

# Variation of Muscle Stiffness with Force at Increasing Speeds of Shortening

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**ABSTRACT** Single frog skeletal muscle fibers were attached to a servo motor and force transducer by knotting the tendons to pieces of wire at the fiber insertions. Small amplitude, high frequency sinusoidal length changes were then applied during tetani while fibers contracted both isometrically and isotonicly at various constant velocities. The amplitude of the resulting force oscillation provides a relative measure of muscle stiffness. It is shown from an analysis of the transient force responses observed after sudden changes in muscle length applied both at full and reduced overlap and during the rising phase of short tetani that these responses can be explained on the basis of varying numbers of cross bridges attached at the time of the length step. Therefore, the stiffness measured by the high frequency length oscillation method is taken to be directly proportional to the number of cross bridges attached to thin filament sites. It is found that muscle stiffness measured in this way falls with increasing shortening velocity, but not as rapidly as the force. The results suggest that at the maximum velocity of shortening, when the external force is zero, muscle stiffness is still substantial. The findings are interpreted in terms of a specific model for muscle contraction in which the maximum velocity of shortening under zero external load arises when a force balance is attained between attached cross bridges some of which are aiding and others opposing shortening. Other interpretations of these results are also discussed.

## INTRODUCTION

It is now believed that the maximum steady tetanic force a skeletal striated muscle can generate at or beyond its optimum length depends on the number of thick filament cross bridges attached to thin filament sites (Gordon et al., 1966). One of the most important questions remaining regarding the nature of the contractile mechanism concerns the number of attached cross bridges present while a muscle shortens under various loads in the optimum or plateau region of the length-tension diagram (Gordon et al., 1966), where the number of cross bridges available for interaction is constant. There is some evidence (A. F. Huxley, 1971) indicating that at high speeds of shortening the number of attached cross bridges, or equivalently the muscle stiffness,

since the cross bridges act in parallel (Gordon et al., 1966), decreases, but this conclusion needs to be supported by further work.

However, if, as in the classical view (Hill, 1938), muscle can be modeled by a contractile component pulling against a series elastic component (SEC) whose stiffness varies with its extension, then measuring the total muscle stiffness, i.e., the stiffness of the series combination of contractile component and spring, may not provide information about the stiffness of the contractile component. If the stiffness of the series spring is less than that of the contractile component, the spring stiffness will dominate the measurement. Therefore, it must first be determined whether a significant SEC is present before meaningful stiffness measurements can be made. To do this in the work reported here, experiments of the type described by Huxley and Simmons (1971 *a*) were done in which very rapid length changes were applied to active muscle and the transient force responses recorded. The length changes were applied both at full and reduced overlap and during the rising phase of short tetani. An analysis shows that the transient force responses can be simply explained on the basis of varying numbers of cross bridges attached at the time of the length step. This suggests that a significant SEC is probably not present in the preparation used in this study. It is further shown that muscle stiffness, measured by the use of small amplitude, high frequency length oscillations, decreases with decreasing force as the speed of steady shortening increases, and this can be interpreted to indicate a decreasing number of cross bridges attached. A short description of this work has already been given (Julian and Sollins, 1974).

#### METHODS

Single muscle fibers were dissected from the anterior tibial muscles of the frog *Rana pipiens* and were securely attached to a servo apparatus by tying the stout tendons these fibers have to pieces of wire with 10-0 nylon suture at the fiber insertions. The composition of the Ringer's solution was (in mM): NaCl, 115; KCl, 2.5; CaCl<sub>2</sub>, 1.8; Na<sub>2</sub>HPO<sub>4</sub>, 2.15; Na H<sub>2</sub>PO<sub>4</sub>, 0.85. Its temperature was kept at 0°C. The general apparatus and procedures have already been described in detail (Julian, 1971; Julian and Sollins, 1973). Significant improvements were made in the force transducer and servo system so that rapid length changes could be applied and resulting force responses recorded.

The first series of results, shown in Fig. 1, was obtained using the servo motor described in the second of the two references just cited. The length steps were completed in approximately 1 ms, and the resonant frequency of the transducer, which was improved by making the deflecting capacitor plate and stylus lighter and stiffer, was about 1,000 Hz in air. This apparatus was also used in the experiments involving length oscillations during steady shortening, shown in Fig. 5. In these experiments (as well as in the length step experiments) the servo system was controlling muscle length (as opposed to force) at all times. A high frequency (500 or 1,000 Hz), small

amplitude ( $4\ \mu\text{m}$  or about  $5.3\ \text{\AA}$ /half-sarcomere, peak-to-peak) sine wave was used as the command signal in the length control loop of the servo system at all times. While the fiber was maintaining a steady force under tetanic stimulation, additional command signals consisting of a step decrease in length immediately followed by a constant velocity length decrease were superimposed on the sine wave oscillation. The oscillations in force and length were observed while the fiber was developing steady tetanic force and no distortion or asymmetry in the sinusoidal wave-forms was detected. The overall widths of the fast force traces used in calculating relative stiffness were corrected by subtracting the inherent width of the force trace in other records (not shown) in which the length oscillation was absent. The noise in the fast force trace was reduced by using a low pass filter with a 3-dB down frequency of about 5 kHz.

The second series of length steps, shown in Fig. 3, was obtained after making additional improvements in the performance of the servo system. The servo motor was replaced by one having a much lower moment of inertia (General Scanning, Inc., Watertown, Mass., model G-108) which was capable of greater acceleration. With this motor it was possible to make length steps in approximately 0.4 ms. The transducer resonant frequency was increased to approximately 2,000 Hz by further stiffening the deflecting plate of the capacitor and the stylus without producing an appreciable loss of sensitivity.

It is important that the results obtained in this work do not depend in a significant way on the resonant frequency characteristics of the force transducers used. In the case of the small amplitude, high frequency length oscillations, similar results were obtained using frequencies near and below the resonant frequency of the transducer. Only relative force values were used at any one frequency of length oscillation so that constancy of force transducer output as a function of frequency of applied oscillation is not necessary. In the case of recording the  $T_1$  and  $T_2$  force responses after sudden length changes, the force transducer resonant frequency component is small compared to the total response. When the force transducers used in this work were deflected in air and then suddenly released, the output voltage response consisted of an immediate large change in DC level followed by a small amplitude, rapidly decaying oscillation at the resonant frequency. The force transducer with the approximately 1,000-Hz resonant frequency was further tested by firmly connecting the transducer wire directly to the servo motor wire. Very small, constant amplitude length oscillations of various frequencies from near zero to about 2,000 Hz were then applied and the force transducer output recorded. The results suggest the presence of a small resonant peak near 1,000 Hz which is consistent with the transducer response to sudden unloading just mentioned. Since the rise time of the transducer with the lower resonant frequency should have been less than 1 ms, a significant limitation was the 1-ms time interval required to complete the length change. In addition, the initial drop in force in phase with the applied length change is not instantaneous while the recovery from  $T_1$  to  $T_2$  is very rapid. This suggests that the in-phase drop in force would have been larger than was recorded. The main effects of these limitations would be to indicate a decreased muscle stiffness, i.e., decreased slope of the curve passed through  $T_1$  points. The fact that, for similarly sized large length de-

creases, the  $T_1$  points in Fig. 4 fall considerably below those shown in Fig. 2 suggests that the true  $T_1$  points would fall along a steeper and more nearly linear path than those shown in Fig. 4 if faster length changes and higher resonant frequency force transducers had been used.

#### RESULTS

Fig. 1 shows typical records from an experiment designed to test whether a significant amount of series elasticity is present in the preparation. As can be seen in the slow traces, the fiber is tetanized and held isometric until the force reaches a nearly steady level. Then the muscle length is suddenly altered by a small amount. The details of the length step and force response are shown in the upper traces which are recorded at a fast sweep speed. The values of interest are measured from the records in the way shown in part F. Two additional series of length steps were performed, one while the force was in the rising phase of a tetanus, and the other in the steady phase of a tetanus after the fiber was stretched so that the steady tetanic force was decreased.

The force values  $T_1$  and  $T_2$ , as defined in Fig. 1 F are plotted against size of the length step in Fig. 2 for the rising and steady phases of contractions at optimal overlap and the steady phase of contraction at reduced overlap. The method by which the points obtained under these different conditions are plotted is described in the legend. In the Discussion, the results shown in this plot will be used to show that  $T_1$  and  $T_2$  can be explained on the basis of varying numbers of cross bridges attached at the time of the length step.

As described in the Methods, the performance of the servo system was improved and this made it possible to complete the length changes in approximately 0.4 ms. Records showing the resulting length changes and force responses are presented in Fig. 3. The records are similar to those in Fig. 1 except that the slow traces have been omitted, and also some stretches are included. The  $T_1$  and  $T_2$  force values are plotted against the size of the length steps in Fig. 4. Also shown are the  $T_1$  responses of a model for muscle contraction (Julian et al., 1974) to instantaneous length steps and to slower length changes which took about 0.4 ms to complete.

Fig. 5 presents records of the type of experiment used to measure stiffness at various shortening velocities. A tetanically stimulated single fiber was constrained to develop force isometrically until a nearly steady level was attained. Then the muscle length was suddenly reduced and subsequently made to decrease at a constant velocity. The force response, after a transient, maintains a constant level less than  $P_o$  until the shortening is stopped at which point the force redevelops to the isometric  $P_o$ . The important point is that throughout the entire process the servo is applying a constant amplitude, high frequency sinusoidal length oscillation to the muscle. The amplitude of the length oscillation, about 4  $\mu\text{m}$  peak-to-peak, is too small to be seen in

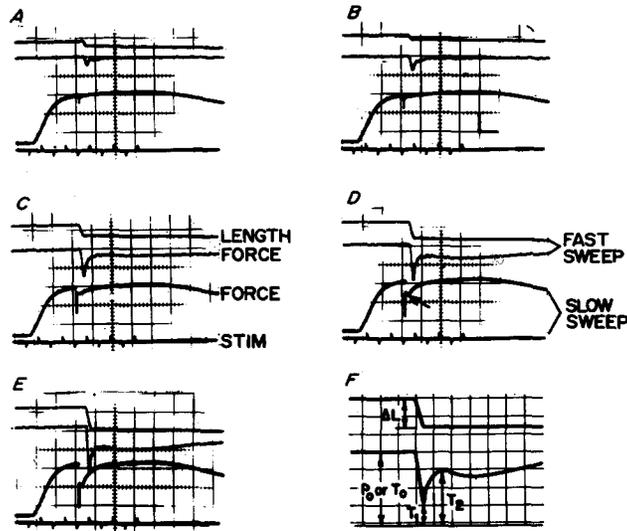


FIGURE 1. Force responses to sudden decreases in length of single fiber during tetanus. Average sarcomere length:  $2.2 \mu\text{m}$ . Force gain:  $40 \text{ mg}$  or  $1.3 \text{ kg/cm}^2/\text{division}$ . Length gain:  $0.05 \text{ mm/division}$  or  $83 \text{ \AA/half-sarcomere/division}$ , except in A,  $0.025 \text{ mm/division}$  or  $41 \text{ \AA/half-sarcomere/division}$ . Fast sweep:  $5 \text{ ms/division}$ . Slow sweep:  $100 \text{ ms/division}$ . Fiber length:  $6.7 \text{ mm}$ . Temperature:  $0^\circ\text{C}$ . Stimulus frequency:  $16/\text{s}$ . Fast sweep begins just before length changes are applied. The length decreases were completed in approximately  $1 \text{ ms}$ . The recovery of the force after the release has two distinct phases as shown in part F: a rapid recovery from  $T_1$  to  $T_2$  (best seen in the fast sweeps) followed by a slower rise in force back to the original  $P_0$ . Note the dip in the force traces immediately after the quick recovery, as indicated in part D by the arrow. This feature of the response is also produced by our model (Julian et al., 1974) in which it results from detachment of cross bridges in the force-generating position. Part F shows how the size of the shortening step,  $\Delta L$ , and the force levels,  $T_1$  and  $T_2$ , were measured from the experimental records. The rapid recovery phase from  $T_1$  to  $T_2$  was retouched in the slow sweeps of parts C, D, and E.

the length traces. However, when the muscle fiber is stimulated and develops force, the oscillation is easily detected in the force records where it appears as a widening of the traces. Since the length oscillation is maintained at a constant amplitude, the variation in width of the force traces can be taken as a measure of stiffness relative to the isometric state. It is apparent that during the phases of steady shortening the force traces are narrower than during the isometric phases. This indicates a decreased stiffness during shortening. In addition, the force trace becomes narrower as the velocity of shortening increases.

In Fig. 6, stiffness, as measured by the length oscillation method shown in Fig. 5, is plotted against force. The data were obtained from three different fibers. The stiffness obtained from the slopes of the curves fitted to the  $T_1$

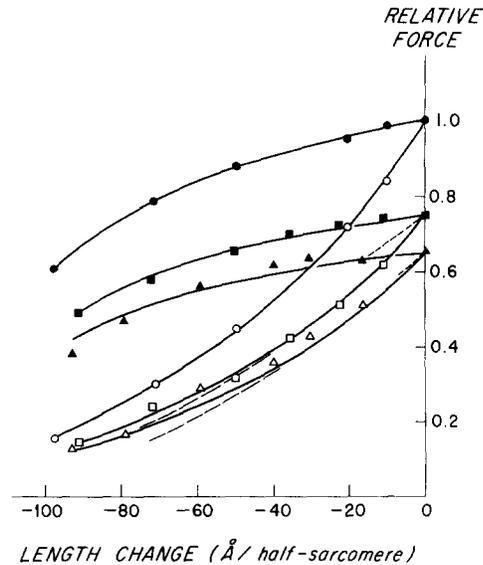


FIGURE 2.  $T_1$  (open symbols) and  $T_2$  (filled symbols) plotted against size of length change. Forces are expressed relative to the steady-state isometric value with full overlap. The circles are the values obtained from the records in Fig. 1 during the steady phase of the tetanus with average sarcomere length  $2.2 \mu\text{m}$ , i.e., full overlap. The squares show the values obtained during the steady phase of tetanic contraction with average sarcomere length  $2.5 \mu\text{m}$  (partial overlap) where the tetanic force expressed relative to that obtained at full overlap was 0.75. The triangles indicate the values obtained during the rising phase of the tetanus with full overlap when the force had reached a value of 0.65 expressed relative to the steady tetanic level. The solid curves through the open and filled circles ( $T_1$  and  $T_2$  at full overlap) are third-order polynomials fitted by the method of least squares. For the open ( $T_1$ ) and filled ( $T_2$ ) circles respectively, the equations are

$$Y = 0.996 + 0.0160X + 1.186 \times 10^{-4}X^2 + 4.358 \times 10^{-7}X^3,$$

and

$$Y = 1.002 + 2.381 \times 10^{-3}X + 1.251 \times 10^{-5}X^2 + 3.062 \times 10^{-7}X^3.$$

The solid curves through the filled squares and triangles were plotted by multiplying each point on the full overlap  $T_2$  curve by the factors 0.75 (squares) and 0.65 (triangles). The solid curves through the open squares and triangles were similarly constructed by multiplying points on the full overlap  $T_1$  curve by the factors 0.75 (squares) and 0.65 (triangles). The two short-dashed curves were constructed by shifting the full overlap  $T_2$  curve to the right so that it crossed the force axis at 0.75 and at 0.65. In a similar manner the two long-dashed curves were constructed by shifting the full overlap  $T_1$  curve to the right so that it crossed the force axis at 0.75 and at 0.65.

points in Fig. 2 (at full overlap) and in Fig. 4 are also plotted against force. In addition, the calculated values for stiffness are shown for a muscle model (Julian et al., 1974) during the steady force phases while shortening at various constant velocities.

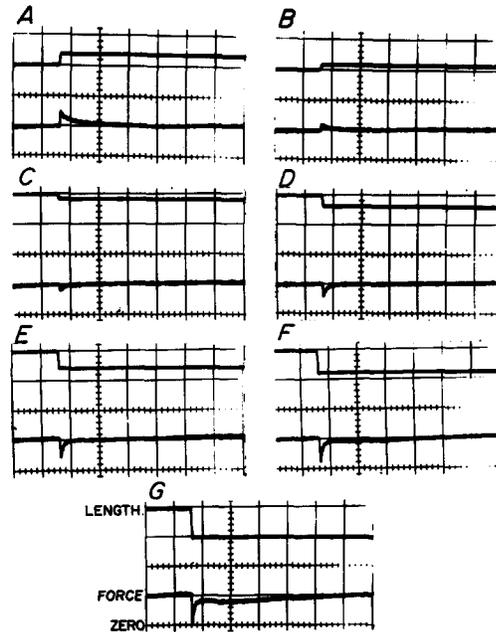


FIGURE 3. Force responses to sudden length changes applied to single fiber during tetanus. Average sarcomere length:  $2.2 \mu\text{m}$ . Force gain: 100 mg/division. Length gain: 0.05 mm/division or  $81 \text{ \AA}$ /half-sarcomere/division. Sweep speed: 10 ms/division. Fiber length: 6.9 mm. Temperature:  $0^\circ\text{C}$ . Only the fast sweep force and length traces are shown here. The length changes were completed in approximately 0.4 ms. In all records, the force zero is the same as that indicated in part G. Note that parts A and B show length increases, while the rest are length decreases.

#### DISCUSSION

The argument first presented by Huxley and Simmons (1971 *a*) will now be used to show how the transient force responses observed after sudden length changes can be simply explained on the basis of cross-bridge interactions. Shortening steps are applied at various muscle lengths corresponding to full and partial overlap of the thick and thin filaments.  $T_1$  and  $T_2$  (see Fig. 1 F for definitions) curves are obtained as shown in Fig. 2. If a force generator is connected to a SEC consisting of damped and undamped springs in series, then with partial overlap the generator would develop less steady force than that developed at full overlap. The extension of the SEC would be less than at full overlap so that in order to drop the force to zero ( $T_1 = 0$ ) the shortening step would not need to be as large as at full overlap. The effect of reducing the overlap would be to shift the  $T_1$  and  $T_2$  curves toward the right, i.e., the curves fitted to the full overlap points could be made to fit the partial overlap points by adding a suitable constant to the length coordinate of each point on the full overlap curves.

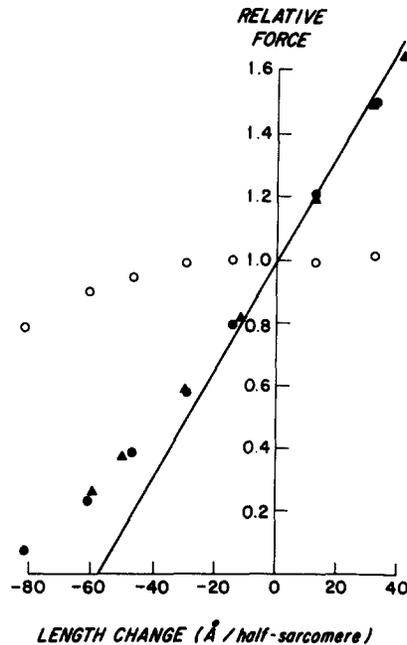


FIGURE 4.  $T_1$  and  $T_2$  plotted against size of the length change for the data shown in Fig. 3 and for a muscle contraction model. The  $T_1$  and  $T_2$  force levels are plotted relative to the force present just before the length changes. The filled and open circles are, respectively, the  $T_1$  and  $T_2$  responses obtained from the data shown in Fig. 3. The filled triangles are the  $T_1$  responses of the model for the case in which the length changes are made slowly so that 0.4 ms is required for completion. This was done to simulate the conditions imposed on the experimental preparation. Note that the model and muscle points fall very close to each other. The straight line shows the model's  $T_1$  responses to instantaneous length changes.

If, however, the only elasticity is in a series combination of damped and undamped springs residing in the cross bridges themselves, and the filaments, Z disks and fiber insertions are much stiffer, a negligible SEC will be present. Then, on decreasing overlap, each individual cross bridge attached to a thin filament develops the same force and stretches its own damped and undamped spring combination the same distance as it would at full overlap. In this case, the same size length step is required to reduce the force to zero regardless of the degree of overlap. The effect of reduced overlap on the  $T_1$  and  $T_2$  curves is to scale down the curves by a factor given by the ratio of partial overlap  $P_o$  to full overlap  $P_o$ , or, in other words, simple vertical scaling. The length axis intercepts are, of course, unchanged by this procedure. In Fig. 2, the vertical scaling procedure was used on the curves fitted to the full overlap  $T_1$  and  $T_2$  points (average sarcomere length, 2.2  $\mu\text{m}$ ). It can be seen that all the rising phase and reduced overlap points fall very near the scaled curves in confirmation of results already presented by Huxley and Simmons (1973).

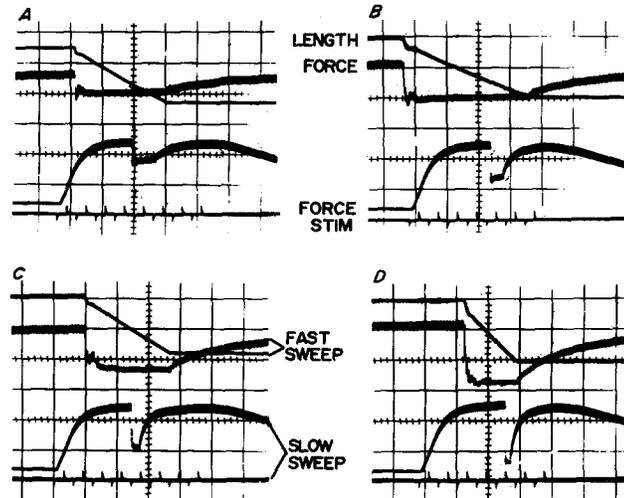


FIGURE 5. Length oscillation applied to tetanically stimulated muscle fiber during isometric contraction and during shortening at various constant speeds. Average sarcomere length:  $2.2 \mu\text{m}$ . Fiber length ( $L_0$ ): 8.3 mm. Force gain: 40 mg/division or 1.4 kg/cm<sup>2</sup>/division. Length gain: 0.25 mm/division or 3%  $L_0$ /division. Fast sweep: 20 ms/division, except in A, 50 ms/division. Slow sweep: 200 ms/division. Temperature: 0°C. Stimulus frequency: 16/s. A 500-Hz, 4- $\mu\text{m}$ , or 5.3-Å/half-sarcomere amplitude (peak-to-peak) oscillation was applied to the fiber at all times (not visible in length records). With the fiber in the isometric steady state, a sudden decrease in fiber length was imposed to bring the force to the desired level followed by a constant velocity length decrease to maintain the force at that level. After approximately 0.5 mm of shortening the fiber was again held isometric. Stiffness was obtained by measuring the width of the fast force trace in the way described in the Methods section; it was expressed relative to the width of the fast force trace in the isometric state just before shortening occurred.

As indicated in Fig. 2, horizontal shifting of the full overlap  $T_1$  and  $T_2$  curves did not fit the reduced overlap and rising phase points as well. This is certainly obvious for  $T_2$  at all force levels, while for  $T_1$  the failure to fit by shifting becomes apparent only at the lower force levels. The good fit to the rising phase points obtained by vertically scaling the full overlap  $T_1$  and  $T_2$  curves implies, as pointed out by Huxley and Simmons (1973), that the rise of tension during an isometric tetanus corresponds directly to an increasing number of cross bridges attached to thin filaments.

The preceding experiments have indicated that the cross bridges are most likely responsible for the  $T_1$  and  $T_2$  responses. It is of further interest to determine whether the  $T_1$  curve is a reflection of a nonlinear cross-bridge spring characteristic or whether there are factors distorting what would otherwise be a linear relation. The results shown in Figs. 2 and 4 suggest that recovery of force from the  $T_1$  to the  $T_2$  level during the length change may be mainly responsible for the  $T_1$  plot deviating from a straight line. As mentioned in

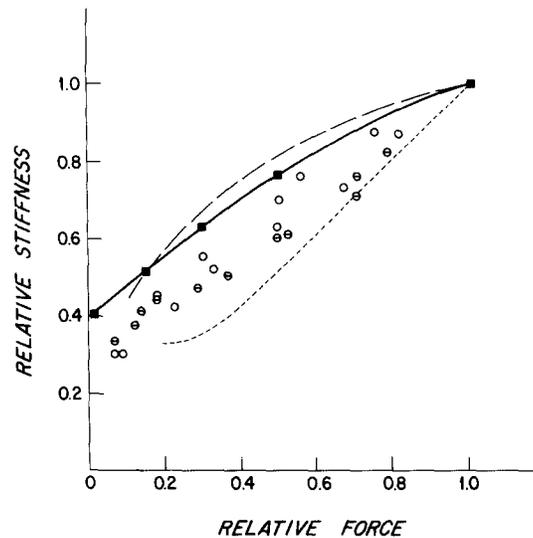


FIGURE 6. Stiffness plotted against steady force at varying speeds of steady shortening in the plateau region of length-tension diagram. Stiffness and force are expressed relative to the isometric steady-state values. The circles show stiffness values obtained from single muscle fibers by the use of small amplitude sinusoidal length oscillations superimposed on steady shortening. The oscillation frequency was 500 Hz for the circles with a bar and 1,000 Hz for the open circles. The filled squares show the variation in stiffness with steady force in a model for muscle contraction obtained while shortening was allowed at various constant speeds. In the model, the stiffness was obtained directly from the number of cross bridges attached during shortening; it was expressed relative to the number attached in the isometric steady state. The curve passed through the filled squares was drawn by eye. The dashed curves show the stiffness as measured by the slope of the  $T_1$  curves. The short-dash curve was obtained from the full overlap  $T_1$  curve in Fig. 2 and the long-dash curve from the  $T_1$  points in Fig. 4 in the following way. Cubic polynomials were fitted by the method of least squares to the plots of  $T_1$  force against size of length change. In the case of the short-dash curve, the equation is given in the legend for Fig. 2. For the long-dash curve, the  $T_1$  points shown in Fig. 4 were fitted by the equation

$$Y = 1.008 + 0.0152X + 2.232 \times 10^{-5}X^2 - 2.881 \times 10^{-7}X^3.$$

These equations were differentiated with respect to length to obtain the slope stiffness. This stiffness was then replotted in this figure as a function of force with the values expressed relative to the stiffness value obtained where the length change was equal to zero.

the Methods section, the time taken by the servo system to complete the length changes was about 1 ms in Fig. 1, but only about 0.4 ms in Fig. 3. It is reasonable to suppose that this is the basis for the higher values for  $T_1$  in Fig. 2 as compared with Fig. 4 for equal sized length steps. In Fig. 4 the  $T_1$  points fall nearly along a straight line over the range of +30 to -30 Å/half-sarcomere. Presumably, if the length steps were made still faster, the

straight line relation would hold out to larger releases, provided that a force transducer with a sufficiently high resonant frequency were used. A dependence of  $T_1$  on the speed of the applied length change would not be expected from a simple passive SEC. Results obtained from a model for muscle contraction (Julian et al., 1974) provide some insight into the way in which an apparent nonlinear cross-bridge characteristic could arise. The model assumes a linear cross-bridge spring characteristic, and, when the length changes are made instantaneously, the resulting  $T_1$  values trace out the straight line shown in Fig. 4. However, when the length changes are made to simulate those applied to the experimental preparation, i.e., constant speed length changes completed in 0.4 ms, the  $T_1$  values from the model deviate from the straight line as shown in Fig. 4 in a way very similar to that observed experimentally. The reason for this behavior in the model is that considerable change in cross-bridge configuration (and, therefore, force recovery) occurs during the slow length decreases. This effect becomes more pronounced at large length decreases so that low force level  $T_1$  points deviate progressively away from the instantaneous linear relation.

The  $T_2$  points in Figs. 2 and 4 are also different in that the points obtained using the faster steps and improved transducer fall slightly above those obtained using slower steps. In the range of length decreases down to about 80 Å/half-sarcomere, the behavior does not appear to be significantly different from that reported by Huxley and Simmons (1971 *a, b*) and Huxley (1974) as the speed of their steps was progressively increased. Beyond length decreases of about 80 Å/half-sarcomere, a range in which we have hardly any data, Huxley and Simmons' results show that the  $T_2$  points obtained using faster steps fall below slower step  $T_2$  points. The  $T_2$  responses of our model to length changes of various speeds have not yet been investigated. There is no apparent reason, however, for believing that these variations in  $T_2$  have serious consequences regarding the conclusions drawn in this work.

Evidence has been presented suggesting that no appreciable SEC is present and that the true cross-bridge force-length relation is more nearly linear than our results indicate. Therefore, measurement of total muscle stiffness can be taken simply as a measure of the relative number of cross bridges attached to thin filament sites. The plot of relative stiffness against relative force shown in Fig. 6 indicates that stiffness falls as the steady force decreases with increasing shortening speed, and it appears that as the force tends to zero (shortening velocity approaches  $V_{max}$ ) relative stiffness does not. That is, even though the external force would be zero, a significant fraction of the number of cross bridges generating force in an isometric steady contraction would still be attached. One way in which this situation might arise can be seen in the model mentioned previously (Julian et al., 1974). Here it is assumed that cross bridges are capable of bearing a compressive force, i.e., a

force tending to oppose shortening. In the isometric state none of the bridges experiences such forces. However, when shortening is allowed in the model the configuration of some of the cross bridges changes to one that opposes shortening. In particular, at  $V_{max}$ , the total external force is zero because the force generated by the cross bridges aiding shortening is exactly balanced by that of the cross bridges opposing shortening. The model has a stiffness-force relation similar to that obtained experimentally as shown by the curve presented in Fig. 6. It should be noted that this similarity occurs only as a consequence of fitting the model's response to data from other types of experiments.

The fall in relative stiffness as the steady force decreases with increasing shortening speed shown in Fig. 6 could be explained in other ways. One case which has been worked out in some detail by Podolsky and Nolan (1973) has a force generator which actually becomes stiffer, i.e., more cross bridges attached, as the speed of steady shortening increases. Clearly, our results are incompatible with this kind of model. However, Podolsky and Nolan proceed to connect their force generator to an external SEC having an exponential force-extension relation. This combination of force generator and SEC would lead to a decrease in total stiffness as the steady force decreases with increasing shortening speed. The result is still not compatible with our data since the Podolsky-Nolan combination would produce a stiffness-force characteristic passing through the origin in Fig. 6.

It could be proposed that a decrease in stiffness without any change in the number of attached cross bridges could be the result of force recovery from  $T_1$  to  $T_2$  occurring more rapidly during a length change the faster a muscle is shortening. If this were the case, then more force recovery would take place during the oscillations at 500 Hz as compared to those at 1,000 Hz. The force change for a given length change would be less using the 500-Hz oscillation, or, in other words, muscle stiffness would appear to be less using the lower frequency. However, such an effect is not evident in the data shown in Fig. 6 since both circles with a bar and open circles fall along the same path. The fact that similar results were obtained using both the 500- and 1,000-Hz oscillations suggests very little force recovery occurred during the very low amplitude length changes used. This means that the frequency of the oscillations was sufficiently high to give a valid indication of number of cross bridges attached.

The decrease in stiffness shown in Fig. 6, where force was varied by shortening at constant velocity, may be contrasted with the results to be expected when steady tetanic force is varied by changing overlap. In this case, stiffness and force would decrease in strict proportion so that stiffness would extrapolate to zero as the force approached zero. This relation was confirmed by measuring muscle stiffness using the length oscillation method at average sarcomere lengths 2.2, 2.6, and 2.8  $\mu\text{m}$ . It was found that the tetanic force

and the stiffness did maintain the same ratio in these three cases. The difference in the stiffness-force relation depending on whether the force is varied by changing the overlap or by varying the speed of shortening could be explained in the following way. In the case of steady shortening, force would be decreased both by a decrease in the number of attached cross bridges together with a redistribution of the remaining attached cross bridges. In the case of overlap changes, force would vary only as a result of changes in the number of attached cross bridges.

Up to this point, the results have been explained, with one exception, using models in which the structures responsible for the  $T_1$  and  $T_2$  responses have been located either entirely within the force generator, i.e., in the cross bridges, or entirely in an external SEC. Vertical scaling and horizontal shifting have been used to decide which model is more appropriate. The analysis can become much more complex if it is assumed that a SEC is present and that its tension-extension relation is exponential down to the lowest force levels reached in this work. In this case, it is possible to obtain a perfect fit by vertical scaling and even a complete lack of fit by horizontal shifting, and this would lead to the conclusion that a SEC was absent. In turn, this could lead to erroneous conclusions regarding number of attached cross bridges based on measurements of total muscle stiffness. However, our results indicate that, as faster length changes and improved force transducers are used, the measured  $T_1$  curve becomes increasingly steeper and more nearly linear. This means that in order to obtain an exponential SEC relation an unusual force generator instantaneous elasticity characteristic would have to be assumed. This would include a region in the characteristic where it bends back toward the origin at low force levels. There does not seem to be any evidence available which would indicate the presence of such generator characteristics. It seems more reasonable to assume a nearly linear generator characteristic and this would lead to a nonexponential SEC and failure by vertical scaling.

Another difficult situation arises if it is assumed that the SEC characteristic changes, becoming less stiff with decreasing overlap as a result of, e.g., decreasing lengths of filaments linked together by cross bridges. In particular, if the SEC changes in such a way that the reduced overlap SEC characteristic corresponds more nearly to a vertically scaled rather than horizontally shifted full overlap curve, then it might be possible to explain our results obtained at different degrees of overlap without having to conclude that a significant SEC was absent. The reason for this is that  $T_1$  curves obtained at reduced overlap could be fitted by vertical scaling of the full overlap curve. It would then be concluded that a significant SEC was not present, but, in this case also, it would be impossible to draw any conclusions regarding number of attached cross bridges from measurements of total muscle stiffness. As pointed out by Simmons and Jewell (1974), it is unlikely that the filament

stiffness decreases to zero with decreasing overlap as would be necessary to fit reduced overlap SEC characteristics by vertical scaling of the full overlap SEC curve. If the limiting value for filament stiffness reached at no overlap were comparable to generator stiffness at full overlap, it would not be expected to obtain the finding previously mentioned in the Discussion that total muscle stiffness decreases in proportion to force with moderate decreases in overlap.

Even in the absence of a significant SEC, our results indicating a decrease in stiffness during shortening might be explained using a model in which the number of cross bridges attached at various speeds of shortening remains constant. This could be done by having the individual cross-bridge force-length characteristic become less stiff with decreasing force level. The small amplitude length oscillation method would then measure the stiffness, i.e., the slope, of a curve passed through the  $T_1$  points. Evidence against this view is presented in Fig. 6 where the slopes of the curves passed through the  $T_1$  points shown in Figs. 2 and 4 are plotted. In neither case is a good fit to the length oscillation data obtained. In addition, it seems clear that the stiffness of  $T_1$  increases as the time taken to complete the length step is decreased and the force transducer is improved, while the stiffness measured using length oscillations does not vary much with frequency. It would be difficult to explain the variation in stiffness of  $T_1$  with speed of length step if the cross-bridge force-length characteristic varied only with force level. It must also be kept in mind that the  $T_1$  curve obtained using length steps applied in the isometric steady state cannot simply be assumed to apply during steady shortening. An indication of this has already been presented by Huxley and Simmons (1973). In their Fig. 14 *b*, it can be seen that the slope of the  $T_1$  curve obtained during steady shortening is less than the slope of the isometric  $T_1$  at the force level equal to the load applied during steady shortening. This also indicates a decrease in relative muscle stiffness with increasing speed of shortening. Huxley and Simmons' figure in addition shows that the decrease in relative stiffness occurring during shortening is less than the decrease in relative force, and our results agree with this finding.

It has been argued that the number of attached cross bridges mainly determine the stiffness properties of the contracting fibers used in this work. The question can be put as to whether this is a plausible conclusion in view of what is now known about the microstructure of skeletal muscle. It would appear that the *A*, *I*, and *Z* filaments are principally in tension during a contraction, while the attached cross bridges act more nearly in a transverse bending mode. This mode is inherently less stiff than is the tension-compression mode. The cross bridges act in parallel so that the total stiffness depends on the number attached, but, according to the X-ray results of Huxley and Brown (1967), at any given moment during a contraction only a small proportion of cross bridges appear to be attached to actin filaments. It is also

commonly believed that the LMM-S<sub>2</sub> and S<sub>2</sub>-S<sub>1</sub> junctions of the myosin molecule are rather flexible (H. E. Huxley, 1971). It seems reasonable to conclude, therefore, that there is no apparent structural basis for rejecting the idea of number of attached cross bridges dominating stiffness measurements. White (1970) has shown that during rigor contractions the stiffness of muscle fibers is much higher than during calcium-activated contractions. Huxley and Brown (1967) interpret their X-ray diagrams to indicate that in rigor a much larger proportion of cross bridges are attached, so that White's findings can be explained on the basis of stiffness being determined by number of cross bridges attached. Further X-ray studies of Elliott et al. (1967) and Huxley and Brown (1967) have indicated that no detectable changes occur in the spacings of the actin reflections from the thin filaments during contraction (within an experimental error of about 0.2%). Elliott et al. (1967) also find no detectable change in the myosin subunit repeat from the thick filaments during contraction, while Huxley and Brown (1967) report an increase of about 1%. It is not at all certain that the increase in the myosin period is the result of stretch caused by the isometric tension. An increase could be caused by activation-related changes in the thick filaments, or by a change of position of the myosin heads relative to the backbone of the thick filament during contraction (Huxley and Brown, 1967).

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