u.v. oscillograph. At each test site, a gradually increasing force was applied up to a maximum of 40 N. The subject was asked to indicate verbally when the sensation of pressure changed to one of discomfort, whereupon the probe was immediately withdrawn. If no indication was given at a deflection on the oscillograph proportional to 40 N, tenderness was considered not to be present at that site. Each site was tested in a defined order, enabling a record to be made of the degree of tenderness over the whole surface of the muscle. From these records maps were drawn showing the degree and distribution of muscle tenderness. Although the accuracy of localization by muscle nociceptors is not well defined, the fact that the receptive areas of mechanical nociceptors are spot-like [20] and also that subjects are well able to localize the sites of contusions and needle biopsies in muscle suggests that the degree of localization is adequate to indicate the sites of muscle trauma.

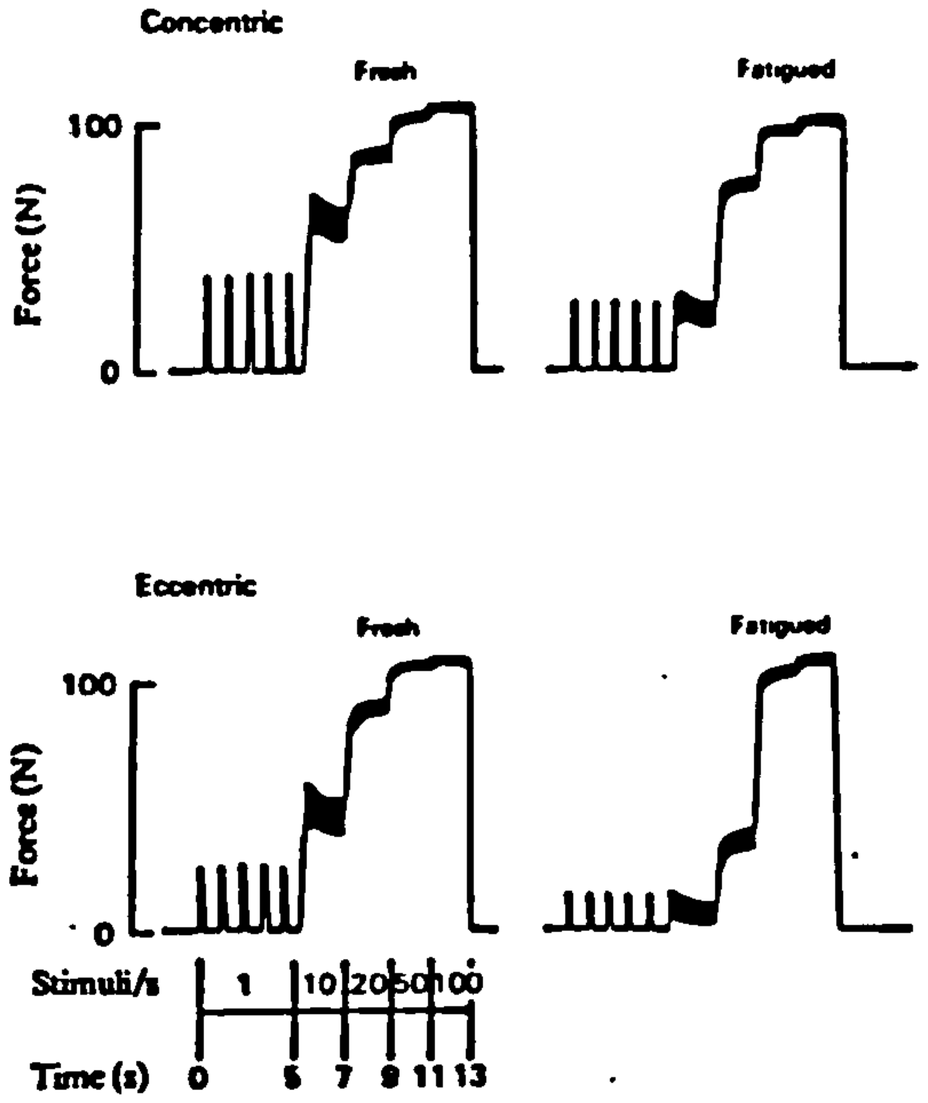

FIG. 1. Force generation in response to electrical stimulation at 1, 10, 20, 50 and 100 Hz in the quadriceps before and 10 min after a 20 min period of stepping in which one muscle contracted concentrically and the other eccentrically. Female subject, 32 years.

Results

No significant difference was found between the 15 and 20 min exercise periods, therefore the following are combined results of both periods.

Force changes

(a) Maximal voluntary force. Maximal voluntary force was reduced in both legs after exercise, the reduction being significant only in the muscle which had contracted eccentrically when pre-exercise values were compared with those at 2 and 10 min after exercise ($P < 0{\cdot}001$). Force did not recover to pre-exercise values until 24 h after exercise.

(b) Stimulated forces. As an index of low-frequency fatigue, we have used the ratio of the forces produced by a low stimulation frequency (10 Hz) to the force produced by a high stimulation frequency (50 Hz), expressed as a percentage (T10/50%).

A significant decrease in T10/50% was found in the quadriceps of both legs ($P < 0{\cdot}001$); the fall was more marked in the muscle which had contracted eccentrically in the exercise period (Fig. 1). The difference of the T10/50% between the two muscles was significant at 2 min ($P < 0{\cdot}02 > 0{\cdot}01$) 10 min ($P < 0{\cdot}025 > 0{\cdot}002$) after exercise and most highly significant 1 h after exercise ($P < 0{\cdot}001$) as the muscle which had contracted concentrically began to recover. Twenty-four hours after exercise there was no significant difference between the two muscles, although when compared with the pre-exercise values, the T10/50% had not fully recovered (Fig. 2).

Electromyography

In both of the two subjects studied no significant increase was seen in the IEMG of the concentrically contracting muscle during the stepping period. In contrast, a progressive increase in the IEMG of all sites monitored was found in the eccentrically contracting muscle throughout the exercise period (Fig. 3).

Similar changes in electrical activation were seen during the submaximal knee extension test when pre- and post-exercise data were compared (Fig. 4); only the muscles which had contracted eccentrically showed increased electrical activation for the generation of a given muscular tension.

There was no significant change in the ratio of the contributions of the three muscle components to that of the total measured quadriceps activity,

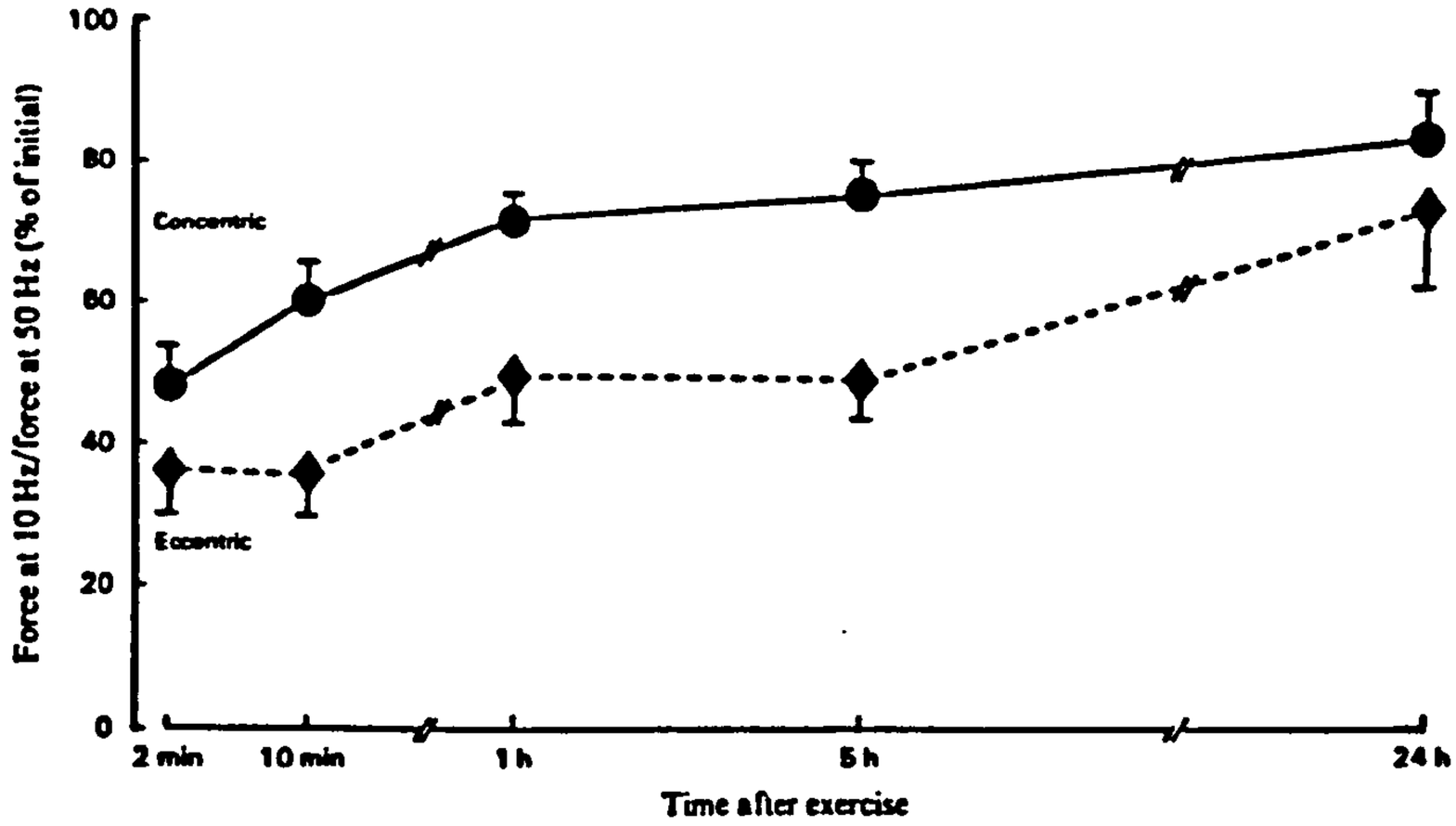

FIG. 2. Changes in the relationship between force generated by 10 and 50 Hz stimulation (expressed as a percentage of pre-exercise values) after 15 or 20 min stepping. Mean values ± SEM are shown for seven subjects.

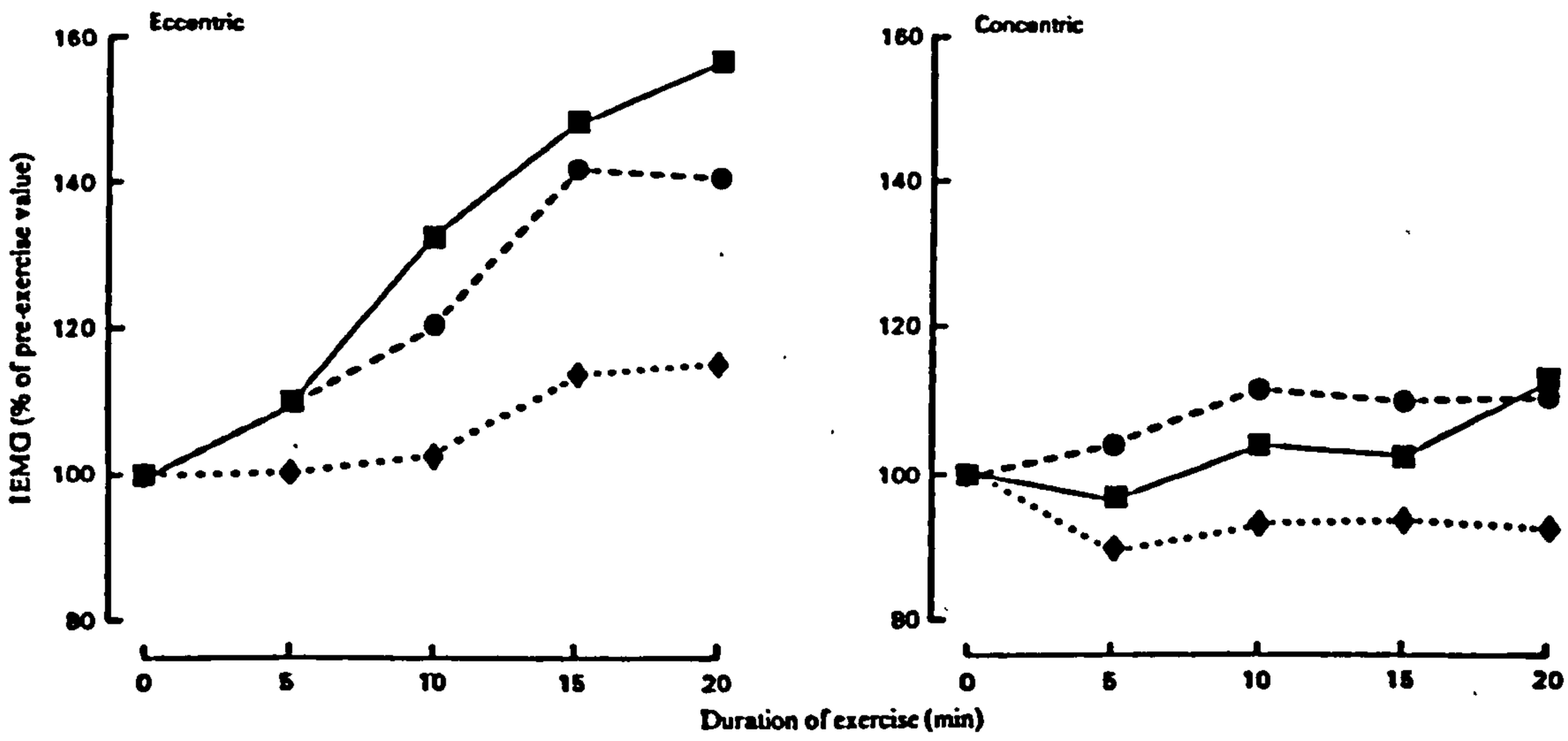

FIG. 3. Integrated electromyogram (IEMG) from three sites of both quadriceps recorded at 5 min intervals during a 20 min period of stepping. Each point is the mean of three consecutive concentric or eccentric contractions and is expressed as a percentage of the pre-exercise value. ■, Rectus femoris; ●, vastus medialis; ♦, vastus lateralis. Male subject, 45 years.

in either leg, during stepping, in the immediate post-exercise phase or during the period when the leg was painful. The simultaneous recording of joint angle and IEMG during stepping (Fig. 5) in the concentrically contracting muscle showed that the main peak of electrical activity occurs during the stepping up phase, with a smaller burst as the opposite muscle lowered the body weight to the ground, to be supported by the former.

The eccentrically contracting muscle showed two peaks in each cycle which were approximately similar in amplitude to each other, and shorter in duration than the main peak in the concentrically contracting muscle. One peak occurred during the eccentric contraction itself, and the other at the time when the same leg was taking part of the body weight after the opposite leg had raised the body up on the step.

The submaximal extension test showed an increase in IEMG at all knee-joint angles be-

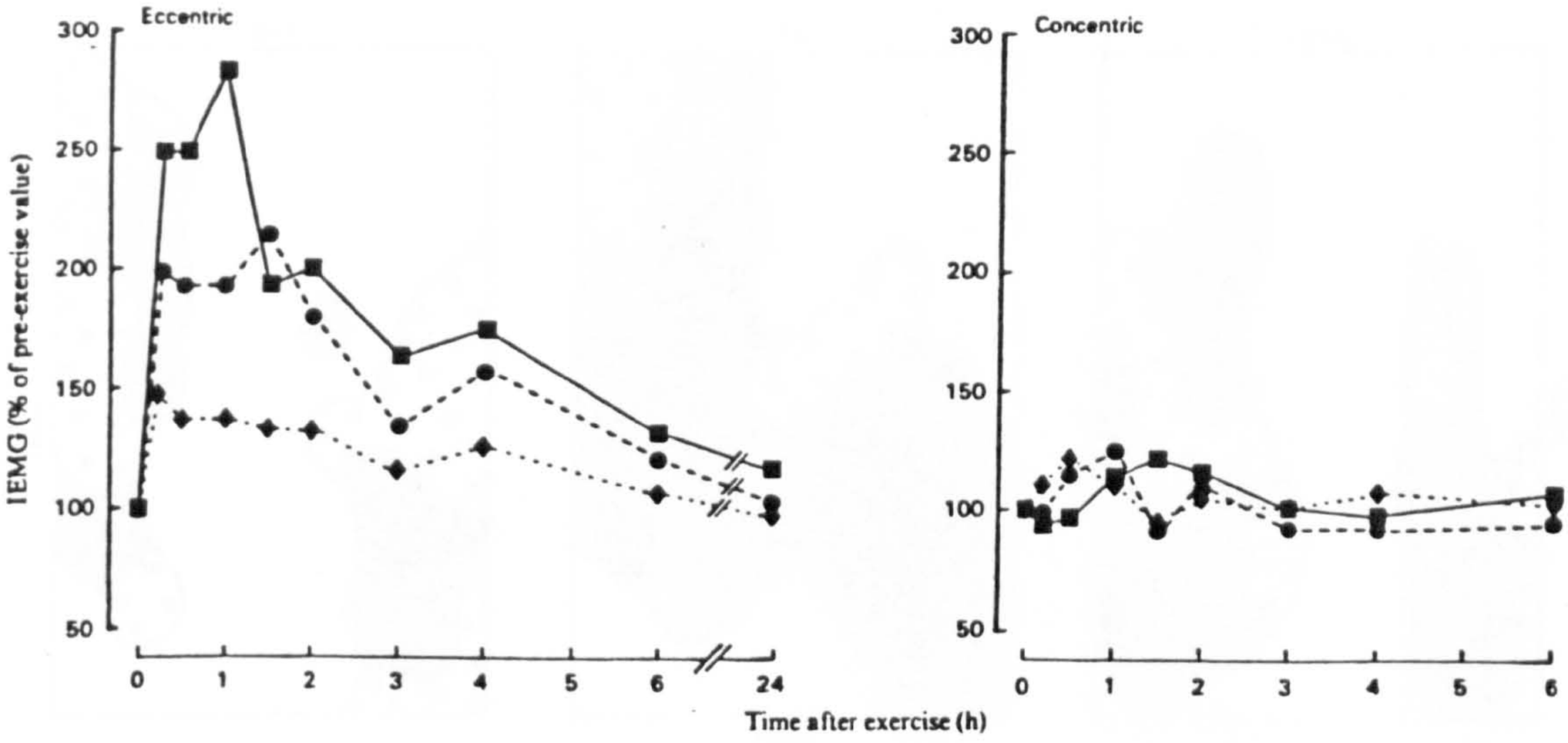

FIG. 4. Integrated electromyograph (IEMG) from three sites of both quadriceps during a submaximal knee extension held for 2 s, after a 20 min period of stepping. Each value is expressed as a percentage of the pre-exercise value. See Fig. 3 for explanation of symbols. Male subject, 45 years.

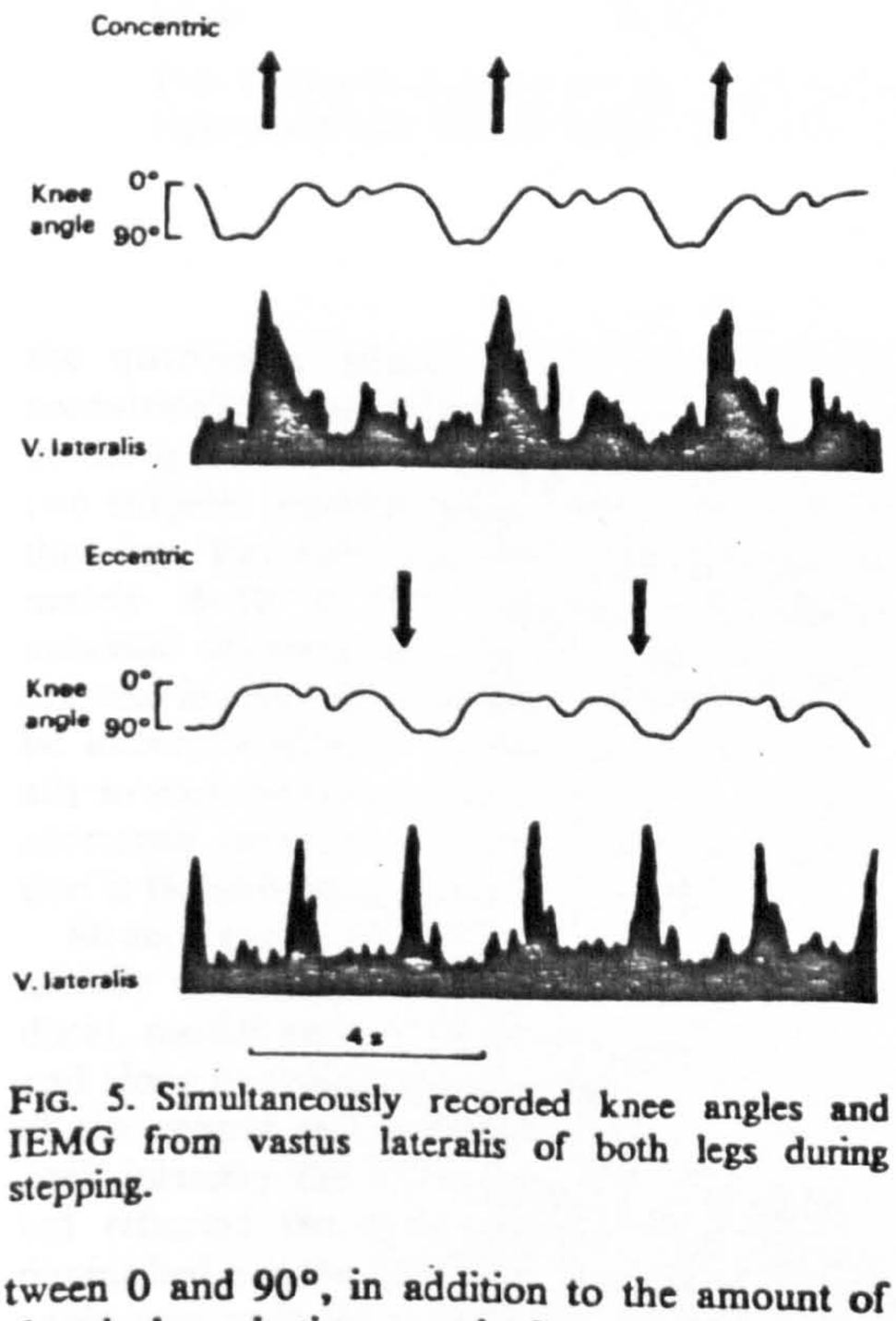

FIG. 5. Simultaneously recorded knee angles and IEMG from vastus lateralis of both legs during stepping.

tween 0 and 90°, in addition to the amount of electrical excitation required to maintain full extension over a 2 s period (Fig. 6).

At no time throughout the testing period was there evidence of spontaneous electrical activity when the muscle was at rest.

Muscle pain and tenderness

Subjective pain was reported by all subjects in

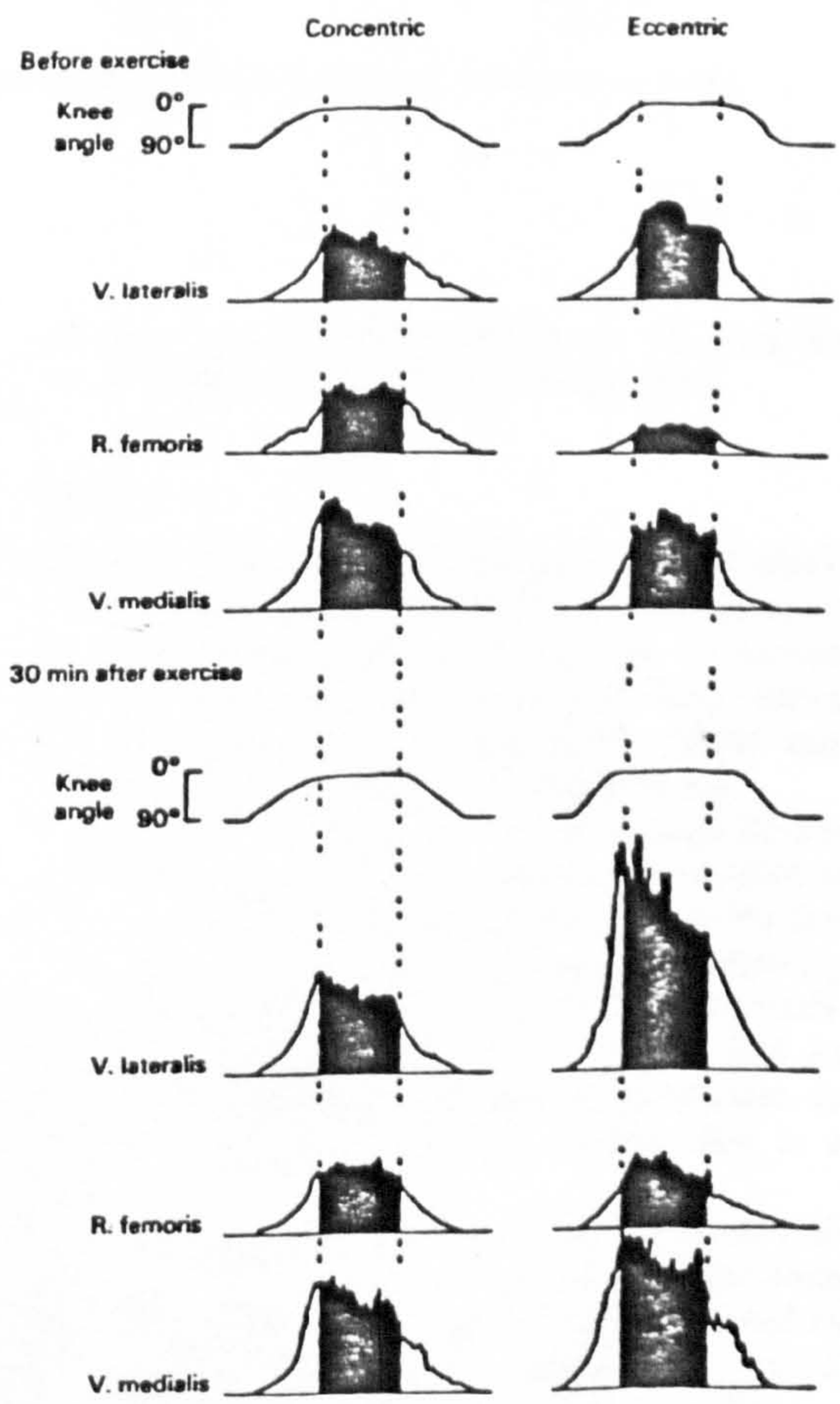

FIG. 6. Integrated EMG from three sites on both quadriceps during knee extension before and 30 min after stepping. Male subject, 45 years.

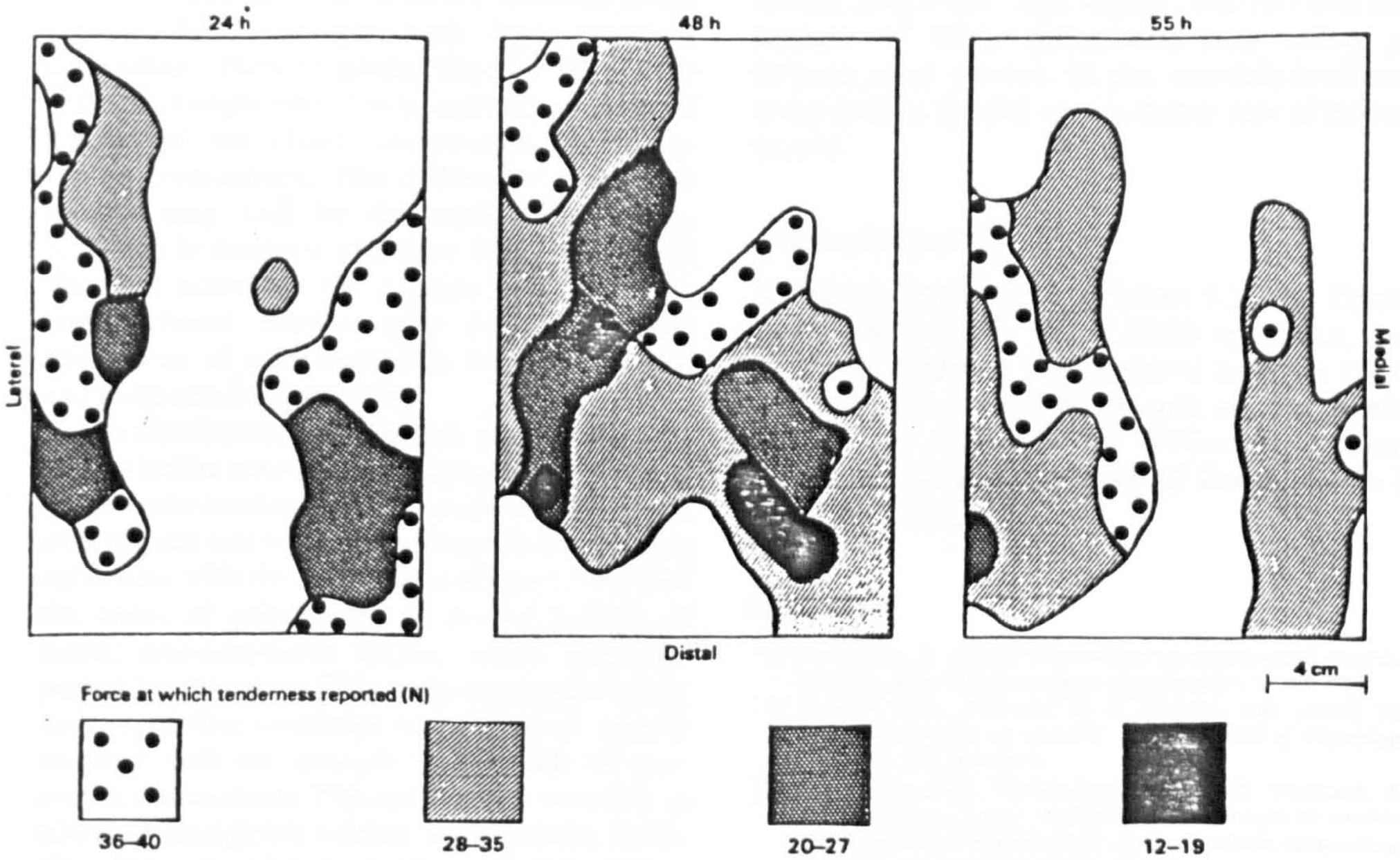

FIG. 7. Distribution and severity of tenderness 24, 48 and 55 h after eccentric contractions in the right quadriceps. Female subject, 32 years.

the quadriceps muscle which had contracted eccentrically in the step test. No pain was noticed in the quadriceps of the opposite leg, although two subjects reported pain in the calf muscles of that leg. The pain was first apparent approximately 8–10 h after exercise and reached maximal intensity between 24 and 48 h after exercise in different subjects. It was also found to be uncomfortable to descend stairs and especially to contract the muscle isometrically in a fully shortened position, although isometric contraction in the mid-length position was less painful.

Strain gauge measurements showed that initially tenderness was primarily located at the distal, medial and lateral parts of the quadriceps and along its lateral margin, with relative sparing of the central and proximal medial regions. At peak intensity the tenderness was more diffuse, but reflected the same pattern. As tenderness diminished a more clear regional localization was again seen as in the early stages of pain (Fig. 7). Both soreness and tenderness had disappeared in all subjects by the fourth day after exercise.

All subjects experienced a feeling of weakness and instability in the immediate post-exercise period only in the muscle which had contracted eccentrically. This sensation was noted at the end of exercise and lasted for approximately 2 h. It was particularly noticeable on performing eccentric contractions, i.e. descending stairs.

Discussion

Despite the relatively low energy cost of eccentric contractions, they are capable of causing more profound changes in some aspects of muscle function, especially the force:frequency curve, than concentric contractions, which clearly cannot be explained in simple metabolic terms.

The fact that greater tension per muscle fibre is generated under eccentric contraction conditions [21, 22] provides a situation where relatively few fibres are recruited and are producing relatively large forces. In this situation the uneven mechanical stresses produced in the muscle and its attachments can be imagined to predispose to physical damage as with the weakest link in a chain.

Mechanical damage to the sarcoplasmic reticulum resulting in less calcium release for each excitatory action potential has been suggested as the cause of low-frequency fatigue [23], and if this is the case it is consistent with our results.

Komi & Rusko [24] reported that with isokinetic exercise at the forearm flexors eccentric contractions cause a greater reduction in

maximal voluntary force than concentric contractions, and in contrast to our findings found similar IEMG changes with both types of contraction. They concluded that the differences in force changes were due to extreme mechanical loading of the elastic components during eccentric contractions. This mechanical stress and trauma may well be the explanation of the reduction in maximal voluntary force, increase in electrical activation for a given muscle tension and profound low-frequency fatigue, changes which were all more marked in the muscle which had contracted eccentrically.

The distribution of tenderness revealed that the muscle bellies are relatively spared, and the areas of musculo-tendinous attachment are the main sites of pain and tenderness. These findings are in agreement with the conclusions of Asmussen that the cause of pain is due to over-stretching of elastic non-contractile tissues, which was supported by Abraham [25], who reported rises in hydroxyproline:creatinine ratios at peak muscle soreness with no changes as a result of concentric contractions. The same author was able to correlate myoglobin release with exercise intensity, but not with soreness, and this argues against the theory that the muscle itself is not the sole tissue responsible for this type of muscle pain.

In agreement with other workers we were not able to detect any evidence of localized muscle spasm during pain as reported by de Vries. The relative contribution of rectus femoris, the medial and lateral vasti to the total measured electrical activity of the muscle did not significantly alter during either short-term fatigue or delayed onset pain, thus providing no evidence of changes in recruitment patterns with fatigue or inhibition of painful areas.

An interesting, but poorly defined, phenomenon is the feeling of weakness and instability experienced for a few hours immediately after exercise only in the muscle which had contracted eccentrically. Further work (unpublished) has indicated that this sensation is an indication of pain to follow, and is presumably a reflection of profound low-frequency fatigue with inappropriate forces being generated by the relatively low normal physiological firing frequency [26, 27].

In conclusion, eccentric muscle contractions have marked effects, initially on the contractile properties and force generating capabilities of muscle, and result in pain of delayed onset. These findings are accountable for in terms of the high forces generated by relatively few muscle fibres and the transmission of these uneven forces to the non-contractile tissues with resultant mechanical damage, and are not related to the metabolic energy cost of the contractions. The different time courses of these phenomena may reflect an inflammatory process in the musculo-tendinous areas and/or the different turnover rate of the two tissues.

Acknowledgments

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Muscle changes have been examined in 16 normal subjects (eight female) after both a 20-minute and a prolonged step test. Stepping differs from most exercise tests in that it involves eccentric contractions (negative work) in which the active muscle is lengthened. Plasma creatine kinase (CK), muscle force, contractile properties, and tenderness in the quadriceps were measured for up to 9 days after the exercise. Muscle tenderness was experienced only in the muscles that had performed eccentric contractions (i.e., stepped down). All subjects showed some early rise in CK (<400 IU/liter) but eight (both male and female) showed a much greater response (up to 34,500 IU/liter) which took a long time to reach peak levels (4–5 days after stepping). It is suggested that eccentric contractions involved in this form of exercise result in some particular form of muscle damage which, in susceptible subjects, may initiate changes giving rise to a large delayed release of muscle enzymes.

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LARGE DELAYED PLASMA CREATINE KINASE CHANGES AFTER STEPPING EXERCISE

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The presence of muscle enzymes in the plasma is commonly taken as an indication of some form of muscle damage either in muscle disease[25] or following exercise in normal subjects.[12,14,26,29] The release after exercise is thought to be analogous to the release of cardiac enzymes after an infarct where the magnitude of the release is an indication of the size of the infarct and the plasma levels reach a maximum some 24 hours after the damage has occurred.[15] Similarly, the release of skeletal muscle enzyme is thought to be proportional to the intensity and duration of the exercise,[2,4] and in the majority of studies, the evidence suggests a similar time course to that seen after cardiac damage.

Most previous reports of muscle enzyme release after exercise have used either treadmill or cycle exercise in which the energy cost is proportional to the work load. Although tiring at the time, normal subjects rapidly recover from this type of exercise with few lasting effects. We have been interested in a different form of exercise test, box stepping, in which the quadriceps of the leg that steps up works "concentrically" as the muscles shorten, while in the leg that steps down the muscles contract "eccentrically" absorbing energy as they lengthen. It is well known that eccentric contractions result in delayed development of pain which is the muscle stiffness and tenderness experienced after unaccustomed exercise.[3,7,9,20,28]

The pain is generally thought to be the result of some form of muscle trauma occurring during the eccentric contractions and the present study was undertaken to look for evidence of this.

In about half of the subjects there was a large and dramatic rise in the plasma creatine kinase (CK) activity—the peak of which occurred several days after the exercise. This appears qualitatively different to the release of enzyme after exercise such as running or cycling, which consists predominantly of concentric contractions.

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METHODS

Thirteen normal healthy subjects (mean age 28 years, range 21–54 years) performed a step test[23] for 20 minutes. Eight subjects were female (mean age 29 years, range 21–54 years) and five were male (mean age 27 years, range 22–38 years). The step was adjusted to be the same relative height for

each subject, being 110% of the lower leg length (knee joint line to ground in the shoes worn for the exercise). The quadriceps muscle of one leg contracted concentrically throughout by stepping up, while the contralateral muscle contracted eccentrically by stepping down. Each contraction lasted for 1 second and a stepping frequency of 15 cycles/min was determined by an electronic metronome.

Venous blood samples were taken immediately before and after exercise and thereafter at 24-hour intervals. Plasma CK activity was measured by the automated Boehinger-Mannheim activated method.

In addition, three male subjects (mean age 27 years, range 25–30 years) who had not participated in the studies above, performed a step test continued to fatigue. The relative step height and stepping frequency was the same as that used by the group of 13 subjects.

The force generated by maximal voluntary contractions and percutaneous electrical stimulation of both quadriceps was measured before, and at intervals after exercise, using techniques previously described.[11] The force:frequency characteristics were monitored by stimulation at 1Hz (for 5 seconds) and 10, 20, 50, and 100 Hz (for 2 seconds each).

Tenderness was quantified by the application of a steadily increasing force (up to 40 N) through a round ended wooden probe to sites 2 cm apart over the surface of the quadriceps muscle.[24] The subject indicated when the sensation of pressure changed to one of discomfort, whereupon the probe was withdrawn. If tenderness had not been elicited by a force of 40 N it was considered not to be present at that site. In this way it was possible to measure the presence and severity of tenderness over the surface of the muscle. For measurement purposes, the tenderness at each site was graded from 0–10, with 0 being no tenderness and 10 being discomfort elicited by a force of less than 4 N. An overall tenderness score was obtained by summing the scores at all sites tested.

RESULTS

Biochemical Changes after 20 Minutes of Stepping. All subjects showed a small CK rise immediately after the exercise and the activity continued to rise during the next 24 hours, reaching two to three times the initial levels. Thereafter, in seven of the subjects, plasma CK returned to pre-exercise values within the next 24 hours. The remaining five subjects showed an unexpected delayed rise in plasma CK activity which was one to two orders of

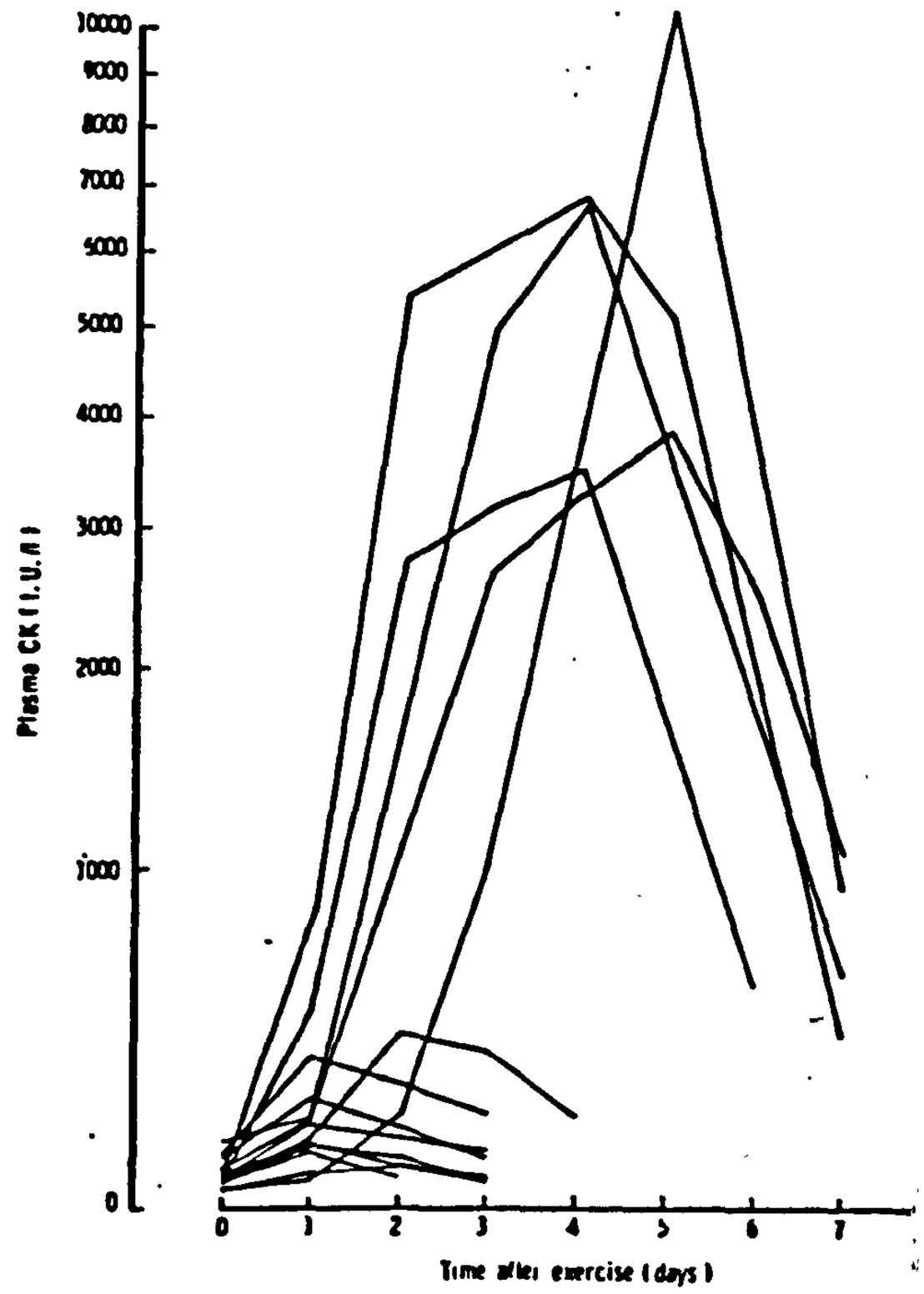

Figure 1. Plasma creatine kinase in 13 normal subjects after stepping for 20 minutes. Note the two groups of response; those showing smaller increases peaked at 1–2 days after exercise while the larger responses peaked at 4–5 days.

magnitude greater than the rise seen after 24 hours (Fig. 1). This much greater rise did not begin until the second or third day after exercise, reached a peak between 4 and 5 days and returned to normal between 7 and 9 days after exercise.

Of the five subjects showing this large delayed rise four were female and their ages spanned the whole range of the sample. Changes in muscle force, tenderness, and plasma CK in two subjects are shown in Fig. 3. Despite similar changes in force and tenderness, the different pattern of CK efflux is evident. Comparing those subjects who showed the large delayed response with those who did not, there was no difference in the height, weight or quadriceps muscle strength/body weight ratios. The height/weight ratio for the subjects who did not show the large delayed CK rise ranged from 1.91–3.59 cm/kg (2 SD about the mean) and for those showing a delayed response the ratio was 2.34–2.98 cm/kg. The quadriceps force/weight ratio for the group not showing the delayed response was 0.63–0.99 N/kg and for those with a delayed response was 0.58–0.94 N/kg. The sub

jects were therefore exercising at very similar absolute work rates and making similar relative demands on their quadriceps. Of the subjects who showed the large delayed response, only one could be described as unfit, the others all participated in regular exercise. In this respect the group did not differ from the subjects who did not respond with the large delayed enzyme efflux.

Biochemical Changes after Prolonged Stepping. Of the three subjects in this group, two were fatigued after 50 and 60 minutes and were limited by the failure of the eccentrically working quadriceps to control the descent from the step. The third subject was stopped after two hours although he could have continued, but, in common with the other two subjects, he found himself unable to straighten against gravity the leg that had been working eccentrically. No trouble was experienced straightening the leg that had been working concentrically.

All three subjects showed the large delayed elevation in plasma CK levels after exercise (Fig. 2). The highest value of 34,500 IU/liter was seen in the subject who had stepped for the shortest time (50 minutes) and occurred on the fifth day after exercise. Peak values occurred 4 days after exercise in the other two subjects, being 1,698 IU/liter in the subject who stepped for 1 hour and 2,643 IU/liter in the subject who was stopped after 2 hours.

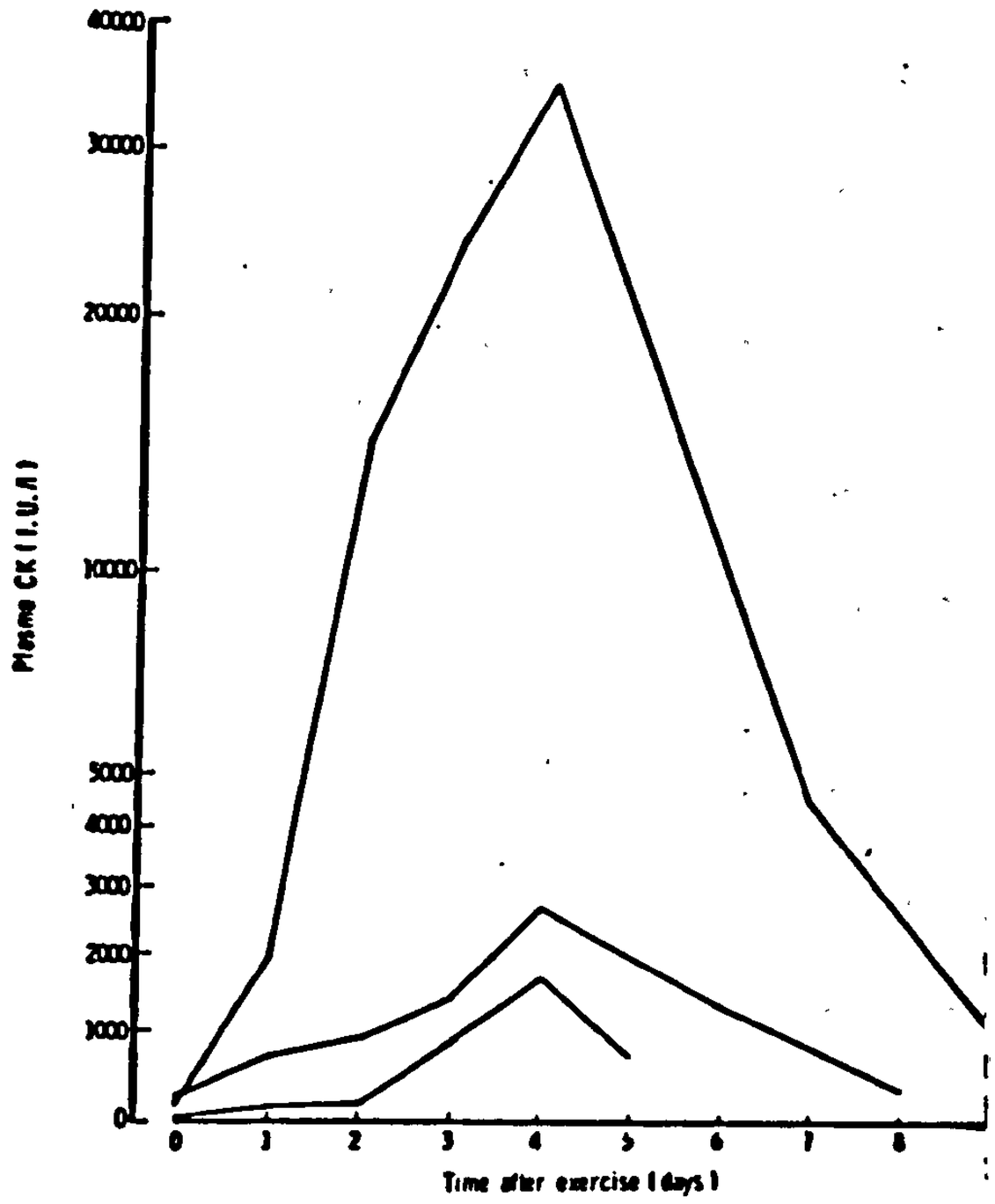

Figure 2. Plasma creatine kinase in three normal male subjects after prolonged stepping (50 minutes to 2 hours). Note the difference in scale compared to Fig. 1.

Muscle Contractile Properties. Changes in maximum voluntary force and the response to stimulation at different frequencies were essentially the same as previously reported.[10] There was approximately a 20% deficit of maximum voluntary force immediately after the exercise, which was somewhat greater in the leg that had worked eccentrically. Force returned to normal within 24–48 hours (Fig. 3). Likewise the force generated at low frequencies was found to be depressed relative to that generated at higher frequencies. This also was greater in the eccentrically contracting leg and returned to normal within 24 hours. Changes in contractile properties were very similar in all subjects irrespective of whether they went on to show small or large releases of muscle enzymes.

Muscle Pain and Tenderness. Muscle pain and tenderness was only experienced in those muscles that had worked eccentrically, that is the quadriceps and gluteal muscles of the leg that had stepped down and the calf of the contralateral leg where the subject had stepped down onto pointed toes. The magnitude of the pain was very variable, some subjects having mild discomfort, while others were severely affected, being in considerable pain for as long as a week. The distribution of tenderness over the quadriceps was as previously reported[9] being localized predominantly over the lateral and distal-medial aspects of the thigh. The peak tenderness occurred at 2 days and in most subjects had returned to normal within about 5 days (Fig. 3).

DISCUSSION

We report here plasma CK changes after stepping exercise in approximately half of the subjects that were markedly different to those reported for other types of exercise in terms of the time course and magnitude of the response. As the result of stepping, some subjects showed a very large delayed release of CK. There was a great deal of variation between subjects in their sensitivity to this form of exercise which was not obviously related to sex, body composition, or general fitness. The delayed rise was associated with eccentric contractions and it is possible that this form of stress may be a useful model for exploring the factors that predispose to the release of muscle enzymes.

Most groups who have investigated CK efflux from muscle after exercise measured the circulat-

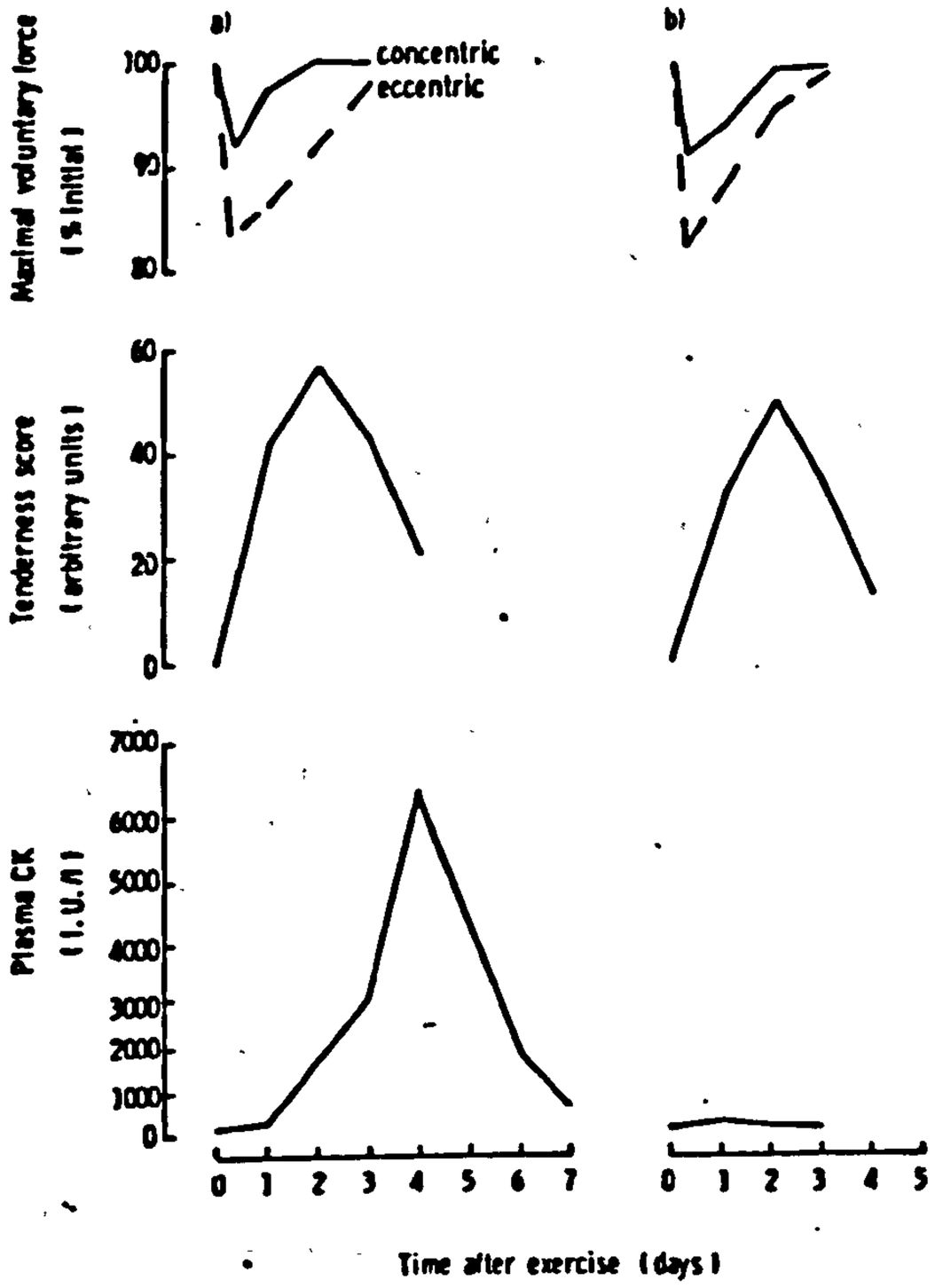

Figure 3. The relationship of muscle force, tenderness, and plasma creatine kinase changes in two subjects after stepping for 20 minutes. Note similar changes in force and tenderness but very different CK changes. (a) Shows the large delayed response while (b) shows a smaller and faster change. Maximum voluntary force was measured in both legs. Muscle tenderness was only experienced in the muscles that had worked eccentrically and the tenderness scores are the sum of the scores at individual sites over the muscle.

ing levels only in the immediate postexercise period.[12,14,22] Those who have studied the time course report peak values occurring less than 24 hours after exercise.[4,5,26] In our study, a number of subjects who exercised for 20 minutes showed the same time course reaching a maximum of three to four times the initial level within 24 hours and returning to normal in a similar time. The other subjects, however, showed very large rises that were characteristically delayed in onset, reached a peak at 4–5 days after exercise and returned to normal in 7 to 9 days. Examples of the two types of response are shown in Fig. 3.

The extent of the delayed CK rise found in this study was generally much greater than has been previously reported for other forms of exercise. Brooke et al.[5] recorded peak values of 1,600 IU/liter occurring 10–20 hours after cycling at 50% maximal oxygen uptake for 2 hours, and Griffiths[14] reported a 24-fold increase immediately after a 53-mile walk, but did not follow the time course. Although large, these increases are still at the lower end of the range of delayed CK rise seen in this study after stepping.

It has been reported that women have less marked enzyme changes after exercise than men.[12,14,26,29] The results of the 20-minute stepping experiment clearly demonstrate that female as well as male subjects can show the large delayed release and the prolonged stepping experiment suggests that, given sufficient stress, all subjects may respond in this way.

The muscle pain and tenderness were variable in magnitude. All the subjects that had large delayed CK rises were also very tender but there were subjects who experienced considerable discomfort without showing the large CK response. The time course of the tenderness and CK release is interesting. In those subjects with a delayed CK rise the tenderness was decreasing at a time when the CK was reaching a peak (Fig. 3), suggesting that a pain producing substance might be acting as a precursor for the subsequent rise in enzyme release.

The reason why stepping should evoke a response that differs from that seen after more usual exercise such as running or cycling must lie in the additional eccentric component that is peculiar to the step test. Although in this work it was not directly possible to decide which leg was responsible for the enzyme release, a comparison with other work makes it clear that the eccentric leg must be responding since the eccentric component is the only difference between stepping and other forms of exercise such as cycling and walking.

When active muscle is stretched, the fibers generate greater tension than when allowed to shorten.[3,18] The metabolic cost of generating a given tension is also lower during eccentric contractions.[1,6,7,17,19,21] Consequently, not only may there be fewer motor units active during eccentric contractions but the metabolic cost of performing the same work is much less. In these circumstances the large delayed rise in plasma enzyme reported here cannot have been a consequence of the energy expenditure of stepping.

It is known that eccentric exercise causes morphological damage to skeletal muscle. Friden et al.[13] found extensive areas of Z-line streaming after downhill running and preliminary results of our own[23] indicate that this occurs specifically after eccentric rather than concentric exercise. It is possible that eccentric contractions, by virtue of the high forces generated, cause an unusual type of

damage either to the contractile elements or the membrane systems of the muscle fiber. This may lead first to the release of algesic substances causing the pain and tenderness and then, in some subjects, initiate a process leading to the release of large quantities of enzyme.

The factors causing enzyme release from the damaged heart have been extensively studied using isolated cardiac muscle preparations and there have been a few similar studies on skeletal muscle.[8,16,27,30,31] These studies have, in general, demonstrated that metabolic depletion either directly causes, or initiates, changes which allow enzyme efflux. This energy dependence is in contrast to the characteristics of the large delayed CK release described here. The work with isolated preparations has also shown that physical damage can give rise to enzyme release, but this process is very rapid, occurring within minutes[16] and is therefore not compatible with the delay of several days reported here.

In isolated preparations, the energy-dependent and damage-related release, with the relatively rapid time course, may well be analogous to the early rise in CK immediately after and during the 24 hours after exercise seen both in this work and in that of many other investigators.

The magnitude of the plasma CK rise seen after running or cycling is normally no more than a fourfold increase. These levels of 200–300 IU/liter are in contrast to the levels found in, for instance, Duchenne muscular dystrophy where the values may range from 1,000–10,000 IU/liter. These are, however, very similar to the large delayed increases seen in some subjects after eccentric exercise and this raises the possibility that the mechanism involved in this form of enzyme release may be more akin to the processes occurring in diseased muscle than are the changes occurring after running or cycling.

There are a number of unexplained features of the large delayed CK rise after stepping, such as the reason for the delay itself and the greater sensitivity of some subjects to this form of exercise. Nonetheless, it appears to be a phenomenon that warrants further investigation and could be valuable in exploring the mechanisms of skeletal muscle damage and repair in man.

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ULTRASTRUCTURAL CHANGES AFTER CONCENTRIC AND ECCENTRIC CONTRACTIONS OF HUMAN MUSCLE

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SUMMARY

Four normal subjects performed a 20 min step test using a step of the same relative height. During the test the quadriceps muscle of one leg contracted concentrically throughout by stepping up, while the contralateral muscle contracted eccentrically by controlling the step down. Thus both muscles performed the same amount of work.

Three subjects had bilateral needle biopsies just prior to exercise. All four had bilateral biopsies immediately after exercise, and 24–48 hours later when the muscles which had contracted eccentrically were painful. The samples were examined by light and electron microscopy.

No abnormalities were seen in pre-exercise samples nor after exercise in muscles which had contracted concentrically.

The muscles which had contracted eccentrically showed some damage immediately after exercise. In the samples taken 24–48 hours after exercise the damage was more marked and involved a greater percentage of fibres.

In view of the known differences between these types of contractions it is suggested that the initial damage is mechanically induced. The exacerbation of damage with time could be due to mechanical or chemical factors.

Key words: *Eccentric contractions – Muscle damage – Muscle ultrastructure*

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INTRODUCTION

In human skeletal muscle the morphological presence of Z-line streaming and disruption of the internal architecture is taken to be a non-specific indicator of damage. These changes have been reported in a variety of pathological conditions including Duchenne muscular dystrophy (Milhorat et al. 1966; Fardeau 1969) malignant hyperpyrexia (Harriman et al. 1977) motor neurone disease (Afifi and Aleu 1966) and various myopathies (Rewcastle and Humphrey 1965; Engel 1966; Gonatas et al. 1969; Dubowitz and Roy 1970; Armstrong et al. 1971; Engel et al. 1971).

It has been shown that eccentric contractions – in which the active muscle is lengthened, result in greater changes of both voluntary and electrically stimulated force generation in human skeletal muscle than do concentric contractions – in which the active muscle shortens (Davies and White 1981; Edwards et al. 1981a; Newham et al. 1983), also that post-exercise muscle pain and tenderness are associated with eccentric rather then concentric contractions (Asmussen 1956; Komi and Buskirk 1972; Talag 1973; Cobb et al. 1975; Edwards et al. 1981b). As this type of muscle pain is not apparent until several hours after exercise we were interested in establishing whether any morphological changes could be seen in the immediate post-exercise period when there was no discomfort and 24–48 h later when pain and tenderness were present.

It has been reported by Fridén et al. (1981) that heavy negative work resulted in morphological evidence of damage in normal human subjects, but a comparison of the effects of performing equal amounts of external work by the two types of contraction has not been investigated before. Fridén et al. performed muscle biopsies before heavy eccentric exercise and then 2 and 7 days afterwards. We report here the morphological investigation of the consequences of performing the same amount of work by means of either eccentric contractions (negative work) or concentric contractions (positive work). These two types of dynamic muscle contractions are particularly interesting as during eccentric contractions both the metabolic energy cost (Asmussen 1953; Nagle et al. 1965; Margaria 1968; Knuttgen et al. 1971; Curtin and Davis 1973) and the electromyographic activity (Bigland and Lippold 1954; Bigland-Ritchie and Woods 1973, 1976) per unit tension are significantly less than during concentric contractions. This occurs because active muscle fibres generate greater tension when stretched than when they shorten (Katz 1939; Asmussen 1956; Komi 1973).

METHODS

Four normal healthy subjects (3 male, aged 22, 23 and 45 years and one female aged 22 years) performed a step test for 20 min. The step was adjusted to be the same relative height for each subject, being 110% of the lower leg length (lateral knee joint line to ground). The quadriceps of one leg contracted concentrically throughout by stepping up while the contralateral muscle contracted eccentrically

by stepping down. Each contraction lasted 1 s and a stepping frequency of 15 cycles/min was determined by an electronic metronome.

Muscle samples were taken bilaterally from the quadriceps using the needle biopsy technique (Edwards et al. 1980). Three of the subjects had biopsies taken immediately prior to exercise and all four were biopsied immediately afterwards and again at between 24 and 48 h later, at a time when the muscles that had contracted eccentrically were painful and tender. The biopsy samples were fixed in phosphate-buffered glutaraldehyde, post-fixed in osmium tetroxide and embedded in Araldite. For histology, semi-thin sections (0.75 μm) were stained with 0.25% toluidine blue. For electron microscopy, ultra-thin sections (60–90 nm) were stained with uranyl acetate and lead citrate and examined in a Philips EM 200 electron microscope. The microscopist was aware of whether each sample was from the right or left quadriceps of each individual and the time of the biopsy in relation to stepping, but did not know which type of contraction the muscles had performed.

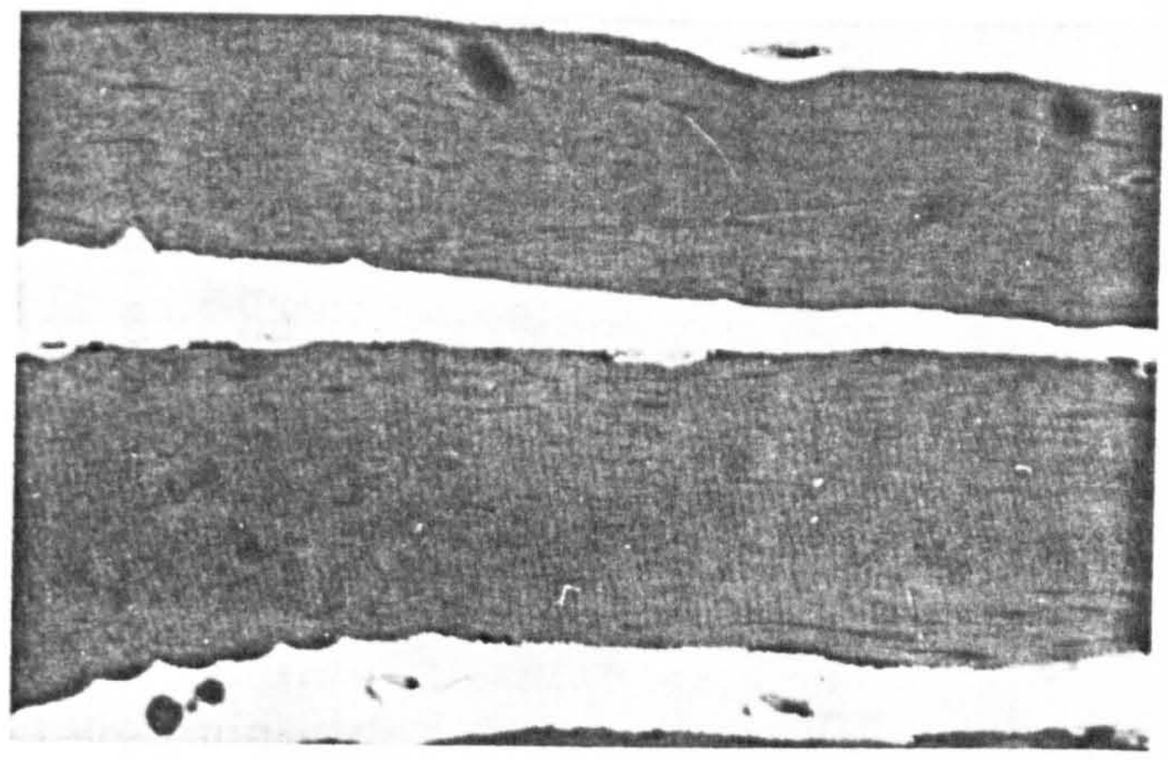
Morphological changes were quantified by careful examination of the toluidine blue sections. About 50 (30–90) individual fibres from each biopsy were studied, and the number of fibres with myofibrillar disruption counted. Areas of disruption affecting one or two adjacent myofibrils and one or two adjacent sarcomeres were designated "focal". Disruption affecting more than two adjacent sarcomeres and more than two adjacent myofibrils were designated "extensive". If a fibre did not contain any areas of this size, but contained more than ten focal areas, the damage was also designated as extensive. In fibres which contained more than one extensive area the damage was designated "very extensive".

RESULTS

The effects of the exercise on force generation and subsequent muscle pain and tenderness have been described elsewhere (Newham et al. 1983a). None of the subjects found the exercise particularly strenuous. At the end of the 20-min exercise period pulse rates varied from 115 to 145 beats/min. During exercise all the subjects reported that the eccentric quadriceps contraction felt virtually effortless while the concentric contractions up onto the step did require some effort, especially towards the end. However, immediately after exercise they were all easily able to extend the knee fully against gravity using the quadriceps which had contracted concentrically, but felt that this required considerably more effort when using the muscles which had contracted eccentrically, and indeed two subjects were unable to gain the last few degrees of extension.

No morphological abnormalities were seen in any of the samples taken either before exercise or afterwards in the muscles which had contracted concentrically. Abnormalities were seen only in muscle samples taken after eccentric contractions. The changes were present in the immediate post-exercise period and were more marked 1–2 days afterwards (Fig. 1).

Light microscopy showed that immediately after eccentric contractions a mean of 16% of the total fibres counted showed focal changes, a similar number



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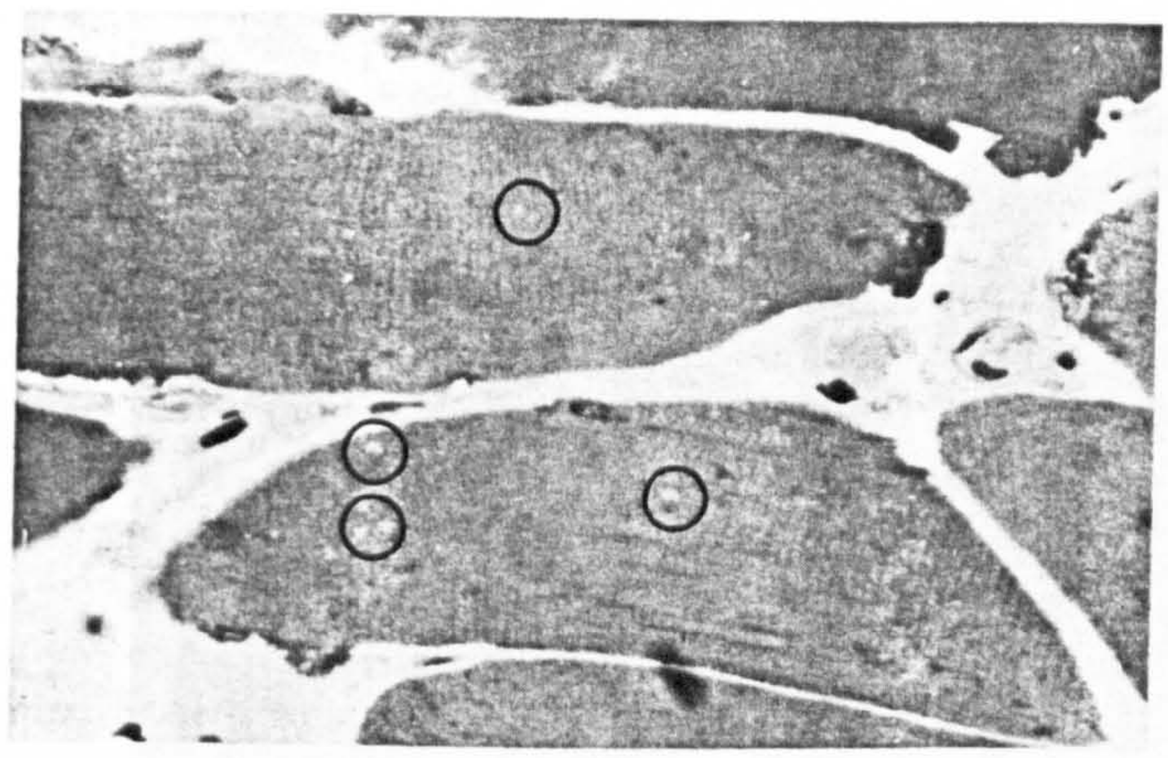

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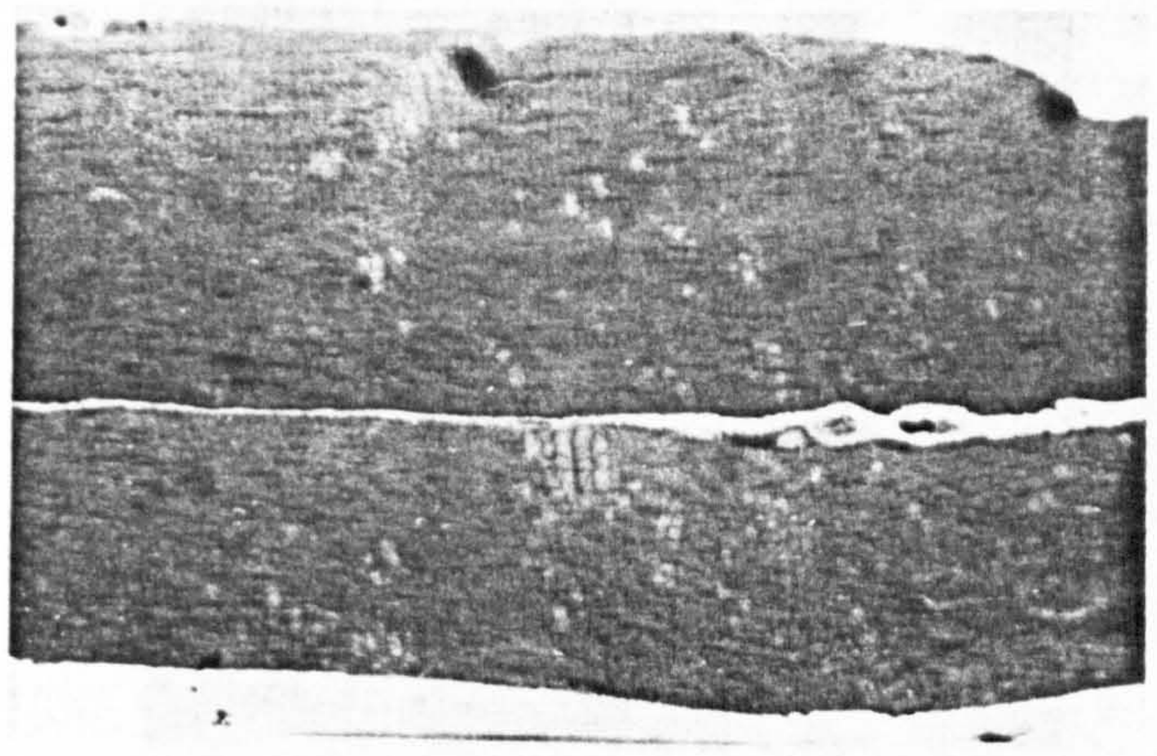

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Fig. 1. Typical histological appearance of muscle biopsy samples in one subject, *a*) before, *b*) immediately after (circles indicate areas of damage) and *c*) 30 h after eccentric contractions. Original magnification × 1000.

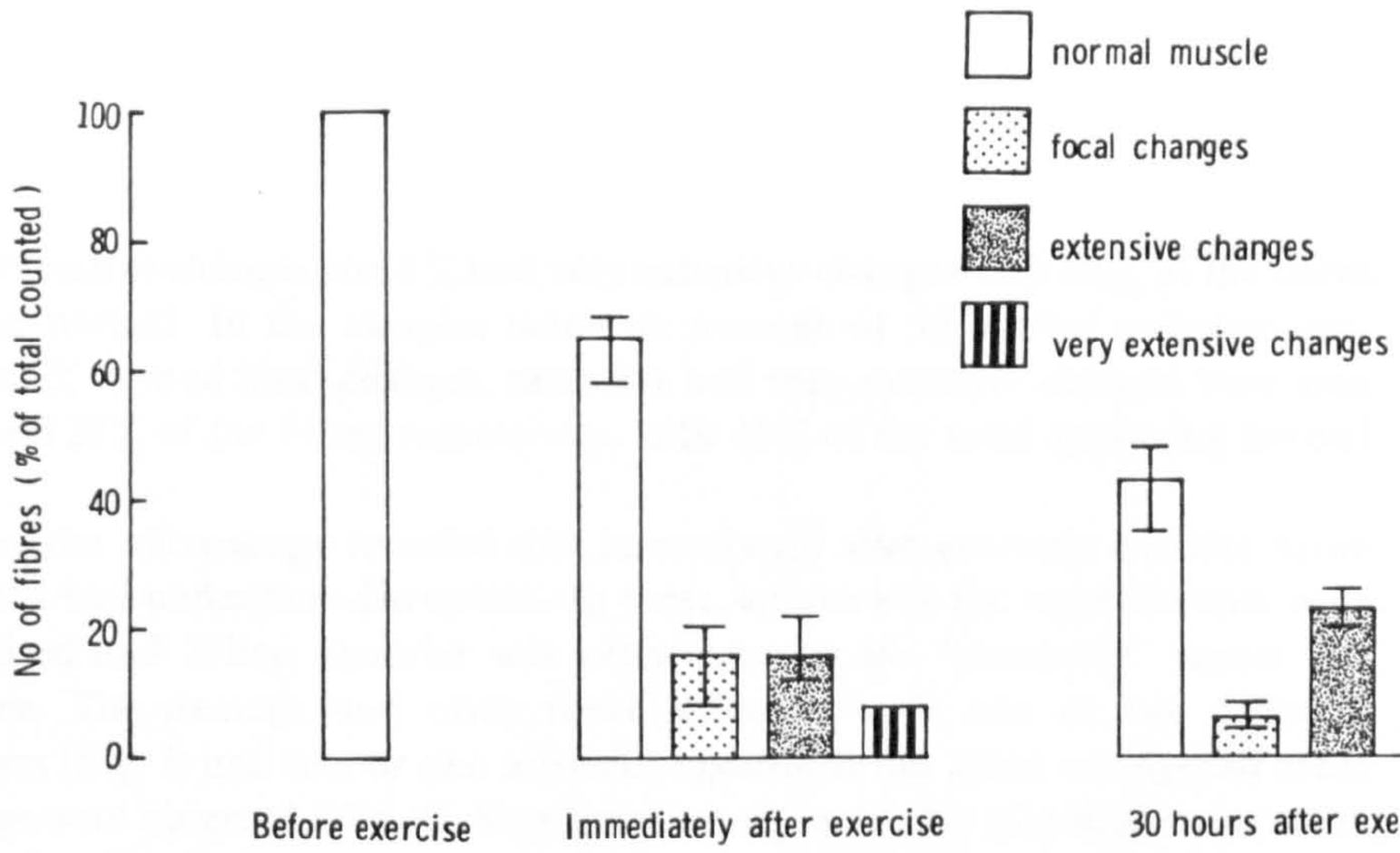

Fig. 2. Quantitation of histological changes in 4 normal subjects (mean and range). Immediately after exercise very extensive changes were only seen in one biopsy sample. See text for details of quantitation method. No changes were seen after concentric contractions.

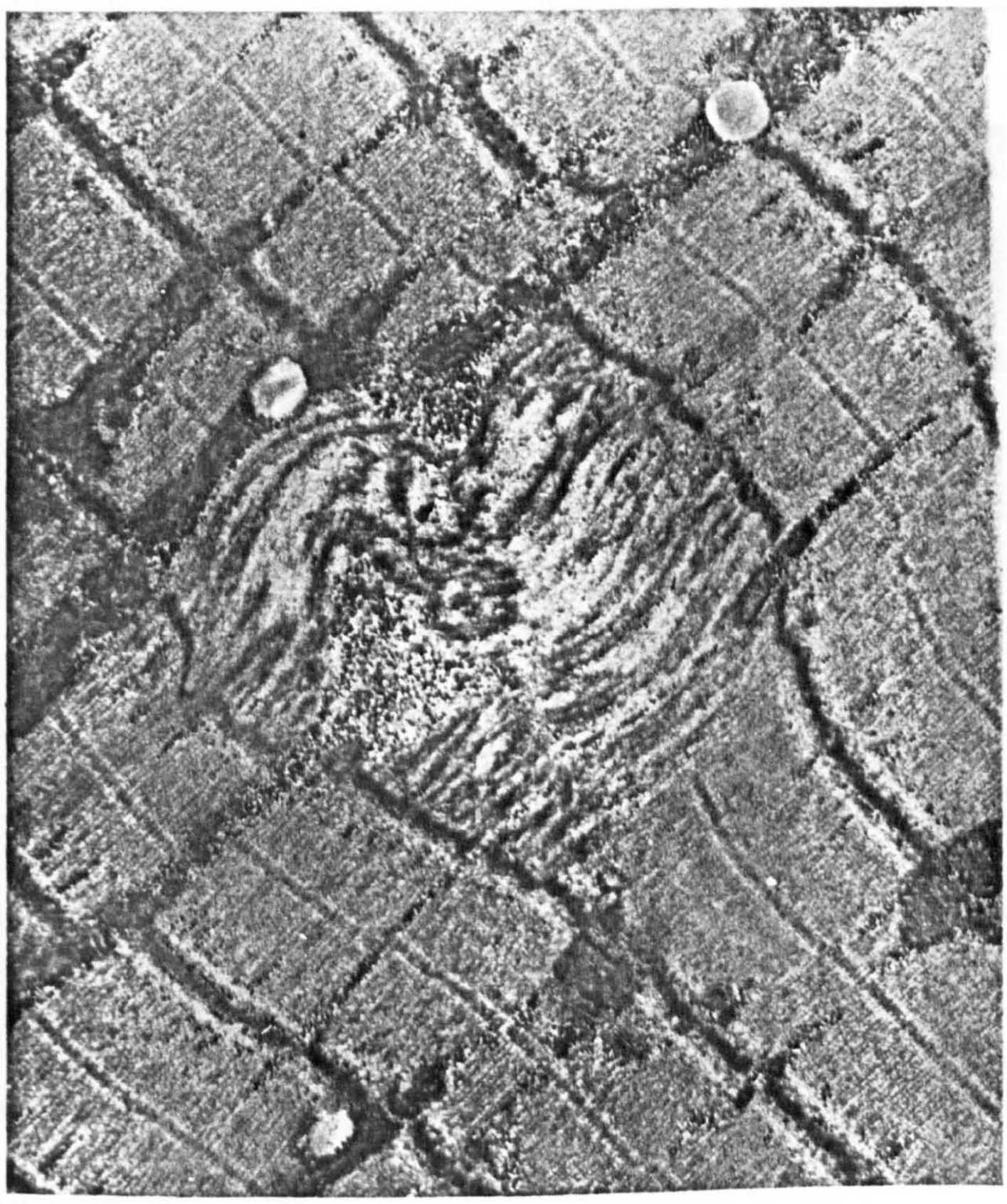

Fig. 3. Focal area of disruption immediately after eccentric contractions affecting one sarcomere and two adjacent myofilaments. The myofilaments are disorganised and there is displacement of the Z-lines. Original magnification × 19,000.

showed extensive changes and 8% had very extensive changes with 58% of the fibres appearing normal. In the samples taken an average of 30 h after eccentric contractions 6% showed focal changes, extensive and very extensive changes were seen in 23% and 28% of the fibres respectively, with 45% of the total appearing normal (Fig. 2).

Electron microscopy revealed that immediately after eccentric exercise many sarcomeres had undergone disruption. In these sarcomeres the myofilaments were disorganised and Z-line material was often seen to be "streaming" across the sarcomere. The damage was often focal, affecting only one or two adjacent sarcomeres (Fig. 3) and one or two adjacent myofibrils but more widespread areas of damage were observed (Fig. 4). Very occasionally only part of a single sarcomere was affected (Fig. 5). In the larger areas there was occasionally complete disruption of the architecture, leaving the myofilaments randomly orientated and the organelles displaced from their usual position. Loss of Z-lines was also seen (Fig. 6). Sarcomeres adjacent to these areas were usually undamaged but occasionally showed mild damage in the form of Z-line streaming over part of the sarcomere (Fig. 7) or an

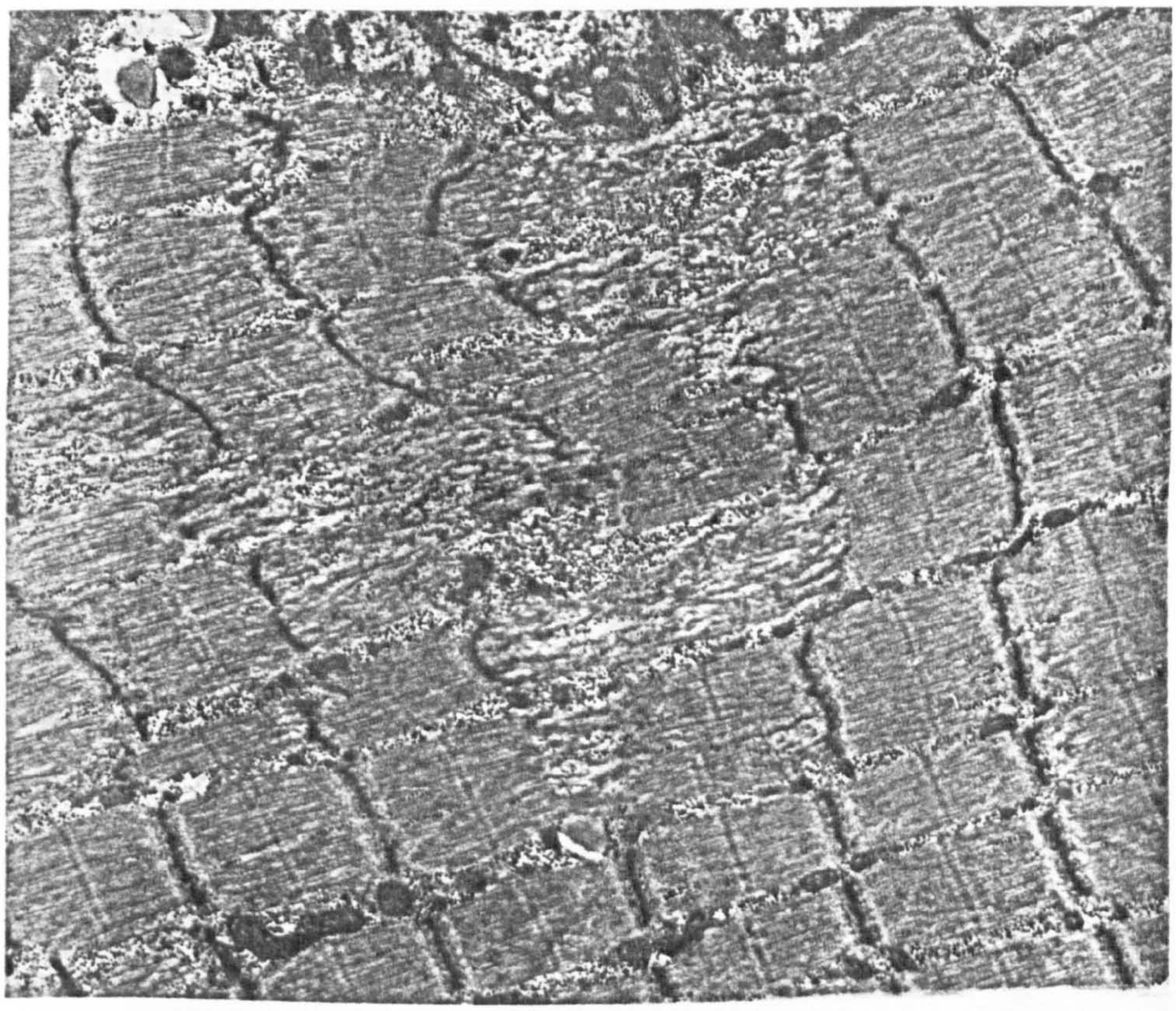

Fig. 4. Extensive area of sarcomere disruption. The damage is similar to that in Fig. 3, but covers a larger area. Original magnification × 19,000.

Fig. 5. Focal area of disruption affecting only part of sarcomere although both Z-lines appear slightly distorted. Immediately after eccentric contractions. Original magnification × 56,000.

apparent widening of the distance between thick and thin myofilaments in the A-band (Fig. 8).

There were a very few areas where groups of adjacent sarcomeres were over-contracted causing a widening of the Z-lines and disorganisation of myofilaments (Fig. 9).

In the biopsies taken a mean of 30 h after eccentric exercise, the damage was essentially the same as that in the immediate post-exercise period but had developed to involve more sarcomeres. Fewer focal areas and more widespread areas were observed.

As previously reported pain and tenderness developed only in those muscles which had contracted eccentrically and changes in force generation were also greater in these muscles than in those which had contracted concentrically (Newham et al. 1983a).

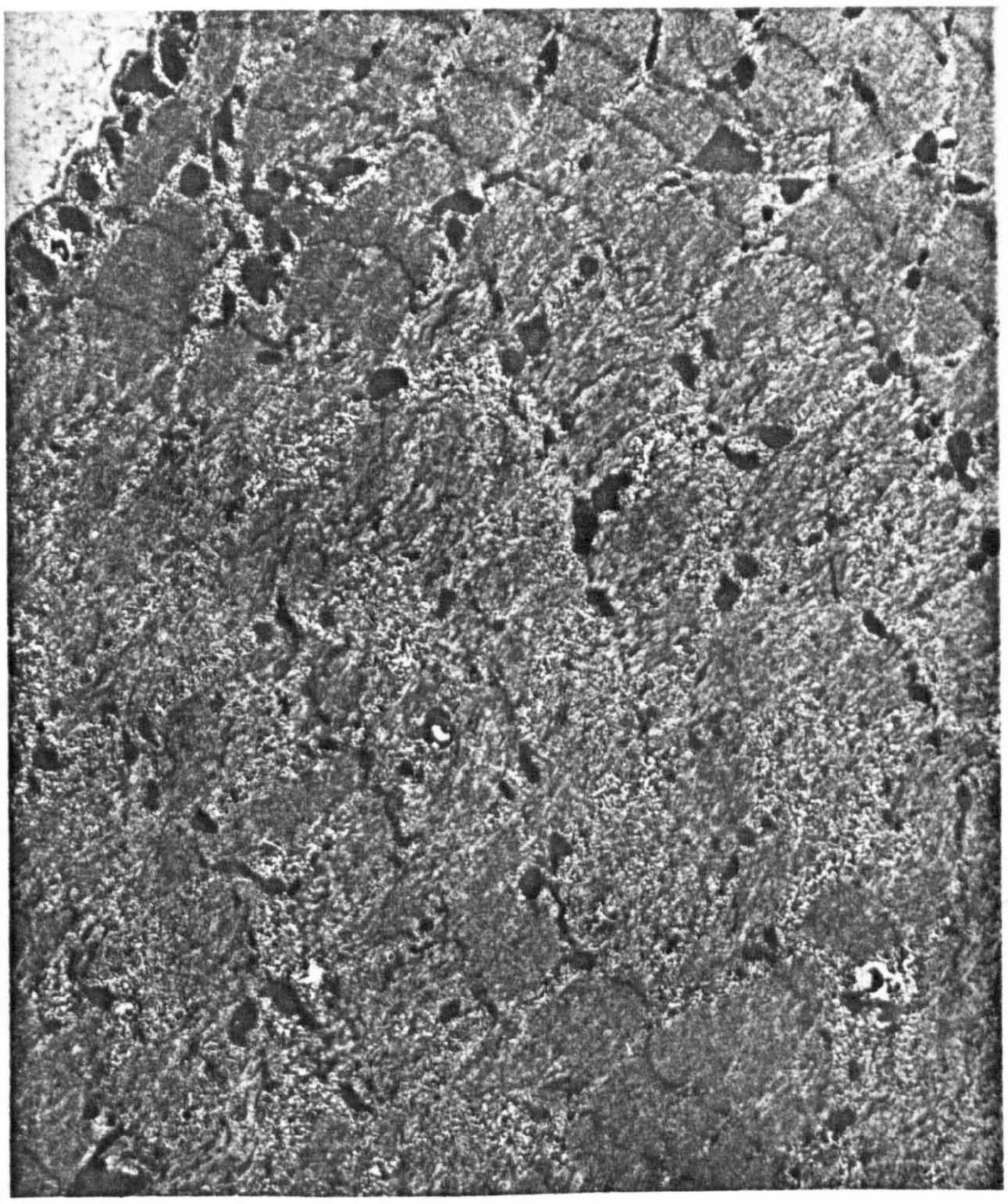

Fig. 6. Very extensive area of architectural disruption. The myofilaments are in disarray and the Z-lines are distorted or absent. Organelles are displaced. Original magnification × 9000.

DISCUSSION

Electron microscopy of needle biopsy samples taken after performing the same total work by means of either concentric or eccentric quadriceps contractions revealed morphological changes only in the muscles which had contracted eccentrically. These changes were present immediately after exercise but unexpectedly were more extensive in the samples taken a mean of 30 h later. Immediately after eccentric contractions, localised areas of damage were seen while in samples taken 24–48 h after exercise there were more extensive areas of damage where the internal architecture of the fibre was disorganised.

Changes of this nature are taken to be a non-specific indicator of damage, and have also been reported as occurring in normal subjects by Meltzer et al.

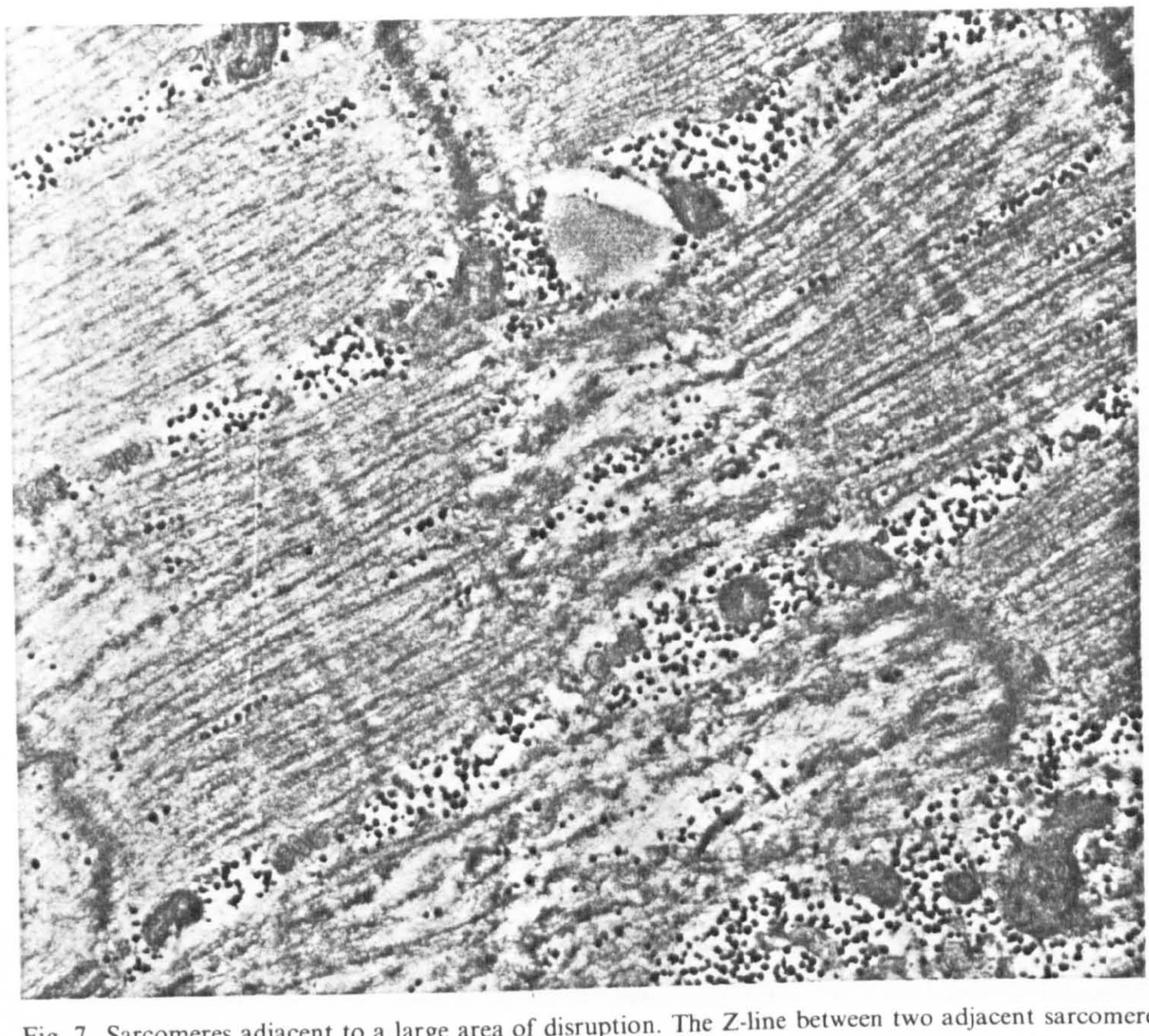

Fig. 7. Sarcomeres adjacent to a large area of disruption. The Z-line between two adjacent sarcomeres is disrupted but the sarcomeres are only partially disrupted. Original magnification × 56,000.

(1976); they have also been seen by ourselves in athletes. In these particular subjects, however, no such abnormalities were seen in the samples taken before exercise nor after exercise in muscles which had contracted concentrically. As the changes reported here were confined to the muscles which had contracted eccentrically they may be taken to be a consequence of this type of contraction. Fridén et al. (1981) reported similar changes after exhausting eccentric contractions, but the morphological consequences of performing the same total amount of work by either concentric or eccentric contractions has not previously been investigated. In the study reported by Fridén et al. biopsies were taken 2 and 7 days after exercise and the area of disruption was at least 3 times less in the samples taken a week after exercise than in those 2 days afterwards and therefore it seems that considerable repair had taken place, as would be expected. It might be thought that the damage is caused only during the exercise period and thereafter the overall trend is towards recovery. However, from our results it appears that the damage develops considerably in the post-exercise period. It is interesting, and previously unreported, that morphological changes are present immediately after exercise at which time there is no muscle pain, but force generation is reduced. One or two days after exercise,

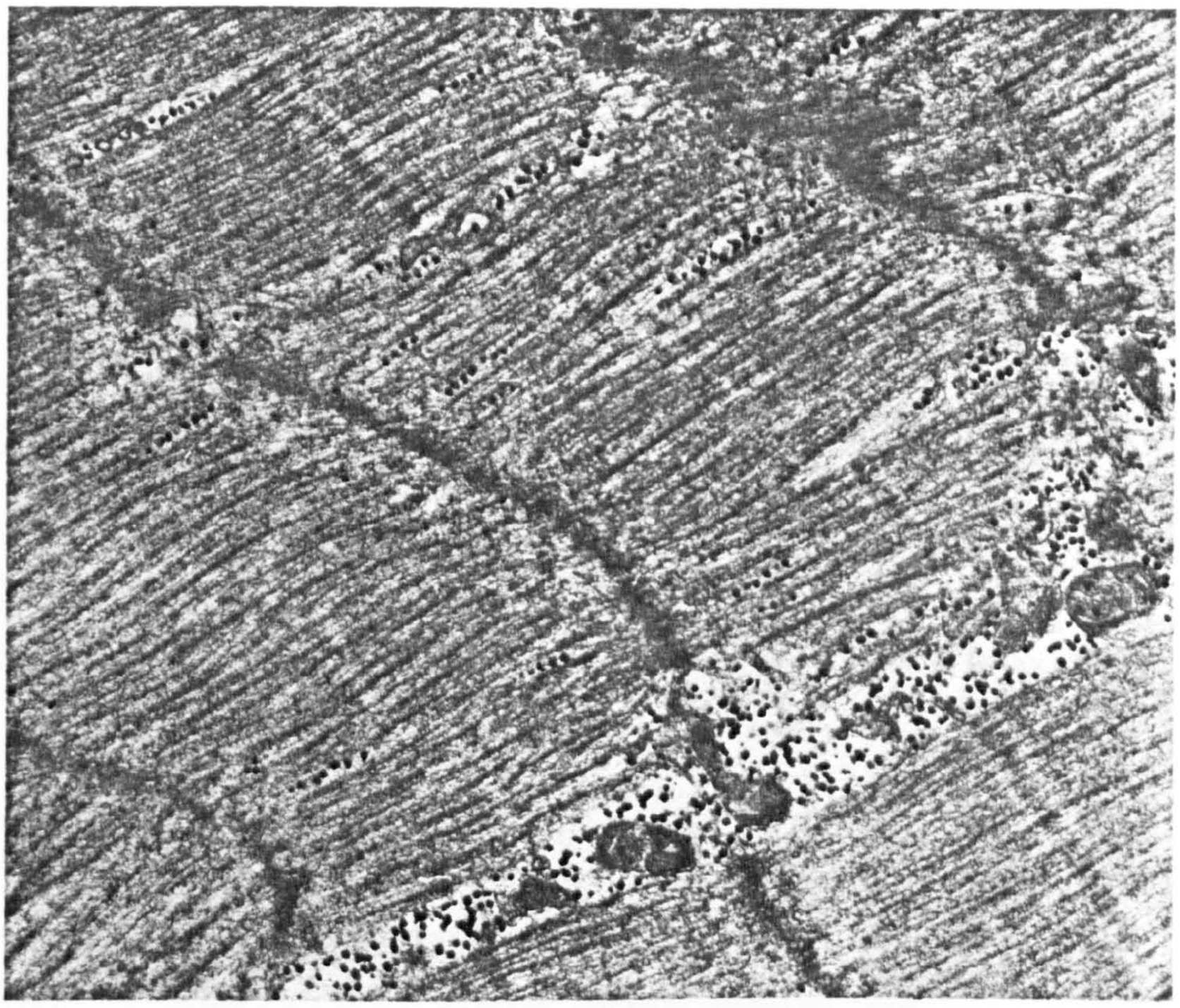

Fig. 8. Sarcomeres adjacent to a large area of disruption. There is apparent widening of the distance between thick and thin myofilaments and the Z-lines are disrupted. Original magnification × 56,000.

muscle pain is at its most severe, force generation has mostly recovered (Newham et al. 1983a) and the morphological damage is more extensive than in the immediate post-exercise period. The time course of the morphological changes seems to reflect better the unexpectedly large and delayed release of creatine kinase into the circulation found in some subjects after the step test (Newham et al. 1983b).

During eccentric contractions there is a reduced metabolic cost coupled with enhanced tension generation in comparison to concentric contractions. Therefore the changes reported here cannot have been determined by metabolic factors – if this were the case they would have occurred predominantly in the muscles which had contracted concentrically. The implication of this is that the initial damage is mechanically induced by the high tensions generated during an eccentric contraction. The amount of integrated electromyographic (IEMG) activity during positive and negative work has been measured (Bigland-Ritchie and Woods 1976; Newham et al. 1983a) and it has been found that at similar work loads and presumably similar muscle forces, the IEMG during negative work is approximately half that recorded during positive work. If the IEMG is taken to reflect the total fibre activity, then during negative work about half the number of fibres are being

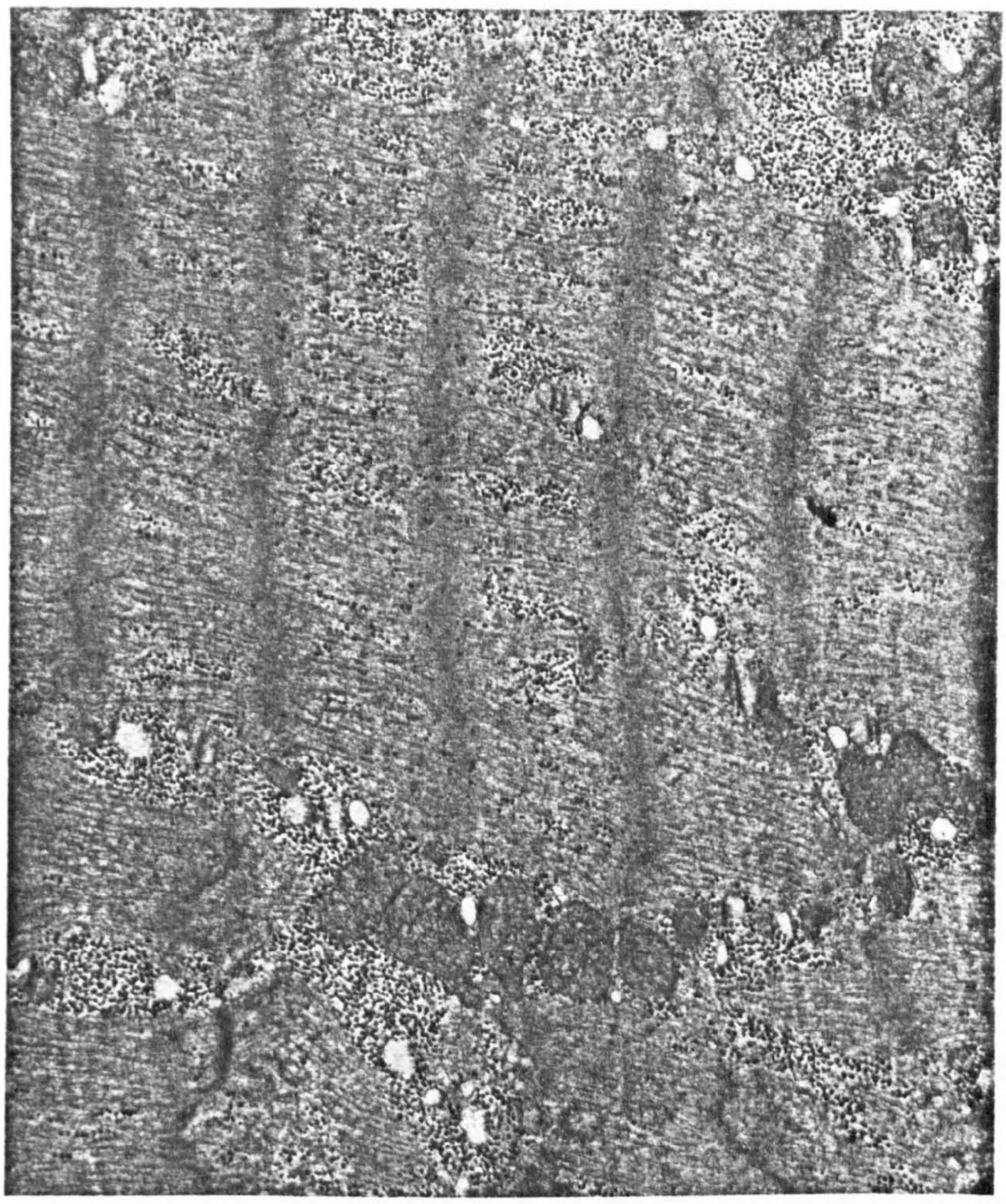

Fig. 9. Large area of over-contracted muscle in which the myofilaments are disorganised and the Z-lines are widened. Note the absence of M-lines and the prominent triads. Original magnification × 19,000.

activated to generate a given tension and therefore the tension per active fibre is doubled.

On electron-microscopic examination the proportion of severely damaged fibres increased in the 24–48 h after exercise (see Fig. 2) as did the total number of affected fibres. The increased severity of the damage implies that the damage progressed from mild to severe in the 1–2 days between the biopsies. Thus the focal lesions seen immediately after exercise were the precursors of the more extensive damage seen in the later biopsies. The increase in total number of affected fibres suggests that some damage occurs *de novo* during the time after the exercise. However, close EM examination of the samples taken immediately after exercise showed the presence of many areas of damage often affecting only half a sarcomere (Fig. 5). These would not be visible on light-microscopic examination of the toluidine blue sections and thus the estimates of unaffected fibres would have been

too low. It seems likely that the initial exercise causes microscopic damage, focal lesions at the level of individual sarcomeres which become progressively more extensive during the next few days. Why the damage should progress is not known. It may be that the apparent increase in damage is part of the inevitable process of repair and regeneration; alternatively it could be that activity of the muscle following the initial changes simply exacerbates the damage. The effects of bed rest following exercise might answer this point.

The cause of the initial damage is also not clear. From the appearance of the sarcomeres it might be supposed that the actin and myosin filaments had been pulled apart. This might occur if there were local inhomogeneities in the sarcomeres in series. The damage could also be the result of damage to the surface membrane with the entry of extracellular calcium and activation of proteases and lipases.

Vihko et al. (1978, 1979) have shown that the activities of certain acid hydrolases are increased 5–7 days following heavy exercise in mice. The increase was associated with fibre degeneration and necrosis with a marked inflammatory response. There exists in animal skeletal muscle a calcium-activated protease enzyme which causes specific removal of Z-lines (Busch et al. 1972) and is thought to be involved in myofibrillary protein turnover (Reveille et al. 1976). It is not known whether such an enzyme exists in man, but if it does it is possible that an increase in intracellular calcium during subsequent exercise may activate the enzyme to increase the damage.

This study has produced some interesting results which may have important clinical relevance. Many myopathies have a proximal distribution and it is those muscles which regularly perform eccentric contractions in their postural, anti-gravity role. In contrast some distal muscles (e.g. in the hands) seem to be involved in very few eccentric contractions during normal activity. It may be that the regular performance of eccentric contractions may render muscles more susceptible to myopathic processes. The extent of the morphological abnormalities reported here in normal subjects is surprising, particularly in view of the fact that the exercise was neither exhausting nor of long duration. If similar stresses are imposed upon myopathic muscle it is interesting to speculate about the ability of the muscle to recover. Many myopathic patients are prescribed strengthening exercises and it may be that a significant involvement of eccentric contractions in these exercises may not be beneficial but actually harmful.

Further work is required to determine the susceptibility of patients with various myopathies to such exercise-induced changes. In both patients and normal subjects it would be useful to determine the time course of both the damage and recovery processes. Such work would lead to a greater understanding of these mechanisms and also the effects and therapeutic role of specific types of muscle contractions.

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Force, Contraction Frequency and Energy Metabolism as Determinants of Ischaemic Muscle Pain

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Summary

Ischaemic muscle pain was studied in a total of 78 experiments on 3 normal subjects. The development of pain induced by intermittent voluntary isometric contractions was recorded whilst force and frequency of contraction were varied. As the muscle progressively expends energy, pain (P) develops in an exponential fashion and can be expressed by the function $P = (F \times t)^k / C$ where $(F \times t)$ is the integral of force with time, k, an exponent, and C, a constant. The exponent is fairly constant despite changes in frequency. The time of pain onset and energy expenditure at the point of intolerable pain depend on frequency. At a given frequency, energy expenditure at intolerable pain is independent of the force of contraction.

Introduction

Ischaemic muscle pain and the factors which govern its development were first studied by Lewis et al. [2]. Subjects performed isometric handgrips at various rates and forces under ischaemic conditions and the times of pain onset and intolerable pain were noted. Lewis found that pain was related to the amount of exercise, in that handgrips performed once every 4 sec produced pain later than once every 2 sec, and handgrips of submaximal force produced pain later than those at full force. Because there was no relief of pain between handgrips, Lewis concluded that pressure on nerve elements played no part in pain generation. He also concluded that since up to 20 min pre-ischaemia made no difference to the time of pain development that hypoxia played no role. His final conclusions were that a stable factor accumulated in active muscle cells which diffused slowly into the extracellular space to stimulate pain nerve endings. Although Lewis implied a linear relation between energy

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expenditure and pain development, this was not demonstrated clearly, and in his comparison of different frequencies of contraction, the work/rest ratio was not constant and average energy expenditure was therefore not comparable between experiments.

McArdle and Verell [4] used a similar experimental model and showed that the time to intolerable ischaemic muscle pain was related to contraction frequency; however, contractions were isotonic and energy expenditure was not estimated. They did not confirm the linear relation between energy expenditure and pain implied by Lewis, but described a decreasing relationship, with high frequencies causing relatively more pain than lower ones. Parke and Rodbard [5] also examined the effect of load and frequency of contraction on the development of forearm ischaemic muscle pain. They confirmed the non-linear relation between pain and contraction frequency and fitted an empirical polynomial equation to the curve, the physiological significance of which is not clear. Again the contractions used were not isometric, energy expenditure was not estimated and the average work rate at different contraction frequencies was not kept constant.

From these studies it is clear that the pain developing in an intermittently contracting muscle depends on the frequency of contractions, the force of contraction, and the time for which the contractions continue. The interrelations of these factors and the role of total energy expenditure are not apparent.

We have re-examined the problem by estimating energy expenditure during exercise, by limiting activity to a single muscle acting isometrically, and by recording subjective pain perception throughout the experiment.

Methods

In 2 subjects, 48 experiments each were performed at 4 force levels (25%, 50%, 75% and 100% maximum voluntary contraction force) and at frequencies of 2, 3, 6 and 30/min, each combination being repeated 3 times. In 3 subjects, experiments at 10 frequencies (from 3/min to 120/min) at 50% maximum force were performed.

The left hand and forearm were immersed in a water-bath at 45°C for 10 min. The limb was immobilised on a hand-board [1] and the force of isometric contractions of the adductor pollicis recorded on an UV recorder (SE Labs Type 3006/DL).

Energy expenditure was estimated using the integral of force with time, electronically derived from the force signal. The maximal voluntary contraction force was determined and from this a visual target was displayed which was 25, 50, 75 and 100% of the maximum. Contractions were performed in time with an auditory signal at 10 frequencies ranging from 3 to 120/min. In all cases the contraction was followed by an equal period of rest, i.e., the work/rest ratio was unity. At each force level continuous contractions to intolerable pain were also made.

Subjects were asked to assess pain continuously in the active muscle by using a sliding potentiometer which was reset at random intervals, effectively rendering its scale infinitely long (Fig. 1). The force and frequency used in each experiment were presented in random order.

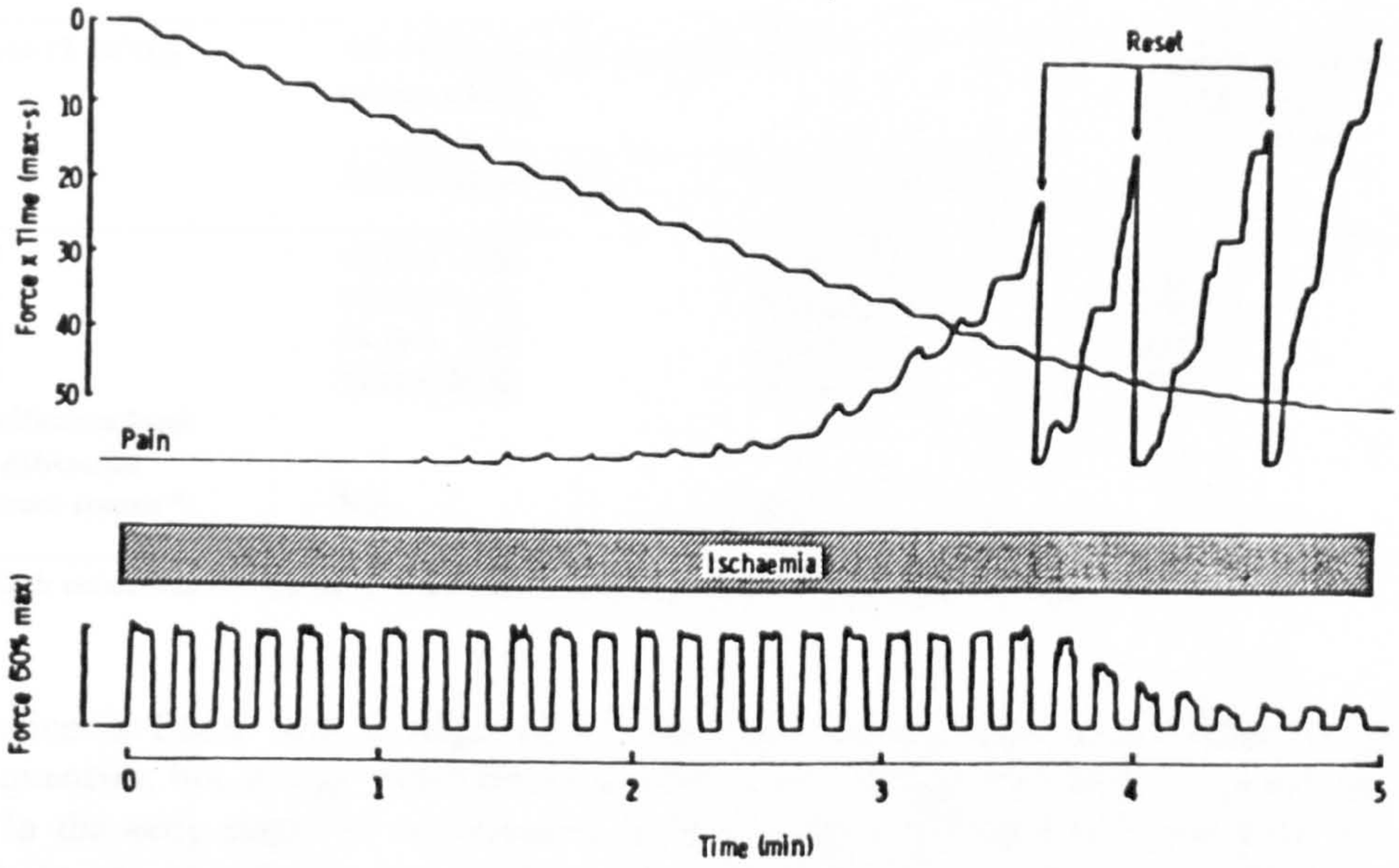

Fig. 1. Records of force, force-time integral (F×t) and pain during an experiment in which the subject performed 50% maximal contractions at a rate of 6/min. The random resets of the pain potentiometer can be seen. Note how pain initially returns to zero between contractions but then increases exponentially.

At the start of each experiment a pneumatic cuff around the upper arm was inflated to 200 mm Hg and contractions started immediately. When the subject reported intolerable pain, contractions stopped, the cuff was deflated and the experiment terminated. A typical record is shown in Fig. 1.

A minimum of 2 h elapsed between successive experiments on the same subject.

Results

The integral of force with time (F×t) is expressed in equivalent seconds of maximum force (Max − s), derived by dividing the integral in Newton-seconds by the maximum voluntary force in Newtons.

At a given frequency F×t at intolerable pain was independent of the force produced during the experiment. Table I shows data obtained from 1 subject at 2 frequencies and 4 force levels.

The relationship between F×t at intolerable pain and frequency of contractions is shown in Fig. 2 in which F×t is expressed as a percentage of the F×t at intolerable pain with a continuous contraction. It can be seen that at frequencies above 60/min F×t at intolerable pain is only some 40% of that achieved with a continuous contraction or with very low frequencies such as 3/min.

The relationship between energy expenditure (F×t) and pain (P) can be expressed as $P = (F \times t)^k$/Constant (Fig. 3). When plotted on logarithmic scales, the

TABLE I

Force (% MVC)	F×t at intolerable pain (max−s) (mean±S.D.)		Significance level for difference between means
	3/min contractions	30/min contractions	*P*
100	50.53± 9.80	29.70±7.61	0.05
75	52.70±12.12	34.80±5.25	N.S.
50	54.37± 1.22	41.26±6.42	0.05
25	51.43±14.32	31.94±8.57	N.S.
Significance level for difference between means *	N.S.	N.S.	

* Each mean was compared with all others at that frequency using a paired *t* test.

relation is linear with a slope of k. The slopes of the lines were similar at all frequencies, but energy expenditure at pain onset was lower at higher frequencies.

In the early stages of experiments at frequencies of 10/min and less, pain was noted to develop during contractions and to diminish during rest periods, initially to zero (Fig. 1). In later phases of these experiments, and throughout experiments at higher frequencies the pain increased continuously.

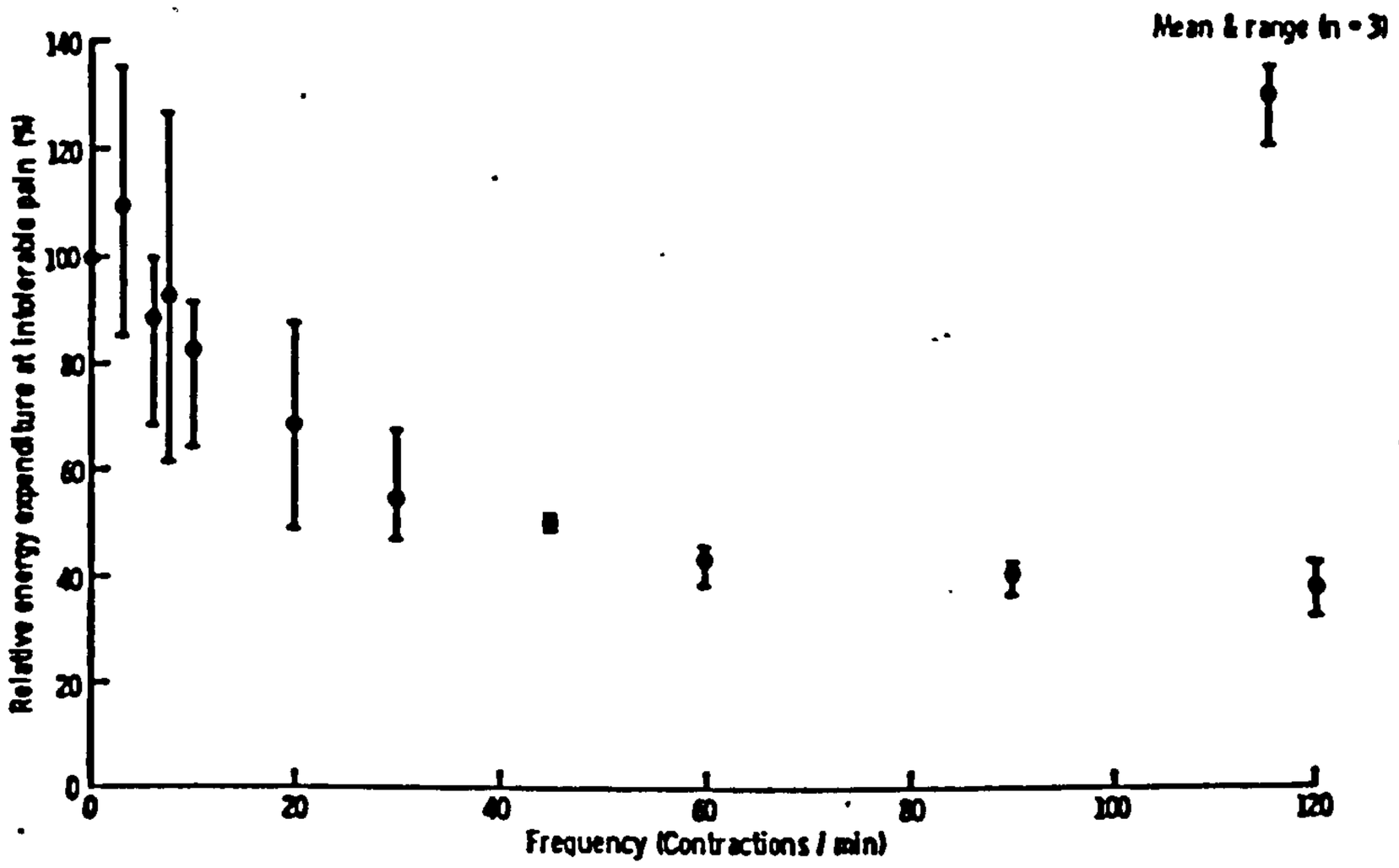

Fig. 2. The relationship between energy expenditure at intolerable pain and frequency of 50% maximal contractions. Energy expenditure (estimated as F×t) is expressed as a percentage of that of a continuous contraction at 50% maximal force.

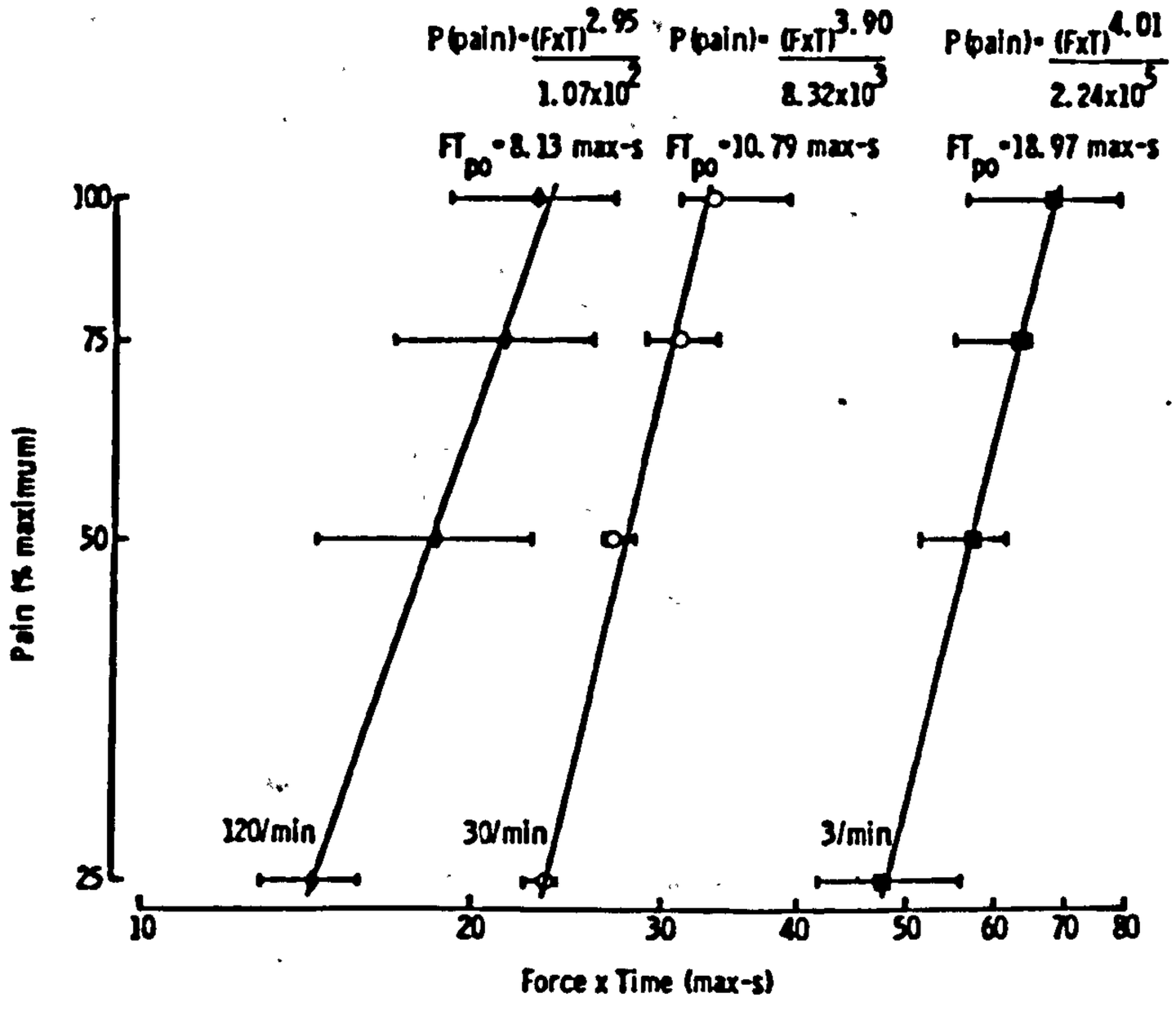

Fig. 3. Pain as a function of energy expenditure at frequencies of contraction of 120/min, 30/min and 3/min. The variables are plotted on logarithmic scales and produce straight lines ($r = 0.99$, $P < 0.001$) in all 3 cases. Symbols represent means and horizontal lines the ranges. Energy expenditure at pain onset (FT_{po}) is indicated.

Discussion

The major factors governing the development of pain during intermittent ischaemic muscular contractions are the energy expended by the muscle and the frequency of the contractions. Force of contraction plays only a minor role. If pain is estimated at the same energy expenditure but at different frequencies, the higher the frequency, the more severe the pain. The fact that pain comes on earlier at higher frequencies suggests that pain generation is influenced by rate of change of force rather more than by absolute force.

It may be argued that at higher frequencies of contraction the muscle would perform more work against its own internal elastic component and that this would cause underestimation of the energy expenditure as measured here. However, it can be seen from Fig. 2 that energy expenditure at intolerable pain was almost the same at 60/min as at 120/min, whilst internal work must have been considerably more at higher frequency.

The mechanism of the effect of frequency of contraction on pain development is not clear, but it may reflect central interaction of muscle chemo- and mechano-

nociceptors shown to be present in cat muscle [3]. Some of these receptors provided a signal related to rate of change of force which could provide the information about frequency of contraction, as could muscle spindle receptors.

The development of pain along an exponential function is expected from the general psychophysical law [7]. If energy expenditure at a given frequency during the experiment is assumed to progress linearly with time, then this would represent a continuously increasing stimulus which is interpreted centrally in an exponentially increasing fashion. An alternative explanation is that a pain producing factor accumulates peripherally, concentrating not in a linearly increasing fashion but accelerating towards the termination of the experiment.

The importance of the frequency component has implications in many test situations, as force is often considered the key factor in pain production. Any test designed to measure ischaemic muscle pain should take into account the effect of contraction frequency.

Acknowledgements

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References

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The author has, in partial fulfilment of the requirements for the degree, attended and contributed to the weekly Medical Research Seminars at The Department of Medicine, University College London School of Medicine, as well as meetings of The Physiological Society, The European Society of Clinical Investigation and The Medical Research Society.