J. Physiol. (1986), **375**, *pp.* 435–448
With 8 text-figures
Printed in Great Britain

EXPERIMENTAL HUMAN MUSCLE DAMAGE: MORPHOLOGICAL CHANGES IN RELATION TO OTHER INDICES OF DAMAGE

BY D. A. JONES, D. J. NEWHAM, J. M. ROUND AND S. E. J. TOLFREE*
*From the Departments of Medicine and *Nuclear Medicine, University College London, The Rayne Institute, University Street, London WC1E 6JJ*

(*Received 6 June 1985*)

SUMMARY

1. The effects of eccentric exercise have been examined in human calf and biceps muscles. Release of muscle creatine kinase and uptake of technetium pyrophosphate have been followed for up to 20 days after the exercise and the results are related to the morphological changes seen in needle biopsy samples.

2. The response to exercise was variable, all subjects developing pain and tenderness in the exercised muscles after 1–2 days and this was followed, in most subjects, by a large increase in plasma creatine kinase 4–6 days after the exercise. This was paralleled by an increased uptake of technetium pyrophosphate into the exercised muscle.

3. Biopsies of the affected muscles showed little or no change in the first 7 days after the exercise but later degenerating fibres were seen, as well as infiltration by mononuclear cells and eventually, by 20 days, signs of regeneration. Very extensive changes were seen in the calf muscle of one subject; changes in the biceps were qualitatively similar but not so severe. In the severely affected calf muscle type II fibres were preferentially damaged.

4. Mononuclear cell infiltration both between and within degenerating fibres was maximal well after the time of peak plasma creatine kinase and it is likely that in eccentrically exercised muscle infiltrating mononuclear cells act to scavenge cellular debris rather than to cause damage to the muscle

INTRODUCTION

It is well established that contractions which involve stretching active muscle (often known as eccentric exercise or negative work) result in muscle pain and tenderness which develops some hours after the exercise and may last for several days (Asmussen, 1953, 1956; Brendstrup, 1962; Komi & Buskirk, 1972; Davies & Barnes, 1972; Newham, Mills, Quigley & Edwards, 1983*a*). Recent work has shown that this type of activity, especially if severe or unaccustomed, also causes damage to muscle fibres. There have been reports of disruption of sarcomere architecture (Friden, Sjostrom & Ekblom, 1981, 1983; Newham, McPhail, Mills & Edwards, 1983*c*) and damage to the surface membrane, as indicated by the release of soluble muscle enzymes, most notably creatine kinase (CK) (Schwane, Johnson, Vandernakker & Armstrong, 1983; Newham, Jones & Edwards, 1983*b*, 1986).

There are two features of the CK release that are of particular interest. The first is the magnitude of the response; in normal subjects the resting plasma CK is around 100 i.u./l while in severe myopathic conditions this may rise to about 10000 i.u./l (Pennington, 1981). In untrained normal subjects eccentric exercise has given rise to circulating levels ranging from 1000 i.u./l to as high as nearly 40000 i.u./l (Newham *et al.* 1983*b*), implying a very considerable degree of muscle damage. The second feature is the delay between the exercise and enzyme release. In the first 1 or 2 days, when the pain is maximal, the circulating CK is relatively normal but it then rises to a maximum between 3 and 6 days after the exercise (Newham *et al.* 1983*b*). This is in contrast to increases in circulating CK as the result of more conventional forms of exercise, such as running on the level or cycling, which involve very little eccentric exercise. Here the rise in CK has generally been reported to be smaller and the time course more rapid, usually reaching a peak within the first 24 h (Kagen, 1972; Thomson, Sweetin & Hamilton, 1975; Shumate, Brooke, Carroll & Davis, 1979; Brooke, Carroll, Davis & Hagberg, 1979).

The reason for the delay following eccentric exercise before changes in membrane permeability become apparent is not known. One possibility is that the initial exercise causes relatively minor damage to the muscle but that this is sufficient to activate the immune system, resulting in attack and destruction of muscle fibres; the time required for this could account for the delay in the release of soluble muscle enzymes. In this case a coincidence might be expected between infiltration of the muscle by mononuclear cells and release of soluble enzymes into the circulation. There is some evidence from animal work to suggest this is the case. Armstrong, Ogilvie & Schwane (1983) found evidence of delayed muscle damage in rats exercised by running down an inclined treadmill and this was accompanied by fibre necrosis and invasion of the muscle by macrophages. In human muscle, however, although disruption of sarcomere structure has been seen, there have been no reports of muscle fibre degeneration or cellular infiltration at 2 or 7 days after eccentric exercise of the calf muscles (Friden *et al.* 1981) or at 3, 5 or 10 days in the quadriceps after stepping (Newham *et al.* 1983*c* and our own unpublished observations). Stepping exercise is a complex movement in which a number of muscle groups are exercised eccentrically, and it is not certain that in the earlier study (Newham *et al.* 1983*c*) the biopsied quadriceps were the muscles most severely damaged by the exercise. We have therefore investigated the effects of eccentric exercise on two other muscle groups that can be safely biopsied, the forearm flexors and the calves. Measurements have been made of the uptake of radio-labelled technetium pyrophosphate to visualize the damaged areas, the release of CK, an index of the extent and time course of the damage, and the histological appearance of biopsy specimens from these muscles.

METHODS

Subjects. The subjects were either the authors themselves or colleagues, aged between 23 and 55 years. All were normally active but not undergoing any regular training programme and had not been involved in any experimental work involving eccentric exercise of the muscle group studied within the previous 6 months. The subjects were fully informed of the nature and possible risks of the various procedures, all of which had the approval of the Committee for Ethical Procedures, University College Hospital.

Exercise. To exercise the forearm flexors the subject sat in an adjustable chair with the upper arm supported on a shelf, the height of which could be adjusted to bring the arm to 90 deg with the body. From a cuff around the wrist an inextensible cord passed through a pulley attached to a strain gauge fixed to the wall in front of the chair and thence to the experimentor, who could forcibly extend the forearm using a winch with a mechanical advantage of about 10:1. Details of the procedure have been given by Jones & Newham (1985*a*).

The calf muscles were exercised by the subject walking backwards down an inclined treadmill. As the subject extended the leg backwards contact with the belt was first made by the plantar-flexed foot, and as the weight was transferred to the ball of the foot and heel the active calf muscles were stretched. Details of the procedure have been given by Newham *et al.* (1986).

Muscle biopsy. Muscle samples were obtained by the percutaneous needle biopsy technique described by Edwards, Jones & Round (1983) using a suction attachment. Biopsies of the calf (gastrocnemius) and biceps were taken from the belly of the muscle. Repeated biopsies were taken from adjacent sites separated by 1–2 cm. Muscle fibre bundles were orientated under a binocular microscope, frozen in isopentane cooled in liquid nitrogen, and transverse sections were stained with haematoxylin and eosin, for acid phosphatase activity, NADH tetrazolium reductase and for myosin ATPase after pre-incubation at pH 9·4, 4·3 or 4·6 (Round, Matthews & Jones, 1981).

Plasma creatine kinase. This was determined by the Department of Chemical Pathology, University College Hospital, using a Boehringer Mannheim kit method with NAC activation. The normal range of plasma CK using this method is 60–120 i.u./l.

Radionuclide uptake. ^{99m}Technetium pyrophosphate (TPP, 'Pyrolite', New England Nuclear Ltd.; 1·5 mCi in 0·1 ml saline) was injected into an antecubital vein. 3 h later the muscle group of interest was positioned in front of a Siemens 37 ZCL gamma camera and an image recorded by collecting 200 K counts. Depending on the extent of the muscle damage this took 20–40 min. The image was stored as a 128 x 128 matrix which could be manipulated for purposes of quantitation. The densities of areas of interest were compared with that of bone not overlaid by muscle (e.g. the olecranon process or patella) and the uptake into muscle expressed as a ratio of muscle counts per pixel/bone counts per pixel. ^{99m}Technetium has a short half-life of 6 h and the normal single dose for diagnostic purposes is around 15 mCi. Subjects received approximately 1·5 mCi on each occasion.

RESULTS

Biceps exercise

Seven subjects performed eccentric contractions of the biceps with their non-dominant arm. The first (D. C.) made maximum voluntary contractions while the forearm was forcibly extended at a rate of once a minute for 20 min. The other subjects performed the same exercise except that after 20 min the frequency was doubled to two contractions a minute and this was continued until the force generated was less than 50 % of the force at the start of the exercise. Biopsies of the biceps were taken from five subjects at intervals after the exercise.

Pain and tenderness in the biceps developed and was maximal 24–48 h after exercise. The discomfort extended to include the brachioradialis muscle. The impression was that the brachioradialis tenderness developed with a longer time course than that in the biceps.

All subjects had a delayed increase in plasma CK which was maximum at about 4–6 days (Fig. 1*A*).

In three subjects repeated TPP scans were performed spanning the time when maximum CK release was seen. The results for subject D.J. are shown in Fig. 2. The area of increased TPP uptake was largely restricted to the biceps on days 3 and 4. This then spread to include the brachioradialis on days 7 and 8. The time course of TPP uptake into the biceps in the three subjects was similar to that of CK release (Fig. 1*A*).

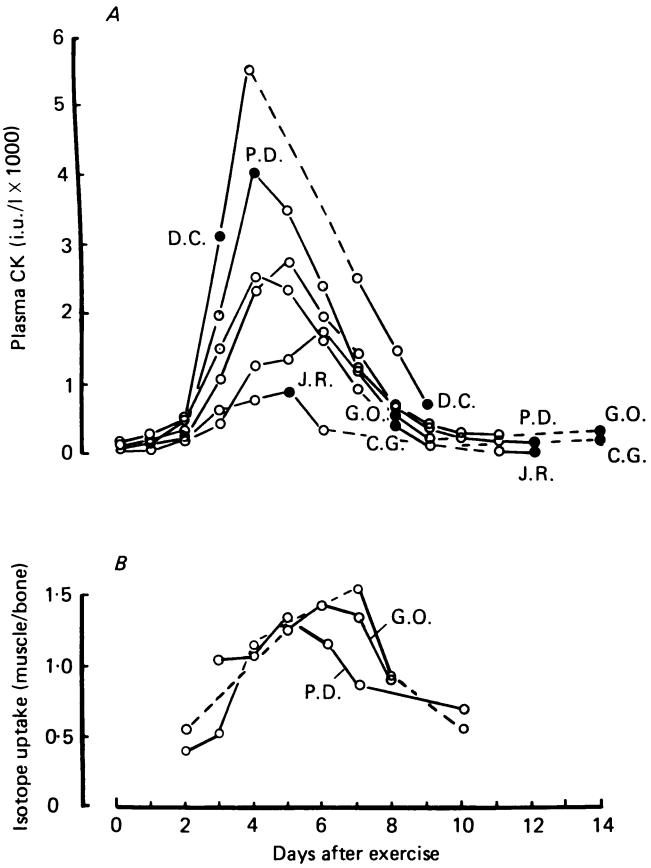

Fig. 1. Plasma CK (*A*) and muscle TPP uptake (*B*) after eccentric exercise of the biceps. Six subjects exercised until the maximum voluntary force was reduced to 50%. Five subjects had biopsies at times indicated by the filled symbols and are identified by initials which correspond with those in Table 1. Values for TPP uptake (*B*) are given for three subjects, two of whom also had biopsies. Dashed lines join points separated by more than 24 h.

Two biopsies were taken from each of five of the subjects, one close to the time when peak plasma CK was expected, and the other about 1 week later.

In three of the five subjects, the first biopsy showed essentially normal muscle (Fig. 3*A*); in the other two there were minimal changes, with a single small area of damage in each biopsy where a few degenerating cells showed high acid phosphatase activity, indicating lysosomal activity. In all five cases the second biopsy showed more extensive changes (Fig. 3*B*) with mononuclear cell infiltration, particularly in perifasicular areas and increased intra- and extracellular acid phosphatase activity. Some regenerating cells were also seen. Details of the histochemical appearance are summarized in Table 1 together with the time of peak plasma CK. The major cellular infiltration occurred in the second biopsies which were always sometime after the peak of enzyme release.

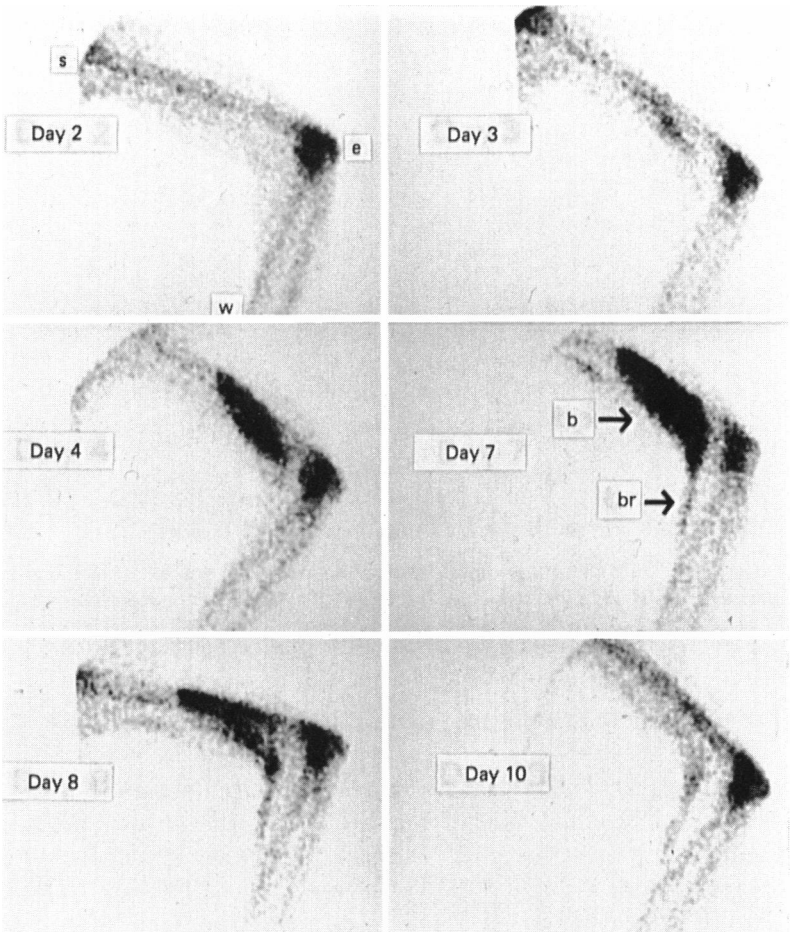

Fig. 2. Anterior scans of the left arm up to 10 days after eccentric contractions of the elbow flexors. Subject D.J. s, shoulder; e, elbow; w, wrist; b, biceps; br, brachioradialis.

Exercise of the calf muscles

Two subjects performed bilateral eccentric contractions of the calf muscles by walking backwards down an inclined treadmill. The first subject (D.J., male, 40 years) exercised for 1·5 h, the second, D. N. (female, 35 years) exercised for 2 h. The second subject made a deliberate effort to place as much strain as possible on the calf by landing heavily on the extended leg.

At the end of the exercise there was a great deal of tremor in the calf and neither subject was able to raise their heel off the ground when standing on one leg. Both subjects developed appreciable pain in the calf muscles; for subject D.J. this was maximal on day 2 after the exercise. Subject D. N. was very severely affected to the extent that on days 3 and 4 after the exercise she had difficulty in walking unaided.

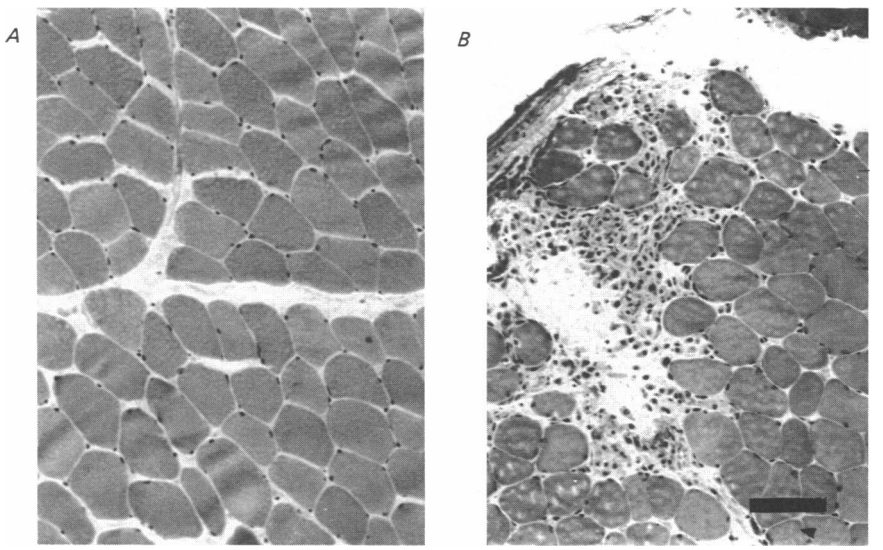

Fig. 3. Biopsies of biceps muscle after eccentric exercise. *A*, subject P. D., first biopsy 4 days after exercise. *B*, subject, C. G., second biopsy 14 days after exercise. Sections stained with haematoxylin and eosin, scale bar in *B* 100 μm.

The pain had completely disappeared by day 4 in subject D. J. and day 8 for subject D. N.

The first subject, D. J., showed no increase in circulating CK activity or any additional uptake of TPP into the calf muscles. In the other subject, D. N., the plasma CK rose until the sixth day after the exercise, when the circulating level reached a value approaching 80000 i.u./l (Fig. 4). It was not until about 12 days after the exercise that the circulating level had fallen to a value approaching the normal range.

Uptake of TPP into the calf muscle was measured in subject D. N. on days 3–7 and on day 12. Between days 3 and 6 there was an increase in the intensity of the uptake and the area affected. Initially the uptake was restricted to the belly of the muscle, but later, on days 5 and 6, this extended to include both the origins and insertions (Figs. 5 and 6). On the lateral view (Fig. 5) it can be seen that the uptake occurred in both the gastrocnemius and the deeper soleus, while on the posterior views (Fig. 6) both heads of the gastrocnemius are seen to be affected.

The time courses of CK release and TPP uptake in this one subject were very similar, maximum activity of both occurring on day 6, returning to near normal values by day 12 (Fig. 4).

Muscle biopsies were taken from subject D. J. on days 3, 5 and 7 after the exercise. The first biopsy was essentially normal, the second biopsy showed some increase in cellularity accompanied by increased interstitial acid phosphatase activity. The third biopsy was again essentially normal but contained three degenerate fibres displaying high acid phosphatase activity. This minor degree of damage was reflected in the absence of any rise in circulating CK and lack of additional TPP uptake. Muscle biopsies were taken from subject D. N. 4, 7, 12 and 20 days after exercise. The biopsy

TABLE 1. Summary of changes seen in the biceps biopsies following eccentric exercise

	Peak plasma, CK, day	First biopsy, Day	First biopsy, Appearance	Second biopsy, Day	Second biopsy, Appearance
D.C.	5–6	4	Normal	9	D,CI,AP
G.O.	6	8	D,AP*	14	D,CI,AP,R
C.G.	3	8	Normal	14	D,CI,AP,R
P.D.	4	4	Normal	12	D,CI,AP,R
J.R.	5	5	D,AP*	12	D,AP

D, small degenerate fibres; AP, increased acid phosphatase activity; CI, increased cellular infiltration; R, regenerating fibres.

* In these two biopsies a single small area comprising two or three degenerating fibres was seen. The time after the eccentric exercise is given for each biopsy together with the time of the maximum plasma CK (see Fig. 4).

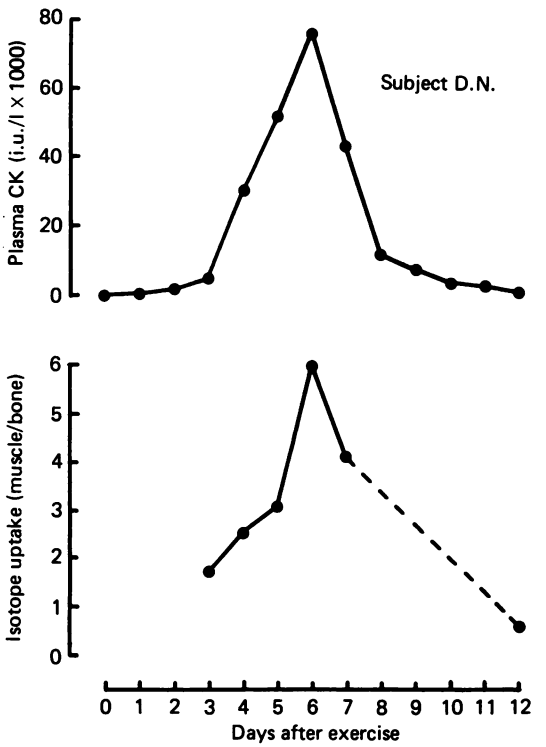

Fig. 4. Plasma CK and muscle TPP uptake following eccentric exercise of the calf muscles in one subject.

taken on day 4 (Figs. 7*A* and 8*A*) showed minimal changes, the fibres were of an even size and there was a normal chequer-board of fibre types when stained for myosin ATPase after pre-incubation at pH 9·4. There was a slight increase in interstitial cellularity and one fibre was seen containing mononuclear cells with high acid phosphatase activity. There was no generalized increase in acid phosphatase activity.

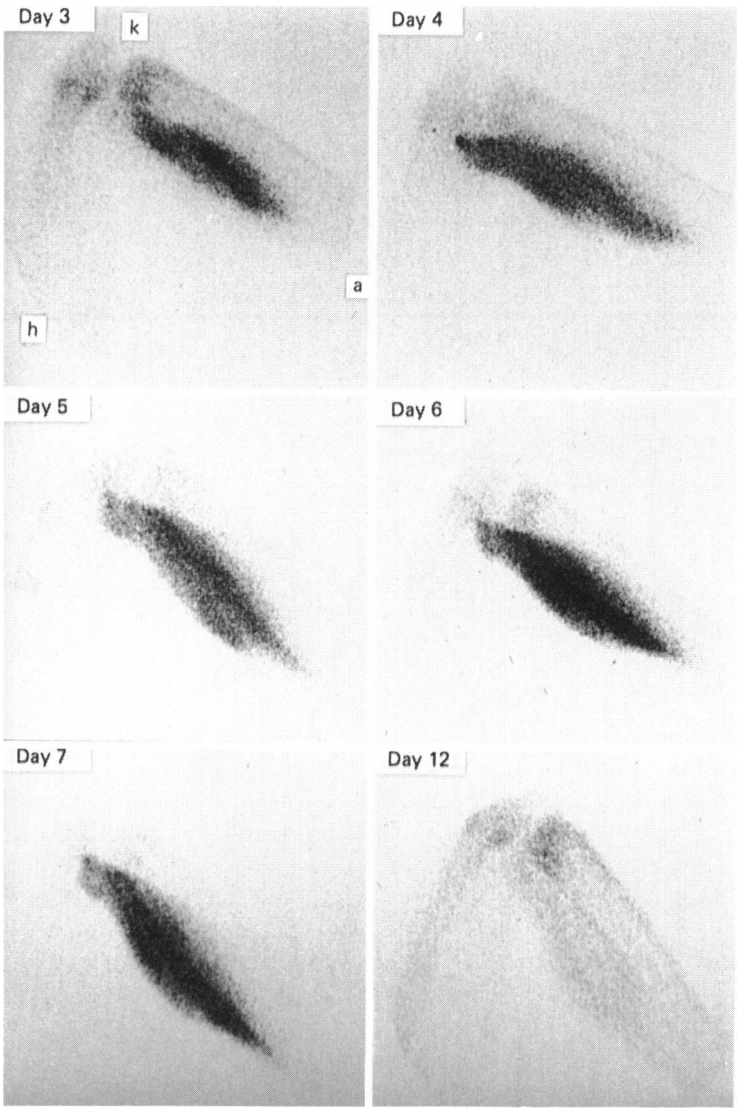

Fig. 5. Lateral scans of the right leg showing TPP uptake 3–7 and 12 days after eccentric contractions of the calf muscles. h, hip; k, knee; a, ankle. Subject D. N.

The biopsy taken on day 7 (Figs. 7*B* and 8*B*) showed differences in fibre size with many cells infiltrated with mononuclear cells. These were also seen in the endo- and perimysial areas where generalized deposition of acid phosphatase product was seen. The ATPase stain after pre-incubation at pH 9·4 showed many fibres with an uneven staining. In general, the type II fibres were more severely affected than the type I fibres. The biopsy on day 12 (Figs. 2*C* and 3*C*) showed massive infiltration of endo- and perimysial areas with mononuclear cells with high levels of acid phosphatase.

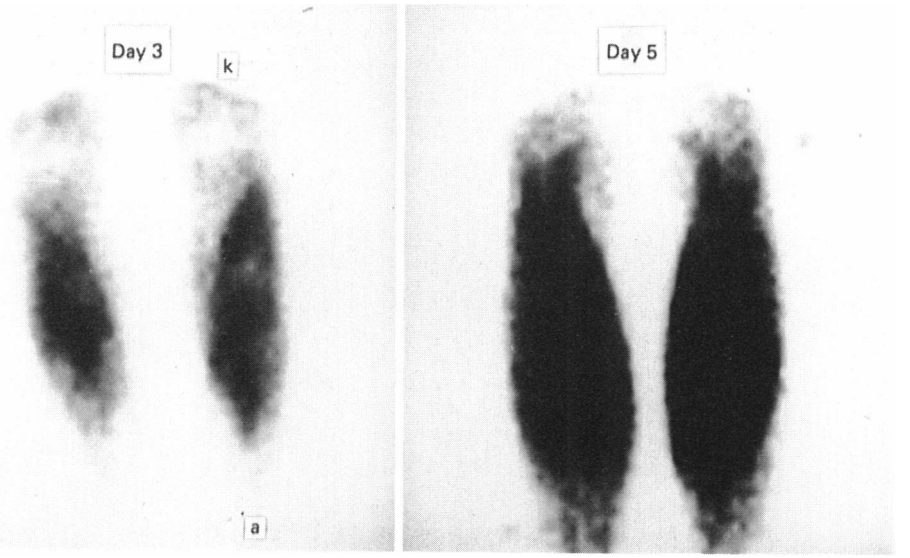

Fig. 6. Posterior scans of both calves 3 and 5 days after eccentric exercise. Note increase in area and density of uptake on day 5. k, knee; a, ankle.

Numerous smaller basophilic fibres were present which stained darkly with the ATPase reaction after pre-incubation at pH 9·4 (Fig. 8*C*). The fourth and final biopsy taken on day 20 after the exercise showed a very few mononuclear cells (Figs. 7*D* and 8*D*). There were many smaller regenerating fibres with basophilic cytoplasm and internal nuclei. There was acid phosphatase activity both in the interstitial spaces and within the regenerating fibres, but the over-all activity was much less than seen in the biopsy taken on day 12. ATPase staining showed that there was substantial recovery of type II fibre size although they were, on average, smaller than the type I fibres. Myosin ATPase after pre-incubation at pH 4·3 and 4·6 and staining for oxidative activity showed the majority of type II fibres to be of the type IIC subgroup. There was no evidence of fibre type grouping or increased fibrosis. The maximum mononuclear cell infiltration of the muscle occurred at day 12, a considerable time after the maximum values for plasma CK and muscle uptake of TPP. On the fourth day after exercise, at a time when the plasma CK was 30000 i.u./l, only minimal changes were seen in the biopsy specimen. At 12 days, when there was maximum mononuclear cell infiltration and highest acid phosphatase activity, the plasma CK had fallen to 3000 i.u./l, and by the last biopsy, when there was a great deal of regeneration and growth, the CK was back to normal.

On the second and third days after exercise subject D. N. noticed myoglobinuria. This was presumably a result of the high degree of damage to the large calf muscle group and has not been observed in any other of our studies of eccentric exercise.

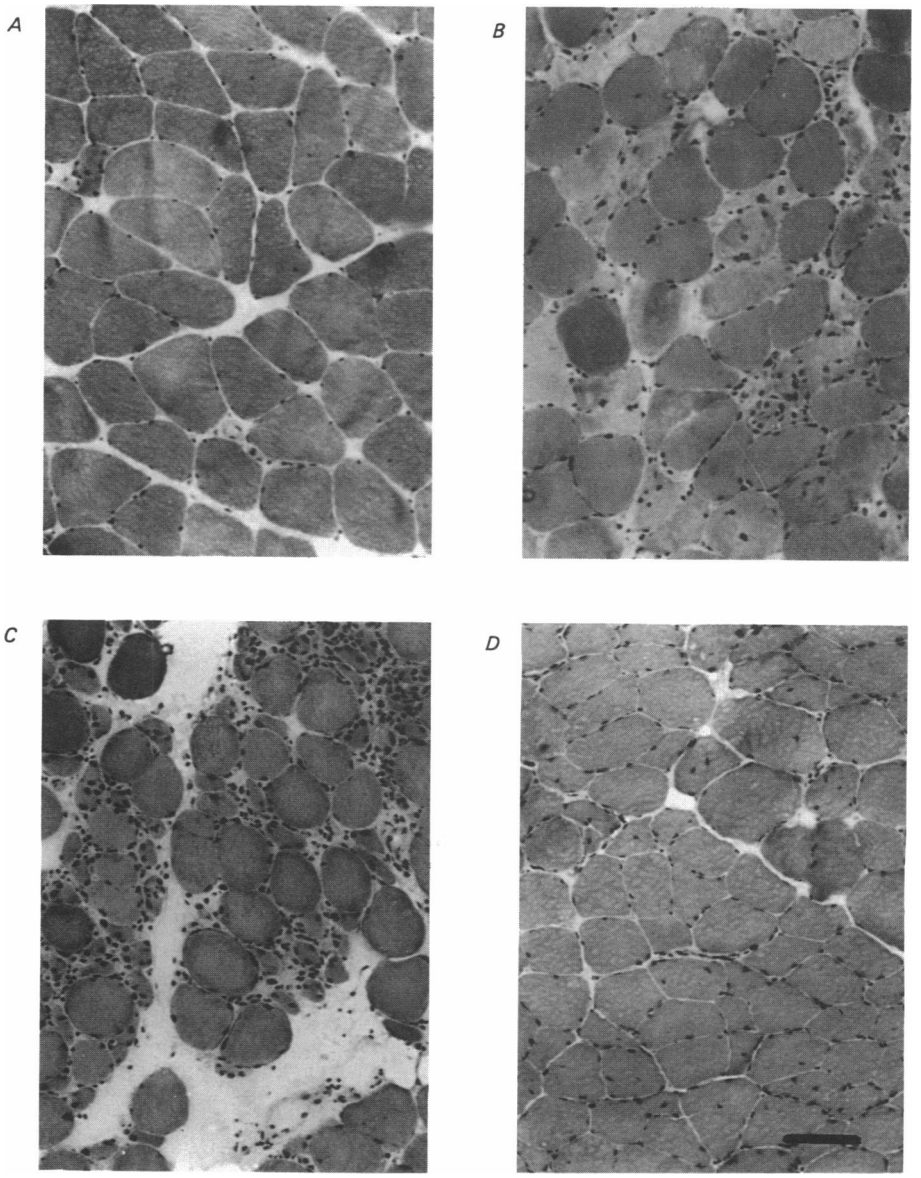

Fig. 7. Calf muscle biopsies after eccentric exercise. Biopsies taken from subject D. N. 4 (*A*), 7 (*B*), 12 (*C*) and 20 (*D*) days after eccentric exercise. Sections stained with haematoxylin and eosin, scale bar in *D* 100 μm.

DISCUSSION

The damage which can occur in muscle after it has been exercised eccentrically is of interest for a number of reasons. There are practical considerations, such as the extent and consequences of muscle damage that may occur during training or rehabilitation. There is also the possibility that such experimental damage can cast light on the mechanisms of damage and repair that could be defective in muscle

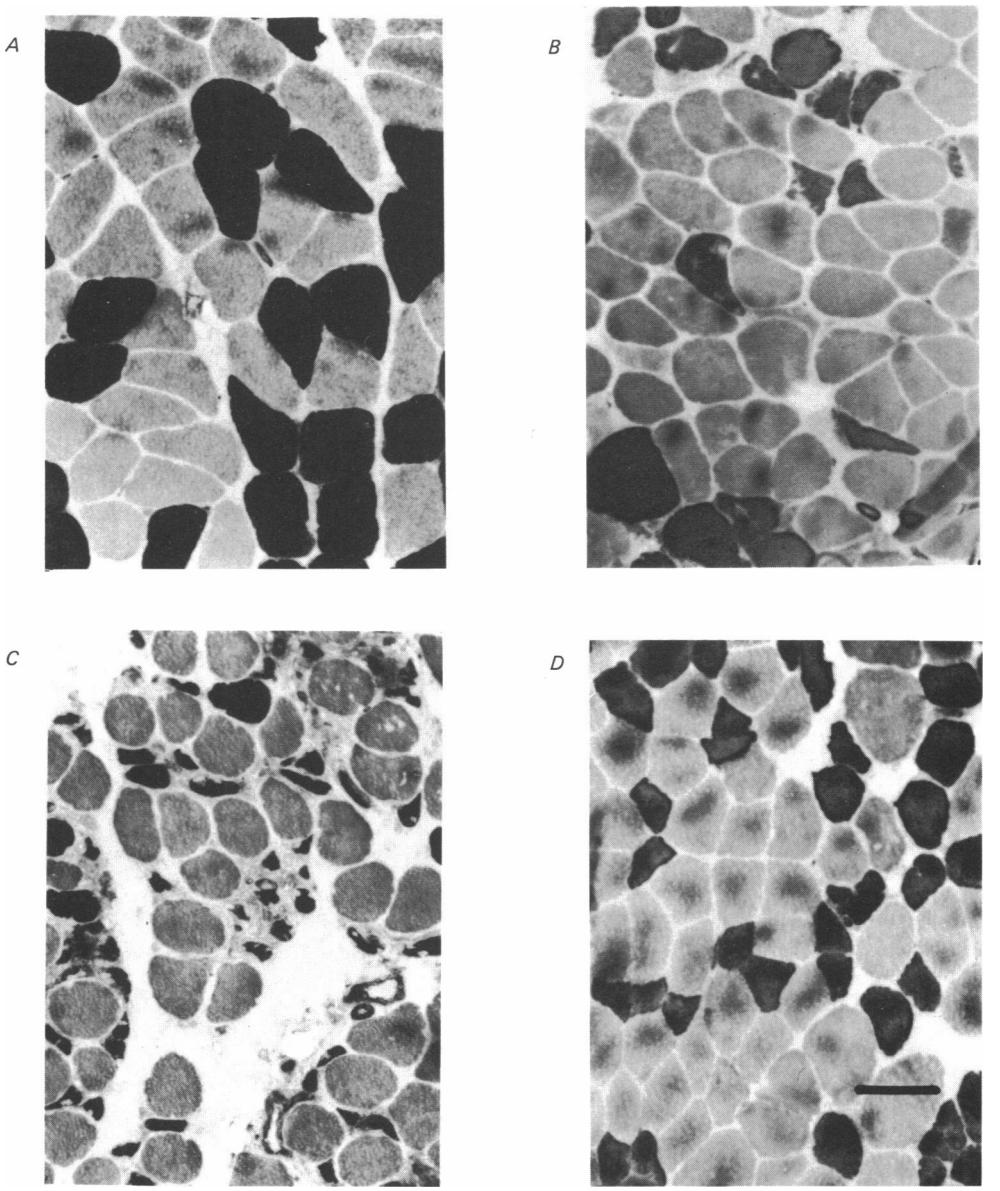

Fig. 8. Calf muscle biopsies after eccentric exercise. Biopsies taken from subject D. N. 4 (*A*), 7 (*B*), 12 (*C*) and 20 (*D*) days after eccentric exercise. Sections stained for ATPase at pH 9·4; scale bar in *D* 100 μm.

disorders. The objective of the present study was to see whether there is any evidence of an inflammatory response with cellular infiltration in human muscle after exercise and, if so, whether this could account for the characteristic delay between the eccentric exercise and the time when gross damage becomes evident.

We have examined human biceps and calf muscles which can be both exercised eccentrically and biopsied. As in our previous studies of exercise-induced damage

there was considerable variability in the severity of the responses in different subjects (Newham *et al.* 1983*b*, 1986). This may be due in part to differences in habitual activity or training which we have found to protect muscle against such damage (Jones & Newham, 1985*b*) but it is unlikely to be the full explanation since no obvious correlation has been seen between susceptibility and factors such as sex, age or general level of activity.

In this study we have not directly compared the effects of eccentric with those of isometric or concentric contractions but there is considerable evidence that eccentric contractions cause significantly more damage than other types of contraction. In previous work with stepping exercise (Newham *et al.* 1983*c*) we found that the ultrastructural changes were limited to the muscles that had worked eccentrically. These observations are supported by the animal work of Armstrong *et al.* (1983) where no abnormalities were found after concentric exercise. In addition, Sjostrom, Friden & Ekblom (1982) were unable to detect myofibrillar damage in subjects who had completed a 30 km race while just 30 min of eccentric exercise of the calf muscles produced marked disruption of sarcomere architecture (Friden *et al.* 1981).

Although the data is somewhat limited, the time course of increased uptake of TPP into muscle after exercise appears similar to that of CK release both for the biceps (Fig. 1) and the calves (Fig. 4), the implication being that CK release and TPP uptake are indicators of the same aspect of muscle damage, and that TPP uptake can be used to identify the muscles from which CK is being released.

An interesting feature of the TPP uptake is the distribution within the muscle. In the calf the damage on day 3 was restricted to the belly part of the muscle and subsequently spread to include the whole muscle up to its origin above the knee (Figs. 5 and 6). With the forearm flexors the damage seemed to progress in a similar way in the biceps and later included the brachioradialis (Fig. 2). The significance of the spread of damage is not known.

In inflammatory muscle diseases the immune system is implicated in the pathogenesis of the damage and it is possible that the infiltrating cells seen in the experimentally damaged muscle could be the cause of further fibre degeneration. Alternatively the invading cells may be a consequence of the damage, being there to scavenge and remove the debris of degenerate cells.

Our findings on this point are quite clear. The peak of enzyme release and of increased TPP uptake occur some days before the time of maximum tissue infiltration with mononuclear cells. In subject D. N. the calf biopsy showed only small areas of infiltration 7 days after the exercise while the plasma CK and muscle TPP were maximum on day 6. By day 12, when the plasma CK was nearing normality, the mononuclear cell infiltration was at its greatest. The results of the biceps study are consistent with this (Table 1). The first biopsy was taken close to the time when the plasma CK was maximal and showed little or no evidence of abnormality. The second biopsy, taken some time later when the CK was returning to normal, always showed more extensive cellular infiltration. The infiltration is therefore seen to be a response to damage rather than the cause. The invading mononuclear cells are associated with high acid phosphatase activity and it is likely that the majority will prove to be macrophages, although work is in hand to fully characterize the nature of the cellular infiltrate.

Since the invading cells cannot be held responsible for the damage to muscle fibres, the primary cause and the reason for the delay between the exercise and appearance of overt damage remains unknown.

An interesting feature of the damage in the calf muscle is that the type II fibres were more severely affected than the type I. This differs from the findings of Armstrong *et al.* (1983) with rats but Friden *et al.* (1983) have reported greater ultrastructural damage in type II fibres, identified by narrower Z-lines on electron microscopy. It remains to be seen whether the preferential involvement is due to different patterns of use during exercise or a greater intrinsic susceptibility to damage of the type II fibres.

Although there is now a good deal of descriptive information about the pain and damage associated with eccentric exercise, the major questions about the causes and factors that determine the time course of events remain unanswered, which makes this type of exercise of continuing interest. The fact that damage can be induced by an acceptable form of exercise means that there is the opportunity of studying not only the mechanisms underlying damage but also the processes of adaptation and regeneration in human muscle.

We are grateful for support from the Muscular Dystrophy Group of Great Britain.

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