Journal of Physiology (1989), **413**, *pp.* 159–174
With 10 text-figures
Printed in Great Britain

EFFECTS OF FATIGUE AND REDUCED INTRACELLULAR pH ON SEGMENT DYNAMICS IN 'ISOMETRIC' RELAXATION OF FROG MUSCLE FIBRES

BY N. A. CURTIN* AND K. A. P. EDMAN†

*From the *Department of Physiology, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF and †Department of Pharmacology, University of Lund, S-223 62 Lund, Sweden*

(*Received 15 August 1988*)

SUMMARY

1. Longitudinal movements of marked segments of single fibres from the anterior tibialis muscle were recorded during tetanus and relaxation under isometric (fixed-end) conditions.

2. During relaxation, shortening and lengthening of different segments occurred simultaneously, starting at about the same time as the end of the linear fall of force (shoulder on the force record).

3. Variations in intracellular pH, measured with pH-sensitive microelectrodes, along the length of fibres were not statistically significant, and are unlikely to be responsible for the non-uniform behaviour of different segments.

4. As expected from earlier studies, both fatigue (produced by increasing tetanus duration or decreasing the time between tetani) and intracellular acidification (produced by raised extracellular CO_2), reduced the tetanus force and prolonged the linear phase of force decline in relaxation. Each treatment delayed the start and markedly reduced the amount of segment movement in relaxation.

5. Fatigue and intracellular acidification have a smaller effect on force during stretching than on force produced under isometric conditions. This may contribute to making the segments behave in a more uniform way during relaxation under these conditions.

6. Changes in the Ca^{2+} uptake mechanisms are also discussed as possible causes for the changes in segment behaviour in relaxation.

INTRODUCTION

Various techniques have shown that longitudinal movements occur during relaxation after an isometric (fixed-end) tetanus of intact single muscle fibres (Cleworth & Edman, 1969, 1972; Huxley & Simmons, 1970, 1973). During these relatively large and rapid movements some regions of the fibre shorten while others are being stretched. The movements start at about the time of the 'shoulder' on the force record, when the rate of fall of force increases and its time course changes from being linear to a form that resembles, more or less depending on the conditions, an exponential decline.

Huxley & Simmons (1973) found that when the length of the central 50 % of a fibre was kept constant ('length clamped') the relaxation of force was strikingly prolonged and the shoulder on the force record was delayed. This demonstrates an association between the shape of the force record in relaxation and movements of different regions of the fibre. This is supported by the finding that the time of the shoulder and the start of sarcomere movements in relaxation respond in the same way when temperature is changed (Edman & Flitney, 1982).

Our previous work has shown that both fatigue and intracellular acidification prolong the relaxation of force and delay the shoulder on the force record (Edman & Mattiazzi, 1981; Curtin, 1986*a*). The aim of the experiments reported here was to see how these treatments affect longitudinal movements of marked segments of single fibres during relaxation. The results are discussed in terms of changes in cross-bridge properties and Ca^{2+} uptake during relaxation. Some of these results have been described in a communication to the Physiological Society (Curtin & Edman, 1987).

METHODS

Experiments were done on single fibres dissected from the anterior tibialis muscle of frogs, *Rana temporaria*, that had been killed by decapitation followed by destruction of the spinal cord. The Ringer solution contained (mM): NaCl, 115·5; KCl, 2·0; $CaCl_2$, 1·8; $NaH_2PO_4 + Na_2HPO_4$, 2·0; pH 7·0 at 2 °C, equilibrated with O_2. In some experiments the Ringer solution contained (mM): NaCl, 108·0; KCl, 2·8; $CaCl_2$, 1·8; and $NaHCO_3$, 10; and was bubbled with mixtures of CO_2 and O_2 to give pH 7·0 or 6·5 at 0 °C.

The fibre was mounted in a Perspex bath between a force transducer and an arm extending from the moving coil of an electromagnetic puller. An aluminium foil clip was attached to each tendon 0·1–0·2 mm from the insertion of the fibre. The side parts of the clips were folded tightly around the hooks and positioned appropriately to minimize any lateral or vertical movements of the fibre during stimulation (Edman & Reggiani, 1984). The bath was thermostatted to about 2 °C (measured in each experiment) and Ringer solution flowed through the bath continuously.

Stimulation

The fibre was stimulated transversely via platinum plate electrodes and stimulus voltage was adjusted to about 1·2 times the threshold value. Pulse duration was constant at 0·2 ms. Stimulus frequency was adjusted to give just fused tetanus (usually 18–20 pulses s^{-1} under control conditions) and was changed as appropriate to maintain fusion during the experiments.

Segment length

Pieces of black dog hair were placed on the fibre at intervals of about 0·65 mm (measured in each case). Sarcomere spacing was set to 2·25 μm in the resting fibre using laser diffraction.

The optical system which detected changes in segment length was essentially the same as that described by Edman & Reggiani (1984). Briefly, laser light was shone through the fibre and the magnified image of the fibre projected onto a photodiode array (Reticon CCPD 1024, time resolution 0·25 ms, or Fairchild CCD 133, time resolution 0·04 ms). An analog circuit converted the output from the array to a signal proportional to the percentage change in segment length (distance between the two selected markers on the fibre). The system detected and recorded (with an accuracy of 0·2 % of the segment length) the length of only one segment at a time, so the tetanus and interval pattern was repeated until records were made from all segments along the fibre. Results of repeat measurements are reported in the Results section. The distance between the outermost marker and the tendon was not recorded during tetanus because the tendon did not provide a suitable signal to the optical recording system. The length of this part of the fibre was smaller than the other segments (outermost segment = 0·24 mm, other segments = 0·65 mm, fibre length = 8·29 mm, number of segments = 11, average values for nine fibres). The separation between the markers was measured using the eye-piece graticule of the stereo-microscope at 40 × magnification.

Measurement of the records

Tension, segment length and stimulus marks were recorded on a storage oscilloscope, photographed and measured on the film using a Nikon Profile Projector. As shown in Fig. 1, measurements were made of maximum force in the tetanus and time from the last stimulus to the end of the linear phase of tension decline in relaxation (relaxation time). On the record of segment length the following measurements were made: the time from the last stimulus to the start of

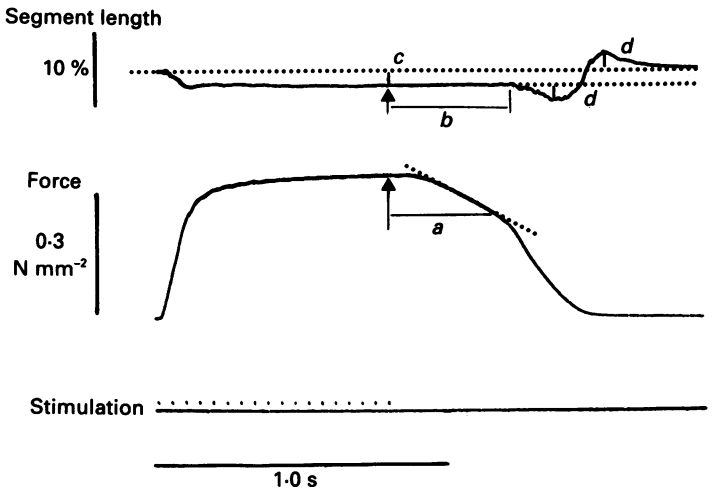

Fig. 1. Example records of the length of one segment and force during a 0·8 s isometric (fixed-end) tetanus. Records are marked to show how they were measured; *a* is the time from the last stimulus (indicated by the triangle) to the end of the linear decline of force in relaxation; *b* is the time from the last stimulus to the start of segment movement in relaxation; *c* is the difference in segment length at the last stimulus compared to before stimulation; *d* is the movement overshoot, that is, movement beyond what would return the segment to its initial length during relaxation. See Fig. 3 for other patterns of length change in relaxation. The maximum force in the tetanus was also measured. Fibre length 8·40 mm at sarcomere length 2·20 μm; cross-sectional area 0·0271 mm^2.

movement, the net movement during stimulation (measured at the last stimulus), and movement 'overshoot' during relaxation. Overshoot is defined as change in segment length beyond what would be required to return the segment to its original length. Some segments, like that shown in Fig. 1, shortened more after the end of stimulation and then lengthened beyond the resting length (overshoot in both directions). In Fig. 2, the distance moved in each direction by the segment is shown separately. The *total* overshoot is the sum of the absolute values of shortening and lengthening (Fig. 7*B*).

Experimental protocol

Control conditions were phosphate-buffered Ringer solution and a series of brief tetani (0·5–1·0 s and constant for each fibre) with an interval of 120 or 300 s between the start of the tetani. Two experimental conditions were used, either separately or together. (1) The fibre was fatigued by reducing the interval between the start of the tetani to between 120 and 15 s, and/or increasing the duration of tetanic stimulation to between 1·0 and 2·0 s. (2) The Ringer solution was changed from one buffered with phosphate and equilibrated with O_2, to one buffered with $HCO_3^- + CO_2$. Entry of the permeant acid CO_2 results in a sustained acid shift of intracellular pH, pH_i (Bolton & Vaughan-Jones, 1977). Abercrombie, Putnam & Roos (1983) found that pH_i recovered slowly during exposure to CO_2 (0·025 pH units h^{-1}) at 22 °C. Under our conditions, pH would decrease by about 0·15 and 0·30 pH units in response to the two levels of CO_2 used here, 2·1 and 6·0 mmol l^{-1}

(assuming a buffering power of 40 mmol l^{-1} pH $unit^{-1}$, Curtin, 1986*b*) and contractile performance changed as soon as the solution change was complete. We did not observe any evidence of spontaneous recovery of the contractile performance during exposures to CO_2 lasting a few hours. Thus it seems that the amount of pH_i recovery was too small to have a detectable effect on contraction, or pH_i recovery was even slower than in the experiments of Abercrombie *et al.* (1983) because of some difference in conditions, such as the lower temperature or intermittent stimulation used here.

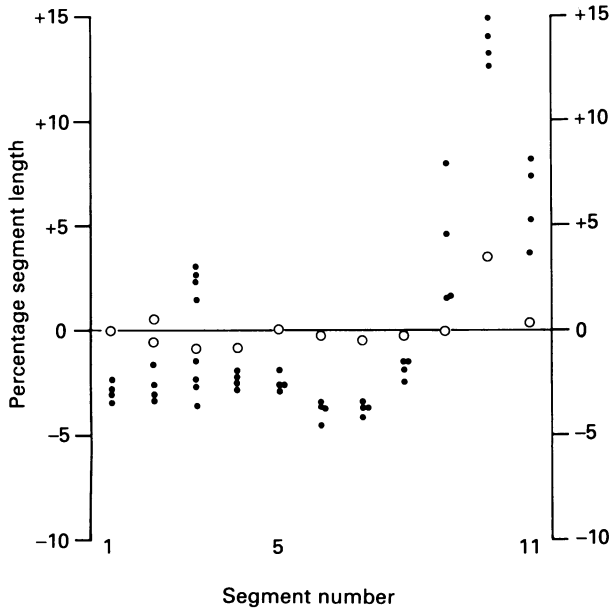

Fig. 2. Extent of overshoot movement (percentage segment length) during relaxation in all eleven segments of a fibre. Positive values indicate that the segment was stretched beyond its pre-tetanus length, and negative values shortening beyond pre-tetanus length. For segment 3 there is both a negative and a positive value because it shortened and then was stretched as shown in the time course records for this experiment shown in Fig. 3. Filled symbols are from four complete sets of measurements (each set included a measurement on every segment) under control conditions (0·8 s tetanus, 120 s interval). Open symbols, fatigued state, 0·8 s tetanus, 30 s interval.

Under each condition, the fibre was stimulated at exact intervals until the force was constant in successive tetani (under control conditions, this required about twelve cycles of a tetanus+ interval). Having reached this steady state, the segment length was recorded during tetanic stimulation and relaxation.

Stretch experiments

The fibre was stretched during the tetanus after force was well developed. The velocity and amplitude of the stretch were adjusted so that the force increased and then remained, as nearly as possible, constant during the stretch. The velocity and amplitude were then kept constant for each fibre. Force was recorded under control conditions, and under the experimental conditions described above. Force was measured just before stretch (isometric force) and at the end of the initial rapid phase of force increase during stretch (stretch force, see Fig. 8); both were measured from the baseline of zero force.

Intracellular pH

Intracellular pH was measured with liquid-membrane, pH-sensitive microelectrodes (eccentric, double-microelectrode design of Thomas, 1986). Other conditions were as described in Curtin,

Kometani & Woledge (1988) except that the Ringer solutions were as described here and temperature was 22 °C. Repeat measurements were made at between two and eight locations along each fibre. In each case the distance between the electrode tip and the left-hand end of the fibre was measured with an eye-piece graticule in the stereo-microscope.

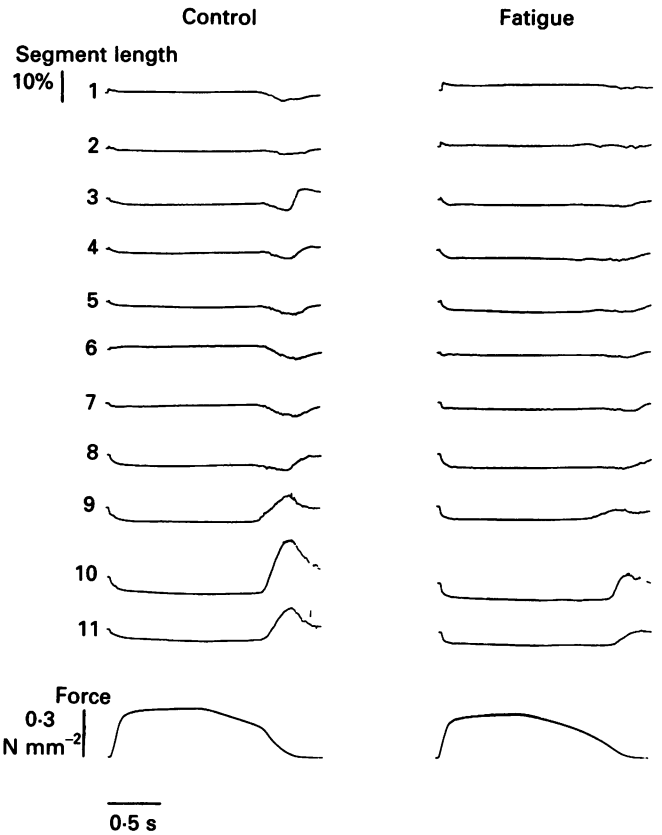

Fig. 3. A complete set of records of length for all the segments in a fibre and the force record for stimulation under control conditions (for this fibre 0·8 s tetani with 120 s intervals between tetani) and experimental conditions (fatigue due to 0·8 s tetani with 30 s intervals). The segment number is shown to the left of the control records.

Statistical tests

Values are reported as mean ±S.E.M. or S.D., as indicated. Student's *t* test was used to test the significance of differences; a probability value of 0·05 was taken as statistically significant.

RESULTS

Repeated measurements of segment movement

An experiment was done to investigate how constant segment behaviour was in repeated tetani. The length of each segment in the fibre was recorded during a 0·8 s tetanus with 120 s intervals between tetani. The entire set of recordings was repeated four times. The segment overshoot during relaxation varied between segments, but as is obvious from Fig. 2, the behaviour of an individual segment was

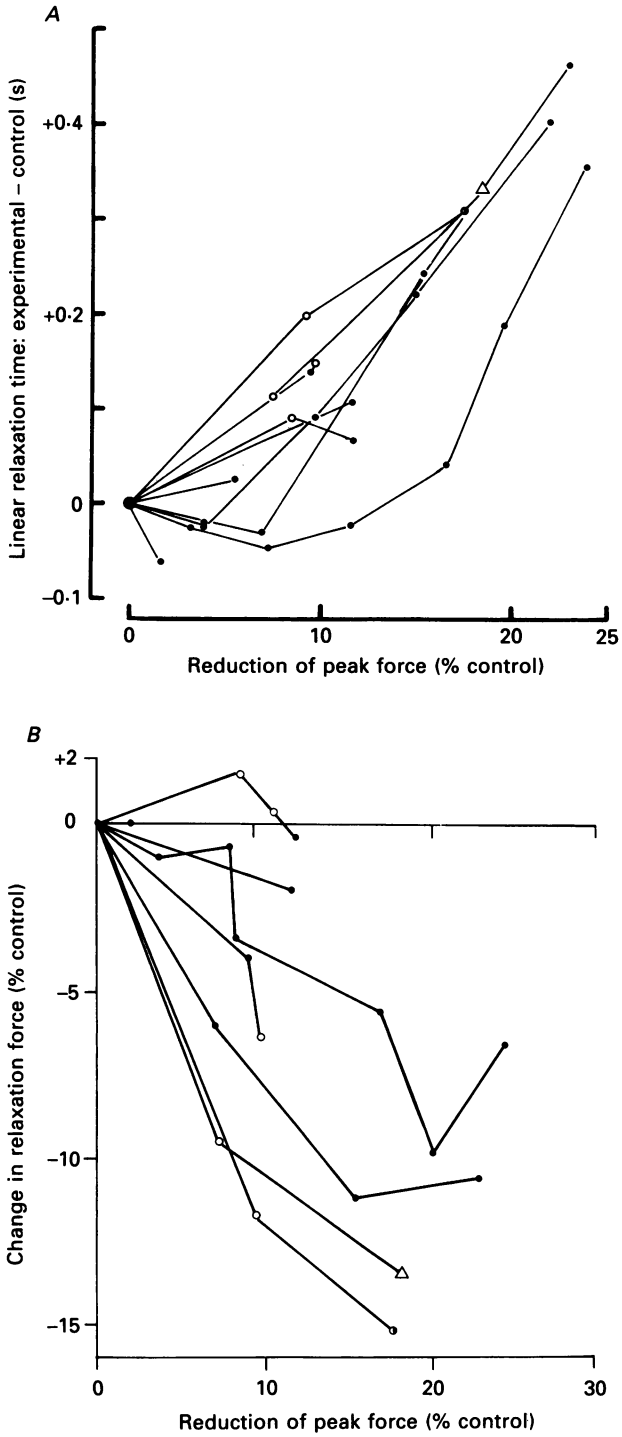

Fig. 4. For legend see facing page.

relatively constant. The mean values for an individual segment ranged from 1·8 to 13·6% of segment length ($n = 4$ for each segment), and the overall average of the mean value for each segment was 4·4% ± 3·3 (S.D.), $n = 11$; coefficient of variation = S.D./mean = 0·75. For the four repeats on the same segment, the coefficient of variation ranged from 0·08 to 0·78 with a mean value of 0·24.

Tetanus force and the time course of relaxation

Figure 3 shows records of the length changes for all eleven segments and force under the control conditions (0·8 s tetanus with 120 s intervals between tetani) and for a fatiguing pattern of stimulation (0·8 s and 30 s intervals) in the same fibre. In the fatigued state the maximum force during stimulation was 11·6% lower than under control conditions and relaxation was slower, as expected from earlier studies (Edwards, Hill & Jones, 1975; Dawson, Gadian & Wilkie, 1980; Edman & Mattiazzi, 1981).

The time from the last stimulus to the end of the linear phase of relaxation (relaxation time) includes (1) the time from the last stimulus to the start of fall of force and (2) the time during which force declines relatively linearly. The first component was only slightly prolonged, by 10 ms in the example in Fig. 3 and 13 ± 0·019 ms (S.D.) in fifteen experiments performed on eight fibres. The linear phase was considerably longer and less steep in the fatigued state and after reduction of pH_i. In the example shown in Fig. 3 the linear phase was 190 ms longer in the fatigued state than under control conditions. Mean values of the change in relaxation time for ten fibres are shown in Fig. 4*A* plotted against the reduction in peak tetanus force expressed as percentage of the control force. Clearly there is variation between fibres, but in all fibres when maximum tetanic force was reduced by more than 13%, linear relaxation time was longer than for the control condition. In four fibres small reductions (< 13%) in tetanus force were associated with a shorter relaxation time; in three of these fibres, further depression of force was accompanied by an increase in the linear relaxation time.

During the linear part of relaxation, force declined to a lower level (as a percentage of force at the last stimulus) with fatigue and acidified pH_i than under control conditions in all but two cases (Fig. 4*B*). It follows from the results in Fig. 4 that the rate of decline of force was slowed by fatigue and reduced pH_i. This was verified by measurements of the time required for force to drop from 95 to 80% of its value at the last stimulus (results not shown).

In summary, the linear part of relaxation is longer under the experimental conditions because the rate of decline of force is lower and because the force reaches a lower level before the shoulder.

Fig. 4. *A*, the relation between change in relaxation time and reduction in maximum tetanic force due to fatigue (●), acidified pH_i (at extracellular pH 7·0, ○; 6·5 △), fatigue and acidified pH_i (◑). Force reduction is expressed as percentage of the force under control conditions. Linear relaxation time is the time from the last stimulus to the end of the linear decline of force in relaxation. The lines join values for the same fibre and each point is the mean of values from between ten and twelve tetani under the same conditions. *B*, changes in the force remaining at the end of the linear fall of force in relaxation *vs.* reduction in maximum tetanic force. Symbols as in *A*.

Segment movement

As expected from earlier observations (Cleworth & Edman, 1969, 1972; Huxley & Simmons, 1970, 1973; Julian & Morgan, 1979; Edman & Flitney, 1982), redistribution of segment length started at the shoulder during relaxation. As described above, the linear fall of force in relaxation is prolonged in the fatigued state

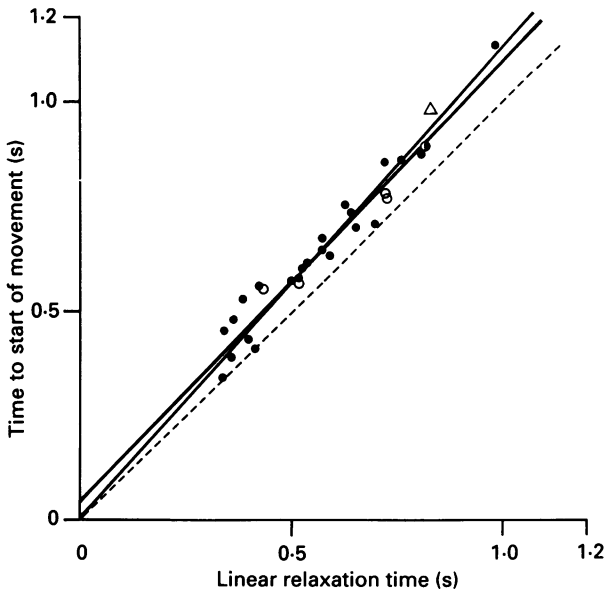

Fig. 5. Relation between time from the last stimulus to the start of segment movements and to the end of the linear decline of force (linear relaxation time). Each point is a mean value: for start of segment movement, it is the mean for all segments in the fibre in which movement overshoot occurred, and for relaxation time, the mean from the corresponding force records. Control and fatiguing patterns of stimulation (●), reduced pH_i (extracellular pH 7·0, ○; 6·5, △), fatigue and reduced pH_i (◑). The dashed line is the line of identity and the continuous lines are regression lines for x on y and y on x for all the points. Results for nine fibres.

and after reduction of pH_i compared to the control. The segment movements in relaxation were also delayed, and overshoot movements started at about the same time as the end of the linear part of force relaxation. Figure 5, which is a summary of all the experiments, shows good correlation ($r = 0{\cdot}968$, $n = 29$, $P < 0{\cdot}001$) between these variables.

Extent of segment movement during stimulation

Measurements were also made of the extent of segment movement during stimulation and during relaxation. During stimulation most of the segments shortened at the expense of the external connections, but a few segments were stretched (see Fig. 3, control, segments 1 and 6). The locations of the stretched segments varied from fibre to fibre. Figure 6 shows changes in the lengths of all segments in one fibre under control conditions and with six different degrees of

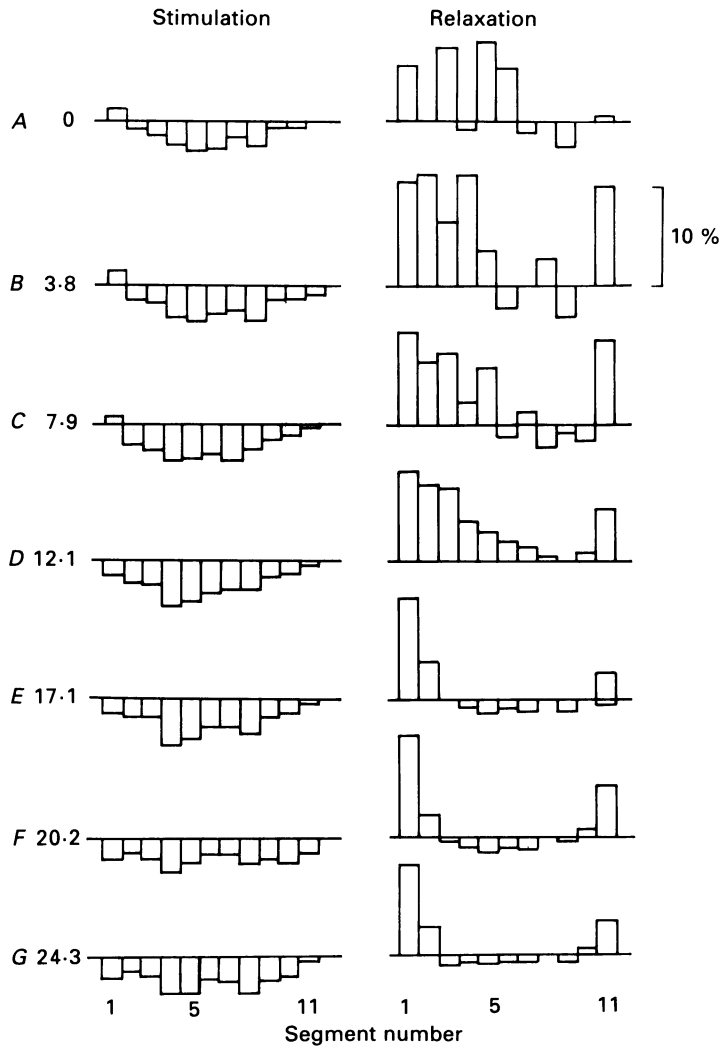

Fig. 6. Histograms showing changes in the length of all segments in a fibre during stimulation (left column) and overshoot during relaxation (right column). See Methods and Fig. 1 concerning measurement of segment length changes. Shortening is indicated by bars below the baseline and stretching by bars above it. Results are shown for different patterns of stimulation (tetanus and interval durations in seconds): *A*, 0·6, 300; *B*, 0·6, 120; *C*, 0·6, 60; *D*, 0·6, 30; *E*, 0·6, 15; *F*, 1·0, 15; *G*, 2·0, 15. The value next to each letter is the percentage reduction in the maximum tetanic force compared to that in *A*.

fatigue. For all segments there were slight differences in segment movement during stimulation, but these did not appear to be related to the extent of fatigue.

To examine total movement in the whole fibre, the change in length of each segment was expressed as distance (in millimetres) and the sum of the absolute values for all segments was calculated. As shown in Fig. 7*A*, the extent of movement during stimulation was not consistently changed by conditions that reduced the maximum isometric force.

Segment movements during relaxation

Figure 3 shows that none of the segments simply returned to their original lengths during relaxation under control conditions. In contrast under fatiguing conditions the segment length generally returned to its original length without much overshoot

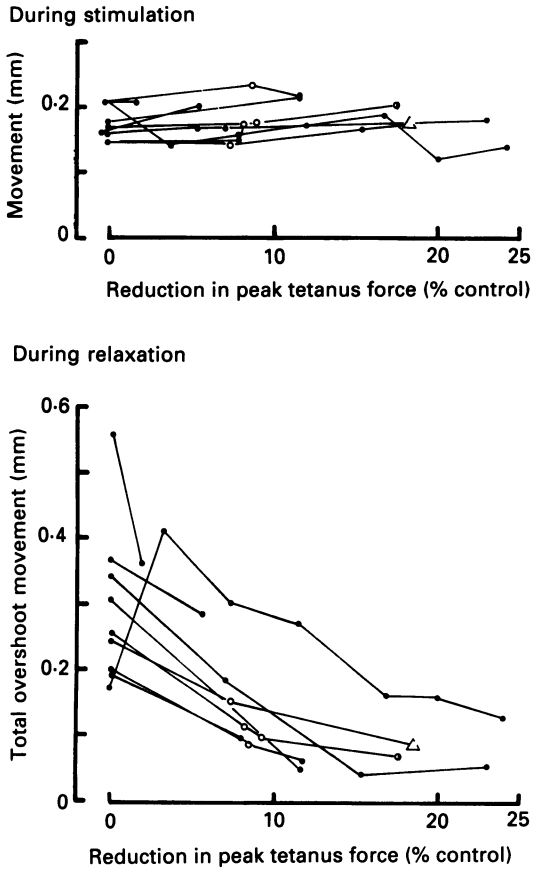

Fig. 7. Relation between the total length change for all segments and the reduction in maximum tetanic force due to fatiguing pattern of stimulation (●), reduced pH_i (extracellular pH 7·0, ○; 6·5 △), fatigue and reduced pH_i (◑). Lines join results for the same fibre. *A*, total change in length during stimulation; *B*, total overshoot movement during relaxation.

(see also Fig. 6). The absolute values of the total overshoot (in millimetres, calculated as described above) for all experiments are summarized in Fig. 7*B*. The fibres varied in the amount of overshoot movement under control conditions. The main feature of the results is that when the tetanus force decreased by a moderate amount (up to 25% of the force under control conditions), there was less segment movement in relaxation.

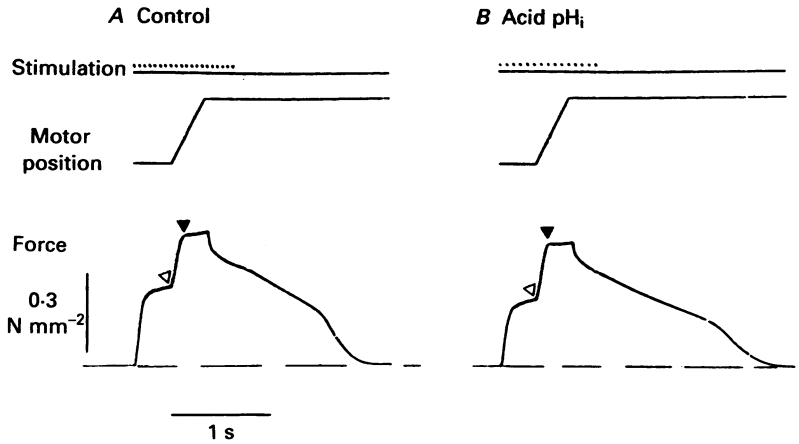

Fig. 8. Examples of force recorded during an isometric tetanus including an isovelocity stretch (upward movement of the motor position signal indicates stretch); stimulus pattern 1·0 s tetanus and 120 s interval between tetani. Force was measured from the baseline (dashed line) at the times indicated; △, isometric; ▼, stretch. *A*, control conditions (phosphate Ringer solution, extracellular pH 7·0); *B*, experimental conditions (bicarbonate + CO_2 Ringer solution, extracellular pH 7·0). Distance stretched, 0·348 mm. Initial fibre length, 6·75 mm, and sarcomere length 2·05 μm. Fibre cross-sectional area, 0·0204 mm^2.

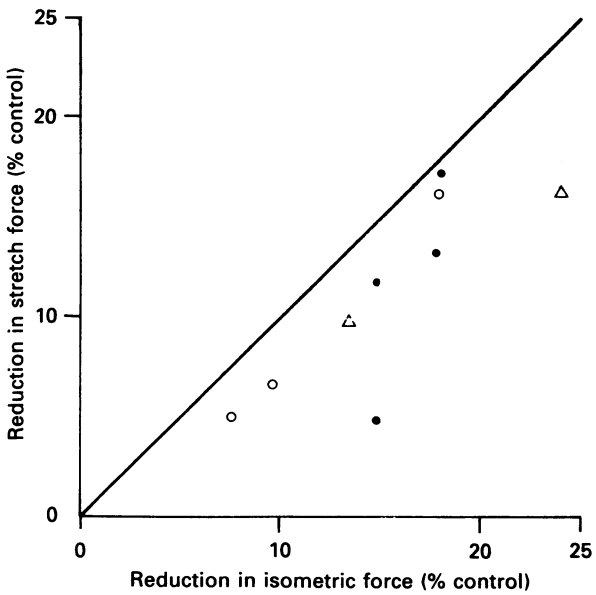

Fig. 9. Summary of the mean values of relative force (experimental/control) during stretch and under isometric conditions. The continuous line is the line of identity. Each point is based on the mean of values from two or three tetani under the same conditions: fatigue (●); reduced pH$_i$ (extracellular pH 7·0, ○; 6·5 △). Results from six fibres.

Recovery

In experiments on four fibres the measurements were repeated under the control conditions after the fatiguing pattern of stimulation or after an acid shift of pH_i.

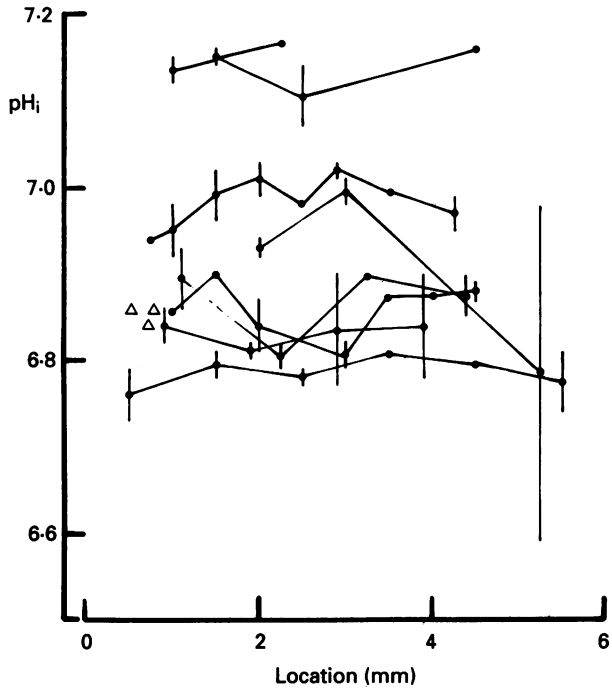

Fig. 10. Measurements of pH_i at different locations along muscle fibres. Each filled symbol (●) is the mean of the two measurements at the same location. The vertical lines show the range of the two measurements when it was greater than the size of the symbol. The location is the distance from the point of insertion of the pH-sensitive microelectrode to the left end of the fibre, measured using an eye-piece graticule in the stereo-microscope. The lines join mean values for the same fibre. The double triangle marks the fibre for which variance of measurements at different locations was significantly greater than the variance of measurements at same location. The single triangle marks the fibre for which the variance for the same location was significantly greater than the variance for different locations.

After recovery periods of between 15 min and 3 h in the control solution, the maximum force, relaxation time, time to start of segment movement, and movement overshoot in relaxation all returned towards their original values.

Force during stretch of active fibres

Because resistance to stretch is probably a major factor which stabilizes the sarcomere and prevents changes in the lengths of fibre segments, six experiments were done in which a fibre was stretched during tetanus to see whether force during stretch was reduced to the same extent as the isometric force was by fatiguing stimulation and/or acid shift of pH_i (Fig. 8). The percentage reduction of the total

force during stretch and of the isometric force by fatigue and reduced pH_i are shown in Fig. 9. All the points fall below the line of identity, showing that the total force during stretch is less affected by fatigue and acid pH_i than the isometric force is.

Intracellular pH in different segments

Measurements of pH_i were made with pH-sensitive microelectrodes to investigate the possibility that variations in pH_i along the fibre could be responsible for the non-uniform movement during relaxation.

Two measurements of pH_i were made at between two and eight locations along the length of eight fibres in control Ringer solution. The values are shown in Fig. 10. The variance ratio test was used to assess the significance of differences between different locations in each fibre. Estimates of the variance of the population were based on differences between locations and on differences between repeat measurements at one location. The statistical analysis showed that the null hypothesis could not be rejected, and the extent of variation between different locations in a fibre was due to random sampling fluctuations and does not reflect real differences in pH_i. Similarly, the null hypothesis could not be rejected on the basis of testing the pooled estimates of the variance of the population based on differences between repeat measures and on differences between locations ($F = 1{\cdot}0005$, $P > 0{\cdot}05$).

In contrast, differences between fibres were highly significant compared with differences between locations ($F = 42{\cdot}2$, $P < 0{\cdot}01$). The differences in fibre pH_i may be responsible for some of the variation in behaviour among fibres under the control conditions, which can be seen, for example, in Fig. 7*B*.

DISCUSSION

Our results extend earlier work (Cleworth & Edman, 1969, 1972; Huxley & Simmons, 1970, 1973; Julian & Morgan, 1979; Edman, 1980; Edman & Flitney, 1982) by showing that even when the time to the shoulder was greatly extended by either fatigue or reduced pH_i there is an excellent correlation between the time when segment movements start and the force shoulder in relaxation (Fig. 5). This adds support to the idea that these two events are causally related. We also found that these conditions reduce the segment movement in relaxation (Fig. 7).

Under control conditions, large movements occur during relaxation when a segment(s) is able to shorten while producing enough force to stretch another segment(s). This must be due to some non-uniformity among the segments under control conditions, which is reduced by fatigue and acidification of pH_i. The following factors will be considered as possible causes of this non-uniformity: passive elasticity, pH_i, cross-bridge number arising from differences in net detachment rate or rate of Ca^{2+} uptake. The dependence of cross-bridge force on direction of movement is also considered.

Passive elasticity

Differences in the passive elasticity along the fibres, in parallel with the myofibrils, are ruled out by the results of Edman, Reggiani & te Kronnie (1985). Although they found that the velocity of segment elongation during ramp stretches of *resting* fibres

did vary slightly from one segment to another, the variations were not correlated with the much larger variations in unloaded shortening velocity, V_0, during stimulation. Thus the passive force at the sarcomere lengths used here is too small to affect movements due to cross-bridge activity.

Intracellular pH

The non-uniformity in relaxation could arise if the pH_i varied along the fibre because contractile processes are influenced by pH in the physiological range (for example, Fabiato & Fabiato, 1978; Edman & Mattiazzi, 1981; Curtin *et al.* 1988; Cooke, Franks, Luciani & Pate, 1988). However, the measurements reported here do not show significant variation in pH at different locations. The variation was no greater than that between repeat measures at the same location and was very much smaller than the variation in fibre means (see Fig. 10).

Cross-bridge properties

Another possibility is that a difference in some aspect of cross-bridge properties is responsible for the difference in segment behaviour. The finding that V_0 varies between segments (Edman *et al.* 1985), suggests that there may be differences in cross-bridge kinetics between segments. If this resulted in a sufficient discrepancy in the number of active bridges between segments in relaxation, segments containing a larger number of active bridges could shorten and stretch segments with fewer. Both fatigue and reduced pH_i have been shown to reduce V_0 (Edman & Mattiazzi, 1981); a slowing of cross-bridge kinetics could slow the build-up of the discrepancy between segments and thus delay the start of segment movements.

The fact that more force is produced by bridges during stretch than shortening (force–velocity relation) will stabilize the fibre against *some* discrepancy between the number of active bridges in different segments. However, segment movement will occur when the reduction in number of bridges in a segment can no longer be compensated for by the increase in force when its bridges are stretched by another segment. From our results it seems likely that this mechanism operates since fatigue and reduced pH_i, conditions which delay segment movements, also reduce isometric force more than force during stretch (Fig. 9). This effect would tend to further stabilize the fibre during relaxation. Thus under these conditions, a greater discrepancy between the number of active cross-bridges in different segments would have to develop before rapid segment movements could start in relaxation.

Ca^{2+} uptake

Since large segment movements only start in relaxation, it is natural to speculate that differences in segment strength arise because of the rate of Ca^{2+} uptake from the sarcoplasm is greater, thus leaving fewer active bridges in some segments than others.

We know of no direct evidence for variations along a fibre of either sarcoplasmic reticulum or parvalbumin, which are believed to be responsible for Ca^{2+} uptake. Parvalbumin has been found to diffuse freely, albeit slowly, within skinned fibres (Maughan & Wegner, 1987), whereas stable gradients would require that it is bound in a fixed location. However, it may be that slight, and thus difficult to detect,

differences in the sarcoplasmic reticulum or parvalbumin between segments could lead to large segment movement in relaxation.

Although our experiments were not designed to give direct evidence about changes in Ca^{2+} uptake during relaxation, the slowing of force decline we observe is consistent with known effects of fatigue and reduced pH_i. The fatiguing pattern of stimulation used here would be expected to reduce the effectiveness of parvalbumin as a 'relaxing factor' binding Ca^{2+} during relaxation (reviewed by Gillis, 1985, see also Cannell, 1986; Peckham & Woledge, 1986). Ca^{2+} is thought to bind to parvalbumin during stimulation and relaxation, and then be released and pumped back into the sarcoplasmic reticulum during the intervals between tetani. In our experiments the intervals between tetani were reduced relative to tetanus duration, and this would result in progressive saturation of the parvalbumin with Ca^{2+}.

The changes we found in relaxation with acidified pH_i are consistent with the pH dependence of the activity of the sarcoplasmic reticulum ATPase. MacLennan (1970) showed that this activity was 40% lower at pH 6·5 than at pH 7·0. With our Ringer solution (phosphate buffer at pH 7·0, 22 °C) the mean pH_i was $6{\cdot}89 \pm 0{\cdot}05$ (S.E.M., $n = 8$ fibres). From previous observations of the effects of CO_2 on pH_i (Curtin, 1986*b*), an acidification of 0·14 and 0·29 pH units would occur on exposure to the two CO_2 concentrations used here (2·1 and 6·0 mmol l^{-1}). The sarcoplasmic reticulum ATPase activity would be reduced by 12 and 24%, respectively, compared to control conditions and significant reduction in the Ca^{2+} pumping rate probably occurred.

Therefore, fatigue and reduced pH_i could both lead to a slower fall in free Ca^{2+}, and therefore a longer time to reach the threshold for large segment movements.

Extent of segment movement in relaxation

The extent of segment movement in relaxation is reduced when the fibre is fatigued and/or acidified (Fig. 7). The movement may be reduced because only small differences among the segments ever develop under these conditions. This could be due to the increased uniformity of Ca^{2+} uptake accompanying fatigue and intracellular acidification. The relative increase in resistance to stretch of the cross-bridges could also reduce the extent of segment movements in relaxation under these conditions.

This work was supported by grants from the Swedish Medical Research Council (project No. 14X-184, KAPE) and the Wellcome Trust and European Molecular Biology Organization (to N.A.C.). N.A.C. thanks R. C. Thomas for instruction in making the eccentric pH microelectrodes.

REFERENCES

ABERCROMBIE, R. F., PUTNAM, R. W. & ROOS, A. (1983). The intracellular pH of frog skeletal muscle: its regulation in isotonic solutions. *Journal of Physiology* **345**, 175–187.

BOLTON, T. B. & VAUGHAN-JONES, R. D. (1977). Continuous direct measurement of intracellular chloride and pH in frog skeletal muscle. *Journal of Physiology* **270**, 801–833.

CANNELL, M. B. (1986). Effect of tetanus duration on the free calcium during the relaxation of frog skeletal muscle fibres. *Journal of Physiology* **376**, 203–218.

CLEWORTH, D. R. & EDMAN, K. A. P. (1969). Laser diffraction studies on single skeletal muscle fibers. *Science* **163**, 296–298.

CLEWORTH, D. R. & EDMAN, K. A. P. (1972). Changes in sarcomere length during isometric tension development in frog skeletal muscle. *Journal of Physiology* **227**, 1–17.

COOKE, R., FRANKS, K., LUCIANI, G. B. & PATE, E. (1988). The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *Journal of Physiology* **395**, 77–97.

CURTIN, N. A. (1986*a*). Effects of carbon dioxide and tetanus duration on relaxation of frog skeletal muscle. *Journal of Muscle Research and Cell Motility* **7**, 269–275.

CURTIN, N. A. (1986*b*). Buffer power and intracellular pH of frog sartorius muscle. *Biophysical Journal* **50**, 837–841.

CURTIN, N. A. & EDMAN, K. A. P. (1987). Force and segment movements during relaxation of frog muscle fibres; effects of fatigue and intracellular acidification. *Journal of Physiology* **390**, 153*P*.

CURTIN, N. A., KOMETANI, K. & WOLEDGE, R. C. (1988). Effect of intracellular pH on force and heat production in isometric contraction of frog muscle fibres. *Journal of Physiology* **396**, 93–104.

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

EDMAN, K. A. P. (1980). The role of non-uniform sarcomere behaviour during relaxation of striated muscle. *European Heart Journal* **1**, suppl. A, 49–57.

EDMAN, K. A. P. & FLITNEY, F. W. (1982). Laser diffraction studies of sarcomere dynamics during 'isometric' relaxation in isolated muscle fibres of the frog. *Journal of Physiology* **329**, 1–20.

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.

EDMAN, K. A. P. & REGGIANI, C. (1984). Redistribution of sarcomere length during isometric contraction of frog muscle fibres and its relation to tension creep. *Journal of Physiology* **351**, 169–198.

EDMAN, K. A. P., REGGIANI, C. & TE KRONNIE, G. (1985). Differences in maximum velocity of shortening along single muscle fibre of the frog. *Journal of Physiology* **365**, 147–163.

EDWARDS, R. H. T., HILL, D. K. & JONES, D. A. (1975). Metabolic changes associated with the 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 muscle. *Journal of Physiology* **276**, 233–255.

GILLIS, J. M. (1985). Relaxation of vertebrate skeletal muscle. A synthesis of the biochemical and physiological approaches. *Biochimica et biophysica acta* **811**, 97–145.

HUXLEY, A. F. & SIMMONS, R. M. (1970). Rapid 'give' and the tension 'shoulder' in the relaxation of frog muscle fibres. *Journal of Physiology* **210**, 32*P*.

HUXLEY, A. F. & SIMMONS, R. M. (1973). Mechanical transients and the origin of muscular force. *Cold Spring Harbor Symposium on Quantitative Biology* **37**, 669–680.

JULIAN, F. J. & MORGAN, D. L. (1979). Intersarcomere dynamics during fixed-end tetanic contractions of frog muscle fibres. *Journal of Physiology* **293**, 365–378.

MACLENNAN, D. H. (1970). Purification and properties of an adenosine triphosphatase from sarcoplasmic reticulum. *Journal of Biological Chemistry* **245**, 4508–4518.

MAUGHAN, D. & WEGNER, E. (1987). Diffusivity of parvalbumin and other proteins in freshly skinned frog skeletal muscle fibers. *Biophysical Journal* **51**, 322a.

PECKHAM, M. & WOLEDGE, R. C. (1986). Labile heat and changes in rate of relaxation of frog muscles. *Journal of Physiology* **374**, 123–135.

THOMAS, R. C. (1986). Eccentric double micropipette suitable both for pH_i micro-electrodes and for intracellular iontophoresis. *Journal of Physiology* **371**, 24*P*.