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THE METABOLIC CAUSES OF SLOW RELAXATION IN FATIGUED HUMAN SKELETAL MUSCLE

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SUMMARY

1. The relationship between slowing of relaxation and changes of intracellular pH and phosphorous metabolites has been examined in human skeletal muscle during the development of fatigue and subsequent recovery. Results obtained with normal subjects have been compared with those from a subject with myophosphorylase deficiency (MPD) who produced no H^+ from glycolysis during exercise and therefore afforded the opportunity of assessing the role of H^+ in the slowing of relaxation.

2. Subjects fatigued the first dorsal interosseous muscle in a stepwise fashion under ischaemic conditions, with intervals between the fatiguing contractions during which the relaxation rate was measured from brief tetanic contractions and the muscle phosphorous metabolites and pH were measured by nuclear magnetic resonance spectroscopy.

3. After 21 s maximal voluntary contraction under ischaemic conditions, relaxation in the MPD subject slowed to approximately 50% of the rate in the fresh muscle at a time when the intramuscular pH had not changed. This demonstrates that there is a mechanism causing slowing of relaxation that is independent of H^+ accumulation.

4. The normal subjects showed a slow recovery of relaxation compared to the MPD subject when the circulation was restored. The main difference in the intracellular metabolite concentrations between MPD and normal subjects at this time was that, for the latter, the pH remained low (around 6·5) for at least 60 s after the circulation was restored. The results suggest that the slow recovery is a consequence of continuing acidosis, i.e. the existence of a pH-dependent mechanism of slowing.

5. The existence of a pH-dependent mechanism was further indicated by the fact that for the normal subjects, for a similar intracellular concentration of phosphocreatine, relaxation of the recovering muscle was approximately half that of the fatiguing muscle. This was at a time when the pH of the recovering muscle was 0·3–0·4 units less than in the partially fatigued muscle.

6. The results show that in normal muscle there are at least two processes that

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lead to slow relaxation in fatigued muscle: one due to H^+ accumulation, the other being independent of H^+.

INTRODUCTION

One of the characteristic features of skeletal muscle fatigued by high force contractions is a slowing of the rate of relaxation from an isometric contraction. The cause of the slowing remains unknown and this, in part, reflects an ignorance about the underlying mechanism determining the characteristic exponential decay of force at the end of a contraction. Jewell & Wilkie (1960) showed that the rate constant for relaxation is not a function of the elastic properties of the passive elements of the muscle but rather represents the progress of some underlying biochemical reaction or reactions. Broadly, the suggested mechanisms for the regulation of relaxation fall into two categories. Slowing may reflect a reduced rate of cross-bridge detachment in the fatigued muscle (Edwards, Jones & Hill, 1975), the evidence being a reduced rate of ATP turnover in fatigued muscle, although this finding has been disputed (Dawson, Gadian & Wilkie, 1980). The other main explanation is that slow relaxation is a consequence of reduced rates of reaccumulation of Ca^{2+} by the sarcoplasmic reticulum, as has been reported to occur during short trains of twitches in amphibian muscle (Blinks, Rudel & Taylor, 1978; see also Dawson *et al.* 1980, for discussion).

Slowing of relaxation is undoubtedly associated with metabolic change in the muscle but there is uncertainty as to which particular metabolite is responsible. Some investigations point to a critical role for H^+ accumulation (Sahlin, Edström, Sjöholm & Hultman, 1981; and see Renaud, Allard & Mainwood, 1986), although work with metabolically inhibited muscles contradicts this (Parkinson, 1933; Edwards *et al.* 1975). It has also been suggested that a combination of metabolite changes leading to a reduction in the free energy of ATP hydrolysis could be the cause of the slowing (Dawson *et al.* 1980).

The work described here is an examination of the relationship between the rate of relaxation and metabolite levels measured in human muscle by nuclear magnetic resonance spectroscopy (NMR). Measurements have been made during the onset and recovery from fatigue and the results for normal subjects are compared with those from a subject with myophosphorylase deficiency (MPD). This muscle enzyme deficiency prevents the accumulation of lactate and H^+ during exercise and therefore allows an assessment of the role of H^+ in causing slow relaxation. The results presented here are an extension of the study of force and muscle metabolites reported in the preceding paper (Cady, Jones, Lynn & Newham, 1989).

METHODS

Subjects

Six normal subjects (two male, four female, age 21–43 years) were studied. The subject with MPD was female, 65 years of age, who had previously been diagnosed on the basis of clinical symptoms and the absence of the enzyme in a biopsy specimen from the quadriceps muscle. The

subjects were those who participated in the study of metabolite levels and force (Cady *et al.* 1989). The investigation was approved by the local Ethics Committee.

Measurement of force

In experiments conducted outside the magnet, isometric force of the first dorsal interosseous was measured with a strain gauge applied to the side of the first finger at the first interphalangeal joint (Jones, Rutherford & Whiteson, 1987; Cady *et al.* 1989). For the fatiguing exercise the subjects performed maximal voluntary contractions (MVCs) with visual feedback of the force signal and verbal encouragement while, for testing, the muscle was stimulated for 0·5 s at 100 Hz by supramaximal square-wave pulses applied to the ulnar nerve at the elbow. For the parallel experiments conducted inside the magnet of the NMR spectrometer, the hand was held in the apparatus described by Aldridge, Cady & Newham (1987) which has a strain gauge mounted in the same position as that for experiments outside the magnet, so that the fatiguing voluntary contractions involved the same movement. There was visual feedback of the force signal and similar verbal encouragement to ensure that the efforts were truly maximal. The half-time ($t_{0·5}$) of the late phase of relaxation (from 0·5 to 0·25 maximum force) was measured from the records of the test tetani (see Fig. 1) and expressed as a rate constant ($0·693/t_{0·5}$).

Measurement of muscle metabolites

Relative concentrations of muscle metabolites were measured by NMR spectroscopy using an Oxford Research Systems–Bruker spectrometer equipped with a 1·9 tesla, 26 cm clear-bore superconducting magnet (Aldridge *et al.* 1987). Details of the analytical methods and the assumptions made in estimating intracellular concentrations are given in the preceding paper (Cady *et al.* 1989).

Experimental protocol

The muscles were fatigued in a stepwise fashion under ischaemic conditions, leaving intervals between the bouts of voluntary exercise so that contractile properties could be tested while the muscle was in a fatigued but relatively stable condition. These values were compared with muscle metabolites measured during the same intervals in parallel runs inside the magnet. After an initial 3 min, during which measurements were made in the fresh resting condition with an intact circulation, the muscle was made ischaemic by inflating a pressure cuff around the upper arm. In the first series of experiments (see Cady *et al.* 1989), the fatiguing exercise consisted of a 15 s maximum voluntary contraction followed by approximately 3 min rest, during which time NMR data were collected or the muscle was tetanically stimulated for 0·5 s at intervals of 1 min. This was repeated three times by the normal subjects, giving a total of 45 s MVC. The MPD subject exercised and was tested in the same way except that the fatiguing contractions were restricted to three 7 s MVCs. After the third period of ischaemic rest the circulation was restored and muscle function and metabolites were followed during recovery for up to 10 min. In the second series of experiments the protocol was changed in three ways. There was a 3 min period of ischaemia with the muscle at rest before the first fatiguing contraction. During the fatiguing phase the MPD and normal subjects all began with two short 7s contractions followed by a longer third contraction to deplete the phosphocreatine (PCr) stores; for the MPD subject the third contraction was 10 s and for the normal subjects 30 s. Recovery was then allowed to proceed in steps. The circulation was restored for 30 s and then occluded for 3 min while the muscle was tested and then the procedure was repeated with the muscle allowed to recover for another 30 s before the circulation was again occluded.

In this first series of experiments for the normal subjects there were nineteen runs in which metabolites were measured and sixteen runs outside the magnet where relaxation was tested. The MPD subject undertook four runs for metabolite measurements and four for assessment of contractile changes. In the second series of investigations three normal subjects undertook two runs each for metabolite measurements and between four and seven (total of sixteen) runs for measurements of relaxation. The MPD subject undertook two runs for metabolites and two for measurements of relaxation in the second series.

The hands were all warmed for 10 min in hot water before the start of each experiment to ensure that the muscles were close to core temperature on each occasion they were tested.

RESULTS

Typical records of the 0·5 s test tetani for fresh, fatigued and recovering muscles are shown in Fig. 1. The half-time of relaxation for fresh muscle of the normal subjects was around 40 ms, while the MPD subject relaxed somewhat more

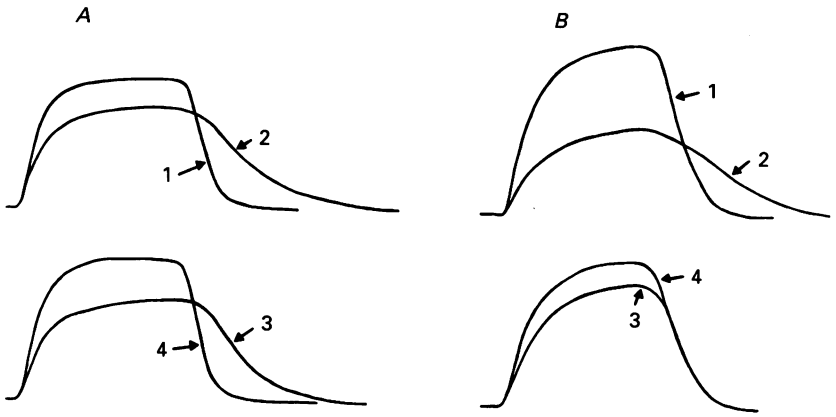

Fig. 1. Tracings of force records for *A*, one of the normal subjects and *B*, the MPD subject, during the development of fatigue and recovery; see Fig. 3 for experimental protocol. Records are of 0·5 s tetani at 100 Hz; 1, fresh muscle with intact circulation; 2, after the third fatiguing contraction under ischaemic conditions; 3, after 30 s recovery with an intact circulation and ischaemia reimposed; 4, after a further 5 min recovery with an intact circulation.

slowly with a half-time of about 60 ms. The rate constants for relaxation were $17{\cdot}9 \pm 0{\cdot}07\ s^{-1}$ (mean ± S.E of mean, $n = 16$) and $12{\cdot}5 \pm 0{\cdot}8\ s^{-1}$ ($n = 4$) for the normal and MPD subjects respectively. To facilitate comparisons between subjects and to demonstrate relationships between change in relaxation rate and change in metabolite concentrations, the data for relaxation are expressed as a percentage of the value in the fresh muscle before the start of ischaemia.

Relaxation from the brief test tetani slowed as a result of the fatiguing exercise. For the normal subjects in the first series of experiments the rate constant for relaxation decreased progressively, so that after 45 s of maximum voluntary contraction the rate was 24 ± 3·3 % (mean ± S.E. of mean, $n = 16$) that of the fresh muscle (Fig. 2*A*). With the MPD subject the relaxation rate did not change as a result of the first voluntary contraction but then fell to 45 ± 4 % ($n = 4$) of the fresh value after 21 s fatiguing exercise (Fig. 2*A*). It was particularly noticeable that relaxation in the MPD subject recovered very much more rapidly than in the normal subjects when the circulation was restored. Relaxation was fully recovered in the MPD subject within 1 min of restoring the circulation whereas the normal subjects had returned to only about 40 % of the fresh rested value at this time (Fig. 2*A*). Estimates of intracellular pH (Cady *et al.* 1989), both for the normal and MPD subjects, are shown in Fig. 2*B*. For the MPD subjects there was no significant change in pH either during the fatiguing exercise or the subsequent recovery, while for the

normal subjects pH fell to approximately 6·5 at the end of the exercise and then remained at this value for the next 2 min before returning to near normal values by 5 min.

A comparison of the data in Fig. 2*A* and *B* shows that slowing of relaxation can

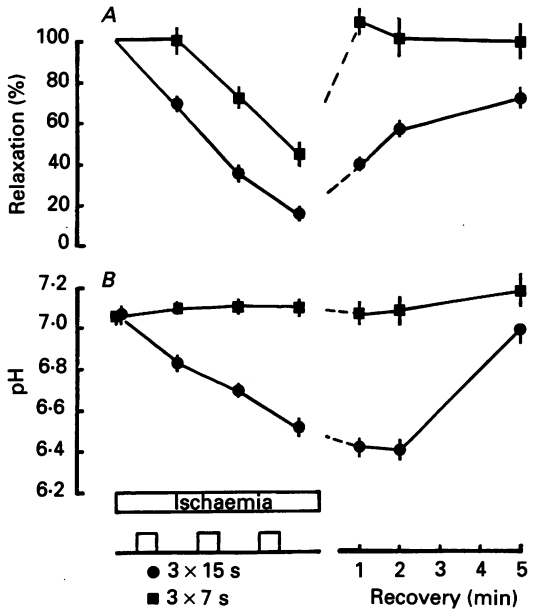

Fig. 2. Relaxation rate (*A*) during the fatiguing protocol and subsequent recovery compared with intracellular pH (*B*) in normal subjects (●) and the MPD subject (■). The muscles were ischaemic while the subjects carried out three maximum voluntary contractions separated by 3 min rest. During the rest period the muscle was either stimulated and the relaxation rate measured, or muscle metabolites and pH were measured. Measurements were made at intervals during recovery. Values are the mean ± S.E. of mean. For measurements of relaxation there were sixteen runs for three normal subjects and four runs for the MPD subject; for pH measurements there were nineteen runs for normal subjects and four for the MPD subject.

occur in the absence of a fall in pH (H^+-independent mechanism) and suggests that the slow recovery in the normal subjects may be a consequence of the prolonged acidification of the muscle after the end of the ischaemic exercise (pH-dependent mechanism). The experimental design was not, however, best suited for a detailed comparison of relaxation and metabolic changes, since the normal subjects carried out much longer contractions than the MPD subject. During the recovery phase NMR collections were made at 1 min periods and the 32 scans obtained gave less reliable spectra than the 96 collected in the 3 min intervals during the fatiguing exercise. During recovery the metabolites were changing rapidly and the NMR measurements reflect the average concentrations over the preceding minute. The values do not, therefore, correspond well with the measurements of contractile properties which were made at the end of each minute. Consequently, in the second series of experiments the protocol was changed, as detailed in the Methods section.

Figure 1 shows force records from the second series of experiments and Fig. 3 shows the combined data for sixteen runs on three normal subjects. There was a progressive decline in the rate constant for relaxation, which was very similar to that seen in the first series of experiments (Fig. 2*A*). A feature of these results, seen but

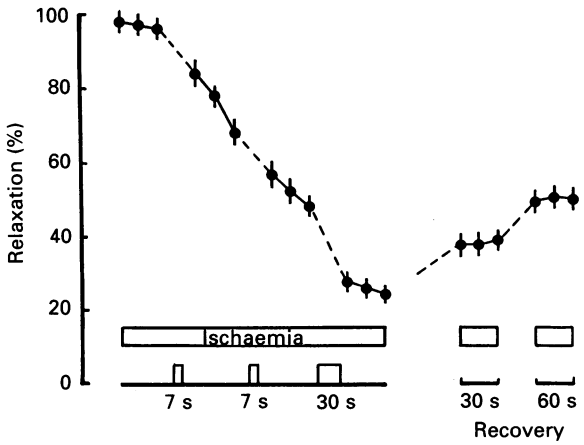

Fig. 3. Change in relaxation rate during fatigue and recovery for normal subjects in the second series of experiments. Relaxation was measured from brief tetani recorded every minute during the intervals between the fatiguing MVCs and between the periods of recovery. Data are given as mean ± S.E. of mean ($n = 16$) and expressed as a percentage of the value of the fresh muscle before the start of ischaemia.

not illustrated in the series shown in Fig. 2*A*, was that during the ischaemic rest periods between the fatiguing MVCs, relaxation measured from the three test tetani continued to slow. It is unlikely that this was due to a fatiguing effect of the test contractions since a series of nine such tetani of an ischaemic muscle, spaced at 1 min intervals, produced no significant change in relaxation. However, during the recovery phase there was no change in relaxation rate during the 3 min test intervals (Fig. 3). The MPD subject showed the same slowing of relaxation rate during the rest intervals. As in the first series, the normal subjects showed only a modest recovery of the relaxation rate during the first 30 s with a restored circulation (compare Figs 2*A* and 3).

Resting values for muscle metabolites and changes during activity were generally as previously described (Cady *et al.* 1989) and only data for PCr, pH and ADP are given here. Metabolites were calculated from NMR scans collected over the whole ischaemic rest period, and to compare changes in relaxation and metabolites levels the relaxation rates were averaged over the same interval. Figure 4*A* shows the data from Fig. 3 treated in this way. There were only two observations on the MPD subject in this series of experiments but the results from the second series confirm the pattern shown in Fig. 2*A*. Estimates of intracellular pH and measurements of PCr are shown in Fig. 4*B* and *C*. The pH measurements were comparable with those shown in Fig. 1*B*, with a clear difference between the MPD subject and the normal subjects, while the PCr measurements showed a very similar pattern of depletion for the MPD and normal subjects, both in the initial stages of the fatiguing exercise and at the end of the third fatiguing voluntary contraction (Fig. 4*B*). During recovery

the resynthesis of PCr was slower in the normal subjects at a time when there was continuing acidosis (Fig. 4*B*).

A comparison of the normal and MPD subjects suggests that relaxation is affected by two factors, one related to intracellular pH, the other to metabolic depletion of

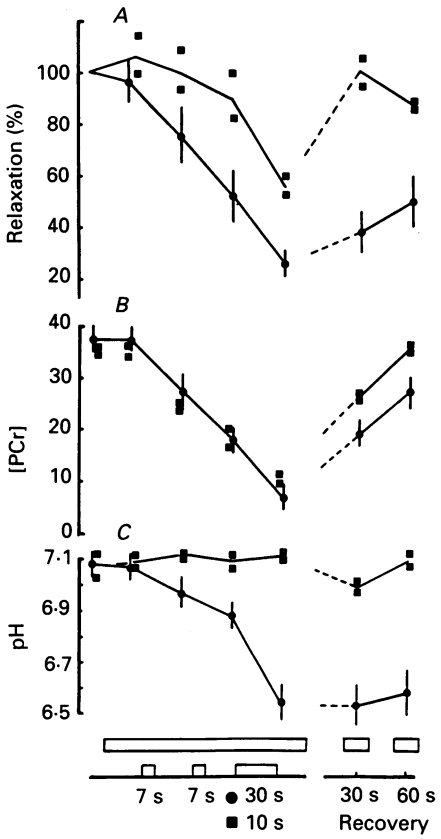

Fig. 4. Relaxation rate (*A*) during the fatiguing protocol and subsequent recovery compared with intramuscular phosphocreatine (*B*) and pH (*C*) in normal (●) and MPD subjects (■). The muscles were ischaemic while the subjects carried out three maximum voluntary contractions separated by three minutes rest; the first two contractions were of 7 s duration and the third was of 10 s duration for the MPD subject and 30 s for the normal subjects. During the rest periods the muscle was either stimulated and relaxation rate measured, or muscle metabolites and pH were measured. The muscles were allowed to recover for 30 s with an intact circulation before ischaemia was reimposed for 3 min while measurements of relaxation and metabolites were made. The muscle was then allowed to recover for a further 30 s and the process repeated. For measurements of relaxation there were nineteen runs for three normal subjects and two runs for the MPD subject; for PCr and pH measurements there were six runs for normal subjects and two for the MPD subject. Individual values are given for the MPD subject with a line connecting the mean points, otherwise values are the mean ± S.D.

the tissue. These two facets can also be seen in the results from the normal subjects if the relationship between slowing and metabolic depletion is compared during the fatiguing and recovery phases (Fig. 5). After 30 and 60 s recovery the PCr values had returned to levels which were similar to those found in the fatiguing muscle after the

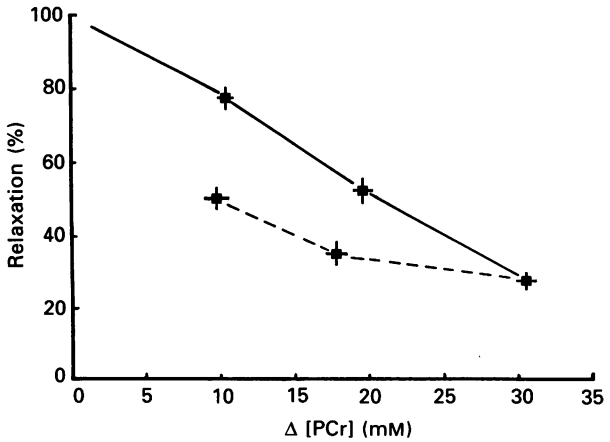

Fig. 5. The relationship between depletion of phosphocreatine and relaxation rate in normal subjects. Data are taken from Fig. 4 and show the change in relaxation rate in relation to the depletion of phosphocreatine during the fatiguing phase of the protocol (continuous line) and after 30 and 60 s recovery (dashed line). Data are given as mean ± S.E. of mean.

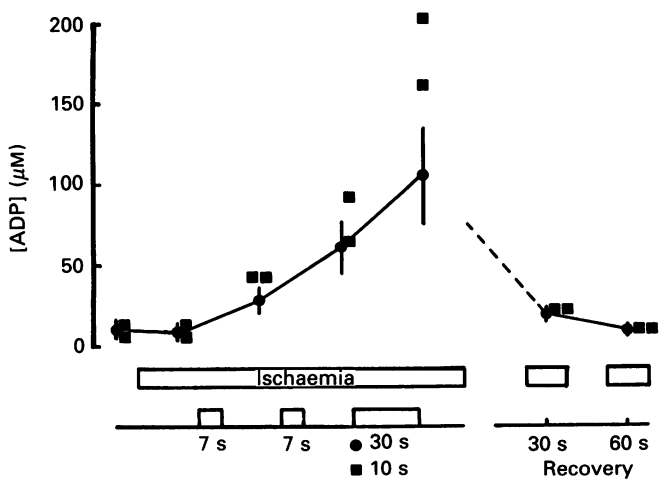

Fig. 6. Calculated muscle free ADP concentrations during the fatiguing exercise and subsequent recovery. Data are from the series illustrated in Fig. 4. Values are mean ± S.D. for normal subjects (●) and the individual values from two runs with the MPD subject (■).

second and first voluntary contractions. Nevertheless, the relaxation rates remained depressed. The main difference between the fatiguing and recovery phases was the continuing acidosis in the latter phase, the muscles being between 0·3 and 0·4 pH units more acidic during recovery (see Fig. 4*C*). During the recovery phase relaxation was approximately half the value that might have been predicted from the muscle content of PCr at a pH of around 7·0.

The muscle free ADP content was calculated (see Cady *et al.* 1989) and the results are shown in Fig. 6. The values for the MPD subject were higher than those for the normal subjects after the third fatiguing MVC. There are two interesting features of these results: the ADP concentration increased most rapidly towards the end of the fatiguing activity and then returned to near resting levels during the first 30 s recovery. This time course was similar to the changes in relaxation seen in the MPD subject (see Fig. 4*A*), which suggests a possible cause of the H^+-independent mechanism of slowing.

One consequence of the accumulation of the reaction products P_i and ADP is a reduction in the affinity or free energy of ATP hydrolysis, which may influence the rate of relaxation (Dawson *et al.* 1980). Free energies were calculated from the metabolite measurement (making the assumptions described by Taylor, Styles, Matthews, Arnold, Gadian, Bore & Radda, 1986) at intervals during the second series of experiments. The values obtained are critically dependent on the ATP and ADP levels and estimates of these have the greatest variance. Consequently, free energies have only been estimated from the mean metabolite data and can only be used as an indication of trends. In the fresh normal muscle the free energy of ATP hydrolysis was calculated to be approximately 46 kJ/mole while for the MPD the value was 43 kJ/mole. As a result of the fatiguing activity the free energy fell to about 35 kJ/mole for the normal muscle and to a similar value for the MPD subject. Values returned to about 40 and 43 kJ/mole after 30 and 60 s recovery respectively for both normal and MPD subjects.

DISCUSSION

The present investigation of metabolite levels and contractile properties of fatigued muscle in normal and MPD subjects suggests that there are two mechanisms acting to slow the relaxation from an isometric contraction.

The effect of H^+ on muscle relaxation was most clearly demonstrated during the recovery phase. There was a marked difference between the normal subjects and the MPD subject in the rate of recovery of relaxation when the only major difference in muscle metabolite content was the continuing acidosis in the normal subjects (Figs 2 and 4*A*). The second feature emphasising the role of H^+ was the dissociation between relaxation rate and PCr levels in the normal subjects when comparing the onset of fatigue with recovery (Fig. 5). For similar PCr levels, the relaxation was appreciably slower at low pH. The present results cast no light on the question as to which process is affected by the decrease in intracellular pH. Most attention has been directed to the possibility of inhibition of Ca^{2+} uptake by the sarcoplasmic reticulum (Fabiato & Fabiato, 1978), but the results of Edman & Mattiazzi (1981) for intact muscles and Chase & Kushmerick (1988) for skinned preparations, suggest that H^+ may also decrease the maximum velocity of shortening. During fatigue, slowing of relaxation and changes in the force:velocity characteristics of skeletal muscle occur with a similar time course (deHaan, Jones & Sargeant, 1989; Lännergren & Westerblad, 1989).

The existence of a pH-independent mechanism for slowing of relaxation was clearly demonstrated by the fact that relaxation in the MPD subject did become slow

with fatigue. This observation confirms earlier work with poisoned mouse muscle (Edwards *et al.* 1975) but differs from the conclusions of Sahlin *et al.* (1981) who also worked with poisoned animal muscle. The discrepancy, however, is probably due to the fact that in the latter work relaxation was only measured from twitches which do not always have the same time course of relaxation as seen from tetanic contractions.

The pH-independent mechanism must have the same time course as the slowing of relaxation demonstrated by the MPD subject, that is a delay before the onset of slowing and a rapid recovery within 30 s after restoration of the circulation. The ATP content did not change significantly as a result of the fatiguing exercise, and changes in P_i have the wrong time course (Cady *et al.* 1989) to account for changes in relaxation. The calculated ADP concentration showed larger changes in the later part of the fatiguing exercise and also recovered rapidly when the circulation was restored (Fig. 6). Accumulation of ADP is therefore a possible cause of the H^+-independent form of slow relaxation. A mechanism for this effect is not clear. With skinned fibre preparations Cooke & Pate (1985) have shown that ADP reduces the maximum rate of shortening and such a change might underlie the slow relaxation seen in fatigued muscle. However, the concentrations required for this effect with the skinned preparations were between 1 and 8 mM, at least ten times the free ADP concentrations estimated in the present work.

Another way in which accumulation of ADP could act is by contributing to a decrease in the free energy available from ATP hydrolysis. Dawson *et al.* (1980) have suggested that Ca^{2+} pumping by the sarcoplasmic reticulum may be sensitive to the reduction in free energy. The calculation of free energies compounds a number of errors and assumptions and the values obtained in this study therefore lack the precision required for a critical evaluation of the relationship between relaxation and free energy of ATP hydrolysis. The general trend for change in free energy was similar to that of the slowing of relaxation in the MPD subject.

There is the possibility that the pH-independent slowing is a consequence of some modification of the activity of the actomyosin cross-bridge or the process of Ca^{2+} pumping. Covalent modification of the myosin subunits has been suggested as a cause of reduced cross-bridge cycling (Crow & Kushmerick, 1983) which could lead to a slow relaxation. It is also possible that the activity of the Ca^{2+} pump could be similarly altered. Such covalent modification could be initiated by changes associated with contraction and fatigue and may be simply related to the intracellular concentrations of metabolites.

In the past there has been a tendency for discussions of the role of H^+ in muscle fatigue to become polarized, with opinions suggesting that it is either the major cause (e.g. Sahlin *et al.* 1981) or is irrelevant (Edwards *et al.* 1975). The present results provide a synthesis of these two views, showing that for relaxation there are at least two mechanisms that lead to slowing, one that is pH dependent, while the other has some other cause. Our investigation of factors affecting force generation in fatigued muscle (preceding paper, Cady *et al.* 1989) led to the same conclusions, namely that there are two mechanisms, one involving H^+, the other not. It will be interesting to see whether this is an indication of common mechanisms involving the control of force and relaxation.

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REFERENCES

ALDRIDGE, R., CADY, E. B. & NEWHAM, D. I. (1987). Apparatus for recording ^{31}P NMR spectra and force from a functionally and anatomically discrete human muscle. *Journal of Physiology* **390**, 3*P*.

BLINKS, J. R., RUDEL, R. & TAYLOR, S. R. (1978). Calcium transients in isolated amphibian skeletal muscle fibres: detection with aequorin. *Journal of Physiology* **277**, 291–323.

CADY, E. B., JONES, D. A., LYNN, J. & NEWHAM, D. J. (1989). The relationship between the fatigue of force and intracellular metabolites in human skeletal muscle. *Journal of Physiology* **418**, 311–325.

CHASE, P. B. & KUSHMERICK, M. J. (1988). Effects of pH on contraction of rabbit fast and slow skeletal muscle fibres. *Biophysical Journal* **53**, 935–946.

COOKE, R. & PATE, E. (1985). The effects of ADP and phosphate on the contraction of muscle fibres. *Biophysical Journal* **48**, 789–798.

CROW, M. T. & KUSHMERICK, M. J. (1983). Correlated reduction of velocity of shortening and the rate of energy utilization in mouse fast twitch muscle during a continuous tetanus. *Journal of General Physiology* **82**, 703–720.

DAWSON, M. J., GADIAN, D. G. & WILKIE, D. R. (1980). Mechanical relaxation rate and metabolism studied in fatiguing muscle by phosphorous nuclear magnetic resonance. *Journal of Physiology* **299**, 465–484.

DEHAAN, A., JONES, D. A. & SARGEANT, A. J. (1989). Changes in velocity of shortening, power output and relaxation rate during fatigue of rate medial gastrocnemius muscle. *Pflügers Archiv* **413**, 422–428.

EDMAN, K. A. P. & MATTIAZZI, A. R. (1981). Effects of fatigue and altered pH on isometric force and velocity of shortening at zero load in frog muscle fibres. *Journal of Muscle Research and Cell Motility* **2**, 321–334.

EDWARDS, R. H. T., JONES, D. A. & HILL, D. K. (1975). Metabolic changes associated with slowing of relaxation in fatigued mouse muscle. *Journal of Physiology* **251**, 287–301.

FABIATO, A. & FABIATO, F. (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *Journal of Physiology* **276**, 233–255.

JEWELL, R. B. & WILKIE, D. R. (1960). The mechanical properties of relaxing muscle. *Journal of Physiology* **152**, 30–47.

JONES, D. A., RUTHERFORD, O. M. & WHITESON, J. (1987). A technique for simultaneous measurement of contractile properties of the human adductor pollicis and first dorsal interosseous. *Journal of Physiology* **390**, 5*P*.

LÄNNERGREN, J. & WESTERBLAD, H. (1989). Maximum tension and force–velocity properties of fatigued, single *Xenopus* muscle fibres studied by caffeine and high K^+. *Journal of Physiology* **409**, 473–490.

PARKINSON, J. L. (1933). The effect of activity on the form of the muscle twitch. *Journal of Physiology* **78**, 106–112.

RENAUD, J. M., ALLARD, Y. & MAINWOOD, G. W. (1986). Is the change in intracellular pH during fatigue large enough to be the main cause of fatigue? *Canadian Journal of Physiology and Pharmacology* **64**, 764–767.

SAHLIN, K., EDSTRÖM, L., SJÖHOLM, H. & HULTMAN, E. (1981). Effects of lactic acid accumulation and ATP decrease on muscle tension and relaxation. *American Journal of Physiology* **240** C121–126.

TAYLOR, D. J., STYLES, P. O., MATTHEWS, P. M., ARNOLD, D. A., GADIAN, D. G., BORE, P. & RADDA, G. K. (1986). Energetics of human muscle: exercise induced ATP depletion. *Magnetic Resonance in Medicine* **3**, 44–54.