

TENSION TRANSIENTS DURING STEADY SHORTENING OF FROG MUSCLE FIBRES

BY L. E. FORD*, A. F. HUXLEY† AND R. M. SIMMONS‡

*From the Department of Physiology, University College London,
Gower Street, London WC1E 6BT*

(Received 14 September 1984)

SUMMARY

1. Single intact fibres from frog muscle at 0–1 °C were stimulated to produce isometric tetani at a sarcomere length of about 2.25 μm , using a spot-follower apparatus to control the length of the central part of a fibre. When the plateau of the tetanus was reached the fibre was forced to shorten by applying a step and ramp length change in an approximation to an isotonic release.

2. When tension had reached a steady level, T_1 , during shortening, tension transients were elicited by applying step changes of length, complete within 0.2 ms, ranging from a stretch of 1.5 nm per half-sarcomere to a release of 6 nm per half-sarcomere.

3. The tension transients recorded during shortening were qualitatively similar to those previously recorded in isometric tetani. There were four phases: phase 1, the change of tension during the step; phase 2, a rapid partial recovery of tension; phase 3, a delay or reversal of recovery; phase 4, a slower recovery of tension to the level before the step was applied.

4. Measurements were made of the extreme tension, T_1 , attained during a step, and the level, T_2 , to which tension recovers in phase 2. The excursion of tension, $|T_1 - T_2|$, during a small step of given size, fell with increase of shortening velocity, reaching about 40% of the isometric value near the maximum velocity of shortening. T_2 fell as shortening velocity was increased and the fraction of steady tension recovered, T_2/T_1 , also decreased, so that the proportion of tension recovery in phase 4 increased. All the recovery phases became progressively more rapid with increase of shortening velocity.

5. The early tension response was matched with a delay-line simulator so as to estimate the value of the instantaneous stiffness. Stiffness during shortening was found to decrease approximately linearly with tension, reaching about 35% of the isometric value as tension approached zero. It was impossible to match the early tension response in a rapidly shortening fibre without assuming decreased stiffness. The decline of stiffness is interpreted as due largely to reduced number of attached cross-bridges, but quantitative estimates would be affected by possible filament compliance and non-linearity of cross-bridge stiffness.

Present addresses: * Department of Medicine, University of Chicago, Chicago, IL 60637, U.S.A.;

† The Master's Lodge, Trinity College, Cambridge CB2 1TQ (address for reprint requests);

‡ Department of Biophysics, King's College London, 26–29 Drury Lane, London WC2B 5RL.

6. The decrease in T_2 also suggests fewer cross-bridges are attached as shortening velocity increases, but uncertainties about the processes determining phase 2 during shortening do not permit a precise estimate of stiffness to be made. The changed T_2 levels also suggest that cross-bridges are less extended in their average attachment positions during shortening and that the distribution of attachments is wider than in the isometric state. The increase in the rate of recovery in phase 2 is also consistent with shortening of the cross-bridge links.

7. The results are shown to be consistent with the theory of force generation proposed by A. F. Huxley & Simmons (1971) and with the general cross-bridge cycle of A. F. Huxley (1957).

INTRODUCTION

This paper is the third in a series describing tension transients recorded during experiments on isolated muscle fibres from the frog performed in 1973–1974. The first paper gave a general account of the tension response to small step changes of length during the plateau of an otherwise isometric tetanus at sarcomere lengths where there is complete overlap (Ford, Huxley & Simmons, 1977) and showed the presence of an undamped and nearly linear elasticity within the fibre. The second (Ford, Huxley & Simmons, 1981) described similar responses when the overlap of the thick and thin filaments was reduced. It was found that the whole time-course of tension change per cross-bridge was almost the same at all degrees of overlap, implying that the transients are due to events in the cross-bridges and that compliance in the filaments is almost negligible. As a corollary, measured instantaneous stiffness is directly proportional to number of attached cross-bridges in this type of experiment. The present paper describes the tension responses to step changes of length imposed during steady shortening at various speeds, performed in the same series of experiments. The results are shown to be consistent with the cross-bridge theories of A. F. Huxley (1957) and A. F. Huxley & Simmons (1971).

METHODS

Apparatus and procedure

These were described in detail by Ford *et al.* (1977, 1981).

Single muscle fibres were isolated from the tibialis anterior muscle of the frog *Rana temporaria*. A fibre was mounted horizontally in Ringer solution at 0–1 °C with one end attached to a force transducer (natural frequency 10.8 kHz) and the other to the arm of a motor under servo-control. The response of the motor to a small step input was complete in 0.2 ms. The length of the central region of the fibre was monitored by means of a spot-follower apparatus to give a signal that was proportional to change of sarcomere length. This signal was used in conjunction with the motor position signal to control the sarcomere length of the central part of the fibre ('length control', Ford *et al.* 1977).

Induction of steady shortening

A muscle fibre was tetanized to produce an isometric contraction at a sarcomere length of about 2.25 μm , and a shortening ramp was applied when the tension had reached its plateau. The sequence of operations is shown in Fig. 1. During the first 3 ms of the ramp, its speed was raised above the steady value in order to bring the tension to its new level as early as possible. When tension was steady, a step change was imposed. The duration of the ramp before the step was inversely

proportional to the ramp speed so that the step was applied at nearly the same sarcomere length, about $2.2 \mu\text{m}$, at all shortening velocities.

Alterations to apparatus

Recordings. The same signals were recorded as described previously, except in the case of the signal proportional to change of sarcomere length recorded on Polaroid film (OSC 2 of Fig. 2 of Ford *et al.* 1977). The ramp used to command the steady shortening was subtracted from this signal to display the step in sarcomere shortening on a flat base line. This display was then used to adjust the gain, G_L , of the sarcomere length signal fed into the servo-loop.

Delay-line. The delay-line circuit (Ford *et al.* 1981) used for simulating the tension response was modified in two respects.

(i) Instead of making the motor perform a step during the simulation and feeding the delay line with the differential of the motor position signal (p_M), the input was provided by a digital read-only memory (r.o.m.) programmed to give, in real time, an output proportional to a record of \dot{p}_M taken during a standard motor step shortly after the experiments were done.

(ii) The part of the circuit within dashed lines in Fig. 2 of Ford *et al.* (1981) was not used. The switches S_1 were put to the position shown in that Figure but the lead from the capacitors representing the end compliances was connected to earth and not to the lower switch. This arrangement is equivalent to assuming that the feed-back in the original experiment had worked perfectly, since the feed-back amplifier A_2 causes the total current through the six arms of the circuit representing the fibre itself to be proportional to the r.o.m. output, i.e. simulated length change in the fibre itself is proportional to p_M in a standard step since current represents rate of change of length. In simulations of steps on the plateaux of isometric contractions this mode gave an output almost indistinguishable from the mode used in Ford *et al.* (1981), but the latter did not work satisfactorily when simulating steps on fast ramps where tension was very low and end compliance was raised because of the non-linearity of tendon compliance. The records of length change during the step in the original experiment did not differ detectably from the standard step.

The value for \mathcal{C}_e , the capacitance representing end compliance, was chosen to give the correct ratio to fibre stiffness ($\mathcal{C}_1\mathcal{C}_3/(\mathcal{C}_1+\mathcal{C}_3)$, Ford *et al.* 1981, Appendix B). This ratio was estimated from the value of G_L (Ford *et al.* 1977, p. 453) found to be necessary in the original experiment. All the matches reported in this paper were made with \mathcal{R}_0 (Ford *et al.* 1981, Fig. 2B), representing 'viscosity' in the fibre, set to zero.

RESULTS

Description of the results

Force-velocity relation. An approximation to an isotonic release was produced in these experiments by applying a shortening ramp at the desired velocity, together with an initial more-rapid length change to accelerate the fall of tension. A steady level of tension was reached at each velocity when the fibre had shortened by about 15 nm per half-sarcomere (e.g. Fig. 1).

The steady-state force-velocity data obtained from three fibres measured at a sarcomere length of about $2.2 \mu\text{m}$ are shown in Fig. 2.

Tension transients during steady shortening. Step changes of length were imposed on fibres when the tension had reached a steady level during shortening. The tension responses to a small stretch and several amplitudes of release were recorded in successive tetani at a number of shortening velocities. Records at a slow speed including the whole of each transient are shown in Fig. 3. There are four characteristic phases to the transients, as previously described for the isometric state: phase 1, the instantaneous tension change during the step; phase 2, a rapid partial recovery; phase 3, a delay or reversal of recovery; phase 4, a slower recovery to the tension level before the step.

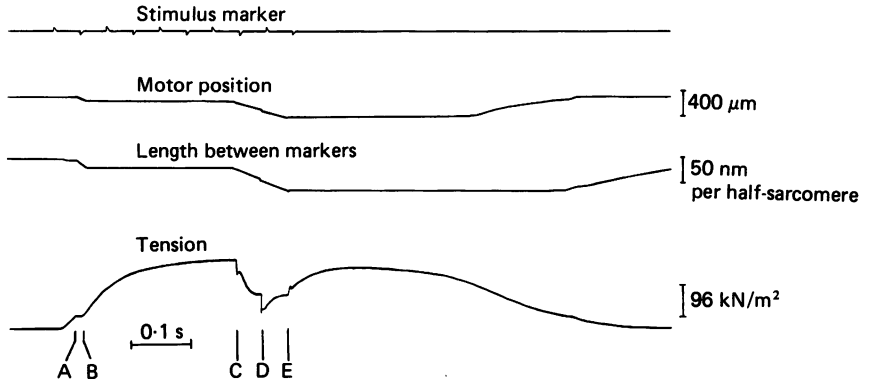


Fig. 1. Sequence of events during a contraction. The change from motor-position control to spot-follower control is achieved by allowing the fibre to shorten during the period from A to B (see Ford *et al.* 1977, Fig. 1). At C a small shortening step and a ramp ($0.4 \mu\text{m/s}$ per half-sarcomere) are applied. After a transient change, tension reached a steady level at D, when a test release of 4.5 nm per half-sarcomere is applied. After the ensuing tension transient the ramp is terminated and the initial shortening step reversed at E. The fibre is then held isometric while tension redevelops. Experiment of 5 December 1973. Tracing of record from U.V. recorder.

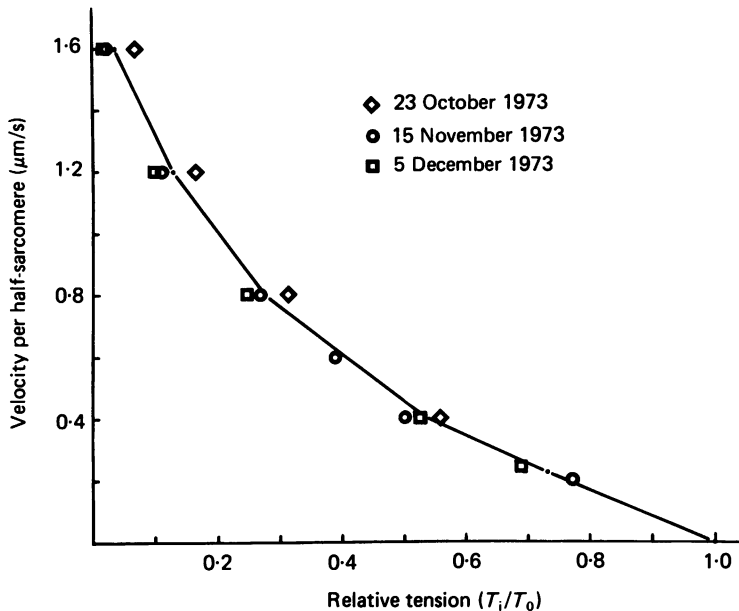


Fig. 2. Force-velocity relation. The results from three experiments are shown. Tension measured from the steady level immediately preceding a step is plotted relative to the isometric value.

The records in Fig. 3 and the higher-speed records in Fig. 4 show that for higher shortening speeds the initial tension changes (phase 1) as well as the partial tension recovery (phase 2) were smaller for a given size of step and the recovery phases (2, 3 and 4) briefer. These changes are described in greater detail in the following sections.

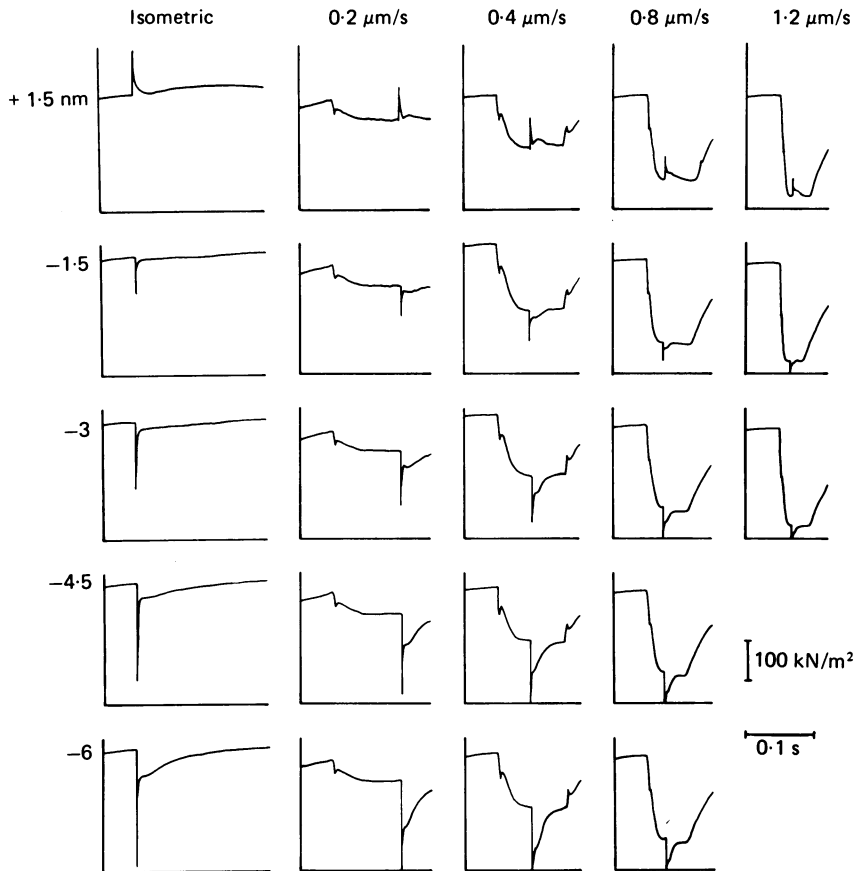


Fig. 3. Slow time-base records of the tension response to steps at different shortening velocities. The velocity is shown above each set of records in units of $\mu\text{m/s}$ per half-sarcomere. The records are tracings of the U.V. recordings. Experiment of 5 December 1973.

Phase 1. Measurements of T_1 , the extreme tension reached during the step, were made from oscilloscope records at 0.2–1.0 ms/cm. A selection of records from one experiment is shown in Fig. 4. The magnitude of the initial tension response to a step of a given size decreased with increasing shortening velocity. There was some variation in the magnitude of the responses, and the results for the two fibres showing the smallest and largest responses are shown in Fig. 5A and B, where T_1 is plotted against the size of step (continuous lines). A third fibre gave responses that were intermediate. The T_1 curves become progressively less steep with increasing shortening velocity, but the slopes of the curves change less than in proportion to the tension before the step, T_1 . This is shown more clearly in Fig. 5C, in which the mean T_1 values

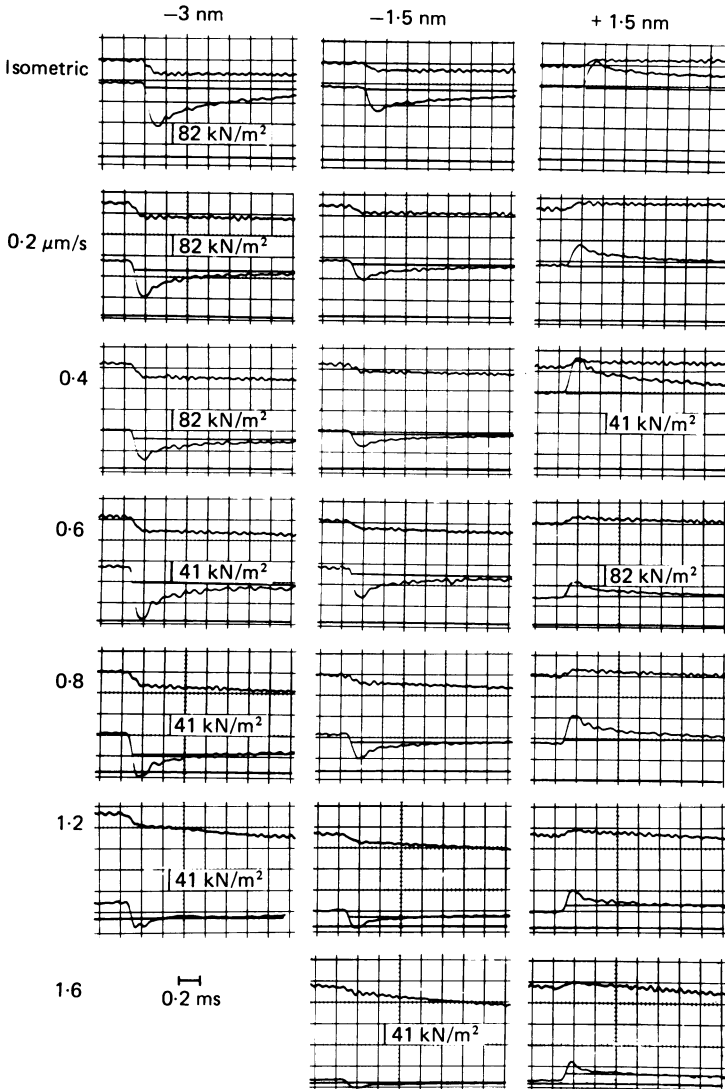


Fig. 4. Oscilloscope records on a fast time base (0.2 ms/cm) of the spot-follower signal (upper traces) and tension (middle traces) for steps applied during shortening at various velocities. Bottom traces: tension base line. The velocity is shown beside each record in units of $\mu\text{m/s}$ per half-sarcomere. Experiment of 15 November 1973.

from three fibres have been normalized to T_1 . The normalized curves become steeper with increasing velocity.

These results suggest at first sight that the stiffness of a fibre is lower during shortening than in the isometric state. A measure of the slope of the T_1 curve, $|T_1 - T_i|$, for a small stretch and an equal small shortening step, is plotted against shortening velocity in Fig. 6A and against steady tension in Fig. 6B. The value of the slope falls with increasing velocity to reach about 40% of the isometric value at the maximum

velocity of shortening. The slope of the T_1 curve is, however, not a reliable estimate of the instantaneous stiffness because of recovery of tension during the step itself (Ford *et al.* 1977) and because of effects of fibre inertia. The rate of tension recovery in phase 2 is higher with higher shortening speeds (see Figs. 4 and 7), so that the

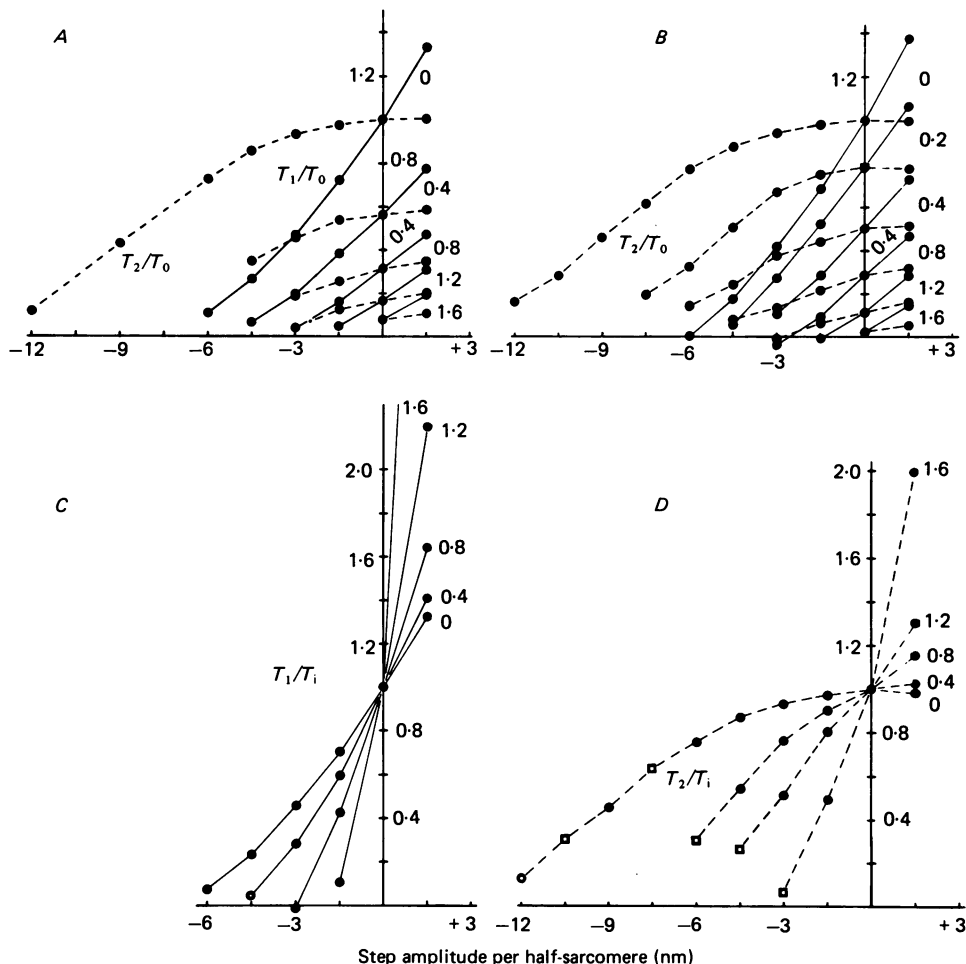


Fig. 5. T_1 and T_2 curves from steps applied during shortening at various velocities. The velocity of shortening is shown against each curve in units of $\mu\text{m/s}$ per half-sarcomere. T_1 continuous lines. T_2 dashed lines. *A* and *B*, curves from two experiments (23 October and 15 November 1973, respectively) in which tension is plotted relative to the isometric tension. *C* and *D*, averages from three experiments. T_1 and T_2 tensions divided by the steady tension before the step are plotted in *C* and *D*, respectively. Open symbols denote points where data were available only from one (squares) or two (circles) experiments.

excursion of tension in phase 1 is more truncated, causing a progressively larger underestimation of the true value of the stiffness at higher speeds. On the other hand, an overshoot due to fibre inertia becomes larger when stiffness is diminished, causing the peak tension change to increase. These effects are taken into account in the analysis described on pp. 140–142.

The results do, however, establish that stiffness does not fall to zero during shortening and that even near the maximum velocity of shortening the stiffness is a substantial fraction of the value in the isometric state.

In several of the records for shortening steps, tension was momentarily reduced below the base line (for example, -3 nm step for $1.2 \mu\text{m/s}$ speed in Fig. 4). Such undershoots were also observed for the isometric state (Ford *et al.* 1977, pp. 484–485) and support the proposition that cross-bridges are capable of bearing negative tension (A. F. Huxley, 1957).

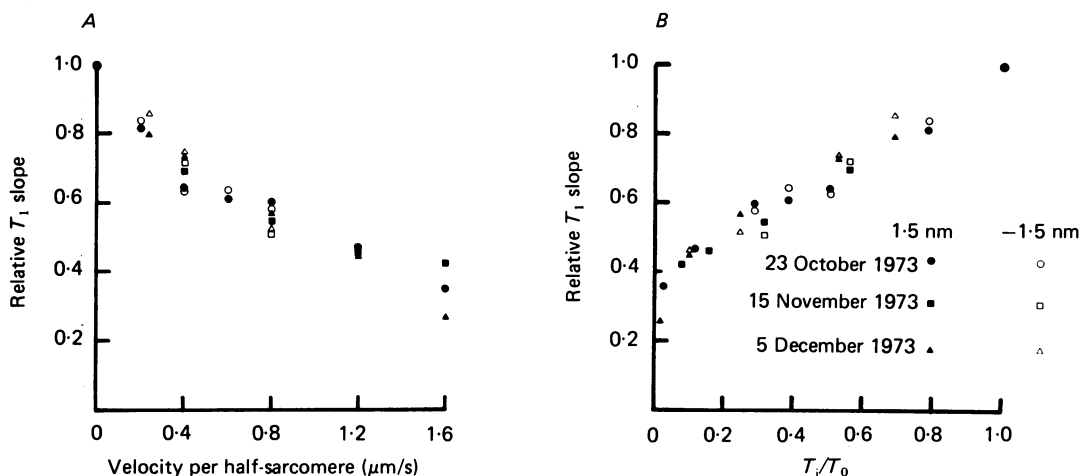


Fig. 6. Initial slopes of T_1 curves, plotted against shortening velocity in *A* and against isotonic tension in *B*. The values of $|T_1 - T_1|$ for 1.5 nm stretches and 1.5 nm releases obtained during shortening have been divided by the corresponding values obtained in isometric control contractions. To normalize for variations in isometric force production between contractions, all values have been multiplied by the ratio of isometric force in the control contraction to isometric force immediately before the onset of shortening in the isotonic contraction.

Phase 2. The level, T_2 , to which tension recovers in phase 2, was measured from the records by fitting an asymptote by eye to the final part of the tension recovery in phase 2 and extrapolating back to the time of the step ($T_a(0)$ of Ford *et al.* 1977, pp. 475–476). The asymptote is shown in each record of Fig. 4. T_2 is shown plotted against step size in Fig. 5; the results from two experiments are shown in Fig. 5*A* and *B* where T_2 is expressed relative to the isometric tension, T_0 , and the average of three experiments is shown in Fig. 5*D* where T_2 is expressed relative to the steady tension before the step, T_1 . The lower part of the T_2 curve becomes progressively less steep with increasing speed of shortening (Fig. 5*A* and *B*), but the slope of the curve decreases less than in proportion to the steady tension (Fig. 5*D*).

The rapidity with which tension recovers during phase 2 is higher for a given size of step the greater the shortening velocity (Fig. 4). The time course of phase 2 cannot be described by a single exponential, and we have used as a measure of the rapidity of force recovery the reciprocal of the half-time for tension to approach the asymptote of the early recovery (Ford *et al.* 1977, p. 479). We shall refer to this quantity as the

rate coefficient. This measure is likely to underestimate progressively the rapidity of the faster recoveries because more of the faster components are lost during the step itself. Its dependence on shortening velocity is shown for three sizes of step in Fig. 7. As in isometric contractions, recovery in phase 2 is faster for larger releases at a given shortening velocity. The rate coefficient increases in roughly linear fashion with velocity, the slopes of the relation being approximately the same for the three sizes of step.

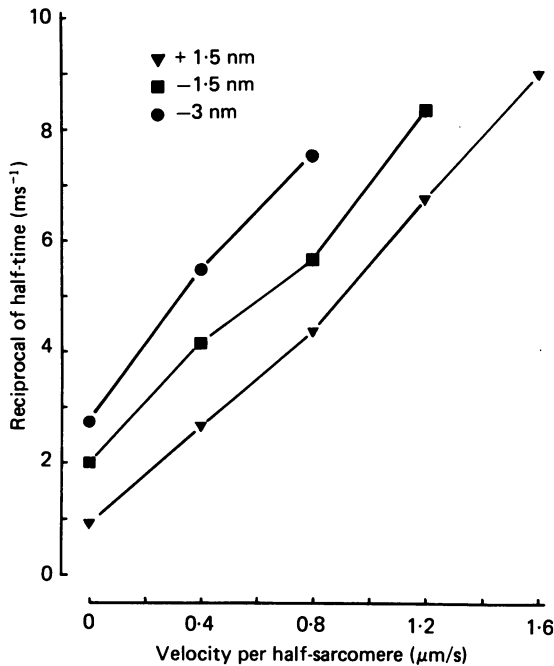


Fig. 7. Rate coefficients (estimated as reciprocal of half-time) of force recovery during phase 2. Each point is the mean from three measurements in different experiments. The range of each group of three averaged $\pm 13\%$ of its mean.

Phase 3. In several of the records of Fig. 3, phase 3 appears as a more prominent feature of the transients during shortening than in isometric contractions. The end of the phase is more sharply defined, probably because a greater fraction of the total tension recovery occurs in phase 4 during shortening, resulting in a steeper onset. There is also a clear reversal of recovery for some of the shortening steps that is not apparent in isometric contractions. For both stretches and shortening steps the time course of reversal, where it occurs, is faster the higher the shortening velocity, but the amount of reversal is small and it is not possible to make accurate measurements of the rate constant. The total duration of phase 3 decreases approximately in inverse proportion to shortening speed. Changes in phase 2 and phase 4 must contribute to change of duration of phase 3, but it seems likely that the rate constant of the process that results in the reversal or slowing of tension recovery in phase 3 increases progressively with shortening velocity.

The interval between the step and the end of phase 3 decreases with shortening

velocity in such a way that during this interval the total shortening (ramp plus step) remains within the range of 4–7 nm per half-sarcomere.

Phase 4. The dependence on shortening velocity of the time course of phase 4 is shown in Fig. 8. Phase 4 cannot be fitted by a single exponential, and we have used the reciprocal of the half-time ('rate coefficient') as a measure of its rapidity. There is a tendency in Fig. 8 for the rate coefficient to be higher for a shortening step of

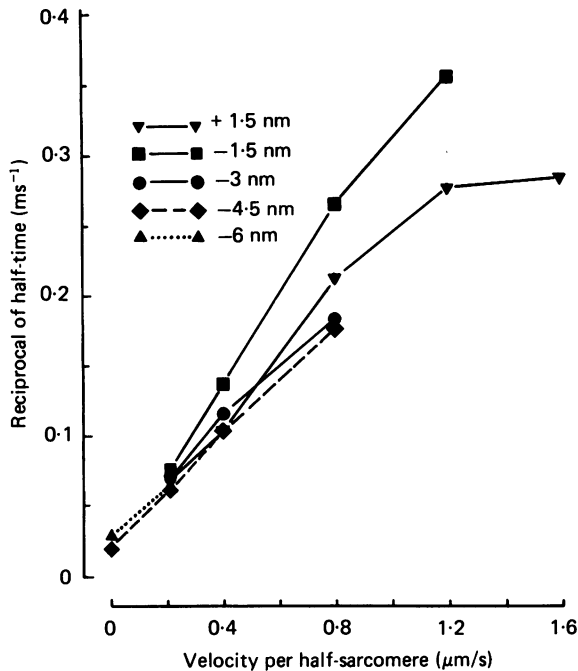


Fig. 8. Rate coefficients (estimated as reciprocal of half-time) of force recovery during phase 4. Each point is the mean from three measurements in different experiments except that the points at a velocity of $0.22 \mu\text{m/s}$ per half-sarcomere are the means of two measurements. The range of each group of three averaged $\pm 22\%$ of its mean.

1.5 nm per half-sarcomere than for other steps, but otherwise it does not seem to depend on the amplitude of the step. As with phase 3, the duration of phase 4 varied inversely with shortening velocity so that $3\text{--}4 \text{ nm}$ of shortening occurred for all steps during the half-time of recovery.

Instantaneous stiffness

The instantaneous stiffness of the fibre was estimated by matching the tension change during phase 1 and the early part of phase 2 with the delay-line described by Ford *et al.* (1981), modified as described in the Methods section. Records at a fast-enough time-base speed (0.2 ms/cm ; Fig. 4) were taken in only one experiment (15 November 1973).

Satisfactory matches were obtained in all cases. Examples are shown in Fig. 9, which also illustrates the striking change in shape of the transient at high values of the speed of shortening. This was well imitated on the delay-line by the combination

of low stiffness with the fibre inertia (Fig. 9*B*) but could not be matched without the inertia (Fig. 9*D*).

Fig. 10 shows the values of stiffness (represented by the sum of $1/\mathcal{C}_1$ and $1/\mathcal{C}_3$; Ford *et al.* 1981, Appendix B) that were needed to match steps of various sizes

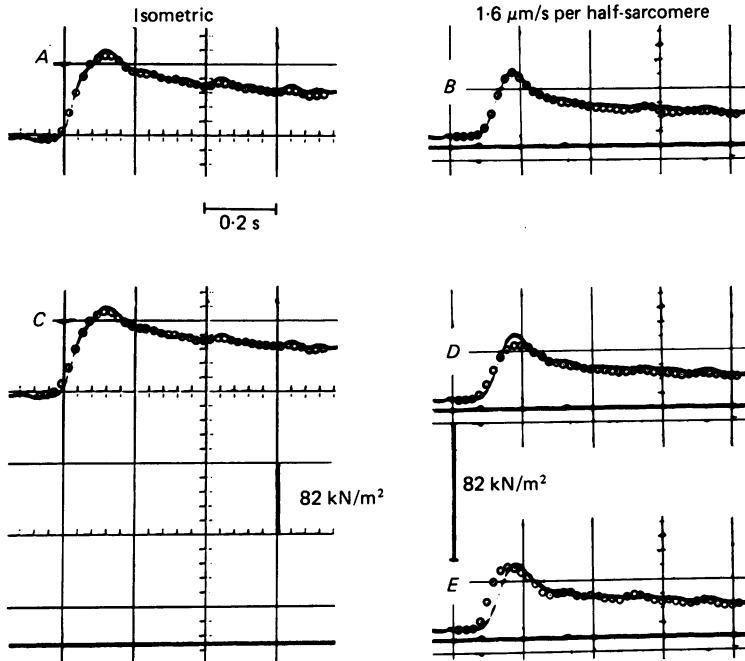


Fig. 9. Delay-line matching of 1.5 nm stretches during isometric contractions (*A* and *C*) and during isotonic shortening at nearly maximum velocity, 1.6 $\mu\text{m/s}$ (*B*, *D* and *E*). Matched values are indicated by circles superposed on the original oscilloscope records obtained in the experiment. The best matches that could be obtained are shown in *A* and *B*. The same parameters used to obtain the best matches in *A* and *B* were used in *C* and *D*, respectively, except that the inductors, representing fibre inertia, were omitted from the delay line. Both Figures show that the early tension responses are delayed by inertia. In the isometric contraction the peak tension change is increased by about 1% by the inertial overshoot while it is increased by about 15% when fibre stiffness is decreased by rapid shortening. In *E*, the peak height of the response of the rapidly shortening muscle was matched by setting the largest capacitances (lowest stiffness) with which the isometric transient could be matched and reducing the time constants of recovery. The simulated initial tension change comes much too early, and could only be brought into coincidence with the original record by setting larger capacitances, i.e. lowered stiffness. Experiment of 15 November 1973.

superposed on shortening at various speeds. Stiffness fell roughly linearly with tension, approaching about one-third of the isometric value as tension approached zero. The matches became noticeably less good if the stiffness was increased or decreased by about 8% from the values shown.

As would be expected, the size of the simulated peak tension change could be reduced by reducing the time constant of the early tension recovery, without altering the fibre stiffness, confirming that peak tension change by itself is not a good measure

of stiffness. Fig. 9E shows that the peak height of the tension response to a 1.5 nm stretch superposed on shortening at almost the maximum velocity could be matched in this way while the stiffness was kept equal to that in an isometric contraction. However, the response is too early and the shape is not well matched, and the discrepancies are far outside the uncertainties of the method (a few microseconds in time to half peak height), showing that the apparent decrease of stiffness cannot be explained solely by the changes in the recovery time constants.

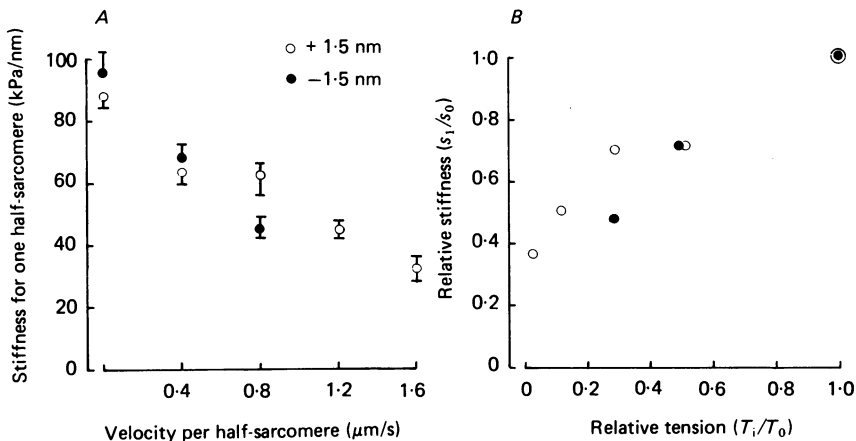


Fig. 10. Instantaneous fibre stiffness estimated from delay-line matches plotted against shortening velocity in *A* and against isotonic tension in *B*. The ends of the error bars in *A* represent settings which gave matches appreciably less good than were given by the best settings. In *B* the values of stiffness giving the best matches to responses obtained during isotonic contractions have been normalized to the value obtained from the best match to the response from the isometric muscle for the corresponding size of step. Same experiment as Fig. 9.

The capacitance \mathcal{C}_e representing end compliance was not set arbitrarily but was chosen to give the correct ratio to fibre compliance as deduced from settings used in the experiment (see Methods section). The value required was about $5 \times$ greater during shortening at almost maximum velocity than in an isometric contraction, showing that the compliance in the tendons and fibre attachments was very non-linear. A high degree of non-linearity of tendon compliance at low forces has been found in whole-muscle preparations (e.g. Rack & Westbury, 1984).

Further analysis of phase 2

Ford *et al.* (1981) showed that fibres stretched so as to reduce overlap gave T_2 curves that scaled with overlap and isometric tension, while the time scale of phase 2 recovery was barely affected. The changes in the T_2 curves and in the rates of tension recovery in phase 2 during shortening (this paper) cannot be explained similarly by a reduction in the number of attached cross-bridges with no other change from the isometric state. During shortening the T_2 curves changed shape and the rate coefficient of recovery in phase 2 was increased.

The following analysis interprets the changes in the T_2 curves on the basis of a model

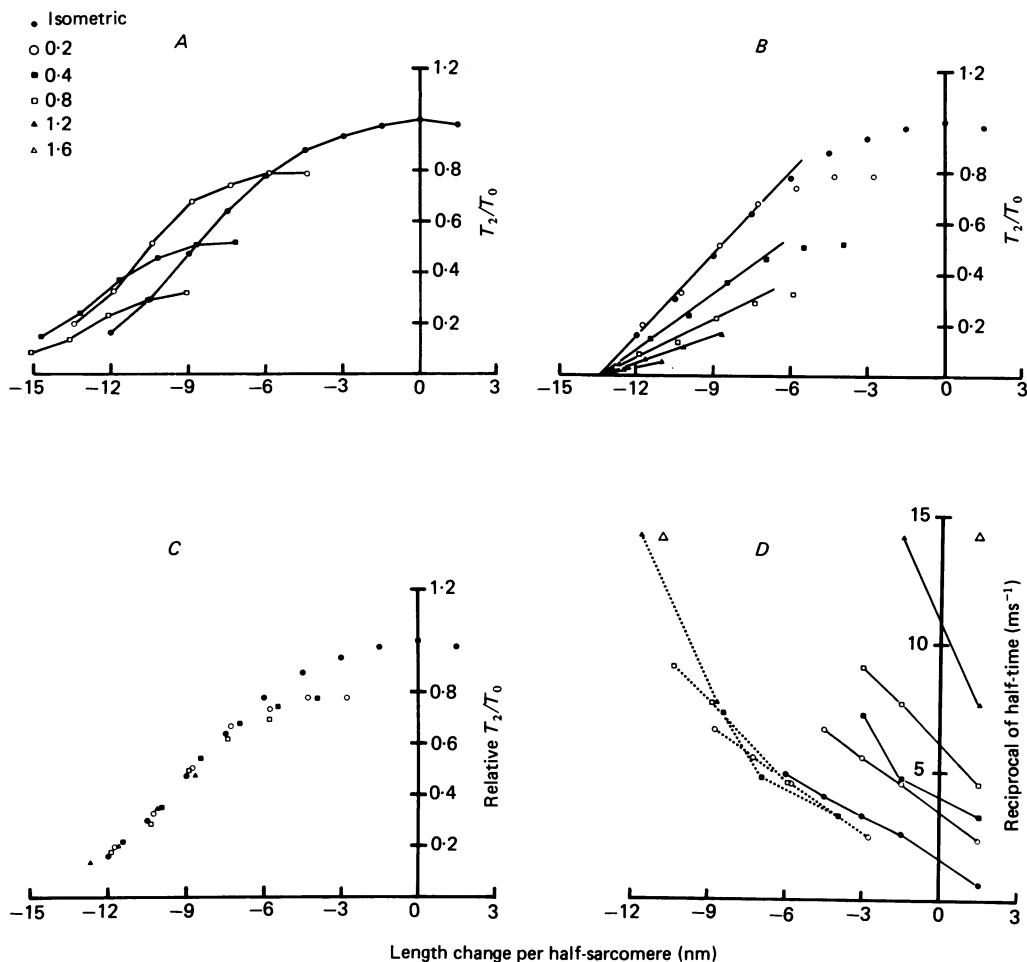


Fig. 11. Shifted and scaled values of T_2 (A–C) and phase 2 recovery speeds (D). In A, the T_2 values have been shifted horizontally so that the tension before the step in an isotonic contraction coincides with the T_2 levels in an isometric contraction. The curves do not superpose. In B, the T_2 curves have been shifted horizontally so that their zero-tension intercepts coincide. Again, the curves do not superpose. In C, the shifted T_2 curves from B have been scaled to superpose their lower, straight parts, indicated by the continuous lines in B. The curves are more nearly similar than in A or B but deviate systematically from the isometric. The phase 2 recovery speeds, plotted as continuous lines against step size in D, are shifted by the same amount as the T_2 curves in B and C to form a nearly continuous curve. Experiment of 15 November 1973.

in which each cross-bridge is composed of an undamped nearly linear elastic component in series with a damped, non-linear elastic component, and detachment and reattachment of cross-bridges during phase 2 are negligible (Huxley & Simmons, 1971; Ford *et al.* 1977). The T_2 curve then describes the average, steady-state force–extension relation of the cross-bridges. The observation (previous section) that instantaneous stiffness declines by less than the steady tension as shortening speed is raised suggests that there is less average force per cross-bridge, and consequently

that the total population of cross-bridges is at a shorter average extension. If this were the only change from isometric, a horizontal shift of the T_2 curve corresponding to the change in average extension of the cross-bridges should bring the curves into coincidence. In Fig. 11 *A* the T_2 curves at different shortening velocities are replotted from Fig. 5 *B* so that each initial tension point (T_1) lies on the isometric T_2 curve. The lower parts of the curves obtained during shortening lie successively further to the left of the isometric curve, suggesting that the average extension of a cross-bridge is greater than would be expected from the steady tension; that is, the steady-state stiffness due to the cross-bridges is lower.

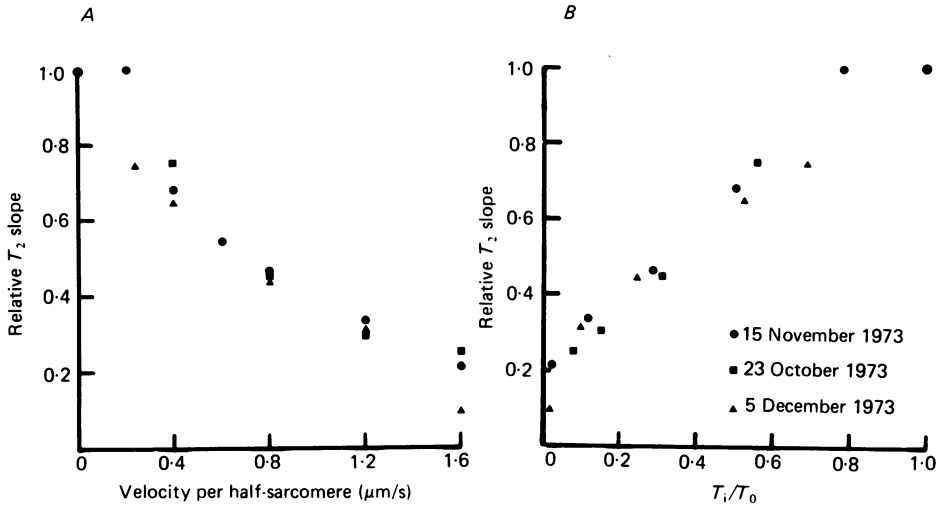


Fig. 12. The slopes of the lower straight parts of the T_2 curves in Fig. 11 *B* normalized to the isometric values and plotted against shortening velocity in *A* and against isotonic tension in *B*. As with the slopes of the T_1 curves in Fig. 6 and the instantaneous fibre stiffness in Fig. 10, these values decline nearly linearly with isotonic force but reach a lower value at maximum velocity (about 20 %).

If one regards the T_2 curves as representing a force-extension property of the cross-bridges, the differences between individual curves should be more apparent if they are plotted so that their origins, at zero tension, coincide. In Fig. 11 *B* the T_2 curves during shortening have been shifted so that their zero-tension points, obtained by extrapolation, coincide with the intercept of the isometric curve on the abscissa. The lines that were used in the extrapolations are also shown. The shifts are given in the Figure legend, and the average shifts for three experiments for shortening speeds per half-sarcomere of 0.4, 0.8, 1.2 and 1.6 $\mu\text{m/s}$ were 4.7, 6.3, 9.5 and 11.3 nm respectively. The decrease in slope of the lower part of the T_2 curve with increase of shortening speed is evident, as noted previously in connexion with Fig. 5 *A* and *B*, and on the present model it may be attributed to a decrease in steady-state stiffness and hence a decrease in the number of cross-bridges attached. The slopes of the lines of Fig. 11 *B* together with the results for the two other experiments are plotted against shortening velocity in Fig. 12 *A* and against T_i/T_0 in Fig. 12 *B*. Stiffness apparently decreases more steeply with increase of shortening velocity than measurements of

stiffness in phase 1 would suggest, and this point is dealt with in detail in the Discussion on p. 147.

The difference between the T_2 curves with increased shortening velocity cannot, however, be wholly explained by a decrease in the number of cross-bridges attached. If variations of stiffness are taken into account by scaling the curves of Fig. 11 *B* in inverse proportion to the slopes of the lines fitted to their lower points, there is a systematic tendency for the T_2 levels during shortening to lie below the isometric curve at higher tensions. This is shown in Fig. 11 *C*. It is not obvious from this Figure whether the effect is progressive with increase of shortening velocity, but another experiment suggested more clearly that this is the case. The effect can be matched by adding together a number of isometric T_2 curves that have been shifted along the abscissa over a total range of about 5 nm, implying that the distribution of cross-bridge extension is broadened during steady shortening.

The analysis so far suggests that a large part of the change in the T_2 curve during shortening can be explained partly by a reduction in the number of cross-bridges attached and partly by a decrease in the average force, and hence extension, per cross-bridge. We now consider the effects of these changes on the rate of recovery of tension in phase 2. A reduction in the number of cross-bridges attached would have no direct effect on the rate coefficient of recovery, provided that the cross-bridges act independently. If, however, the rate coefficient is a function of cross-bridge extension at the time when the step is completed (Huxley & Simmons, 1971), then, for a given size of release, a reduction in the mean extension before the step (during the steady shortening) would lead to an acceleration of the recovery in phase 2 in the same way as when the size of release from the isometric state is increased (Fig. 7). If so, the curves relating rate coefficient to step size at different shortening speeds should be brought into coincidence by correcting for the reduction of average cross-bridge extension with shortening speed. This is shown to be the case in Fig. 11 *D*, where the rate coefficients at the various shortening speeds are plotted against step size, with no allowance for cross-bridge extension (continuous lines) and with the same shifts as used in Fig. 11 *B* (dotted lines). There is a fairly close agreement between the shifted curves themselves, and the curves are also in reasonable coincidence with the isometric curve where there is overlap in the values along the abscissa.

DISCUSSION

These experiments are interpreted in terms of a cross-bridge theory in which phase 1 is due to the instantaneous compliance of the attached cross-bridges, phase 2 is due to changes within attached cross-bridges, phase 3 is due (in a release) to a detachment of cross-bridges, and phase 4 is due to cross-bridge attachment and detachment (Huxley, 1957; Huxley & Simmons, 1971, 1973; Ford *et al.* 1977, 1981). Before describing this interpretation, we will discuss some limitations of the measurements and some general conclusions about the properties of cross-bridges.

Limitations and general conclusions

Uncertainty of estimates of stiffness. The chief deficiency in our method is that the spot-follower signal, indicating length of the fibre segment between the markers, was

too noisy to be used directly as a measure of change of length during the step itself and could only be used as a check on the performance of the servo-system. It is hoped that much more precise estimates of stiffness, and of any non-linearity or viscosity that it may show, will be obtained with the striation follower developed by A. F. Huxley, Lombardi & Peachey (1981 *a, b*). Direct use of the signals for movement at each end of the segment under study will also eliminate the need for several of the approximations in the delay-line simulator. Nevertheless, we believe that the uncertainties in our estimates of stiffness are not much greater than the limits for satisfactory matches with the delay line indicated in Fig. 10.

Relation of stiffness to number of attached cross-bridges. It is likely that nearly all of the instantaneous compliance of the sarcomeres lies within the cross-bridges (Ford *et al.* 1981), and that the compliance is nearly linear (Ford *et al.* 1977). Changes of fibre stiffness can therefore be taken *prima facie* as a measure of changes in the number of cross-bridges attached.

If 20% of the instantaneous compliance in isometric contraction is in the filaments etc. and is linear (upper limit found by Ford *et al.* 1981), the reduction of total stiffness to 35% in rapid shortening (this paper) would correspond to a reduction of cross-bridge stiffness to 30%, i.e. the observed stiffness change would underestimate the change in number of attached cross-bridges.

If the elasticity of the cross-bridges is non-linear in the direction of becoming less stiff at low force, part of the observed decrease of stiffness would be attributable to the reduced average cross-bridge extension (p. 144) and the observed stiffness change would be an over-estimate of the change in number of attached cross-bridges. The degree of non-linearity described by Ford *et al.* (1977, p. 489) as 'the most curved relation that could reasonably be assumed' gives a stiffness at zero force equal to 70% of that in isometric contraction; even in that case our observed reduction of stiffness to 35% would still imply a twofold reduction in the number of cross-bridges attached and contributing to the stiffness.

Earlier estimates of stiffness during shortening. These relied on the amplitude of the tension response to small oscillations of length imposed on a fibre (Julian & Sollins, 1975; Julian & Morgan, 1981). The frequency of oscillation used was of the same order of magnitude as the equivalent predominant frequency of the steps used in our experiments, but no allowance was made for tendon compliance, truncation of tension change by early recovery, or effects of inertia in the fibre. Their results and ours are in fairly close agreement. It was shown on p. 137 and in Fig. 9 that the effects of truncation and of inertia are in opposite directions, so the agreement is probably in part fortuitous.

As Julian & Sollins point out, a decrease of stiffness with increase of shortening velocity would rule out theories of the cross-bridge cycle in which stiffness increases during shortening, such as that of Podolsky, Nolan & Zaveler (1969). An increased number of attached cross-bridges is predicted on this theory because of a very high rate constant for attachment. A more recent version of this theory (Podolsky & Nolan, 1973) has included, for other reasons, a compliance with non-linear properties in series with the cross-bridges, so the net stiffness decreases with increase of shortening velocity, at least when stiffness is measured over a restricted range of tensions about the isotonic tension level.

Estimates of number of attached cross-bridges from X-ray diffraction. The intensities of the equatorial reflexions in the X-ray diffraction pattern of whole frog sartorius muscles have also been used as a measure of cross-bridge attachment during shortening. A roughly linear relation between I_{11}/I_{10} and isometric tension has been found in contractures where the level of activation was altered (Yu, Hartt & Podolsky, 1979) and this relation can therefore be used to provide a measure of the fraction of attached cross-bridges during shortening, relative to the corresponding number in a maximal isometric contraction. At moderate loads, $0.14\text{--}0.4 P_0$, the change in I_{11}/I_{10} reported by Podolsky, St. Onge, Yu & Lynn (1976) yields an estimate of $0.8\text{--}0.9$ for the fraction of cross-bridges attached relative to the isometric value, considerably higher than our estimate of $0.4\text{--}0.7$ from the instantaneous stiffness over the same isotonic tension range. At smaller loads, $0.1 P_0$ and less, the corresponding value from X-ray diffraction is about 0.6 (calculated from results of H. E. Huxley, 1979), to be compared with a stiffness measurement of $0.35\text{--}0.5$. The discrepancy between the two sets of measurements might in part be explained by non-linearity of the force-extension curve as explained above, but it is also possible that the interpretation of the X-ray diffraction results is complicated by changes in cross-bridge configuration during shortening (Lynn, 1978) or that a fraction of attached cross-bridges contributing little to stiffness is larger during shortening than in the isometric state.

Other evidence for reduced number of attached cross-bridges during shortening. It has been known for a long time that when a load equal to the isometric tension is applied immediately after a muscle (Katz, 1939, Figs. 7 and 8) or an isolated fibre (A. F. Huxley, 1971, Fig. 6) has been shortening under low load, the tissue does not merely stop shortening but lengthens at a speed several times greater than the speed of unloaded shortening. The simplest explanation for this phenomenon is that the number of cross-bridges attached, and therefore able to resist lengthening, is reduced during rapid shortening (A. F. Huxley, 1957, p. 291). However, the phenomenon might alternatively be explained by cross-bridge attachments being weaker than in the isometric state, so it is not conclusive evidence in favour of a reduced number of attached cross-bridges.

T_3 curves. The analysis on pp. 142–145 showed that the changes in the T_2 curve with increase of shortening speed can be explained by a combination of (a) reduced number of attached cross-bridges, (b) reduced average extension of a cross-bridge, and (c) broadened range of cross-bridge extension. All these effects were predicted in the theory of A. F. Huxley (1957, Fig. 7). This or any related theory would also imply that more of the cross-bridges would be brought by the step into positions of attachment where they exert a large negative force. These cross-bridges might detach very rapidly, or might buckle, and the consequent removal of their negative contribution to the total force would add to the recovery of tension in phase 2. Thus, the T_2 curve would be less steep than if these cross-bridges had remained attached. This would contribute to the decline of steepness of the T_2 curve and would reduce the part of this effect that has to be explained directly by a reduction in the number of attached cross-bridges; it might thus account for the decline in T_2 slope (Fig. 12) being somewhat steeper than the decline of instantaneous stiffness (Fig. 10).

Rate of recovery in phase 2. The tension recovery in phase 2 became very much faster

for a given size of step as shortening velocity increased. It is difficult to make accurate estimates of the rate constant, first because the time course cannot be fitted with a single exponential, secondly because of noise on the records, and thirdly because of difficulties in making precise estimates of the asymptote to the recovery. It is shown on p. 145 that the acceleration of recovery may be explained by reduced average cross-bridge extension. Some part of the increase in recovery rate might, however, be due to a rapid detachment of cross-bridges as considered in the preceding section, and a firm distinction of the relative contributions of cross-bridge movement and cross-bridge detachment cannot be made until more precise estimates of stiffness are available.

Interpretation of the data

Phase 1. The cross-bridge theory of A. F. Huxley (1957) postulated that the inverse relation between force and velocity was caused partly by a decreased number of attached cross-bridges during shortening and partly by a decreased average tension per cross-bridge. At maximum velocity the average tension per cross-bridge was zero but there was still a substantial number of attached cross-bridges (26.4% of the number in the isometric state). Those that were attached and exerting tension were exactly balanced by cross-bridges that had been pulled beyond the range where they aid contraction and were resisting further shortening. The present results agree with these several different aspects of the theory. The observation that stiffness diminishes with shortening suggests a decreased number of attached cross-bridges. The stiffness does not, however, decrease in proportion to force so that the average force per attached cross-bridge is decreased. The presence of a finite stiffness at maximum velocity, together with the observation that the fibres can support negative tension, suggests that there is a substantial number of attached cross-bridges at maximum velocity and that some of these cross-bridges are capable of resisting further shortening.

Phase 2. On the basis of the theory of force generation proposed by Huxley & Simmons (1971), phase 2 is explained by transitions between distinct mechanical states of the attached cross-bridges. The rates of transition between states are determined, in part, by the tension on the cross-bridges. Thus, when the average force per cross-bridge is low, as during shortening, the rates of transition between states, and consequently the rapidity of recovery in phase 2, will be high. According to this theory the phase 2 recovery rates should increase progressively with increased velocity since the average force per cross-bridge declines progressively. The present findings are consistent with this aspect of the theory.

The cross-bridge theory of A. F. Huxley (1957) proposed that cross-bridges would begin to detach rapidly after a definite amount of sliding. The range of movement was later defined as being about 13 nm (Huxley & Simmons, 1971; Ford *et al.* 1977). Several aspects of the present experiments are consistent with this length of cross-bridge stroke; all features of the transient tension responses occur within the period required for the muscle fibre to shorten at its steady rate by less than 13 nm.

The original cross-bridge theory predicted a broader distribution of attached cross-bridges during shortening than in the isometric state (A. F. Huxley, 1957,

Fig. 7). As discussed above, the changes in the T_2 curves suggest such a broader distribution.

Phase 3. Huxley & Simmons (1973) offered three possible explanations of phase 3. These were (1) distribution functions for attachment and detachment of cross-bridges such that the detachment rate is substantially increased following shortening steps, and decreased following stretches; (2) a refractory period for cross-bridges such that they cannot contribute to force generation for a period after detachment; and (3) transiently decreased activation following releases and increased activation following stretches. The three mechanisms are not mutually exclusive, and all depend on cross-bridge detachment to produce phase 3 following releases. Since detachment is increased by shortening, the greater prominence of phase 3 during shortening is consistent with the theory. The observations of Iino & Simmons (1982) make it likely that phase 3 after a release does indeed involve detachment.

Phase 4. Final tension recovery following a step is attributed mainly to detachment of cross-bridges from positions appropriate to the old length and reattachment at positions appropriate to the new length, with rearrangement of the cross-bridge distribution to establish that which existed before the step. The predominant recovery rate constant is then likely to be determined by the sum of the prevailing mean attachment and detachment rate constants. The net detachment rate constant is likely to rise as a result of the shift of distribution of attached cross-bridges during shortening so that the net rate constant of phase 4 would be expected to increase, as observed.

REFERENCES

- FORD, L. E., HUXLEY, A. F. & SIMMONS, R. M. (1977). Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *Journal of Physiology* **269**, 441–515.
- FORD, L. E., HUXLEY, A. F. & SIMMONS, R. M. (1981). The relation between stiffness and filament overlap in stimulated frog muscle fibres. *Journal of Physiology* **311**, 219–249.
- HUXLEY, A. F. (1957). Muscle structure and theories of contraction. *Progress in Biophysics and Biophysical Chemistry* **7**, 255–318.
- HUXLEY, A. F. (1971). The activation of striated muscle and its mechanical response (Croonian Lecture). *Proceedings of the Royal Society B* **178**, 1–27.
- HUXLEY, A. F., LOMBARDI, V. & PEACHEY, L. D. (1981*a*). A system for fast recording of longitudinal displacement of a striated muscle fibre. *Journal of Physiology* **317**, 12–13*P*.
- HUXLEY, A. F., LOMBARDI, V. & PEACHEY, L. D. (1981*b*). A system for recording sarcomere longitudinal displacements in a striated muscle fibre during contraction. *Bollettino della Società italiana di biologia sperimentale* **57**, suppl. 1, 57–59.
- HUXLEY, A. F. & SIMMONS, R. M. (1971). Proposed mechanism of force generation in striated muscle. *Nature* **233**, 533–538.
- HUXLEY, A. F. & SIMMONS, R. M. (1973). Mechanical transients and the origin of muscular force. *Cold Spring Harbor Symposia on Quantitative Biology* **37**, 669–680.
- HUXLEY, H. E. (1979). Time resolved X-ray diffraction studies on muscle. In *Cross-bridge Mechanism of Muscle Contraction*, ed. SUGI, H. & POLLACK, G. H., pp. 391–401. Tokyo: University of Tokyo Press.
- IINO, M. & SIMMONS, R. M. (1982). Tension responses to double step length changes in frog skinned fibres. *Journal of Physiology* **332**, 54–55*P*.
- JULIAN, F. J. & MORGAN, D. L. (1981). Variation of muscle stiffness with tension during tension transients and constant velocity shortening in the frog. *Journal of Physiology* **319**, 193–203.
- JULIAN, F. J. & SOLLINS, M. R. (1975). Variation of muscle stiffness with force at increasing speeds of shortening. *Journal of General Physiology* **66**, 287–302.

- KATZ, B. (1939). The relation between force and speed in muscular contraction. *Journal of Physiology* **96**, 45–64.
- LYMN, R. W. (1978). Myosin subfragment-1 attachment to actin. Expected effect on equatorial reflections. *Biophysical Journal* **21**, 93–98.
- PODOLSKY, R. J. & NOLAN, A. C. (1973). Muscle contraction transients, cross-bridge kinetics and the Fenn effect. *Cold Spring Harbor Symposia on Quantitative Biology* **37**, 661–668.
- PODOLSKY, R. J., NOLAN, A. C. & ZAVELER, S. A. (1969). Cross-bridge properties derived from muscle isotonic velocity transients. *Proceedings of the National Academy of Sciences of the U.S.A.* **64**, 504–511.
- PODOLSKY, R. J., ST. ONGE, R., YU, L. & LYMN, R. W. (1976). X-ray diffraction of actively shortening muscle. *Proceedings of the National Academy of Sciences of the U.S.A.* **73**, 813–817.
- RACK, P. M. H. & WESTBURY, D. R. (1984). Elastic properties of the cat soleus tendon and their functional importance. *Journal of Physiology* **347**, 479–495.
- YU, L. C., HARTT, J. E. & PODOLSKY, R. J. (1979). Equatorial X-ray intensities and isometric force levels in frog sartorius muscle. *Journal of Molecular Biology* **132**, 53–67.