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Stiffness and Tension During and After Sudden Length Changes of Glycerinated Single Insect Fibrillar Muscle Fibres

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Abstract. Rapid length changes were applied (within 0.2 ms or 0.4 ms) to single isometrically contracted glycerol extracted muscle fibres of the dorsal longitudinal muscle of *Lethocerus maximus* suspended in an Ca^{2+} and ATP containing solution at 20–23° C. Force transients and the fibre stiffness were measured during and after rapid length changes.

At length changes *below 0.5%* of the initial fibre length (~ 2.4 μm sarcomere length) the mechanical transients were characterized as follows: (1) After stretch and after release the force regains at least partly the value of tension before the length change within a quick phase of tension recovery. The quick phase induced by stretch was nearly completed within 1–2 ms. (2) A pulse in length of 1.5 ms duration, i.e., a stretch followed by a release to the initial length or a release followed by a stretch to the initial length, was applied to the fibre. The force transient induced by this procedure regains after the second length change the value of the isometric tension before the procedure. (3) The stiffness was constant during each length change of the "pulse" and was equal during the first and the second length changes.

These findings are predicted by the muscle contraction model of Huxley and Simmons (1971):

The identical force before and after a length pulse may indicate that the rotation of cross bridges after the first length change is followed by a rotation into the original position after the second length change. The constancy of the stiffness during the length changes may indicate a Hookean elastic element of the cross bridge. The similarity of the stiffness during the first and the second length changes, i.e., before and after the quick phase, gives evidence that the quick phases after stretch and after release are not accompanied by a change in the net number of attached cross bridges.

If stretches of *more than 0.5%* of the initial length were applied, the mechanical transient of the muscle fibre changed as follows: (1) An ultra fast tension decay phase (duration < 0.4 ms) was observed in addition to the slower decay phase induced by the smaller stretches. (2) If the initial stretch was followed by a release to the initial length, no fast recovery phase was observed, which returns

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the force to the value before the stretch. The reduced tension value persists for a longer period in time than 10 ms. (3) If the muscle was stretched and released repetitively an ultra fast quick phase was induced only by the first stretch. (4) The stiffness increased during stretch, but was found to be the same in the isometrically contracting muscle and after the quick tension decay phase following a large stretch.

These findings indicate that the contraction model of Huxley and Simmons has to be extended by a further process additional to cross bridge rotation in case of large stretches ($> 0.5\%\ L_i$). The findings are taken to indicate a rapid detachment and reattachment of overstrained cross bridges, i.e., a cross bridge slippage induced by large stretches.

Key words: Actomyosin interaction – Muscle mechanics – Cross bridge slippage – Contraction mechanism.

Introduction

Recent contraction models (Huxley and Simmons, 1971; Julian et al., 1974) suppose that force generation is due to a three or more state process involving the myosin cross bridges:

1) bridges attach to the actin filament,

2) rotation of perpendicularly attached cross bridges into an acute angled position,

3) detachment of the cross bridge.

Rotation is assumed to cause a displacement of the actin filament if the muscle is allowed to shorten; in the case of isometric contraction an elastic element within the cross bridge is assumed to be strained as a consequence of the rotation. According to the prediction of the model, a rapid stretch of the muscle will force cross bridges into the upright position and a rapid release would tend to cause a reverse rotation into the acute angled position. If the length change is performed sufficiently fast (duration less than 1 ms) the proposed cross bridge rotation is expected to cause the following force transient: after an initial elastic phase during the length change, the force ought to recover within 1 or 2 ms during which the bridges supposedly rotate on the actin filament.

The model predicts in more detail that cross bridges which rotated in consequence of a release into the acute angled position, will be forced to rotate back into their original position if the muscle is restretched to its initial length. Furthermore the model predicts that the cross bridges which are forced into the upright position by stretching the muscle will rotate back into the original position as soon as the length change is reversed by a subsequent release to the initial length. In agreement to this consideration we will show that after the procedure of stretch-release and release-stretch the initial tension is exactly restored if and only if the stretch amplitudes were smaller than 0.5%.

According to the model the stiffness of a muscle ought to be constant during the whole transient since it is proposed that the transient is not associated with rapid attachment or detachment processes of cross bridges (cf. Podolsky and Nolan,

1973, for an opposing view). Constancy of stiffness during tension transients will be demonstrated in this paper as long as the length step does not exceed 0.5% L_0 (cf. also Ford et al., 1974). Stiffness was measured by determining the slope of the force-extension plot obtained during the length step (cf. also Güth and Kuhn, 1976; 1978).

At larger stretches however, the experimental findings no longer obey the strict predictions of the model. Evidence will be presented that a further process – cross bridge slippage – contributes to tension transients when ever stretching exceeds 0.5% of the initial length. Preliminary evidence for such a process has been described in two previous papers (Güth and Kuhn, 1978; Güth et al., 1978) and further evidence for cross bridge slippage has recently been presented by Flitney and Hirst (1978a, b), and Sugi (1972).

Methods

Preparation and Solutions

In order to investigate the mechanical properties of the contractile system under defined chemical conditions, we used a glycerol extracted preparation: The dorsal longitudinal muscle (DLM) of *Lethocerus maximus* was – still fixed to the thorax – extracted in a 50% glycerol solution at pH 7 (cf. Jewell and Rüegg, 1966). After extraction for 24 h the muscle was stored in 50% glycerol solution containing 2 mM EGTA at pH 7. A single fibre of DLM (5 mm length) was clamped between a length step generator and a force transducer (for further details see Appendix) and immersed into the test solutions. After washing the fibre for 5 min in rigor solution containing 20 mM imidazole, 4 mM EGTA, 4 mM EDTA, 50 mM KCl, 1 mM NaN_3 and 37 mM HDTA, it was transferred into relaxation solution containing 20 mM imidazole, 4 mM EGTA, 10 mM NaN_3, 7.5 mM ATP, 7.5 mM $MgCl_2$, 15 mM HDTA and 100 mM KCl. The relaxed fibre was prestretched until passive force was 0.02 mN. This corresponds to a sarcomere length of about 2.4 μm (Reedy, 1968). To transfer the fibre into the contracting state it was immersed into contraction solution containing: 7.5 mM ATP, 7.5 mM $MgCl_2$, 20 mM imidazole, 100 mM KCl, 10 mM NaN_3, 4 mM CaEGTA, 15 mM HDTA at pH 6.7, $T = 20–23°$ C.

Some experiments were done using a contraction solution, which contained an ATP regenerating system: 15 mM ATP, 15 mM $MgCl_2$, 50 mM imidazole, 10 mM KCl, 5 mM NaN_3, 4 mM CaEGTA, 10 mM phosphocreatine and 20 mg/ml creatine kinase at pH 6.7. The presence of the ATP regenerating system did not affect the properties of the muscle, which are interpreted to be due to cross bridge rotation and cross bridge slippage.

Rapid length changes could be applied to the fibre by a relay type length step generator. The length change was completed within 0.2 ms for amplitudes below 30 μ and within 0.4 ms up to an amplitude of 150 μ. The shape of the length change could be chosen to be either parabolic or *S*-shaped (further details see Appendix).

The force was measured by a semiconductor type force transducer provided by Aksjeselskapet Mikro-Elektronikk (Type 801). The resonance frequency of the

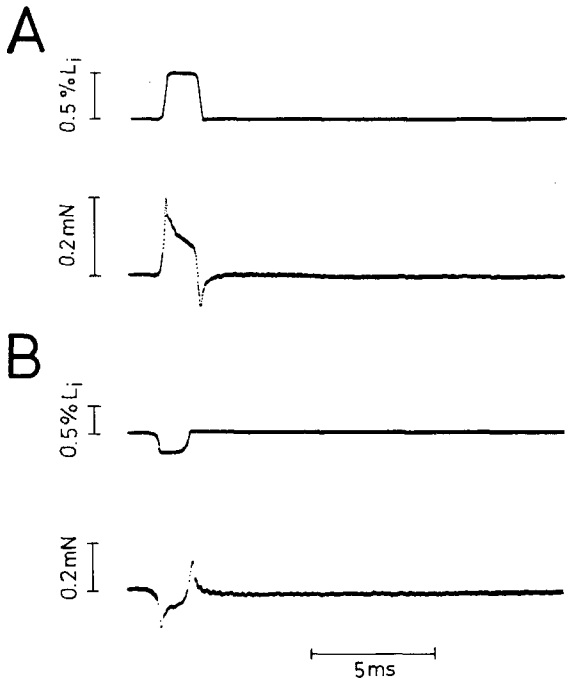

Fig. 4. Force transient induced by a sequence of stretch-release pulse and release-stretch pulse respectively. The delay between stretch and release and between release and stretch was 1.5 ms. Single fibre of DLM isometrically contracted (~ 0.2 mN) in 7.5 mM ATP, 7.5 mM $MgCl_2$, 20 mM imidazole, 100 mM KCl, 10 mM NaN_3, 4 mM CaEGTA, 15 mM HDTA at pH 6.7 and 23° C. Resonance frequency of the force transducer was 15 kHz

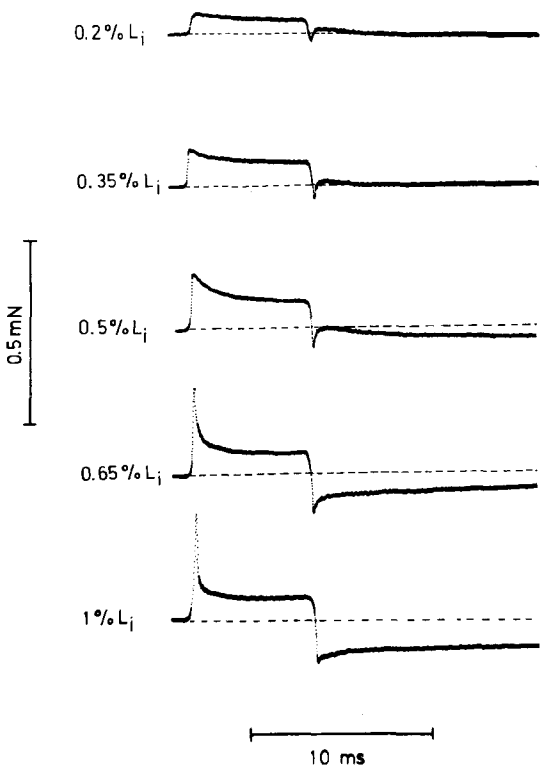

Fig. 5. Influence of the amplitude of the length change on size and shape of the induced force transients. The muscle fibre was stretched and 7 ms later released to its initial length. The dotted line specifies the level of the isometric tension. Isometric tension approximately corresponds to 0.15–0.2 mN. Single fibre of DLM isometrically contracted in 7.5 mM ATP, 7.5 mM $MgCl_2$, 20 mM imidazole, 100 mM KCl, 10 mM NaN_2, 4 mM CaEGTA, 15 mM HDTA at pH 6.7 and 23° C. Resonance frequency of the force transducer was 15 kHz

seen from the figure, the force is after the pulse in length the same as before the procedure. Note that the length changes didn't exceed 0.5%. If the length changes exceed 0.5% the behaviour of the fibre changes. The corresponding force transients are shown in Figure 5. As can be seen from the figure the stretch-release responses appear to be qualitatively different for the length changes below 0.5% L_0 compared to those above:

At small length changes the initial elastic response in phase with the length change was proportional to the length change and the subsequent quick phase appears to be governed by a single time constant. Furthermore the initial tension could be restored within about 1 ms after a quick release to the initial length. By contrast, imposing length changes greater than 0.5% caused an elastic tension response which was no longer proportional to the extent of stretch. Moreover the quick tension decay following stretch could no longer be described with a single time constant. Instead there appears an ultra quick phase in tension decay with a time constant of less than 0.3 ms. This can be more clearly seen from Figure 6: If the time course of the force transient is due to a single exponential, it can be described by

$$F = A_0 + A_1 e^{-\lambda t}, \tag{1}$$

where F is the measured force, λ the rate constant of the process and A_1 its amplitude. A_0 must be introduced because after stretch the force remains higher than before (A_0 can be different for different stretch amplitudes). Consequently a linear relationship is expected if ln $(F - A_0)$ is plotted against time. Because A_0 is not

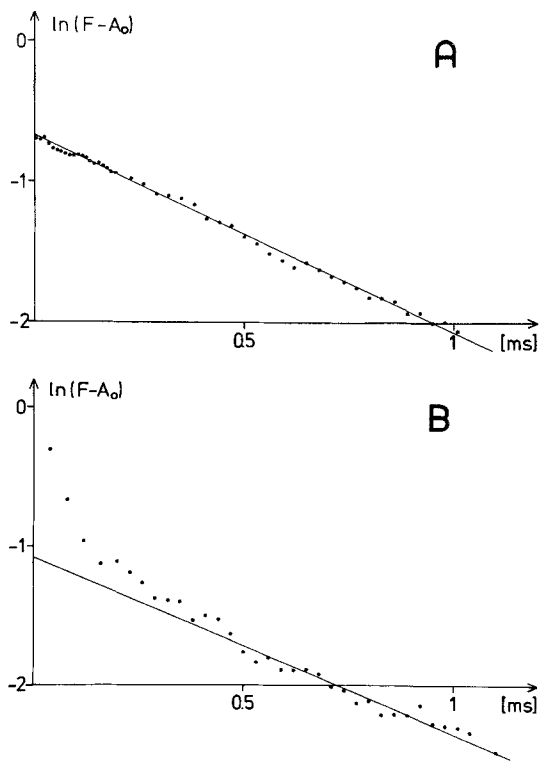

Fig. 6. Time course of force during the quick phase following a small stretch (0.4% L_i; **A**) and large stretch (1% L_i; **B**). For specification of F and A_0 see Eq. (1). Single fibres of DLM isometrically contracted in 7.5 mM ATP, 7.5 mM $MgCl_2$, 20 mM imidazole, 100 mM KCl, 10 mM NaN_3, 4 mM CaEGTA, 15 mM HDTA at pH 6.7 and 23° C

known a plot must be made for several A_0 and by trial and error the A_0 corresponding to the most linear plot can be taken to be the correct one. The best plot for a 0.4% stretch is shown in Figure 6A. Since the curve is fairly linear the transient may be described by Eq. (1).

For a large stretch of 1% it was impossible to get a linear dependence between ln $(F - A_0)$ and time over the whole range. However, the ultra fast quick phase observed at these large stretches was mainly completed after 0.4 ms. Therefore an attempt was made to describe the transient for a time later than 0.4 ms after stretch by a single exponential, i.e., by Eq. (1). As can be seen from Figure 6B for times greater than 0.4 ms after stretch a fairly linear relationship between ln $(F - A_0)$ and time resulted.

The reciprocal of the slopes of the straight lines in Figure 6A and B, i.e., the time constants, are with 0.7 ms for the small and 0.8 ms for the large stretch rather similar. Also, the intersection of the lines with the ordinate, i.e., the amplitude A_1 appears to be not very different.

Abbott and Steiger (1977) found for small stretches two components in the quick tension decay phase (with time constants of 1 ms and 4 ms respectively). The faster component corresponds to that reported here. The slower one may be detected (only) in transients lasting for more than 2–3 ms (cf. Fig. 5). Computer fits to force transients over a sufficiently long period in time showed in good agreement to the findings of Abbott and Steiger (1977) indeed an additional slower component of the quick phase.

In addition to the appearance of the ultra fast quick phase of the force transient a "large" stretch induced a further feature (Fig. 5): After the subsequent release the tension did not quickly recover to its initial value as it did after small amplitude stretch-release cycles. Instead tension remained lower than the initial tension even 12 ms after the release and in case of very large releases (1% L_i) a recovery phase was nearly completely absent.

The force transient induced by the small releases of about 0.2 to 0.3% L_i overshoots after the rapid tension recovery (quick phase) the initial tension (dotted line in Fig. 5) and subsequently decays to the steady state value. This tension fall occurs within a time of about 5 ms and may perhaps be comparable to the somewhat slower deactivation phase observed already by Jewell and Rüegg (1966). The "deactivation" was not noticable at larger releases (cf. Fig. 5) or even in the case of small release if this occurs already 1.5 ms after a preceding stretch (Fig. 4).

Another interesting feature of the sequence of force transients shown in Figure 5 is the level in force, which was maintained after stretch at a time when the quick tension decay phase was almost over: Whereas up to a stretch amplitude of 0.5% this level increased with stretch amplitude, the level remains constant or even decreased after increasing the stretch above 0.5%.

3. Evidence for Constant Number of Attached Cross Bridges During Stretch-Induced Quick Phase

In order to assess the relative number of attached cross bridges during or after length changes the immediate fibre stiffness was measured. The immediate stiffness

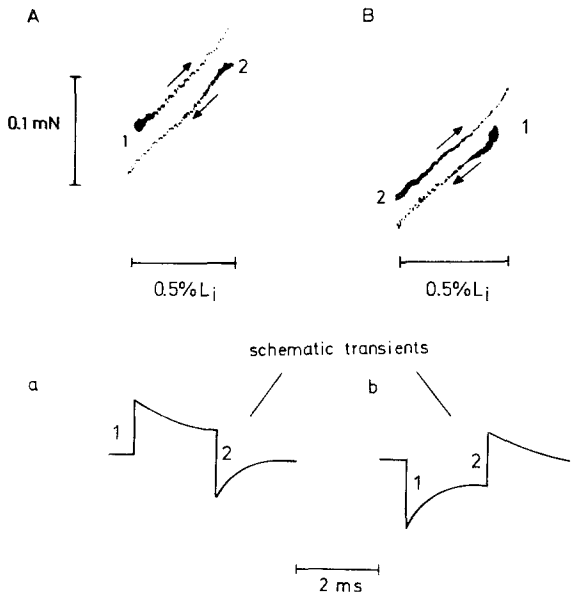

Fig. 7. Force-length diagram for an isometrically contracted fibre which was stretched and 2.5 ms later released (part **A**) or released and 1.5 ms later restretched (part **B**). Below: scheme of the corresponding force transients. Curves were plotted during the corresponding length changes completed within 0.2 ms. The isometric tension was approximately 0.15 mN. For conditions, see legend to Figure 5. Resonance frequency of the force transducer was 15 kHz

is assumed to be proportional to the number of cross bridges attached to actin at any one moment (cf. Huxley and Simmons, 1971). Stiffness was estimated from plots of length versus tension recorded during a single length change.

Figure 7A shows such a length force plot obtained during a 0.5% stretch of 0.3 ms duration, as well as a plot obtained during the release to the initial length performed 2.5 ms after the stretch. Note that both tension-length plots are approximately linear indicating hookean elasticity. During release, tension values were lower at the same length than during stretch, but the slopes of the tension length plots were identical: there is a parallel shift of the force-length plots obtained during stretch or release, suggesting that the number of attached bridges remains constant during stretch (curve due to the stretch is linear), the subsequent quick phase (curves due to stretch and release have the same slope) and during the release to the initial length (curve of the release is linear). Furthermore the linearity of the curves may be taken as evidence for the hookean nature of cross bridge elasticity (cf. Ford et al., 1977).

4. Constancy of Number of Attached Cross Bridges During the Quick Tension Recovery (Quick Phase) Following a Quick Release

When the isometrically contracted fibre was first released by 0.5% L_0 and restretched to the initial length 1.5 ms later, the following transients were observed (cf. Fig. 4B). First there was a quick fall in tension followed by a fast recovery nearly completed within about 1 ms. This recovery is probably comparable to the quick phase described by Huxley and Simmons in frog muscle. Restretching the fibre to the initial length caused an immediate tension increase followed by a quick tension decay approximately to the initial force.

In Figure 7B the corresponding measurement of stiffness during the release and the subsequent (1.5 ms later) restretch is shown. It appears from the figure that the force generation during the quick recovery phase obviously occured without any change in the number of attached cross bridges, for the force length plot obtained during release has a nearly constant slope, which is almost the same as the slope of the force tension plot obtained during restretching the fibre. However, in the latter plot the forces were larger at a given length than during release. Thus there is a parallel shift of the tension length diagrams due to the recovery phase after the release.

The tension length plots obtained during the length change may be comparable to the T_1 curves described by Huxley and Simmons (1971) and Ford et al. (1977). The plots shown here intersect the x-axis (zero force) approximately at 1% L_i. This is not contrary to the value of ca. 0.5% reported at low temperature ($\sim 3°$ C) by Ford et al., because at the low temperature we found similar values.

5. Analysis of Processes During Ultra Fast Quick Phase Following Large Stretches

Figure 8 shows the force length diagram obtained during a 1% stretch and during a release to the initial length performed 3 ms later. Note that during stretch the slope of the tension length plot increases (as has been described by Güth and Kuhn, 1978)

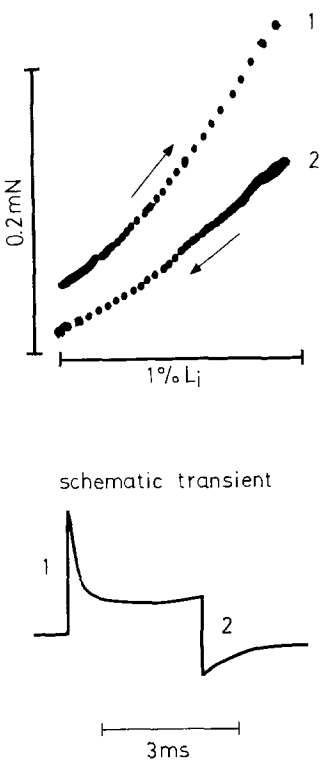

Fig. 8. Force-length diagram for a sequence of length changes involving a comparentively large stretch and release. Below the schematic force transient is shown. Single fibres of DLM isometrically contracted in 7.5 mM ATP, 7.5 mM $MgCl_2$, 20 mM imidazole, 100 mM KCl, 10 mM NaN_3, 4 mM CaEGTA, 15 mM HDTA at pH 6.7 and 23° C. Resonance frequency of the force transducer was 15 kHz

however, the slope is similar at the beginning of stretch and at the beginning of the release. This is taken to mean that the ultra quick tension decay following large stretches does not involve a reduction in the number of attached cross bridges below that of the isometric contraction before the stretch.

Figure 9 shows force transients induced by a sequence of two subsequent stretch-release cycles (length pulses). Above, an experiment involving 0.5% length changes and below one with 1% length changes is shown. It can be seen that at large amplitude length changes irreversible effects occur: the first stretch induced an ultra fast quick phase, whereas the ultra fast quick phase was almost missing after the second stretch. This may indicate that the processes responsible for the ultra fast component of the quick phase are not reversible.

If the amplitude of the stretch was increased to more than 2%, the processes responsible for the ultra quick phase seemed to become so fast, that the force stopped rising before the length step was completed. This phenomenon can clearly be seen in Figure 10A: After exceeding a certain extension, the fibre (the cross bridges) yielded and plastic flow or slippage occured.

Note that force increased only during the first 0.3 ms and subsequently remained constant though the extension increased further from 1–2%. The yield point is quite sharp as shown in the extension force diagram (Fig. 10B) obtained during the length change performed within 0.4 ms.

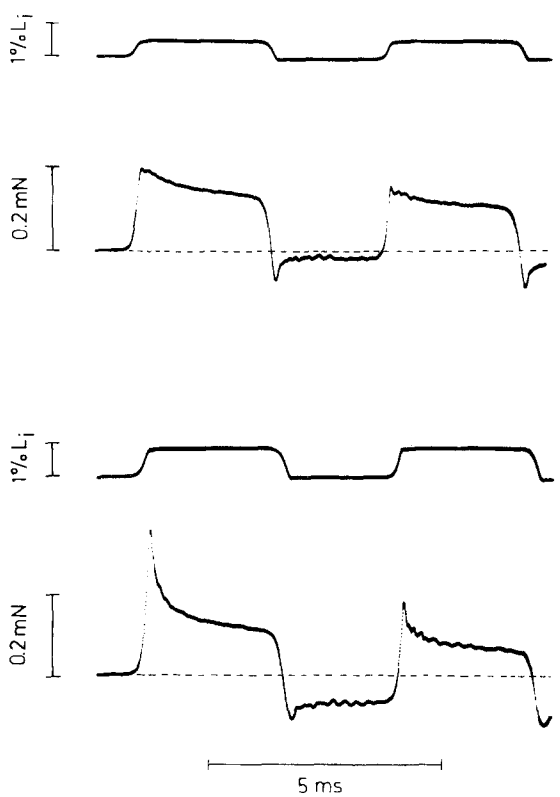

Fig. 9. Different effects produced by a sequence of two subsequent length pulses involving "small" (0.5% L_i) or "large" (0.8% L_i) stretches and releases to the initial length. Single fibres of DLM isometrically contracted in 7.5 mM ATP, 7.5 mM $MgCl_2$, 20 mM imidazole, 100 mM KCl, 10 mM NaN_3, 4 mM CaEGTA, 15 mM HDTA at pH 6.7 and 23° C. Resonance frequency of the force transducer was 15 kHz. The oscillations of the force transient are due to oscillations of the surface of the incubation liquid

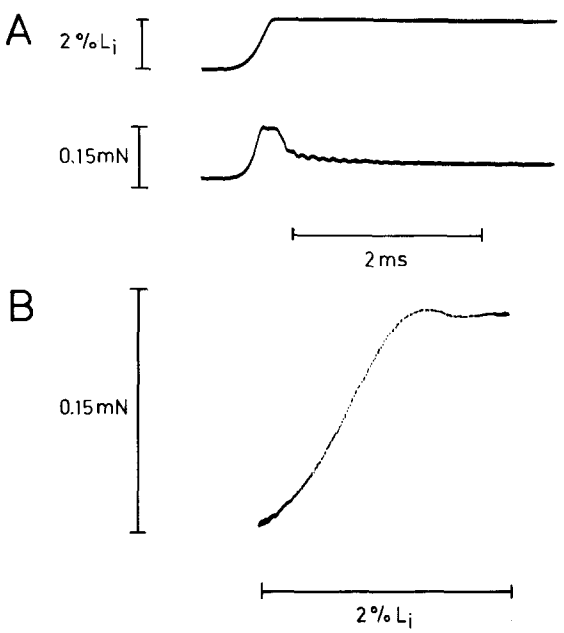

Fig. 10. Force transient and length-force diagram for a 2% L_i stretch. Figure 10A shows length signal above and corresponding force transient below. Figure 10B shows the corresponding length-force diagram recorded during length step. Single fibres of DLM isometrically contracted in 7.5 mM ATP, 7.5 mM $MgCl_2$, 20 mM imidazole, 100 mM KCl, 10 mM NaN_3, 4 mM CaEGTA, 15 mM HDTA at pH 6.7 and 23° C. Resonance frequency of the force transducer was 15 kHz

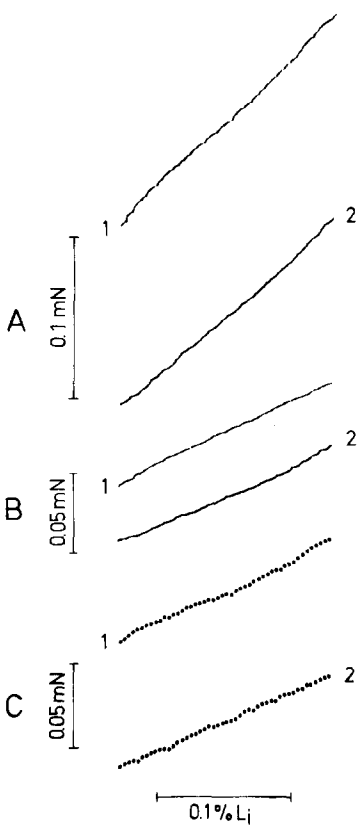

Fig. 11. Tension-length diagrams obtained during small length changes of single fibres of DLM. The curves correspond to a stretch (1) and a release (2) to the initial length. The delay between stretch and release was 2.5 ms. **A:** fibre in contracted state (conditions as Fig. 10); **B:** fibre in relaxed state; **C:** difference curve of A and B: the force obtained in relaxed state was subtracted from the corresponding force in contracting state. The fibre stretch (1) was started from the stretch activated state. The tension at the beginning of the stretch was 0.6 mN

6. *The Resting Elasticity*

The elastic properties of relaxed muscle were investigated in order to rule out the possibility that the particular mechanical properties described above were due to special mechanical features of the passive parallel elastic components. Figure 11 shows an experiment in which a single fibre was stretched (by 0.2% L_i) in relaxing solution and subsequently (i.e., 2.5 ms later) released to the initial length (traces B). Note that the force extension curve obtained during stretch (1) was fairly linear and very similar to that obtained during release (2). In comparison, in contracted muscle fibres (Fig. 11A) stiffness was higher. Furthermore the parallel shift of the curves obtained during stretch (1) and release (2) is much more distinct than in the released state. These features were also seen in the "difference curves" obtained by subtracting the passive force extension curve of relaxed fibres from the active curve of fibres suspended in contraction solution (Fig. 11C). According to White et al. (1977) tension (or stiffness) of the contractile component is given by the difference between tension (or stiffness) measured in contracted and relaxed muscle. Consequently the difference curve shows that the elasticity of the contractile component is "Hookean".

Figure 12 shows force transients induced by two cycles of stretch and release in contraction solution (B) and in relaxing solution (C). The difference curve plotted in

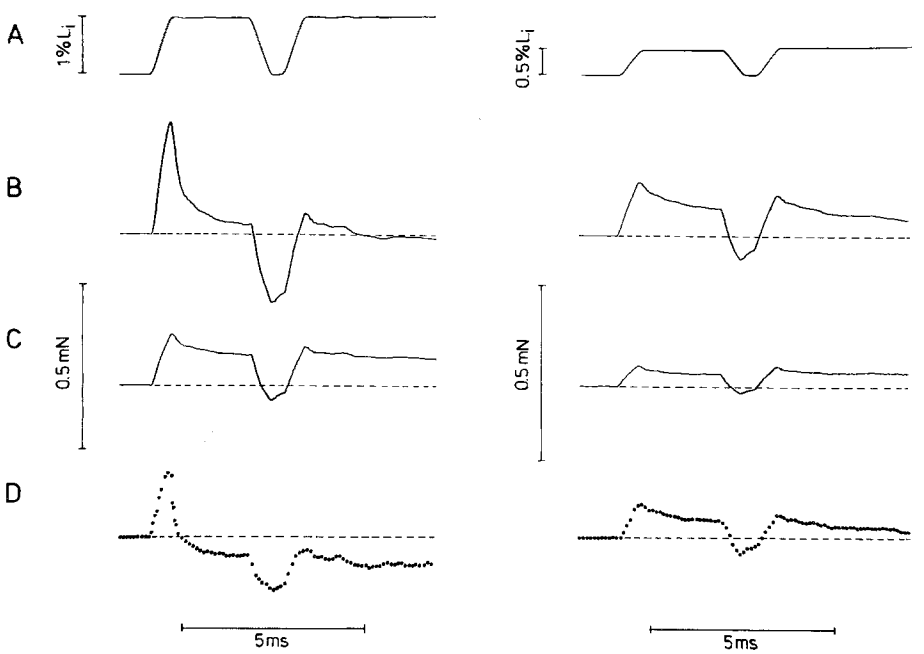

Fig. 12. Force transient of repetitively stretched and released muscle in the relaxed and in the contracted state. A single fibre (DLM of *Leth. max.*) was subjected to two cycles of stretch and release. *Left side:* transients induced by large length changes (1% L_i); *right side:* transients induced by small length changes (0.4% L_i). The traces show from top to bottom: the length change (**A**), corresponding force transient of the contracted muscle (**B**), transient of the relaxed muscle (**C**) and the difference curve of trace B and C (**D**)

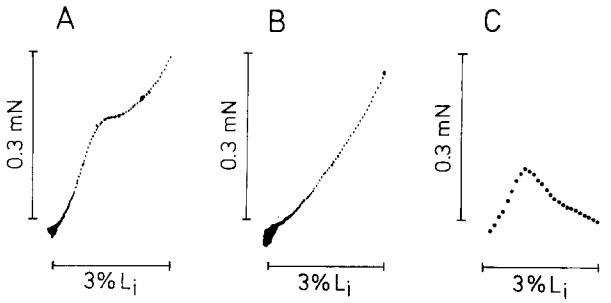

Fig. 13. Tension-length diagram of single fibre obtained during a large stretch (3% L_i) in contracted state (**A**) and in relaxed state (**B**). Curve **C** is the difference curve of A and B

(D) exhibits the same features as the tension transients of active muscle: an elastic response in phase with the length change followed by a quick tension recovery nearly completed within 1–2 ms in the case of a small stretch ($\leq$ 0.5% L_i). In the case of a larger stretch ($>$ 0.5% L_i), the quick recovery phase was after the first stretch preceded by an ultra fast phase nearly completed within 0.3 ms. This feature was not observed in stretched relaxed muscle (traces C).

In Figure 13 the force extension curve obtained during large stretches (3% L_i) of contracted fibres (A) and relaxed fibres (B) are compared with the difference curve (C). As can be seen from the figure, the declining slope of the force extension curve above a critical extension value of approximately 1% L_0 is a feature characteristic of activated muscle, and is missing in relaxed muscle.

In conclusion the slippage phenomena described in this paper must be attributed to the contractile structure; probably the cross bridges. These results may be compared with those obtained by Flitney and Hirst (1978) and by Sugi (1972) who showed that in living frog muscle fibres about $1/10$ or $1/5$ of the fastest stretch induced force transients can be ascribed to properties of the relaxed muscle.

Discussion

The Nature of Force Transients Induced by "Small" Stretches or Releases

The measured force transients induced by length changes up to 0.5% L_i can be interpreted in terms of the Huxley and Simmons model of muscle contraction (Huxley and Simmons, 1971; Huxley, 1974): A stretch induces a rotation of acute angled attached cross bridges into the upright position; a release causes upright attached cross bridges to rotate into an acute angled position. As predicted by the model the quick tension decay after the elastic phase induced by a stretch and a quick tension recovery after the elastic phase induced by a release has been observed. Furthermore it could be shown that these changes in force are reversible: The force after a sequence of stretch and subsequent release regains within the recovery phase a value rather similar to that before the initial stretch (Fig. 4A). Obviously cross bridges which have rotated after stretching into the upright position rotate back into the original position after the release to the initial length.

The reversibility of the rotation into the opposite direction could also be shown: Figure 4B shows that after a sequence of release and subsequent restretches to the initial length the force regains during the decay phase almost exactly the value before the procedure. This indicates, according to the model, that cross bridges which have rotated after release into the acute angled position rotate back after the subsequent restretch.

Moreover during the tension transients no change in fibre stiffness (that is in the net number of attached cross bridges) could be detected within the quick tension recovery and the quick tension decay phase following the length step (cf. Fig. 7). This analysis of a quick release recovery provides thus new evidence supporting a model, which claims that force is generated by a conformational change in the cross bridges while they are attached to actin as suggested by Huxley and Simmons.

After a cycle of stretch and release to the initial length quick tension recovery is usually followed by a somewhat slower tension decay, which may be interpreted as deactivation by release. This deactivation phenomenon is, however, only observed if the interval between stretch and release exceeds 2 ms. This may indicate that following a stretch the muscle fibre must be "primed" by a time consuming process before it is capable of this release induced deactivation.

Evidence for Cross Bridge Slippage Induced by Large Stretches

If the muscle fibre is stretched with an amplitude larger than 0.5% L_i the shape of the corresponding force transients is markedly changed:

1) In addition to the quick phase described in the small stretch experiments an ultra fast component appears which is almost completed within 0.4 ms after the stretch (Fig. 5; cf. also Güth and Kuhn, 1978).

2) The force after the subsequent release to the initial length does no longer regain a value similar to that before the stretch, but it remains low for at least 10 ms.

3) If a muscle is stretched and released repetitively by "small" length changes the corresponding force transient of the first cycle is similar to the subsequent cycles. If, however, the amplitude of repeated stretches and releases are larger than 0.5% it is only the first stretch which induces a pronounced ultra fast quick phase.

To account for these phenomena in terms of the Huxley and Simmons model, the model must be extended to include a further process in addition to cross bridge rotation. This process might be considered as involving detachment of strongly overstrained cross bridges. If this were so, the stiffness would be expected to be less after stretching than during isometric contraction. This is not the case (Fig. 8): The slope of the length-force plot is just as high at the beginning of the stretch as at the beginning of the subsequent release. Consequently the net number of attached cross bridges is not changed in the time interval between the beginning of stretch and after completion of ultra fast and fast quick phases. Therefore we must assume that any possible detachment of overstrained cross bridges is compensated by rapid reattachment of presumably the same cross bridges with partly discharged elastic elements. Such fast detachment and reattachment processes (within a fraction of 1 ms) would

be equivalent to a slippage of overstrained cross bridges along the actin filament (cf. Güth and Kuhn, 1978).

It should be pointed out that the cross bridge slippage does not seem to replace the cross bridge rotation; rather slippage seems to be an additional process. This is clearly indicated by Figure 6: Amplitude and time course of the quick phase following small stretches is similar to that of the slower component in the quick phase induced by a large stretch. The slow component has been ascribed to cross bridge rotation in the case of low stretch amplitudes. Thus it is reasonable to assume that this interpretation applies also for the slow component of the large stretches and that an additional ultra fast process (cross bridge slippage) must be proposed to account for the fast component of the transient (ultra fast quick phase). Further evidence for cross bridge slippage is provided by the finding that the release of an overstretched fibre to its initial length reduces tension below the initial level and that tension does not quickly recover to the initial tension but remains below that level for at least 10 ms. A reasonable interpretation is that cross bridges which have slipped have partly discharged their elastic elements during a process of detachment and reattachment involving conversion from slipping contacts to stable contacts. After the fibre has been released to the initial length these slipped cross bridges would be expected to generate less froce than the elastically more strained cross bridges prior to slippage. The slippage hypothesis is also supported by the observation that an ultra quick phase is only observed after the first stretch but not after subsequent stretches of repetitive stretch-release sequences. Clearly, overstrained cross bridges slipping during the first cycle of stretch and release cannot again be overstrained by an identical stretch in a second stretch release cycle. Consequently no further slippage of bridges would occur after that second stretch.

Further slippage does occur, however, if the first stretch is not followed by a release but by a second and third stretch, as has been pointed out before (Güth et al., 1978). While for small stretches and releases the length-force relationship is quite linear, indicating hookean elasticity of the cross bridges, the slope of the length-force curve increases in the case of larger stretches (cf. Fig. 8). As yet it could not be clarified experimentally whether this increase in stiffness reflects non-hookean elastic properties of overstrained cross bridges or whether it is a indication of fast attachment and detachment processes occurring even during stretch and during the quick tension decay following the stretch. In any case: if fibres are stretched by more than 2% L_i the force values reach a maximum level before the length step is completed (cf. Fig. 10). According to the slippage hypothesis this finding may be taken to indicate that attached cross bridges break if the cross bridge elasticity is overstrained, and that they quickly rebind (see above) as soon as the elastic elements are sufficiently discharged. In this interpretation the "yield point force" reflects the maximum force of myosin cross bridge-actin interaction. Cross bridge slippage processes involving fast attachment and detachment of bridges may account for the fact that muscle can be overstretched quickly without breaking of molecular structures.

So far the "give" of overstretched muscle has been described in terms of cross bridge slippage along the actin filament. However, other sites of yielding such as thick filaments themselves ought to be taken into account as well. In this connection we recall the lengthening of the A-band observed in quickly stretched active skeletal muscle by Klein (1972). Recently the possibility has been considered that the neck-

region of a cross bridge corresponding to the *S*-2 fragment of myosin may change from an helical configuration into a melted configuration under certain conditions (Harrington, 1971; Mason, 1978; Holmes, 1977). Such a melting process might indeed account for many of the phenomena described in this paper including the ultra fast tension decay observed after stretches exceeding 0.5% of the muscle fibre length. However, a "give" due to such a process would by necessity be limited: The give per half sarcomere should not then exceed a value corresponding to that of the uncoiled *S*-2 fragment.

Detachment and reattachment of cross bridges involving ATP splitting as it occurs during the cross bridge cycle might also account for a give of overstretched muscle. Hnwever, such a mechanism would be feasible only as long as the elongation rate dnes not exceed about 10% muscle length per second, for this rate would correspond to a cross bridge cycle time of about 0.1 s deduced from ATP consumption measurements of a living frog muscle under isometric conditions (Curtin et al., 1974). Indeed the cycle time might be even longer during stretching since Curtin and Davies (1973) found a reduction in ATP consumption during stretch. These authors already suggested that during stretching cross bridges may infact detach and reattach without a concomitant ATP splitting as suggested in this paper.

Preliminary evidence for cross bridge slippage in vertebrate striated muscle has been presented already (Güth and Kuhn, 1978; Güth et al., 1978). Recently Flitney and Hirst (1978a, b) found a "give" of the muscle force if they stretched tetanized sartorius muscles of rana temporaria relatively slowly (within 50 ms) but by a large amount ($\sim$ 5% L_i). They found a treshold in the region of 1% L_i stretch for the onset of the muscle "give". It seems reasonable to assume the same process to be responsible for the phenomena described in this paper. This point of view is supported by the fact that the results of Flitney and Hirst (1978a, b) would be qualitatively expected from our results, as one can see from Figure 5: The force after the quick tension decay phase of the rapidly stretched fibre (in Fig. 5, the force measured immediately before the release), increases with increasing stretch amplitude but only up to 0.5% L_i. For larger stretches it remains constant or it may even decrease. Therefore one would predict – just as reported by Flitney and Hirst (1978a, b) for frog muscle – that the force generated by a slowly stretched muscle increases up to a certain treshold ($\sim$ 0.5–1% L_0) and remains nearly constant when the stretch amplitude exceeds this value.

Appendix

Calculation and Plotting of the Original Fibre Force from the Oscillating Force Transducers Signal

Assuming the force transducer to be a free oscillating system the relationship between the force (f) applied to the transducer and the recorded signal (x) is

$$f = m\ddot{x} + R\dot{x} + Dx \tag{2}$$

where m is the mass and D the stiffness of the force transducer. R corresponds to friction of the oscillating system. In order to calculate f (the force developed by the fibre) we solved the differential Eq. (2) electronically. The circuit used is shown schematically in Figure 14.

Figure 15 compares the force transducers signal to the recalculated original signal: transients are shown with and without the force recalculating circuit. The transients were induced by stretching a rubber string followed (1 ms later) by a release to the initial length. The length signal was on purpose not ideally damped in order to show that the recalculated force changes (lower trace) follows even very small and fast changes of the length (upper trace). These changes are masked by the oscillation of the force transducer in the directly recorded signal (middle trace). It is interesting to note that the delay of the force signal with respect to the length signal is of the same order of magnitude as one would expect from the calculated transmission time. For further information about transmission time effects see Güth and Kuhn (1978).

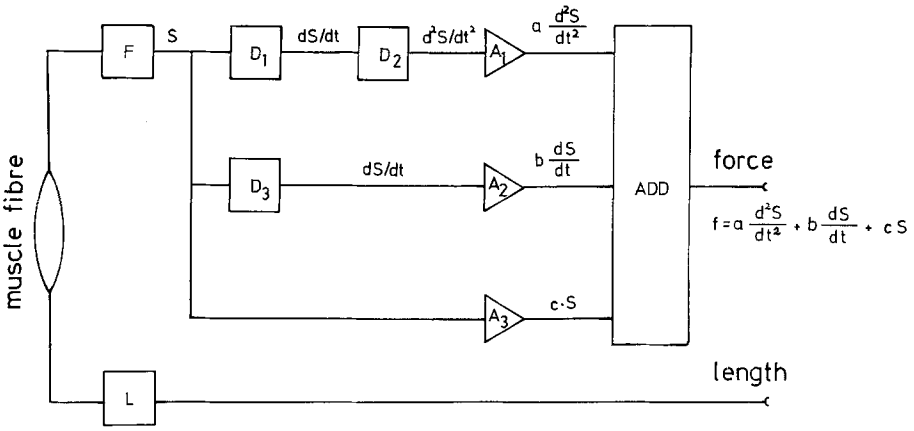

Fig. 14. Scheme of the circuit used to calculate the force (*f*) developed by the fibre from the measured signal (*S*). *D1 ... D3* are differentiating circuits. *A1 ... A3* are adjustable amplifiers. *ADD* is an adding circuit

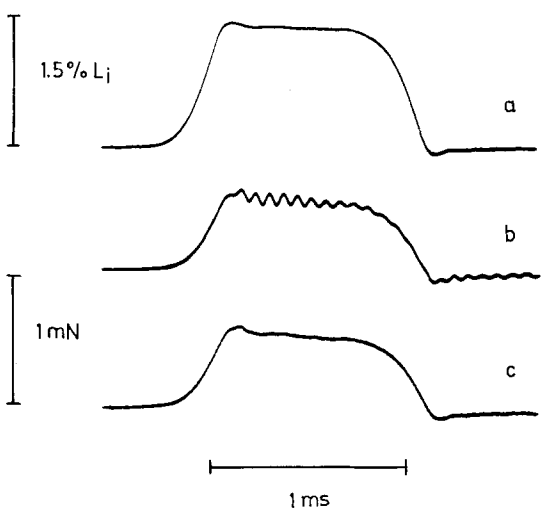

Fig. 15. The effect using a force recalculating system as shown in Figure 14 (the muscle fibre was replaced by a rubber string). *Upper trace:* length signal; *middle trace:* direct recorded signal of the force transducer; *lower trace:* recalculated force signal using the circuit shown in Figure 14. The length signal was not ideally damped in order to show that the recalculated signal follows even very faint length changes

Fixation of the Fibre at Force Transducer and Length Step Generator

To avoid uncertainties in the determined fibre length and disturbance of the mechanical behaviour of the measuring system due to the mass of glue, we glued the fibre neither at the tip of the length step generators glass rod nor at the tip of the force transducer. On the end of the force transducer the fibre was not glued directly at the transducer rod because the mass of the glue would have lowered the resonance frequency considerably.

How the fibre was fixed at the length step generator and the force transducer is shown in Figure 16. The fibre is bent around the tip of the glass rod of the length step generator and glued ca. one millimetre behind the kink. A notch cut into the end of the rod prevents the fibre from slipping off the tip of the rod. At the tip of the force transducer it was not desirable to glue the fibre in the same manner (the sensitive areas of the transducer would have been destroyed). Hence the fibre was wound around the tip of the force transducer and then attached to a separate pin. Slippage of the fibre around the tip of the glass rod of the length step generator or around the tip of the force transducer, may disturb the force measurement. Therefore we checked whether the force signal was the same if the fibre was glued at the very top of the length step generator glass rod or if the fibre was wound around the force transducer pin before glued to the separate pin: It could be shown that the fibres were effectively fixed at both the tip of the length step generator and at the tip of the force transducer.

The Force Transducer

The force transducer was provided by Aksjeselskapet Mikro-Elektronikk, Horten Norway (Type 801). Its pin was filed as it is shown in Figure 16 in order to prevent the fibre from slipping away from the pin and in order to remove mass. Due to the removal of mass a transducer resonance frequency of 15 kHz was achieved if the fibre was fixed. The sensitive areas of the transducer pin are not only sensitive against mechanical deformation but also against heat and light. Therefore the whole transducer was mounted in a tube filled with grease. Only the tip of the transducer pin sticked out of the tube. This protection of the transducer from light and heat and an extremely low noise and high speed amplifier resulted in a short time (several seconds) signal to noise ratio of 4 for forces in the range of 0.01 mN, in spite of the high time resolution of 15 kHz.

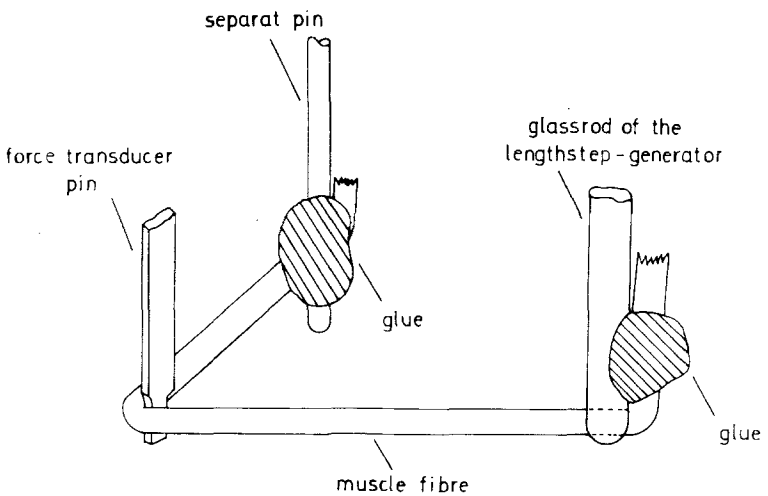

Fig. 16. Fixation of the muscle fibre at the length step generator and the force transducer. The fibre is glued ca. 1 mm above the tip of the glass rod of the length step generator. In the tip of the glass rod is a notch cut in order to prevent the fibre from slipping off the tip. At the force transducer the fibre is not glued in order to obtain a high resonance frequency (15 kHz) of the system. Instead it is bend by 90° or wound around the transducers pin and glued at a separate pin

The Length Step Generator

The length changes were performed by a relay type length step generator, which is schematically shown in Figure 17. If a current flows through coil *A* the anchor *C* is drawn from stop *b* to stop *a* and vice versa if the current flows through the other coil. The friction of the bearing bush *D* is so high that the anchor moves only if one of the coils is in action. The muscle fibre is fixed as described in Figure 16 at the pin *p* (only a point in the drawing, because it is seen from the top). The amplitude of the length change can be adjusted with the micrometer screws *M* by displacement of the core of the coil. After a displacement of the coil it is locked by the screws *F*.

Great care must be taken to avoid oscillations of the anchor after a step. Thus it is advantageous to ensure that the surface of the anchor is exactly parallel to the surface of the stop in the moment of contact. The magnetic flow through coil and anchor should be as high as possible. Therefore gaps in the magnetic yoke should be avoided, except the gap between anchor and stop. These requirements – no additional gap and parallel surfaces of stop and anchor – are fulfilled by the construction:

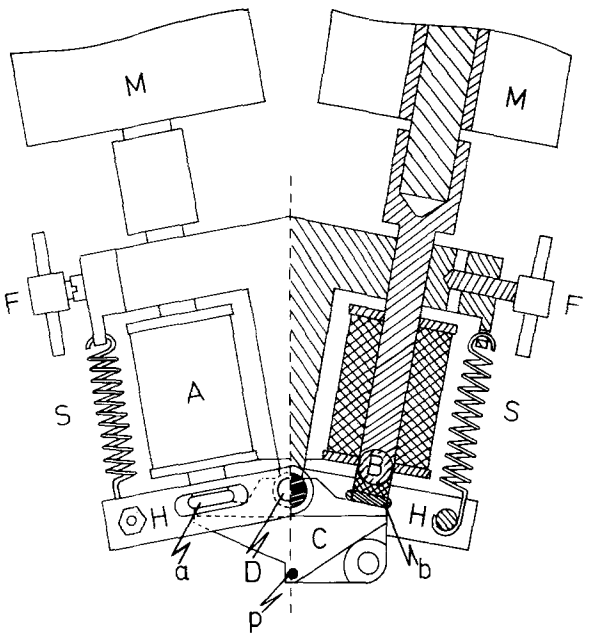

Fig. 17. Length step generator seen from above. Right: section through the axis of the coils core. *A:* Magnetic coil; *B:* Ball; *a, b:* Stop; *C:* Anchor; *H:* Mounting support for the stops a and b; *p:* Glass rod (cross-section), where the muscle fibre is fixed; *S:* Spring; *F:* Screw to lock the core of the coil; *M:* Micrometer-screw; *D:* Bearing bush of the anchor *C* and of the mounting support *H*

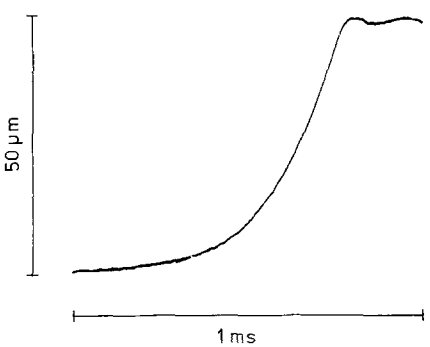

Fig. 18. Length change performed by the undamped length step generator. This continuously accelerated length change was used for the length tension plots reported in the paper

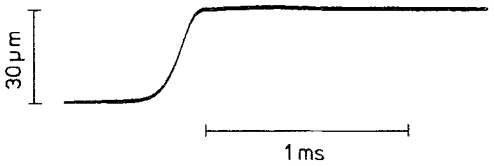

Fig. 19. Length change performed by the damped length step generator. This *S*-shaped length change was used for the force transients reported in the paper

First, because the anchor and the holder of the stop turn around the same axis, their surfaces are parallel to each other, independent on the adjusted amplitude of the length step. Second, if the stop is moved, i.e., another amplitude of the length step is adjusted, no gap appears in the magnetic yoke: The part of the magnetic yoke next to the stop is pivoted on a ball (*B*) and pressed by a spring (*S*) against the rearward surface of the stop. Consequently it adjusts itself so that a gap is prevented.

For stiffness measurement during the length change it is preferable to use a continuously accelerated length change (cf. Güth and Kuhn, 1978). The undamped length step generator provides such a length change, as can be seen from Figure 18. The duration of the length change could be adjusted between 0.2 ms and 0.5 ms for stretches up to 30 µm and between 0.4 ms and 1 ms for length changes up to 150 µm.

In case of the continuously accelerated length change it was not possible to avoid the oscillation of the length completely, after the anchor is stopped. Because this oscillation disturbs the force transient following the length change, the apparatus is damped by a drop of liquid (butanol) filled into the gap between the stop and the anchor. A correspondingly ideally damped S-shaped length change is shown in Figure 19.

The length was measured by a slit, which was narrowed proportionally to the length change. Consequently a light beam passing through the slit was broadened or narrowed proportionally to the length change. The intensity of the light was measured with a photodiode.

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