J. Physiol. (1984), **351**, *pp.* 169–198
With 14 *text-figures*
Printed in Great Britain

REDISTRIBUTION OF SARCOMERE LENGTH DURING ISOMETRIC CONTRACTION OF FROG MUSCLE FIBRES AND ITS RELATION TO TENSION CREEP

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(*Received 8 April 1983*)

SUMMARY

1. Changes in length of successive 0·5–0·8 mm segments along single muscle fibres of *Rana temporaria* were recorded during 3 s isometric (fixed fibre ends) tetani at 2·15 and 2·60 μm sarcomere length. The measurements were performed by means of a photo-electric detector system which recorded the distance between opaque markers (*ca.* 60 μm in width) that were attached to the upper surface of the fibre. The segment length change had an initial rapid phase (1) which coincided with the steep rise of force and a subsequent slow phase (2) which coincided with the upper, rounded portion of the force myogram and the 'plateau' of the tetanus.

2. At 2·15 μm sarcomere length the majority of the central segments (comprising approximately 90% of the fibre) shortened to various degrees during phase 1. A considerable redistribution of length occurred during phase 2 in that some segments shortened at the expense of others which were forcibly stretched. The central region, taken as a whole, shortened by 0·1–0·5% during phase 2. The end segments were consistently found to elongate during phase 1. However, they were able to hold the tension, without further elongation, during phase 2.

3. The pattern of length changes within the central region of the fibre observed at 2·15 μm sarcomere spacing remained largely the same after increasing the sarcomere length to 2·60 μm. However, in contrast to the situation at 2·15 μm sarcomere length there was an over-all (0·4–1·5%) *elongation* of the central region of the fibre during phase 2 at the great fibre length. This elongation of the central region was associated with marked shortening of the end segments.

4. The sarcomere length of the end segments ($s.l._e$) was compared to that of the central region of the fibre ($s.l._c$) at various fibre rest lengths. There was no significant difference between $s.l._e$ and $s.l._c$ when the fibre was just taut, i.e. at approximately 2·1 μm sarcomere length. The following relationship between $s.l._e$ and $s.l._c$ was found to apply for values of $s.l._c$ ranging between 2·2 and 2·7 μm: $s.l._e = 0{\cdot}636\ s.l._c + 0{\cdot}744$ (correlation coefficient, 0·93).

5. The possibility was explored that redistribution of sarcomere length along the fibre causes the slow climb of force ('tension creep') that occurs during a tetanus at great (> 2·2 μm) sarcomere lengths. Tension creep could be reproduced, after peak

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force had been attained, during an isometric tetanus by releasing the fibre to shorten within the range 2·6–2·3 μm sarcomere length. Under these conditions shortening at a low, constant velocity caused a steady increase of force whereas shortening against a high, constant load caused acceleration of the fibre. Similar shortening protocols performed within the plateau of the length–tension relation did not enhance the contractile performance.

6. The duration and magnitude of the tension creep increased with the sarcomere length at which the tetanus was initiated. Peak force occurred when the end segments had shortened to just below 2·2 μm sarcomere length. This is explained by the fact that the end segments, being the main generators of tension creep, do not further improve their strength by shortening once they have reached the plateau of the length–tension relation.

7. Tension creep could be eliminated, at any fibre rest length, by holding a small (0·5–0·7 mm) segment at constant length during the tetanus. This finding provides evidence that tension creep is not a feature of the contractile process at sarcomere level.

8. A computer model (described in the Appendix) was used to simulate segment length changes and tension creep during fixed-end tetani.

INTRODUCTION

When vertebrate skeletal muscle is stimulated to produce an isometric tetanus at a pre-stretched length, tension increases rapidly at first and rises thereafter slowly to a maximum value. This second, slow phase of tension rise, generally referred to as 'tension creep', is exhibited by single fibres (Ramsey & Street, 1940; Gordon, Huxley & Julian, 1966*a*,*b*; Edman, Elzinga & Noble, 1978; Julian, Moss & Sollins, 1978*a*; Julian, Sollins & Moss, 1978*b*; ter Keurs, Iwazumi & Pollack, 1978, 1979) as well as whole muscle (Abbott & Aubert, 1952; Deleze, 1961) and becomes a prominent feature of the force myogram at great fibre lengths.

So far there has been no general agreement concerning the mechanism underlying tension creep. Gordon *et al.* (1966*a*) advanced the idea that the slow rise of force during the isometric tetanus is based on internal redistribution of length in the fibre. In support of this view it was demonstrated (Gordon *et al.* 1966*a*) that sarcomeres close to the tendon insertion, at least at a high degree of stretch of the fibre, were able to shorten during the isometric tetanus at the expense of sarcomeres in the middle portion of the fibre which elongated to a corresponding degree. The greater strength of the end sarcomeres could be explained by the earlier finding (Huxley & Peachey, 1961) that in greatly stretched fibres (50 % or more above slack length) the sarcomere spacing is considerably smaller near the tendon junctions than in the middle of the fibre. More recent studies (Julian *et al.* 1978*a*,*b*; Julian & Morgan, 1979) have provided further support for the view that the slow climb of tension during the tetanus is generated by the end sarcomeres as these shorten toward the plateau of the length–tension relation.

Arguments against the idea that tension creep is due to internal length changes during contraction have been raised by ter Keurs *et al.* (1978). These authors, using laser diffraction techniques to monitor changes in sarcomere length in single muscle

fibres, did not observe any sarcomere movements during the isometric tetanus that were considered large enough to account for the tension creep. On the basis of these negative findings ter Keurs *et al.* (1978) put up the alternative hypothesis (also see ter Keurs *et al.* 1979 and Pollack, 1983) that tension creep reflects the true isometric force development at sarcomere level.

In the present investigation we have further explored the occurrence of internal length changes in isolated frog muscle fibres during isometric activity. For this purpose a technique has been used by which length changes of short consecutive segments could be monitored throughout the course of a tetanus. Evidence will be presented to show that there is a considerable redistribution of sarcomere length during the isometric tetanus not only between the ends and the middle portion of the fibre but also between various segments within the central region. Experiments have been specifically directed toward the question of whether this redistribution of sarcomere length is responsible for the slow increase in force during isometric tetanus at a pre-stretched length. Attempts have furthermore been made to find out if tension creep can be eliminated by length clamping very short fibre segments during tetanic contraction. Some of the results have been presented in a preliminary communication (Edman & Reggiani, 1984).

METHODS

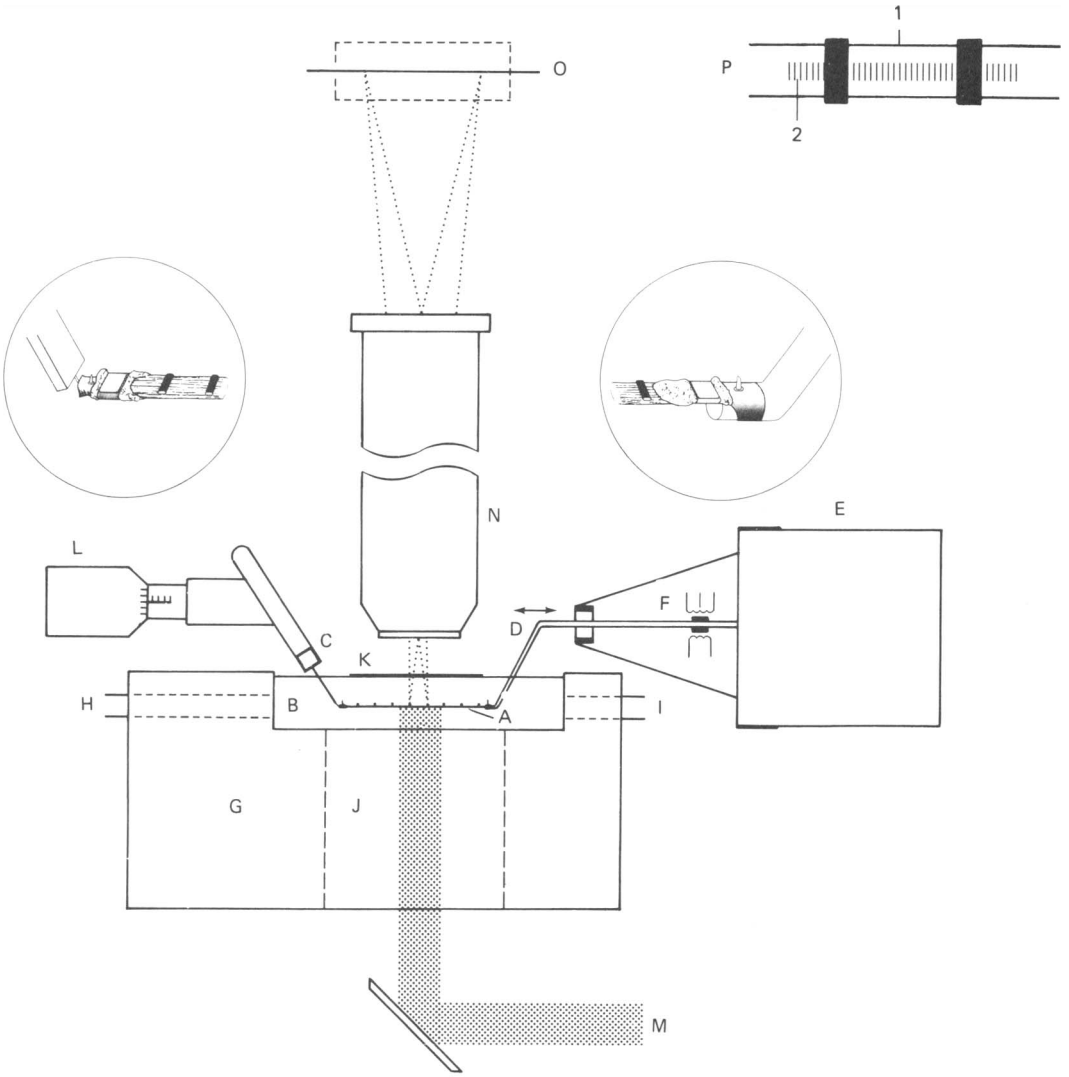
Preparation and mounting

Single fibres were dissected from the tibialis anterior muscle of *Rana temporaria*. The frogs had been stored at about +4 °C for at least 7 days before use. The dissection was carried out by means of a fine pair of scissors and care was taken to avoid any appreciable stretching of the fibre during the dissection procedure. Only fibres which could be carefully cleaned from connective tissue along their entire length were selected for this study. Fig. 1 illustrates a schematic drawing of the experimental arrangement. The fibre was mounted horizontally in a thermostatically controlled Perspex chamber between a force transducer and an arm extending from the moving coil of an electromagnetic puller. In its mounted position the fibre had no turn, which means that one tendon insertion was facing upwards, the other downwards. For mounting the fibre a small clip of aluminium foil, similar to that described by Ford, Huxley & Simmons (1977), was attached to each tendon 0·1–0·2 mm from the insertion of the fibre (Fig. 1; A). The clips were provided with a hole to fit a small hook of stainless-steel wire (0·1 mm diameter) attached to the tip of the tension transducer and the puller arm. The side parts of the aluminium clips were folded tightly around the hooks to prevent any change in position of the clip during the experiment.

For the purpose of the present experiments it was essential to minimize any lateral and vertical movements of the fibre during isometric contraction. Such movements could be almost completely eliminated by carefully adjusting the angle at which each aluminium clip was finally attached to the steel hook. It was ensured by inspection of the fibre at 40 × magnification (using a Zeiss Stereo II microscope provided with an ocular micrometer) that the sideways movement of the fibre (measured near the tendon insertions) was $< 15\ \mu m$. Any tendency of the fibre to twist during contraction was also minimized. This was achieved by appropriately twisting the part of the aluminium clip that held the tendon.

Bathing solution and temperature

The bathing solution had the following composition (mM): NaCl, 115·5; KCl, 2·0; $CaCl_2$, 1·8; Na_2HPO_4–NaH_2PO_4, 2·0; pH 7·0. The pre-cooled solution was continuously perfused through the muscle chamber at a speed of approximately 5 ml min^{-1}. The temperature of the bathing solution (recorded by a digital thermistor probe) was maintained constant to ±0·1 °C throughout an experiment. No temperature gradient greater than ±0·1 °C was measurable along the length of the fibre during perfusion of the chamber. The temperature varied between 1·3 and 4·9 °C in the entire series of experiments.



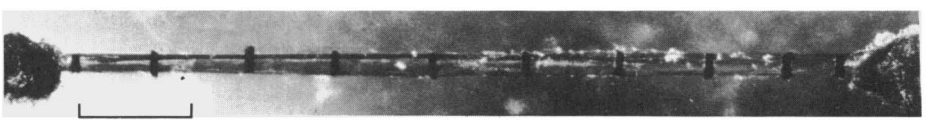


Fig. 1. Schematic illustration of experimental arrangement. A, muscle fibre; B, muscle fibre chamber; C, force transducer; D, shaft movable in the horizontal plane; E, electromagnetic vibrator; F, displacement transducer; G, jacket for circulation of thermostatically controlled water–glycol mixture; H, I, inlet and outlet for bath solution; J, air space for passage of laser beam; K, glass slide on top of Ringer solution; L, micrometer screw; M, path for laser beam; N, monocular microscope; O, horizontal stage for Reticon CCPD 1024 photodiode assembly; P, image of fibre segment (1) with two markers projected onto photodiode assembly (2). Encircled insets: detail of attachment of fibre to force transducer (left) and puller arm (right). Note that the tendons are clamped by aluminium foil and that the side parts of the foil are folded around the end of the puller shaft and the steel hook of the force transducer. Lower inset: photomicrograph of resting muscle fibre illustrating position of markers.

Stimulation

The fibre was stimulated by passing current between two platinum plate electrodes placed on either side of the preparation approximately 2 mm from it. Rectangular pulses of 0·2 ms duration were used and the stimulus strength was approximately 25 % above threshold. A train of pulses of a frequency of 14–22 Hz was given to produce a fused tetanus of 1–3 s duration. The stimulation frequency used was just sufficient to produce mechanical fusion in the individual fibre. Care was taken to keep the interval between the tetani constant. The interval was 2 min for 1 s long tetani and 4 min for tetani of 3 s duration.

Force transducer

Tension was recorded by means of a semiconductor strain-gauge transducer (AE 801, Aksjeselskapet Mikroelektronikk) that has previously been described (Edman, 1979). The resonant frequency of the transducer submerged in the Ringer solution was about 6 kHz.

Electromagnetic puller

The electromagnetic puller used in the present study was similar to that previously described (Edman, 1975) but was capable of producing faster movements. The rise time of a 100 μm step, allowing 5–10 % overshoot, was 0·3 ms. The position of the puller arm was monitored by a differential transformer type transducer. The frequency response of the transducer was approximately 10 kHz. A rectilinear response was obtained for movements within a range of 2 mm. The noise level of the transducer was 1·6 mV which corresponded to 0·5 μm movement of the puller arm. It was possible to change rapidly the mode of operation of the puller from fibre length control to force control or to segment length control. The segment length signal was obtained from a photo-electric detector system which is described below. The change in mode of operation of the puller was achieved by means of CMOS analog switches.

Determination of sarcomere length

Generally the sarcomere length was determined by the laser diffraction technique previously described (Cleworth & Edman, 1972). In one series of experiments (section B, Results) changes in sarcomere length were compared in the end regions (near the tendon insertions) and the middle portion of the fibre at various degrees of stretch of the preparation. In these experiments the sarcomere pattern was photographed on 35 mm panchromatic film (Kodak Tri-X) in a Zeiss Standard Universal microscope at 100 × magnification. The sarcomere length was measured from such records at an over-all magnification of 1000 × in a Nikon model 6C profile projector using the stage micrometer reading. Sequences of about fifteen sarcomeres were measured and a mean value of the sarcomere spacing within such a sequence was formed. A grand mean of the sarcomere length was calculated from three to seventeen sequences of sarcomeres.

Determination of fibre length and cross-sectional area

The distance between the insertions of the fibre to the tendons was measured to the nearest 0·05 mm in a Zeiss Stereo II microscope at 10 × magnification. The measurement was performed while the fibre was mounted in the bath and the resting sarcomere length was set to 2·25 μm.

The cross-sectional area was calculated from the largest and smallest diameters measured along the fibre at 40 × magnification after the fibre had been twisted one-half turn while still mounted in the bath at 2·25 μm sarcomere length. For this calculation the cross-sectional area was assumed to have an elliptical shape.

Segment length recording

Markers. Opaque markers approximately 60 μm wide and 200 μm long were cut from black dog's hair and were placed at 0·5–0·8 mm intervals on the upper surface of the muscle fibre along its entire length (Fig. 1). The outermost markers were placed *ca.* 0·2 mm from the edge of the tendon, i.e. 0·3–0·4 mm from the fibre–tendon junctions. The markers had been flattened to a thickness of approximately 25 μm by prior compression of the hairs between two Plexiglas slides so as to obtain a larger contact surface with the fibre. Provided that the fibre was carefully cleaned from connective tissue the markers could be firmly attached to the fibre by gently pressing the marker against the fibre surface with a thin glass rod. Care was taken to fix each marker in a perpendicular position relative to the long axis of the fibre. On microscopic inspection the markers could be seen to have

a rugged surface formed by partly overlapping scales. This feature of the hair surface probably accounted for the good adhering properties of the markers. As a rule the markers maintained their position on the fibre, without any significant change in distance between the markers (see further below), over several hours of experimentation including numerous releases and stretches of the fibre.

Optical arrangement. The fibre was illuminated from below by a laser beam (He–Ne, Spectra-Physics, model 124 A) that was expanded to cover a *ca.* 4 mm long portion of the fibre. A cylindrical lens parallel to the fibre axis was used to make the width of the laser beam approximately correspond with the fibre diameter. A 10× image of the fibre was projected onto a photodiode array (Reticon CCPD 1024) which was mounted on an adjustable stage above the eyepiece of a microscope (Fig. 1). A glass slide (0·1 mm thickness) was placed on the surface of the Ringer fluid below the objective lens to prevent surface vibration. The image of the fibre was carefully aligned with the photodiode array. The laser beam, the microscope and the stage holding the photodiode array could be translated together along the preparation to enable recordings from any part of the fibre. The laser light intensity was adjusted by means of a polaroid filter to be just sufficient to saturate the photodiodes except at marker image locations.

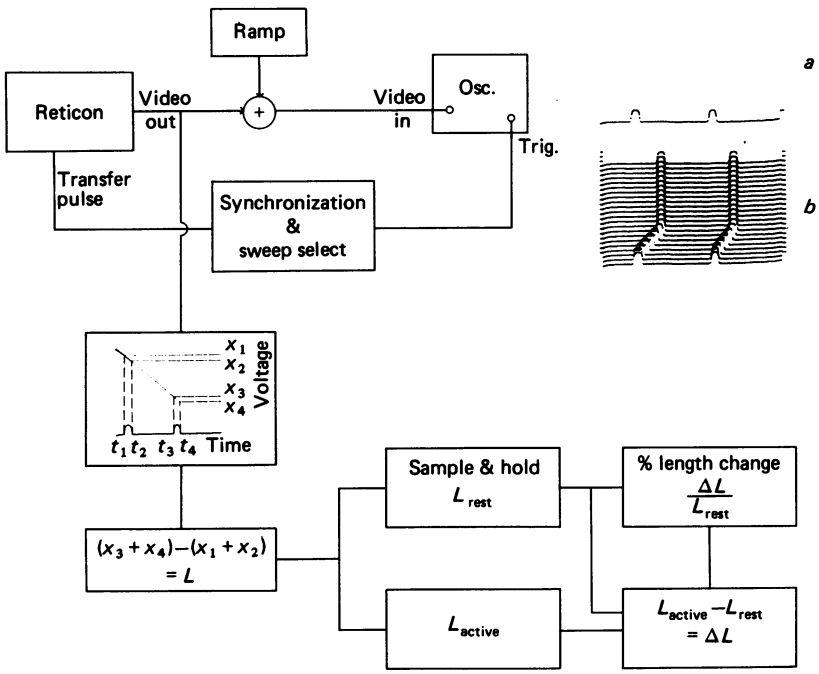


Fig. 2. Block diagram of circuitry used for recording marker position and for computation of distance between two adjacent markers. Insets: *a*, oscilloscope record of single scan of Reticon CCPD 1024 illustrating relative position of two adjacent markers; *b*, selected scans of the photodiode assembly displayed as staggered sweeps on the oscilloscope during shortening ramp of resting fibre. For further explanation, see text.

Recording circuitry. Fig. 2 shows a block diagram of the system used for recording marker position and for computing the distance between adjacent markers. The photodiode array was scanned at a rate of 4 kHz and the signal was displayed on a Tektronix 5113 N storage oscilloscope. Two markers at a time were studied as shown in Fig. 2 (inset *a*). The video signal of the marker had a rectangular shape with steep flanks. The latter had a staircase-like contour where each step represented the output of an individual photodiode. These irregularities of the signal were smoothened by appropriate filtering (time constant of filter approximately 1 μs). As a check that the marker signal was distinct throughout a contraction selected scans of the photodiode array were

displayed as staggered sweeps on the oscilloscope (Fig. 2, inset *b*). This was achieved by adding a voltage ramp to the video signal.

The mean distance between two selected markers was determined by transforming the time base of the photodiode scan into a voltage signal. For this purpose each scan was synchronized with a voltage ramp (see voltage–time diagram, Fig. 2). The values x_1, x_2 and x_3, x_4 existing at times t_1, t_2 and t_3, t_4 when each marker passed a given voltage during its rising and falling phases were collected. From these values the mean distance (L) between the two markers was computed by an analog circuit. The distance, L_{rest}, computed immediately before stimulation was stored in a 'sample & hold' circuit. This value was subtracted from the inter-marker distance, L_{active}, that was derived from each subsequent scan during a pre-set period usually covering the entire contraction–relaxation cycle. The value so obtained provided a measure of the *change* in distance, ΔL, between the two markers. Finally, by computing the ratio $\Delta L/L_{rest}$ the fractional change of inter-marker distance was produced for each scan of the photodiode array.

The value of L (L_{rest} and L_{active}) was continuously displayed on a 3 1/2 digit voltmeter. The 'percentage signal' ($\Delta L/L_{rest}$) was displayed on a storage oscilloscope (Tektronix 5113N) together with the signals from the force transducer and the displacement transducer of the electromagnetic puller.

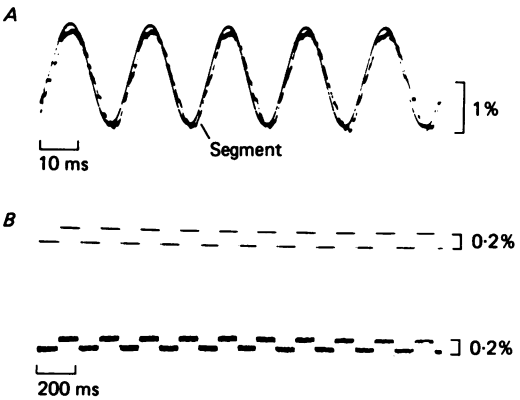


Fig. 3. *A*, superimposed oscilloscope records of puller movement and segment length change during 50 Hz sinusoidal oscillation of resting muscle fibre. Peak-to-peak amplitude of puller movement 2% of total fibre length. *B*, segment length recording (lower trace) during low amplitude length steps of resting muscle fibre. Peak-to-peak amplitude of puller movement (upper trace) adjusted to be 0·2% of total fibre length. Fibre pre-stretched to approximately 2·6 μm sarcomere length in both *A* and *B*.

Performance of segment length recorder. The computation on the photo-electric signal described above was performed while a new scan of the CCPD 1024 was going on. This means that the information on L, ΔL and $\Delta L/L_{rest}$ was generated with a constant time lag of one scan. With the scanning frequency used (4 kHz) the time resolution of the segment length measurement was therefore 0·25 ms. Fig. 3*A* illustrates simultaneous recordings of the puller movement and the change in length of a 0·8 mm long segment during 50 Hz sinusoidal length oscillation of a resting fibre. As can be seen there is no appreciable distortion or phase shift ($< 0{\cdot}5$ ms) of the segment length signal. Fig. 3*B* shows that the resolution of the segment length measurement was better than 0·2% of the distance between two markers, corresponding to approximately 2 μm.

The segment length recording (linearity 0·2%) was generally set to cover a 0–20% length change of a segment. The measuring range could be extended, if required, at the expense of reduced sensitivity of the measurement.

Length control of fibre segments

The 'percentage signal' from the photodiode array (see above) was used for feed-back control of the electromagnetic puller in experiments where the length of individual fibre segments was held constant during contraction (segment length clamp). The switch-over to segment length control

was made before the onset of stimulation, and the puller was usually maintained in the 'segment length clamp' mode until the 'shoulder' of the relaxation phase of the tetanus had been reached.

Cine photographic recording of markers

The portion of the fibre located between the end markers and the fibre's insertion to the tendon was not accessible for study by using the photo-electric recording technique. Changes in length within these regions of the fibre were investigated by means of cine photographic recording of surface markers. For this purpose a marker of black dog's hair was placed approximately 0·4 mm from the fibre–tendon junction as previously described. Another two markers of opaque Nylon filaments (13 μm wide and approximately 150 μm long) were placed on the fibre surface, one close to the tendon insertion, the other halfway between the fibre–tendon junction and the dog's-hair marker. The markers were placed perpendicularly to the long axis of the preparation. The ends of the fibre were thus divided into two segments of approximately 0·2 mm length. The position of the markers was recorded on 16 mm film (Kodak Tri-X) using a Bolex cine camera fitted on a Zeiss Stereo II microscope. The film speed was 24 frames s^{-1} and the exposure time was 6 ms. Tracings of the photographic records were produced at an over-all magnification of 425 × using a Kodak analysing projector. The inter-marker distances could be measured from such tracings with an accuracy of $\pm 0{\cdot}5\%$.

RESULTS

A. Length changes within the central portion of the fibre, excluding the fibre ends

Changes in length of consecutive 0·5–0·8 mm segments were studied during 3 s fused isometric tetani by recording the distance between adjacent markers placed on the surface of a single muscle fibre. The measurements described in this section exclude the end segments of the fibre, i.e. the portions, 0·3–0·4 mm in length, located next to the tendon insertions (see Methods). Only sarcomere lengths below 2·7 μm were considered to avoid complications due to resting tension. Fig. 4*A*, *B* shows a series of segment length recordings performed at two different sarcomere lengths, at the plateau (2·25 μm) and on the descending limb (2·60 μm) of the length–tension relation, respectively. The records refer to repeated tetani induced at 4 min intervals. The behaviour of any given segment was found to be remarkably consistent during repeated contractions provided the fibre was stimulated at regular intervals. Thus virtually indistinguishable records were obtained in many cases when the same segment was tested on different occasions during the experiment, even several hours apart (Fig. 4*B*). The force record also remained constant under these conditions (lower traces). A series of records like those shown in Fig. 4 may therefore be used to evaluate the over–all pattern of length changes along the fibre during any given tetanus, although the information on each individual segment originates from successive contractions.

In the analysis of the segment length changes distinction was made between the initial length change which coincided with the steep rise of tension (covering approximately 90 ms) and the subsequent slow length change which coincided with the later (rounded) portion of the rising phase and the 'plateau' of the tetanus. The approach used for measuring the amplitude of the two phases is shown in Fig. 4 (panel *C*). As can be seen the amplitude (and direction) of the second slow phase was determined from the total length change of the segment at the last stimulus *minus* the amplitude of the initial phase.

Fig. 5 shows the amplitude of the initial length change in different segments of six fibres that were stimulated to produce a 3 s isometric tetanus at 2·15 μm (●) and

2·60 μm (○) sarcomere lengths. The amplitude and direction of the subsequent slow length changes are also indicated for comparison in the same diagrams (vertical bars). The results show that nearly all segments shortened during the initial phase at 2·15 μm sarcomere length. However, the degree of shortening varied substantially along the fibre. In some preparations one or more segments remained stationary and a few (four out of sixty-four segments in six fibres) elongated. The pattern of length changes observed along any given fibre during the initial phase at 2·15 μm sarcomere length was largely maintained as the sarcomere spacing was increased to 2·60 μm.

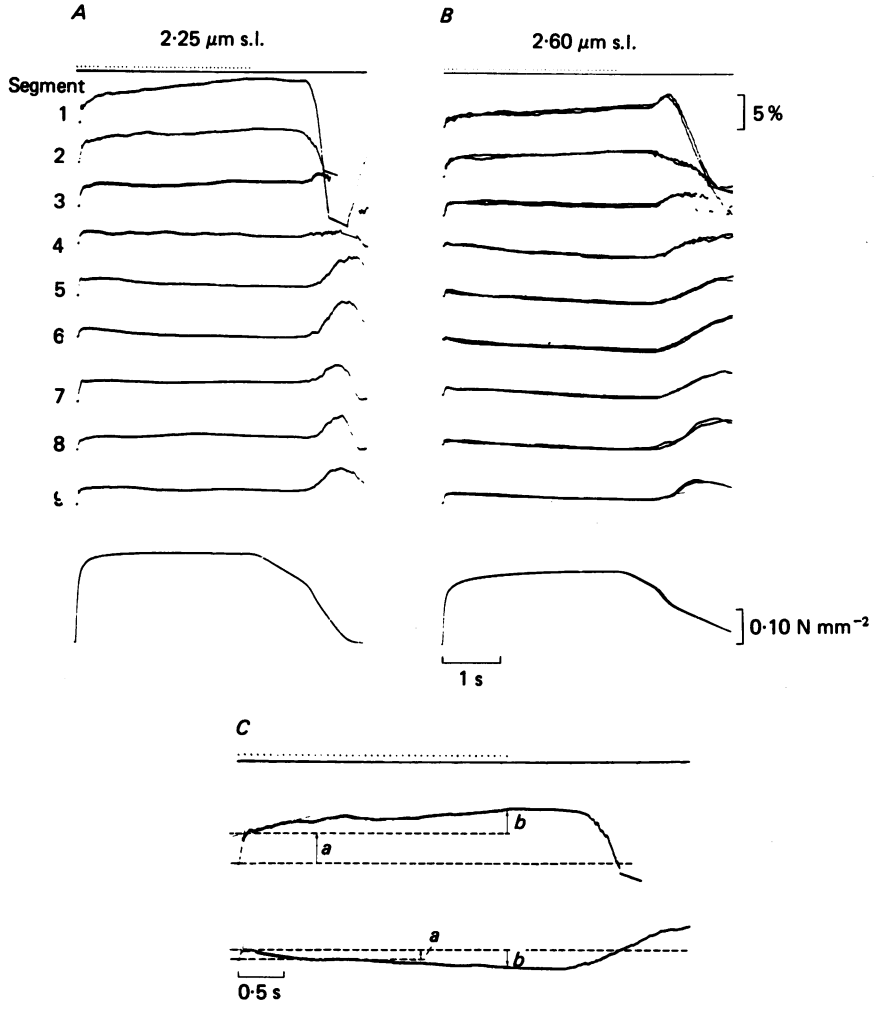


Fig. 4. Oscilloscope records illustrating changes in length (shortening upwards) of nine consecutive segments of single muscle fibre during 3 s isometric tetanus at 2·25 μm (*A*) and 2·60 μm (*B*) resting sarcomere length (s.l.). Bottom traces in *A* and *B*, force myograms. The segment length records refer to separate tetani repeated at 4 min intervals. Superimposed traces in *B* are separated in time by 1·5–2·5 h. *C*, approach used for measuring amplitude of length change during the initial steep phase (*a*) and the subsequent slow phase (*b*) illustrated by two examples. Cross-sectional area, $44{\cdot}67 \times 10^{-3}$ mm^2. Temperature, 1·3 °C.

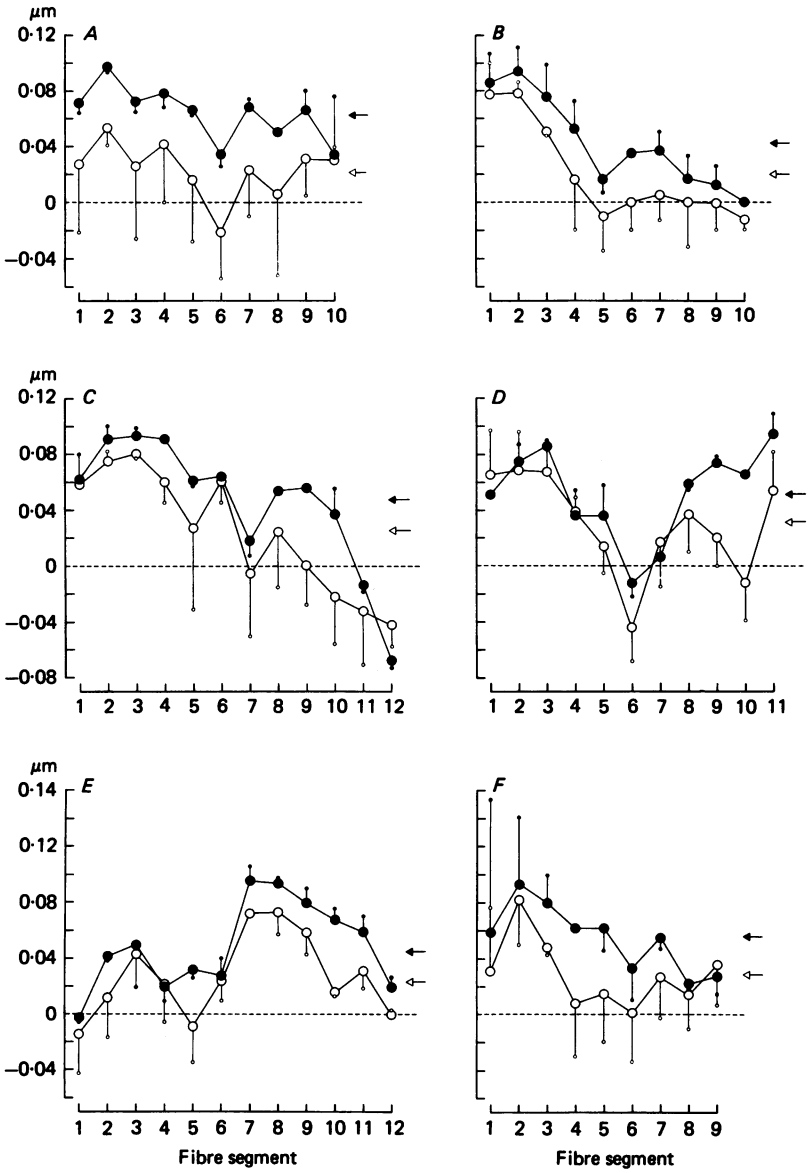


Fig. 5. Amplitude of *initial* length change (shortening positive) of central segments during fixed-end tetanus of single muscle fibres at 2·15 μm (●) and 2·60 μm (○) sarcomere length. Diagrams *A–F* show results from separate fibres. Vertical bars indicate amplitude and direction of the subsequent slow length change in respective segment. Horizontal arrows indicate mean value of the initial length change during contraction at 2·15 μm (filled) and 2·60 μm (open) sarcomere length, respectively. Abscissa: number of consecutive segments along the fibre. Ordinate: segment length change expressed in μm sarcomere^{-1}. Mean resting length of segments at 2·15 μm sarcomere length, 0·74 ± 0·10 mm (mean ± S.D., $n = 64$).

However, the performance of the segments was 'weaker' in the latter case, i.e. the amplitude of shortening was reduced and a greater number of segments (eleven out of sixty-four segments in six fibres) elongated during the initial phase at the stretched length. The data in Fig. 5 furthermore show that, at either sarcomere length, there was no strict correlation between the length changes during the first and the second phases. A given length change during the initial phase can be seen to be associated with different amounts of movement during the subsequent slow phase in the various segments. Quite frequently segments which shortened during the initial phase were found to elongate during the second phase.

Fig. 6 illustrates the amplitude of the slow length changes at 2·15 and 2·60 μm sarcomere lengths using data from the same six fibres as shown in Fig. 5. It is of interest to note that there was a substantial redistribution of length along the fibre even at 2·15 μm sarcomere length where no appreciable tension creep occurred during the tetanus plateau. Furthermore, there was a small (0·1–0·5 %) *net shortening* of the central portion of the fibre if the length changes of all segments are taken together.

The pattern of length changes during the second phase was quite well maintained after stretching the fibre from 2·15 to 2·60 μm sarcomere length, i.e. the distribution of 'stronger' and 'weaker' segments was similar at the two lengths. However, only a few segments were able to shorten at the stretched length; the majority of the segments were found to elongate to various degrees resulting in a *net* (0·4–1·5 %) *increase in length* of the central portion of the fibre. This implies that the tips of the fibre must have shortened considerably in order to account for the 'tension creep' (with further extension of the tendons) that occurred during the tetanus at this sarcomere length (cf. Fig. 4, lower right isometric myogram). Direct measurements of the length changes within the end regions of the fibre during the tetanus were performed in separate experiments and are described in the following section.

B. Length changes within the ends of the fibre

Resting fibres. There is evidence from previous studies (Julian *et al.* 1978*a*; Huxley & Peachey, 1961; Carlsen, Knappeis & Buchthal, 1961) that sarcomeres near the fibre–tendon junctions have a shorter length than those located in the central part of the fibre. This was demonstrated in isolated muscle fibres that had been passively stretched to sarcomere lengths (middle region) of 2·8–3·2 μm. For the purpose of the present study it was of interest to find out how the striation spacing at the ends of the fibre changed relative to that in the central part as the fibre was extended at rest from slack length to a sarcomere spacing (middle region) of approximately 2·7 μm (cf. section *A*).

A mean value of the sarcomere length was determined from photographic recordings of the striation pattern in two segments located at each end of the fibre close to the tendon insertions (see Methods). These segments, referred to as *outer* (next to the tendon insertion) and *inner* end segments, respectively, had a length of 2·0–3·0 % of the total fibre length. The sarcomere spacing in the central portion of the fibre was determined from similar recordings made at two or more locations in the middle of the fibre. Fig. 7 shows results from experiments in which the fibres were passively extended to various degrees within the range 2·1–2·8 μm sarcomere length (middle

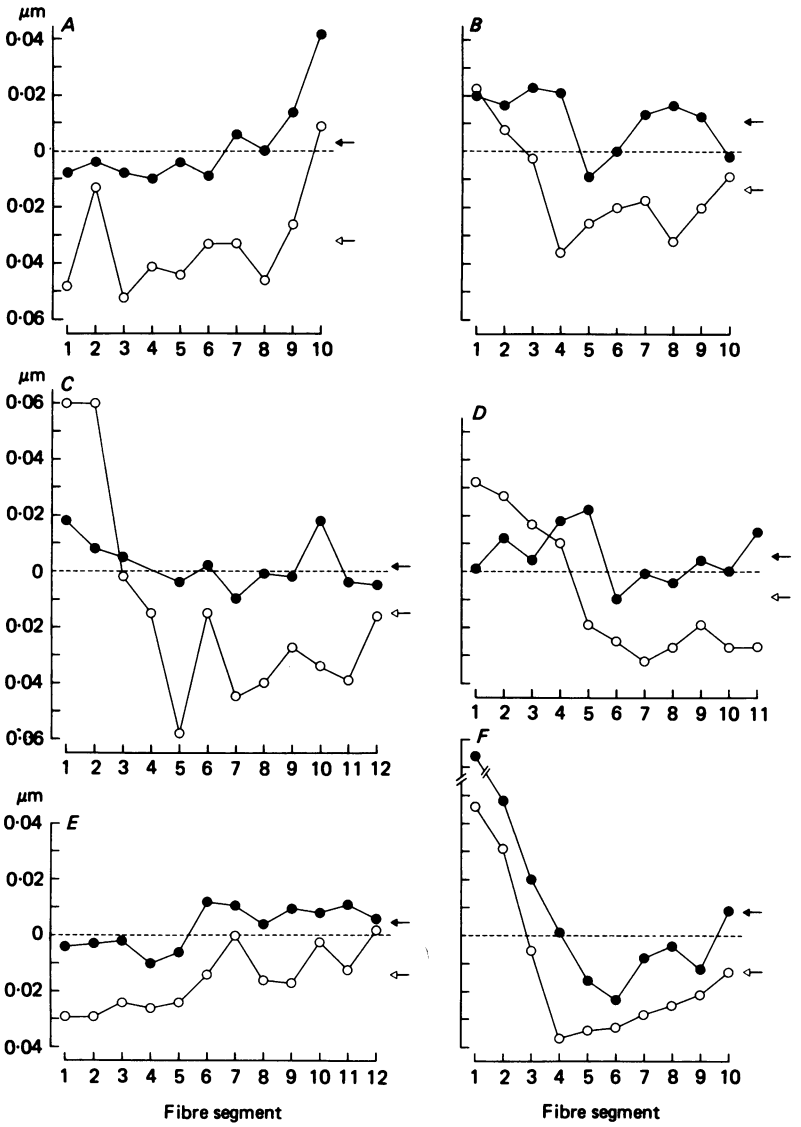


Fig. 6. Amplitude of slow length change of central segments during fixed-end tetani at 2·15 μm (●) and 2·60 μm (○) sarcomere length. Data in diagrams *A–F* refer to same experiments as illustrated (with corresponding lettering) in Fig. 5. Horizontal arrows indicate mean value of slow length change during contraction at 2·15 μm (filled) and 2·60 μm (open) sarcomere length, respectively. For further information, see Fig. 5.

region). It can be seen that there was no consistent difference in sarcomere spacing between inner and outer end segments and the central parts of the fibres at slack length, i.e. at about 2·1 μm sarcomere spacing. Fig. 7*A* gives an example (fibre *c*) where the sarcomere length in one outer segment was substantially *larger* ($P < 0·01$) than that in the middle of the fibre, whereas the opposite was true for fibre *b*, in which one end segment had a significantly shorter sarcomere length ($P < 0·05$) than the central region. However, stretching the fibre above slack length led to a clear

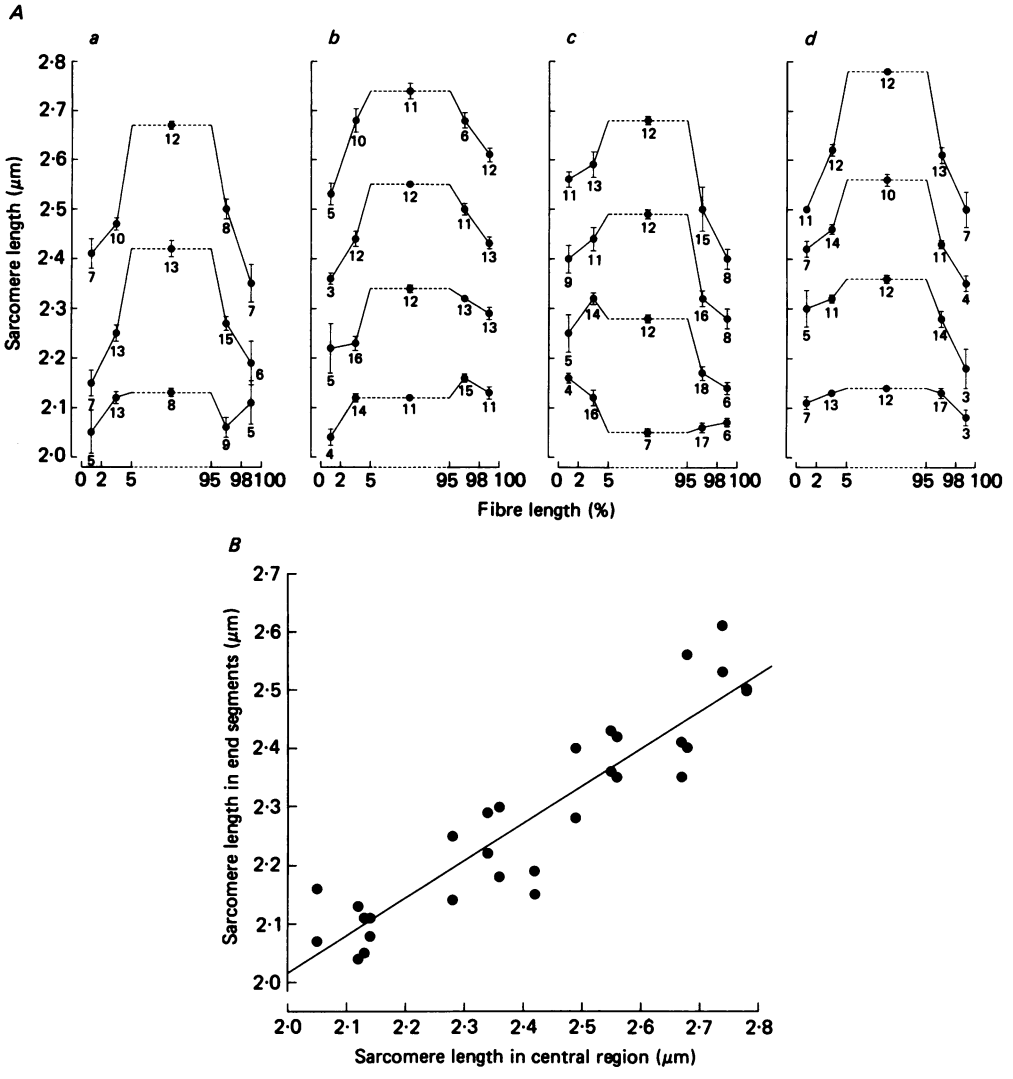


Fig. 7. *A*, sarcomere length in end segments and in central region of the fibre at varied fibre rest length. Data connected by line refer to the same fibre rest length. Panels *a–d* represent different fibres. Abscissa: intervals of fibre studied, expressed in percentage of fibre length. Each data point is the mean of three to seventeen sarcomere length determinations as indicated by the number at respective point. Bars, ±S.E. of mean. *B*, relationship between resting sarcomere length in central region ($s.l._c$) and outer end segments ($s.l._e$) of single muscle fibres. Data from the same four experiments as shown in *A*. Regression line: $s.l._e = 0{\cdot}636\,.\,s.l._c + 0{\cdot}744$. (Correlation coefficient, 0·93.)

dissociation of the sarcomere length along the fibre in that the ends were consistently found to have shorter sarcomeres than the middle portion of the fibre. Of the two segments studied at each end the one located next to the tendon insertion exhibited the shortest sarcomeres. The data in Fig. 7*B* show that on stretching the fibre to a sarcomere length of 2·67–2·79 μm in the middle region there was a 0·23±0·07 μm ($n = 8$) shorter mean sarcomere length in the outer end segments.

Active fibres. Changes in length of the fibre ends during 3 s isometric tetani were studied by cine photographic recording of markers placed on the fibre's surface. These markers divided the end segments (which were excluded in the experiments presented in section *A*) into two shorter segments of approximately the same length (0·2 mm) as those studied in the resting fibres. Fig. 8 summarizes results from four such experiments (eight fibre ends) at various resting fibre lengths. It can be seen (Fig. 8*A*) that the outer end segments elongated by approximately 3 % during the onset

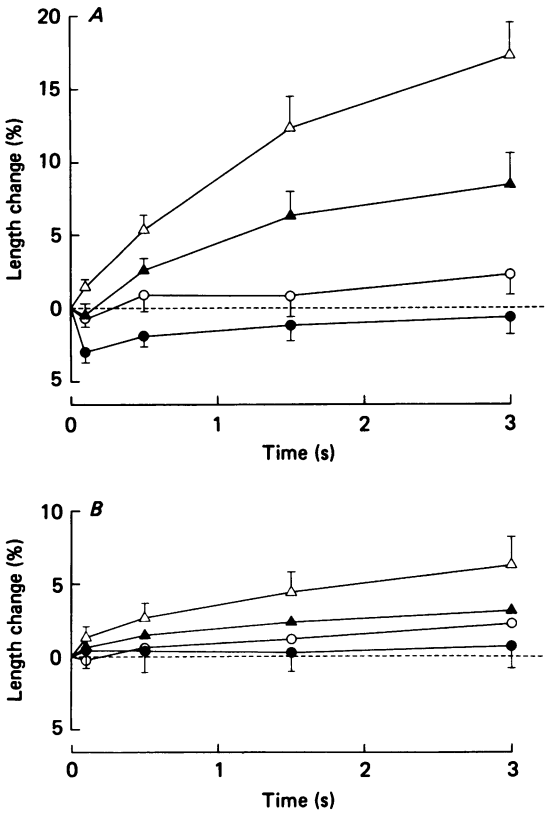


Fig. 8. Length changes of end segments during isometric tetani at different fibre rest length. *A*, outer end segments; *B*, inner end segments (definitions, see text). Mean values (bars, S.E. of mean) of four fibres (eight fibre ends). Abscissa: time after onset of tetanus, seconds. Ordinate: length change of end segments in percentage of their resting length. Resting sarcomere length in the middle of the fibre: ●, 2·15 μm; ○, 2·30 μm; ▲, 2·50 μm; △, 2·70 μm.

of the isometric tetanus at slack length. This initial elongation was largely reversed during the plateau phase of the tetanus. At more stretched fibre lengths (> 2·30 μm sarcomere spacing in the middle region) the initial elongation of the outer end segments was small or absent, and there was a marked shortening of these segments throughout the tetanus plateau. The amount of shortening increased with increasing resting fibre length. At a fibre length corresponding to a sarcomere spacing of 2·70 μm (middle region) the amplitude of slow shortening of the outer end segments was

$17{\cdot}30 \pm 2{\cdot}22\,\%$ ($n = 8$) during a 3 s isometric tetanus. This means that the sarcomeres in these segments, having a resting length of approximately 2·5 μm according to Fig. 7*B*, were able to shorten to below 2·1 μm during the plateau phase of the tetanus. Length changes similar to those exhibited by the outer end segments were also recorded in the inner end segments (Fig. 8*B*). However, the amplitude of shortening was considerably smaller in the latter case.

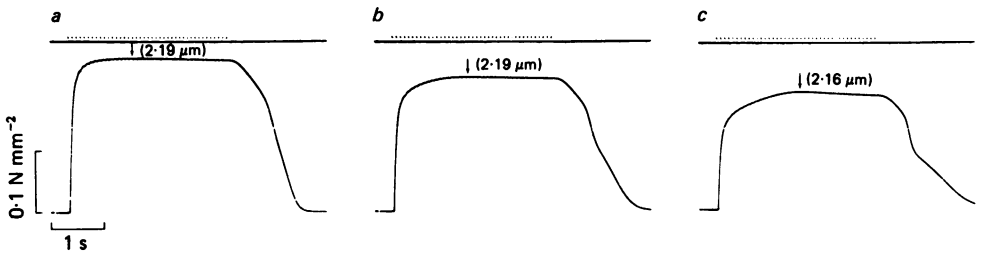


Fig. 9. Effect of altering fibre rest length on time course of tension creep during isometric tetanus. Resting sarcomere length in central region of the fibre: *a*, 2·30 μm; *b*, 2·50 μm; *c*, 2·70 μm. Estimated sarcomere length of end segments at the attainment of peak force (arrow) is given in parentheses at each myogram. Note that (1) peak force is reached at a later time when contraction is initiated at a greater fibre length and (2) peak force occurs when end sarcomeres have shortened to just below 2·2 μm. Cross-sectional area, $32{\cdot}99 \times 10^{-3}$. Temperature, 1·3 °C.

In Fig. 9 the tension creep during a 3 s isometric tetanus is compared at three different sarcomere lengths between 2·3 and 2·7 μm. It can be seen that peak force occurred at a later and later time during the tetanus as the resting sarcomere length was increased. From the data presented in Fig. 7*B* and Fig. 8*A* it was possible to estimate the sarcomere length in the end segments at the time when maximum force was attained. The results of the analysis are given in brackets above each myogram in Fig. 9. It is evident that in each case when maximum force was attained, the end sarcomeres were estimated to have shortened to a length close to 2·20 μm. A similar observation has been reported by Julian *et al.* (1978*a*) who recorded sarcomere shortening in the end segments of a fibre during isometric tetanus at a highly stretched length (3·16 μm in the middle of the fibre).

C. Increase in the mechanical performance during shortening on the descending limb of the length–tension relation

The idea that tension creep during isometric contraction is due to redistribution of sarcomere length along the fibre rests on the assumption that sarcomeres which shorten within the range of the descending limb of the length–tension relation steadily improve their contractile performance during the motion. The following experiments were performed to test this point. Fig. 10*A* (myogram *a*) shows the force development during a 3 s isometric tetanus at 2·60 μm sarcomere length. Typically at this sarcomere length there was a substantial amount of tension creep during the first 1·4 s of the tetanus. The superimposed record (myogram *b*) shows a tetanus initiated at the same sarcomere length, but in this case the fibre was released to shorten at a low, constant speed *after* the isometric force had reached its maximum. Shortening reduced the force below the isometric level as expected from the force–velocity

relation. It is clear, however, that the force was steadily increased during the shortening phase. The myograms in Fig. 10*B* show that no such improvement occurred as the fibre shortened across the plateau of the length–tension relation. On the contrary, there was a slight decrease in force during shortening in the latter case.

Experiments were also performed in which the fibre was released to shorten against a high *constant* load ('load clamp') after maximum force had been attained during the tetanus. Fig. 10*C*, *D* illustrates oscilloscope records from such an experiment. It can be seen (Fig. 10*C*) that shortening under constant load from a pre-stretched length (2·60 μm sarcomere spacing) led to *acceleration* of the fibre. By contrast, similar load-clamp recordings performed between 2·20 and 2·00 μm sarcomere lengths resulted in a slight *deceleration* of the fibre (Fig. 10*D*).

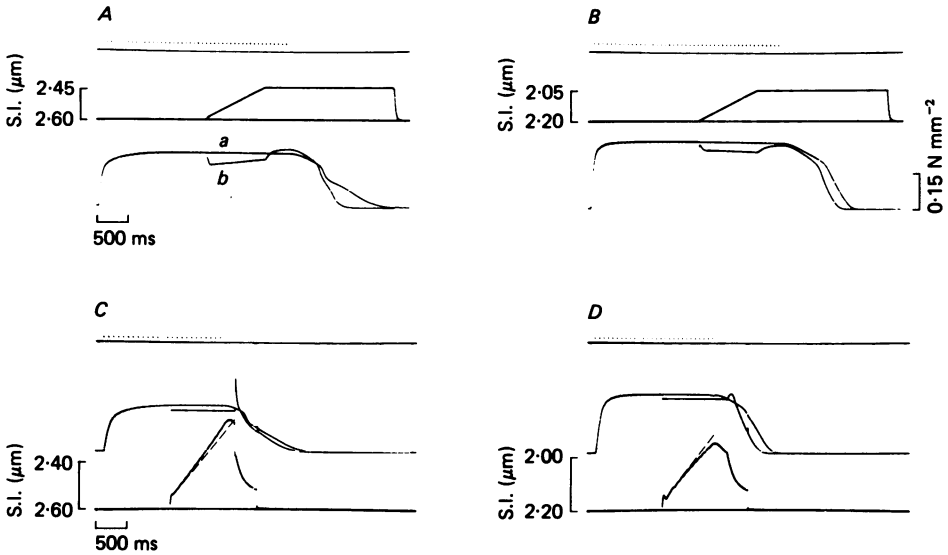


Fig. 10. Change in contractile performance during shortening at 2·60 μm (*A*, *C*) and 2·20 μm (*B*, *D*) sarcomere length (s.l.). Myograms *a*, isometric tetanus with no shortening. Myograms *b* (superimposed on *a*), shortening phase interposed during plateau of isometric tetanus. *A*, *B*, shortening at constant velocity; *C*, *D*, shortening at constant load. Dashed lines on displacement records in *C* and *D* serve as a reference to visualize change in shortening velocity during load-clamp recording. Note that shortening from 2·60 μm sarcomere length leads to progressive increase in force production or, if the load is held constant, to accelerated shortening. Records in *A*, *B* and *C*, *D* refer to two different fibres. Temperature, 2·0 °C.

The results presented in Fig. 10 clearly show that the fibre's ability to produce force and its ability to shorten actively against a high load are both steadily increased during shortening within the range of the descending limb of the length–tension relation. This improvement in mechanical performance may be attributed to the increase in area of overlap between the A and I filaments that occurs when the fibre shortens at these sarcomere lengths. There is reason to believe, however, that the rise in contractile strength that results from increased myofilament overlap is to some extent counteracted by a 'deactivating' effect of shortening (Edman, 1975, 1980). The existence of such a depressant effect of shortening is supported by the finding

that force and loaded velocity of shortening are both slightly reduced when the fibre shortens within the plateau of the length–tension relation (Fig. 10*B*, D).

D. Tetanic force produced by length-clamped segments of single fibres

If tension creep is due to redistribution of sarcomere length along the fibre, one might expect that the creep would be less pronounced when force is recorded from a short, length-clamped segment of a muscle fibre. Attempts have been made in the past to test this point by using a 'spot-follower' technique by which it was possible to hold 2–6 mm segments of an intact fibre at constant length during contraction (Gordon *et al.* 1966*a*; Julian *et al.* 1978*a*,*b*). It was found in these studies that tension creep still existed in such length-clamped segments, but the amount of creep was generally smaller than during an ordinary isometric (fixed fibre ends) tetanus. In this section experiments are described in which considerably shorter fibre segments, 0·5–0·7 mm in length, were kept stationary during contraction. This was achieved by holding the distance between two adjacent markers on the fibre surface constant by feed-back control during a 1 s tetanus (see Methods).

Fig. 11*A* (left-hand panel) shows ordinary isometric tetani (fixed fibre ends) recorded at 2·20, 2·50 and 2·95 μm sarcomere spacings. The length change recorded in one typical segment is also shown for each tetanus. The right-hand panel in Fig. 11*A* illustrates length-clamp recordings performed at the same sarcomere lengths. It can be seen that tension creep became more and more pronounced during standard isometric recording at longer sarcomere lengths. By contrast, there was no significant tension creep at any sarcomere length, when one of the fibre segments was held at constant length during the tetanus.

Creep-free tension records like those presented in Fig. 11*A* could generally be obtained from one or more segments in a given fibre. However, segments were also regularly found in each fibre which did produce some tension creep. In some of these segments there was a distinct force plateau in the beginning of the tetanus followed by a slow rise of force (Fig. 11*B*, record *a*). The mechanical behaviour of the segments, irrespective of whether tension creep occurred or not, was remarkably consistent over many hours of experimentation. It is of interest to note, however, that as a fibre began to deteriorate after long usage, segments that had previously produced flat tension records now started to develop tension creep. This observation is in line with the view that tension creep is due to sarcomere instability leading to redistribution of sarcomere length.

DISCUSSION

1. Experimental approach

In the present study a technique was used to record changes in length of short consecutive segments of single muscle fibres during isometric tetanus. The segments, 0·5–0·8 mm in length, were defined by markers of hair that were firmly attached to the fibre surface. The length of one segment, i.e. the distance between two adjacent markers, could be determined with an accuracy of 1–2 μm, corresponding to 0·2–0·3 % of the segment length, and with a time resolution of 0·25 ms. The method was thus well suited for a study of the relatively slow, low-amplitude length changes that occur during isometric contraction at the temperature (1–2 °C) considered. The time

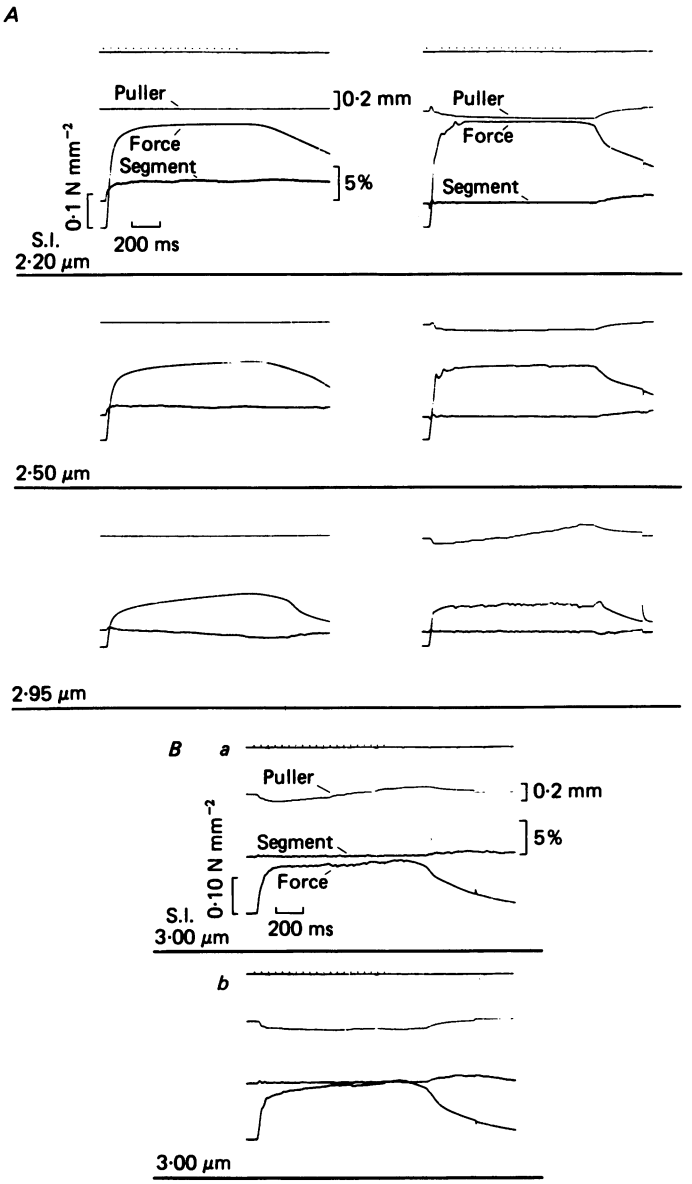


Fig. 11. *A*, oscilloscope records of tetanic contractions at three different sarcomere lengths illustrating force production during isometric recording (fixed fibre ends, left panel) and during length clamp of a fibre segment (right panel). Upward deflexion of puller signal (over-all fibre length) and of segment length recording indicates shortening. Length of whole fibre and of fibre segment at 2·20 μm sarcomere spacing, 8·70 mm and 0·60 mm, respectively. Cross-sectional area, $18{\cdot}41 \times 10^{-3}$ mm^2. Temperature, 2·2 °C. Note absence of tension creep when segment is held at constant length during contraction. *B*, examples of length-clamped segment recording exhibiting tension creep. In (*a*) force is quite stable over approximately 500 ms after which tension starts to climb whereas in (*b*) no force plateau is formed. Sarcomere length, 3·0 μm. Length of whole fibre and of fibre segment at 3·0 μm sarcomere spacing, 11·80 mm and 0·67 mm, respectively. Cross-sectional area, $18{\cdot}90 \times 10^{-3}$ mm^2. Temperature, 1·7 °C.

resolution of the recording system was also sufficient to enable length clamping of individual segments during tetanic contraction. Except for a small transient at the onset of contraction the segment could be held at constant length to within 0·2% throughout a tetanus.

By recording the distance between two adjacent markers on the fibre surface the length measurement was insensitive to any longitudinal translation of the fibre during contraction provided the same pair of markers was held within the domain of the photodiode assembly. The marker technique thus had a clear advantage over a laser diffraction measurement in that the length signal referred to the same portion of the fibre throughout the contraction period. This feature of the recording technique was of particular significance during length-clamp recording of an individual segment, as this recording sometimes involved a large compensatory movement of the puller and therefore a substantial longitudinal translation of the segment.

The technique allowed measurements to be made from only one fibre segment per contraction. This limitation, however, was fully compensated by the fact that the mechanical response of any particular segment was consistent during repeated contractions, even over several hours of experimentation. A series of length records obtained from the various segments therefore depicted the complete pattern of length changes along the fibre during any given contraction, in spite of the fact that the individual records stemmed from different tetani.

A change in distance between two adjacent markers may be presumed to provide a useful index for evaluating a change in mean sarcomere length within this segment. This is suggested by the fact that each myofibril is connected to the plasma membrane, via neighbouring myofibrils, both at the Z-line and at the M-line (Brown & Hill, 1982). Any change in distance recorded on the fibre surface may therefore be used as a measure to estimate a change in mean sarcomere length within the underlying segment of the fibre.

2. Pattern of segment length changes during isometric tetanus

The present technique has enabled a detailed study of the interplay between various regions along a muscle fibre during isometric contraction. The results have shown that there is a considerable redistribution of length along the fibre during an isometric tetanus both on the plateau and on the descending limb of the length–tension relation. Furthermore, this redistribution of length was found to occur between 0·5–0·8 mm long segments along the *entire* fibre. The problem of sarcomere instability during isometric contraction is thus more complex than assumed in previous studies (Gordon *et al.* 1966*a*; Julian *et al.* 1978*a*,*b*; Julian & Morgan, 1979) in which only length changes of the fibre ends and of *one* large central segment were considered. The results do confirm, however, that the end segments have mechanical characteristics that make these segments particularly effective as force generators above slack length.

Performance of end segments. The sarcomere spacing of the end segments was not significantly different from that of the central segments when the fibre was just taut, i.e. at approximately 2·1 μm sarcomere length. Under these conditions the end segments produced approximately the same amount of shortening during the plateau of the tetanus as did the central segments (cf. Figs. 6 and 8). However, the end

segments were consistently found to elongate during the rising phase of the tetanus near slack length (Fig. 8). The nature of this initial yielding is unclear at the present time. The mechanical behaviour of the end segments suggests that full activation was reached at a later time at the ends than in the middle of the fibre. This was probably not due to delay in membrane excitation at the fibre ends in view of the fact that field stimulation was used and it was ensured that the stimulus strength was well above the threshold.

In confirmation of previous results (Gordon *et al.* 1966*a*; Julian *et al.* 1978*a*,*b*) the end segments exhibited a greater amount of shortening during isometric tetanus at extended fibre lengths. This relative increase in contractile potential of the end segments may be accounted for by a smaller degree of extension of the fibre ends resulting in shorter sarcomere length in the end segments than in the middle region of the fibre (Huxley & Peachey, 1961; Carlsen *et al.* 1961). The present results show (Fig. 7) that a difference in sarcomere spacing between the ends and the middle region of the fibre comes into existence already when the sarcomeres in the central segments are elongated to a point (2·3 μm) just outside the plateau of the length–tension relation. This difference in sarcomere length was found to increase by further elongation of the fibre. The shorter sarcomere spacing of the end segments will render the fibre ends relatively stronger than the central segments. In accordance the tips of the fibres were found to shorten more extensively than any other region during isometric contraction at stretched lengths causing an over-all elongation of the central portion of the fibre during the tetanus plateau (cf. Figs 6 and 8).

Performance of central segments. The central portion of the fibre exhibited a complex pattern of length changes during isometric contraction both on the plateau (2·1 μm sarcomere length) and on the descending limb (2·6–2·7 μm sarcomere length) of the length–tension relation. The general pattern of length changes was quite similar at both these sarcomere spacings suggesting that the underlying mechanism of the length redistribution was the same in both cases. The different behaviour of the individual segments is not readily explainable on the basis of sarcomere non-uniformity along the fibre. The variability in sarcomere length within the fibre would not exceed $\pm$0·035 μm according to previous measurements (Edman, Mulieri & Scubon-Mulieri, 1976). This means that at an average sarcomere length of 2·1 μm all sarcomeres along the central segments would be within a range (2·05–2·15 μm) where the contractile strength would be expected to be the same. The irregular pattern of length changes along the fibre would therefore seem to reflect *inherent* differences in the contractile performance of the individual segments. The possible nature of these differences will be considered in some further detail. For this discussion it is necessary to distinguish between the initial length change, which coincides with the rapid rise of force, and the subsequent slow length change which occurs during the plateau phase of the tetanus.

It is reasonable to assume that *the slow length changes* occurring during the plateau of the tetanus are due to local differences in the fibre's ability to produce force and to carry a load. Such a variability in contractile strength along the fibre may arise from a variation in fibre width, i.e. from an uneven distribution of the myofibrillar mass along the fibre. Previous studies have shown (Blinks, 1965; Edman *et al.* 1976) that the cross-sectional area varies by $\pm$(5–7) % from one region to another along

frog muscle fibres. No attempt was made in the present study to correlate the length changes during the isometric tetanus with measurements of the mean cross-sectional area of the individual segments. However, a computer analysis showed (Fig. 12, Appendix) that a variation in maximum isometric force (P_0) of $\pm$(5–7)% along with fibre would indeed be sufficient to explain the redistribution of length within the central part of the fibre during the plateau phase of the tetanus.

As there is reason to believe that the contractile system is mechanically saturated by the end of the rising phase of the tetanus (Blinks, Rüdel & Taylor, 1978), it is unlikely that the observed length redistribution during the tetanus plateau was due to differences in myofibrillar calcium concentration along the fibre. In accordance with this view caffeine in a twitch potentiating concentration (0·5 mM) did not have any significant influence on the pattern of length changes during the isometric tetanus (K. A. P. Edman & C. Reggiani, unpublished observations).

If all segments were activated uniformly and the kinetic properties of the contractile system were the same along the fibre, the amplitude of *the initial length change* would be proportional to the length change occurring during the plateau phase of the tetanus. This is clearly not the case as is demonstrated in Figs. 4 and 5. For example, segments which shortened during the initial phase were in many instances found to elongate during the plateau phase. Thus, a segment being at an advantage over a neighbouring segment at the onset of contraction might end up in a weaker position during the plateau, or the reverse may occur.

The different behaviour of the segments during the rising phase and the plateau of the tetanus is consistent with the recent finding that maximum velocity of shortening differs along the length of a muscle fibre. Evidence has been presented (Edman & Reggiani, 1982, 1984) indicating that unloaded velocity of shortening (V_0) may vary by 25–50% from one part to another of a muscle fibre. For instance, a segment having a high V_0 and low P_0 (relative to the fibre mean) would be expected to exhibit an initial phase of shortening followed by elongation during the plateau of the tetanus.

The above hypothesis was tested by using the computer model described in the Appendix. For this analysis V_0 and P_0 were assumed to vary independently by $\pm$15% and $\pm$5%, respectively, along the fibre. As can be seen in Fig. 12 (Appendix) it is possible on the basis of these assumptions to simulate the characteristic features of the segment length changes during a 3 s isometric tetanus. The situation may in reality be more complicated as both the latent period and the rate of onset of activation are likely to vary to some extent between different segments. In the present model analysis these parameters were held constant along the fibre.

3. Redistribution of sarcomere length and tension creep

An important aspect of the present study is the demonstration that shortening above 2·2 μm sarcomere length leads to a steady increase in the fibre's ability to produce force. This was tested by allowing the fibre to shorten at a constant speed (*ca.* 0·1 length s^{-1}) that was adjusted to approximately correspond to that recorded in shortening segments during an isometric tetanus. Shortening reduced the force output below the isometric level according to the force–velocity relationship. The results clearly show, however, that there was a steady increase of force as the fibre

shortened within the range of the descending limb of the length–tension relation. This force enhancement was similar in magnitude to the tension creep recorded during an isometric tetanus. The increase in contractile potential by shortening was also demonstrated by allowing the fibre to shorten against a high *constant* load. In this case shortening resulted in acceleration of the fibre. The rise in force producing capability was attributable to the steady increase in area of overlap between the A and I filaments that occurs as a fibre shortens from a pre-stretched length. In accordance with this view there was no enhancement of force during shortening within the plateau of the length–tension relation.

In order to explain tension creep on the basis of sarcomere instability it is necessary to postulate that there is an equally large increase in force in all segments along the fibre, i.e. also in segments undergoing elongation. Sarcomeres which elongate would become progressively weakened by going to a smaller and smaller area of filament overlap and, at sarcomere lengths shorter than 2·7, stretch would not be expected to recruit any appreciable passive force (cf. resting tension, Fig. 6 in Edman, 1979). However, the effect of decreasing the area of overlap is overridden by the force enhancement that occurs when a muscle is stretched during contraction. This force enhancement during stretch increases with the velocity of stretch (Flitney & Hirst, 1978) and represents the negative portion of the force–velocity relation (Katz, 1939; Aubert, 1956). The magnitude of force enhancement during stretch and its dependence on the velocity of stretch has recently been characterized in some detail in the single muscle fibre preparation (Edman *et al.* 1978, 1981). It has furthermore been demonstrated (Cavagna & Citterio, 1974; Edman *et al.* 1978; Sugi & Tsuchiya, 1981) that, at sarcomere lengths greater than 2·3 μm, stretch causes a shift of the force–velocity curve towards higher force values. Both these components of force enhancement during stretch would contribute to the rise of force in the elongating segments during tension creep.

As an attempt to further evaluate the above hypothesis that tension creep results from a redistribution of sarcomere length, a computer analysis was performed the details of which are described in the Appendix. The analytical model comprised a single fibre composed of twelve segments, i.e. a system simulating the intact fibre preparation used in the present study. The various segments were given sarcomere length characteristics similar to those described in section B (Results). That is, the sarcomere length was assumed to be the same in all segments at 2·1 μm sarcomere spacing. At more extended fibre lengths the end segments were assumed to have a shorter sarcomere length than the middle segments according to the experimental findings described in section B. The assumptions concerning the force–velocity relationship (during shortening and elongation) and the length–tension relationship were based on experimental data previously obtained in studies of intact single muscle fibres.

Fig. 12 (Appendix) illustrates that tension creep and segment length changes during an isometric tetanus can be simulated well by the model. Similar conclusions were reached in a recent computer study by Morgan, Mochan & Julian (1982). The following observations made in experiments on living fibres were reproduced by the present model:

(1) Active force continues to rise until the shortest sarcomeres have reached a

length of approximately 2·2 μm. Force thereafter begins to decline slowly. Furthermore, the attainment of peak force occurs at a later and later time during the tetanus the longer the sarcomere length at which contraction is initiated (Fig. 9 and Fig. 13, Appendix). This accords with the fact that the stronger segments have a longer distance to shorten before they reach 2·2 μm sarcomere length. The decrease in force that occurs after maximum can be explained by the fact that the speed of elongation of the central segments is steadily reduced after the end segments have reached 2·2 μm sarcomere length and begin to shorten on the plateau of the length–tension relation.

(2) No tension creep occurs during a tetanus performed on the plateau of the length–tension relation in spite of the fact that there is a substantial redistribution of sarcomere length along the fibre also in this case. The absence of tension creep can be explained by the fact that the stronger segments do not gain in strength by shortening within this region of the length–tension relation. The velocity of elongation of the yielding segments therefore will remain constant during the tetanus plateau (Fig. 12, Appendix).

(3) Tension creep may develop at sarcomere lengths $> 2{\cdot}2$ μm even if force is recorded from a limited region of the fibre as occurs when the central portion of the fibre is held at constant length during contraction (Fig. 14, Appendix; also see Fig. 11*B* in this paper and Fig. 9 in Gordon *et al.* 1966*a*). Tension creep is also here attributable to redistribution of sarcomere length. However, with the end segments excluded there is a higher degree of sarcomere stability in the preparation to start with, and tension creep therefore tends to be less pronounced in this situation than during conventional isometric recording.

Conclusive evidence that tension creep is a complication caused by sarcomere instability is provided by the finding that tension creep can be completely eliminated when recording is made from a very short segment of the fibre (Fig. 11*A*, right panel). The absence of tension creep during such recording suggests that there is a high degree of sarcomere uniformity within the segment so that no substantial length redistribution occurs during contraction. The creep-free force so produced is therefore likely to be quite close to the true sarcomere isometric force during tetanic activity.

The slow length changes along the muscle fibre during a fixed-end tetanus can also be visualized by means of laser diffraction technique (K. A. P. Edman & C. Reggiani, unpublished observations) using streak photography to monitor the zero- to first-order diffraction pattern (Cleworth & Edman, 1972). However, laser diffraction can be presumed to be less accurate than the surface marker technique, as the sarcomere population within the laser beam does not remain constant during the course of a contraction (cf. p. 187). Ter Keurs *et al.* (1978) who used laser diffraction to monitor sarcomere movements did not observe any redistribution of length that they considered large enough to account for the tension creep recorded in their experiments. The present results have clearly demonstrated, however, that a considerable redistribution of length does occur along a muscle fibre during a fixed-end tetanus, and the analysis leaves little doubt that this redistribution of length is indeed responsible for the tension creep. It is therefore reasonable to conclude that the length–tension curve derived by ter Keurs *et al.* (1978, 1979), based on data including tension creep, does not reflect the contractile behaviour at sarcomere level.

APPENDIX

Computer simulation of force development and segment length changes in single muscle fibre

Description of computer model

A computer model has been used to simulate force development and segment length changes during fixed-end tetani of single muscle fibres. For this calculation the fibre is assumed to consist of twelve consecutive segments of length L_1, $L_2 \ldots L_i$ acting in series with an elastic component. The sarcomere length, contractile properties (force–velocity characteristics) and time course of activation are assumed to be uniform within any given segment, i.e. only non-uniformities *between* segments are considered. The numerical value of the different parameters used in the model have been selected from studies of isolated muscle fibres at the low temperature (1–2 °C) considered.

The contractile properties of each segment are defined by the force–velocity relation using Hill's (1938) hyperbolic relation

$$v_i = (P_{0i} - F).b_i/(F + a_i), \tag{1}$$

where v_i is the velocity of sarcomere shortening in a given segment (i), F active force and P_{0i} the maximum ability of the segment to produce force. The numerical values of constants $a_i = 0{\cdot}17\ P_{0i}$ and $b_i = 0{\cdot}17\ V_{0i}$ are obtained from Edman *et al.* (1976). V_{0i} represents the unloaded velocity of shortening and is assumed to be independent of sarcomere length (between 1·65 and 2·8 μm) and state of activation of the contractile system (Edman, 1979). For elongating segments the force–velocity relation is assumed to be a straight line with a slope of 0·5 (μm sarcomere^{-1}) s^{-1} P_{0i}^{-1} derived from Edman *et al.* (1981).

The isometric force of each segment, P_{0i}, is determined by the following function:

$$P_{0i}(\text{s.l.}, t, C) = O(\text{s.l.}).A(t).C. \tag{2}$$

Here O(s.l.) is the effective area of overlap between the thick and thin filaments which is assumed to vary with sarcomere length (s.l.) as described by Gordon *et al.* (1966*b*), that is:

when $1{\cdot}80\ \mu\text{m} < \text{s.l.} < 2{\cdot}00\ \mu\text{m}$, $O(\text{s.l.}) = 0{\cdot}0304 + 0{\cdot}4848.(\text{s.l.})$;
$2{\cdot}00\ \mu\text{m} < \text{s.l.} < 2{\cdot}20\ \mu\text{m}$, $O(\text{s.l.}) = 1$;
$2{\cdot}20\ \mu\text{m} < \text{s.l.} < 3{\cdot}65\ \mu\text{m}$, $O(\text{s.l.}) = 2{\cdot}5172 - 0{\cdot}6896.(\text{s.l.})$.

$A(t)$ is the level of activation of the contractile system and is defined by the empirical function:

$$A(t) = \alpha.t/(\tau + t) \quad \text{for} \quad 0 < A(t) \leqslant 1, \tag{3}$$

where α is the asymptote which has been arbitrarily set to 1·2 and τ the half-time of the function. With the upper limit used for the function, activation becomes constant after attainment of $A(t) = 1$. A numerical value of $\tau = 20$ ms is used to provide full activation in approximately 100 ms (cf. Cecchi, Colomo & Lombardi, 1978).

The third factor, C, expresses the relative capacity of an individual segment to

produce force at a given area of overlap and a given state of activation. This factor is used to express inherent differences in P_{0i} between individual segments caused, for instance, by variation in cross-sectional area (myofibrillar mass) along the fibre. Factor C is required in the model to account for differences in contractile performance between individual segments during contraction within the plateau of the length–tension relation.

In the computer simulation P_{0i} is assumed to increase by $1{\cdot}15\,P_0.(\mu\text{m sarcomere}^{-1})^{-1}$ when a segment is stretched during contraction at sarcomere lengths $>2{\cdot}3\ \mu$m. This increase represents the shift of the force–velocity curve to higher force values during stretching that has previously been demonstrated (Edman *et al.* 1978). Furthermore, *shortening* during tetanic activity is assumed to cause a slight decrease of P_{0i} amounting to $0{\cdot}25\ P_0.(\mu\text{m sarcomere}^{-1})^{-1}$ (Edman, 1980).

The load–extension relationship of the series elastic components is defined by the following function:

$$F = \gamma_{se}.\exp(\beta_{se}.L_{se}) - \gamma_{se}, \tag{4}$$

in which L_{se} denotes the elongation of the series elastic component and β_{se} and γ_{se} are constants. The numerical values of the constants are chosen so as to make the series elastic component elongate by approximately 2 % of the total fibre length at maximum isometric force, i.e. at $F = 1$. This is consistent with the over-all length changes of the fibre observed in the present experiments (see Fig. 5, ●).

Within the range of sarcomere lengths considered in this study ($< 2{\cdot}8\ \mu$m) resting tension is small (< 5 % of maximum tetanic force, see Edman, 1979). Resting tension has therefore been omitted in the computer analysis.

The following approach is used to calculate the segment length changes during contraction at fixed fibre ends:

$$\mathrm{d}L_1/\mathrm{d}t + \mathrm{d}L_2/\mathrm{d}t \ldots + \mathrm{d}L_i/\mathrm{d}t = \mathrm{d}L_{se}/\mathrm{d}t. \tag{5}$$

To account for differences in sarcomere length between individual segments a parameter $k_i = \mathrm{d}L_i/\mathrm{d}t/v_i$ is introduced, where v_i is the velocity of sarcomere shortening. Eqn. (5) can therefore be rewritten as follows:

$$k_1.v_1 + k_2.v_2 \ldots + k_i.v_i = \mathrm{d}L_{se}/\mathrm{d}t. \tag{6}$$

Substitution of $\mathrm{d}L_{se}/\mathrm{d}t$ according to the differentiated form of eqn. (4) yields the following function:

$$\mathrm{d}F/\mathrm{d}t = \beta_{se}(F+\gamma_{se})\ (k_1.v_1 + k_2.v_2 \ldots + k_i.v_i). \tag{7}$$

In this function v_i is computed according to eqn. (1) when the segment shortens. For force values greater than P_{0i}, i.e. during segment elongation, v_i is computed according to the linear negative portion of the force–velocity relation (see above). The value of F is computed from eqn. (7) at 1 ms intervals. At each interval F is used to compute the segment's velocity and integrated length change. This procedure is iterated over a time period of 3 s. In each iteration the determination of $\mathrm{d}L_i/\mathrm{d}t$, L_i and F is based on the values that these variables have at the end of the nearest preceding interval.

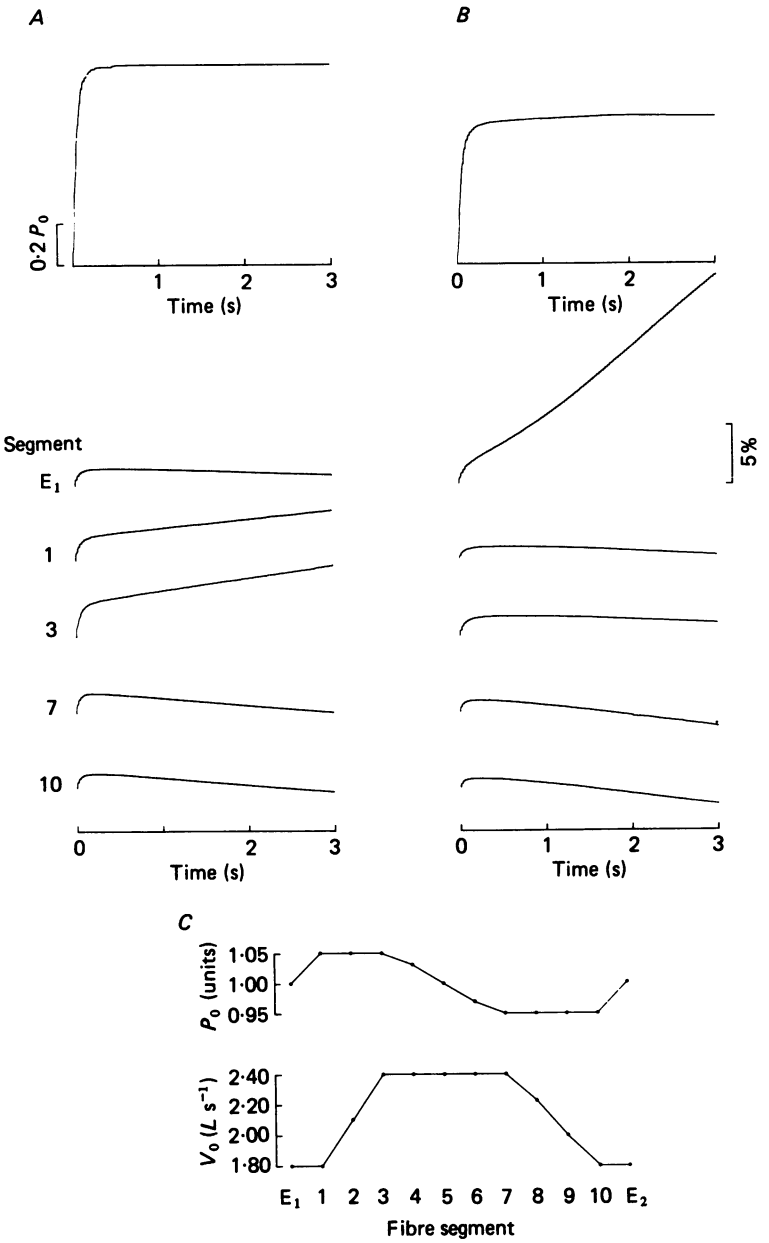


Fig. 12. Computer simulation of fixed end tetani at 2·15 μm (A) and 2·70 μm (B) sarcomere length. Upper traces: force. Lower traces: percentage length change of one end segment (E_1) and four selected central segments. Vertical scales in this and following Figures: force, percentage of maximum tetanic force (P_0) at 2·15 μm sarcomere length; length change, percentage of segment rest length. C indicates the numerical values of V_0 and P_0 for the two end segments (E_1 and E_2) and the central segments numbered 1–10. In A sarcomere length is the same in all segments including the fibre ends. In B sarcomere length of end segments is 2·46 μm. Note tension creep and extensive shortening of end segments in pre-stretched fibre. No tension creep occurs on the plateau of the length–tension relation in spite of marked redistribution of length between segments.

Results of computer simulation

Fig. 12 shows simulations of fixed-end tetani on the plateau (*A*) and at a point on the descending limb of the length–tension relation (*B*). In Fig. 12*A* the sarcomere length is the same, 2·15 μm, in all segments. In Fig. 12*B* the sarcomere spacing is 2·70 μm in the central segments and 2·46 mm in the end segments in accordance with

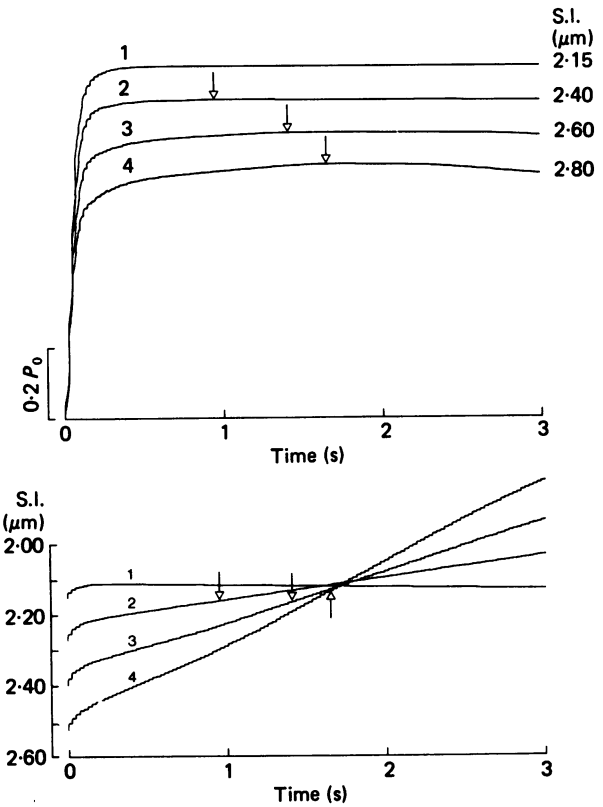


Fig. 13. Computer simulated fixed-end tetani at four different sarcomere lengths. Resting sarcomere length of central segments indicated at respective force trace in upper diagram. Length change of one end segment is illustrated for each contraction in lower diagram (the same numbering, 1–4, as in upper diagram). Resting sarcomere length of end segment determined according to Fig. 7. The contractile properties of the individual segments are the same as in Fig. 12. Note that peak force of contractions 2–4 occurs when end segments have shortened to 2·16–2·18 μm.

the experimental data presented in Fig. 7. Length changes of one end segment (E_1) and four central segments are illustrated. Different combinations of numerical values of V_{0i} and P_{0i} are used in the various segments as illustrated in Fig. 12*C*. This variation of V_{0i} and P_{0i} between individual segments ($\pm 15\%$ and $\pm 5\%$, respectively) is consistent with observations made in experiments on intact fibres (see pp. 188, 189). As can be seen, maximum force is reached approximately 0·6 s after the onset of contraction at 2·15 μm sarcomere length. Tension thereafter remains constant. By

contrast tension continues to climb slowly for another second during contraction at 2·70 μm sarcomere length. After this point there is a slow decline of tension.

In accordance with observations in the living fibre there is a considerable redistribution of length both between the end segments and the centre of the fibre and between individual central segments. This redistribution of length occurs on the plateau as well as on the descending limb of the length–tension relation. Fig. 12

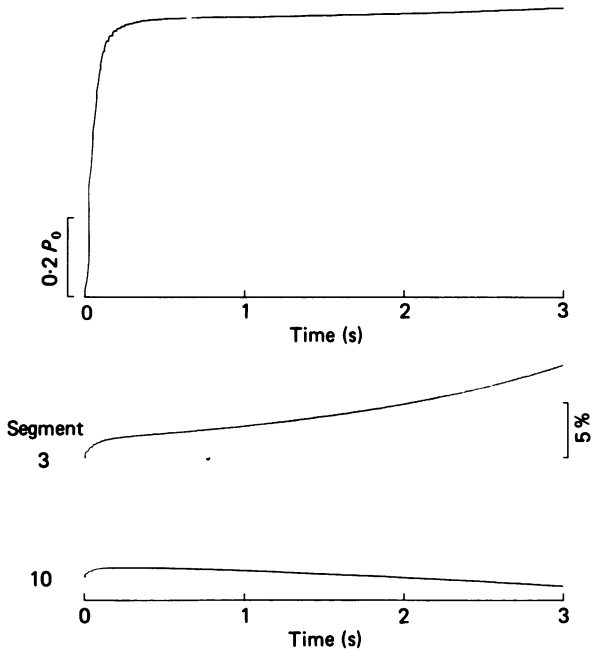


Fig. 14. Computer simulated tetanic contraction of fibre in which the central portion (consisting of ten segments) is held at constant length. Resting sarcomere length is the same, 2·70 μm, in all segments. The contractile properties of the individual segments are those used for segments 1–10 in Fig. 12. The length changes of two central segments are shown. Note tension creep and redistribution of length between the segments.

furthermore confirms that the amplitude of the initial length change of the segments is related to the value of V_{0i} whereas the performance during the second slow phase is determined by P_{0i}, the segment's capacity to produce force. The shorter sarcomere length of the end segments in Fig. 12*B* makes P_{0i} of these segments considerably larger than that of the central segments. This leads to pronounced shortening of the fibre ends during the tetanus at the expense of the middle portion of the fibre which elongates. However, one or more of the central segments may also be able to shorten (not illustrated), or remain stationary (segment 3, Fig. 12*B*) during the second phase depending on the relative values of P_{0i} given to the segments (cf. Figs. 4–6). This requires that other segments are stretched considerably beyond their resting length. Results similar to those shown in Fig. 12 may be obtained by varying the rate of activation (τ in eqn. (3)) along the fibre, combined with variation of P_0 *while* V_0 is kept constant. By assuming slower activation of the fibre ends it is possible to

simulate the initial yielding of the end segments that occurs during a fixed-end tetanus on the plateau of the length–tension relation (cf. Fig. 8).

Fig. 13 illustrates the time course of force development during simulated fixed-end tetani at different sarcomere lengths using the same assumptions as stated in Fig. 12. Peak force can be seen to be attained later and later after the onset of contraction as the resting sarcomere length is increased from 2·15 to 2·80 μm. The lower panel of Fig. 13 shows that in each case when peak force is attained during a tetanus at a pre-stretched length, the end sarcomeres have shortened to a point just below 2·2 μm. The decrease in force after attainment of the maximum is associated with a slight decrease in velocity of the shortening segments.

Fig. 14 shows a simulation of a 3 s tetanus at 2·70 μm sarcomere length while the central portion of the fibre is held at constant length, i.e. the end segments are here omitted. In this simulation the ten middle segments are given the same contractile properties as used in Fig. 12. The resting sarcomere length is the same, 2·70 μm, in all segments. It is clear that tension creep does occur also in the absence of end segments when the contractile properties of the clamped portion are non-uniform and contraction is initiated at a sarcomere length that is greater than 2·2 μm. However, the tension creep can be seen to be less pronounced under these conditions than in the presence of the end segments. This is due to the fact that, at pre-stretched fibre lengths, the end segments have a greater ability to produce force than any of the central segments.

The authors wish to thank Mrs Britta Kronborg and Miss Gunilla Einarsson for their excellent technical assistance and Mr Ove Höglund for building the electronic equipment. This study was supported by grants from the Swedish Medical Research Council (project 14X-184) and the Muscular Dystrophy Association. C.R. was supported by fellowships from the Blanceflor Boncompagni-Ludovisi Foundation and the Rusconi Foundation.

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