Effects of passive tension on unloaded shortening speed of frog single muscle fibers

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ABSTRACT Experiments were performed to determine the influence of sarcomere length and passive tension on the velocity of unloaded shortening (V_u) as measured by the slack test technique. Slack test results were obtained from intact twitch fibers isolated from the frog (Rana temporaria). Measurements were made both in the absence and presence of passive tension using two different protocols. In one, all releases were initiated from the same sarcomere length and passive tension level; in the other, all releases ended at the same sarcomere length. In the absence of passive tension, no difference was observed between the results from the two slack test protocols. When passive tension was present, performing all releases from the same initial sarcomere length and passive tension level resulted in linear step size–slack time relationships in which the slopes (V_u) were independent of length over a sarcomere length range extending to 3.1 μm, and the intercepts increased with increasing sarcomere length. Performing all releases to the same final sarcomere length in the presence of passive tension produced nonlinear step size–slack time relationships. The results presented here show that, in the presence of significant levels of passive tension, the traditional interpretation of the slope of the slack test plot as the constant unloaded shortening velocity is only correct when all length steps are initiated from the same initial sarcomere length and level of passive tension.

INTRODUCTION

Gordon et al. (1966) showed that the developed tetanic tension is proportional to the overlap between thick and thin filaments of isolated frog skeletal muscle fibers. This observation is widely accepted as showing that the regularly spaced cross-bridge projections from the thick filaments are responsible for tension generation. If the cross-bridges are identical and act independently, the speed of unloading shortening (V_u) should not be influenced by the degree of filament overlap. Gordon et al. (1966) proceeded to demonstrate that, under lightly loaded isotonic conditions, shortening speed is nearly constant for sarcomere lengths ranging from 2.1 to 3.0 μm.

Edman (1979) introduced a technique for measuring unloaded shortening speed which consists of suddenly releasing an activated fiber, thus inducing slack, and then measuring the time elapsed before force redevelopment begins. A straight line fitted to a series of data points obtained from varying the size of the release has a slope of V_u. The intercept of the line with the release-size axis provides an estimate of the extent of recoil of series elastic elements upon release. The "slack test" technique has since been widely adopted as a standard method for estimating V_u. Using the slack test, Edman (1979) con-

firmed that V_u does not vary with filament overlap over a sarcomere length range extending to 2.7 μm. At sarcomere lengths longer than 2.7 μm, where resting fibers develop increasing amounts of passive tension, Edman reported substantial increases in V_u. He proposed that the mechanism responsible for increasing V_u involved passive forces acting as a negative load on the contractile component, increasing the rate of cross-bridge cycling.

Our efforts to pursue further work in this area were motivated by initial experiments indicating that, in the presence of substantial levels of passive tension, values for V_u obtained using the slack test method depended on the protocol used to apply this method. Our results show that V_u is independent of sarcomere length, in confirmation of Edman's results (1979), and of slack test protocol as long as passive tension levels are very low. However, when passive tension levels are high, values for V_u obtained using the slack test method become dependent on the way the test is done. Evidence is presented indicating that V_u remains constant even at long sarcomere lengths, extending to 3.1 μm, and that slack test speeds are reliable in the presence of passive tension, provided that the protocol used imparts the same passive tension effect to all releases in a test series. A preliminary report of this work was presented at a meeting of the Biophysical Society (Claflin et al., 1987).

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METHODS

Dissection, mounting, and apparatus

Long (6–8 mm) and short (1.0–1.5 mm) twitch fibers were isolated from the anterior tibialis and lumbricalis digiti (II and IV) muscles, respectively, of frogs (*Rana temporaria*). Short fibers were used exclusively for experiments in which sarcomere shortening was monitored photographically. Dissections were performed under dark field illumination at room temperature in a Ringer solution with the following composition (in millimolar): NaCl 115; KCl 2.5; CaCl$_2$ 1.8; Na$_2$HPO$_4$ 2.15; NaH$_2$PO$_4$ 0.85; pH 7.2.

For most experiments, fibers were mounted in a chamber by inserting the wires protruding from a force transducer and an arm attached to a servomotor through holes cut in the tendons remaining at each end of the fiber. The tendons were secured to each wire using loops of 9-0 monofilament nylon suture. Fibers were then stretched until taut. Sarcomere length was determined from photographs of at least four different areas along the length of the fiber.

In some experiments, the attachment to the motor wire was made via a 2–6-mm length of a single strand of silk (diam. ~14 μm) teased from a section of braided 10-0 silk suture. The strand of silk was tied to the wire and to a well-trimmed tendon, then clamped into place with knots of 9-0 monofilament nylon at the wire and single silk strands at the tendon. Fibers attached directly to the motor wire became buckled immediately after a step release, the deviation from a straight line representing the induced "slack" (see Fig. 1 *B*). In contrast, fibers attached by a link of silk remained straight as the silk strand buckled (see Fig. 8 *A*).

The temperature of the Ringer solution in the experimental chamber was maintained at 5.0 ± 0.5°C for all experiments. The experimental chamber, stimulation circuitry, force transducer, servomotor, and recording system have been described (Julian and Morgan, 1979; Julian et al., 1986).

Slack test

Julian et al. (1986) showed that, near optimal length, the removal of slack (monitored photographically) coincided with the point at which the rate of rise of tension was 5–10 P_o/s, where P_o is tetanic tension generated at a sarcomere length of 2.1 μm. In the present study, tension records were differentiated using a digital oscilloscope (model 4094, Nicolet Instrument Corp., Madison, WI) and slack times were read as the time required for the rate of tension redevelopment to reach 20% of maximum. This criterion corresponds closely to that used previously.

At long lengths, the presence of passive tension had a large effect on the slack time of a fiber. For a fixed step size at a particular sarcomere length, slack time decreased as the level of passive tension increased. Because of this, all releases within a given slack test were initiated from the same passive tension when the test consisted of length steps from a fixed initial sarcomere length. Passive tension was controlled by varying the time interval between restretch and stimulation without significantly altering the time between stimulations.

Photomicrography

Photomicrographs were taken to determine the sarcomere length response of a fiber to step length releases. To increase the proportion of sarcomeres sampled, short fibers (1.0–1.5 mm) from the lubricalis digiti muscles in the toe of the frog were used. Releases were imposed from fiber lengths corresponding to passive sarcomere lengths near 3.35 and 2.75 μm. Active fibers were photographed before release and at a series of times after the step was applied. One tetanus was required for each image and two images were required at each time point in order to photograph the entire length of the fiber. A typical toe fiber showing complete end-to-end striation patterns just before and immediately after a slack-producing release is shown in Fig. 1. Images were recorded on 35-mm film (Technical Pan, Eastman Kodak Co., Rochester, NY). Illumination was provided by a high-speed (~6 μs in duration) xenon
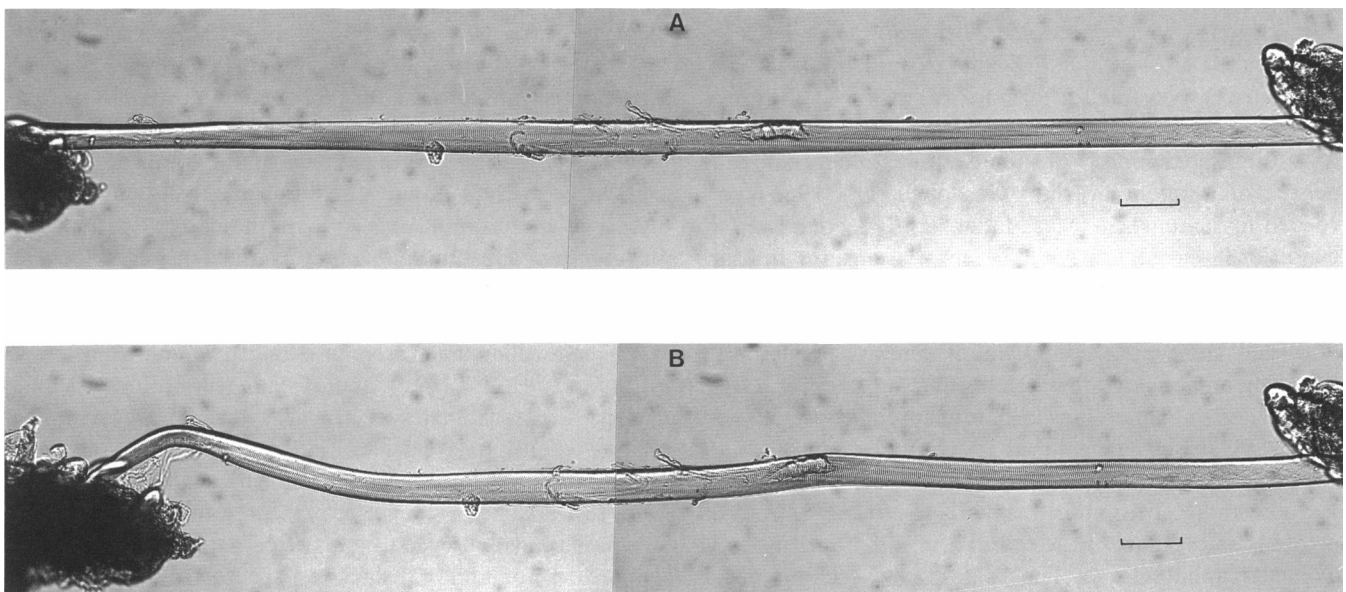

FIGURE 1 (*A*) Photograph of a tetanically contracting lumbricalis digiti fiber with average (active) sarcomere length of 3.31 μm. (*B*) Same fiber 0.5 ms after slack-producing release of 0.25 μm per sarcomere. Static passive tension level before stimulation was 10% of P_o. Calibration bar indicates 0.1 mm. Left end of fiber attached to servomotor. Fiber length at sarcomere length of 2.1 μm was 1.32 mm. In both *A* and *B*, two exposures were required using two tetani in order to obtain composite photograph of entire fiber length.

flash lamp (EG&G Electro-Optics Div., Salem, MA). The consistency of sarcomere length measurements was verified by repeating several time points. The size of the slack inducing step had no effect on the magnitude or time course of the early rapid phase of sarcomere shortening. An intermediate step size of 0.25 μm per sarcomere was selected since larger steps caused more buckling, decreasing the likelihood that the entire fiber would remain within the plane of focus.

Negatives were enlarged to a final magnification of ×357–482. Each fiber image was divided into 10 segments of equal length and the average sarcomere length was determined within four to six of the segments by measuring the length of strings of 40 sarcomeres per segment. Mean sarcomere lengths from individual segments were then averaged at each time point. Consistency was ensured by noting naturally occurring landmarks on the fiber so that sampling from the same sarcomere population within each fiber segment from image to image was possible.

RESULTS

Slack tests

A typical set of slack test responses recorded from one fiber in the presence of passive tension is shown in Fig. 2.

In Fig. 2 A the protocol used was a series of length steps to a fixed final sarcomere length. In Fig. 2 B the steps were initiated from a fixed initial length. In applying the slack test, the sarcomere length range spanned by the smallest step is common to all steps within each protocol. The slope of the line fitted to the step size–slack time relationship contains no information regarding the speed at which a fiber shortens over this range. Rather, the slope of this line indicates the average speed at which a fiber shortens over the range of sarcomere lengths included in the largest step but not the smallest. For steps to a fixed final length (Fig. 2 A), this range extends from the longest initial length to the initial length of the smallest step. For steps from a fixed initial length (Fig. 2 B), this range extends from the final length of the smallest step to the final length of the largest step. These critical ranges (see also Fig. 3, inset) will be referred to as "relevant length ranges." In Fig. 2 A note that fixed increments in step size produce progressively decreasing increments in slack times. The slack test results obtained from the records shown in Fig. 2 are plotted in Fig. 3 (triangles).
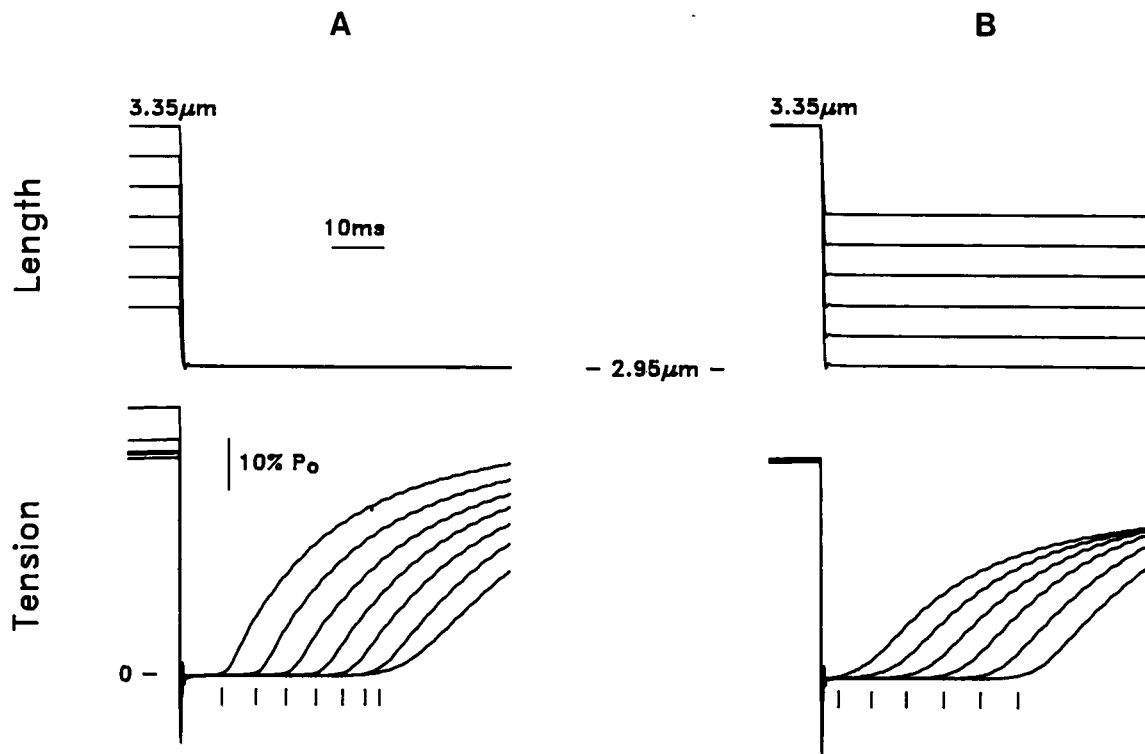

A

B

FIGURE 2   Slack test records from one fiber. (A) Upper traces: step releases initiated from a series of sarcomere lengths ranging from 3.35 to 3.05 μm to a fixed final sarcomere length of 2.95 μm. Lower traces: tension responses for releases shown above. The vertical marks beneath the tension records indicate slack times as measured according to the criterion described in Methods. Using this protocol, note that the tension level preceding the step varies with the highest level corresponding to 2.95 μm and the lowest to 3.35 μm. This is due to a decrease in total tension, since active tension over this sarcomere length range decreases more rapidly than passive tension rises. (B) Upper traces: step releases initiated from a fixed initial sarcomere length of 3.35 μm to a series of sarcomere lengths ranging from 3.20 to 2.95 μm. Passive tension level prior to stimulation was 28% of P_o for each step. Lower traces: tension responses for releases shown above with slack times indicated as described in A.
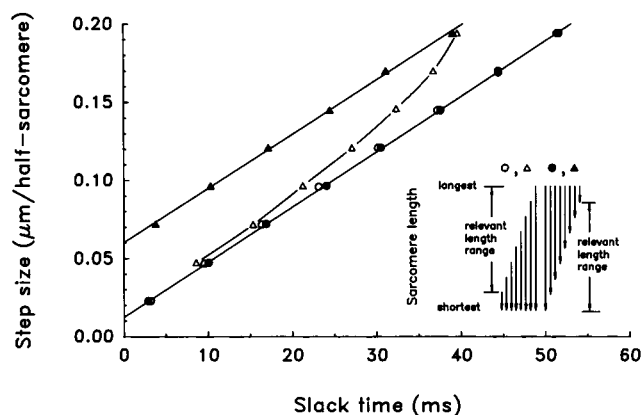
FIGURE 3 Slack test plots for fixed final length and fixed initial length protocols. Results are from same fiber as in Fig. 2. *Filled circles:* results for releases from a fixed initial sarcomere length of 2.35 μm. The *line* represents the best least-squares fit to the filled circles with step size as the independent variable (V_u = 3.47 μm per half-sarcomere/s). *Open circles:* releases to a fixed final sarcomere length of 1.95 μm. The initial sarcomere length corresponding to the largest step was 2.35 μm. *Open triangles:* results for releases to a fixed final sarcomere length of 2.95 μm. The initial sarcomere length corresponding to the largest step was 3.35 μm. The *curve* is a cubic spline interpolation of the data points. *Filled triangles:* results for releases from a fixed initial sarcomere length of 3.35 μm and passive tension level of 28% of P_o. The line represents the best least-squares fit (V_u = 3.48 μm per half-sarcomere/s). Original oscilloscope records for *open and filled triangles* are shown in Fig. 2. (*Inset*) Diagram of length step protocols used, together with indication of "relevant length ranges."

In the absence of passive tension, slack test results do not depend on which of the protocols described above is used (Fig. 3, *circles*). In contrast, the presence of substantial passive tension produced results that differed markedly depending on which protocol was selected. For releases to a fixed sarcomere length (Fig. 2 *A*), the relationship between slack time and step size fell on a curved line that increased in slope as step size increased (Fig. 3, open triangles). The order in which the steps were introduced had no effect on slack time as long as a consistent level of passive tension was maintained at each initial sarcomere length. Note that tangent lines (not shown) to the curve fitted to the *open triangles* would intersect the step-size axis at decreasing values as step size is increased. For the largest steps, the tangent line intercepts would be negative, a result inconsistent with the usual interpretation of the intercept as an indicator of the strain of series elastic elements.

The *filled triangles* in Fig. 3 show the slack times from a series of steps in which the same fiber was released from a constant sarcomere length (Fig. 2 *B*). Again, when a constant initial passive tension level was maintained, the slack time for any step size was highly repeatable and independent of step order. Two characteristics were observed in all fibers in which releases were performed

from a constant initial sarcomere length and passive tension level in the presence of substantial passive tension: (*a*) the slope of a line fitted to the step size–slack time data points was not significantly different from those of lines fitted to points collected over sarcomere length ranges in which passive tension was negligible; however, (*b*) the intercept of the line was markedly increased, as shown in Fig. 3. These two characteristics are shown in Fig. 4 for five different fibers in which the fixed initial sarcomere length protocol was used. The initial sarcomere lengths ranged from 2.35 μm, where passive tension was negligible, to 3.35 μm, where substantial passive tension was present.

Double-length steps

To confirm that V_u does not change at long sarcomere lengths, a series of double-length step experiments were performed as shown in Fig. 5. From each of two initial sarcomere lengths, one in the absence (2.35 μm) and one in the presence (3.35 μm) of passive tension, identically sized single-length steps were applied and the time of tension redevelopment was noted. Next, the same initial step was applied and, at the instant tension redevelopment
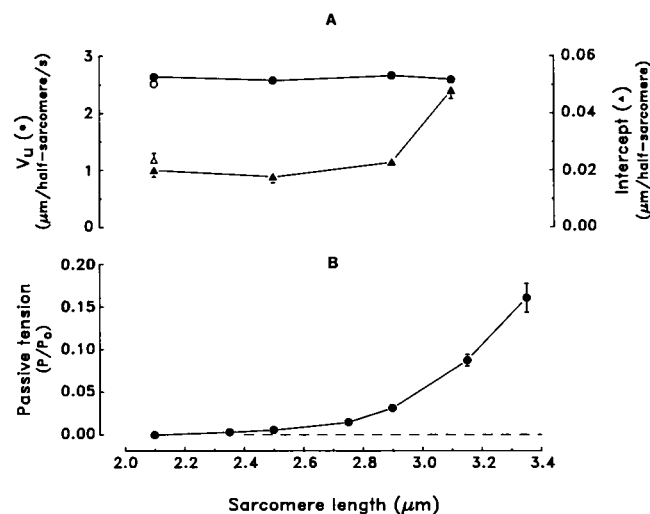

FIGURE 4 Plots of (*A*) slack test slopes (V_u) and intercepts and (*B*) static passive tension levels against sarcomere length. In *A*, results are plotted against the sarcomere length at the center of the corresponding relevant length range (see text and *inset* in Fig. 3). In *B*, the results show fixed-length passive tension levels measured 1 min after stretch to indicated sarcomere length. Results are from five fibers and show mean ± SEM. Slack tests were performed in order of increasing sarcomere lengths followed by a repeat of the test series at the shortest length (*open symbols*). Error bars are omitted where they fall within the mean symbol. Note that some of the rapid initial shortening required to produce slack takes place in series elastic material outside the sarcomeres. Consequently, the intercept values, although reported in micrometers per half-sarcomere, are an overstatement of the actual amount of rapid shortening that occurred within a half-sarcomere.
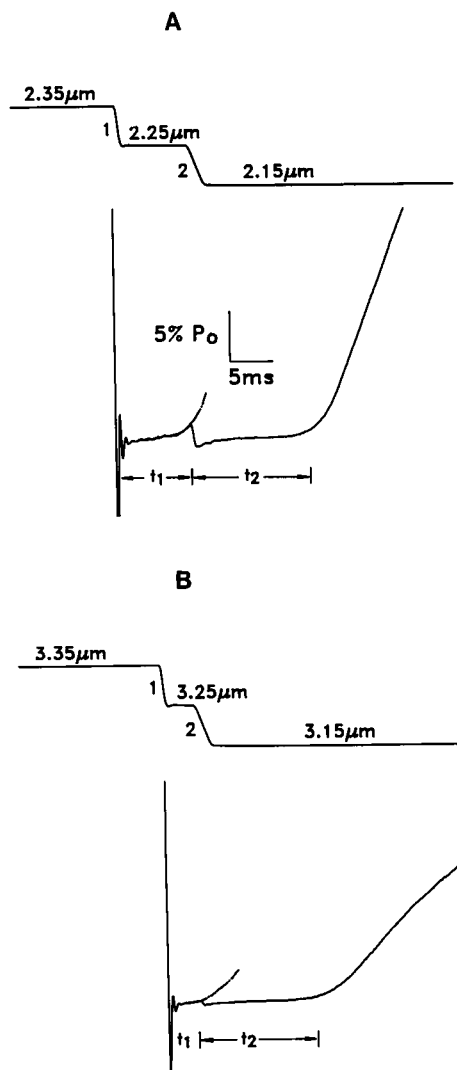
A

B

FIGURE 5 Records showing results from double-length step experiments. In both parts, *upper trace* shows length-step sequence; *lower trace* shows tension response. All tension records are on the same tension scale; all records are on the same time scale. In A, fiber was initially released by 0.10 μm per sarcomere from a sarcomere length of 2.35 μm, and then released again at a time corresponding to the end of the slack time for the first release (indicated by t_1). The time required to remove the slack produced by the second step is indicated by t_2. In B, a similar protocol was followed, but the initial sarcomere length was 3.35 μm. Static passive tension level at 3.35 μm was 16% of P_o. The *broken lines* in the force traces at the end of the t_1 periods show the time course of force redevelopment in the absence of a second step. Note that t_1 in B is much shorter than t_1 in A, whereas t_2 is unchanged.

began, a second step of the same size was added. The second steps were thus applied to fully activated, just taut fibers in which the series elastic elements were unstressed. Since there was no series elastic recoil, the size of the second length step divided by the time required to remove the slack it introduced gives a direct measure of the

average unloaded speed of shortening over the sarcomere length range covered by the second step. As shown in Fig. 5, the time required to take up the slack introduced by the second length step (t_2) was the same at both sarcomere lengths. In a series of similar double-step experiments, slack times for first length steps of 0.17 μm per sarcomere from sarcomere lengths of 2.35 and 3.35 μm were 19.3 $\pm$ 0.3 and 15.2 $\pm$ 0.4 ms (mean $\pm$ SEM, n = 8), respectively, whereas slack times for second length steps of the same size were 29.8 $\pm$ 0.4 and 29.4 $\pm$ 0.4 ms, respectively.

When the size of the second length step was varied and plotted against its associated slack time, the result was a line passing very nearly through the origin with the same slope regardless of initial sarcomere length. This slope was the same as that obtained in the usual way with a series of single steps.

Sarcomere shortening after step release

Sarcomere shortening was recorded photographically both in the presence and absence of passive tension. Typical results are shown in Fig. 6. In the presence of high levels of passive tension (Fig. 6 A), active shortening (*filled circles*) proceeded in two distinct phases: an initial phase of very rapid shortening, complete within 1 ms, followed by a phase of much slower shortening. The size of the rapid shortening phase was repeatable from step to step and independent of the size of the length step when care was taken to perform all releases from the same level of passive tension. When a passive fiber was released in the presence of high levels of passive tension (Fig. 6 A, *open circles*), sarcomere shortening took place at a rate even greater than that recorded during the fast phase of active shortening.

When passive tension levels were very low (Fig. 6 B), active sarcomere shortening after a step release proceeded at a nearly constant rate and did not include a detectable initial rapid phase. The dotted lines in both A and B show the time courses of shortening expected if the sarcomeres simply began shortening immediately upon release at the V_u measured in the absence of passive tension. Careful examination of the magnified images of stretched sarcomeres after a slack-inducing release failed to reveal any sign of the wavy myofibrils described by Gonazáles-Serratos (1971).

Factors affecting the initial rapid shortening phase

The rapid shortening phase revealed by the photographs resulted in a significant reduction in slack times at long lengths. Experiments were performed to determine the
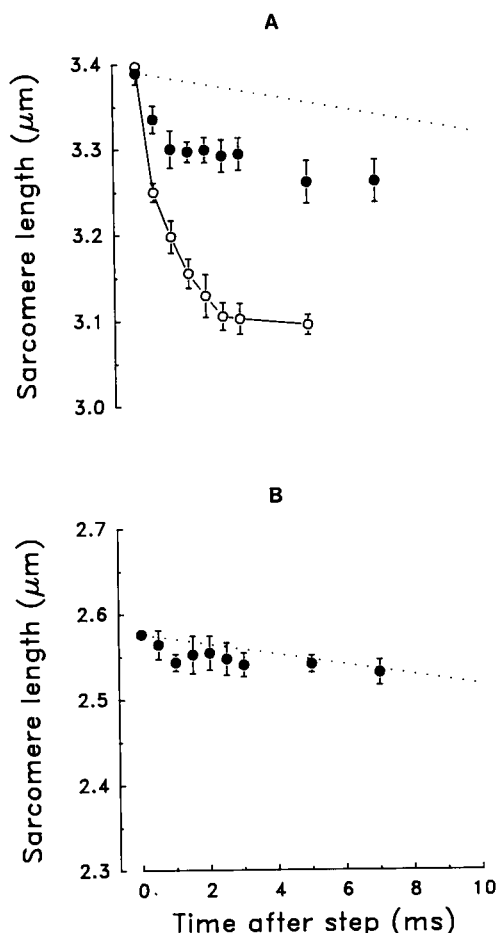
FIGURE 6 Plots of sarcomere shortening against time. Values for sarcomere length were obtained using the flash photography technique described in Methods. The results shown in A and B are from two different fibers. Each point is composed of the mean and SEM of measurements from four fiber segments comprising ~40% of the fiber length. The sarcomere length associated with zero time indicates the active length just prior to the length step, 3.39 and 2.58 μm, respectively, for the long and short lengths. At the long length, the static passive tension level was 10% of P_o whereas at the short length, passive tension was negligible. The *dotted lines* indicate the time course expected if sarcomere shortening began immediately upon release and proceeded at a constant rate equal to the value of V_u obtained in each fiber using the slack test at a sarcomere length of 2.1 μm. In A, the response of the fiber while passive (*open circles*) is also shown. Note the phase of high-speed active shortening immediately after the length step in A. Also note in A that the fiber shortens most rapidly when passive. Step size for both fibers was 0.25 μm per sarcomere.

influence of three factors on this reduction in slack time: (a) the passive tension level at the time of the release, (b) the degree of overlap of thick and thin filaments, and (c) the push of the motor on the fiber.

At long sarcomere lengths, both passive tension and the degree of overlap of thick and thin filaments had significant effects on slack time. Passive tension levels were

varied at a given sarcomere length by varying the time between stretch and stimulation, which takes advantage of the characteristic tension decay after stretch of a passive fiber. For a fixed step size and initial sarcomere length, slack time was inversely related to the level of passive tension present. At a fixed level of passive tension, slack time was directly related to the degree of filament overlap. These effects are shown in Fig. 7 A in which four tension responses to a constant-amplitude length step (0.15 μm per half-sarcomere) are superimposed. The response with the longest slack time corresponds to a release from a sarcomere length of 2.35 μm, where passive tension is negligible. The two intermediate slack times resulted from releases from a sarcomere length of 3.25 μm in the presence of two different levels of passive tension. An increased passive tension level resulted in a decreased slack time. When the fiber was stretched to a sarcomere length of 3.35 μm and the passive tension allowed to decline to the same level as that producing the shortest slack time from 3.25 μm, an even shorter slack time was observed. These results show that slack time decreases as either the passive tension level or the sarcomere length increases. Since passive tension rises as sarcomere length is increased, the two effects combine to result in a marked decrease in slack time for a fixed step size at long lengths.

The decreases in slack time shown in Fig. 7 A affect slack test plots as shown in Fig. 7 B. For a constant step size, the effect of an increase in passive tension, decrease in filament overlap, or both, is a shift of the plotted points toward the step-size axis. The magnitude of the shift is independent of the size of the step. Consequently, a line fitted to a set of shifted points has an increased intercept but no significant change in slope. These results are consistent with an increase in the rapid initial phase of sarcomere shortening in the presence of high levels of passive tension and reduced filament overlap, with no change in the rate of the subsequent, slow phase of unloaded shortening.

The effect of the push of the motor was tested by making connections between the fiber and the motor through a flexible link (see Methods and Fig. 8 A). The results of one of three such experiments are summarized in Fig. 8 B. The plots show that the slack times for releases at short sarcomere lengths, where passive tension levels are low and the degree of overlap of thick and thin filaments is high, were decreased. The decreases are consistent with an increase in mounting compliance associated with the silk link. In contrast, the slack times for releases from a long sarcomere length increased when a flexible link was used, but remained shorter than those for the short length with flexible link. This result suggests that a fraction of the increase in the initial rapid shortening phase at long lengths is due to pushing by the motor.
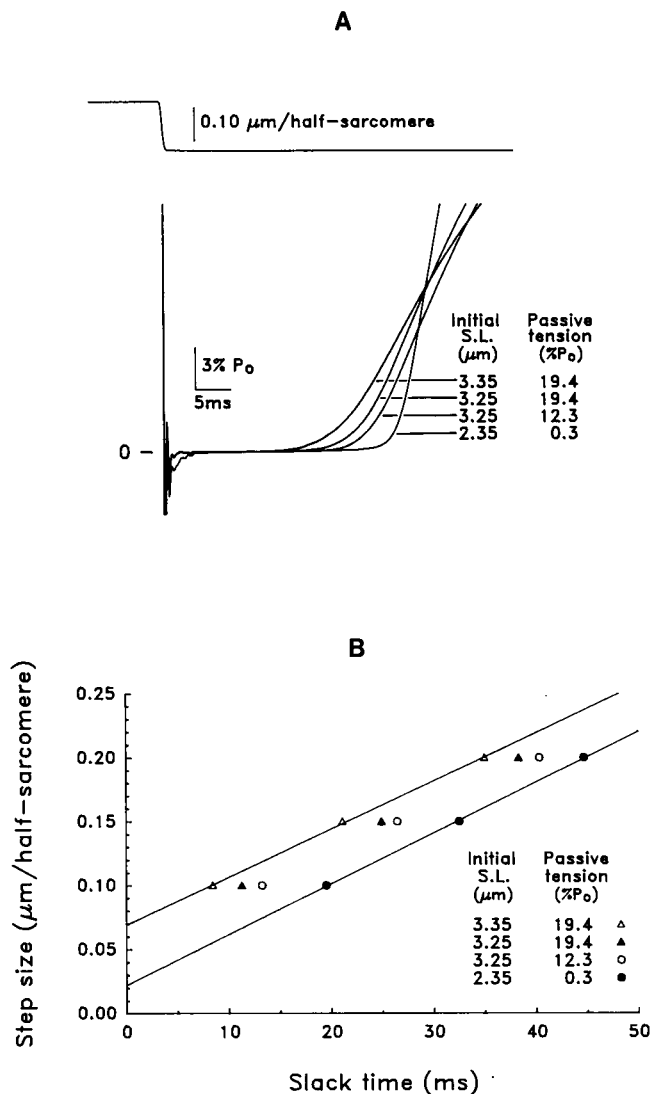
A

B

FIGURE 7 The effects of passive tension and sarcomere length (S.L.). (A) Effects on slack time. *Upper trace:* length record. Step size was 0.15 μm per half-sarcomere for all tension responses shown below. Initial sarcomere lengths lengths are indicated on the corresponding tension traces. *Lower traces:* superimposed tension responses. The record indicating the longest slack time occurred in the absence of significant passive tension in response to a step from a sarcomere length of 2.35 μm. The remaining three tension records, in order of decreasing slack times, represent responses to steps from the following sarcomere lengths and passive tension levels: 3.25 μm, 12.3% of P_o; 3.25 μm, 19.4% of P_o; and 3.35 μm, 19.4% of P_o. The time calibration bar corresponds to both length and tension records. (B) Effects on slack test plots. Stretching the fiber from a sarcomere length of 2.35 μm, where passive tension is negligible, to 3.25 μm, where a constant passive tension of 12.3% of P_o was maintained, resulted in an increase in intercept from 22.3 to 51.7 nm per half-sarcomere. Increasing passive tension to 19.4% of P_o at a sarcomere length of 3.25 μm further increased the intercept to 58.3 nm per half-sarcomere. Finally, stretching the fiber to a sarcomere length of 3.35 μm and maintaining passive tension at 19.4% of P_o resulted in the largest slack test intercept of 69.2 nm per half-sarcomere. The range of V_u values is 7% of the average value.
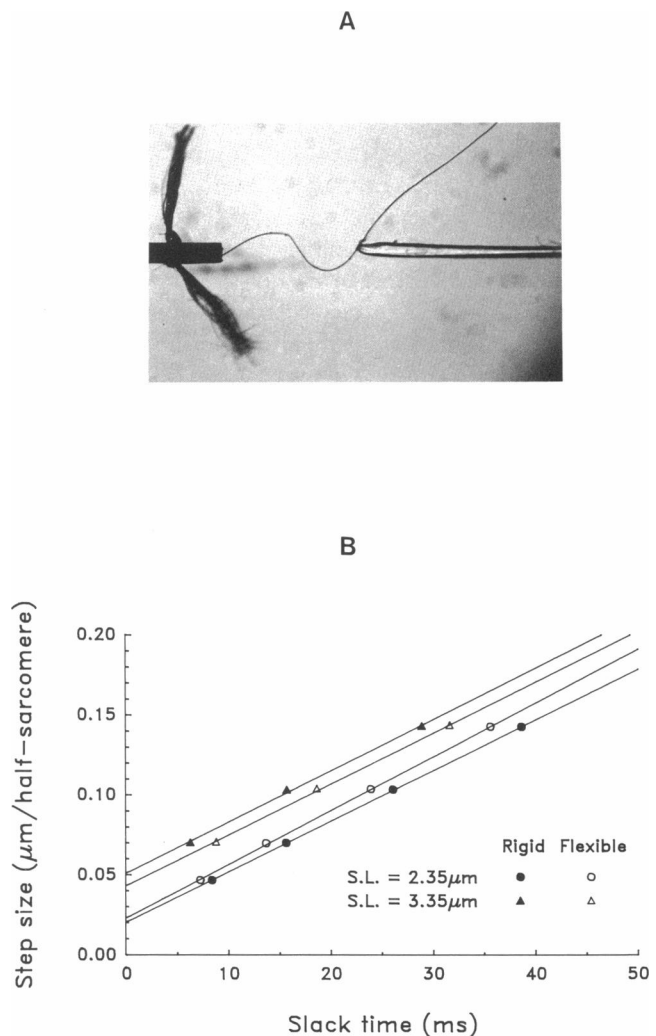
A

B

FIGURE 8 The effect of adding a flexible link. (A) Photograph of a tetanically contracting tibialis anterior fiber 2 ms after a step of 0.17 μm per sarcomere. Fiber is attached to motor wire via 2 mm of a single strand of silk. Note that the silk strand buckles while the fiber remains straight. Calibration bar indicates 1.0 mm. (B) Slack test plots. Adding the flexible link increased the intercept from 20.7 to 23.3 nm per half-sarcomere at the short length but decreased it from 51.3 to 43.4 nm per half-sarcomere at the long length. The range of V_u values is 6% of the average value. S.L., sarcomere length.

The slope of the plot remained unchanged, indicating that V_u was not affected.

Static and dynamic passive tension

The passive tension present at the center of the relevant sarcomere length range (3.1 μm) for slack tests initiated from 3.35 μm was about 7% of P_o (see passive tension curve in Fig. 4 B). If this large compressive force were

present in an actively contracting slack fiber, it should increase the speed of sarcomere shortening. However, the static passive length–tension characteristic of a nonactivated fiber may not be relevant after stimulation. A more relevant estimate of the residual compressive force could be found by shortening a passive fiber at speeds near V_u and recording the tension response. This gives a much-reduced passive tension level that depends on the length at which the release begins. In view of this, an experimental protocol was devised to test whether such variations in dynamic passive tension could affect the measured V_u.

For these experiments, slack tests were performed in the usual way beginning from sarcomere lengths 2.35, 3.35, and 3.55 μm. Step sizes were chosen so that the relevant length ranges for releases from 3.35 and 3.55 μm were the same. Results from one such series of slack tests are shown in Fig. 9 A. In agreement with results presented earlier, the slopes of the fitted lines were not significantly different while the intercepts increased with increasing sarcomere length. At each of the two longer
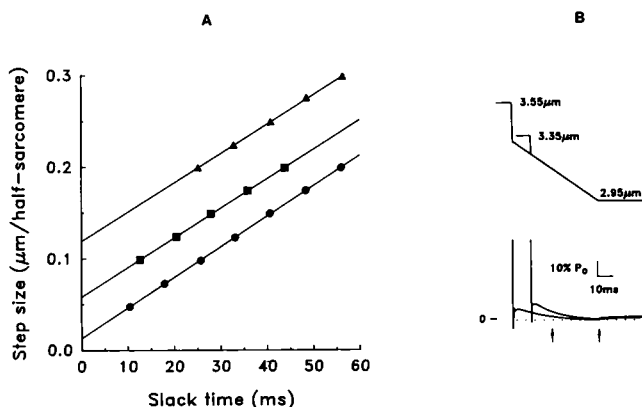

A   B

FIGURE 9 The effect on slack test V_u of varying the amount of dynamic passive tension present over the relevant length range at long sarcomere lengths. Three initial sarcomere lengths were chosen: short, 2.35 μm, *filled circles* in A; long, 3.35 μm, *filled squares;* and very long, 3.55 μm, *filled triangles.* Passive static tension was negligible at the short length, whereas it was 23% of P_o at 3.35 μm and 36% of P_o at 3.55 μm. Slack tests were performed in the usual way using the fixed initial length protocol, and the results are plotted in A. Step sizes were chosen to make the relevant length ranges coincide for steps from 3.35 and 3.55 μm. The slope of the fitted lines is essentially the same for each of the three lengths, whereas the intercepts increase markedly with increasing initial sarcomere length. (B) Passive responses to step-ramp length releases from the long and very long sarcomere lengths. The step releases shown in the upper part were selected to match the corresponding slack test intercepts, whereas the ramp had the same slope as the lines shown in A. Corresponding passive force responses are shown in the lower part of B where it is evident that less passive force was present over the relevant length range (marked by *vertical arrows*) in the case of the step-ramp release from 3.55 μm. All results from one fiber. Results from two other fibers were essentially identical.

sarcomere lengths, after the slack test results had been obtained, a combination step-ramp release was imposed on the passive fiber. The magnitude of the step and speed of the ramp were selected to match the intercept and slope of the corresponding slack test plot. The ramps were terminated at the end of the relevant length range. The step-ramp releases were thus designed to simulate, in a passive fiber, the length changes of actively contracting sarcomeres after a slack-inducing length step. Typical responses to these step-ramp maneuvers are shown in Fig. 9 B.

In contrast to the passive tension levels observed under static conditions, the average dynamic passive tensions maintained over the relevant length range for step-ramp releases from 3.35 and 3.55 μm were very small: 0.98 $\pm$ 0.11% and 0.32 $\pm$ 0.08 P_o, respectively (mean $\pm$ SEM, $n = 3$). These values for dynamic passive tension levels are about 14% and 4%, respectively, of the amount present at the center of the relevant length range under static conditions. Much of this decrease during dynamic shortening can be attributed to the hysteresis in the passive length–tension relationship that is evident even under static conditions (McCarter et al., 1971). These results show that the compressive passive tension remaining in an active fiber following a slack-inducing release may be much smaller than that predicted from a static length–tension curve.

DISCUSSION

Slack test in the absence of passive tension

In the absence of significant levels of passive tension, the response of a contracting fiber to a step-length release that induces slack consists of a short initial phase of very high speed shortening, interpreted as recoil of stressed series elastic elements, followed by a phase of much slower shortening at a constant rate against zero external load (Edman, 1979). When the speed of sarcomere shortening against zero load is to be determined, the effect of the recoil of the series elastic material must somehow be nullified. To do this, Edman (1979) applied several steps of different amplitudes. He showed that, in the absence of significant levels of passive tension, plots of step size against slack time could be fitted well by a straight line which intersected the step-size axis at a positive value. Edman interpreted these results by assuming that the magnitude of the rapid recoil phase was independent of the size of the applied slack-inducing step. Based on this assumption, the slope of the fitted line indicates the speed of the constant, slow phase of sarcomere shortening (V_u). The positive intercept of the line is due to the rapid phase of shortening, and is taken as an index of the extent to

which series elastic elements were stretched by the preceding fixed-length contractions.

Edman (1979) and Julian et al. (1986) have shown that V_u, as estimated by the slack test in the absence of significant passive tension, is in agreement with estimates of unloaded shortening speed obtained from application of load clamps and length ramps. The present study provides support for Edman's interpretation when passive tension is absent. The effect of the presence or absence of the rapid elastic recoil phase can be seen directly in Fig. 5 A where double-step experiments show that t_1, the slack time resulting from a release during maximal tension generation, is shorter than t_2, the slack time produced by the same-sized release applied to a just taut, unstressed fiber. When a slack test is performed on a just taut, unstressed fiber, the intercept is very nearly zero but the slope is equal to that obtained from releases applied during maximal tension generation.

It should be noted that the assumption of a constant rapid-recoil phase common to all responses is not strictly correct for slack test protocols that do not consist of releases from a fixed sarcomere length, since active tension and, therefore, strain of elastic elements depends upon length. Comparison of results from slack tests consisting of steps from a fixed sarcomere length and to a fixed sarcomere length over the sarcomere length range 1.95 to 2.35 μm, where passive tension levels are small, indicate that this effect is negligible.

Slack test in the presence of passive tension

Slack tests performed in the presence of significant levels of passive tension using the fixed final length protocol resulted in step size–slack time relationships that were not linear, indicating that one or both of the assumptions applied in the absence of passive tension (rapid recoil common to all releases followed by constant, slower shortening of sarcomeres) are no longer valid. Slack tests performed in the presence of passive tension using the fixed initial length protocol produced step size–slack time relationships that could still be fitted well by straight lines. The slopes of the fitted lines were not different from those obtained in the absence of passive tension. The intercepts, however, were increased significantly. Since, in these stretched fibers, total tension (active plus passive) was lower at the time the step releases were applied, it would be expected that the intercepts should be slightly lower rather than much higher. These results indicate that the simple interpretation of the slack test intercept as an index of the strain of series elastic elements is not valid when passive tension levels are high. Two separate series of experiments were undertaken to determine whether either slack test protocol produced valid results in the presence of passive tension: (a) measurement of V_u using double-length steps, and (b) photography of striation spacings after a step-length release.

Double-length steps

By applying a pair of identically sized, slack-inducing length steps to a contracting fiber, with the second step added just as the slack produced by the first step is taken up, it is possible to separate an effect on slack time caused by rapid recoil from that associated with steady sarcomere shortening. While the response to the first step is strongly influenced by rapid recoil, the response to the second step, applied to a just taut, unstressed fiber, should reflect the active unloaded shortening behavior. The observation that t_2, the time required to remove slack caused by the second step, is the same in Fig. 5 A, where the range covered is from 2.25 to 2.15 μm, as in Fig. 5 B, where the range is from 3.25 to 3.15 μm, confirms that the speed of unloaded shortening is the same regardless of the markedly different sarcomere length ranges.

Another feature to be noted in Fig. 5 concerns t_1, the time required to remove the slack produced by the first step. It is much shorter when passive tension levels are high as shown in Fig. 5 B. Since total tension at the time of the first step is lower in the stretched fiber, this result cannot be interpreted as a series elastic recoil determined by tension, followed by sarcomere shortening at the speed determined from the second step. At long lengths the steady speed is the same, the recoil of elastic components is smaller, but the slack time is reduced.

Sarcomere shortening after step releases

One possible interpretation of Fig. 5 is that the initial rapid shortening phase of a fiber is much larger in the presence of high levels of passive tension. This could occur if the rapid shortening phase had, in addition to a component of recoil from stressed series elastic elements, another component contributed by rapid sarcomere shortening. This possibility was examined using flash photography to record the time course of sarcomere shortening, with emphasis on the early events, following slack-inducing releases in the presence and absence of passive tension.

Analysis of the photographs showed that, in the presence of passive tension, a significant fraction of the imposed length change, much greater than the recoil of series elastic structures, was taken up by an initial, very rapid phase of sarcomere shortening. The extent of this phase did not depend on the size of the slack-inducing step provided that initial sarcomere length and level of passive tension were fixed. This rapid phase was not observed

after releases applied at sarcomere lengths associated with very low levels of passive tension. It could be interpreted as a transient effect of passive tension acting to increase the speed of sarcomere shortening as proposed by Edman (1979). This interpretation is supported by the observation, shown in Fig. 7, that increasing the level of passive tension without changing the degree of overlap of thick and thin filaments results in a slack test plot that indicates an increase in the rapid initial phase of sarcomere shortening. Furthermore, when this transient effect of passive tension is aided by the push of the rapidly moving motor, a further increase in the rapid phase of shortening is indicated as shown in Fig. 8 B.

It should be noted in Fig. 6 that the rapid phase of shortening was almost as fast as passive shortening, so that it could be interpreted as shortening opposed by very few cross-bridges. This interpretation is consistent with the observation, shown in Fig. 7, that decreasing the degree of overlap between filaments, thus decreasing the number of cross-bridges available for attachment, without changing the level of passive tension results in a slack test plot that indicates an increase in the magnitude of the rapid shortening phase. This view would suggest that most of the cross-bridges became detached at the time of the release, and only when the fast phase ended did they reattach in large numbers. At that point the speed of shortening returned to its full overlap value (V_u), independent of fiber length.

Influence of protocol on slack test results

The different results obtained in the presence of passive tension using either the fixed initial or fixed final sarcomere length protocols for slack tests can be explained as follows. When slack tests are performed by releasing from increasing sarcomere lengths, each step is initiated from an increased level of passive tension and a decreased amount of filament overlap. Consequently, the initial shortening response to each increasingly larger step contains a rapid sarcomere shortening phase of nonlinearly increasing magnitude, resulting in slack test plots that can be fitted with a curved line of continuously increasing slope.

If the releases that constitute a slack test span a small range of sarcomere lengths, this curvature is much less apparent than that shown in Fig. 3 (*open triangles*). When a straight line is fitted to data obtained in this way, both the slope and the intercept are affected by the increasing magnitude of the rapid initial phase of shortening. The speed of shortening indicated by the slope of the fitted line is intermediate between the rapid initial and slower, secondary phases after release. The speeds obtained in this way do not, however, correspond to a steady rate of sarcomere shortening. Consequently, attempts to interpret these results as increases in steady rate shortening due to a constant negative load provided by passive tension are not appropriate.

The interpretation of the intercept of the fitted line is also complicated when slack test releases are initiated from different sarcomere lengths in the presence of passive tension. The result of the increasing magnitude of the rapid shortening phase as release size is increased is a decrease in the intercept. At moderate to high levels of passive tension, the intercepts of the fitted lines can, in fact, fall below zero. A negative intercept can be obtained, for example, by fitting a line to the largest three releases in Fig. 3 (*open triangles*). The reduced and negative intercepts are not consistent with the traditional interpretation of slack test results.

In contrast, when slack tests are performed from a fixed initial sarcomere length and level of passive tension, the length response to each increasingly larger step contains a brief rapid phase of sarcomere shortening which is essentially the same. The rapid shortening phase is followed by a phase of much slower sarcomere shortening at a rate near the full overlap V_u. Slack test plots in this case can be fitted well by straight lines. The slopes of the lines indicate that V_u is constant as sarcomere length is increased to values as high as 3.1 μm. The rise in the intercepts of the fitted lines as initial sarcomere length is increased is due to an increase in the magnitude of the rapid sarcomere shortening phase. This results in each data point on the slack test plot being shifted a constant amount along the time axis toward smaller values, producing a plot that can still be fitted with a straight line whose slope is unchanged, whereas the intercept is increased in proportion to the magnitude of the initial rapid phase of fiber shortening.

Static and dynamic passive tension

The fact that V_u remains constant at sarcomere lengths associated with high levels of passive tension, which should act as a negative, compressive load, requires explanation. In the passive fiber, the tension level present at a particular sarcomere length depends on the way the length is attained. When a step-ramp length decrease, which simulated the time course of active sarcomere shortening observed in stretched fibers, was applied to a passive fiber, it was found that the average tension maintained over the relevant length range for releases from 3.35 μm decreased markedly to a level of only 14% of that corresponding to the static level associated with the center of this length range. Average dynamic passive tension level over the same relevant length range fell even further to 4% of the static level when the step-ramp

combination was applied from the longer initial length of 3.55 μm. Nevertheless, the dynamic levels are not insignificant if account is taken of the force–velocity relation of muscle. Assuming that a hyperbola (Hill, 1938) can be reliably extrapolated to include negative loads, then a speed increase of 15% could be expected corresponding to the negative load indicated by the mean passive dynamic level noted for step-ramp releases from 3.35 μm.

Our data do not reveal a speed increase of this magnitude. The fact that V_u was unchanged when slack tests were initiated from 3.55 μm instead of 3.35 μm, which produced more than a threefold decrease in dynamic passive tension level over the relevant length range, argues that the residual passive tension present under these circumstances does not significantly influence unloaded speed of shortening. One possibility is that the tension level maintained by passive elements during rapid shortening in a stimulated fiber is even lower than the level indicated by the dynamic passive curve.

Conclusion

This work shows by several complementary methods that step releases in the presence of passive tension lead to two phases of sarcomere shortening. The initial rapid phase has an amplitude dependent on the preceding level of passive tension, sarcomere length, and the method of attachment. The later phase of steady shortening has a speed independent of the sarcomere length. Edman (1979) reported large increases in the steady shortening speed at lengths where passive tension is significant. In that study, however, no distinction was made between the two phases of shortening, leading to the adoption of a protocol unsuitable for determining the speed of the steady shortening phase. Plotting the slack test results presented by Edman (1979, Fig. 7) produces a regression line with a negative intercept, a finding that is inconsistent with his interpretation that the intercept is a measure of the total series compliance, but is in agreement with the present results for releases to a constant final sarcomere length in the presence of passive tension.

In addition, we did not find the decrease in velocity with increasing sarcomere length reported in skinned fibers (Metzger and Moss, 1987; Tsuchiya, 1988). In fibers with intact surface membranes, the lateral spacing between filaments varies as the inverse square root of fiber length, indicating that a constant fiber volume is maintained (H. E. Huxley, 1953). At a sarcomere length of 3.1 μm, the diameter of a fiber is reduced by ~18% compared with the full-overlap diameter at 2.1 μm. Our results indicate, therefore, that unloaded sarcomere shortening speed does not depend in any critical way on reductions of lateral filament spacing of this magnitude. Skinned skeletal muscle fibers that are compressed to a similar degree, using osmotically active long-chain polymers, show marked decreases in unloaded shortening speed (Metzger and Moss, 1987; Tsuchiya, 1988). However, as recently commented on by Edman (1988), results from skinned fibers require further elucidation before their relevance to results from fibers with intact surface membranes can be determined.

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