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Stiffness and Tension During and After Sudden Length Changes of Glycerinated Rabbit Psoas Muscle Fibres

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Abstract. A sudden stretch (within 0.3 ms) of glycerol-extracted rabbit psoas fibre bundle suspended in ATP-salt solution caused an immediate tension increase followed by a rapid tension decay (quick phase) which was nearly completed within 3 ms. The quick phase was missing or much reduced in the absence of ATP when the fibres were in rigor. Since the immediate stiffness of the fibres was nearly the same at the onset and at the end of the quick phase, the latter cannot be due to cross-bridges detachment per se. However, it may be ascribed to a conformational change (e.g. rotation) of attached bridges as suggested by Huxley and Simmons. Alternatively it might be explained by a slippage of attached cross-bridges. This mechanism would presuppose fast detachment and reattachment of strongly strained cross-bridges during the quick phase. Evidence for such a process was obtained by analysing the tension transients obtained when fibre bundles subjected to a large stretch were subsequently (within 10 ms) released to the initial length, as well as from stiffness measurements during the sudden length change: The stiffness was not found to be constant either during stretch or during the release. This may be taken to mean that the number of attached cross-bridges does not remain constant even during a rapid length change. In view of these results, the model proposed by Huxley and Simmons might be extended to take account of rapid attachment and detachment of cross-bridges.

Key words: Actin-myosin interactions – Muscle mechanics – ATP hydrolysis in skeletal muscle – Rigor state of muscle – Cross-bridge slippage.

Electron micrographs and X-ray diffraction pattern indicate that cross-bridges protrude perpendicularly from the myosin filaments in the relaxed muscle, i.e. when the cross-bridges are detached; in rigor state the cross-bridges are in an acute angle in relation to the myosin filaments (Huxley, 1969; Reedy et al., 1965). On this basis, Huxley and Simmons (Huxley and Simmons, 1971; Huxley, 1974) developed a model of muscular contraction. According to this model, development of force and

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shortening of a muscle fibre is caused by rotation of the cross-bridges after they have attached in the perpendicular position to the actin: the cross-bridge is thought to contain an elastic element which is in a discharged state when the cross-bridge attaches in the perpendicular position and which becomes strained when the cross-bridge rotates. These strained elastic elements connected between the actin and the myosin filament pull at the actin filament and try to shorten the muscle fibre by discharging themselves.

The cross-bridges that have rotated into an acute angle can be forced to rotate back into the perpendicular position by stretching the muscle fibre. This stretch induced rotation will discharge the elastic elements of these cross-bridges. Therefore a force transient induced by a stretch rises at first in phase with the length change because the elastic elements are strained (elastic phase) and then returns nearly to its initial values because the elastic elements are discharged by the rotation into the perpendicular position (quick phase). It is assumed that the cross-bridges remain attached during the quick phase. A basic assumption in the formulation of the model is therefore that the attachment and detachment process is slow compared to the process of rotation. This assumption is based on investigations of the ATP hydrolysis cycle performed by Lymn and Taylor (Lymn and Taylor, 1971). These authors concluded from their results that the attachment step is rate limiting within the cycle.

However, more recent investigations of Eisenberg and White (Eisenberg and Kielley, 1972; White and Taylor, 1976) gave evidence that probably not the attachment of cross-bridge is the rate limiting step but a previous step within the cycle. On the basis of this investigation even a fast (faster than cross-bridge rotation) attachment and detachment process is possible, a process demanded for instance by the two state model of Podolsky (Podolsky and Nolan, 1972). Such fast attachment and detachment processes are not inconsistent with the concept that muscle force is produced by cross-bridge rotation (as proposed by Huxley and Simmons, 1971).

According to Huxley and Simmons the stiffness of a muscle fibre is under certain preconditions (cf. Appendix) a measure of the number of attached cross-bridges. In previous experiments stiffness was measured by determining $\Delta T/\Delta L$ (cf. Huxley and Simmons, 1971). This values could be distored because of truncation effects caused by rotation or detachment of bridges occurring already during stretch. Consequently, we measured the stiffness of a glycerol-extracted muscle fibre *during* a stretch and *during* a subsequent release after the quick tension decay in force (quick phase of the force transient). This experiment allows comparison of the net number of attached cross-bridges at the end of the stretch and at the end of the quick phase of the force transient. Furthermore it can be tested, whether a change of the net number of the attached cross-bridges due to fast attachment and detachment processes occurs even during the length change. Since the processes responsible for the quick phase need ATP, a comparison of those stiffness measurements between a muscle in contracting and in rigor state should be interesting. Therefore glycerinated fibres are used, which can be reversibly transferred into rigor state.

Methods

In order to analyse the role of ATP it was necessary to expose the myofilaments of the muscle fibre to different ATP concentrations. For this purpose fibre bundles of rabbit psoas were extracted with 50% glycerol in order to render the cell membrane permeable. The "chemically skinned" fibres obtained were then suspended in the desired test solutions (pH 6.7) containing calcium-EGTA buffer (4 mM), sodium azide (10 mM), imidazole buffer (20 mM) either without added ATP (rigor solution) or with 15 mM MgATP (contraction solution, pCa ~ 5) or with 15 mM MgATP but without Ca^{+2} (relaxation solution). To adjust the ionic strength at I = 0.08, KCl was used. The bath temperature was 20–23° C. For mechanical measurements a fibre bundle (6–7 fibres of 0.5 cm length) was fixed between a capacitance type force transducer and a relais type length step generator.

The force transducer has a resonance frequency of 10 kHz. The signal to noise ratio was better than 500 for measured forces of about 300 mg. Up to this force the linearity was better than 5%. The duration of the length step could be varied between 0.2 and 1 ms. The length measurement was performed by an inductive system. The shape of the length step could be altered from a S-shape (relais stop damped by oil, cf. Fig. 4) to a parabolic shape (relais undamped, cf. Figs. 7 and 8).

The apparatus used to measure stiffness is shown schematically in Figure 1. The force and the length signal were differentiated. The subsequent division of the one differential by the other leads to a differential dK/dL (= stiffness), which when multiplied by the length of the fibre results in the elastic modulus of the fibre.

The advantage of measuring the stiffness in this way is that a single length change of a certain amplitude ΔL_0 is sufficient to obtain all the slope stiffness values corresponding to lengths between $\Delta L = 0$ and $\Delta L = \Delta L_0$ (cf. also Ford et al., 1976).

For the discussion of the influence of viscosity of the tissue, resonance frequency of the force transducer and transmssion time on the measurement, see Appendix.

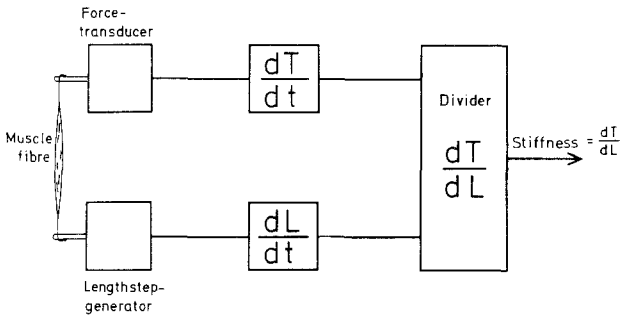

Fig. 1. The apparatus used to measure the stiffness of a muscle fibre during a length change. The length change can be performed within a time period which can be varied between 0.2 and 1 ms. The resonance frequency of the force-transducer was 10 kHz. The shape of the length change for stiffness measurements was parabolic

Results

Figure 2 shows the force developed by a single glycerol-extracted fibre of rabbit psoas after incubating in contraction solution (Fig. 2A) and in rigor solution (Fig. 2B). When the fibre was fully contracted, first a stretch of 1.3% of the initial length and then a subsequent release to the initial length followed by a further stretch were performed (cf. upper traces of Figs. 2A and B).

Figure 3 shows (expanded) the force transient of a released contracting fibre bundle of six fibres, which was subsequently restretched after 120 ms to the initial length. The stretch amplitude was 0.2% initial length in Figure 3 (I) and 2% initial length in Figure 3 (II). In order to make it easy to compare the features of the two transients, the force signals of the smaller length steps were amplified. As it can be seen from the figure, in case of the smaller release the elastic phase is followed by a quick phase which has a time constant comparable to the quick phase of the corresponding stretch. This phase is followed by a plateau, which is also observed by Huxley (Huxley, 1974) and Abbott (Abbott and Steiger, 1976) for small releases. However, in case of the large release the plateau is missing and the time constant of the quick phase is obviously much larger than the time constant of the corresponding stretch (cf. also Abbott and Steiger, 1976). But it should be mentioned (as discussed in more detail later on) that, in the case of the large release, there is some evidence that there exists a much faster quick phase in adddition to the recorded quick phase, this may be truncated in the force transient of the large release.

Figure 4 shows with an even more expanded time scale the force transients induced by stretches (also completed within 0.4 ms) for glycerol-extracted psoas fibre bundles in the presence of ATP and Ca ions (pCa 5, Fig. 4A), as well as in the absence of ATP (rigor, Fig. 4B). The corresponding length change (1% L_i) is shown in the upper trace of Figure 4. When the bundle (2 × 6 fibres) was incubated in contraction solution, the 1% L_i stretch induces a rise of tension from about 1.5

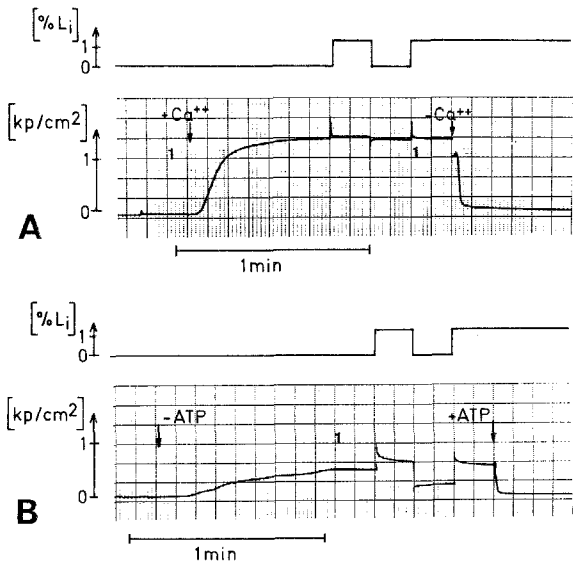

Fig. 2. Force developed by a single glycerol-extracted fibre of rabbit psoas after incubation in contraction solution (**A**) and in rigor solution (**B**) at 24° C. The upper traces show length changes which were performed when the fibre was in a full force generating state. The length changes are presented in per cent of the initial length of the fibre. *Contraction solution:* 15 mM ATP, 15 mM $MgCl_2$, 20 mM imidazole, 10 mM NaN_3, 4 mM CaEGTA, pH 6.7. *Rigor solution:* 55 mM KCl, 20 mM imidazole, 10 mM NaN_3, 4 mM EGTA, pH 6.7

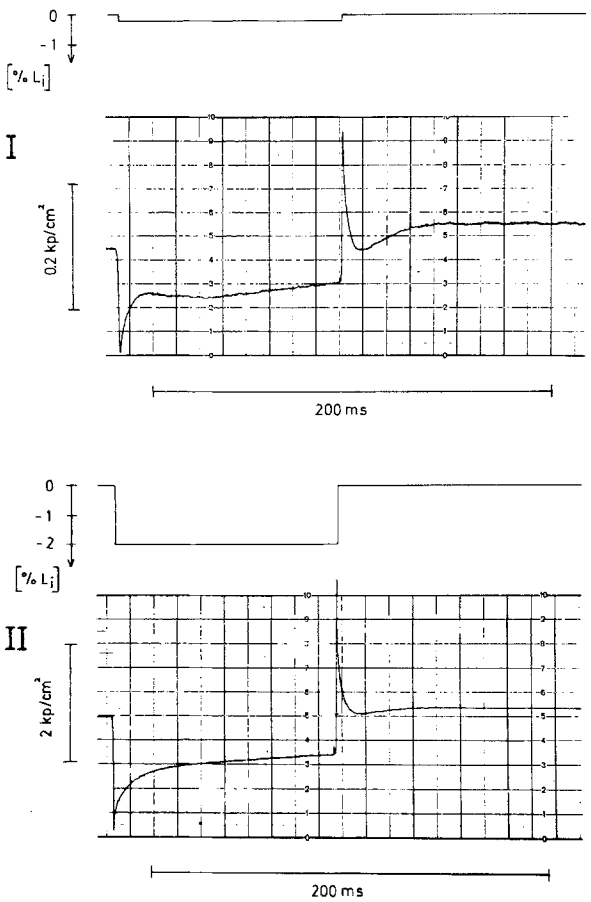

Fig. 3. Force transients of a contracting glycerol-extracted psoas fibre bundle (6 fibres) induced by a release and a subsequent restretch to the initial length. The upper traces show the corresponding length changes in percent of the initial length (L_i). In order to make it easy to compare the features of the two force transients, the force signal due to the 0.2% L_i length steps is more amplified than the force signal of the 2% L_i length steps. *Conditions:* Isometric tension 1.4 kp/cm^2; 15 mM MgATP; pCa 4.9; $I = 0.1$ M; 22° C

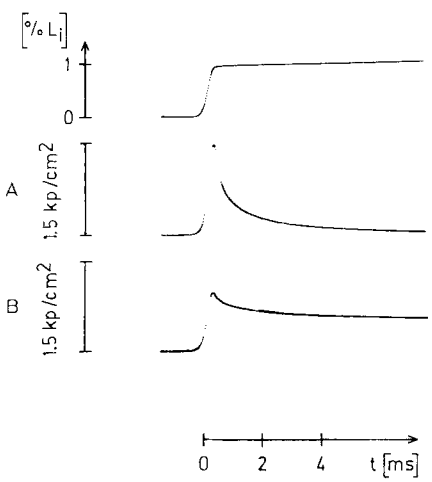

Fig. 4. Force transients of glycerol-extracted psoas fibre bundles (6 fibres) induced by stretches completed within 0.4 ms in the presence of ATP and Ca^{+2} ions (trace **A**) as well as in absence of ATP (trace **B**). The upper trace shows the corresponding length change. *Conditions:* pH 6.7; 23° C; $I = 0.1$ M

kp/cm² to a maximal tension value of about 3 kp/cm². Immediately after completion of the length change the force falls rapidly, reaching nearly the value it had before stretch. This quick phase of force decay is already up to 50% completed about 0.3 ms after the force reached its peak value. However, when a fibre bundle is incubated in rigor solution the force decays only to a small extent after completion of a 1% L_i stretch (Fig. 4B): the quick phase is practically missing in rigor (cf. also Heinl et al., 1973; Yamamoto and Herzig, in press). This indicates that the presence of ATP is a basic requirement for the occurrence of a quick phase in skeletal muscle.

In order to obtain information on the number of attached cross-bridges and their stiffness, Huxley and Simmons measured the peak height of the force transients induced by stretches and releases of various amplitudes and plotted the results in a length-force diagram (T_1 curve). For stretches and small releases they found linear dependence on the peak heights and the corresponding amplitudes of the length changes. We supplemented these measurements of peak heights by recording force transients *during* the elastic phase with a sufficient accuracy to plot corresponding values of force and length during the length change itself. The result for a stretch and a release is shown in Figure 5. The straight line in Figure 5 intersects the length coordinate at $-1.2\%\ L_i$. For a muscle in rigor state Figure 6 shows a similar plot to that in Figure 7, but for a stretch only. All features of this plot are equivalent to those of the contracting muscle, except that the deviation of the length-force curve from a straight line is even more pronounced. This may be caused by the lower force in rigor as compared to the contracting state.

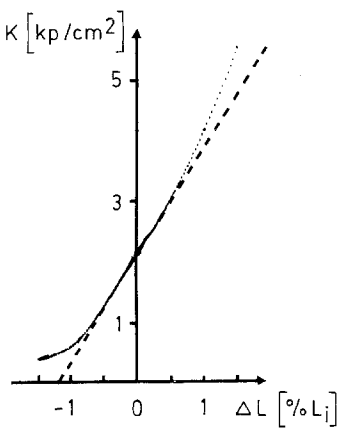

Fig. 5

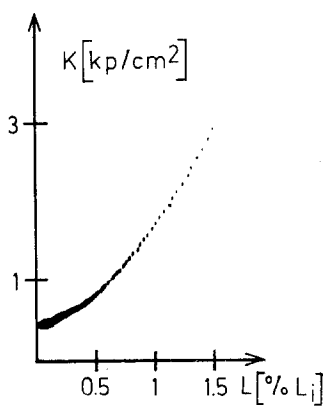

Fig. 6

Fig. 5. Force-length diagram of a contracting bundle of rabbit psoas fibres. The length is plotted in percent of the initial length L_i of the fibre before the length change. The force values and the corresponding length values are recorded during a single stretch and a single release from the same initial length. *Same conditions as in Figure 3*

Fig. 6. Force-length diagram of a rabbit psoas fibre bundle in rigor state. The length is plotted in percent of the initial length L_i of the fibre before the length change. The force values and the corresponding length values were recorded during a single stretch. *Conditions:* Five fibres; pH 6.7; $I = 0.1$ M; 25° C

To investigate this deviation in more detail the slope of the length-force curve (i.e. the slope stiffness of the fibre, cf. Simmons und Jewell, 1974) was measured (see Methods).

Figure 7 and 8 show such stiffness plots in case of a fibre bundle in contracting and in rigor state. It can be seen from Figure 7 that the stiffness of a contracting

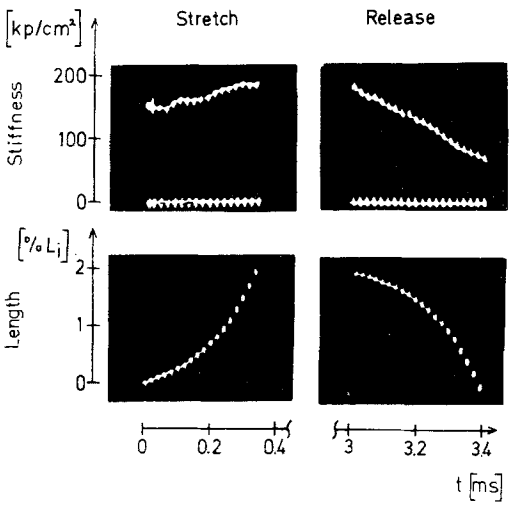

Fig. 7. Slope stiffness of a rabbit psoas fibre bundle in contracting state. In the upper trace the slope stiffness is plotted and in the lower trace the corresponding length change. *Conditions:* Isometric tension 1.6 kp/cm²; 15 mM MgATP; pCa 4.9; $I = 0.1$ M; 22° C

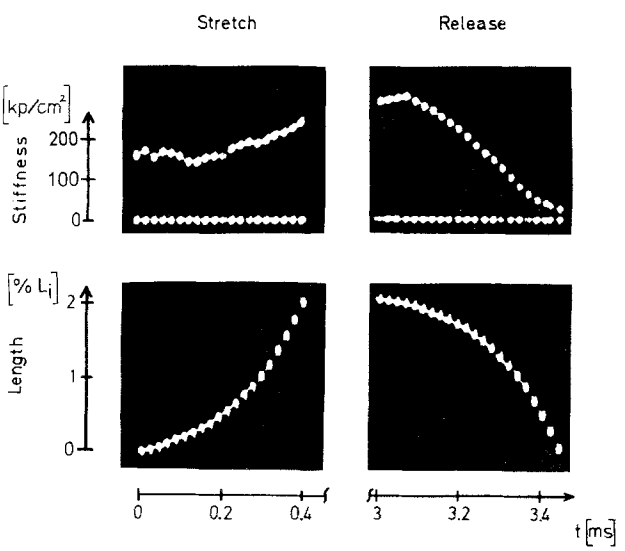

Fig. 8. Slope stiffness of a rabbit psoas fibre bundle in rigor state. In the upper trace the slope stiffness is plotted and in the lower trace the corresponding length change. *Conditions:* pH 6.7; $I = 0.1$ M; 22° C

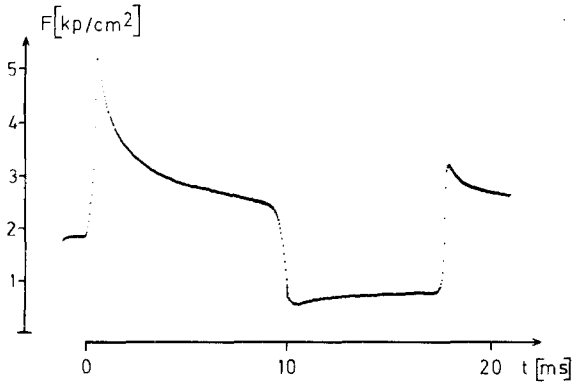

Fig. 9. Force transient of contracting rabbit psoas fibre bundle which was stretched and released to its initial length after 10 ms. The amplitude of the length change was 1.5% of the initial length of the fibre bundle. *Conditions:* 15 mM MgATP; pCa 4.9; $I = 0.1$ M; pH 6.7; 26° C

fibre bundle increases slightly during the stretch and decreases markedly during the subsequent release. But the values of the slope stiffness at the end of the stretch and at the beginning of the release are equal. Since the release was performed immediately after the quick phase it can be concluded that probably no change in the net number of attached cross-bridge occurs during the quick phase. (It should be mentioned that the large decrease of stiffness during 2% L_i release is difficult to interpret in terms of the number of attached cross-bridges, because the fibre might become slack.) Figure 8 shows the corresponding experiment for a fibre bundle in rigor state. As it can be seen from the figure the increase and decrease of the slope stiffness during stretch and release is even more pronounced than in contracting state. Furthermore, the stiffness at the beginning of the release seems somewhat higher than at the end of the preceding stretch.

Figure 9 shows a force transient of a contracting fibre bundle induced by a stretch and a release to the initial length performed 10 ms later. After another 10 ms the fibre was restretched again. It appears from the figure that a very pronounced quick phase is observed after the first stretch, whereas the second stretch of the same amplitude produces a smaller tension increase and only a weak quick phase. Furthermore the force was found to remain low after the release over the time of about 10 ms until another stretch was performed.

Discussion

As described previously (Fig. 4) a quick stretch was found to produce a nearly instantaneous increase of tension in phase with the length change (elastic reponse). This was followed by a quick decay of tension (quick phase) in which the tension readjusts within about 3 ms almost to the tension level before stretching. While the elastic phase was found to be rather similar in contraction and rigor, the quick phase is missing in ATP depleted fibres (rigor), as has been found previously by Heinl (Heinl et al., 1974).

Elastic Response

The length tension diagram recorded during the length change contains a fairly linear portion in the range −0.5 to +0.5% initial length (cf. also Huxley and Simmons, 1971). However, it is important to point out that stiffness does not remain constant during the entire length change of 1.5% initial length. This finding may account for a truncation of the quick phase caused by quick tension recovery due to cross-bridge rotation as predicted by the Huxley and Simmons model. However, this seems to be unlikely since the effect is even more pronounced in rigor state, where no quick tension recovery takes place.

But the very similarity of stiffness measurements in rigor and in contracting state may indicate a formation of rigor linkages due to incomplete saturation with ATP. However, this possibility seems unlikely because of the observation that the stretch induced force transient after the elastic phase decays very quickly and almost completely to the initial value before the stretch. Rigor linkages would have been not discharged after the elastic phase (cf. Fig. 4). If therefore rigor linkages are present, no complete recovery in force is expected during the quick phase.

It seems furthermore unlikely that viscous phenomena are responsible for the stiffness changes, since then the stiffness should increase during both stretch and release. Likewise, a non-Hookean behaviour of the system would give rise to an increase in stiffness during stretch equal to the decrease in stiffness during the subsequent release to the initial length. By process of elimination a third process may be considered to be the cause of changes in the stiffness observed during the length change: a rapid change in the number of attached bridges caused by very fast attachment and detachment processes. Evidence for this may be obtained more indirectly from analysing the fast tension transients following the elastic response.

Nature of the Quick Phase Induced by Large Stretches ($> 0.5\%\ L_i$)

The tension transients shown in Figure 3 are very similar to those obtained by Huxley and Simmons (1971) in tetanized frog muscle and obtained by Abbott and Steiger (1977) in glycerinated psoas fibres. These transients can be easily explained by the Huxley and Simmons model (1971).

In contrast the transient shown in Figure 9, which is produced by a large stretch ($1.5\%\ L_i$) followed by a subsequent release to initial length and another stretch ($1.5\%\ L_i$), does not admit of straightforward interpretation in terms of the Huxley and Simmons model.

As mentioned already, the rapid decrease in tension (quick phase) following the tension increase induced by quick stretch may be described in terms of a "backward" rotation of cross-bridges while they remain attached to actin (Huxley and Simmons, 1971). If cross-bridges indeed remain attached during the quick phase as supposed, the immediate stiffness ought to be the same just before and just after the quick phase. It was proved experimentally in this paper that this is so. However, a tension transient corresponding to a quick phase with constant stiffness might also be described in terms of a slippage of attached cross-bridges. Further experiments are necessary in order to test the cross-bridge rotation model extended to cross-

bridge slippage for large stretches as compared to the cross-bridge rotation model without slippage. The latter model would predict that a release of stretched fibres to the inital length should quickly restore the initial state of the cross-bridge population (to that before the quick stretch). Consequently the force ought to recover rapidly to the tension level it had before stretch. Furthermore another stretch should again produce a pronounced quick phase. In contrast to this expectation the force remains low at least for more than 10 ms after release: there is an obvious difference between the forces before and after the release. Moreover, only a weak quick phase is induced by the second stretch. Part of the tension loss might be due to cross-bridge detachment caused by the release. This possibility is supported by the observation that a restretch causes a smaller tension increment than the first stretch. (Since the stiffness at the beginning and at the end of the quick phase was found to be equal, the mentioned detachment of cross-bridges at the most can be induced by the release).

A second possible explanation for the missing quick phase following the second stretch is the formation of rigor linkage due to incomplete saturation with ATP: in rigor a quick phase is missing and tension would not be expected to recover after a quick release. However, the possibility of formation of rigor linkage even when the fibre is immersed in contraction solution was already excluded (see above). Since neither of the two possiblities accounts convincingly for the low force after release followed by a large stretch, a third mechanism must be considered e.g. that of Davies (1963) and Holmes (1977). A further attractive mechanism might be detachment of tension bearing cross-bridges and reattachment of the bridges at a new site, but in a non tension generating state (cross-bridge slippage) during the tension loss observed within the quick phase. As long as the total number of attached bridges remains constant, stiffness is not expected to change. This cross-bridge slippage would account for the marked loss in tension developed by a stretched and a subsequently released contracting fibre. Furthermore it explains that this loss in the tension occurs without stiffness change. The drastically reduced quick phase of the second stretch can be easily explained: the cross-bridges had already slipped during the first stretch. In contrast to the first stretch there is no need for further slippage during the second stretch, because the elastic elements of the cross-bridges were discharged in the meantime so that they are only restrained during the second stretch.

The non-constancy of the elastic modulus during a length change may indicate that the new equilibrium has not yet been established. However, such a cross-bridge slippage nevertheless implies a comparatively rapid establishment of equilibrium between attached and detached cross-bridges. Indeed White and Taylor concluded from biochemical measurements that the association process

$A + M\text{-}Pr \rightleftharpoons AM\text{-}Pr$

A = actin;

$M\text{-}Pr$ = myosin-product complex;

$AM\text{-}Pr$ = actomyosin product complex

is a rapid equilibrium. In the absence of ATP, the myosin-product complex is not formed and not present in muscle fibres so that the rapid equilibration reaction described cannot take place.

Appendix

Problems of Stiffness Measurements During Length Changes

In general, stiffness measurements on tissues entail some problems in interpreting the data obtained:

1. Viscosity of the Experimental Material: A viscous component may be involved in the elastic elements of the experimental material. In this case the stiffness data obtained will depend on the velocity of the applied length change.

2. Resonance Frequency of the Force Transducer: If the rise time of the force transducer is comparable to the duration of the length-step, it will influence the stiffness measurement.

3. Transmission Time of the Experimental Material: Because of the finite velocity of a sound wave of the experimental material, the length change at the one end of the experimental material will not immediately result in an "force response" at the other end.

In the following the three points mentioned are discussed in detail with respect to the parabolic shape of the length change as used in our measurements.

1. Viscosity of the Experimental Material

Since no experimental material with defined viscous and elastic properties was available for a test, some calculations were done to estimate the influence of viscous behaviour on our measurements. The results are shown in Figure A1. (It was assumed that the experimental material can be represented as a set up of a spring and a dashpot as shown in the inset of Fig. A1.)

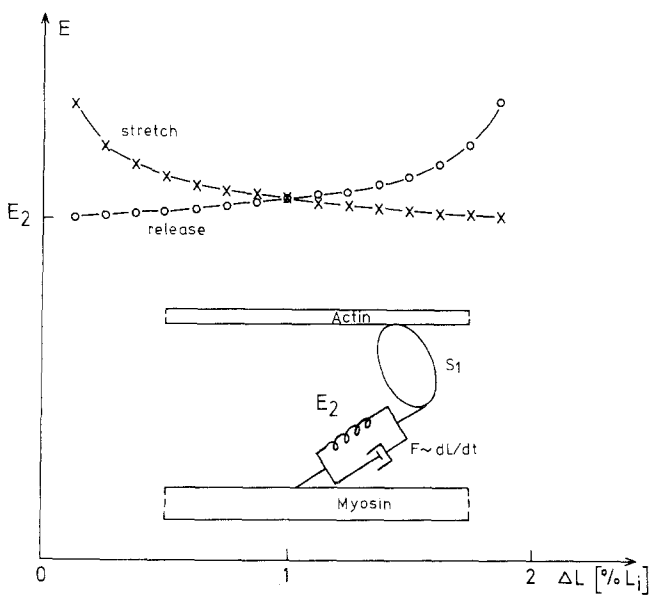

Fig. A1. Calculation of the influence of a viscous component within the cross-bridge elasticity. The plotted curves represent the stiffness, which would be recorded by the apparatus shown in Figure 1, if the elastic elements of the cross-bridges are damped by dashpots as shown in the inset of the figure

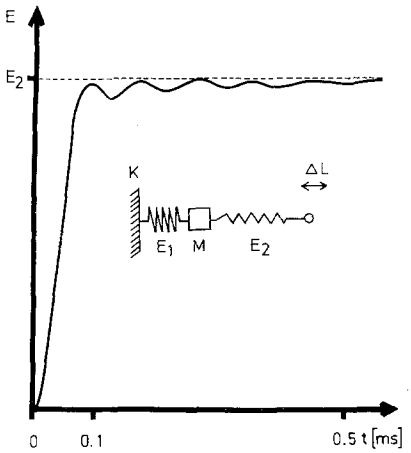

Fig. A2

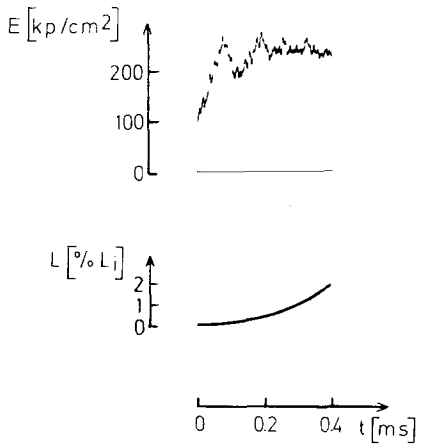

Fig. A3

Fig. A2. Calculation of the influence on the stiffness measurement of a limited resonance frequency of the force transducer. The force transducer is assumed to be a strong spring of the stiffness E_1 connected with a mass M at its end. The soft spring with a stiffness E_2 represents the muscle fibre, which is elongated by a certain amount ΔL at the end which is not fixed to the force transducer. The stiffness curve plotted shows the result which an apparatus as shown in Figure 1 should record according to calculation

Fig. A3. Stiffness measurement of a rubber band. The apparatus shown in Figure 1 was used to measure the stiffness of a rubber band. The upper trace represents the measured stiffness. The lower trace shows the corresponding length change

The most important feature of the calculated stiffness is the difference between the value at the end of a stretch and that at the beginning of a subsequent release. Furthermore the calculated stiffness decreases during stretch *and* during release.

2. *Resonance Frequency of the Force Transducer*

To estimate the influence of the force transducer, the system (force transducer and fibre) was assumed to be a harmonic oscillator composed of a stiff spring (the force transducer), a soft spring (the fibre) and of a mass.

When such a harmonic oscillator with a resonance frequency of 10 kHz is stretched by $\Delta L = \text{const.}\ xt.^3$ stiffness values are calculated as (dK/dL) plotted in Figure A2.

As can be seen from the figure, even after 0.1 ms, the "measured" stiffness differs only by less than 10% from the real stiffness of the spring S_2, i.e. the fibre, indicating that the inertia of the force transducer affects the measurement of stiffness (dK/dL) significantly only during the first oscillation period of the force transducer. Figure A3 shows in the upper trace the result of a stiffness measurement performed with a rubber band instead of a muscle fibre. The lower trace shows the corresponding length change. The stiffness measurement of the rubber band shows a behaviour very similar to that predicted by the calculation (cf. Fig. A2).

3. *Transmission Time of the Experimental Material*

If a sarcomere of a muscle fibre is thought of as Hookean spring with a mass at its end, the whole fibre may be thought of as a chain of such elements.

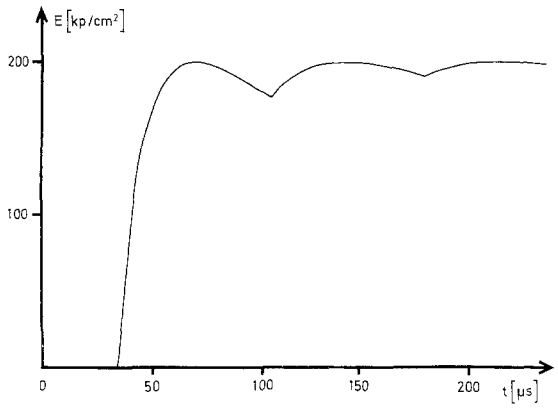

Fig. A4. Calculation of the influence of the transmission time on the stiffness measurement. The calculation is based on a fibre density of 1 g/cm^3, an elastic modulus of the fibre of 200 kp/cm^2 and a fibre length of 0.5 cm

The stiffness of a single link and its mass can be estimated from the "ringing frequency" of a fibre (i.e. the resonance frequency of the fibre), its density (1 g/ml) and the sarcomere length (2.5 µ). The ringing frequency was found to be approximately 10 kHz at 1 cm fibre length and was extrapolated to 20 kHz at the 0.5 cm fibre length. On this basis the force at the one end of the chain was calculated when the other end was stretched by $l(t) = \text{const.}\ xt^3$. Furthermore the resulting "measured" stiffness was calculated by differentiating force and length and dividing one differential by the other, as is done by the apparatus shown in Figure 1.

The result is shown in Figure A4. As one can see from the figure, the "measured" stiffness approaches the real value (E_0) nearly completely within 50 µ. The real value (E_0) is that expected without masses at the ends of the springs. It is interesting to note that the delay between the start of the length signal and the first rise of the recorded stiffness equals the transmission time, i.e. the time required by a sound wave to pass along the fibre.

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References

Abbott, R. H., Steiger, G. J.: Temperature and amplitude dependence of tension transients in glycerinated skeletal and insect fibrillar muscle. J. Physiol. (Lond.) **266,** 13–42 (1977)

Davies, R. E.: A molecular theorie of muscle constraction: calcium dependent contractions with hydrogen bond formation plus ATP-dependent extensions of part of the myosin cross bridges. Nature (Lond.) **199,** 1068–1074 (1963)

Eisenberg, E., Wayne Kielley, W.: Evidence for a refractory state of heavy meromyosin and subfragment-1 unable to bind to actin in the presence of ATP. Cold Spr. Harb. Symp. quant Biol. **37,** 145–152 (1972)

Ford, L. E., Huxley, A. F., Simmons, R. M.: Tension responses to sudden length change in stimulated frog muscle fibres near slack length. J. Physiol. (Lond.) **269,** 441–515 (1977)

Heinl, P., Kuhn, H. J., Rüegg, J. C.: Tension responses to quick length changes of glycerinated skeletal muscle fibres from the frog and tortoise. J. Physiol. (Lond.) **237,** 243–258 (1974)

Holmes, K. C.: The myosin cross bridge as revealed by structure studies. Int. Symp. Rottach-Egern/Tegernsee (eds. G. Rücker, et al.). Berlin-Heidelberg-New York: Springer 1977

Huxley, A. F.: Muscular contraction. J. Physiol. (Lond.) **243,** 1–43 (1974)

Huxley, A. F., Simmons, R. M.: Proposed mechanism of force generation in striated muscle. Nature (Lond.) **233,** 535 (1971)

Huxley, H. E.: Electron microscope studies of the organisation of the filaments in striated muscle. Biochim. biophys. acta (Amst.) **12,** 387–394 (1953)

Huxley, H. E.: The mechanism of muscular contraction. Science **164,** 1356–1366 (1969)

Julian, F. J., Sollins, K. R., Sollins, M. R.: A model for the transient and steady-state mechanical behaviour of contracting muscle. Biophys. J. **14,** 546–562 (1974)

Lymn, R. W., Taylor, E. W.: Mechanism of adenosine triphosphate hydrolysis by actomyosin. Biochemistry **10,** 4617–4624 (1971)

Podolsky, R. J., Nolan, A. C.: Muscle contraction transients, cross bridge kinetics, and the Fenn effect. Cold Spr. Harb. Symp. quant Biol. **37,** 661–668 (1972)

Reedy, M. K., Holmes, K. C., Tregear, R. T.: Induced changes in orientation of the cross bridges of glycerinated insect flight muscle. Nature (Lond.) **207,** 1279 (1965)

Simmons, R. M., Jewell, B. R.: Mechanics and models of muscular contraction. In: Recent advances in physiology (ed. R. J. Linden). Edinburgh-London: Churchill Livingstone 1974

White, H. D., Taylor, E. W.: Energetics and mechanism of actomyosin adenosine triphosphate. Biochemistry **15,** 5818–5826 (1976)

Yamamoto, T., Herzig, J. W.: Series elastic properties of skinned muscle fibres in contraction and rigor. Pflügers Arch. (in press)

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