EFFECT OF ACTIVE PRE-SHORTENING ON ISOMETRIC AND ISOTONIC PERFORMANCE OF SINGLE FROG MUSCLE FIBRES

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SUMMARY

1. We studied the effects of shortening history on isometric force and isotonic velocity in single intact frog fibres. Fibres were isometrically tetanized. When force reached a plateau, shortening was imposed, after which the fibre was held isometric again. Isometric force after shortening could then be compared with controls in which no shortening had taken place.

2. Sarcomere length was measured simultaneously with two independent methods: a laser-diffraction method and a segment-length method that detects the distance between two markers attached to the surface of the fibre, about 800 μm apart.

3. The fibre was mounted between two servomotors. One was used to impose the load clamp while the other cancelled the translation that occurred during this load clamp. Thus, translation of the segment under investigation could be minimized.

4. Initial experiments were performed at the fibre level. We found that active pre-shortening reduced isometric force considerably, thereby confirming earlier work of others. Force reductions as large as 70% were observed.

5. Under conditions in which there were large effects of shortening at the fibre level, we measured sarcomere length changes in the central region of the fibre. These sarcomeres shortened much less than the fibre's average. In fact, when the load was high, these sarcomeres lengthened while the fibre as a whole shortened. Thus, while the fibre-length signal implied that sarcomeres might have shortened to some intermediate length, in reality some sarcomeres were much longer, others much shorter.

6. Experiments performed at the sarcomere level revealed that isometric force was unaffected by previous sarcomere shortening provided the shortening occurred against either a low load or over a short distance. However, if the work done during shortening was high, force after previous shortening was less than if sarcomeres had remained at the final length throughout contraction. The correlation between the force deficit and the work done during shortening was statistically significant ($P = 0.0001$).

7. Interrupting the tetanus for 0.5–3.0 s did not reverse the effects of shortening on

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isometric force; at least 5–10 min of rest were required before force recovered completely.

8. Sarcomeres accelerated during the period of shortening under constant load, indicating that the sarcomeres became progressively stronger. However, the acceleration was less than that predicted from the force–velocity relation applicable at each of the sarcomere lengths transversed during shortening.

9. Velocity of shortening appeared to be much more sensitive to previous shortening than isometric force.

10. Results obtained with the diffraction method were the same as those obtained with the segment method. Therefore, it is unlikely that heterogeneous behaviour of sarcomeres within the sampled region underlies any of the observed effects.

11. As for the mechanism, the increase of the proton and inorganic phosphate concentration that occurs during shortening might underlie the deficit in contractile performance. However, our finding that velocity is more sensitive to previous shortening than isometric force is not expected from this hypothesis, and remains to be explained.

<h2 style="text-align:center">INTRODUCTION</h2>

The cross-bridge model (A. F. Huxley, 1957; H. E. Huxley, 1969) is widely believed to represent the mechanism of muscle contraction, despite numerous experimental results that do not concur with its predictions (Pollack, 1983). We decided to follow up one of these apparent conflicts: the effect of shortening history on isometric force and isotonic shortening.

According to the cross-bridge model, maximal isometric force is determined by the instantaneous degree of overlap, not by the degree of overlap when contraction started. However, reducing overlap by stretching tetanized fibres results in more force than predicted; force is enhanced. This phenomenon is now well documented (Abbott & Aubert, 1952; Edman, Elzinga & Noble, 1978, 1982, 1984; Sugi & Tsuchiya, 1981). On the other hand, the effect of increasing overlap by imposing shortening is less clear. Some investigators (Buchthal, Kaiser & Rosenfalk, 1951; Abbott & Aubert, 1952; Délèze, 1961; Edman, 1964; Maréchal & Plaghki, 1979) reported a substantial decrease of isometric force: reductions as large as 50–75 % of the predicted values have been measured (Buchthal *et al.* 1951). Other investigators found that the reduction in force after shortening was only a few per cent, and could be neglected (Edman, 1966; Gordon, Huxley & Julian, 1966; Edman, 1980). Based on this conflicting evidence it is not clear whether previous shortening does or does not affect isometric force. Therefore, we reinvestigated this issue.

In addition, we studied the velocity of sarcomere shortening during the load clamp. According to the cross-bridge model the velocity should increase progressively since the number of cross-bridges that generate force increases, and thus the average load per cross-bridge decreases. Again, experimental results conflict. Edman & Reggiani (1984) found a modest increase of velocity with increased overlap, while records obtained by Buchthal *et al.* (1951) and Gordon *et al.* (1966) show essentially constant velocity. Hence, we reinvestigated this issue by measuring the course of shortening velocity during the load clamp. Furthermore, we compared these

velocities with those obtained at the same sarcomere length without pre-shortening (Granzier, Burns & Pollack, 1989), thereby assessing the effect of pre-shortening on shortening velocity.

It was found that contractile performance did not just depend on the instantaneous degree of filament overlap, but also on pre-shortening. However, several characteristics of the pre-shortening effect (e.g. time required to reverse the effect on isometric force was at least 5–10 min) appear to suggest that the underlying mechanism might not be found in the contractile proteins themselves, but possibly in some long-term effect such as accumulation of metabolic products.

METHODS

General

Frogs (*Rana temporaria*; body length 4–5 cm) were cold adapted at 4 °C for 1–2 weeks prior to use. Frogs were quickly killed by decapitation. Single intact fibres were dissected from the semi-tendinosus muscle. Slack length was approximately 10 mm (mean ± S.D., 10.3 ± 0.8 mm; $n = 20$). Small holes were punched in each tendon, 200–600 μm away from the myotendinous junction. Fibres were mounted horizontally between two servomotors, one of which had a force transducer attached to its movable arm. The fibre itself sat immersed in an experimental chamber whose volume was about 600 μl. The walls of the chamber were constructed of small strips of glass. This allowed us to illuminate the same region of the fibre from two directions: vertically for laser-diffraction and horizontally for segment length detection (see Fig. 1).

Temperature control was achieved by blowing cold, pre-dried air around the chamber. The top of the chamber was covered with a thin piece of glass (after the fibre had been mounted) and cooled by an independent jet of cold air. The air flow required to maintain the temperature of the saline in the chamber at about 0 °C did not result in measurable vibration. Temperature distribution in the chamber was determined by measuring the temperature at different locations with a small thermocouple. At a mean temperature of 0 °C, the variation was less than 0.5 °C. Temperature control was achieved by using the signal from a thermocouple placed close to the centre of the fibre as a feedback control signal for the thermoelectric heat modules that cooled the air. During long-lasting experiments temperature did not fluctuate by more than ± 0.1 °C.

Fibres were kept in a physiological salt solution of the following composition (in mM): NaCl, 115.5; KCl, 2.0; CaCl$_2$, 1.8; MgSO$_4$, 1.0; Na$_2$HPO$_4$, 6.3; NaH$_2$PO$_4$, 1; glucose, 5.0. The pH was 7.1 at 2 °C. The salt solution was refreshed after each contraction.

Diffraction

To diminish the likelihood of artifact we measured sarcomere length with two fully independent methods: one based on segment length detection (segment method), the other on optical diffraction (diffraction method). In the diffraction method, sarcomere length was determined from the distance between the zeroth and first-order diffraction maxima. A region of the fibre was illuminated by a collimated, randomly polarized, He–Ne laser-light beam (CW Radiation, Inc., LLS5R). Beam diameter was 800 μm. The diffraction pattern was collected with a long working distance (5.5 mm), bright field objective (Leitz L32/0.6). A telescope (Zeiss, 464822-9902), focused on the back focal plane of the objective, projected the diffraction pattern, after compression with a cylindrical lens, onto a photodiode array (Reticon RL 256 C/17); see Fig. 1A.

Median position of the first-order intensity profile was taken as first-order position. Locating the median was accomplished as described previously for locating marker position (Granzier, Myers & Pollack, 1987). Uncertainty in the position of the median corresponded to one-sixteenth of the width of a single photodiode element. At a sarcomere length of 3.6 μm this corresponded to ± 0.5 nm/sarcomere, and at 1.6 μm to ± 0.08 nm/sarcomere.

We recorded the first-order position signal on a digital oscilloscope with a 15-bit analog-to-digital converter. The least significant bit of the A/D corresponded at a sarcomere length of 3.6 μm to 0.015 nm/sarcomere; at shorter sarcomere lengths it was less. Sarcomere length was determined
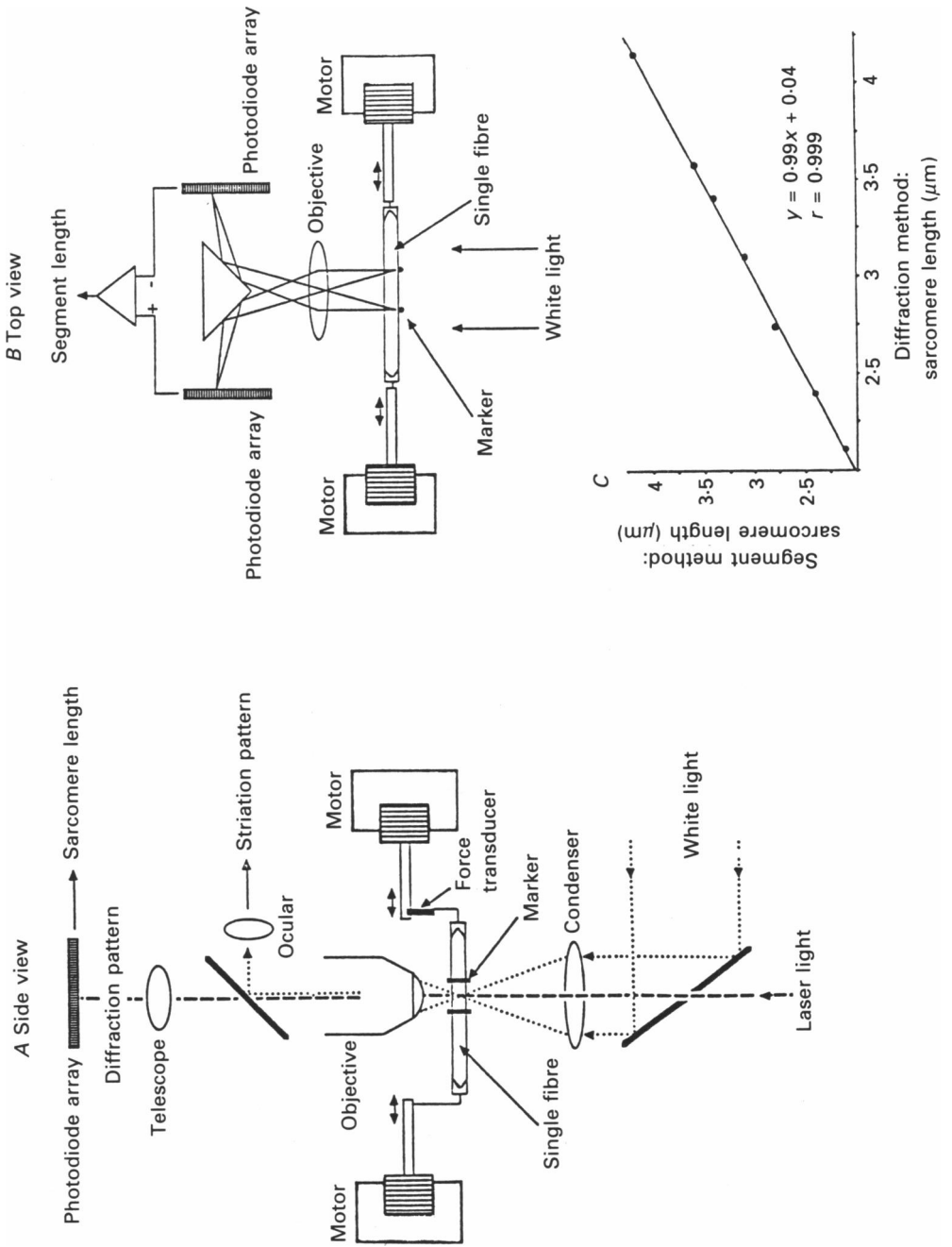
Fig. 1. For legend see facing page.

from calibration gratings mounted in the chamber at the same level as the fibre. Using the least-squares method we computed the linear equation that describes the relation between the positions of the first-order peaks on the photodiode array and the inverse of the known grating spacings. This equation was determined for each experiment. The measured first-order position obtained from the fibre was then converted to sarcomere length by using this equation.

Accuracy was checked by comparing diffraction-based sarcomere lengths with those measured with the optical microscope. The average of strings of about thirty sarcomeres within the field of laser illumination were determined several times at different depths in the fibre. At sarcomere lengths from 2·0 to 3·0 μm the two values differed typically by 0·5%. Resolution of the sarcomere length measurement was limited by noise. Root mean square sarcomere-length noise (bandwidth 0–2 kHz) measured in five fibres during tetanic contraction (sarcomere lengths range from 2·0 to 3·4 μm) amounted to 0·41 $\pm$ 0·155 nm/sarcomere ($n = 20$).

Segment length

This method detects the distance between two markers positioned along the fibre surface. In some experiments small gold leaves were used (cf. Gordon *et al.* 1966) but in most experiments we used 100–150 μm long segments from human black hair (thanks to Miss J. Wong). Markers were dipped in silicone high-vacuum grease and mounted along the side of the fibre, typically 800 μm apart. For mounting, the fibres were pre-stretched to a sarcomere length of about 2·9 μm. Care was taken to attach the hairs perpendicularly to the fibre axis. Alignment was subsequently checked during slow releases and stretches of the unstimulated fibre, and also during short tetani. If the markers did not remain perpendicular to the fibre axis during any of these manoeuvres, their position was corrected. At slack length, the marker closest to the tendon was about 2 mm from the myotendinous junction (2·3 $\pm$ 0·4 mm; range, 1·5–3·3 mm; $n = 28$).

The fibre region containing a pair of markers was illuminated with a 3 mm beam of collimated white light, obtained from a 150 W xenon light source, passed through a heat filter. The image of the two hairs was magnified by an objective lens (numerical aperture, 0·25), split approximately midway between the hairs with a prism, and directed to two identical photodiode arrays (Reticon RL 256 C/17); see Fig. 1*B*. A cylindrical lens positioned in front of the photodiode array compressed the rod-like image of each hair to a dot. Final magnification amounted to 6·4 $\times$, as determined by placing calibration gratings at the level of the fibre.

The median of the marker image was taken as the marker position. This was measured in the same way as described above for laser diffraction, except that the intensity deficit, not the intensity, was used, as the hair is opaque. Segment length was determined as the difference of the two median positions, obtained from each of the two synchronously scanned arrays. Segment length was expressed in units of sarcomere length by measuring the sarcomere length within the segment using the optical microscope, as described above. We assumed that the measured degree of segment length change corresponded to the same degree of sarcomere length change. Figure 1*C* shows how the sarcomere length obtained this way compares to the sarcomere length determined by laser diffraction from the same segment. The two measurements are highly correlated ($r = 0·999$).

Fig. 1. Experimental apparatus. *A*, the fibre was mounted between two servomotors: the left motor was used to control force and the right motor to control marker position. The striation pattern, visualized with white light, could be observed continuously via a video camera (not shown) mounted on an ocular of the microscope. Numerical apertures of condenser and objective lens were matched (0·6). The condenser had a small, 1 mm hole drilled through its centre; this allowed undisturbed passage of laser light (dashed line) that was used for sarcomere length measurement with laser diffraction. *B*, segment length detection system. The image of each of the two markers was projected onto a separate photodiode array and the distance between markers was measured. *C*, sarcomere length obtained from the same section of a single fibre measured simultaneously by diffraction and segment methods. The two were highly correlated. Cylindrical lenses in front of the photodiode arrays, used for image compression, are not shown. For further details see text.

The segment-length signal had a time resolution of 260 μs. Root mean square noise (bandwidth 0–2 kHz), determined from three fibres over a wide range of sarcomere lengths, was 0.72 ± 0.02 nm ($n = 10$).

Force and length control

The objective was to impose shortening against a predetermined constant force and subsequently to hold the fibre length constant. The fibre was mounted between two servomotors. One of the motors had a force transducer mounted between this hook and its arm; see Fig. 1*A*. Each servomotor consisted of an electromagnetic puller, which produced linear motions (over a range of 6 mm), and a digital control system which allowed us to switch at desired times during the tetanus between control of fibre length (or motor position), force, first-order position, or segment length. Any one of the four signals could be chosen to control the motor. A more complete description of the servomotor may be found elsewhere (Granzier, 1988).

Root mean square noise (bandwidth 0–500 Hz) of the force signal in force control was typically about 0.01 mN (occasionally slightly higher, as in Fig. 11). For the motor position signal in position control, noise (bandwidth 0–2 kHz) was 0.3 μm, or about 0.003 % of the length of a typical, unstrained, fibre. For the sarcomere-length signal during first-order position control the value (bandwidth 0–2 kHz) was 0.9 ± 0.5 nm/sarcomere (mean ± S.D., $n = 10$), while for the segment-length signal during segment-length control root mean square noise (bandwidth 0–2 kHz) was 1.1 ± 0.3 nm/sarcomere ($n = 10$).

Marker-position control and force measurement

In order to prevent the segment from moving out of the field of view during large-scale shortening, the marker closest to the force transducer was maintained at a consistent position within the field of view. This was achieved through compensatory motion of the motor to which the force transducer was attached. Motor motion was effected in either of two ways: first, by estimating the amount of translation that was expected to occur during contraction and then imposing a ramp-like displacement to cancel this translation; second, by using the marker position as a feedback signal to the motor, thus keeping marker position constant. Marker control was performed only during the load clamp period.

An example of a representative contraction with marker-position control may be seen in Fig. 2; marker position is panel *D* of the figure. The slanted dashed line indicates the movement anticipated if position control had not been imposed. This is estimated from the measured displacement of motor 1. Without marker-position control the marker would have left the field of view.

We used a strain gauge (AME 801E, Horten, Norway) as a force transducer. This transducer was attached to the tip of the movable arm of the motor that controlled marker position. To keep the mass of the transducer low we removed the part of the silicon beam that extends beyond the diffused resistors. To prevent 60 Hz noise pick-up, a small strip of aluminium foil was wrapped around the resistors, avoiding mechanical contact. Transducer sensitivity was approximately 2.5 V/mN. The servomotor, to which the force transducer was attached, imposed unavoidable high-frequency, small-amplitude (less than 1 μm) perturbations on the transducer. These movements were picked up at frequencies of 700 Hz and above. To prevent instabilities in the force feedback loop, we filtered the output of the force transducer with a six-pole Bessel filter that had a 3 dB frequency at 500 Hz. After filtering root mean square force noise when the motor was in position control was about 1 μN, or 0.04 % of the maximal force of a typical fibre.

Movement of the motor to which the force transducer is attached will induce a force artifact arising from acceleration of the transducer mass. The magnitude of this artifact was determined by first measuring the amplitude and velocity of movement that occurred during a typical load clamp, and then imposing the same movement on the passive fibre. An example is shown in Fig. 2*B*, lowest force trace. (Note: the sensitivity of this trace is 10 times higher than for the other force traces.) The force artifact was typically less than 5 μN (about 0.25 % of maximal force) and was neglected in our analysis.

Experimental protocol

Fibres mounted in the chamber were carefully aligned relative to the photodiode arrays, using high-resolution *X–Y–Z* translators on which each motor was mounted. The markers were then
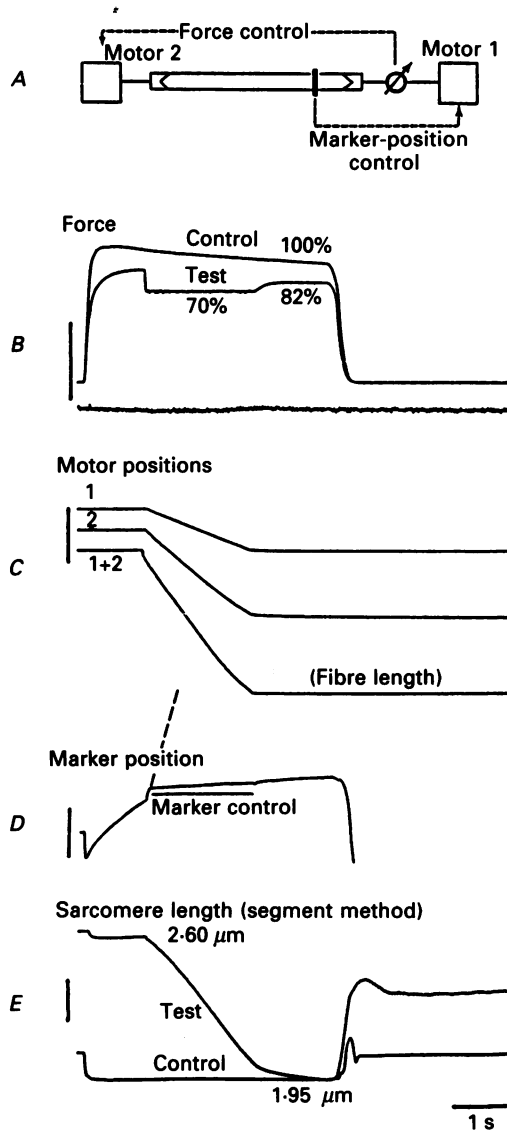
Fig. 2. Example of representative protocol. *A* shows that the fibre was mounted between two motors, the left to control force and the right to control marker position. *B*, force traces of test and control contractions are superimposed. The third trace from the top is the force artifact caused by movement of the force transducer, determined by imposing the same movement that occurred during contraction on the fibre that is now passive. Sensitivity of the third trace is ten times larger than that of the others. The sum of the shortenings imposed by the two motors (*C*) is equal to fibre shortening. *D*, marker position is shown for the test contraction; marker position was controlled during the load clamp. *E*, sarcomere length traces. Maximal force, 36 N/cm²; cross-sectional area, 2·46 × 10⁻³ mm², temperature, 2·4 °C. Calibration bars: force, 0·5 mN; motor position (shortening downwards), 2 mm; marker position (movement towards tendon upwards), 100 μm; sarcomere length, 0·2 μm.

mounted on the fibre surface and the various calibrations made. Sarcomere length was generally detected simultaneously with segment and diffraction methods; occasionally one method was used.

Before each experiment we measured major and minor fibre diameters with a stereomicroscope set at 80 ×. Cross-sectional area, needed to compute stress, was calculated assuming the fibre had an elliptical cross-section. Maximal active stress of all fibres used in this study, measured at 2 °C, was 32 ± 6 N/cm² ($n = 25$).

Experiments were performed at 2·0–2·5 °C. Fibres were stimulated with two platinum electrodes that ran parallel, along the full length of the fibre. Pulses of constant current were used. Frequency was set at about 50 Hz. Successive stimuli of the pulse train had opposite polarities since we found that this pattern prolonged the lifespan of the fibres.

First we performed a *test* contraction. Fibres were pre-stretched to the desired sarcomere length and tetanized. When force reached a plateau, a load clamp was imposed for some set period, after which the fibre was held isometric again. We studied the shortening velocity during the load-clamp period and isometric plateau force after this period. Force was measured typically 1 s after termination of the load clamp. An example of a test contraction is shown in Fig. 2 (superimposed on a control contraction).

After a rest period of about 300 times the duration of the test contraction, we performed a *control* contraction. The control contraction was a fixed-end tetanus (fibre length kept constant) in which the sarcomere length during the force plateau was similar to the one in the test contraction after the load clamp period (see bottom signals in Fig. 2). We then compared the isometric force plateau developed by the test contraction after pre-shortening with the force developed by the control contraction. These forces were measured at the same time during the tetanus.

Data were accepted only if the sarcomere length in test and control contractions differed by less than 50 mm (see also Discussion). This criterion was independently applied to the results of each method. For the same contraction, results from one method might thus be accepted, but not necessarily results from the other. Furthermore, we accepted contractions only if sarcomeres during the force plateau were either isometric or were shortening with a velocity less than 50 nm/s; stretch was not accepted. Initially we had planned to keep sarcomeres isometric after the load clamp, but for technical reasons it turned out to be difficult to obtain stable segment-length clamps after a period of isotonic shortening. Sarcomere-shortening velocity at the time when force was determined was typically about 10 nm/s (see Results), and sarcomeres in test contractions were thus close to isometric.

As for the control contraction, sarcomere length during the tetanic force plateau was typically between 2·0 and 2·1 μm. At such lengths, sarcomeres were either isometric or slowly shortening ($3·8 \pm 1·7$ nm/s, $n = 56$, by diffraction; and $4·9 \pm 2·3$ nm/s, $n = 52$, by the segment method). In some instances the sarcomere length at the end of the shortening phase of the test contraction was longer than 2·1 μm. Control contractions at extended lengths were never isometric: sarcomeres in the central region lengthened slowly. Since this was unacceptable (see above), we imposed sarcomere-isometric control contractions in those instances.

Force and length signals were digitized with 15-bit analog-to-digital converters of a four-channel digital oscilloscope (Nicolet, 4094/4851). Signals were stored on floppy discs for subsequent analysis.

RESULTS

Pilot experiments

Initially, we repeated some of the experiments that had been reported to result in large effects of previous shortening on isometric force. As in the experiments of Buchthal *et al.* (1951), fibres were tetanized at long lengths, and when force had reached a plateau, the load was quickly reduced to the level of the passive force prior to stimulation; see inset of Fig. 3. The fibre was allowed to shorten until a steady length had been reached. If previous shortening had no effect on force development, this terminal length would lie on the ascending limb of the isometric length–tension

relation. The extent of shortening predicted for one load is given by the continuous horizontal line in Fig. 3.

We found that the fibre was indeed able to shorten to the predicted length on the ascending limb, or close to it, but only if shortening had started at lengths that were less than about 160% L_0 (for definition of L_0 see caption of Fig. 3). If contraction started at longer lengths, the ascending limb was never reached (see dashed
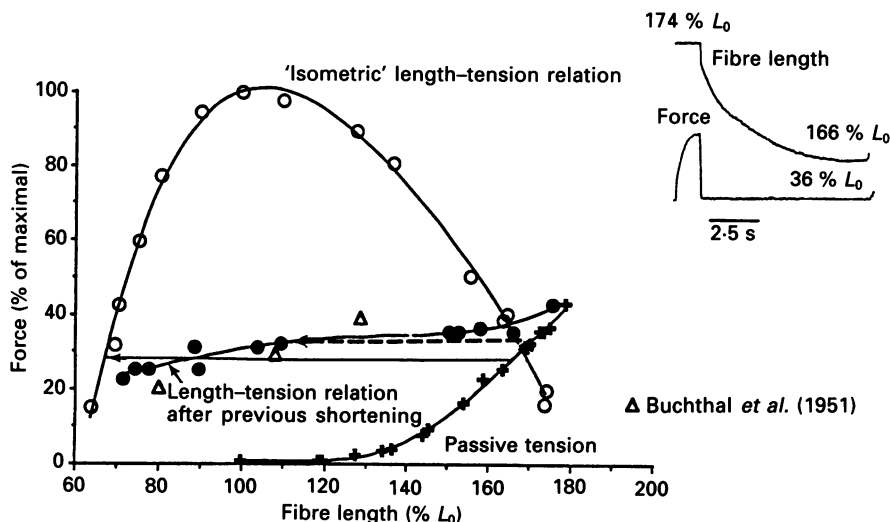

Fig. 3. Isotonic release contractions. The inset shows the protocol. The length–tension relation after previous shortening was obtained by plotting the terminal length during the load clamp against the level of the load clamp. The isometric length–tension relation is shown by open circles. (Measurements at lengths shorter than the slack length were obtained by decreasing the end-to-end distance of the fibre prior to stimulation, and allowing the stimulated fibre to shorten to the desired length.) L_0 is the fibre length at which the passive force is equal to 0·5% of the maximal active force, as defined by Buchthal *et al.* (1951). L_0 corresponded to a sarcomere length of $2·34 \pm 0·05\ \mu m$ ($n = 3$).

horizontal line in Fig. 3). The fibre stopped shortening 'prematurely,' and the amount of shortening that did occur was strongly dependent on the starting length; see Fig. 3. This figure also shows that our results are similar to those of Buchthal *et al.* (1951). Force development can apparently be diminished by shortening. For contractions that stopped shortening at L_0, the deficit in force was largest – force was only 30% of the maximal force measured without shortening.

In order to determine whether experiments done at the fibre level, as above, can be extrapolated to the sarcomere level, we measured the dynamics of the sarcomeres in similar protocols. We studied fifteen isotonic releases in three fibres under conditions in which we anticipated large effects of shortening on force development at the fibre level, i.e. at starting length > 170% L_0. An example is shown in Fig. 4*A*. This fibre shortened by 16%. However, apart from 60 nm of shortening that occurred simultaneously with the load step, the interrogated sarcomeres did not shorten at all. This result is in agreement with the finding of Huxley & Peachey (1961) and of Podolsky (1964) that sarcomeres stretched to lengths above about
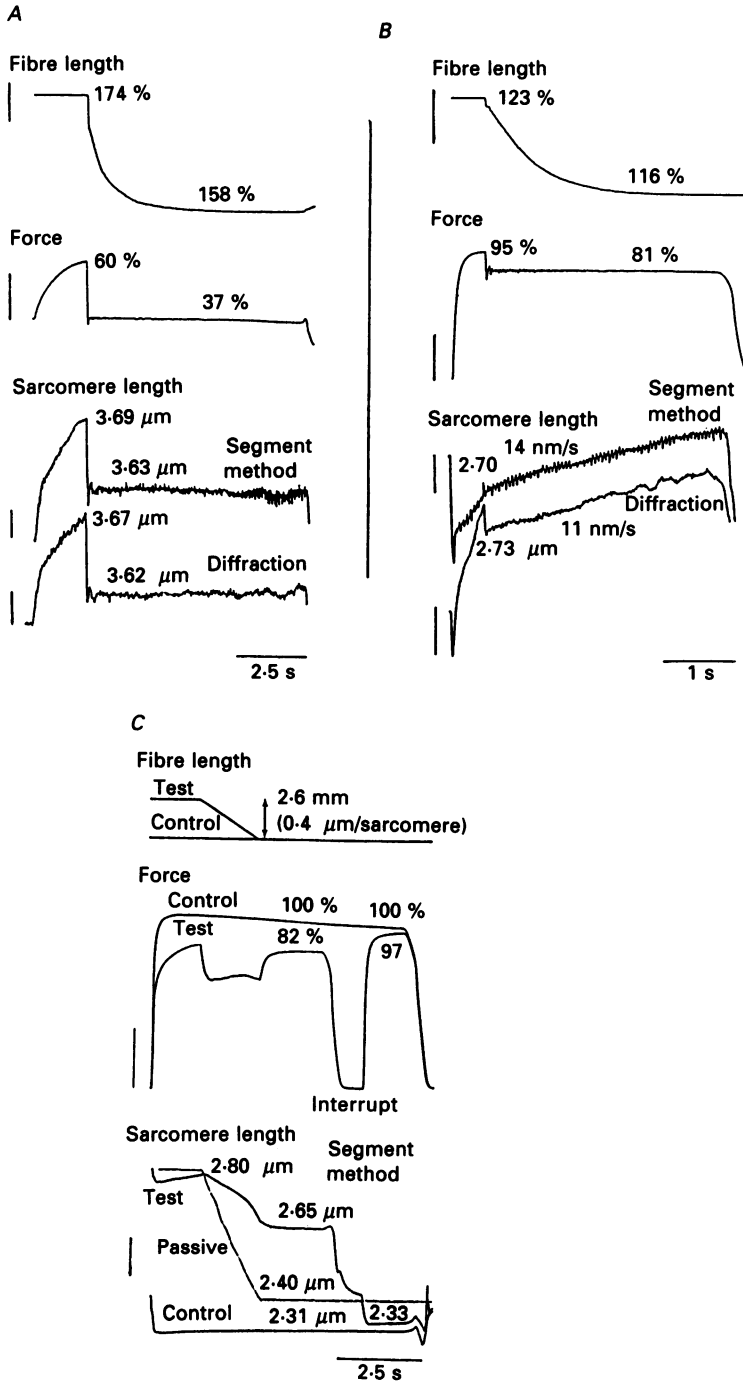
A

Fibre length

174 %

158 %

Force

60 %

37 %

Sarcomere length

3·69 μm

3·63 μm   Segment method

3·67 μm

3·62 μm   Diffraction

2·5 s

B

Fibre length

123 %

116 %

Force

95 %   81 %

Sarcomere length   Segment method

14 nm/s

2·70

Diffraction

11 nm/s

2·73 μm

1 s

C

Fibre length

Test

Control   2·6 mm
(0·4 μm/sarcomere)

Force

Control   100 %   100 %

Test   82 %   97

Interrupt

Sarcomere length   Segment method

2·80 μm

Test   2·65 μm

Passive

2·40 μm

Control   2·31 μm   2·33

2·5 s

Fig. 4. For legend see facing page.

3·6–3·7 μm are not able to shorten actively. The observed fibre shortening in Fig. 4*A* apparently resulted from shortening of the end regions of the fibre. Thus, shortening at the fibre level is no guarantee that proportional shortening occurs at the sarcomere level.

We repeated these experiments at shorter sarcomere lengths (about 2·7 μm), and at higher loads. As reported by Délèze (1961), the extent of fibre shortening was extremely small – far less than predicted by the isometric length–tension relation; see the two top panels of Fig. 4*B*. When sarcomere length was measured in the central region of the fibre, however, we again found anomalous sarcomere behaviour. As the fibre shortened, the interrogated sarcomeres were slowly stretched; see the two bottom traces of Fig. 4*B*.

Finally, we conducted experiments similar to those performed by Abbott & Aubert (1952), Maréchal & Plaghki (1979) and Julian & Morgan (1979), in which a moderately pre-stretched fibre is isometrically tetanized and then constrained to shorten at constant velocity. Shortening is then stopped and force development at this shorter length is compared to that of a control contraction at the same fibre length. We found that force after shortening was less than that of the control contraction (Fig. 4*C*). Only after stimulation had been interrupted for 1–2 s was force restored to that of the control. A similar result was obtained by Julian & Morgan (1979).

We then measured the corresponding sarcomere length change in the central region of the fibre. When the release was imposed on the unstimulated fibre, sarcomere shortening was linearly related to fibre shortening; see Fig. 4*C*. However, when the active fibre was similarly shortened, the interrogated sarcomeres shortened much less than anticipated; other sarcomeres evidently shortened more. This non-homogeneity may explain why force after shortening was less than in the control: during the short interruption of stimulation the difference in sarcomere length between test and control was largely rectified, and when the fibre was tetanized again, sarcomere length was similar to the test contraction, as was force (Fig. 4*C*).

It is clear from the sample records of Fig. 4 that erroneous conclusions could result if fibre-length signals are converted directly into sarcomere length. The fibre-length signal implied that sarcomeres had shortened to some intermediate length, while in reality they were either much shorter or much longer. Although test and control

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Fig. 4. Examples of difficulties extrapolating fibre shortening to the sarcomere level. *A*, isotonic quick-release contraction. Based on observed fibre shortening, sarcomeres are predicted to shorten by about 0·6 μm. Actual sarcomere shortening is only one tenth of that. *B*, isotonic quick-release contraction to high load. Based on observed fibre shortening, sarcomeres are predicted to shorten by about 0·2 μm. Instead, sarcomeres are stretching. *C*, the fibre is released at constant velocity during the plateau of the test tetanus. After release, test and control contractions have the same fibre length. Force is less in the test contraction. However, sarcomere lengths are not the same (2·65 μm for the test and 2·31 μm for the control). After brief interruption of the tetanus, force returns to control, as does sarcomere length (2·33 *vs.* 2·31 μm). Calibration bars: fibre length, 1 mm in *A* and 0·5 mm in *B*; sarcomere length, 25 nm in *A* and *B* and 100 nm in *C*; force, 0·5 mN in *A* and 1 mN in *B* and *C*. Cross-sectional area, 7·9, 9·2 and 7·5 × 10^{-3} mm^2 in *A*, *B* and *C*, respectively. Temperature, 2·1, 2·2 and 2·4 °C in *A*, *B* and *C*, respectively.

contractions had the same fibre length when force was compared, sarcomere-length distributions were very different. Such non-homogeneity could potentially underlie the effect of fibre shortening on force development. It is for this reason that we felt measurements needed to be carried out at the sarcomere level.
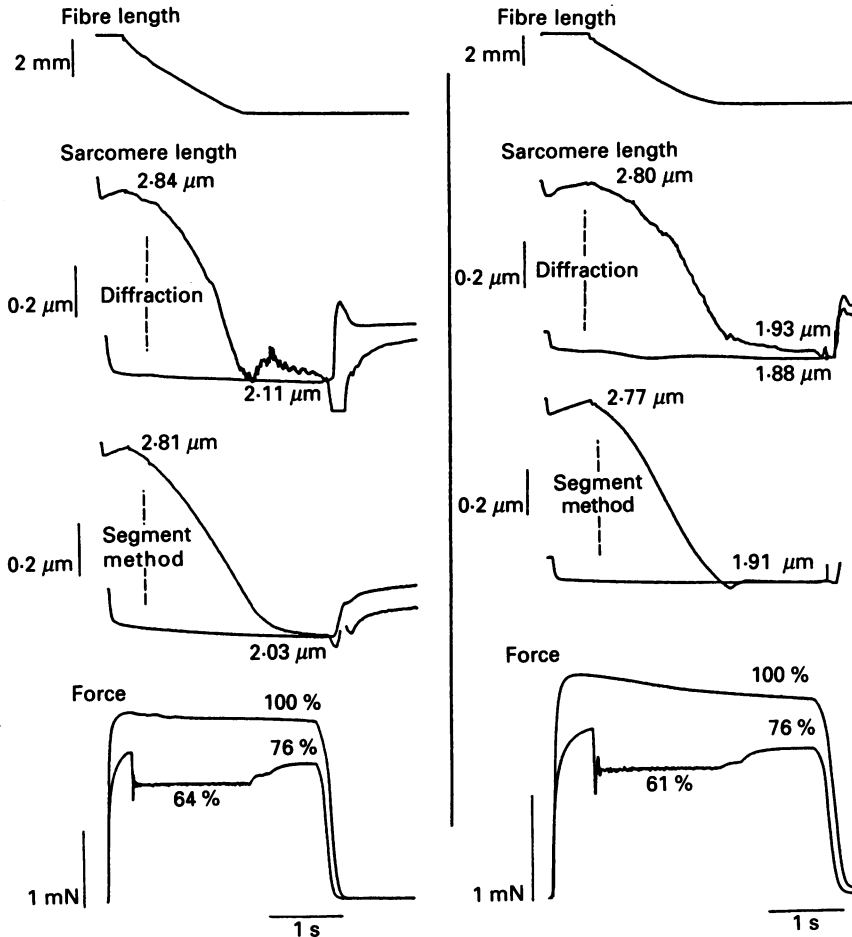

Fig. 5. Examples of contractions in which force is reduced after shortening. Control and test contractions are superimposed. Cross-sectional area, 8·6 and 6·4 × 10⁻³ mm²; maximal force, 30 and 37 N/cm² for left and right panels respectively; temperature, 2·3 °C.

Effect of previous shortening on isometric force

Examples of representative experiments are shown in Fig. 5. Terminal sarcomere lengths were similar in test and control contractions. Yet isometric force was often much lower following shortening than the force of the control contraction. Test contractions were occasionally repeated and similar degrees of depression were found to that of the initial test contraction. Thus, force reduction after shortening did not result from fibre deterioration.

Force rise after the end of the load clamp was often not smooth, as in Fig. 5. A possible reason for non-smooth force rise is that sarcomeres in the end regions of the fibre stopped shortening soon after the end of the load clamp; see Discussion. Alternatively, non-smoothness may arise from changes in calcium concentration that have been detected immediately after the end of a load clamp (Allen, 1978; Cecchi, Griffiths & Taylor, 1984).

In some instances, force was not seriously affected by previous shortening; see Fig. 6*A* and *B*. Comparison of contractions with and without large force deficits gave the preliminary impression that the force deficit might depend on the level and/or duration of the load clamp; see for example Fig. 6*C* in which two contractions with

TABLE 1. Relation between the force deficit and conditions of the load clamp

	Diffraction method ($n = 50$)			Segment method ($n = 46$)		
x	Equation	*r*	*P*	Equation	*r*	*P*
Load (% maximal force)	$y = -0.23x + 1.2$	0.48	0.0006	$y = -0.36x + 1.2$	0.47	0.0008
Shortening distance (μm/sarcomere)	$y = -18.9x + 1.3$	0.45	0.0015	$y = -23.3x + 0.9$	0.47	0.001
Work (% maximal force × μm/sarcomere)	$y = -0.46x + 3.3$	0.71	0.0001	$y = -0.46x + 0.8$	0.71	0.0001

y = force deficit (% of maximal force); r, correlation coefficient; P, probability.

different force deficits are superimposed.

To determine whether the force deficit was related to the conditions of the load clamp, we performed linear least-squares fits on the relation between force after shortening and each of the following three parameters: level of the load clamp, extent of shortening, and work done during the load clamp. Results are shown in Table 1. The correlation coefficients for shortening distance and load were moderate (about 0.45), and their P values were low enough to imply satistical significance. The product of shortening distance and load, the work during the load clamp, had a higher correlation coefficient (0.71) than either load or distance, and its P value (0.0001) indicated high significance.

A scattergram of the work done during the load clamp and the force deficit after shortening is shown in Fig. 7. The 99.99% confidence intervals of the slope of the regression line did not include zero. A slope of zero is expected if shortening history were not to affect force. Results were similar for both segment and diffraction methods. Thus, the force deficit is clearly related to the amount of work done during previous shortening.

Mechanism of shortening-induced force reduction

Although results obtained by the two methods were similar, we first investigated whether the small differences that did exist might be related to the force deficit. We calculated the linear least-squares fit of the relation between size of the force deficit and the difference in sarcomere length obtained by the two methods at the moment when the deficit was determined. The resulting equation was: force deficit (% maximal active force) = 0.014 × sarcomere length difference (nm/sarcomere) + 15.6;

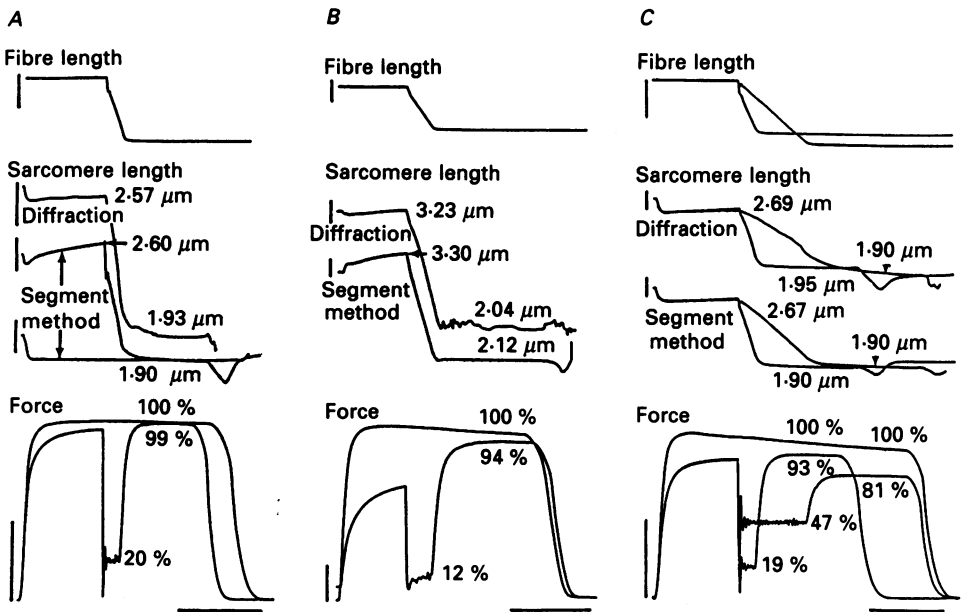Fig. 6. A and B show examples of contractions in which force after shortening is similar to that of the control. C shows two contractions in which the force deficit after shortening varies with the level of the load clamp. Sarcomere length of the control contraction is shown in panel A only; in other cases control traces are omitted for clarity. Calibration bars: fibre length, 2 mm; sarcomere length, 0·2 μm; force, 1 mN; time (horizontal bar), 1 s. Cross-sectional area, 2·5, 11·1 and 5·4 × 10^{-3} mm^2 for A, B and C, respectively. Maximal force, 36, 46 and 40 N/cm^2 for A, B and C, respectively. Temperature, 2·1, 2·3 and 2·3 °C in the same order as above.

the correlation coefficient was 0·044 ($P = 0·79$). Because of the low value of the correlation coefficient and high P value it seems unlikely that sarcomere length difference underlies the force deficit. Furthermore, since the sarcomeres were often slowly shortening when the force deficit was measured (12 ± 9 nm/s, $n = 62$, as found with the segment method and 7·2 ± 12·2 nm/s, $n = 55$, with the diffraction method) we performed the same analysis on the relation between maintained shortening velocity and force deficit. The resulting equation was: force deficit = 0·075 × shortening velocity (nm/s) + 14·827; correlation coefficient 0·078 ($P = 0·47$). Again, the statistics imply no correlation. Maintained shortening does not appear to underlie the deficit in force.

Next, we considered the involvement of the activation mechanism. If the level of activation were to decrease during the load clamp, subsequent force development might be diminished. This possibility was investigated by adding caffeine to the bathing solution. Caffeine increases the concentration of free calcium (Lopez, Wanek & Taylor, 1981). Initially, we injected caffeine into the experimental chamber during the isometric phase immediately after shortening. The final concentration was 10 mM. In the absence of electrical stimulation, caffeine in such concentration triggers a contraction with large force, close to the maximal force obtained with electrical stimulation. We found that force was unaltered after caffeine injection. However,
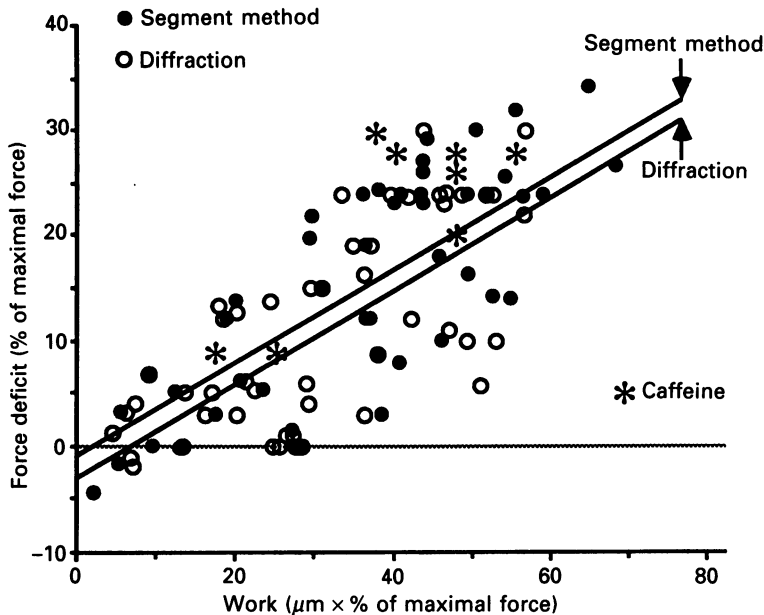
Fig. 7. Relation between work performed during the load clamp and the force deficit. Sloped lines are linear regression lines (for equations see bottom of Table 1). The horizontal line shows where data are predicted to fall if the force after shortening is not affected by previous shortening. Data points shown with asterisk were obtained in the presence of 0·2 mM-caffeine. For the possible significance of the residual variability, see Discussion.

this intervention damaged the fibre: the diffraction pattern was permanently lost and the force of the subsequent control contractions was reduced to a very low level. Therefore, we adopted an alternative protocol. The fibre was bathed continuously in saline with a low concentration of caffeine. This did not damage the fibre. We used a concentration of 0·2 mM, which we determined just below the threshold for force production in absence of electrical stimulation. This threshold is lower than the one in the experiments of Lopez *et al.* (1981). The difference most probably results from the lower temperature used in our experiments, 2 °C as opposed to 15 °C; see also Sakai (1979). Caffeine greatly enhanced isometric twitch force at all lengths tested, from about 2·0 to 3·2 μm. On the other hand, caffeine had little effect on isometric tetanic force; force was only slightly higher in contractions at sarcomere lengths shorter than about 2·2 μm (Granzier, 1988). The effect of caffeine on isometric force was similar to that measured by Lopez *et al.* (1981).

We tested eight contractions in three fibres and found that in the presence of caffeine force after shortening was still reduced: the relation between the force deficit and the work performed is shown in Fig. 7. Caffeine apparently does not reverse the effect of shortening on force development.

Next we considered whether the effect of shortening against high load could be reversed by a subsequent phase of rapid shortening (see Discussion for underlying reasoning). Thus, we quick-released the fibre during the post-shortening isometric
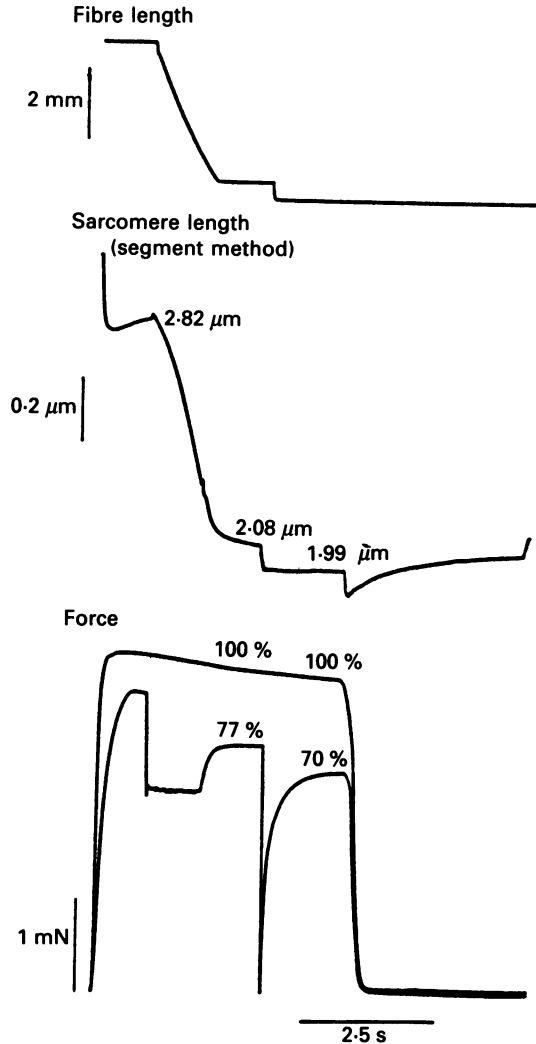
Fibre length

Sarcomere length
(segment method)

2·82 μm

0·2 μm

2·08 μm

1·99 μm

Force

100 %   100 %

77 %

70 %

1 mN

2·5 s

Fig. 8. Enhancement of force deficit by imposition of quick length step. Cross-sectional
area, $9·2 \times 10^{-3}$ mm²; maximal force, 37 N/cm²; temperature, 2·3 °C.

plateau. This allowed the sarcomeres to shorten rapidly under a very low load. We
studied a total of ten contractions in three fibres and found that force after such
additional shortening step was always slightly less (7 ± 4%) than the force developed
before the shortening step; an example is shown in Fig. 8. Thus, the force deficit after
shortening against a high load is not reversed by a subsequent phase of rapid
shortening. Instead the deficit is increased.

Finally, we measured the time course of deficit recovery. Interrupting stimulation
for 0·5–3·0 s after the isometric force after shortening had reached a plateau turned
out to have only a minor effect on the force deficit; see Fig. 9. In two fibres the deficit
remained after the short interruption, while in two others, force was slightly elevated
by 2 and 5% of the control force.

We therefore investigated how long a period of rest had to be imposed before force

of the control contraction would recover fully. A representative result is shown in Fig. 10. In two fibres 5–10 min were required while in two others between 10 and 15 min of rest were required for complete recovery. Force reduction can apparently be induced by only a few seconds of shortening, but minutes of rest are required for recovery.
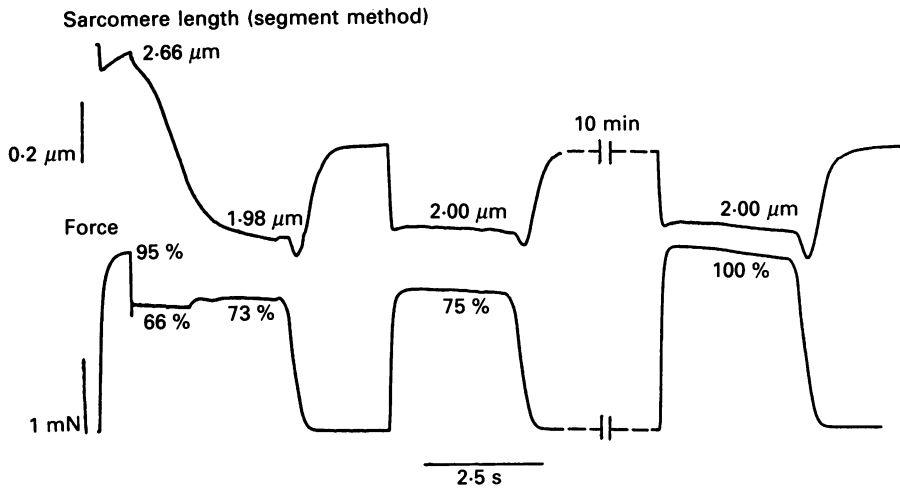

Fig. 9. Persistence of force deficit after brief interruption of tetanus. The percentage values on force trace refer to percentage of force of control contraction (third contraction shown), measured at the same time after the start of stimulation. Cross-sectional area, $5 \cdot 1 \times 10^{-3}$ mm^2; maximal force, 46 N/cm^2; temperature, $2 \cdot 1$ °C.

Effect of previous shortening on shortening velocity

A striking finding was that the velocity of sarcomere shortening increased progressively during the load clamp period. Sarcomeres accelerated. Examples are shown in Fig. 11. Only if shortening occurred against loads that were lower than about 20 % of maximal was sarcomere shortening nearly linear (as in Fig. 6*A* and *B*). We studied a total of fifty-five contractions in which shortening was imposed against loads higher than 20 % of maximal force and in which sarcomeres shortened by at least $0 \cdot 5$ μm: in only two of them was sarcomere shortening linear. In the remaining fifty-three contractions there was clear acceleration. By contrast, the fibre as a whole behaved quite differently from what was seen at the sarcomere level. Although on occasion there was slight acceleration, fibre shortening was typically linear and occasionally there was even slight deceleration; see Fig. 11.

Since fibre shortening took place at approximately constant velocity, acceleration of the interrogated sarcomeres implies that other sarcomeres must have decelerated. The latter sarcomeres are presumably located outside the region of study, i.e. towards the ends of the fibre. The ends are apparently stronger at the start of the load clamp since they shorten faster than the central region. This is in agreement with the findings of Altringham & Pollack (1984) and Edman & Reggiani (1984). Apparently these sarcomeres then weaken and decelerate. No attempt was made to study the ends specifically in this study, mainly for technical reasons.

Sarcomere length (segment method)

2·93 μm

0·2 μm

2·13 μm

Force

20 min

10 min
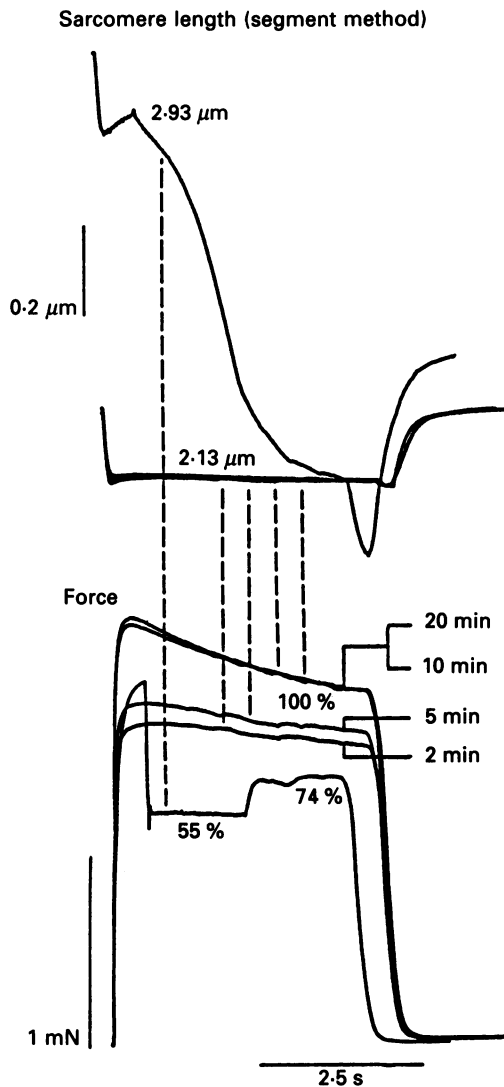
100 %

5 min

2 min

74 %

55 %

1 mN

2·5 s

Fig. 10. Time course of recovery of force deficit. Between 5 and 10 min of rest are required before force returns to control value. Cross-sectional area, $4·6 \times 10^{-3}$ mm²; maximal force, 47 N/cm²; temperature, 2·3 °C.

We compared the degree of acceleration of the central sarcomeres with that predicted if previous shortening were not to affect shortening velocity. Predicted acceleration was estimated as follows. First we measured the force–velocity relation at each of three different sarcomere lengths: 3·15, 2·65 and 2·15 μm. This was accomplished by pre-stretching the passive fibre such that the sarcomere length during the plateau of an isometric tetanus was slightly higher (10–30 nm) than the desired value. A load clamp was imposed during the tetanic plateau and the instantaneous velocity measured immediately after velocity transients gave way to

A

Fibre length

Sarcomere length
Diffraction   0·23 μm/s

3·15 μm

Segment method
0·18 μm/s

1·98 μm/s

2·15 μm

Force

66 %

2·10 μm/s

33 %

B

Fibre length

Sarcomere length
Segment method
0·03 μm

Diffraction   2·70 μm   0·88 μm/s

0·06 μm/s

2·40 μm

Force   96 %

0·98 μm/s
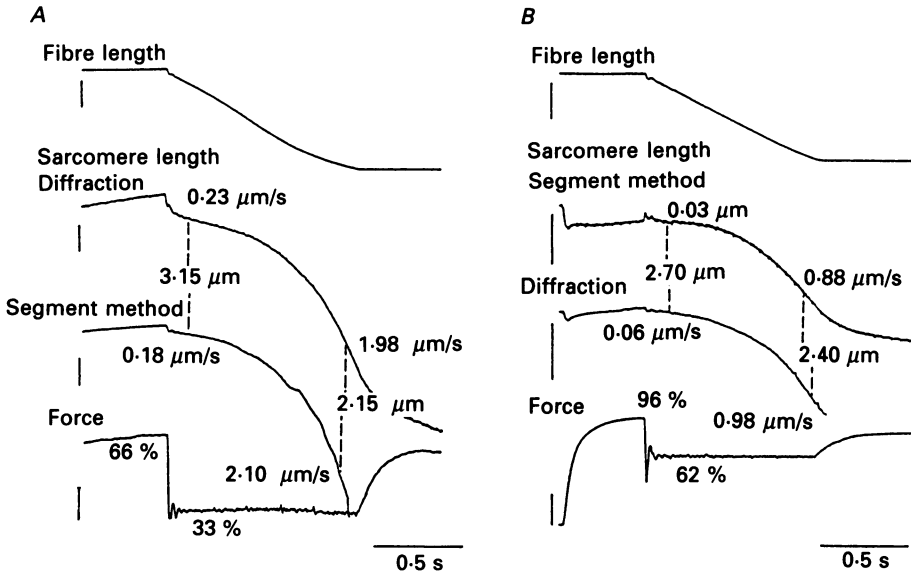
62 %

0·5 s   0·5 s

Fig. 11. Sarcomere acceleration during period of load clamp. By contrast, fibre shortening waveform tends to be nearly linear. Calibration bars: fibre length, 2 mm; sarcomere length, 0·2 μm; force, 0·5 mN; time (horizontal bar), 0·5 s. Cross-sectional area, 6·2 and 6·5 × 10^{-3} mm^2 for *A* and *B*, respectively. Maximal force, 30 and 28 N/cm^2 for *A* and *B*, respectively. Temperature, 2·3 °C for both contractions.

✗ ☐ ■ 3·15 → 2·65 μm

✚ △ ▲ 3·15 → 2·15 μm

$\frac{2·15}{3·15}$→ $\frac{2·65}{3·15}$→

Velocity ratio

12

8

4

0

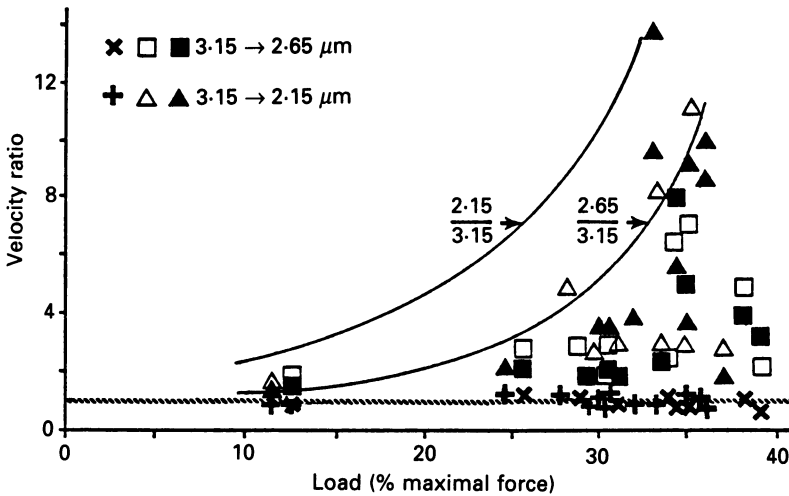0   10   20   30   40

Load (% maximal force)

Fig. 12. Comparison of measured acceleration with that predicted if previous shortening were not to affect velocity. Upper curves indicate predicted acceleration. Acceleration is expressed as the ratio of the velocities at short and initial length. This was determined from force–velocity relations obtained at different sarcomere lengths in absence of pre-shortening (see text for details). Filled symbols are respective data points obtained with segment method, open symbols with diffraction. Sarcomeres accelerate less than predicted. At the same time during the load clamp when the sarcomere velocities were determined, we also measured fibre-shortening velocities. The obtained ratios are given by + and × symbols. Hatched broken line corresponds to ratio of 1·0, i.e. linear shortening. The fibre does not accelerate. Temperature, 2·3 °C.
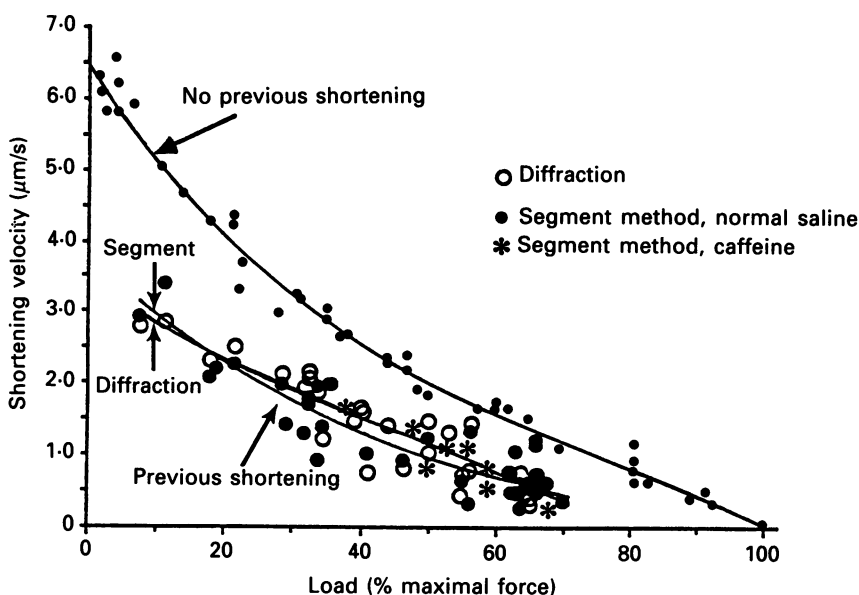
Fig. 13. Depression of shortening velocity after previous shortening. Shortening velocity was measured at a sarcomere length of 2·15 μm after 0·5 μm of uninterrupted isotonic shortening and compared with velocity measured at 2·15 μm without previous shortening. Test shortening started at a sarcomere length of 2·65 μm (10–30 nm longer for shortening against low loads, to ensure that velocity was steady when 2·65 μm was reached). Abscissa can be converted into relative work (μm × % maximal force) by multiplying by 0·5. Data points shown with an asterisk were obtained in presence of 0·2 mM-caffeine. Results obtained from seven fibres.

steady shortening. In this way force–velocity curves were constructed at sarcomere lengths of 3·15, 2·65 and 2·15 μm. Acceleration was then predicted from these curves, assuming shortening started at 3·15 μm and continued against a constant load until sarcomeres reached either 2·65 μm or 2·15 μm. Predicted acceleration was expressed as the ratio of the velocity at the shorter length to the velocity at the starting length; see upper curves in Fig. 12. The measured acceleration, in which sarcomeres started shortening at a length of 3·15 μm and continued to shorten to either 2·65 μm or 2·15 μm, is shown by the data points. If shortening history were irrelevant, these points would lie on the predicted curves. Figure 12 shows that the prediction is not met. Data points generally fall below the predicted curves. Apparently previous shortening affects shortening velocity.

The effect of shortening was studied further by comparing the velocity after 0·5 μm of previous shortening with the velocity obtained at the same sarcomere length, but without any previous shortening. Sarcomere length of 2·15 μm was used as a common basis. We found that the velocity after 0·5 μm of shortening was consistently less than the velocity measured without previous shortening; see Fig. 13. Reduction was seen at all loads. Furthermore, the reduction in velocity was also apparent in the presence of 0·2 mM-caffeine.

Figure 13 also shows that the reduction in shortening velocity did not vary greatly with load, and therefore also not with work done during shortening. For example,

after 0·5 μm of shortening against a load of 70% of maximal force, shortening velocity is reduced by about 65%, and by 50% if shortening occurs against a load of 10% of maximal force. On the other hand, the isometric force after comparable shortening against high and low load is reduced by about 15 and 0%, respectively (Fig. 7). Shortening velocity is thus more sensitive to previous shortening than isometric force. This sensitivity difference may offer a clue as to the mechanism that underlies the effect of previous shortening.

DISCUSSION

Previous shortening apparently results in a deficit of both isotonic velocity and isometric force. Below we consider the implications of these results, particularly *vis-à-vis* the cross-bridge model.

Comparison with previous results

The initial phase of our study consisted of repeating earlier experiments at the fibre length level in which previous shortening had been claimed to exert large effects on force development (Buchthal *et al.* 1951; Abbott & Aubert, 1952; Délèze, 1961; Edman, 1964; Maréchal & Plaghki, 1979). We confirmed those findings. When fibre shortening occurs against high loads or starts at long lengths, force is reduced remarkably. However, sarcomere shortening in the central region of the fibre is different from the average implied by the fibre-length signal: sarcomeres shorten much less, and may even be stretched while the fibre is shortening (Fig. 4). This implies that other sarcomeres are predominantly responsible for the observed fibre shortening. So, although the test and control contractions have the same fibre length when forces are compared, sarcomere-length distributions are very different. Force developed after fibre shortening may then be diminished relative to the control because sarcomeres are either very short or very long.

On the other hand, we found that shortening at the sarcomere level also results in force reduction. Sarcomere heterogeneity is thus not the only reason for the force deficit. Part of the deficit found in earlier experiments may therefore be genuine. How much can be estimated from the equations in Table 1. For example, the 'real' deficit for those sarcomeres in Fig. 2 that stop shortening at L_0 is about 20%. Since the observed reduction was 70% (Fig. 2) it appears that heterogeneity is a serious source of error in the experiments at the fibre level.

As for our study at the sarcomere level, the force deficit after sarcomere shortening was found to depend on the work done during the load clamp (Fig. 7). When work was small, the force deficit was also small and sometimes almost absent (Fig. 6). This explains why Gordon *et al.* (1966) and Edman (1966, 1980) found only a small effect of shortening on subsequent force development: the work done during shortening was small. In the study of Gordon *et al.* (1966) for example, the relative work performed in the contraction that resulted in the largest force deficit (their Fig. 6C), was only 25 units (unit = shortening distance (μm) × force (% of maximal force)). According to our results, this would have resulted in a force deficit of 8–10% (calculated from the equations at the bottom of Table 1), similar to the deficit of 9%

measured by Gordon *et al.* (1966). Hence, where our study overlaps with those of others, results concur. However, our study extends observations to the high-work region, where no systematic experiments have been carried out. When the work done during shortening is high, isometric force appears to be reduced substantially.

Sources of error

In theory, force generated in test and control contractions could be different for a number of reasons. First, sarcomere lengths were not always exactly the same in the two contractions at the time force was measured. The difference in length might underlie the measured difference in force. However, we accepted data only if the difference was less than 50 nm. This results in a force deficit no greater than 3% at any sarcomere length above 2 μm (Granzier, 1988). The measured force reduction was much higher than 3%; values as high as 20–30% were consistently found. Thus, the small difference in sarcomere length between test and control contractions that was tolerated in this investigation is unlikely to explain our findings.

Another reason why the force of test and control contractions might be different is that the sarcomeres were not perfectly isometric when force was measured. Suppose, for example, that sarcomeres were being stretched in the control contraction while in the test contraction they were shortening. Because of velocity effects on force development (Katz, 1939), force would then be less in the control contraction. However, our strategy was the same as above: we accepted data only if the velocity difference was small. We excluded all contractions in which sarcomeres were being stretched, and shortening was tolerated only if the velocity was less than 50 nm/s. Of contractions that passed our selection criteria, average shortening velocity was approximately 10 nm/s (see Results). This would have resulted in a force error (determined from the force–velocity relation at 2·15 μm with 0·5 μm of pre-shortening; Fig. 13) of less than 1%. Furthermore, we determined the linear least-squares fit of the relation between the force deficit and maintained shortening velocity. The correlation coefficient was found to be only 0·078 (P value of 0·48), in support of the view that the slow shortening found at the moment the force deficit is measured does not underlie the observed force deficit.

Finally, sarcomere heterogeneity. For measurements at the fibre length level we concluded that this was a serious source of error. Suppose such heterogeneity persisted even within a small segment of the fibre's central region. Again, this would result in an artifact since sarcomere lengths would then be different in test and control contractions. To exclude this possibility, we measured shortening with both diffraction and segment methods. These methods are based on different physical principles with different sensitivities to sarcomere length heterogeneity. The segment method measures the average sarcomere spacing of *all* sarcomeres between the markers, while diffraction is based on regularity and will reflect principally the most regular sarcomeres. Small populations of sarcomeres at different lengths may therefore escape detection by the diffraction method, but they will be reflected in the segment measurement. Hence, if shortening behaviour were heterogeneous, the results of the two methods would be different.

We found that the results of the two methods were generally very similar (Figs 5, 6, 7, 11, 12 and 13). Furthermore, the small difference in sarcomere length found by the two methods was statistically unrelated to the magnitude of the force deficit

(correlation coefficient between the length difference and force deficit was 0·044; $P = 0·79$). Hence, the force deficit after previous shortening does not seem to arise from local shortening heterogeneity.

We are unable to identify an artifact that can account for the reduction in isometric force after previous shortening. Force diminution appears to arise from shortening itself.

Velocity of shortening

We found that the interrogated sarcomeres accelerated during the load-clamp period (Figs 11 and 12). The degree of acceleration depended on the load and was small or absent at low loads (Figs 6 and 12). This explains why acceleration was virtually absent in the experiments of Gordon *et al.* (1966): the load was very small in those experiments. Furthermore, the absence of acceleration in the experiments of Buchthal *et al.* (1951) and the small degree of acceleration against very high loads measured by Edman & Reggiani (1984) is probably due to the fact that measurements were made at the fibre level where we, too, found shortening to be linear.

For sarcomere acceleration to occur, the imposed load has to become a progressively smaller fraction of the isometric force (Hill, 1938; Katz, 1939; Fig. 13). Since the load was kept constant, acceleration implies that the isometric force capability increases with shortening. However, acceleration was less than predicted by the force–velocity relations measured without pre-shortening (Fig. 12). Thus, previous shortening apparently reduces not only subsequent isometric force but shortening velocity as well.

As for the sensitivity of velocity to previous shortening, we found that when pre-shortening occurred against light loads, and the work performed was small, the velocity after previous shortening was still depressed relative to the isotonic control (Fig. 13). Velocity is thus very sensitive to previous shortening.

High velocity sensitivity to pre-shortening raises question about the isotonic control contraction. Prior to imposition of the load clamp, the fibre was maintained at constant length. This allowed sarcomeres to shorten during the force rise, by stretching the tendons. We computed the work done by these sarcomeres during the force rise, and determined from Fig. 13 that this work would result in a depression of velocity by as much as 50 %. Thus, the isotonic control contraction itself might have a depressed shortening velocity. This intrinsic depression may help explain one of the intriguing observations of Yanagida, Arata & Oosawa (1985). Maximal shortening velocity of intact fibres was found to be substantially less than that of isolated sarcomeres. Isolated sarcomeres do not perform any work prior to velocity measurement. They may therefore give the real, undepressed, maximal shortening velocity. In intact fibres, it may be important to keep sarcomeres length constant prior to the load step, in order to determine the history-free force–velocity relation. Since this has apparently not yet been done, the real force–velocity relation of intact muscle may still be unknown.

Differences between fibre shortening and sarcomere shortening

During long-lasting load clamps the fibre as a whole shortened with nearly constant velocity. Sometimes there was slight deceleration, and occasionally slight acceleration (as found by Edman & Regianni, 1984), but fibre shortening was

typically linear. This is in sharp contrast to the typical behaviour of sarcomeres in the central region of the fibre: they accelerated (Figs 12 and 13). This implies that some unsampled sarcomeres must have behaved oppositely from the interrogated sarcomeres, i.e. they shortened rapidly just after the load step and then decelerated. Since acceleration was found in the central region of the fibre, it is likely that the sarcomeres that decelerate are located in the end regions of the fibre.

Why the ends decelerate was not investigated experimentally, but the issue can be addressed indirectly. It has been found that during the plateau of fixed-end tetani at intermediate lengths, sarcomeres in the ends are able to shorten by stretching the central region of the fibre (Edman & Reggiani, 1984). Sarcomeres in the ends are apparently stronger than those in the centre. When the load clamp begins, end sarcomeres will therefore start shortening faster than those in the centre. Furthermore, at the time the load clamp begins, sarcomeres in the ends are likely to be much shorter than those in the central region. Altringham & Pollack (1984) found for example that during the force rise of fixed-end tetani at 3·0 μm sarcomere length, sarcomeres in the ends shortened to a final length of, typically, 2·2 μm. Thus, sarcomeres in the ends begin the load clamp period at a short length with a velocity that is initially high. As soon they reach very short lengths they are able to generate progressively less force. Hence, the ends will decelerate.

In conclusion, the almost linear fibre velocity seems to result from a summation of accelerating sarcomeres in the central region and decelerating sarcomeres in the end regions.

An intriguing question is whether the linear behaviour of the fibre occurs by chance or to create some advantage for the animal. Fibre shortening against a constant load may be linear in order to make control of fast muscle movement easier. Linear behaviour simplifies prediction of the future and thus simplifies the control of voluntary movement. Furthermore, the constant force that is generated during linear shortening obviates the need for continuous force adjustment, and thus diminishes the chance for instabilities in the feedback control of movement (Houk & Henneman, 1967; McMahon, 1984). Thus, linear overall behaviour may carry functional advantage.

Mechanism

We consider first whether the mechanism underlying force reduction after shortening might be the same as the one that underlies force enhancement after stretch (Abbott & Aubert, 1952; Edman *et al.* 1978, 1980, 1984; Julian & Morgan, 1979; Sugi & Tsuchiya, 1981). The following observations suggest that the mechanisms may be different. (1) Force enhancement after stretch is absent at sarcomere lengths less than about 2·25 μm (Edman *et al.* 1978, 1980, 1984); force reduction, on the other hand, is quite prominent at such lengths (Figs 5–10). (2) Force enhancement is independent of stretch velocity, while force reduction does depend on the velocity of shortening (Fig. 6*C*). (3) Force enhancement after stretch is abolished by a brief (0·5–3·0 s) interruption of the tetanus (Abbott & Aubert, 1952; Julian & Morgan, 1979); this is not the case for the force deficit (Figs 9 and 10). Thus, it seems unlikely that one and the same mechanism is responsible for force enhancement and force deficit.

The mechanism underlying the force deficit may be found in either a direct effect

or an indirect effect of shortening on the contractile proteins. As for an indirect effect, reductions in shortening velocity or isometric force would ensue if the level of activation were to decrease during shortening. This might result, for example, if the calcium affinity of troponin were to decrease with cross-bridge detachment during shortening (for Discussion see Allen & Kurihara, 1982 and Cannell, 1986). A decrease of activation has been shown to underlie the decline in force observed during repeated tetani (Westerblad & Lännergren, 1987). In this situation force is largely restored by adding caffeine – which is supposed to enhance free calcium – to the fibre's bathing solution. On the other hand, we found that both force and velocity remained depressed in the presence of caffeine (Figs 7 and 13). A possible explanation is that activation is reduced by pre-shortening but that caffeine did not increase the calcium concentration in our experiments. Against this explanation is our finding that caffeine did affect isometric force of both twitches and tetani in experiments carried out in a way similar to Lopez *et al.* (1981). These investigators showed that the effects of caffeine (and other potentiators) on isometric force were accompanied by enhanced calcium levels. It thus seems likely that caffeine also increased the calcium concentration in our experiments. Therefore, our results imply that the deficit in contractile performance does not arise from submaximal activation.

This interpretation is supported by measurement of calcium concentration in contractions that are isometric except for a brief shortening phase (Allen, 1978; Cecchi *et al.* 1984). It was found that the calcium level was unaltered during shortening (see, for example, Fig. 1 of Cecchi *et al.* 1984). During the isometric phase following shortening, the concentration fell transiently, but once the new force plateau was reached the concentration had recovered to a level similar to that present prior to shortening. It appears that activation is normal both during shortening and during the plateau of the subsequent isometric phase. Thus, although conclusive results can only be obtained by direct calcium measurement under our experimental conditions, at the present stage evidence suggests that the force and velocity deficits do not result from a diminished activation level.

Other factors that might mediate effects of shortening on the contractile proteins are proton (H^+) and inorganic phosphate (P_i) concentrations, both of which increase during contraction (Kushmerick, 1983). In skinned fibres it has been shown that such increases reduce isometric force and velocity of shortening (Fabiato & Fabiato, 1978; Metzger & Moss, 1987; Cooke, Franks, Luciani & Pate, 1988). The increase of H^+ and P_i most probably results, directly or indirectly, from ATP consumption (Kushmerick, 1983; Woledge, Curtin & Homsher, 1985; Sahlin, 1986). It is therefore to be expected that H^+ and P_i concentrations will increase even further when work has to be performed, since ATP consumption increases steeply with the amount of work done (Kushmerick, 1983; Woledge *et al.* 1985). Thus, force and velocity deficits after previous shortening could result from the enhanced production of protons and P_i during shortening.

In agreement with the above view is the slow recovery of the force deficit (Figs 9 and 10). The return of both H^+ and P_i concentrations to baseline values is a process that takes many minutes (Wilkie, Dawson, Edwards, Gordon & Shaw, 1984; Dawson, 1988; Yamada, Tanokura, Kawano & Kitano, 1988). On the other hand, the finding that shortening velocity is more sensitive to previous shortening than isometric force is in disagreement with the H^+ and P_i hypothesis. The reverse is

predicted: Cooke *et al.* (1988) found in skinned fibres that both H^+ and P_i depress the isometric force considerably more than the unloaded shortening velocity. Thus, the effects of shortening could be mediated through products of ATP consumption, but the evidence is conflicting.

As for direct effects, if contraction were to occur according to the cross-bridge model (A. F. Huxley, 1957; Huxley & Simmons, 1971), force and velocity should be independent of previous shortening. However, velocity and force might be reduced, in theory, if as a result of previous shortening cross-bridge distribution were to be different in the test and control contractions. If some cross-bridges did not detach after their power stroke, but instead 'hung on' for some time, filament sliding would be hampered. Furthermore, such cross-bridges would not be available for force development, and could even generate negative force, i.e. force in the direction of the Z-line. Subsequent isometric force development would then be reduced. This explanation is rendered unlikely, though, by the results of our quick-release experiments. In the example shown in Fig. 8, the release amounted to 45 nm/half-sarcomere. This is about four times the putative cross-bridge stroke (Huxley & Simmons, 1971), and slightly more than the cross-bridge repeat (H. E. Huxley, 1969). Such a shortening step would result in a large negative stress on the S-2 of those cross-bridges that 'hung on' and would strongly promote detachment. Force might then be expected to recover after the length step. The opposite was the case: the force plateau was reduced by the length step (Fig. 8). It seems unlikely therefore, that the cross-bridge distribution at the moment when the force deficit is measured is affected by pre-shortening. This is in agreement with X-ray diffraction measurements (Yagi & Matsubara, 1984; Matsubara & Yagi, 1985): the 14·3 nm meridional reflection during activation does not display a long-lasting effect of pre-shortening but instead returns to isometric values within about 1 s after termination of shortening. Thus, if the deficit in contractile performance were to result from a direct effect of shortening on the contractile proteins, our findings appear to disagree with the cross-bridge theory.

On the other hand, the contractile mechanism might be different from the one proposed in the cross-bridge model. Several alternatives have been proposed (Iwazumi, 1970; Harrington, 1971; Tirosh, Liron & Oplatka, 1978; Pollack, 1984). Those models in which contraction is brought about by a helix-coil phase transition of a small segment of the S-2 (Harrington, 1971; Pollack, 1984) are especially interesting *vis-à-vis* the deficit in contractile performance after previous shortening. The phase transition will have to be reversed after the shortening potential of this S-2 segment has been exhausted. However, this reversal might not occur (or not occur completely) under activating conditions; the S-2 might favour the coil conformation under those conditions, to promote force generation upon activation. Extensive sarcomere shortening might then result in a decrease in the number of force generators that contribute to force generation or shortening. Thus, the deficit in contractile performance after pre-shortening seems, on first sight, to agree with these models. Furthermore, a second shortening step is predicted to result in an increased force deficit, since the number of force generators that reach their shortening limit will increase further during the second shortening phase. Thus, the result in Fig. 8 seems to agree with contraction models based on helix-coil phase transitions.

On the other hand, the rest period of many minutes required before the effects of previous shortening have been reversed (Figs 9 and 10) would then imply that even under relaxing conditions reversal of the helix-coil phase transition is a very slow process, which is not expected. The variability found in the force deficit (Fig. 7) is also surprising if the deficit were to reflect a primary property of the contractile proteins. Thus, the deficit seems to be induced by some factor outside the contractile proteins. If this factor were to be proton concentration, for example, the variability could be a natural consequence of the slightly different proton concentrations found in different resting frog fibres (Curtin, 1988).

Conclusion

Shortening clearly impairs subsequent contractile performance. The slow recovery of this depression is surprising, giving the muscle's need to contract repetitively, and implies that the phenomenon's origin may lie outside the contractile proteins themselves. The increase of proton and inorganic phosphate concentrations predicted to occur with shortening are certainly candidates. However, the sensitivity to previous shortening of the force and velocity deficits are opposite to that anticipated from the H^+ and P_i hypothesis. Clearly, further work will be required to determine the nature of the mechanism.

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