Journal of Physiology (1993), **466**, *pp.* 535–552
With 8 figures
Printed in Great Britain

DEPRESSION OF TETANIC FORCE INDUCED BY LOADED SHORTENING OF FROG MUSCLE FIBRES

By K. A. P. EDMAN, C. CAPUTO and F. LOU

From the Department of Pharmacology, University of Lund, Sölvegatan 10, S-223 62 Lund, Sweden

(*Received 10 August 1992*)

SUMMARY

1. Single fibres isolated from the anterior tibialis muscle of *Rana temporaria* were allowed to shorten against a high load during a 2·5–4·0 s fused tetanus (1–3 °C) and the maximum force produced at the short length was compared with that recorded during a fixed-end tetanus at the same overall fibre length. Changes in length of marked, consecutive segments (*ca* 0·5 mm in length) along the fibre were measured throughout the tetanus using a photoelectric recording system.

2. Loaded shortening (load *ca* 3/4 of maximum tetanic force) starting from approximately 2·55 μm sarcomere length and ending near slack fibre length depressed the tetanic force by 13 ± 2 % (mean $\pm$ S.E.M., $n = 10$) and caused a marked redistribution of sarcomere length along the fibre. Unloaded shortening over the same range caused no force deficit and did not lead to increased dispersion of sarcomere length.

3. Loaded shortening below slack length produced less force depression and less non-uniformity of sarcomere length than did a corresponding intervention above slack length.

4. The force deficit after loaded shortening, both above and below slack fibre length, was positively correlated ($P < 0{\cdot}005$) to the coefficient of variation of the sarcomere length along the fibre.

5. The decrease in active force after loaded shortening, and its relation to increased dispersion of sarcomere length along the fibre, could be simulated closely by a computer model in which the muscle fibre was assumed to consist of eleven discrete segments acting in series with a passive elastic element.

6. Experiments were performed in which the length of an individual segment of the intact muscle fibre was strictly controlled throughout a tetanus. Loaded shortening of such a 'length-clamped' segment caused *no* force depression during the subsequent isometric phase either above or below slack fibre length.

7. The results suggest strongly that force depression after loaded shortening of a single muscle fibre is attributable to non-uniform sarcomere behaviour along the fibre. The experimental evidence supports the view that: (i) the myosin cross-bridges act as independent force generators; and (ii) their steady-state performance during a tetanus is unaffected by the preceding contractile activity.

INTRODUCTION

Striated muscle that is released to shorten against a high load during tetanus produces less force after the end of shortening compared with a fixed-end tetanus at the corresponding length. This phenomenon, observed by Abbott & Aubert (1952) in dogfish muscle, has been further explored in subsequent studies on whole muscle and single muscle fibres of the frog (Délèze, 1961; Maréchal & Plaghki, 1979; Julian & Morgan, 1979; Sugi & Tsuchiya, 1988; Granzier & Pollack, 1989). Abbott & Aubert (1952) discussed the possibility that various parts of a muscle fibre may perform differently during shortening and stretch but considered it unlikely that non-uniform contractile behaviour along the muscle would be the true cause of the decrease in force after loaded shortening. Maréchal & Plaghki (1979) using whole skeletal muscle of the frog, characterized the force deficit after loaded shortening in further detail. They found that the decrease in force was a function of the mechanical work performed during the shortening phase, i.e. the force deficit was found to increase with both load and amplitude of shortening. An intriguing hypothesis, based on strain of the free portion of the thin filament during force production, was advanced by Maréchal & Plaghki (1979) in explaining the phenomenon.

The question of non-uniform sarcomere behaviour as a cause of the force deficit after loaded shortening was taken up afresh by Julian & Morgan (1979) in a study of frog single muscle fibres. A spot-follower technique was used to monitor length changes of a central segment relative to the length of the fibre as a whole. Evidence was presented to show that loaded shortening is unequally distributed along the length of the fibre, and it was proposed on the basis of these findings that non-uniformity of sarcomere length is the main cause of the force deficit. Two subsequent studies, likewise performed on single muscle fibres, have confirmed the occurrence of non-uniform length changes during loaded shortening (Sugi & Tsuchiya, 1988; Granzier & Pollack, 1989). However, the conclusion drawn in the latter studies was that the decrease in force after loaded shortening is not attributable to non-uniform sarcomere behaviour but rather to fatigue, or some equivalent thereof (Granzier & Pollack, 1989), or to some other, as yet unidentified change in the contractile system (Sugi & Tsuchiya, 1988).

The present experiments were performed to further elucidate the role of non-uniform sarcomere behaviour in the development of force depression after loaded shortening in striated muscle. With the technique used it was possible to monitor the complex pattern of length changes that occur along a muscle fibre during tetanic activity and to correlate these changes with the depression of force. Experiments have been performed to find out if the force deficit after loaded shortening can be eliminated by allowing a small segment of the intact fibre to shorten and develop force under strict control of the segment's length. The experimental results, supplemented by computer simulations, would seem to make clear that force depression after loaded shortening is a consequence of non-uniform sarcomere behaviour along the fibre. Some of the results have previously been reported in a preliminary form (Edman & Caputo, 1991).

METHODS

Preparation and mounting

Experiments were performed on muscles from cold-adapted (+4 °C) *Rana temporaria* that were killed by decapitation and destruction of the spinal cord. Single fibres were dissected from the anterior tibialis muscle using fine-tipped scissors. The fibres were mounted horizontally in a thermostatically controlled Perspex chamber between two electromagnetic pullers, one of the pullers being provided with a force transducer to which the fibre was attached. The force transducer, muscle chamber and electromagnetic pullers have been described previously (Edman & Reggiani, 1984). The tendons were held by aluminium clips that were attached to the hooks of the force transducer and puller arm, respectively. By this approach it was possible to minimize any lateral, vertical or twisting movements of the fibre during stimulation (see further Edman & Reggiani, 1984). The lateral displacement of the fibre during isometric contraction was < 15 μm as measured near the tendon insertions.

Fibre length, sarcomere length and cross-sectional area were measured as described by Edman & Reggiani (1984).

The bathing solution had the following composition (mM): NaCl, 115·5; KCl, 2·0; $CaCl_2$, 1·8; $Na_2HPO_4 + NaH_2PO_4$, 2·0; pH 7·0. The solution was precooled and was continuously perfused through the muscle chamber (volume *ca* 2·5 ml) at a rate of approximately 2 ml min^{-1}. The temperature varied between 1–3 °C among the different experiments but was maintained constant to within 0·2 °C during any given experiment.

Stimulation

Two platinum plate electrodes were placed on either side of the fibre approximately 2 mm from it covering its entire length. The fibre was stimulated by passing rectangular current pulses of 0·2 ms duration between the electrodes. The stimulus strength was approximately 15 % above threshold. A train of pulses of appropriate frequency (14–20 Hz) was used to produce a fused tetanus of 2·5–4·0 s duration. The pulse frequency used was just sufficient to produce complete mechanical fusion in the individual fibre at the sarcomere length considered and was adjusted, if necessary, during the experiment. The fibre was tetanized at regular, 3 or 4 min intervals throughout the experiment.

Segment length recording

Changes in length of discrete segments of the muscle fibre were recorded using a modified version of the surface marker technique previously described (Edman & Reggiani, 1984). The segments were delineated by opaque markers of letter-press (Mecanorma, thickness 10 μm) that were placed at 0·5–0·7 mm distances on the upper surface of the fibre. The endmost markers were placed approximately 0·3 mm from the edge of the tendon, i.e. 0·4–0·5 mm from the fibre–tendon junctions. The markers were cut to a size of approximately 75×150 μm and were gently pressed against the upper surface of the fibre with the glue side facing upwards. Care was taken to attach each marker in a perpendicular orientation with respect to the fibre axis. The markers were generally spaced along the entire length of the fibre, each site selected for a marker being carefully freed of connective tissue. With the approach used it was possible to obtain distinct marker signals and excellent adherence of the markers, even during rapid length perturbations of the fibre (see Edman & Lou, 1990). The time resolution of the segment length measurement was 40 μs.

In the majority of experiments the length of individual segments was recorded while the overall length of the fibre was controlled, i.e. either during fixed-end tetani or during tetani in which a shortening ramp of selected speed and amplitude was imposed on the fibre. Recording was made from only one segment during each stimulation period. A given type of contraction was therefore repeated, with strict timing of the individual contractions, until all fibre segments had been explored. In order to keep a given segment within the domain of the photodiode array during long-range shortening it was necessary to induce a movement from both ends of the fibre so as to minimize the longitudinal translation of the fibre segment during shortening. This could be achieved by dividing the movement between the two pullers appropriately. The ramps produced by the pullers had the same speed and were carefully timed to provide a continuous phase of shortening of the fibre during the tetanus.

Some experiments were performed in which the length of a selected segment was governed (segment-length clamp) by adjusting the overall length of the fibre by feedback control of one of the electromagnetic pullers. The switch to segment-length control was made prior to stimulation and the system was maintained in the 'segment-length clamp' mode until the 'shoulder' of the relaxation phase of the tetanus had been passed. Using this approach it was possible to make a segment shorten at a given speed to a preselected sarcomere length to be held stationary at this length for the remainder of the activity period. This was achieved by altering the reference level of the feedback system by means of a voltage ramp. In these recordings the second puller was used to assist in producing the required fibre shortening, a necessity in order to make the segment stay within the range of the photodiode array (cf. above).

The segment-length signal generally exhibited a few small steps during a shortening ramp due to slight differences in the illumination of the markers as the segment was translated within the light field (Fig. 8*A* and *B*). The occurrence of these steps (amplitude 1–2 nm (half-sarcomere)$^{-1}$ caused some irregularity of the tension trace during loaded shortening reflecting the high stiffness of the contractile system under these conditions (Fig. 8*A* and *B*). As previously demonstrated (Fig. 1 in Edman & Lou, 1990), a length perturbation of this amplitude does not, however, affect the fibre's capacity to produce force.

Recordings of signals

The experimental data were recorded and measured using a Nicolet 4094 B digital oscilloscope that was provided with a dual disk recorder (Nicolet, XF44).

Computer model

A computer model of a multisegment muscle fibre preparation was used to simulate isometric and isotonic tetani. The model was essentially the same as that previously described for fixed-end tetani (Edman & Reggiani, 1984) but was supplemented in the present study also to enable a length change of selected speed and amplitude during the tetanus. For the present simulations the fibre was assumed to consist of eleven consecutive segments (approximately 0·5 mm in length) acting in series with an elastic element. The contractile properties of the individual segments (Table 1) were selected to conform with the mechanical performance of intact muscle fibres taking account of the differences in contractile behaviour that normally exist along the length of single muscle fibres. Further details of the computer model are given in the Appendix of Edman & Reggiani (1984). However, no depressant effect of active shortening ('shortening-induced deactivation') was included in the present version of the model.

In the computer model the force–velocity relation was assumed to have the double-hyperbolic shape that has previously been described using the following equation (Edman, 1988):

$$V = \frac{(P_0^* - P)b}{P + a}\left(1 - \frac{1}{1 + e^{-k_1(P - k_2 P_0)}}\right).$$

V here denotes the velocity of shortening, P the load on the muscle fibre, P_0 the measured isometric force, P_0^* the isometric force predicted from the hyperbola derived below 0·78 P_0. The constants a and b represent the asymptotes of the rectangular hyperbola below 0·78 P_0 and have dimensions of force and velocity, respectively. The constant k_1 determines the steepness of the high-force curvature and has the dimension of 1/force. k_2, which is dimensionless, determines the point of transition between the two curvatures of the force–velocity relation. The above force–velocity relation was preferred to the standard single-hyperbolic function (Hill, 1938) as it enabled a more faithful simulation of the time course of the tetanus. The slope of the force–velocity relation at loads greater than P_0 was assumed to be 0·2 μm sarcomere^{-1} s^{-1} P_0^{-1}. This value accords well with the shallow part of the force–velocity relation recorded between P_0 and 1·20 P_0 (Edman, 1988).

Statistics

Student's t test was used for determination of statistical significance. All statistics are given as means ± S.E.M.

RESULTS

Force deficit after loaded shortening studied at the whole-fibre level

Shortening along the descending limb of the length–tension relation

Single muscle fibres, prestretched to a resting sarcomere length of approximately

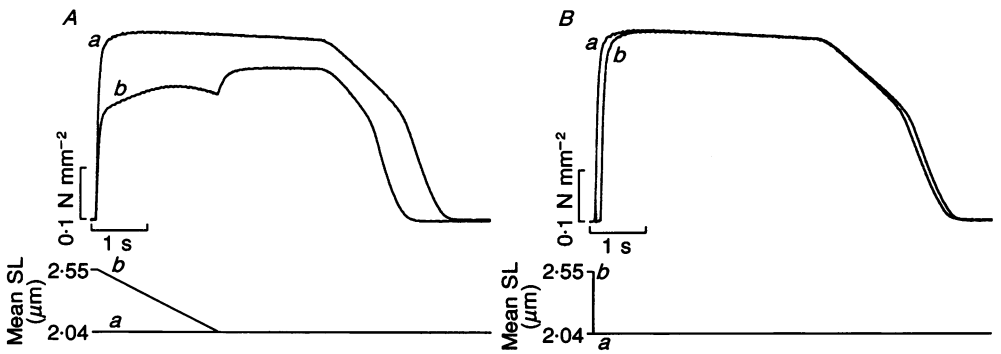

Fig. 1. Superimposed oscilloscope records illustrating force development after an initial phase of loaded (*A*) and unloaded (*B*) shortening of a single muscle fibre above slack length (myograms *b*). Control tetani (myograms *a*) performed at the length where the shortening ended in myograms *b*. Note marked reduction of force after loaded shortening but no depression of force after unloaded shortening. Fibre length at 2·20 μm sarcomere length (SL), 7·00 mm. Temperature, 1·0 °C.

2·5 μm, were stimulated at regular 3 or 4 min intervals to produce a fused isometric tetanus of 2·5–4·0 s duration. During the rising phase of the tetanus the fibre was released to shorten over a given distance, 0·25–0·5 μm sarcomere^{-1}, to redevelop force at the new length. Two velocities of shortening were used in different runs: (1) a relatively low speed, 0·05–0·11 lengths s^{-1}, that constrained the fibre to keep a high tension during the shortening phase; and (2) a high velocity that allowed the fibre to shorten at virtually zero load. As a control the fibre was also stimulated to produce a fixed-end tetanus at the short length. Results from a typical experiment are illustrated in Fig. 1. It can be seen that the force developed after loaded shortening was substantially lower than that produced in the isometric control. In the example given in Fig. 1*A* the force deficit during the isometric phase after shortening amounted to 16 % of the control value. By contrast, the force produced after unloaded shortening was not significantly different from that of the control tetanus at the short length (Fig. 1*B*).

Figure 2*A* illustrates changes in length of consecutive segments along a muscle fibre during loaded shortening above slack length. The tetanus was initiated at a mean sarcomere length of 2·55 μm, and the fibre was released to shorten at a low speed (0·11 lengths s^{-1}) to approximately 2·18 μm sarcomere length. Figure 2*B* shows a series of recordings from the same fibre during a fixed-end tetanus performed at about the same overall fibre length as that reached after shortening in *A*. As can be seen the behaviour of the individual segments was fairly uniform

during the fixed-end tetanus, the sarcomere length varying by merely 0·11 μm, from 2·16 to 2·27 μm, along the length of the fibre. Loaded shortening, on the other hand, led to a wide distribution of sarcomere length in that the speed of shortening varied greatly from one segment to another. The sarcomere length finally attained

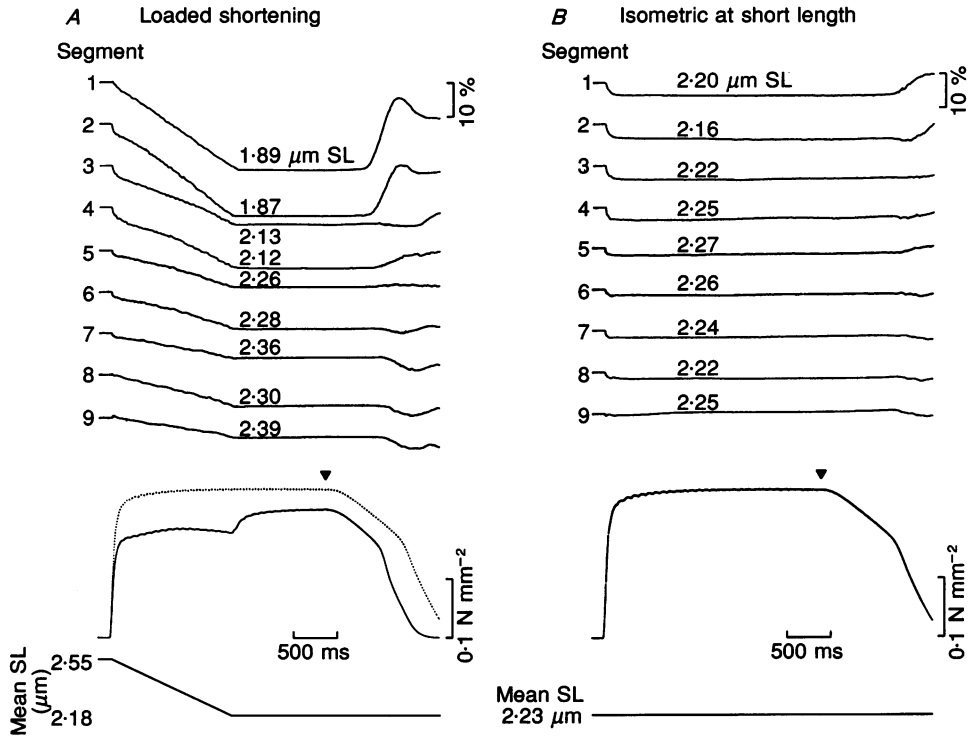

Fig. 2. Oscilloscope records illustrating changes in length of consecutive segments along a muscle fibre during tetanus. *A*, contraction initiated at approximately 2·55 μm sarcomere length and fibre allowed to shorten against a high load to a mean sarcomere length of 2·18 μm, at which point the ends of the fibre were fixed for the remainder of the tetanus period. *B*, fixed-end tetanus at short fibre length without preshortening. Upper sets of records in each panel are segment-length traces. Segment-length scale, percentage of initial sarcomere length. The sarcomere length recorded at the end of the tetanus plateau (indicated by triangle above the force myograms) is given for each segment. Lower sets of records, tetanic force (N mm^{-2}) and puller position (mean sarcomere length calculated from the values given at the segment-length records in each panel). The force myogram of the right-hand panel is superimposed as a dotted line on the left-hand myogram for comparison. Note force depression and marked non-uniformity of the segment length pattern after loaded shortening. Fibre length at 2·20 μm sarcomere length, 6·20 mm. Temperature, 1·5 °C.

after the shortening phase ranged between 1·87 and 2·39 μm among the different segments. The dispersion of sarcomere length was thus nearly 5 times larger than that observed in the isometric control.

After the shortening phase was over and the ends of the fibre were fixed, there was a further, slow redistribution of length along the fibre in that stronger

segments continued to shorten at a very low rate while weaker segments were correspondingly stretched. Measurements from records like those displayed in Fig. 2*A* showed that the speeds of shortening and elongation of individual segments during the 'isometric' phase generally did not exceed 1 % of maximum

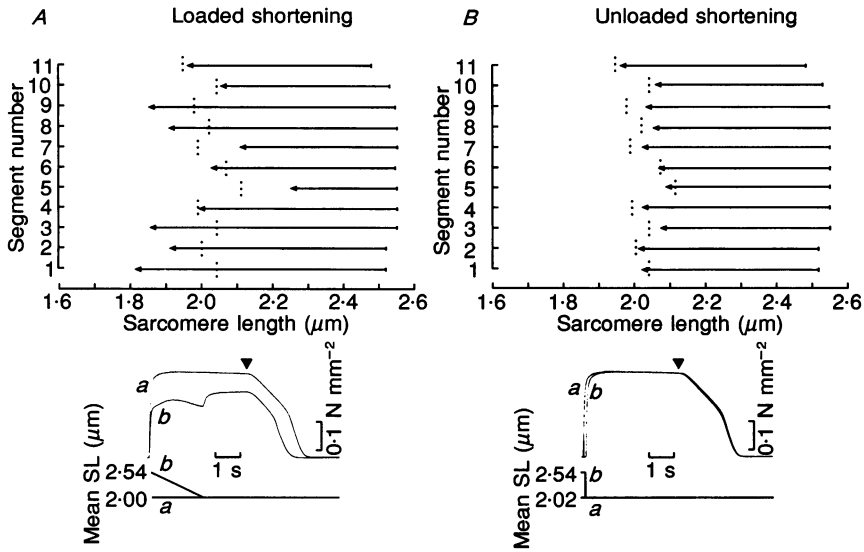

Fig. 3. Differential length changes along a single muscle fibre during tetanus with loaded (*A*) and unloaded (*B*) shortening, respectively. The change in sarcomere length from rest to end of tetanus plateau (triangle above myograms) is indicated by horizontal arrows for 11 consecutive segments. Dotted vertical lines indicate the sarcomere length in the same segments during a fixed-end tetanus at the short fibre length. The superimposed records below the diagrams indicate force and puller position during control tetanus at short length (myograms *a*) and during tetanus with shortening (myograms *b*). Note that whereas loaded shortening leads to a wide redistribution of sarcomere length, the sarcomere pattern after *unloaded* shortening is not markedly different from that of the fixed-end tetanus. Fibre length at 2·20 μm sarcomere length, 7·10 mm. Temperature, 0·8 °C.

velocity (V_{max}). Similar low velocities were measured during the plateau phase of the control tetanus at the sarcomere lengths considered.

The wide distribution of sarcomere length observed after loaded shortening did not appear when the load was small during the shortening phase. This is illustrated in Fig. 3, which demonstrates changes in sarcomere length along a muscle fibre during loaded (*A*) and unloaded (*B*) shortening above slack length. The change in sarcomere length from rest to maximum isometric force at the short length (measured at the end of the tetanus plateau) is indicated here for each of eleven consecutive segments along the fibre. For comparison, the sarcomere length recorded during a fixed-end tetanus at the short fibre length is also shown for the same fibre segments (dotted vertical lines). The results show that the distribution of sarcomere length after unloaded shortening was not markedly different from that recorded in the isometric control. In accordance, the force developed after unloaded

shortening did not differ from that of the control tetanus. Shortening under a heavy load, on the other hand, led to a large scatter of sarcomere length along the fibre associated with a marked depression of the isometric force after the movement.

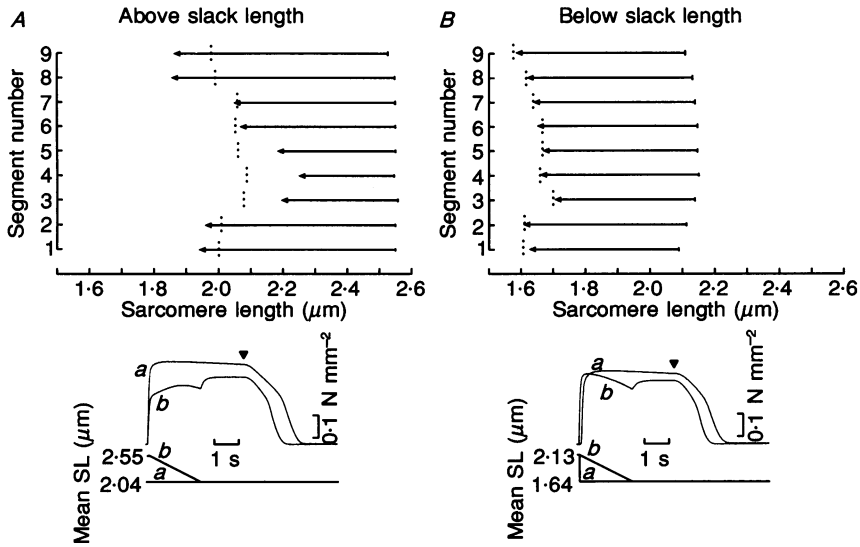

Fig. 4. Differential length changes along a single muscle fibre during tetanus with loaded shortening *above* (*A*) and *below* (*B*) slack fibre length. Experimental design as in Fig. 3 with the addition that the control tetanus below slack length contained an initial phase of unloaded shortening. For further explanation, see legend of Fig. 3. Note that loaded shortening causes less dispersion of sarcomere length below slack length. Fibre length at 2·20 μm sarcomere length, 7·00 mm. Temperature, 1·0 °C.

Shortening below slack fibre length

The force deficit after loaded shortening may be expected to be smaller below slack fibre length where the sarcomere pattern is likely to remain more uniform during contraction than at greater lengths (Edman & Reggiani, 1987). Figure 4 shows an experiment performed to test this point. Contraction was initiated here at either of two sarcomere lengths, 2·55 and 2·13 μm, and the fibre was allowed to shorten by a given amount (approximately 0·50 μm sarcomere^{-1}) during the first part of the tetanus. The low speed of shortening (about 0·10 lengths s^{-1}), which was the same in both cases, made the fibre keep a high tension during the shortening phase. The isometric force produced after *unloaded* shortening was used as a control below slack length based on the previous observation (Figs 1 and 3) that unloaded shortening does not affect the fibre's force-producing capability or increase the dispersion of sarcomere length relative to that produced during a fixed-end tetanus. As can be seen in Fig. 4, loaded shortening below slack length caused a smaller force deficit and a less pronounced redistribution of sarcomere length than did a corresponding movement at greater length. In four experiments, including that

shown in Fig. 4, loaded shortening initiated at a mean sarcomere length of 2·07 μm reduced the force by 6 ± 1 % of the control value. This is to be compared with a force deficit of 13 ± 2 % ($n = 10$) when a loaded shortening of similar speed and amplitude was performed from an initial sarcomere length near 2·5 μm.

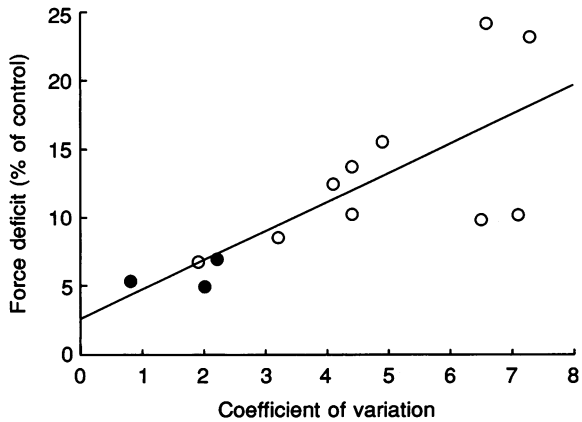

Fig. 5. Relation between force deficit after loaded shortening (ΔF) and coefficient of variation (K_v) of sarcomere length along the muscle fibre. K_v is the standard deviation expressed as percentage of the mean. ○, shortening above slack length; ●, shortening below slack length. The regression of ΔF upon K_v is indicated by line: $\Delta F = 2{\cdot}10\ K_v + 2{\cdot}55$ ($P < 0{\cdot}005$).

In Fig. 5, which summarizes results from thirteen experiments, the force deficit after loaded shortening has been plotted against the variation of sarcomere length along the fibre. The results show that, over the range investigated, there was a positive correlation between the magnitude of force deficit after loaded shortening and the degree of non-uniformity of sarcomere length along the fibre.

Reversal of force deficit induced by loaded shortening

Previous experiments have demonstrated that the decrease in active force after loaded shortening is not maintained when the muscle is restimulated after a brief period of relaxation (Abbott & Aubert, 1952; Julian & Morgan, 1979). In the light of the evidence presented in the foregoing section it was of interest to investigate if restoration of active force after relaxation is associated with a change towards a more uniform sarcomere pattern along the muscle fibre. Figure 6 illustrates an experiment designed to investigate this point.

The protocol was essentially the same as that employed in the preceding experiments, i.e. the fibre was stimulated to produce a 4 s tetanus, with or without loaded shortening, at 4 min intervals. In the present experiments, however, each 4 s tetanus was followed by a second tetanus of 1·5 s duration starting 10 s from the end of the preceding stimulation volley. As is evident from Fig. 6*A*, the second tetanus occurred at the same overall fibre length in both test and control runs.

The results confirm that loaded shortening reduces the tetanic force and leads to

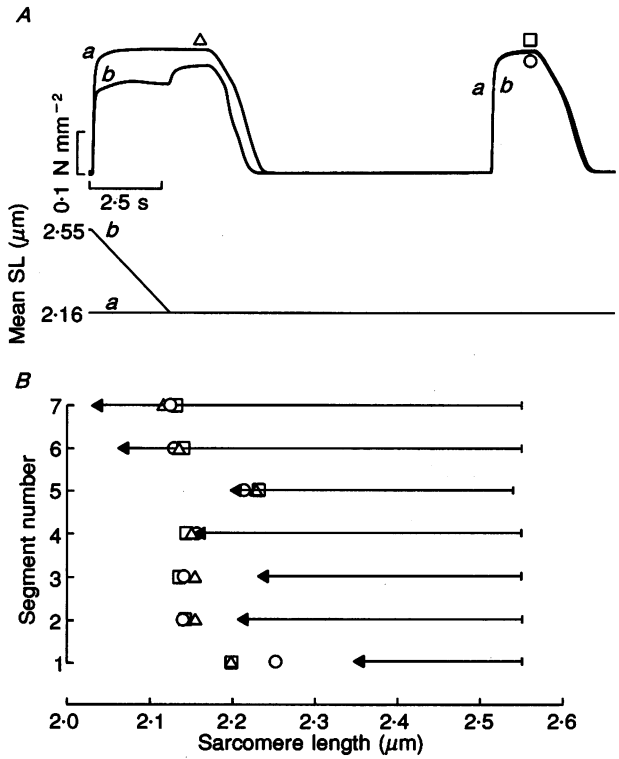

Fig. 6. Disappearance of force depression after loaded shortening by interposing a brief period of relaxation. The superimposed myograms in *A* show the following stimulation sequence which was repeated every 4 min: 4 s tetanic stimulation followed by a 10 s pause and a new, 1·5 s period of tetanic stimulation. In myogram *b* the fibre was allowed to shorten against a high load from a mean sarcomere length of 2·55 to 2·16 μm during the 4 s tetanus. In myogram *a* (control) the fibre was held at the short length throughout. Note that the force deficit produced by loaded shortening during the first tetanus is no longer seen when tetanization is resumed after relaxation. The horizontal arrows in *B* show the change in sarcomere length of successive segments along the fibre during the 4 s tetanus with loaded shortening. △, □, and ○ indicate the sarcomere length in the same segments at the times shown at respective myogram in *A*. Note that the force deficit after loaded shortening is associated with a grossly irregular sarcomere pattern along the fibre and that the length pattern returns to normal, in parallel with restoration of the tetanic force, as the fibre is restimulated after a brief period of relaxation. Fibre length at 2·20 μm sarcomere length, 5·20 mm. Temperature, 1·0 °C.

a considerably wider distribution of sarcomere length than that recorded in the isometric control. The latter point is evident by comparing the arrowheads and the open triangles in Fig. 6*B*. Furthermore, the force-producing capability can be seen to be almost completely restored after a 10 s pause at the short length (compare second tetani in myograms *a* and *b* of Fig. 6*A*). The results also clearly show that the recovery of force was associated with the reappearance of a more uniform

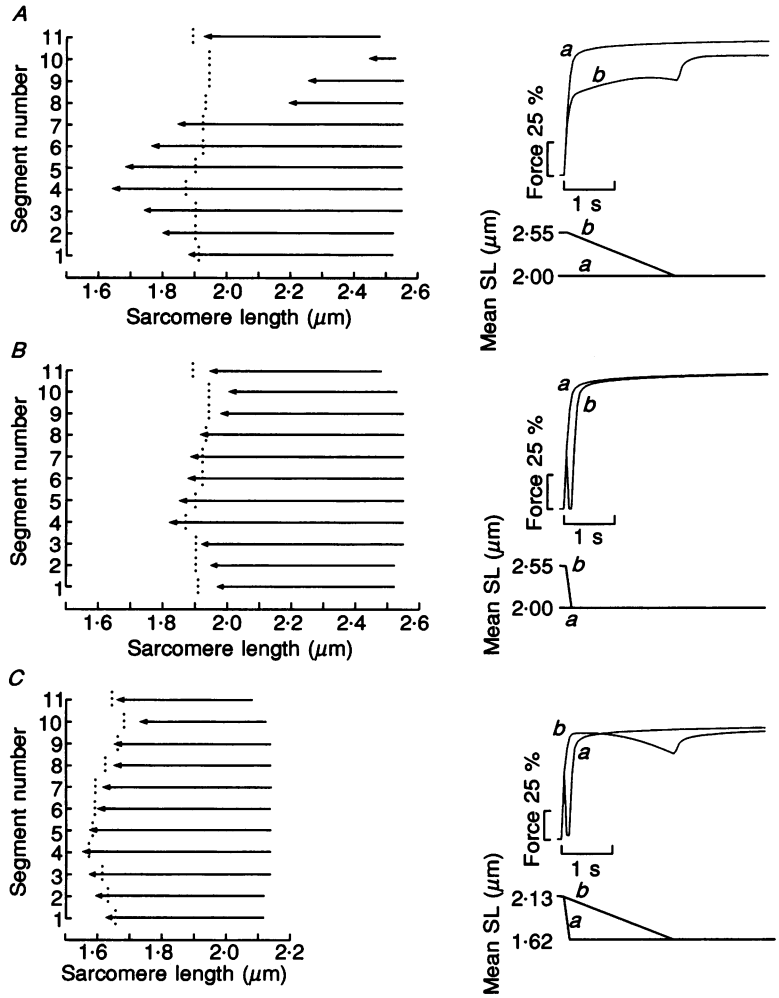

Fig. 7. Computer simulation of force deficit after loaded shortening and the associated redistribution of length along the muscle fibre. The conditions used in *A–C* were chosen to correspond to the experiments illustrated in Figs 3 and 4. Myograms *b* in the right-hand panel indicate a 4 s tetanus with an initial phase of shortening; superimposed myograms *a* are control tetani at the short fibre length. The simulated puller position (calibrated in mean sarcomere spacing) is indicated below the force traces. The horizontal arrows in the left-hand diagrams indicate changes in sarcomere length of consecutive segments as the fibre goes from rest to maximum force during a tetanus that includes shortening. The dotted vertical lines indicate the sarcomere length in the same segments during a fixed-end tetanus at the short fibre length. *A*, loaded shortening above slack length. *B*, unloaded shortening above slack length. *C*, loaded shortening below slack length. The control tetanus below slack length contains an initial phase of unloaded shortening as in Fig. 4*B*. Note excellent agreement between the computer simulation and the experimental results demonstrated in Figs 3 and 4.

sarcomere pattern along the fibre. Apart from some minor deviations the segment length pattern recorded during restimulation after a tetanus with loaded shortening was quite similar to that observed in the control run (compare circles with triangles and squares in Fig. 6 *B*).

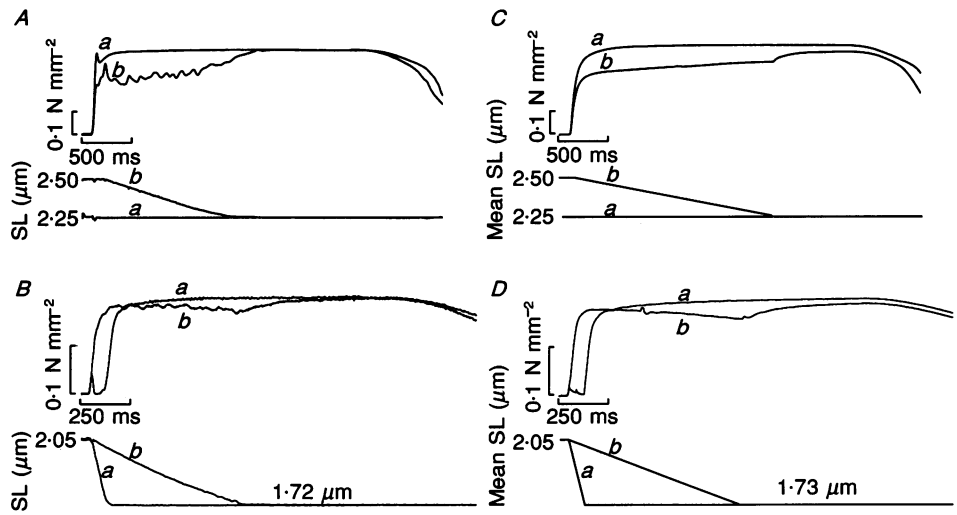

Fig. 8. Lack of force deficit after loaded shortening of length-controlled segment of single muscle fibres above (*A*) and below (*B*) slack length. *C* and *D* show, for comparison, the effects of loaded shortening when recording is made from the fibre as a whole. The same fibre in *A* and *C* and in *B* and *D*, respectively. Myograms *a*, control tetani; myograms *b*, tetani with loaded shortening. Control tetani below slack length contain an initial phase of unloaded shortening. Fibre length at 2·20 μm sarcomere length and temperature were: *A* and *C*, 5·40 mm, 0·8 °C; *B* and *D*, 6·60 mm, 0·9 °C.

TABLE 1. Numerical values of the mechanical parameters used for individual fibre segments in computer simulation

	Segment number										
	1	2	3	4	5	6	7	8	9	10	11
P_0 (units)	1·00	1·03	1·05	1·07	1·04	1·00	0·98	0·96	0·97	0·93	0·96
V_{max} (L s^{-1})	1·60	1·60	1·75	2·00	2·00	2·00	2·00	1·90	1·70	1·60	1·60

The following parameters were given the same numerical values in all segments: $a/P_0 = 0{\cdot}17$; $k_1 = 33\ P_0^{-1}$; $k_2 = 0{\cdot}83$; τ (half-time of function describing onset of activation, see Edman & Reggiani, 1984) = 0·02 s. The resting sarcomere length of the individual segments is indicated in Fig. 7. L, length. For further information, see text.

Computer simulation of force depression by loaded shortening

An attempt was made to simulate the decrease in tetanic force after loaded shortening using a computer model similar to that described by Edman & Reggiani (1984). According to this model the fibre was assumed to consist of eleven consecutive segments, approximately 0·5 mm in length, acting in series with an

elastic component. The mechanical properties of the various segments were chosen to correspond to those previously identified in intact fibres (see Methods). The model thus included segmental differences in maximum speed of shortening, maximum isometric force and resting sarcomere length. Table 1 summarizes the numerical values of the mechanical parameters used in the simulation.

Figure 7 *A–C* shows computer simulations analogous to the experiments presented in Figs 1–3. The results obtained accord remarkably well with the observations made on the intact fibres. Loaded shortening above optimum sarcomere length (*A*) can thus be seen to reduce the contractile force during the subsequent isometric phase in association with a marked redistribution of sarcomere length. Unloaded shortening, on the other hand, does not lead to any substantial increase in dispersion of sarcomere length, nor does it cause any force deficit (*B*). Furthermore, in accordance with the results obtained in intact fibres, loaded shortening below optimum length (*C*) causes less sarcomere redistribution and less force deficit than does the same movement above optimum length.

In the above simulations the segmental variation of P_0 is the main cause of the redistribution of sarcomere length that occurs during loaded shortening. However, the assumed differences in *resting* sarcomere length also contribute to the development of sarcomere non-uniformity during the shortening phase. It is of interest to point out in this connection that local differences in P_0 and in resting sarcomere length similar to those assumed in the model have been shown to exist in living muscle fibres (Edman & Reggiani, 1984; Edman, Reggiani & te Kronnie, 1985).

In agreement with the observations made in the biological preparation (see earlier) the model predicts a continuous slow redistribution of length along the fibre after the shortening phase has been completed and the ends of the fibre fixed. Similar to the situation in the living fibre the speed of the length changes predicted by the model during the 'isometric' phase do not exceed 1 % of V_{max}.

Tetanic force of length-clamped segments

The results presented above, including those derived in the computer simulation, suggest strongly that force deficit after loaded shortening is due to redistribution of sarcomere length along the fibre. Further evidence in support of this view is provided by the following experiments in which the length of individual segments of the muscle fibre was controlled throughout the tetanus. Since one of the pullers was used for the length-clamp manoeuvre in these experiments, the amplitude of loaded shortening was limited to approximately 0·25 μm sarcomere^{-1} in this part of the study. The force deficit after loaded shortening recorded at whole-fibre level (see below) was therefore somewhat smaller than that described at corresponding sarcomere lengths in preceding sections.

Figure 8 *A* shows the development of isometric force in a length-clamped segment at 2·25 μm sarcomere length without preceding shortening (*a*) and after an initial phase of loaded shortening from 2·55 μm (*b*). Figure 8 *B* illustrates similar recordings in which the tetanus was initiated at 2·05 μm sarcomere length. The segment was here allowed to shorten to 1·72 μm against a low (*a*) and high (*b*) load, respectively, during the initial part of contraction. Conventional whole-fibre

recordings from the same preparations are illustrated in Fig. 8*C* and *D* for comparison. The results show that, contrary to the situation at whole-fibre level, loaded shortening of a length-controlled segment does *not* lead to depression of the isometric force. This holds true irrespective of whether the shortening occurs above or below slack length.

The lack of force depression after loaded shortening during tetanus of a length-controlled segment was confirmed in five experiments altogether. In three of these experiments, including those presented in Fig. 8, standard recordings were also carried out on the whole preparation. The force deficit after loaded shortening recorded from the whole fibre in these three experiments was 5, 7 and 11 % of the control isometric force, respectively. In none of the five experiments with segment-length clamp recording was a clear force deficit after loaded shortening observed. The force recorded after the shortening phase in these experiments was in most runs virtually identical to the control force.

DISCUSSION

Characteristics of force depression after loaded shortening, distinction from shortening-induced deactivation

The present study provides new information on the decrease in tetanic force that occurs after loaded shortening of striated muscle. Before discussing the nature of the depressant effect it is necessary first to consider the distinction between the above phenomenon and another, seemingly related manifestation of active shortening that has previously been explored in detail and that is generally referred to as 'shortening-induced deactivation', or 'movement effect' (Edman & Kiessling, 1971; Edman, 1975, 1980; Ekelund & Edman, 1982). The 'movement effect' is likewise characterized by a reduced capacity to produce force after shortening. However, contrary to the phenomenon described in the present paper, the movement effect is of brief duration, active force being restored to normal within 1–2 s after the end of shortening during continuous stimulation. Furthermore, the movement effect is independent of the load during the shortening phase and is inversely related to the state of activity in the muscle when the shortening occurs. The movement effect is thus negligible during a fused tetanus but is prominent during a partially fused tetanus and a single twitch. Results derived from both intact and skinned muscle fibres suggest strongly that the movement effect is based on a transitory decrease in the calcium affinity of the troponin binding sites leading to a temporary deactivation of the contractile system (e.g. Edman, 1975; Ekelund & Edman, 1982; Fuchs & Wang, 1991).

The force deficit after loaded shortening clearly represents a basically different phenomenon from the movement effect. The depressant effect after loaded shortening appears during a *fused* tetanus, it increases with the load during the shortening phase and, most importantly, the force deficit is maintained for as long as the fibre is activated. These differences with respect to the movement effect would seem to rule out that the force deficit after loaded shortening is based on a transitory deactivation of the contractile system (cf. Edman, 1975 and Ekelund & Edman, 1982).

The role of non-uniform sarcomere behaviour during loaded shortening

With the surface marker technique used in the present study it was possible to elucidate the complex pattern of length changes that occurs within a muscle fibre undergoing loaded shortening. The non-uniform shortening behaviour, the precise expression of which is unique to any given fibre, reflects segmental differences in the force–velocity characteristics of the fibre. As previously demonstrated (Edman *et al.* 1985) there exist inherent differences in force-producing capability along a muscle fibre amounting to 7–8 % of the fibre mean. Such variation in isometric force may arise due to local differences in cross-sectional area and myofibrillar mass but may also reflect segmental differences in myosin isoform composition (Edman *et al.* 1985; Edman, Reggiani, Schiaffino & te Kronnie, 1988). By prestretching the fibre above slack length such differences in active force are likely to become more pronounced due to the fact that the degree of extension of the sarcomeres will differ to a certain degree along the fibre, the greatest differences in sarcomere length generally being observed between the end segments (close to the tendon insertions) and the middle region of the fibre (Huxley & Peachey, 1961; Edman & Reggiani, 1984). Previous experiments have furthermore shown that the curvature of the force–velocity relation (as also the maximum speed of shortening) may vary substantially from one region to another along the fibre due to local differences in myosin heavy chain composition (Edman *et al.* 1985, 1988). It is therefore logical that the fibre's ability to shorten against a given load will be different from one segment to another and, furthermore, that this variation in shortening potential along the fibre will become more pronounced as the load is raised and the fibre is prestretched beyond its slack length. Due to the differences in shortening speed along the fibre the individual segments will reach widely different lengths during the shortening phase (Figs 2 and 3). In the extreme case when the load equals or exceeds the force-producing capability of a given segment some part(s) of the fibre may be found to elongate, or remain stationary, while the rest of the fibre shortens (K.A.P. Edman, unpublished observations; also see Julian & Morgan, 1979).

The experimental results suggest strongly that the dispersion of sarcomere length arising during loaded shortening is the principal cause of the force deficit during the subsequent 'isometric' phase. In support of this view there is a positive correlation between the magnitude of force depression and the degree of non-uniformity of sarcomere length (Fig. 5). Furthermore, the disappearance of the force deficit after a brief period of relaxation is associated with return to a normal sarcomere pattern along the fibre.

The decrease in force is explainable as follows. Due to the extensive redistribution of sarcomere length during loaded shortening individual segments along the fibre acquire greatly different capacities to produce force. After the movement, when the ends of the fibre are fixed, the stronger segments will keep on shortening at a very low rate at the expense of the weakest segment(s) which are correspondingly stretched (see earlier). As a result the measured tension will be lower than the true isometric force of the stronger (shortening) segments but higher than the isometric force of the weakest (elongating) segment. The presence of one or more segments that are considerably weaker than any of the segments in the

control run will, according to this mechanism, lead to a decrease of the measured force. The effectiveness of this mechanism in reducing the force output is based on the fact that the force–velocity relationship is exceedingly flat within the range 85–120% of the isometric force (Edman, 1988). Thus even a very slow redistribution of length during a fixed-end tetanus will cause a relatively large reduction in force of the stronger segments to match the force held by the weakest segment.

The above mechanism of force depression after loaded shortening was further evaluated in a computer study. The analytical model (see Methods) contained the essential features of the muscle preparation used. It consisted of eleven segments acting in series with a tendon-like elastic component. The resting sarcomere length and the kinetic properties of the individual segments were assumed to differ within the limits previously determined in living fibres. An important aspect of the present analytical model was the introduction of a double-hyperbolic force–velocity relationship of the type existing in intact fibres (Edman, 1988). This provided for the flat relationship between force and velocity of shortening in the high-force range discussed above. Qualitatively similar results were obtained with the model by assuming a single-hyperbolic force–velocity relation like that used previously (Edman & Reggiani, 1984). The force deficit obtained under these conditions, however, was considerably smaller than that predicted on the basis of a double-hyperbolic force–velocity curve (K.A.P. Edman & F. Lou, unpublished data).

The analytical model simulated the experimental results with remarkable precision (Fig. 7). Thus, given the intrinsic differences in resting sarcomere length and contractile properties normally existing within a muscle fibre (Table 1), the model predicted differential length changes along the fibre during loaded shortening that were similar to those observed in the biological preparation. The model furthermore confirmed that the dispersion of sarcomere length arising during loaded shortening led to a decrease in tetanic force that well matched the force deficit observed in the living fibre. This applies to measurements both above and below the optimum of the length–tension relation.

Conclusive evidence that force depression after loaded shortening is based on non-uniform sarcomere behaviour was provided by the finding that no force deficit appeared when recording was made from only a small portion of the fibre (Fig. 8). In this experiment the length of a short (*ca* 0·5 mm) segment was carefully controlled throughout the tetanus so as to minimize any interference in the force measurement from sarcomere non-uniformity (see further Edman & Reggiani, 1984). The fact that no force deficit occurred after loaded shortening in a length-clamped segment clearly shows that non-uniform sarcomere behaviour is indeed a prerequisite for this phenomenon to occur.

The absence of force depression in length-clamped segments would seem to exclude fatigue as a possible cause of the force deficit observed after loaded shortening at whole-fibre level (Granzier & Pollack, 1989). For the same reason it is also possible to rule out that loaded shortening induces some structural change of the myofilament system that would affect the contractile performance as suggested by Maréchal & Plaghki (1979) and Sugi & Tsuchiya (1988). The present results thus

strengthen the previous conclusion (Edman, 1966) that the contractile system acquires the same potential to produce force at a given degree of filament overlap irrespective of whether the fibre is held at this particular sarcomere length from the very beginning of contraction or the fibre is allowed to preshorten to this length from a more extended position against a low or a high load. This is an important finding as it suggests that the steady-state performance of the individual cross-bridge is unaffected by the preceding contractile activity. The results thus fully support the idea that contraction is based on a sliding-filament mechanism in which the myosin cross-bridges act as independent force generators (Huxley & Niedergerke, 1954; Huxley & Hanson, 1954).

This work was supported by grants from the Swedish Medical Research Council (project no. 14X-184) and the Medical Faculty, University of Lund. C.C. was supported by a Visiting Scientist Fellowship (K89–14V-08899–01) from the Swedish Medical Research Council.

REFERENCES

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.

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. (1966). The relation between sarcomere length and active tension in isolated semitendinosus fibres of the frog. *Journal of Physiology* **183**, 407–417.

EDMAN, K. A. P. (1975). Mechanical deactivation induced by active shortening in isolated muscle fibres of the frog. *Journal of Physiology* **246**, 255–275.

EDMAN, K. A. P. (1980). Depression of mechanical performance by active shortening during twitch and tetanus of vertebrate muscle fibres. *Acta Physiologica Scandinavica* **109**, 15–26.

EDMAN, K. A. P. (1988). Double-hyperbolic force–velocity relation in frog muscle fibres. *Journal of Physiology* **404**, 301–321.

EDMAN, K. A. P. & CAPUTO, C. (1991). Force deficit following loaded shortening in isolated muscle fibres of the frog. *Journal of Physiology* **438**, 148*P*.

EDMAN, K. A. P. & KIESSLING, A. (1971). The time course of the active state in relation to sarcomere length and movement studied in single skeletal muscle fibres of the frog. *Acta Physiologica Scandinavica* **81**, 182–196.

EDMAN, K. A. P. & LOU, F. (1990). Changes in force and stiffness induced by fatigue and intracellular acidification in frog muscle fibres. *Journal of Physiology* **424**, 133–149.

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

EDMAN, K. A. P. & REGGIANI, C. (1987). The sarcomere length–tension relation determined in short segments of intact muscle fibres of the frog. *Journal of Physiology* **385**, 709–732.

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

EDMAN, K. A. P., REGGIANI, C., SCHIAFFINO, S. & TE KRONNIE, G. (1988). Maximum velocity of shortening related to myosin isoform composition in frog skeletal muscle fibres. *Journal of Physiology* **395**, 679–694.

EKELUND, M. & EDMAN, K. A. P. (1982). Shortening induced deactivation of skinned fibres of frog and mouse striated muscle. *Acta Physiologica Scandinavica* **116**, 189–199.

FUCHS, F. & WANG, Y.-P. (1991). Force, length, and Ca^{2+}–troponin C affinity in skeletal muscle. *American Journal of Physiology* **261**, C787–792.

GRANZIER, H. L. M. & POLLACK, G. H. (1989). Effect of active pre-shortening on isometric and isotonic performance of single frog muscle fibres. *Journal of Physiology* **415**, 299–327.

HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proceedings of the Royal Society* B **126**, 136–195.

HUXLEY, A. F. & NIEDERGERKE, R. (1954). Structural changes in muscle during contraction: Interference microscopy of living muscle fibres. *Nature* **173**, 971–973.

HUXLEY, A. F. & PEACHEY, L. D. (1961). The maximum length for contraction in vertebrate striated muscle. *Journal of Physiology* **156**, 150–165.

HUXLEY, H. E. & HANSON, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973–977.

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.

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

SUGI, H. & TSUCHIYA, T. (1988). Stiffness changes during enhancement and deficit of isometric force by slow length changes in frog skeletal muscle fibres. *Journal of Physiology* **407**, 215–229.