

PLATEAU AND DESCENDING LIMB OF THE SARCOMERE LENGTH-TENSION RELATION IN SHORT LENGTH-CLAMPED SEGMENTS OF FROG MUSCLE FIBRES

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SUMMARY

1. The relation between sarcomere length and tetanic tension was determined at 10–12 °C for 70–80 μm long segments of single fibres isolated from the tibialis anterior and semitendinosus muscles of the frog. Measurements of segment striation spacings were performed during fixed-end or length-clamp contractions by means of a laser light diffractometer.

2. At sarcomere lengths of around 2.10 μm tetanic tension rose promptly to a steady plateau, independent of the recording conditions. At greater sarcomere lengths under fixed-end conditions the tension rise occurred in two distinct stages: an initial rapid rise followed by a much slower creep. The tension creep was entirely abolished in length-clamp contractions.

3. The sarcomere length-tension diagram of length-clamped segments of tibialis anterior fibres exhibited a definite flat region between about 1.96 and 2.16 μm where tension varied by less than 1.5%. The highly linear descending limb reached zero tension at about 3.53 μm . The shift to the left by about 0.10 μm , with respect to the length-tension diagram of length-clamped segments of semitendinosus fibres, may be tentatively explained by assuming that thin filament lengths vary in different muscles.

4. The results are in agreement with those of a previous work by Gordon, Huxley & Julian (1966) and support the hypothesis (Huxley, 1957, 1980) that muscle tension is produced by simultaneous action of independent force generators, in proportion to the number of myosin bridges overlapped by actin filaments.

INTRODUCTION

Important evidence supporting the cross-bridge sliding filament theory (Huxley, 1957, 1980; Huxley, 1972) was given by Gordon, Huxley & Julian in 1966. They concluded that the sarcomere length-tension diagram of length-clamped segments of frog semitendinosus muscle fibres has a polygonal shape with a practically flat region between about 2.05 and 2.25 μm and a linear descending limb which reaches zero tension at about 3.65 μm . Because of the presence of a substantial amount of tension

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creep even in length-clamped contractions at sarcomere lengths greater than $2.25\ \mu\text{m}$, Gordon *et al.* (1966) drew the length-tension diagram taking the tension developed at the end of the initial fast phase of tetanus rise on the assumption that the residual tension creep was determined by sarcomere instability within the length-clamped segments. Thus, in accordance with the predictions of the theory, muscle tension appeared to be produced by the simultaneous action of independent force generators, in proportion to the number of myosin bridges overlapped by actin filaments.

In more recent experiments, however, ter Keurs, Iwazumi & Pollack (1978), using semitendinosus muscle fibres selected for high homogeneity in sarcomere spacings, gave evidence that sarcomere instability was unable to account for tension creep. Taking the tension developed at the plateau of a long, fixed-end tetanus, they showed that the length-tension diagram is approximately flat between about 2.00 and $2.40\ \mu\text{m}$, as if the probability of cross-bridge attachment and/or the force developed by each attached cross-bridge depends on the degree of filament overlap and the distance of individual cross-bridges from the centre of thick filaments.

The subject was then reinvestigated by Julian, Sollins & Moss (1978), Altringham & Bottinelli (1985) and Edman & Reggiani (1984, 1987). Using fibres from the tibialis anterior muscle, they confirmed the essential results of Gordon *et al.* (1966), and showed that clamping shorter segments reduced or minimized the amount of tension creep, as would be expected from the idea that creep is an artifact produced by sarcomere instability during contraction. Edman & Reggiani (1984, 1987), however, found, with respect to the length-tension relation of Gordon *et al.* (1966), small but significant differences, not only at the sarcomere lengths usually taken as being on the plateau, but also at greater lengths throughout the descending limb of the relation.

The purpose of the present study was to clarify the discrepancies found among the different experimental results. The sarcomere length-tension diagram was determined for very short length-clamped segments of tibialis anterior and semitendinosus muscle fibres of the frog, selected for high homogeneity in sarcomere pattern. With respect to previous studies the segment length to clamp was reduced to only $70\text{--}80\ \mu\text{m}$. It will be shown that: (i) tension creep is entirely abolished in length-clamped contractions; (ii) the sarcomere length-tension diagram of tibialis anterior muscle fibres exhibits a practically flat region between 1.96 and $2.16\ \mu\text{m}$ as well as a highly linear descending limb which reaches zero tension at about $3.53\ \mu\text{m}$; (iii) the sarcomere length-tension diagram of semitendinosus muscle fibres is shifted to the right by about $0.10\ \mu\text{m}$ with respect to that of tibialis anterior muscle fibres, whereas its general shape, at the level of the plateau region at least, remains unvaried.

Provided that thin filaments in tibialis anterior muscle are about 6% shorter than in semitendinosus muscle, these results are in full agreement with the basic concepts of the cross-bridge sliding filament theory (Huxley, 1957, 1980; Huxley, 1972). A preliminary report of the results has already been published (Bagni, Cecchi, Colomo & Tesi, 1986).

METHODS

Preparation and mounting of the fibres

Frogs (*Rana esculenta*) were obtained from the surrounding countryside throughout the year and were kept in running water tanks at about 6 °C for not more than 2 weeks before use. Single fibres were dissected from either the tibialis anterior muscle or in some experiments the semitendinosus muscle. Dissection was performed at room temperature under Ringer solution with the aid of a stereo-microscope, small knives and scissors. Dark-field illumination was used during the dissection. In order to minimize the shortening of the fibres against the tendons and to improve the performance of the length-clamp apparatus the length of each tendon attachment was made as short as possible and the connections to the force transducer and to the moving coil of the loudspeaker were made by means of aluminium foil clips as described by Ford, Huxley & Simmons (1977). This also reduced the length displacement required for segment length clamping and in turn the movement of the region selected at rest for laser light illumination, especially during the rise of the tetanus. The fibres were placed in an experimental chamber with a glass floor for ordinary and laser light microscope observation, and were mounted between the lever-arms of the force transducer and the loudspeaker coil. Experiments were performed at 10–12 °C. The temperature during an experiment was maintained constant within ± 0.1 °C range by a servo-controlled system driving a thermo-electric module (Thermagotrons, TL-0812, M.L.P. Electronics Ltd) which was mounted between the bottom of the experimental chamber and a water-cooled heat sink. The experimental trough and two micromanipulators, one carrying the loudspeaker coil and the other the force transducer, were mounted on the movable stage of a microscope (Carl Zeiss, Jena, mod. Mikro 532), so as to displace the whole fibre across the optical field of the microscope and to adjust the position of the fibre in the trough as well as its resting length. All the fibres used during the course of the present work gave reproducible tetani at 4 min intervals.

Determination of fibre length, cross-sectional area and resting sarcomere length

All the measurements were made under ordinary light illumination on the movable stage of the microscope. A 40 \times dry objective (Zeiss, F.R.G., NA 0.6, working distance 4.9 mm) and a 10 \times or 25 \times micrometer eyepiece were used. The resting length of the fibres was determined with a dial gauge by measuring the longitudinal displacement required to bring the two tendon ends of the fibre into the centre of the microscope field. Striation spacing was determined by averaging the measurements of sequences of twenty sarcomeres at different points along the fibre. The cross-sectional area of the fibres was calculated as if the section were elliptical, from measurements of the major and minor diameters at about 2.10 μm sarcomere length. The length of the fibres was changed by displacing the micromanipulator arm carrying the loudspeaker coil. Changes in the fibre length were measured by means of the micromanipulator dial gauge.

Laser diffractometer

Resting and dynamic measurements of sarcomere length were performed from the laser diffraction pattern by means of an opto-electronic system similar to that described by Zite-Ferenczi & Rüdél (1978). The beam of a 5 mW He-Ne laser source (Spectra Physics, model 105, U.S.A.) was reduced to 70–80 μm (full width at half-peak intensity) and projected onto the fibre by means of a spherical lens and mirror. The spherical lens was oriented so that at sarcomere lengths of about 2.10 μm the incident angle of the laser beam on the fibre was approximately equal to the diffracted angle. The right first-order diffraction line was reduced to a point by a cylindrical lens and projected at a right angle on the surface of a position-sensitive photodiode (PS-100-10, Quantrad Corporation, U.S.A.) mounted 120 mm above the fibre. The system was calibrated using resting fibres as diffraction gratings of variable spacing and comparing the values of sarcomere length measured with the laser diffractometer to those obtained with ordinary light microscopy. The sarcomere length was calculated by means of an analog computer using the general grating equation. The rise time of the system was less than 5 μs and the noise corresponded to about 0.2 nm peak to peak.

Tension transducer

The tension was measured by means of a capacitance-gauge transducer similar to that described by Huxley & Lombardi (1980). The frequency of resonance of the tension transducers used here

ranged from 35 to 60 kHz when unloaded. The tension produced by the muscle fibre was directly applied to the centre of the moving plate of the transducer by means of a fine glass or aluminium lever-hook which was attached to it. Capacitance changes produced by forces applied to the movable plate of the transducer were detected with a phase-discriminator circuit (Cecchi, 1983), derived from that described by Cambridge & Haines (1959), with a carrier frequency of 8 MHz. With this circuit the sensitivity of the tension transducers ranged from 100 to 300 mV/mN and the noise from 0.2 to 0.5 mV peak to peak.

Loudspeaker coil servo system

The position of the loudspeaker coil (Cecchi, Colomo & Lombardi, 1976) was servo-controlled using an electronic circuit derived from those previously described by Cecchi *et al.* (1976) and by Ford, Huxley & Simmons (1977). Fixed-end conditions were obtained by feeding back the output of the photodiode sensor that signalled the position of the loudspeaker coil. Length-clamp conditions were obtained by feeding back the output from the laser diffractometer so as to hold constant the sarcomere length of the selected segment of fibre, independent of the length changes undergone by the tendons and by the parts of the fibre beyond the segment. The procedure was as follows. The loudspeaker coil was first operated to obtain a fixed-end contraction and then, at a preset time during the rise of tetanic tension, was switched by a command signal to length-clamp mode of operation. A second command signal applied during the relaxation, usually before the shoulder, returned the servo system to the fixed-end mode. A protection circuit ended the length clamp when the movement of the loudspeaker exceeded the maximum allowed value. The sarcomere length signal at the end of the initial period of fixed-end contraction was stored in a sample and hold circuit. During the length clamp, the stored signal was continuously subtracted from the instantaneous sarcomere length, and the difference was used as the feed-back signal in the servo-loop. This avoided changes in fibre length on switching the system to the length-clamp mode. A similar system was used to return the loudspeaker coil to its initial position, at the end of the length-clamp period.

Solutions

The Ringer solution had the following composition (in mM): NaCl, 115; KCl, 2.5; CaCl₂, 1.8; phosphate buffer, 3; at pH 7.1.

Stimulation

Stimuli of alternating polarity were applied transversely to the muscle fibre by means of a pair of platinum plate electrodes (10 mm long, 4 mm apart) across which up to 15 V could be applied with a constant voltage pulse generator. Stimuli of 0.5 ms duration and 1.5 times the threshold strength were used. Tetanic stimulation was applied in brief volleys (0.6–0.8 s duration) of pulses at 4 min intervals. The optimal stimulation frequency required to produce a fully fused maximal tetanus was determined for each fibre.

Recording and measurement of the responses

Tension, fibre length, sarcomere length and intensity of the first-order diffraction line were recorded on a four-channel digital oscilloscope (Nicolet, model 4094, U.S.A.) and stored on floppy discs. The time base of the Nicolet oscilloscope was triggered externally by different pulse frequency trains so as to obtain an adequate temporal resolution of the records. Measurements of the responses were made directly from the oscilloscope using its cursor and digital logic.

RESULTS

Selection of the fibre segments for length clamping

Special care was taken in localizing the segments to illuminate with the laser beam. The segments were consistently localized at the centre of 0.5–1.0 mm long regions which were, excluding the end parts of the fibre with shorter sarcomeres, within the half of the fibre closer to the force transducer. Selection was made on the basis of the striation orientation being perpendicular to the axis of the fibre, uniformity of

sarcomere length and quality of the laser diffraction pattern. Figure 1 shows the sarcomere length variation for two typical resting fibres within the halves closest to the force transducer. It can be seen that within about 200 μm from the laser spots (indicated by the arrows) the variation in sarcomere length was less than 0.20–0.35%. The advantage of selecting segments on the force transducer end of the fibre was that this minimized displacement of the segment due to the longitudinal movement of the

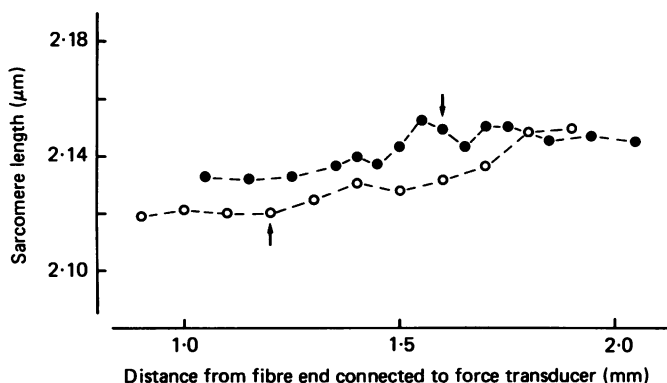


Fig. 1. Longitudinal variation of sarcomere length around the segments selected for length clamping (indicated by the arrows) in two resting fibres, one from the tibialis anterior muscle (○) and one from the semitendinosus muscle (●). The measurements were performed using the laser diffractometer. Fibre lengths: 4550 μm (tibialis anterior muscle) and 10790 μm (semitendinosus muscle). Temperature: 12 °C.

loudspeaker coil to maintain constant sarcomere length during the clamping period. Under these conditions the displacement was not greater than 100 μm , but many times smaller than the 0.5–1.0 mm region in which the laser spot was centred. It is unlikely, therefore, that in active fibres the new sarcomere populations sampled by the laser beam during displacement would exhibit a striation pattern significantly different from those sampled just before the beginning of the movement.

The relation between fibre length and segment sarcomere length at rest

In accordance with the results of previous work (Huxley & Peachey, 1961; Edman, Mulieri & Scubon-Mulieri, 1976) resting fibres at slack length exhibited a remarkably constant striation pattern along most of their length. Apart from the end regions where sarcomeres were shorter (Huxley & Peachey, 1961), the variation in striation spacing was no greater than about $\pm 1\%$. The high linearity of the relation between fibre length and segment sarcomere length as well as the observation that the fitted line extrapolated to the axis origins (Fig. 2) indicate (i) that the variation in average sarcomere length does not change significantly upon changing the fibre length, and (ii) that the contribution to this relation of end regions with shorter sarcomeres is negligible.

The sarcomere length-tetanic tension relation

Figures 3 and 4 show sample records obtained at various sarcomere lengths from two fibres from the tibialis anterior muscle. In order to show the effectiveness of the

length-clamp system at each sarcomere length, tension records obtained under fixed-end conditions were superimposed on those obtained under length-clamped conditions. No appreciable differences were noted at sarcomere lengths around $2.10 \mu\text{m}$ between records obtained under fixed-end and length-clamp conditions: tension rose promptly to a steady plateau independent of the recording conditions.

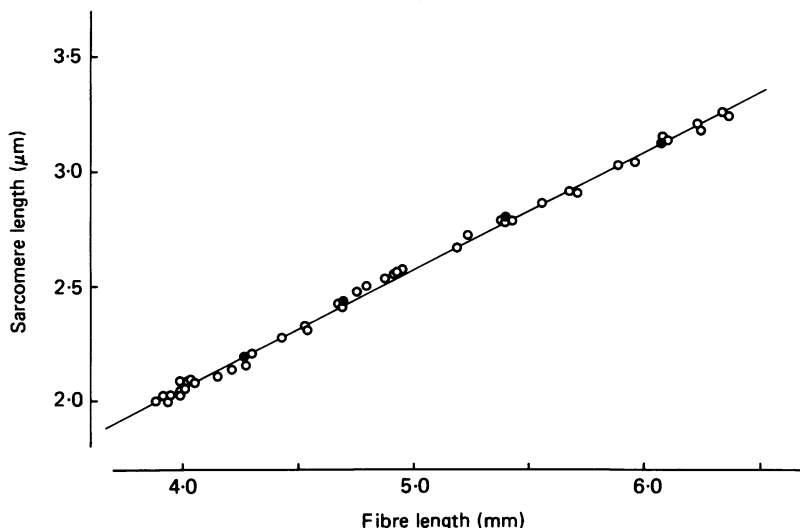


Fig. 2. Segment sarcomere length against fibre length for one tibialis anterior muscle fibre at 11°C . \circ , measurements determined with the laser diffractometer; \bullet , optical microscope measurements at four different sarcomere lengths for calibration of the laser diffractometer. The slack length of the fibre was $3890 \mu\text{m}$. The straight line is the regression line calculated for data points denoted by \circ . The line intercepts the segment sarcomere length axis at $-0.0037 \mu\text{m}$. The correlation coefficient was 0.999 .

Differences became more and more evident as sarcomere length was further increased. In accordance with the results of previous work, under fixed-end conditions the rise in tension occurred in two distinct stages: an initial rapid rise, and then a much slower creep. Under our length-clamp conditions, even at sarcomere lengths greater than $2.50 \mu\text{m}$, the creep was absent.

The relation between steady tetanic tension and sarcomere length for tibialis anterior muscle fibres was determined utilizing one segment of each of twelve preparations studied during the course of the present work. The results are illustrated in Fig. 5. The relation shows (i) a nearly flat region extending for more than $0.2 \mu\text{m}$ around a sarcomere length of $2.05 \mu\text{m}$, and (ii) a highly linear descending limb at longer sarcomere lengths, reaching the length axis at about $3.53 \mu\text{m}$. Figure 6 shows an enlargement of the length-tension diagram in the region near the optimum for tension development, and the corresponding values for tensions and sarcomere lengths are listed in Table 1. It can be seen that relative tension remains constant within a sarcomere length interval from 2.00 to $2.12 \mu\text{m}$ and that it dropped to 99.0% and to 98.5% of the maximum, respectively, at 1.96 and at $2.16 \mu\text{m}$. The regions around $2.05 \mu\text{m}$ of the length-tension relations for three different fibres are illustrated in Fig. 7 to show that tensions produced by individual fibres are remarkably constant within a significant range of sarcomere lengths.

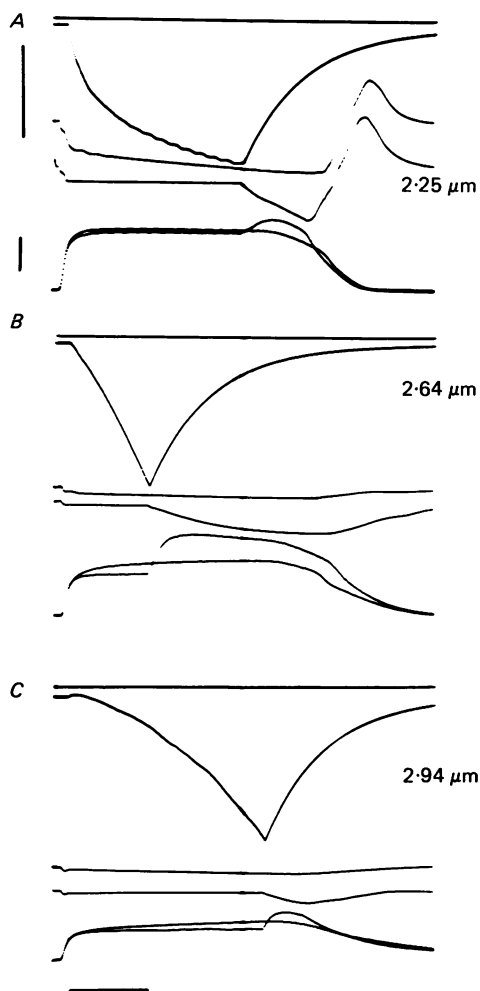


Fig. 3. Oscilloscope records of length changes and tension responses for one fibre from tibialis anterior muscle during fixed-end and length-clamped tetani. Figures close to the records refer to the actual sarcomere length of the segment during the length-clamp period. *A-C*, the upper pair of traces show fibre length under fixed-end conditions (top trace) and under length-clamp conditions (lower trace), downward deflections indicating fibre shortening. The middle pair of traces show segment sarcomere length under fixed-end conditions (top trace) and under length-clamp conditions (lower trace), downward deflections indicating sarcomere stretching. The bottom pair of traces show tensions in fixed-end or length-clamp conditions. Horizontal calibration: 200 ms. Vertical calibration: tension, 200 kN/m²; length, 1.30% of the fibre resting length at 2.25 μm sarcomere spacing (l_0) or 64 nm for *A* and 3.25% l_0 or 160 nm for *B* and *C*. The rise of tension that occurs at the end of the length-clamp period is produced by the servo system slowly stretching the fibre to its original length. Note in *A* that in fixed-end conditions even when the tension had reached a fairly steady plateau the sarcomere population sampled by the laser beam was being slightly stretched by other sarcomeres along the fibre. This effect was sufficient to produce a 5% enhancement of plateau tension compared to that produced under length-clamp conditions.

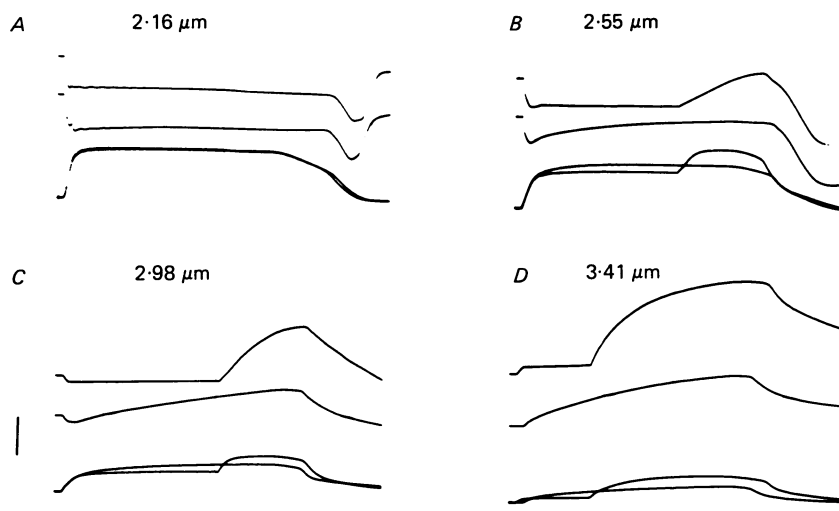


Fig. 4. Oscilloscope records of length changes and tension responses for one fibre from tibialis anterior muscle during fixed-end and length-clamped tetani at 12 °C. Figures close to the records refer to the actual sarcomere length of segment during the length-clamp period. *A-D*, the upper pair of traces show sarcomere length in the segment under length-clamp conditions (top trace) and under fixed-end conditions (lower trace), downward deflections indicating sarcomere shortening. The bottom pair of traces show tension in fixed-end or length-clamp conditions. Horizontal calibration: 250 ms. Vertical calibration: 215 kN/m² or 50 nm. The rise of tension that occurs at the end of the length-clamp period is produced by the servo system slowly stretching the fibre to its original length.

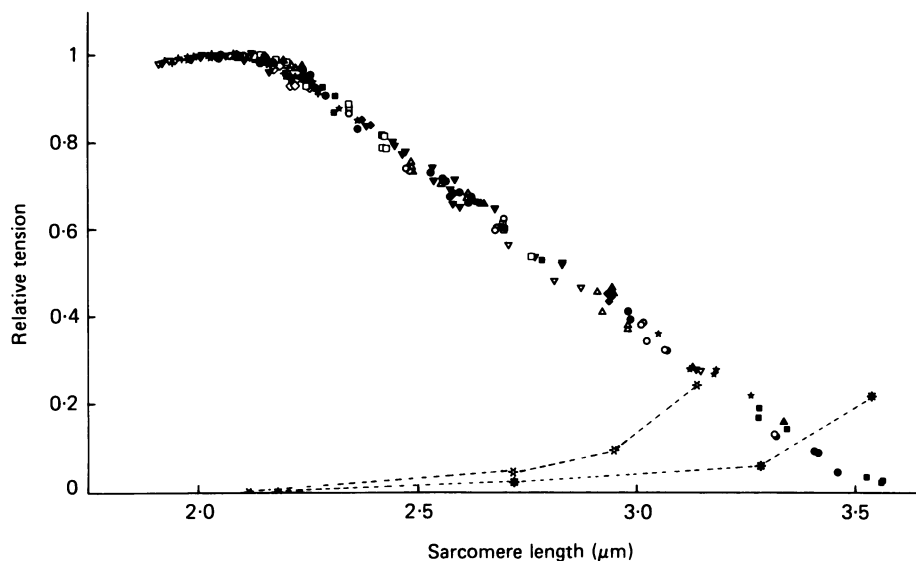


Fig. 5. Sarcomere length-tetanic tension relation for twelve length-clamped segments from as many fibres isolated from tibialis anterior muscle. Tension values (total minus resting tension) were normalized for the maxima obtained at the optimum length. Different symbols refer to segments of different fibres. The interrupted lines show the sarcomere length-resting tension for two different fibres.

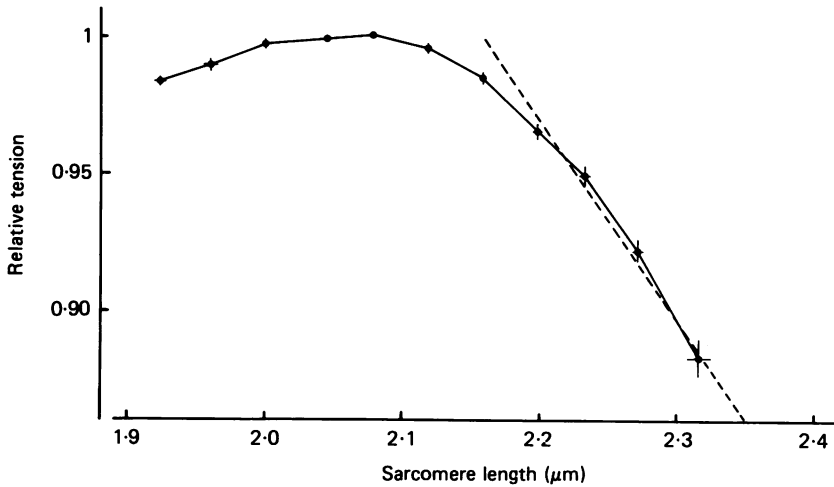


Fig. 6. Tension developed by tibialis anterior muscle fibres at sarcomere lengths near the optimum. Individual data points were obtained by $0.04 \mu\text{m}$ class averaging of tension and sarcomere length values reported in Fig. 5. Tensions were expressed as a fraction of the maximum. Vertical and horizontal bars on data-points are the s.e.s of means. The interrupted line is the regression line calculated for all the data points collected at sarcomere length $\geq 2.20 \mu\text{m}$. The correlation coefficient was 0.998.

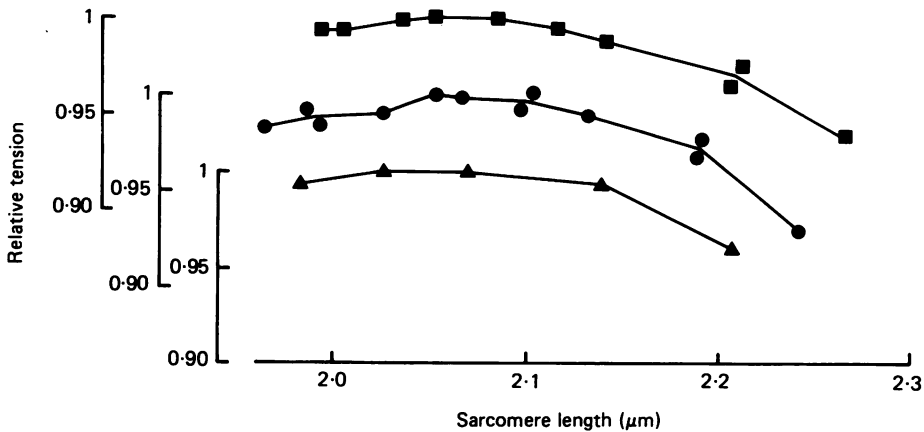


Fig. 7. Tension developed by three individual fibres from tibialis anterior muscle. Tension (total minus resting tension) was expressed as a fraction of the maximum. Different symbols refer to segments of three different fibres.

The interrupted straight line in Fig. 6 is the regression line calculated for all the data points collected at sarcomere length $\geq 2.20 \mu\text{m}$. The line gave an upper length limit for the plateau at $2.16 \pm 0.0260 \mu\text{m}$ (mean \pm s.e.m.) and intercepted the sarcomere length axis at $3.53 \pm 0.0260 \mu\text{m}$ (mean \pm s.e.m.). Thus, apart from a shift to the left by about $0.1 \mu\text{m}$, the length-tension relation for short length-clamped fibre segments from the tibialis anterior muscle was practically identical to that determined by Gordon *et al.* (1966) for much longer length-clamped segments of semitendinosus muscle fibres.

TABLE 1. Tensions developed at different sarcomere lengths by tibialis anterior and semitendinosus muscle fibres, expressed as a fraction of the maximum value

Tibialis anterior muscle			Semitendinosus muscle		
Sarcomere length (μm) (mean \pm S.E.M.)	Relative tension (mean \pm S.E.M.)	<i>n</i>	Sarcomere length (μm) (mean \pm S.E.M.)	Relative tension (mean \pm S.E.M.)	<i>n</i>
1.923 \pm 0.003	0.984 \pm 0.001	7	2.017 \pm 0.001	0.982 \pm 0.001	3
1.961 \pm 0.004	0.990 \pm 0.002	10	2.050 \pm 0.003	0.992 \pm 0.002	8
2.000 \pm 0.002	0.998 \pm 0.001	21	2.084 \pm 0.002	0.998 \pm 0.001	17
2.045 \pm 0.002	0.999 \pm 0.001	20	2.122 \pm 0.003	0.999 \pm 0.001	16
2.078 \pm 0.002	1.000 \pm 0.001	27	2.158 \pm 0.003	0.997 \pm 0.002	16
2.118 \pm 0.002	0.997 \pm 0.001	32	2.197 \pm 0.003	0.990 \pm 0.002	20
2.158 \pm 0.002	0.985 \pm 0.002	28	2.235 \pm 0.003	0.976 \pm 0.002	17
2.198 \pm 0.002	0.966 \pm 0.003	24	2.283 \pm 0.005	0.952 \pm 0.005	8
2.233 \pm 0.003	0.950 \pm 0.004	22			
2.272 \pm 0.003	0.922 \pm 0.004	6			
2.317 \pm 0.007	0.883 \pm 0.007	4			

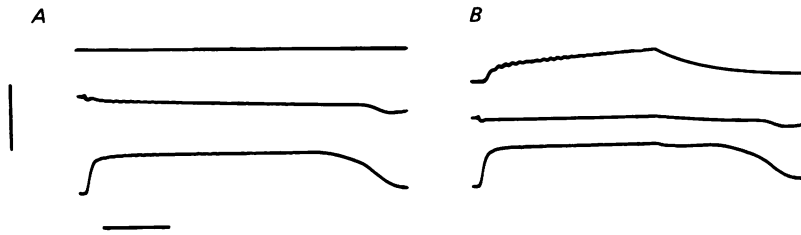


Fig. 8. Oscilloscope records of length changes and tension responses for one fibre from a semitendinosus muscle during fixed-end (*A*) and length-clamped tetani (*B*) at 12 °C. The resting sarcomere length was 2.1 μm . In each panel traces, from top to bottom, are fibre length, segment sarcomere length (downward deflections indicate shortening) and tension. Horizontal calibration: 200 ms. Vertical calibration: 365 kN/m², or 1.60% of the fibre resting length at 2.25 μm sarcomere spacing (l_0), or 120 nm. The small ripple in the sarcomere length trace under fixed-end conditions (*A*) and on the fibre length trace under length-clamp conditions (*B*) results from the fact that stimulus frequency was just under optimal.

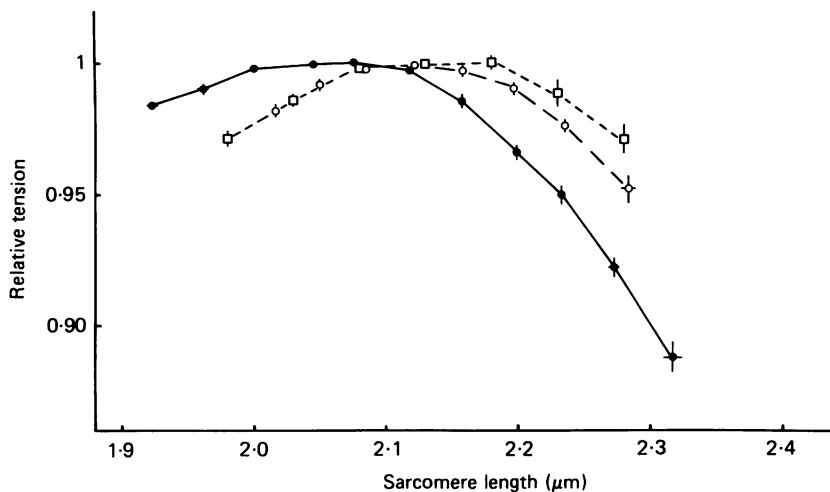


Fig. 9. Superimposition of the sarcomere length-tension relations for tibialis anterior muscle fibres (●) and for semitendinosus muscle fibres (○, □). ○, data obtained in experiments performed during the course of the present work; □, data of Fig. 2 by Gordon *et al.* (1966). Vertical and horizontal bars on data points are the s.e.s. of means.

In order to identify the causes of this shift, the plateau region of the sarcomere length-tension relation was also determined for length-clamped segments of semitendinosus muscle fibres. Figure 8 shows sample records from a typical experiment, and the results from four fibres are shown in Fig. 9, together with those reported by Gordon *et al.* (1966), and listed in Table 1. It can be seen that relative tension remains absolutely constant from 2.08 to 2.16 μm and that it dropped to 99% of the maximum both at 2.05 μm and at 2.20 μm .

DISCUSSION

The tension response in length-clamp contractions

The results reported in the present paper show definitely that tension creep may be entirely abolished by using the sarcomere length-clamp technique. It can be seen in the records of Figs 3, 4 and 8 that tetanic tension settled to a remarkably and absolutely smooth plateau during the length-clamp period, independent of the sarcomere length at which fibres contracted. This effect was probably due to either the short length (70–80 μm) and the high uniformity in sarcomere pattern of the fibre segments selected for the experiments or the quality of the length-clamp apparatus used here and may be taken as further indirect evidence that redistribution of sarcomere lengths during contraction is responsible for tension creep.

Qualitatively similar results have also been obtained in previous experiments in which the length-clamped segments were longer (6–8 mm, Gordon *et al.* 1966; 1.5–3.0 mm, Julian *et al.* 1978; 0.5–0.6 mm, Edman & Reggiani, 1984, 1987; 100–200 μm , Altringham & Bottinelli, 1985). It is interesting to note that comparison of the results reported here and in the papers quoted above shows that tension creep decreases as the length of the clamped region is reduced, disappearing entirely with segments as short as those used here.

The sarcomere length–tension relation

In accordance with the results of previous work (Gordon *et al.* 1966; Julian *et al.* 1978; Altringham & Bottinelli, 1985) and the basic concepts of the cross-bridge sliding filament theory (Huxley, 1957, 1980; Huxley, 1972) the results of the present work show that the sarcomere length–tension relation of frog skeletal muscle exhibits a practically flat region and a highly linear descending limb. The values of 1.96 and 2.16 μm for the plateau ends and of 3.53 μm for the length–axis intercept at zero tension, found in tibialis anterior muscle fibres, are fully consistent with the projections made by Huxley (1980) and Woledge, Curtin & Homsher (1985) on the basis of measurements of myofibril lengths (Page & Huxley, 1963; Page, 1968; Huxley, 1972; Craig & Offer, 1976).

Another result of the present work is that the length–tension diagrams of fibres from different frog muscles may be slightly, but significantly, shifted along the horizontal axis. The shift to the left by about 0.10 μm between the length–tension diagrams of tibialis anterior and semitendinosus muscle fibres may be tentatively explained assuming thin filaments to be about 6% longer in semitendinosus than in the tibialis anterior muscle. It is interesting to note here that Akster, Granzier & ter Keurs (1984) have shown that in the perch thick and thin filaments of different muscles exhibit different lengths and that the sarcomere length–tension diagrams of these muscles are shifted along the length axis approximately in proportion to what is expected from the differences in filament lengths. On the other hand, it is evident that if filaments of tibialis anterior and semitendinosus muscle of the frog are found to be the same length, the result would require a revision of the actual form of the sliding filament cross-bridge theory.

Measurement of striation spacing

The possibility of errors in dynamic measurements of the striation spacing because of changes in length and orientation of sarcomere populations sampled by the laser beam during contraction requires comment. Lieber, Yeh & Baskin (1984) have shown that at rest the apparent sarcomere length within 100 μm long segments varies significantly with the incident angle of the laser and the fibre thickness. This means that during contraction longitudinal displacements of the muscle fibre may introduce an error in the measurements of the striation pattern. The average vertical diameter of the fibres used in the present work was $69.75 \pm 4.10 \mu\text{m}$ (mean \pm S.E.M., $n = 12$, range 48.10–89.70 μm). According to the data of Lieber *et al.* (1984), the maximum error in the preparations used here should not be greater than 2%. Lieber *et al.* (1984), however, did not select the fibre segments for striation uniformity. Presumably, since in the present study segments for length clamping were positioned within much longer regions characterized by high uniformity of sarcomere spacing, the possible error introduced by the longitudinal displacement of contracting fibres should be less than 2%. It is interesting to note here that during contraction the longitudinal displacement of the fibre across the laser spot was not greater than 100 μm , a length 5–10 times smaller than that of the highly homogeneous region from which segments were selected. Moreover, even if an error of 2% were present in the measurements of sarcomere length, this error would be randomly distributed with sarcomere length and therefore would not affect the shape of the length-tension diagram.

Comparison with previous work

The length-tension relation described in the present paper for semitendinosus muscle fibres is similar, at the level of the plateau region at least, to that obtained by Gordon *et al.* (1966) using fibres from the same muscle. The slightly lower tensions found in our experiments at sarcomere lengths above 2.20 μm are likely to be related to differences in experimental procedures. Selection for high homogeneity in sarcomere spacing and conspicuous reduction in length of fibre segments are, in fact, expected to reduce sarcomere length variability within the length-clamped segment and, in turn, the extension of flat region of the length-tension diagram (Gordon *et al.* 1966, p. 190).

The discrepancies between the results described here and those reported by ter Keurs *et al.* (1978) are clearly due to differences in experimental conditions. Their length-tension diagram was drawn by plotting the extreme steady tension reached during a long, fixed-end tetanus *vs.* the actual sarcomere length at that time. This tension includes the creep which follows the initial rapid rise at sarcomere lengths greater than about 2.20 μm . Inspection of the records (Figs 5, 6 and 8 in the paper of ter Keurs *et al.* (1978) shows, that the sarcomere length did not settle to a definite constant value even at times when tetanic tension had already reached a steady plateau. The problem is whether the sarcomere contraction during the plateau phase of a fixed-end tetanus is truly isometric. Actually, the experiments reported here show that even during the plateau of a fairly steady fixed-end tetanus sarcomere contraction could be non-isometric. This can be seen in records in Fig. 3B. In this

tibialis anterior muscle fibre at a resting sarcomere length of $2.23 \mu\text{m}$, the sarcomeres illuminated by the laser beam were being stretched during the tetanus plateau by other sarcomeres along the fibre. The lengthening velocity was only $0.016 \mu\text{m/s}$ per half-sarcomere. This was sufficient to produce a 5% enhancement of steady tetanic tension with respect to that produced under length-clamp conditions, thus extending significantly the plateau region of the length-tension diagram, just as shown by ter Keurs *et al.* (1978).

The sarcomere length-tension diagram described by Altringham & Bottinelli (1985) for tibialis anterior muscle fibres differs from that reported in the present work, in showing the linear decline in tension from a plateau end at $2.20 \mu\text{m}$ to zero at a sarcomere length of $3.65 \mu\text{m}$ instead of $3.53 \mu\text{m}$. It is possible that the greater vertical scatter of length-tension data points in the experiments of Altringham & Bottinelli (1985) is responsible for this discrepancy.

The sarcomere length-tension relation described by Edman & Reggiani (1984, 1987) differs from that reported here in showing a definite peak at about $1.98 \mu\text{m}$ and slight curvatures around 2.40 and $3.30 \mu\text{m}$. The absolute differences are small, but enough to change significantly the shape of the sarcomere length-tension relation. The reason for these discrepancies remains unclear. It cannot be excluded that the discrepancies are related to differences in the experimental procedures, such as the 6-fold reduction in the length of the segments we used and their selection for homogeneity of sarcomere spacing. However, the observation that the shapes of the length-tension relations for 70 – $80 \mu\text{m}$ long segments (this paper) or of much longer segments (6 – 8 mm , Gordon *et al.* 1966) are about the same does not support this possibility.

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