Local Movement in Stimulated Frog Sartorius Muscle

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ABSTRACT Local movement was recorded in tetanically contracting frog sartorius muscle to estimate the nonuniformity in the distribution of compliance in the muscle preparation and the compliance that resides in the attachments of the preparation to the measuring apparatus. The stimulated muscle was also subjected to rapid length changes, and the local movements and tension responses were recorded. The results indicate that during tension development at resting length the central region of the muscle shortens at the expense of the ends. After stimulation the "shoulder" in the tension, which divided the relaxation into a slow decline and a subsequent, rather exponential decay toward zero, was accompanied by an abrupt increase in local movement. We also examined the temperature sensitivity of the two phases of relaxation. The results are consistent with the view that the decrease in tension during relaxation depends on mechanical conditions. The local movement brought about by the imposed length changes indicates that the peak value of the relative length change of the uniformly acting part was ~20% less than the relative length change of the whole preparation. From these observations, corrections were obtained for the compliance data derived from the tension responses. These corrections allow a comparison with data in the literature obtained from single fiber preparations. The implications for the stiffness measured during the tension responses are discussed.

INTRODUCTION

The dynamic properties of the contractile mechanism are often investigated by means of mechanical measurement on whole muscle preparations as well as on fiber bundles and single fibers. Such measurements are complicated by the compliance in the attachments of the muscle tissue to the measuring apparatus and by the nonuniform distribution of the compliance of the preparation. These phenomena can be investigated by studying the local movement in the preparation. Gordon et al. (1966) were the first to use a "spot-follower," which enabled them to measure and to control the length of a single fiber segment between markers. Many workers have used laser diffraction techniques for the registration of movement in a fiber (e.g., Clewort and Edman [1972] and Kawai and Kuntz [1973]). Bethe and Happel (1923) and Jewell and Wilkie (1958) among others, registered movements of markers

in whole muscle during isotonic and isometric contractions. Their measurements suggest that the difference between the results obtained with single fibers in which the compliance in the tendon attachments is eliminated (e.g., Ford et al. [1977]), and the results with whole muscle (e.g., Stienen and Blangé [1980]) largely is due to the nonuniform distribution of the compliance and to the additional compliance present in the attachments.

A number of methods for studying dynamic contractile properties are used with whole muscle only: e.g., x-ray diffraction, nuclear magnetic resonance (NMR) techniques, and heat measurements. Because these investigations benefit by a comparison of the mechanical behavior of muscles and single fibers, the aim of our study was to gain insight into the local movement in a muscle to estimate nonuniformity in the distribution of compliance.

When used to continuously register local movement in whole muscle during contraction and relaxation, the methods mentioned above are hampered in their effectiveness by absorption of light. In the case of laser diffraction measurements, there is also the disadvantage of the differences in the contribution of the different fibers illuminated by the beam. In view of these complications, a follower system was developed that allows the registration of movement of reflecting markers attached to the muscle.

METHODS

Preparation, Mounting, Solutions, and Stimulation

The experiments were performed on the sartorius muscle of the frog (*Rana esculenta*), which was dissected free together with part of the pelvic bone and a very small part of the tibia. These parts of the preparation were tied to the displacement system and the force transducer, respectively. A drop of fast-drying glue was applied to the connections to minimize additional compliance in the attachments and in the tibial tendon. The muscle was mounted horizontally at resting length L_0. The oxygenated bathing solution contained NaCl, 115 mM; KCl, 2.5 mM; $CaCl_2$, 1.8 mM; and 9 mg/liter d-tubocurarine chloride. The temperature was kept at ~0°C by cooling fluid circulating through the walls of the muscle chamber. To allow registration of local movement by the marker-follower system to be described next, one side of the chamber consisted of a thin glass window. The muscle was stimulated electrically by two platinum wire electrodes positioned parallel to the axis of the muscle at a distance of ~2 mm. To obtain a homogeneous field in the preparation, the electrodes ran the full muscle length. Supramaximal pulse trains of 600 ms with a repetition frequency of 30 Hz and a pulse duration of 1 ms were repeated every 6 min.

Displacement System

Position changes of one end of the muscle could be imposed by the position servosystem described by Stienen et al. (1978). Displacements of < 60 μm could be performed with a rise time, from 5 to 95%, of 0.15 ms. Larger changes were performed in a ramp shape with an acceleration and deceleration phase of 0.15 ms. The maximum velocity attained was 0.35 m/s. The performance of the displacement system is illustrated in Fig. 2.

Force Transducer

A strain gauge transducer with a resonance frequency of 7 kHz, a sensitivity of 0.15 V/N, and a compliance of 5 μm/N was used (Blangé et al., 1972).

Marker-Follower System

A marker-follower system that makes use of reflecting markers attached to the muscle has been developed for the registration of local movement in different areas of the muscle. An important requirement for this device is that it be rather insensitive to variations in the intensity of the incident and reflected light. This is achieved by taking the centre of the marker—where the reflected light intensity is at a maximum—as a reference point for the position of the beam. The beam is brought in a fast oscillating motion, which permits detection of movement of the marker relative to the center of the beam from relative intensity changes of the reflected light.

A block diagram of this system is shown in Fig. 1 *A*. The beam of a laser light source (133; Spectra-Physics Inc., Mountain View, Calif.) is made to oscillate by projecting it onto a small surface mirror glued to a piezoelectric element P_1 (PXE5; Philips Industries, Eindhoven, The Netherlands) moving in resonance. The construction of this element is such that application of an electric tension leads to bending of the element. This bending of this element causes an angular movement of the beam in the plane of the drawing, as indicated in Fig. 1*A*. The free end of this cantilever-mounted element is adjusted to allow movement of the ceramic in a first overtone resonance of ~120 kHz.

The oscillating beam is projected onto another mirror attached to a second piezoelectric ceramic P_2 (Philips PXE5) clamped at two locations between pieces of razor blade. The clamps also serve as electric connections. The interdistance of these clamps is 5 mm, i.e., one-half of the length of the ceramic, including the mirror. In this case the resonance frequency for the angular movement of the mirror glued at the tip of this ceramic was raised to 18 kHz. The oscillating beam is projected onto the reflecting marker M by this mirror. The laser beam is focused at the marker by the spherical lens S (focal length = 30 cm). The reflected light is detected by a silicon detector D positioned near the marker, which has a sensitive area of ~1 cm^2.

The direction and extent of the mean deviation of the beam from the marker can be found by correlating the driving signal of P_1 and the detector output. This correlate is obtained by multiplication (multiplier X) of the driving signal and the detector signal and subsequent low-pass filtering T of the output of the multiplier. A schematic illustration of the function of the multiplier is presented in Fig. 1 *B*. The low-pass filter also incorporates a compensation network for the frequency characteristic of P_2 in the range from direct current (DC) to ~30 kHz. After amplification G the filtered output signal of the multiplier is fed back to P_2. In the feedback mode, the position of the beam is adjusted by an angular movement of P_2 in the plane of the drawing so that the center of the beam follows the marker. In this way a DC open-loop gain of X 100 is achieved without the introduction of instabilities into the system.

The driving signal for P_2 is related to the position of the marker. This signal, however, turned out to be a rather inaccurate measure for the position because of the nonlinear deflection-to-voltage relation and the aging of the polarization of the piezoelectric element. Therefore, an independent registration device was incorporated via beam splitter B that projects part of the incident beam on a silicon position sensor (Silicon Detector Corp., Newbury Park, Calif.). This beam was focused in the plane of the drawing by a cylindrical lens C perpendicular to the longer axis of this lateral
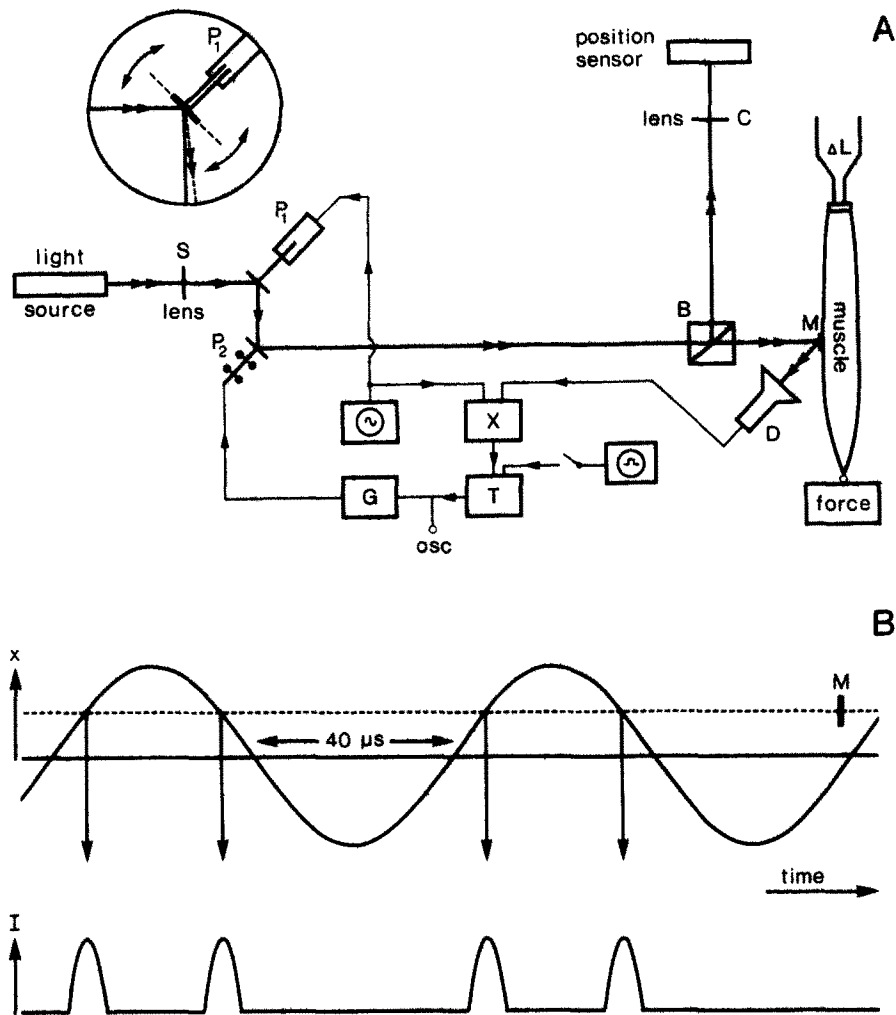
FIGURE 1. (A) Block diagram of the marker-follower system. This device registers movement of reflecting markers attached to the muscle that is horizontally mounted between a displacement system (ΔL) and a force transducer. The basic elements in this device are the piezoelectric elements P_1 and P_2, which have a surface mirror attached at their tip, that cause a deflection of the beam in the plane of the drawing. As indicated in the *inset*, bending of the free end of P_1 leads to a rotation of the mirror and to deflection of the beam. For a description of the components and further details, see Methods. (B) Schematic illustration of the rationale of the multiplier in the marker-follower system. *x* indicates the position of the marker relative to the center of the oscillating beam (120 kHz); the solid line indicates the beam position as a function of time; and I denotes the reflected light intensity as a function of time in the marker position as indicated. Multiplication of the position signal *x* and the reflected signal *I* results in a *positive* signal in the situation drawn. The result is a *negative* signal when the marker *M* is below the base line. It is alternately positive and negative in time when the center of the marker is at the baseline. Near the baseline the integrated (or low-pass-filtered) output of the multiplier is an increasing function of the distance of the marker to the center of the beam.

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photo detector. The 25-kHz bandwidth of the follower system attained in this way corresponds to a rise time, from 10 to 90%, of 25 μs to a step change in the marker position. The bandwidth of the position sensor is 10 kHz.

It is possible to estimate the open-loop gain for the feedback system from the maximum output of the light detector with the center of the beam positioned near the edge of the marker, but this procedure could not be followed during the measurements. Therefore, a check of the gain was incorporated into the device by summation of a small square wave to the output signal of the multiplier. At the oscilloscope (osc) output, the system reacts with a signal that is inversely proportional to the DC gain.

The importance of controlling the gain can be illustrated as follows. At a DC gain of $\times$ 100 a difference of 1% between the center of the beam and the position of the marker after amplification provides for the driving signal of P_2. At a constant gain this difference has been taken into account in the calibration, but if the gain is reduced to, e.g., $\times$ 50 (a typical value for a poorly positioned detector) a difference of 2% is needed. If the marker is kept 0.6 mm from the center of the range of the follower system, this change in gain results in a virtual change of 6 μm.

The range of the follower system depends on the distance between P_2 and the marker and on the range of rotation of P_2. A range of 1.2 mm proved to be convenient and sufficient in the measurements presented. The noise in the driving signal of P_2 corresponds to ~0.5 μm. The main limitation, however, on the accuracy during the time the measurements were made was caused by the noise on the output of the position registration device. This noise corresponds to ~5 μm.

The performance of the system can be tested by attachment of a reflecting marker to the tip of the displacement system. The marker position signal and the position servooutput due to a displacement of 100 μm completed in 0.5 ms and a displacement of 400 μm completed in ~2 ms are shown and compared in Fig. 2.

Markers

Small pieces of aluminium foil ~18 μm thick carefully cut to 1 $\times$ 0.4 mm were used as markers. The markers were carefully flattened to improve the reflection.

Application of the Markers to the Preparation

An α-cyanoacrylate glue (Scotch 202; 3M Company, St. Paul, Minn.), which consists of an ethyl monomer was chosen to fasten the markers. This glue could be used in very small amounts, and its bonding characteristics and its viscous properties before polymerization were conductive to a relatively easy and firm connection. Up to 10 markers were applied to a preparation. The muscles were examined after the experiments with a light microscope. Inspection of the sarcolemma and the striation pattern revealed no damage or irregularities caused by the glue or the marker. The markers were attached in the central region of the *in situ* upper surface of the muscle with their longer axes perpendicular to the fiber direction.

RESULTS

Force Transients

Typical force transients to ramp-shaped displacements completed in 1 ms are shown in Fig. 3. The responses to a rapid shortening show the characteristic phases as indicated in Fig. 3 B (phase 1: decrease in tension during the ramp,

which is followed when the length change is complete by phase 2; phase 2: the fast recovery in tension; phase 3: a plateau in tension where the recovery in tension is absent or slow; and phase 4: the slow or delayed recovery toward isometric tension). During a quick lengthening, tension increases, and when
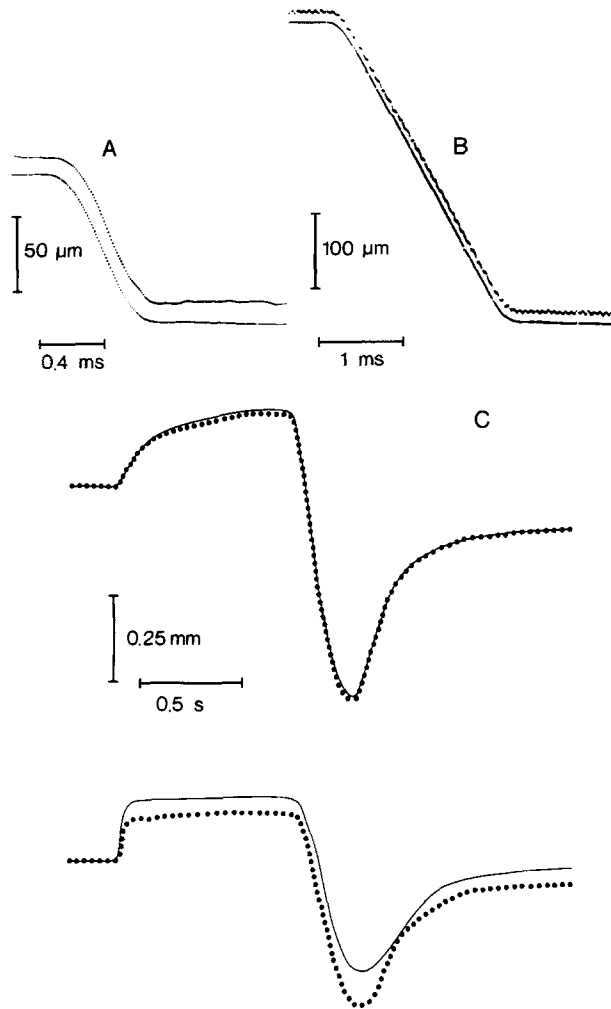
FIGURE 2. Performance of the displacement systems and the marker-follower system (*A* and *B*). The displacement of the position servo (*lower trace*) is registered by an interferometer device. The movement of a marker, attached to the tip of the motor, is registered by means of the follower system. The marker movement, i.e., the output of the position sensor (shown in Fig. 1 *A*) is shown in the *upper trace*. The noise on the marker trace, which mainly originates from the position sensor, corresponds to ~5 μm. In (*B*) a residual oscillation is recognizable that originates from the 18-kHz resonance of P$_2$. Note the differences in scale in *A* and *B*. (*C*) Reproducibility and variability of the marker movements. Example of two subsequent registrations (*upper panel*). Example of repeated registrations in a series of observations (*lower panel*).

the change is complete, tension returns to the isometric level in an exponential fashion. In some experiments the tension course showed a slight undershoot ~50 ms after the lengthening.

By following the procedure described by Ford et al. (1977), the T_1 and T_2 curves can be determined (cf. Fig. 6). T_1 is defined as the extreme tension reached during the ramp. In case of a shortening, T_2 is defined as the intersection of the extrapolated plateau (phase 3) in the force transient and the initial fall in tension. In case of a lengthening, the minimum of the undershoot in tension, if present, is taken as T_2.
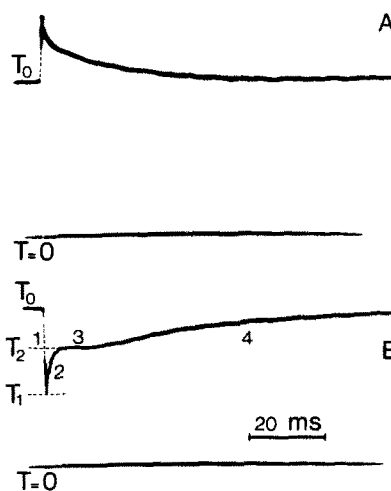

FIGURE 3.   Typical tension recordings to quick, ramp-shaped length changes imposed on tetanically contracting sartorius muscle of the frog. (*A*) Response to lengthening of 200 μm completed in 1 ms. (*B*) Response to a similar shortening. This tension response can be divided in a decrease in tension during the ramp (*1*) followed by the fast tension recovery (*2*), the plateau (*3*), and the delayed or slow recovery (*4*). Experimental conditions: muscle resting length (L_0), 24.5 mm; sarcomere length (s_0), 2.2 μm; isometric tetanic tension (T_0), 2.2 × 10^5 N/ m^2, temperature, 2°C.

Local Movements in the Preparation during the Experiments

It can be expected that the tension responses and, consequently, the T_1 and T_2 curves are influenced by the compliance in the attachments of the muscle to the apparatus and also by the nonuniform distribution of the compliance of the preparation. To estimate the contribution of these properties, which—albeit to varying degrees—are inherent in any type of preparation, local movements were registered with the marker-follower system. The possibility of an extra check on the state of preparation turned out to be an additional benefit of these registrations. Incidentally occurring local damage of the preparation, which often has no recognizable effect on the force traces, showed up especially well in the marker trace as a change in the global pattern of movement of the markers, i.e., generally, as an increase in displacement.

During a measurement, only one marker could be followed at a time, and, accordingly, data from successive tetani of one muscle are combined. The markers were generally observed from the pelvic end to the tibial end and then back. Consequently, every marker was observed at least twice in each experiment. In general, the two registrations at one marker were practically indistinguishable, provided the experimental conditions had not changed (cf. Fig. 2 C). The series of observations in the reverse order provided a check on the stability of the displacement pattern. A set of observations was rejected when repeated registrations of the movements at one marker during activation or relaxation differed by >0.1 mm. This value allowed for the differences that occurred within an experimental series, which sometimes lasted several hours. Experience taught that larger deviations often were accompanied by a drastic change in the total displacement pattern. The amplitude of the change in length induced during the plateau of the tetanus (at 300 ms after the onset of stimulation) was sometimes varied. In these cases, the initial phase of the time-course of the marker movement—up to the displacement—was used as a test of the reproducibility of the traces.

In Fig. 2 C an example is presented of the differences tolerated in a series. In this case, the deviation in displacement on activation is 70 μm, i.e., ~10% of the total displacement of the marker. The patterns, however, are rather similar during relaxation. The variability of the marker displacements due to the imposed length changes in different observations is <5%. This is considerably less than what might be expected from the variability allowed for in the global movements. In Fig. 4 A, the movements of markers in different regions of the muscle are shown during tetanical contraction and subsequent relaxation. One of the features generally observed, which shows up during tetanic contraction, is the local shortening in the central region of the muscle at the expense of one or both end regions. Obviously, the local lengthening was most pronounced in the tibial end region of the muscle. Compared with the displacements during the initial tension development, the markers remained rather stationary during the plateau of the tetanus and the subsequent slow decline in tension, roughly until the time at which the shoulder in the tension response occurs, denoted by the dotted line in Fig. 4 A. A small amount of creep, however, was sometimes observed during these phases, reflecting movement of part of the muscle toward the pelvic end. This movement caused a small extra stretch of the muscle near the tibial end.

It can also be seen that the well-known shoulder in the tension response after termination of stimulation, as seen in single fibers (Huxley and Simmons, 1970), is accompanied by a rather abrupt increase in local movement. Furthermore, it appears that the maximum displacement during relaxation is found when tension has nearly reached its zero (resting) value. In Fig. 5 A are shown the results of an experiment in which, during the tetanic plateau, shortening of 200 μm, complete in 1 ms, and shortenings of 400 μm, complete in 2 ms, were applied.

In Fig. 4 B are shown the displacements of the markers during contraction and relaxation. The displacements during contraction are denoted by the maximum displacement of the markers during stimulation. The displacements

during relaxation are the differences in position at t_1 (the time at which the shoulder occurred) and at t_2 (the mean time of occurrence of the hump in the marker position traces). It follows from the slope of the lines connecting the displacement of each marker that during both phases relative local length changes up to ~6% occur. It can be seen in Fig. 4 B that the local movements that occur in a substantial part of the muscle during the first part of the exponential relaxation reflect a relative shortening of ~2.5%. The counterpart—local lengthening—occurs in the pelvic end region. In Fig. 5 B are shown the maximum displacements during contraction and relaxation for another experiment.

In Fig. 4 C the "profile" of a rapid length change in of L_0 ~1% is shown. It was obtained by combining the marker displacements that occurred as a consequence of this change in length. In some of the marker records (cf. Fig. 5 B), especially near the tibial end, an overshoot that reflects the extensibility of the tibial end region is clearly present. To quantify this we determined the peak marker displacement value and the displacement value 10 ms later (i.e., during the plateau phase in the tension response).

These results indicate that the peak values of the local displacements are almost linearly related to the position of the markers, implying that a major part of the imposed length change is nearly uniformly distributed over the muscle. The intercept at the fixed (tibial) end reflects the extensibility in this part of the muscle. The extra displacement there is compensated by a smaller relative length change in the rest of muscle. Therefore, a correction for the compliance of the uniformly acting region is necessary. As follows from the linear approximation to the peak values in Fig. 4 C, the relative length change is 0.86 ± 0.03 (SD) of the relative length change of the total preparation. This factor was determined by dividing the virtual displacement derived from the slope of the regression line by the total displacement applied. From the linear approximation to the displacement after 10 ms, it follows that the deviation is reduced to 0.97 ± 0.03. This is a result of the local lengthening in the tibial end region during the fast-recovery phase in the tension response. Corresponding values were obtained in cases of similar lengthening. The nonuniformity in cases of shortening of ~0.5 and 1% of L_0 in the same preparation is shown in Fig. 5 C. These data yield a first-order correction at -200 μm of 0.77 ± 0.04 (peak) to 0.96 ± 0.04 (after 10 ms) and at -400 μm of 0.80 ± 0.05 (peak) to 0.90 ± 0.03 (after 10 ms). The latter corrections imply a translation of the T_1 and T_2 values to lower displacements, which is illustrated in Fig. 6. In view of the extrapolation procedure inherent in the definition of T_2, the correction at the T_2 (-400 μm) value was obtained by linear interpolation between the tension value after 10 ms and the T_1 value.

The data presented in this section are from two experiments selected from ~30 experiments. In these two experiments, as well in two others, the position detector was used; in the remaining experiments the position signal was obtained from the driving signal of the piezoelectric element P_2. The calibration of P_2 is not sufficiently accurate for the detailed analysis needed to obtain the corrected T_1 and T_2 curves. In 10 experiments, however, which yielded enough data to reconstruct the global movement of the muscle during
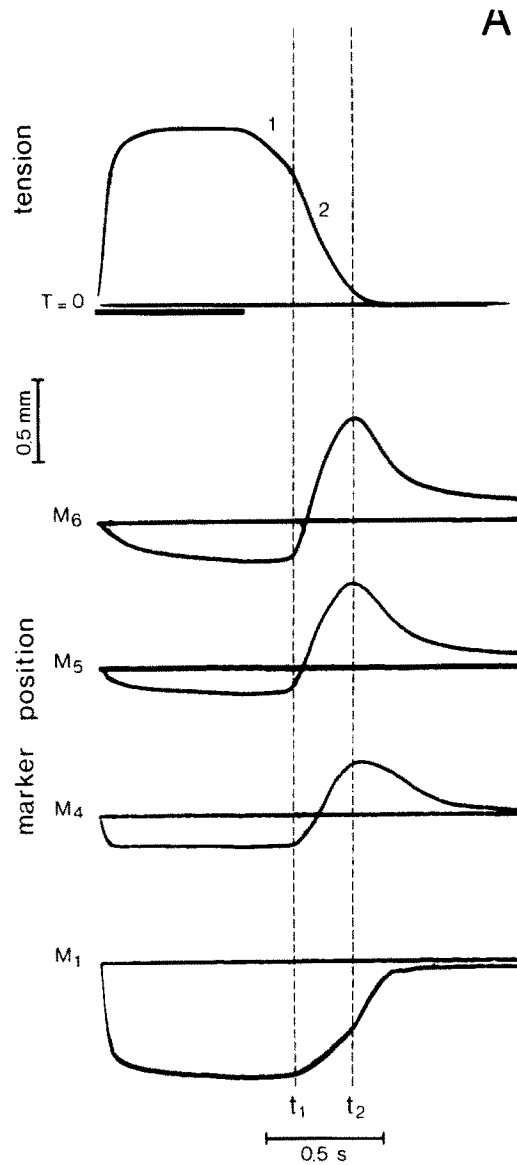
FIGURE 4.   Local movements of the preparation during isometric tetanic contraction and the subsequent relaxation. (A) Tension-course as a result of tetanic stimulation, and the marker movements in different parts of the muscle. The heavy bar indicates the period of stimulation (600 ms). Note the "shoulder" in the tension trace at t_1, which divides the relaxation into 2 phases: 1, the rather linear decline in tension; and 2, the exponential relaxation phase. The end position of the markers at the tetanic level is indicated in B. During tension development the markers move toward the pelvic end of the muscle (downward in the Figure). During relaxation an abrupt increase in local movement occurs coincidently with the "shoulder" in the tension response as is indicated by the
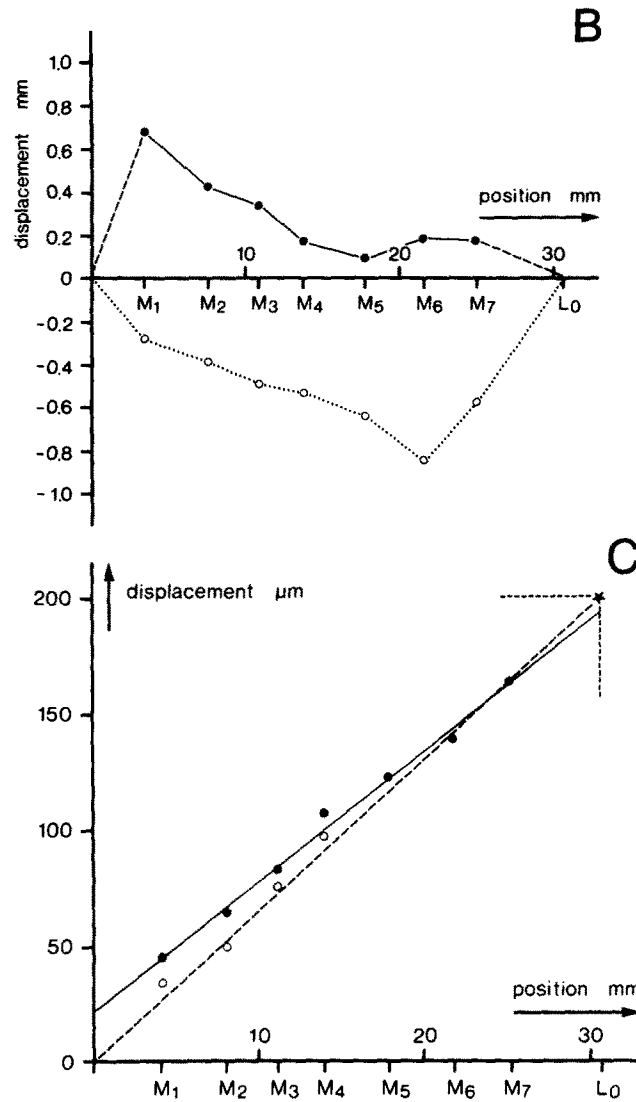
FIGURE 4

interrupted line at t_1. The interrupted line at t_2 indicates the mean time of occurrence of the hump in the position traces. (*B*) Displacements of the markers M_1–M_7 as a function of the original marker positions. The dots indicate the maximum displacement reached during contraction (creep included). The circles indicate the displacements reached during relaxation from t_1 to t_2. (*C*) Displacements of the markers due to a rapid shortening of 200 μm (1-ms duration) (the "displacement profile"). As a description of the overshoot in the position traces, the peak values as well as the displacement values after 10 ms (during the plateau phase; cf. phase 3 in Fig. 3) are shown. The line is the regression line to the peak values. Experimental conditions: L_0, 30.7 mm; s_0, 2.4 μm; T_0, 2.2 × 10^5 N/m²; temperature, 3°C.
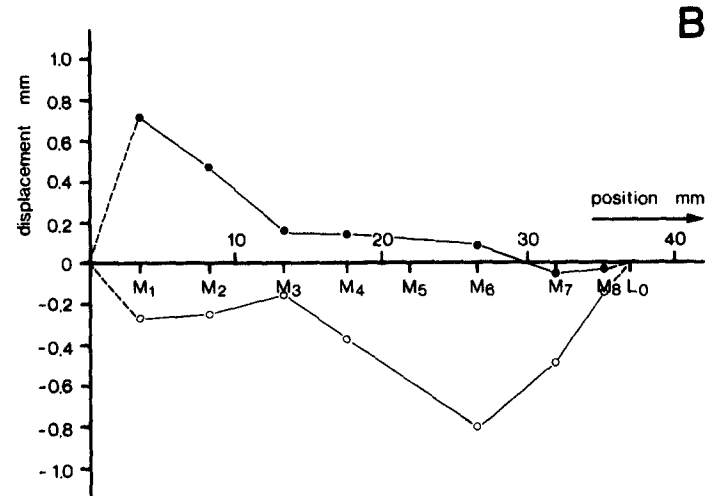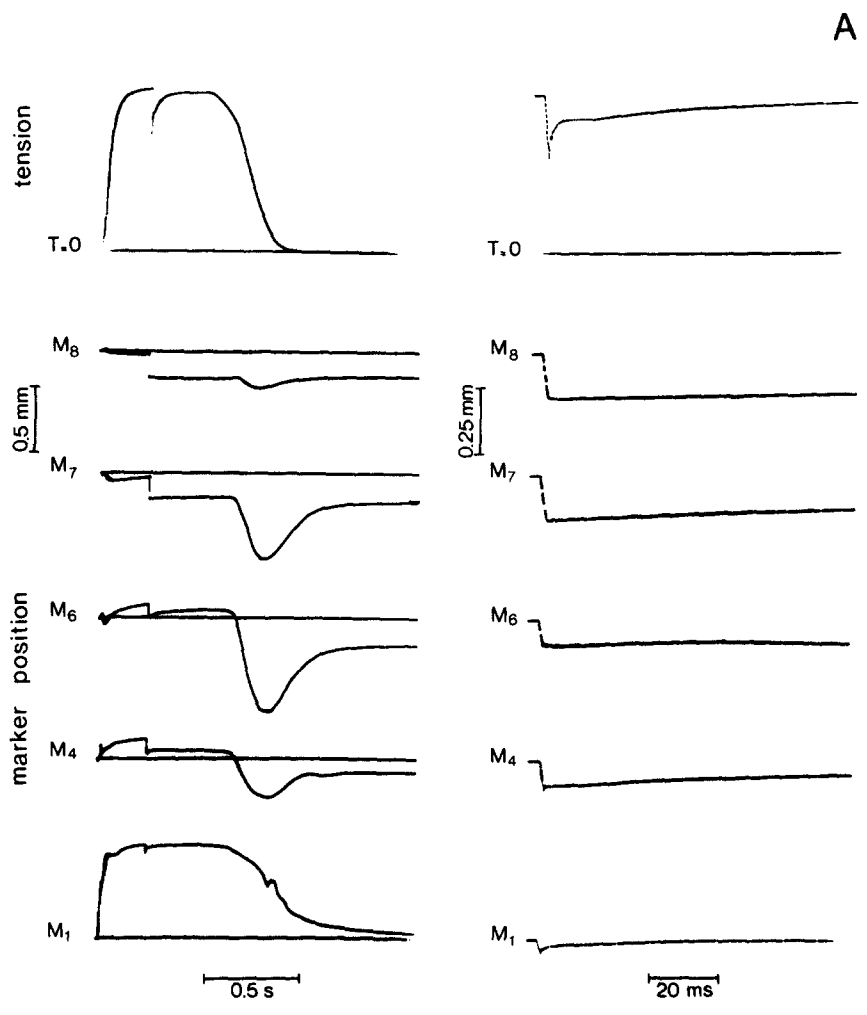
FIGURE 5

contraction and relaxation, the internal shrotening in the central one-half of the muscle during contraction was 2–4% L_0. The variation in the T_1 and T_2 curves, was <5% when the dependence of T_1 on the velocity attained during the changes in length was taken into account (cf. Stienen and Blangé [1980]).
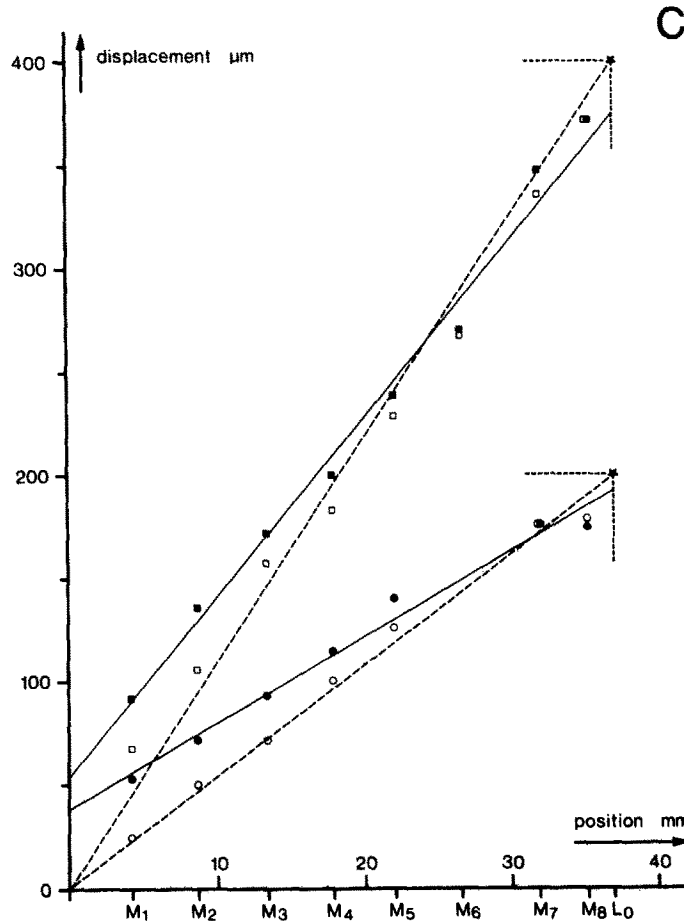

FIGURE 5.   Tension-course and marker movements when rapid shortenings of 200 μm (~1% of L_0) and 400 μm were imposed at the isometric tetanic tension level. Movement toward the pelvic end corresponds with an upward movement in A. The format here is similar to that of Fig. 4, with the exception of C, in which the squares denote the marker displacements as a result of the shortening of 400 μm. Data depicted by the filled and open circles originate from the 200-μm shortening. Note that in B, the open circles indicate the maximum displacement during the whole relaxation period. Experimental conditions: L_0, 37 mm; s_0, 2.4 μm; T_0, 2.3 × 10^5 N/m²; temperature, 3°C.

Momentary Stiffness Measurements

The momentary stiffness was determined during the force transients by applying a second "test" lengthening at different times after the initial change in length (cf. Blangé and Stienen [1979]; Stienen and Blangé [1980]). In summary, we found that the stiffness after shortening *decreased* during the plateau phase of the force response. It reached a minimum value ~15 ms after
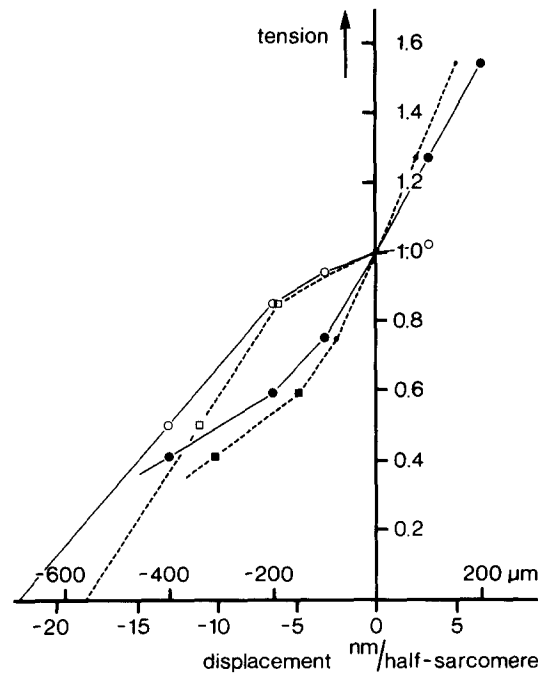

FIGURE 6. T_1 and T_2 curves determined from the tension responses to quick length changes. The T_1 values (filled circles) denote the extreme in tension reached during the length change relative to the isometric tetanic tension T_0. The T_2 values (open circles) denote the plateau in the tension responses normalized to T_0. The corrected values obtained from the regression lines in Fig. 5 are shown in here by the filled (T_1) and open (T_2) squares. In practice, it is impossible to carry out the correction procedure for all the displacements on one muscle and therefore the corrections are completed by the values (dots) expected on the basis of the correction at −200 µm. Experimental conditions: similar to those Fig. 5.

the initial shortening. This minimum value depended on the amplitude of the initial shortening (Fig. 7A). After the minimum, the momentary stiffness recovered to its isometric value in a way similar to the force recovery. It was found after normalization that the relative momentary stiffness was larger than the relative tension at the time the test was made. The stiffness after an initial lengthening increased within ~10 ms. It reached a maximum value that depended on the amplitude of the initial lengthening (Fig. 7B). It then

decreased slowly, but the stiffness clearly remained at a high level during the remainder of the tetanic contraction. We expected that these measurements also were influenced by the nonuniform distribution of compliance of the preparations. Therefore, the marker movements were followed during the stiffness measurements. The delay between the initial and test length changes was varied, and the movements of different markers were compared. From
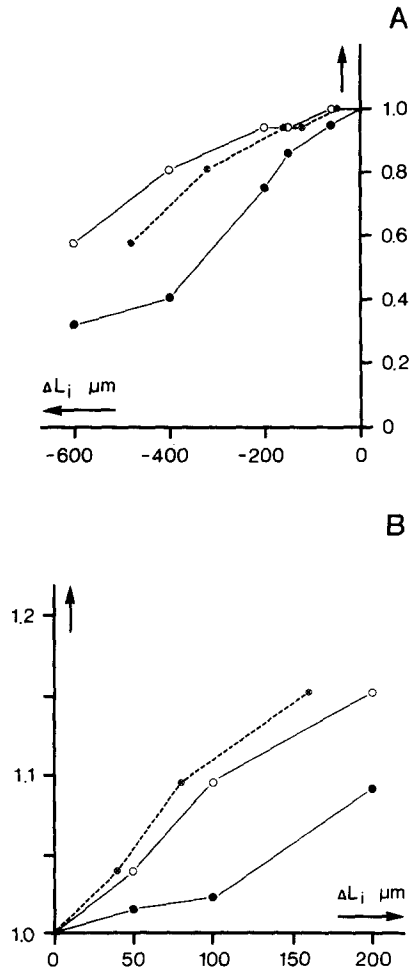

FIGURE 7.   Illustration of the correction for local movement on the momentary stiffness determined during the force transients. The momentary stiffness was determined by means of a test length change (ΔL_t) applied after an initial change in length (L_i). The stiffness is normalized to the isometric value obtained from a similar test length change applied at the isometric tension level. (*A*) Normalized stiffness as a function of the initial shortening after 15 ms. (*B*) Normalized stiffness after 30 ms as a function of the initial lengthening. (*A* and *B*) ○, Relative stiffness; ●, relative tension; ⊗, corrected stiffness.

the similarity of the marker movements it seems unlikely that the nonuniformity of the preparation is an important source of error in the time-course of the momentary stiffness. The noise in the marker traces (~5 μm), which becomes progressively more important for the markers situated near the fixed end, however, impeded further analysis.

The results presented in the preceding paragraph suggest a correction that concerns the relation between the depth of the minimum stiffness that occurred ~15 ms after the initial shortening and the amplitude of the initial shortening. This correction is expressed in the relation illustrated by the dotted line in Fig. 7 A. In Fig. 7 B, the correction is indicated for the momentary stiffness measurements after an initial lengthening.

Concerning the data presented in Fig. 7, it can be noted that the accuracy of the individual measurements is ~5%. The maximum value of the change in relative stiffness varied by 20% among the experiments.

Temperature Effects

To contribute to the identification of the dynamic processes underlying the relaxation phase, the temperature dependence of the tension course in the range from 0° to 15°C was investigated. During such a series the repetition frequency for the tetanic stimulation was adjusted from 30 Hz at 0°C to 60 Hz at 15°C to maintain a fused tetanic contraction. It was observed that the slope of the rather linear decline in tension (cf. Fig. 4 A) that preceded the shoulder in the tension response increased from 1.1 T_0/s at 0°C to 3.0 T_0/s at 15°C. This corresponds to a temperature coefficient Q_{10} of ~2. The following— exponential—relaxation phase was analyzed in a way similar to the method of Jewell and Wilkie (1960), yielding similar results: rate constant at 0°C (6 s^{-1}) and Q_{10} of ~3. In addition, a Q_{10} of 2.7 was found for the exponential rate constant that dominates the slow recovery phase in the tension response after a rapid shortening (rate at 0°C: 26 s^{-1}) (Stienen and Blangé, 1980). For the isometric tetanic tension, a Q_{10} of 1.3 was found.

DISCUSSION

Local Movements in the Preparation during the Experiments

Jewell and Wilkie (1958) examined with a microscope the movements of carborundum particles distributed over the muscle that accompany the transition in tetanus from rest to plateau. Our registrations, in which the movement is followed during activation and relaxation, reveal more detailed information, but a limited comparison is possible.

The displacements in the tibial end region shown are more pronounced than the displacements that Jewell and Wilkie (1958) obtained. It was possible to reduce this movement by mounting the muscle at a somewhat larger length, suggesting that in our experiments the muscle was mounted closer to slack length. The local displacements, especially in the tibial end region, in which a small rotation of the muscle along its central axis was sometimes observed, are of course rather sensitive to differences in the mounting of the muscle in

the measuring device. This is probably a cause of variation in the global movements from one muscle to another, as was also found by Jewell and Wilkie (1958). In general, however, it was found that during tension development the central one-half of the muscle was shortened by ~3%. Our registrations show that the maximum displacements during relaxation are found when tension is near zero, as described for single fibers by Edman and Flitney (1978). Furthermore, our registrations show a correspondence of the time of occurrence of the shoulder in the tension recorded and the start of a rather abrupt increase in local movement. In a single fibers these two phenomena were proven to be related by Huxley and Simmons (1970), who demonstrated that the shoulder could be delayed by means of a length clamp of the central part of the fiber. Julian and Morgan (1979a), have suggested that the movement during relaxation is due to sarcomere length nonuniformity and variations in the rate of decay in tension with sarcomere length. It seems likely, though difficult to test, that local differences in the dynamic strain history of the sarcomeres also are involved in the pattern of local movement.

The clear distinction in the relaxation phases expressed by the shoulder, which remains in the temperature range studied, as well as their distinction in temperature sensitivity—a Q_{10} of 2 for the linear decline in tension and a Q_{10} of 3 for the exponential relaxation toward zero tension—is not inconsistent with a relaxation that depends on the mechanical conditions. Our data are also compatible with the suggestion of Dawson et al. (1980) that the rate of the exponential phase is dependent on the free energy change for ATP hydrolysis, which in turn may be related to the rate of Ca^{++} uptake into the sarcoplasmic reticulum.

To determine the direct cause of the instability of the muscle at the shoulder it probably would be necessary to investigate in more detail the time-course of the force relaxation and the local movement as disturbed by imposed length changes. After the shoulder, a substantial part of the muscle shortens ~2% within 0.25 s. This corresponds to a shortening velocity that is roughly a factor of 10 slower than the maximum shortening velocity of the tetanically contracting muscle. Thus, it follows that the tension generated in the part that shortens is less than the tension that would be generated in an isometrically relaxing muscle. Moreover, as is suggested from the momentary stiffness measurements, such a shortening would accelerate detachment of cross-bridges. In the concept of cooperativity within the actin filament, this could have consequences for the affinity of troponin for Ca (cf. Weber and Murray [1973] and Curtin and Woledge [1978]). It seems plausible, therefore, that the movement directly or indirectly affects the tension-generating capacity of the muscle and in this way enhances relaxation. Because of the magnitude of the length change under consideration, it seems unlikely that a relation with the deficit in the tetanic tension development after release found by Maréchal and Plaghki (1979) exists.

The suggestion of Julian and Morgan (1979a and 1979b) that the rate of the rather slow decline in tension reflects the isometric cross-bridge detachment rate *g* would imply that *g* also has a Q_{10} of 2. In a simple two-state cross-

bridge model, the stationary turnover rate for the cross-bridge is proportional to the isometric detachment g. It follows then that the ATP hydrolysis per unit time associated with cross-bridge cycling also has Q_{10} of 2. Surprisingly, this value is consistent with the Q_{10} for Mg-activated myofibrillar ATPase activity (Bárány, 1967). The Q_{10} of the slow decline in tension, however, is different from the Q_{10} (2.67) for the velocity of unloaded shortening obtained by Edman (1979), which also could be limited by the detachment rate. This last value is in better agreement with the Q_{10} of 2.7 we have found for the slow recovery in tension during phase 4 of the tension transient after a rapid release. The rate of tension decay during relaxation and the temperature dependence of the time-course of the tension decay both seem to be in quantitative agreement with the results of the exponential fit of Mittenthal and Carlson (1971).

Implications for the Force Transients and the Momentary Stiffness Measurements

The amount of peak displacement absorbed in the central part of the muscle is ~20% less than would be expected in the uniform case. This value corresponds to an elastic element located in the end regions, which, under full tetanic tension, lengthens ~100 μm. As a result, the T_1 values in Fig. 6 have to be translated to ~20% smaller displacement values. As follows from the marker-displacement values after 10 ms, the correction for the T_2 values is smaller. As a consequence, the fast-recovery phase (T_1-T_2) is increased. Moreover, the correction of 20% reduces the estimate for the upper limit of the undamped compliance in frog sartorius found by Stienen et al. (1978) from 7 nm/half-sarcomere to 5.6 nm/half-sarcomere. This increases the compatibility of the results presented with the results obtained on single fibers by Ford et al. (1977), in which a value of 4 nm/half-sarcomere was found using very rapid length changes. The remainder could be caused by truncation of the rapid parts in the tension transient due to the large duration of the length changes we imposed.

Our corrected T_2 values are also only slightly different from the T_2 curve given by Ford et al. (1977). Therefore, we are inclined to conclude that, with due precautions or corrections, the results of mechanical experiments on whole muscle and single fibers are compatible. This implies that the effects due to the differences in length of the fibers in the muscle are rather small.

Assuming that the momentary stiffness is a measure of the number of attached cross-bridges (cf. Julian et al. [1974]), the decrease in stiffness after shortening implies that a net loss in the number of attached cross-bridges already occurs during the 15 ms after the shortening. Without additional assumptions it is not possible to estimate the detachment rate from the time-course of the momentary stiffness. In view of the time-course on which stiffness decreased, however, an (average) detachment rate after shortening on the order of 200 s^{-1} is plausible. This seems reasonable in view of the modeling efforts of, among others, Julian et al. (1974) and of Eisenberg et al. (1980).

The difference between the relative tension and the relative momentary stiffness suggests that during the slow-recovery phase the formation of cross-brdiges at high strain prevails.

The increase in momentary stiffness observed after an initial lengthening is more difficult to interpret. A similar phenomenon was observed in insect flight muscle (Herzig, 1977), but there it was accompanied by an increase in tension. The increase in stiffness was found to depend on the initial strain. This suggests that there might be a relation with stretch activation phenomena, which, in the interpretation of Pringle (1978), could be governed by the effective internal strain sensed at the myosin filament. From the results of the tests performed during the momentary stiffness measurements it follows that the time-course of the stiffness after the release is not drastically affected by the nonuniform behavior, i.e., the stiffness passes through a minimum value ~15 ms after the initial shortening. The depth of the minimum depends on the amplitude of the shortening, which has to be corrected by ~20%. The same correction of the initial strain has to be applied to the dependency of the (increased) stiffness on the initial stretch.

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