Journal of Physiology (1988), **407**, *pp.* 215–229
With 8 text-figures
Printed in Great Britain

STIFFNESS CHANGES DURING ENHANCEMENT AND DEFICIT OF ISOMETRIC FORCE BY SLOW LENGTH CHANGES IN FROG SKELETAL MUSCLE FIBRES

BY H. SUGI AND T. TSUCHIYA

From the Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173, Japan

(*Received 23 June 1987*)

SUMMARY

1. The mechanism of the enhancement and the deficit of isometric force by slow length changes in frog fast muscle fibres was studied by recording muscle fibre stiffness changes as measured with sinusoidal vibrations (0·5–1·9 kHz, peak-to-peak amplitude 0·1 % of slack length, L_0).

2. When a tetanized fibre was slowly stretched by 5–9 % from sarcomere lengths 2·4–2·6 μm, the force rose to a peak during the stretch and then decreased towards a steady level higher than that during the ordinary isometric tetanus at the same sarcomere length.

3. The stiffness of the fibre first rose abruptly in response to stretch and then started to decrease linearly while the stretch went on; after the completion of stretch the stiffness decreased towards a steady value which was equal to that during the isometric tetanus at the same sarcomere length, indicating that the enhancement of isometric force is associated with decreased stiffness.

4. If a tetanized fibre was slowly released by 4–12 % from sarcomere lengths 2·55–2·7 μm, the steady force attained after the completion of release was lower than that during an isometric tetanus at the same sarcomere length.

5. The stiffness of the fibre changed in parallel with the force both during and after the applied release.

6. Recordings of the segmental length changes along the fibre with a high-speed video system (200 frames/s) indicated that all segments lengthened in response to the applied stretch.

7. The segmental length changes in response to the applied release were markedly non-uniform; the length of a segment located at the centre of the fibre did not change appreciably both during and after the release.

8. These results are discussed in terms of cross-bridge performance and structure of the myofilament lattice.

INTRODUCTION

It has long been known that, when an active muscle is slowly stretched, the steady isometric force attained after the completion of stretch is higher than the normal

Authors' names are in alphabetical order.

isometric force at the same muscle length despite the decrease in the amount of overlap between the myofilaments; conversely, when an active muscle is slowly released, the steady force after the completion of release is lower than the normal isometric force at the same muscle length (Abbott & Aubert, 1952; Délèze, 1961; Marechal & Plaghki, 1979). These phenomena suggest that the magnitude of isometric force at a given sarcomere length may vary according to the past history of muscle, and are generally considered to be effective in preventing dispersion of sarcomere length along the muscle length, i.e. the longitudinal stability of sarcomere length.

Though more detailed studies on the effect of slow length changes on isometric force have been made using single muscle fibres (Edman, Elzinga & Noble, 1978; Julian & Morgan, 1979), the mechanism underlying the enhancement and deficit of isometric force remains to be elucidated. In the present experiments, we measured the force and the stiffness of tetanized muscle fibres in response to slow length changes to give information about the behaviour of the cross-bridges during the enhancement and deficit of isometric force. The results obtained were correlated with length changes of fibre segments recorded with a high-speed video system. Preliminary accounts of this work have already appeared (Tsuchiya & Sugi, 1986, 1988).

METHODS

Preparation

All experiments were performed with single fast muscle fibres (diameter 80–120 μm) isolated from the tibialis anterior muscles of the frog (*Rana japonica*). The Ringer solution had the following composition (mM): NaCl, 115; KCl, 2·0; $CaCl_2$, 1·8; Na_2HPO_4–NaH_2PO_4, 2·0; pH 7·0. A pair of stainless-steel wire connectors (0·1 mm in diameter and 2–3 mm in length; Civan & Podolsky, 1966; Sugi, 1972) or small clip of aluminium foil (Ford, Huxley & Simmons, 1977) were tied to both tendons. The connectors were attached close to the fibre insertions, so that the length of tendinous material between the connectors was less than 0·1 mm.

Stimulation chamber

The single-fibre preparation (0·4–0·6 cm in slack length L_0) was mounted horizontally in an acrylic plastic chamber (3 ml) by hooking the connectors to a servo-motor and a force transducer respectively. The chamber contained a multielectrode assembly consisting of eight platinum wire electrodes connected as alternate anodes and cathodes. In most experiments, cooled Ringer solution (2–3 °C) was constantly circulated through the chamber at a rate of 2 ml/min with a peristaltic pump, the temperature of Ringer solution being controlled by a thermo-electric device (Coolnix, Yamato Kagaku Co.) with an accuracy of $\pm$0·1 °C.

Servo-motor

Both slow length changes at constant velocities and small sinusoidal length perturbations were applied to the preparation with a servo-motor (G-100PD, General Scanning Inc.) with a position feed-back circuit, the position of the motor arm 0·5 cm in length being sensed by a differential transformer incorporated in the servo-motor. The compliance of the motor arm at the point to which the preparation was connected was about 0·003 mm/N when the feed-back was operating.

Force transducer

The force in the preparation was measured by a force transducer (AE-801, Aksjeselskapet) with a compliance of 0·1 mm/N and a resonant frequency of about 5 kHz. The force changes were recorded with a digital oscilloscope (type 4094, Nicolet) together with the displacement signal from the servo-motor.

Stiffness measurement

The stiffness of muscle fibres was measured by applying sinusoidal vibrations (0·5–1·9 kHz, peak-to-peak amplitude 0·1 % of L_0) with the servo-motor and recording the resulting force changes. The sinusoidal voltages were generated with a waveform generator (model 164, Wavetek Inc.). The fibres were first tetanized isometrically with supramaximal 1 ms current pulses at 17–22 Hz, and when the isometric force reached a maximum steady level they were subjected to slow length changes lasting for 0·3–4 s. The fibres were tetanized for 6–7 s, so that the force changes could be examined over several seconds after the completion of the length changes. Records were taken in pairs; one with and one without sinusoidal vibrations. Electronic subtraction of the records was made in the digital oscilloscope to provide a force record containing only the vibration component. The peak-to-peak forces of more than ten cycles of the vibration were averaged for one data point of stiffness. The amplitude of the force vibrations thus obtained gave a good measure of the muscle fibre stiffness. The experiments were made within the range of fibre lengths where the resting force was negligible. Essentially similar results were obtained in the range of vibration frequencies used.

Sarcomere length measurement

The sarcomere length of the fibres at rest was measured by diffraction of He–Ne laser light (light beam diameter, 1 mm). The diffraction pattern was displayed on a screen 15 cm distant from the fibres. The sarcomere spacings were fairly uniform along the fibre length except for the end regions. The sarcomere length changes in response to various amounts of length changes were estimated by measuring the sarcomere lengths at the middle of resting fibres at lengths before and after the applied length changes.

Recording of length changes of fibre segments

To examine non-uniform length changes along the fibre length during the applied length changes, fine carbon particles were firmly attached to the fibre surface to divide the fibre into several elementary segments of nearly equal lengths. The image of the whole fibre was observed under a Nikon dissecting microscope, and recorded with a high-speed video system (HSV-200, Nac Inc.) at 200 frames/s (see Fig. 1). The videotape records were analysed with a video microscaler (IV-500, For-A. Co.).

RESULTS

Muscle stiffness changes during the enhancement of isometric force by slow stretches

Figure 1 shows typical experimental records of the stiffness measurement in single frog fast muscle fibres when they are first tetanized isometrically and then stretched by 5–9 % of the initial length with velocities of 0·02–0·2 L_0/s. The difference between the force record with sinusoidal vibration and that without sinusoidal vibration was obtained by digital subtraction. The difference records obtained were symmetrical with respect to the force axis, indicating that the force changes in response to applied stretches were not affected by superimposed vibrations. In agreement with the work of Edman *et al.* (1978), the force enhancement after stretch, i.e. a maintained force above control after the completion of stretch, was more marked at long sarcomere lengths than at short sarcomere lengths. To produce the most pronounced force enhancement by stretch with negligible resting force development, the initial sarcomere length of the fibres was chosen to be 2·4–2·6 μm. The fibres were tetanized for 6–7 s, so that the force changes after stretch could be recorded over several seconds.

Figures 2 and 3 illustrate typical results. In the experiment shown in Fig. 2, the fibre was stretched at 1·2 s after the beginning of tetanic stimulation from sarcomere

length 2·6 to 2·75 μm with three different velocities. The force rose to a peak during stretch, and then started to decay exponentially at the completion of stretch. The force recorded during stretch was dependent on the velocity of stretch; the faster the stretch velocity, the higher was the recorded force (Edman *et al.* 1978). In spite of the difference in the peak forces attained by stretches of different velocities, the forces after the completion of stretch were observed to approach towards the same steady

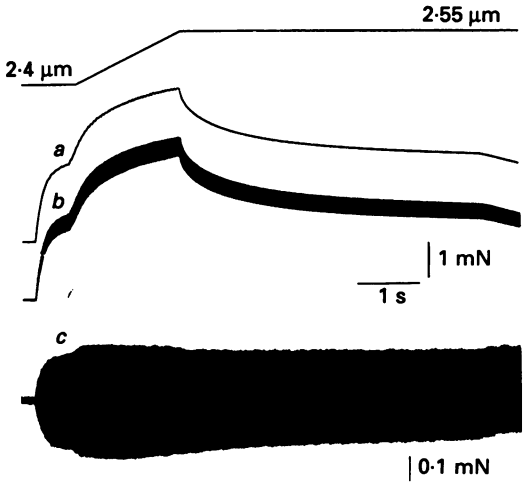

Fig. 1. Stiffness measurement of a single frog fast muscle fibre. The fibre was first tetanized isometrically at 2·4 μm sarcomere length, and then stretched to 2·55 μm sarcomere length in 2 s (trace *a*). This was repeated with superimposed sinusoidal vibration (0·1 % of L_0, 0·9 kHz, trace *b*). The difference between the two records was obtained by digital subtraction, amplified, and plotted (trace *c*) as a measure of stiffness.

level, which was definitely higher than the ordinary isometric force at 2·75 and 2·6 μm sarcomere lengths. This indicates that the degree of the enhancement of isometric force after stretch is independent of the stretch velocities used. With stretches of 5–9 % starting at 2·4–2·6 μm sarcomere lengths, the steady isometric force attained after stretch was about 0–10 % higher than the ordinary isometric force before stretch, and about 20–30 % higher than that at the stretched sarcomere length.

The stiffness changes in response to the applied stretches are shown in the lower part of Fig. 2. During the initial isometric tetanus preceding stretch, both the force and the stiffness increased nearly in parallel with each other to reach their respective steady levels. In response to the subsequent stretch, the stiffness first increased abruptly and then started to decrease nearly linearly with time while the stretch was still going on; after the completion of stretch, the stiffness decreased exponentially towards a steady value. The initial stiffness peak was reached at the time when the amount of stretch exceeded 10–20 mm/half-sarcomere. A most striking feature brought about by the stretch experiments was that, irrespective of the stretch velocities used, the steady stiffness value after stretch was found to be equal to the steady value attained during the ordinary isometric tetanus at sarcomere length

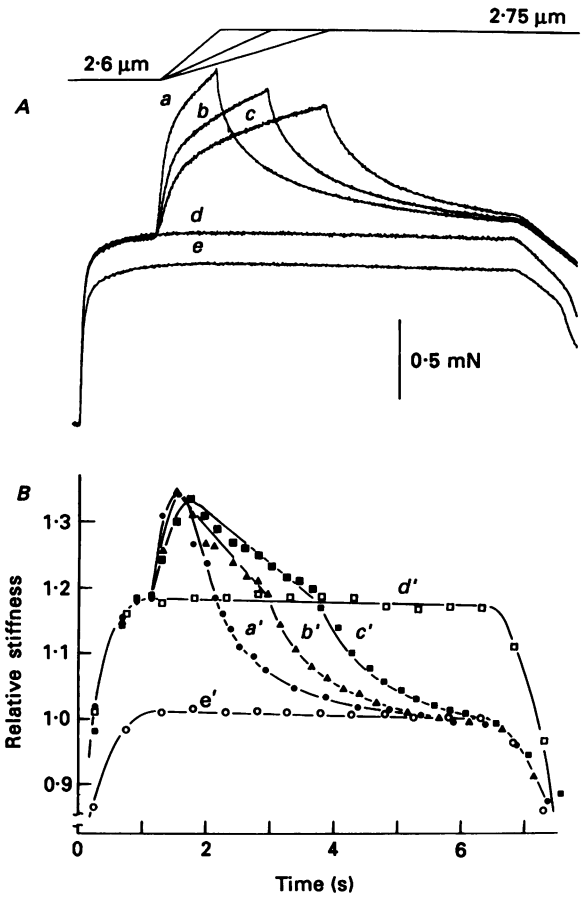

Fig. 2. Force and stiffness changes of a tetanized muscle fibre in response to slow stretches of three different velocities. *A*, force records. The fibre was first tetanized isometrically, and then stretched from 2·6 μm sarcomere length to 2·75 μmin 0·8 s (trace *a*), 1·6 s (trace *b*) and 2·4 s (trace *c*). Traces *d* and *e* are the force records of isometric tetanus at 2·6 and 2·75 μm sarcomere lengths respectively. *B*, stiffness changes. Lines *a'* (●), *b'* (▲), *c'* (■), *d'* (□) and *e'* (○) are the stiffness changes corresponding to the force records *a*, *b*, *c*, *d* and *e* respectively.

2·75 μm, i.e. the sarcomere length after the completion of stretch. Thus, the force and the stiffness changes exhibited completely different time courses in response to the applied stretch; though the steady isometric force after stretch was markedly enhanced despite the resulting decrease in the amount of myofilament overlap, the steady stiffness value after stretch was below the value immediately before stretch, reflecting the decreased myofilament overlap.

In the experiment shown on Fig. 3, the isometrically tetanized fibre was stretched from sarcomere length 2·4 to 2·5 μm and from 2·4 to 2·6 μm with the same velocity. The steady isometric force attained after the completion of stretch was higher when the fibre was stretched to 2·6 μm sarcomere length than when it was stretched to 2·5 μm sarcomere length, indicating that the degree of the force enhancement

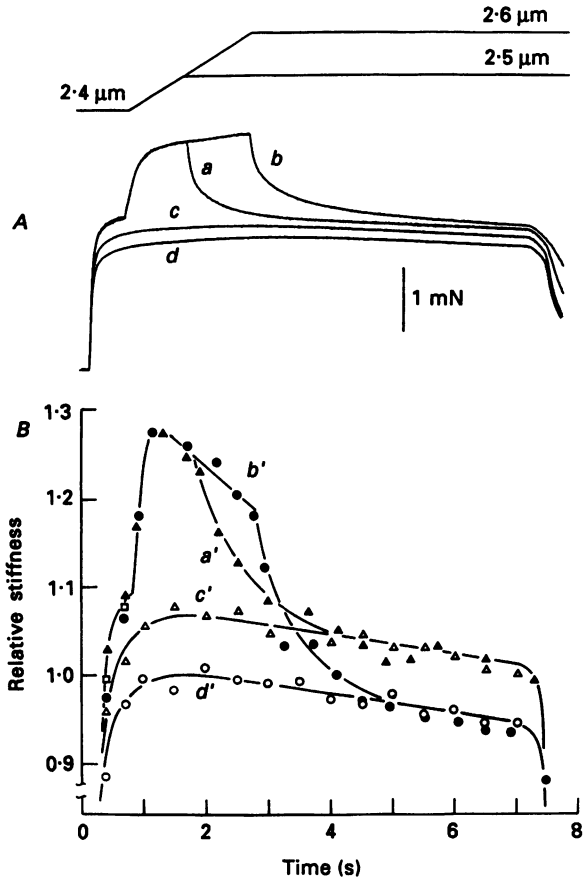

Fig. 3. Force and stiffness changes of a tetanized muscle fibre in response to slow stretches of two different amounts. *A*, force records. The fibre was first tetanized isometrically, and then stretched from sarcomere length 2·4 to 2·5 μm in 1 s (trace a) and from sarcomere length 2·4 to 2·6 μm in 2 s (trace *b*). Traces *c* and *d* are the force records of isometric tetanus at 2·5 and 2·6 μm sarcomere lengths respectively. *B*, stiffness changes. Lines *a'* (▲), *b'* (●), *c'* (△) and *d'* (○) are the stiffness changes corresponding to the force records *a*, *b*, *c* and *d* respectively.

increases with increasing amount of stretch (Edman *et al.* 1978). On the other hand, the steady stiffness values attained after stretch were found to be equal to the values during the ordinary isometric tetanus at the stretched sarcomere lengths. Thus, an increase in the amount of stretch increased the degree of force enhancement, but decreased the steady stiffness value after stretch. Similar results were obtained with ten other preparations studied.

Muscle stiffness changes during the deficit of isometric force by slow releases

The stiffness measurement was also performed when the fibres were first tetanized isometrically and then released by 4–12% of the initial length with velocities 0·02–0·15 L_0/s by the method similar to that shown in Fig. 1. Typical results are

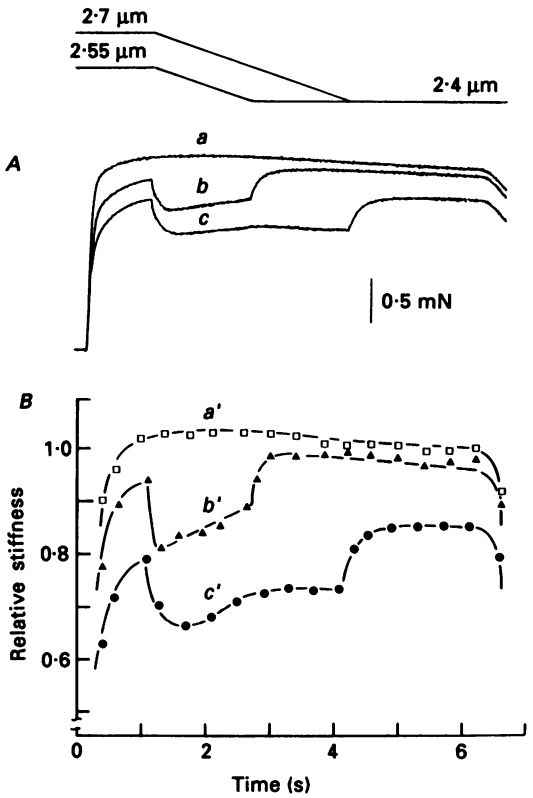

Fig. 4. Force and stiffness changes of a tetanized fibre in response to slow releases of two different amounts. *A*, force records. The fibre was first tetanized isometrically, and then released from 2·55 μm sarcomere length to 2·4 μm in 1·5 s (trace *b*) and from sarcomere length 2·7 μm to 2·4 μm in 3·0 s (trace *c*). Trace *a* is the force record of isometric tetanus at sarcomere length 2·4 μm. *B*, stiffness changes. Lines *a'* (□), *b'* (▲) and *c'* (●) are the stiffness changes corresponding to the force records *a*, *b* and *c* respectively.

shown in Figs 4 and 5. In the experiment shown in Fig. 4, the fibre was released to 2·4 μm sarcomere length from two different initial sarcomere lengths of 2·7 and 2·55 μm. The force first decreased at the beginning of release, and then exhibited a slow increase; if the duration of release was long enough, the slow force increase was followed by a slow decrease, thus giving the humped shape to the force trace (Julian & Morgan, 1979). At the completion of release, the force started to rise to a steady level which was definitely lower than that during the ordinary isometric tetanus at 2·4 μm sarcomere length. The steady isometric force attained after the completion of release was higher when the fibre was released from 2·55 μm sarcomere length than when it was released from 2·7 μm sarcomere length, indicating that the degree of the deficit of isometric force after release is dependent on the amount of release (Marechal & Plaghki, 1979). In the experiment shown in Fig. 5, the fibre was released from 2·6 μm sarcomere length to 2·4 μm with two different velocities. The steady isometric force attained after release was higher when the fibre was released faster, indicating

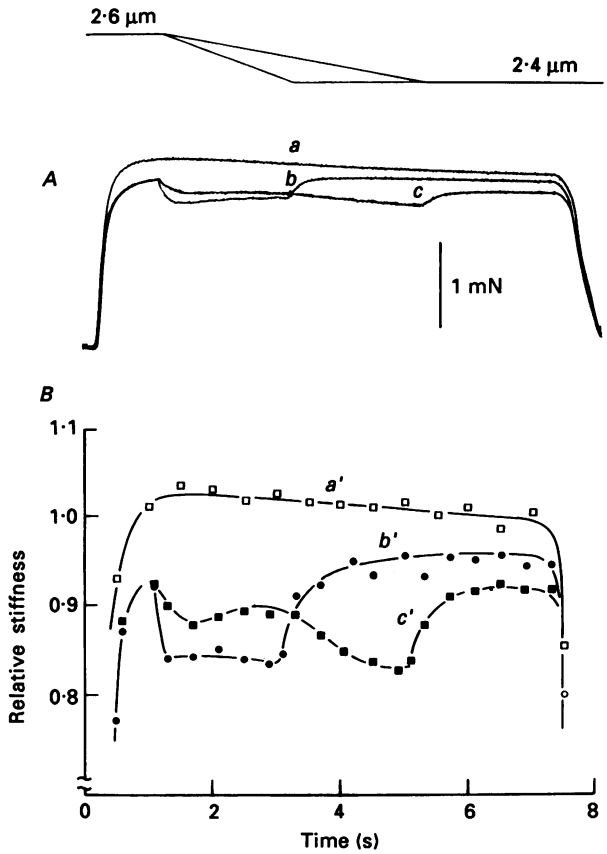

Fig. 5. Force and stiffness changes of a tetanized fibre in response to slow releases of two different velocities. *A*, force records. The fibre was first tetanized isometrically, and then released from 2·6 μm sarcomere length to 2·4 μm in 2·0 s (trace *b*) and in 4·0 s (trace *c*). Trace *a* is the force record at sarcomere length 2·4 μm. *B*, stiffness changes. Lines *a'* (□), *b'* (●) and *c'* (■) are the stiffness changes corresponding to the force records *a*, *b* and *c* respectively.

that the deficit of isometric force is also dependent on the release velocities (Marechal & Plaghki, 1979). As shown in Figs 4 and 5, the muscle fibre stiffness was found to change in parallel with the force both during and after the applied release. Similar results were obtained with fifteen other preparations used.

Length changes of fibre segments

Figures 6 and 7 are typical records of length changes of the elementary segments of the fibre divided by the carbon markers attached to the fibre surface. As shown in Fig. 6*A*, the fibre segments were kept nearly isometric during the ordinary isometric tetanus. In ten other fibres studied, no marked non-uniformity in the segmental length changes was observed during the ordinary isometric tetanus at sarcomere lengths from 2·4 to 2·7 μm. Figure 6*B*, shows the length changes of the

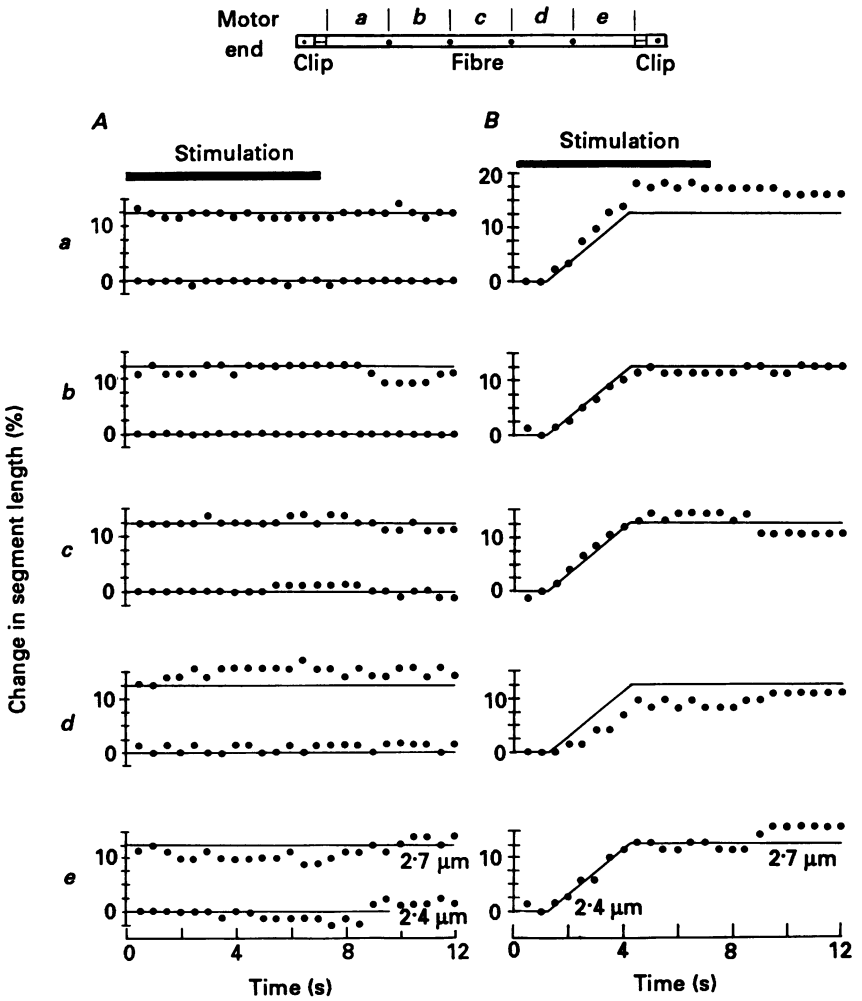

Fig. 6. Segmental length changes of a tetanized muscle fibre recorded by a high-speed video system. *A*, isometric tetanus at sarcomere length 2·4 μm and 2·7 μ. *B*, isometric tetanus followed by stretch from 2·4 μm sarcomere length to 2·7 μm in 3·0 s. The segmental length changes are expressed as a percentage of the initial length (see Fig. 8). The fibre was divided into five elementary segments of nearly equal lengths by carbon markers (inset).

fibre segments when the isometrically tetanized fibre was slowly stretched from 2·4 μm sarcomere length to 2·7 μm. All the segments were observed to lengthen in response to the applied stretch, though the amount of lengthening was not completely uniform among the segments.

On the other hand, a marked non-uniformity in the segmental length changes was seen during slow releases. In the experiment shown in Figs 7*A*, and 8, the isometrically tetanized fibre was slowly released from 2·7 μm sarcomere length to 2·4 μm. No marked length changes were observed in the segment located at the

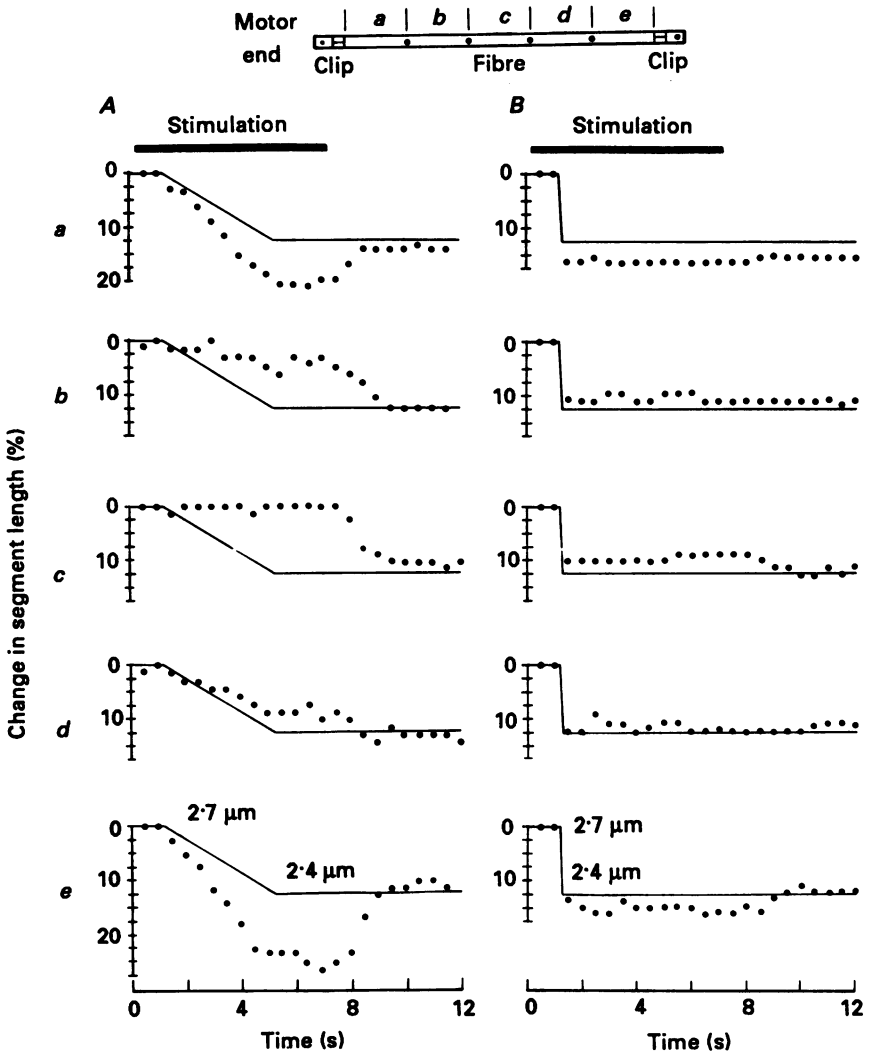

Fig. 7. Segmental length changes of a tetanized muscle fibre in response to slow and quick releases. The isometrically tetanized fibre was released from 2·7 μm sarcomere length to 2·4 μm in 4·0 s in *A* and in 0·1 s in *B*. The fibre was divided into five elementary segments by carbon markers (Fig. 8).

centre of the fibre, while the other segments shortened to variable extents. The marked non-uniformity in the segmental length tended to persist after the completion of release. If a quicker release at 1·2 L_0/s from 2·7 μm sarcomere length to 2·4 μm was applied to the same fibre during an isometric tetanus, the fibre segments shortened fairly uniformly (Fig. 7*B*), indicating that the non-uniform segmental response such as shown in Fig. 7*A* is characteristic of slow release. Similar results were obtained on ten other fibres with respect to both stretches and releases.

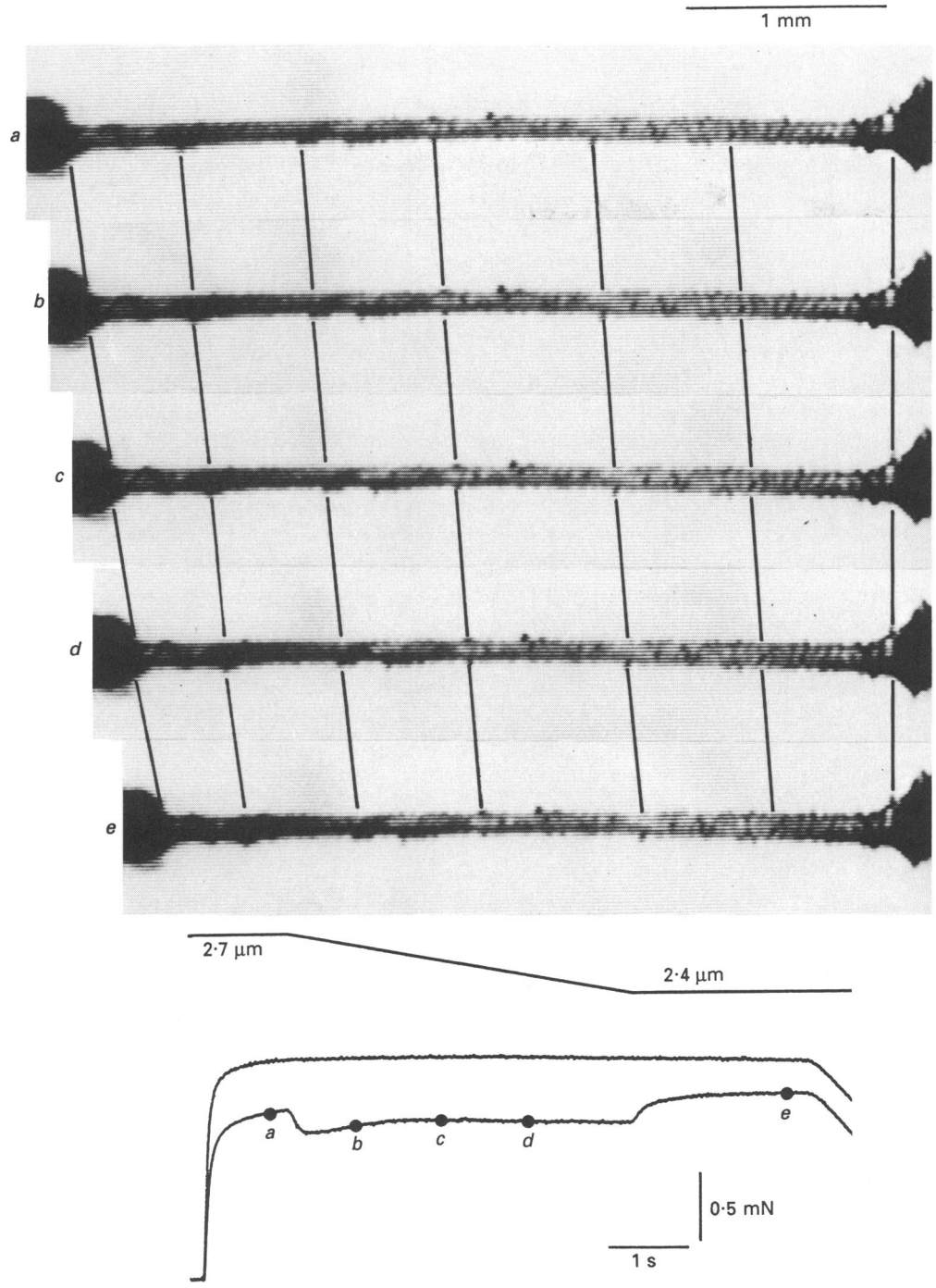

Fig. 8. Selected frames from video record of a tetanized muscle fibre before (*a*), during (*b–d*) and after (*e*) the applied slow release from 2·7 μm sarcomere length to 2·4 μm in 4·0 s. Note non-uniform segmental length changes. In the lower part, the force record of isometric tetanus at 2·4 μm sarcomere length and that during the release from 2·7 μm sarcomere length to 2·4 μm are also shown. The points *a–e* on the force record indicate times when the frames *a–e* were taken.

DISCUSSION

Differences between the force enhancement by stretch and the force deficit by release

The present experiments have revealed a number of interesting features concerning the changes in muscle fibre stiffness in response to slow length changes applied during isometric tetani. Although the enhancement of isometric force by stretch and its deficit by release are symmetrical phenomena in appearance, the stiffness changes indicate that their mechanisms may be completely different from each other. In response to stretches of the same amount, the force and the stiffness changes exhibited different time courses, and approached their respective steady values irrespective of the stretch velocities (Fig. 2). The finding that the steady stiffness value after stretch is determined by the amount of myofilament overlap (Figs 2 and 3) indicates that slow stretches show a hysteresis-like effect on the steady isometric force but not on the steady stiffness. On the other hand, the force and the stiffness changes in response to releases took place in parallel with each other, and their steady values attained after release were influenced by both the distance and the velocity of release (Figs 4 and 5); namely, slow releases exerted a hysteresis-like effect on both the force and the stiffness. Recordings of the segmental length changes of the fibres during the applied length changes have demonstrated a marked non-uniformity of the segmental length changes in response to slow releases (Figs 7 and 8), while the non-uniformity of the segmental length changes in response to slow stretches were not so marked (Fig. 6). In agreement with this, Hill (1977) has reported that sarcomere lengths along the fibre remain uniform during a tetanus plus stretch. All these results seem to indicate that the underlying mechanism for the force enhancement by stretch is completely different from that for the force deficit by release.

Possible mechanism of the force enhancement by stretch

It has long been known that, during a slow stretch applied to an active muscle, its rate of energy output can be reduced close to zero (Abbott & Aubert, 1951; Hill & Howarth, 1959). In accordance with this finding, Curtin & Davies (1973) showed that the breakdown of ATP is markedly reduced during stretch of active muscles despite a high force produced by stretch. These results can be explained qualitatively by assuming that the cross-bridges are forcibly detached from the thin filaments by stretch without breaking down ATP (Huxley, 1957). On this basis, the initial abrupt increase of the stiffness in response to stretch (Fig. 3) may reflect synchronized distortion of attached cross-bridges. The stiffness reaches a maximum when the fibre is stretched by 10–20 nm/half-sarcomere, suggesting that the cross-bridges can remain attached to the thin filaments over a distance of 10–20 nm when they are forcibly displaced in the direction opposite to that of shortening, and that the stiffness of the distorted cross-bridge increases. The subsequent linear decrease of the stiffness during stretch (Figs 2 and 3) might result if the attached cross-bridges displaced beyond 10–20 nm/half-sarcomere start to slip past the thin filaments, and the number of cross-bridges, which can attach to, and then slip past, the thin filaments, decreases with decreasing amount of myofilament overlap.

At the completion of stretch, the stiffness is as large as (Fig. 2), or higher than (Fig.

3), the value immediately before stretch, depending on the amount of stretch. This implies that after the completion of stretch a certain period of time is necessary for the cross-bridges to restore their configuration to that during ordinary isometric contraction.

Since the stiffness is generally believed to be a measure of the number of the attached cross-bridges (Huxley & Simmons, 1973; Julian & Sollins, 1975), the steady stiffness values after stretch (Figs 2 and 3) strongly suggest that the number of the cross-bridges generating isometric force is uniquely determined by the amount of myofilament overlap, not only during the ordinary isometric tetanus (Gordon, Huxley & Julian, 1966) but also during the isometric tetanus preceded by stretch. This implies that the number of the force-generating cross-bridges is smaller after stretch than before stretch, though the isometric force is enhanced after stretch. The stiffness changes observed in the present study differ from the results of Julian & Morgan (1979) who reported that the stiffness stayed substantially constant both during and after a slow stretch in single frog fibres. Their experiments may have given different results partly because the stretches they used were much faster than those in the present study and produced a much less pronounced enhancement of isometric force, and partly because the force changes in response to stretch were obviously affected by superimposed sinusoidal vibrations (Fig. 7 in their paper). Nevertheless, their data (their Fig. 8) suggest a tendency for the stiffness to change in response to stretch in a manner similar to that found in the present study.

Edman *et al.* (1978) have found that the degree of the force enhancement after stretch increases with decreasing amount of myofilament overlap. In the present study, the larger enhanced force was associated with lower stiffness (Fig. 3). Though these results may be accounted for by assuming that each cross-bridge generates more and more force as the amount of myofilament overlap decreases by stretch, it seems difficult to explain how the cross-bridges can remember their past history as they repeat attachment to, and detachment from, the thin filaments.

It has been shown that when the load on the fibre is suddenly reduced after stretch or during isotonic lengthening the fibre shortens with an enhanced velocity (Edman *et al.*, 1978; Sugi & Tsuchiya, 1981). Edman *et al.* (1978) explained the enhanced mechanical performance of the stretched fibre as being due to the recruitment of a spring-like force-bearing system in parallel with the cross-bridges. This idea seems, however, to be inconsistent with the present stiffness data, since the recruitment of such an elastic system should result in an increase of the stiffness comparable to the degree of the force enhancement.

As described in detail in the following paper (Amemiya, Iwamoto, Kobayashi, Sugi, Tanaka & Wakabayashi, 1988), recent time-resolved X-ray diffraction studies on the effect of slow length changes on tetanized frog skeletal muscle have presented evidence that a slow stretch produces disordering of the myofilament lattice in such a way that the thin filaments are displaced from the trigonal position in the thick filament lattice (see Fig. 5 of Amemiya *et al.* 1988) without changing their mean position; this kind of disorder would result in an increase in the overall electrostatic repulsion forces between the filaments, and this would show up as the enhanced isometric force after stretch when coupled with constant-volume behaviour of the myofilament lattice. Thus, the stretch-induced disordering of the myofilament lattice

may serve as a force-bearing system functionally in parallel with the cross-bridges. In the constant-volume myofilament lattice, sinusoidal vibrations of the fibre length (peak-to-peak amplitude 0·1% of L_0) would produce vibrational changes of the distance between the thick and thin filaments of about $\pm 0{\cdot}001$ nm, and the resulting fluctuation of the interfilament repulsion forces would produce force changes too small to be sensed by the force transducer.

Thus, the idea that the stretch-induced disordering of the myofilament lattice is responsible for the enhanced isometric force after stretch can qualitatively explain the reason why the marked enhancement of isometric force is attained with decreased stiffness (Figs 2 and 3). Much more experimental work is needed to prove that this is actually the case.

Possible mechanism of the force deficit by release

A marked non-uniformity in the segmental length was developed in a tetanized fibre by slow releases, the length of the central segment being kept nearly constant both during and after the applied release (Figs 7A and 8). Using a spot-follower apparatus, Julian & Morgan (1979) have made a similar observation. In spite of the above highly non-uniform segmental responses, the force and the stiffness changes in response to releases were parallel to each other (Figs 4 and 5). In the fibre segments shortening during release, the attached cross-bridges would be put into a displaced configuration, so that the force exerted by each cross-bridge would decrease compared to that during the ordinary isometric tetanus. Then, the force in the segments which remain isometric during release should also decrease simply because the fibre segments are connected in series. As the degree of shortening at the completion of release differs markedly from segment to segment, a marked redistribution of the segment lengths along the tetanized fibre may be expected to take place after the completion of release. Contrary to this expectation, no marked segmental length changes were observed after the completion of release; all the segments maintained their lengths fairly well at the completion of release until the cessation of stimulation (Figs 7A and 8). The absence of distinct segmental length redistribution after a slow release suggests that despite the markedly non-uniform segmental length changes developed during release the degree of reduction of force-generating capacity after the completion of release is fairly uniform in every segment. On this basis, the parallel force and stiffness changes in response to a slow release (Figs 4 and 5) imply that in all the segments along the fibre the force-generating capacity, i.e. the number of the cross-bridges available for isometric force generation, is gradually reduced as the force in each segment is slowly decreased during the applied release, irrespective of whether the segment is shortening or remains isometric. At present, we have no definite idea about the mechanism of the above 'deactivation' of the contractile system induced by slow releases.

We wish to thank Mr T. Takei and Mrs Y. Oshimi for their excellent technical assistance.

REFERENCES

ABBOTT, B. C. & AUBERT, X. M. (1951). Changes of energy in a muscle during very slow stretches. *Proceedings of the Royal Society* B **139**, 104–117.

ABBOTT, B. C. & AUBERT, X. M. (1952). The force exerted by active striated muscle during and after change of length. *Journal of Physiology* **117**, 77–86.

AMEMIYA, Y., IWAMOTO, H., KOBAYASHI, T., SUGI, H., TANAKA, H. & WAKABAYASHI, K. (1988). Time-resolved X-ray diffraction studies on the effect of slow length changes on tetanized frog skeletal muscle. *Journal of Physiology* **407**, 231–241.

CIVAN, M. M. & PODOLSKY, R. J. (1966). Contraction kinetics of striated muscle fibres following quick changes in load. *Journal of Physiology* **184**, 511–534.

CURTIN, N. A. & DAVIES, R. E. (1973). Chemical and mechanical changes during stretching of activated frog skeletal muscle. *Cold Spring Harbor Symposia on Quantitative Biology* **37**, 619–626.

DÉLÈZE, J. B. (1961). The mechanical properties of the semitendinosus muscle at lengths greater than its length in the body. *Journal of Physiology* **158**, 154–164.

EDMAN, K. A. P., ELZINGA, G. & NOBLE, M. I. M. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *Journal of Physiology* **281**, 139–155.

FORD, L. E., HUXLEY, A. F. & SIMMONS, R. M. (1977). Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *Journal of Physiology* **269**, 441–515.

GORDON, A. M., HUXLEY, A. F. & JULIAN, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *Journal of Physiology* **184**, 170–192.

HILL, A. V. & HOWARTH, J. V. (1959). The reversal of chemical reactions in contracting muscle during an applied stretch. *Proceedings of the Royal Society* B **151**, 169–193.

HILL, L. (1977). A-band length, striation spacing and tension change on stretch of active muscle. *Journal of Physiology* **266**, 677–685.

HUXLEY, A. F. (1957). Muscle structure and theories of contraction. *Progress in Biophysics and Biophysical Chemistry* **7**, 255–318.

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

JULIAN, F. J. & MORGAN, D. L. (1979). The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. *Journal of Physiology* **293**, 379–392.

JULIAN, F. J. & SOLLINS, M. R. (1975). Variation of muscle stiffness with force at increasing speeds of shortening. *Journal of General Physiology* **66**, 287–302.

MARECHAL, G. & PLAGHKI, L. (1979). The deficit of the isometric tetanic tension redeveloped after a release of frog muscle at a constant velocity. *Journal of General Physiology* **73**, 453–467.

SUGI, H. (1972). Tension changes during and after stretch in frog muscle fibres. *Journal of Physiology* **225**, 237–253.

SUGI, H. & TSUCHIYA, T. (1981). Enhancement of mechanical performance in frog muscle fibres after quick increases in load. *Journal of Physiology* **319**, 239–252.

TSUCHIYA, T. & SUGI, H. (1986). Variation of muscle fibre stiffness during and after slow length changes in tetanized frog skeletal muscle fibres. *Journal of Muscle Research and Cell Motility* **7**, 276.

TSUCHIYA, T. & SUGI, H. (1988). Muscle stiffness changes during enhancement and deficit of isometric force in response to slow length changes. In *Molecular Mechanism of Muscle Contraction*, ed. SUGI, H. & POLLACK, G. H., pp. 503–511. New York: Plenum Press.