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Length Dependent State of Activation – Length Change Dependent Kinetics of Cross Bridges in Skinned Insect Flight Muscle

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Abstract. Stretch induced activation and release induced deactivation of single glycerol-extracted insect flight muscle fibres were investigated.

The results are interpreted to indicate that the muscle length controls the number of acting cross bridges, whereas their attachment-detachment kinetics in mainly determined by the state of strain of the cross bridges. It is concluded that the net detachment rate of the cross bridges is enhanced if the muscle is released thereby "unloading" the cross bridges. This behaviour of the unloaded cross bridge is a basic postulation of most of the molecular muscle contraction models.

1. The delayed tension rise induced by stretches of different amplitudes could be restored to the level before the stretch by a release to the initial length.

2. The delayed tension decrease induced by a release of moderate (up to $\Delta L = 1.5\% \ L_i$) amplitude is quantitatively restored within the delayed increase induced by the restretch to the initial length.

3. Stiffness, which decreased during the delayed tension drop after release, is restored during a delayed stiffness increase effected by a restretch to the initial length.

4. The rate and the extent of the stiffness drop after release increased with increasing amplitude of the release and with increasing temperature.

5. After the deactivation, i.e., after tension and stiffness achieved a new steady level after the release, the attached cross bridges are already in the same state of strain as they were before the release. This finding is interpreted to indicate that within the deactivation phase *all* cross bridges attached prior the release are replaced by cross bridges attached after the release.

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6. The rate of tension and stiffness decay after release does not depend on the absolute muscle length but on the amplitude of the release which induced the deactivation.

Key words: Insect-fibrillar muscle – Cross bridge kinetics – Actin-Myosin interaction – Length dependent activation of muscle

Introduction

The force generated as well as the ATPase activity of a glycerinated contracting insect flight muscle fibre is not only a function of the composition of the incubation solution but also a function of the fibre length (Jewell and Rüegg 1966; Rüegg et al. 1970; Rüegg 1972; Pybus and Tregear 1973; Herzig and Herzig 1974; Abbott and Steiger 1977; Breull et al. 1973; Steiger 1977). The order of magnitude of the length change necessary for full activation or deactivation is only a few percent of the initial muscle length. The phenomenon can therefore not be understood on the basis of the different degree of overlap of the actin and myosin filaments at the different lengths. Particularly since this property of the muscle is to a smaller extent also observed in heart muscle (Steiger 1971, 1977; Bozler 1972) and in skeletal muscle (Heinl 1972; Steiger 1977; Kawai 1979), it is of interest to investigate the nature of the phenomenon in detail (for review see Pringle 1977).

A first theoretical approach to explain the phenomenon of stretch activation and release deactivation was given by Thorson and White (1969). The authors proposed an increasing attachment rate of myosin cross bridges to the actin filament with increasing strain in the myosin filament or with increased displacement between actin and myosin filaments. In insect flight muscle the Z-disc and the myosin filament are assumed to be connected by an additional filament (so called C-filament; Auber and Couteaux 1963; Pringle 1977) which affects the observed high stiffness of the muscle in the relaxed state. When the muscle is elongated, the C-filament strains the myosin filament. On the basis of the Thorson and White model this strain effects the observed, enhanced activation of the muscle as a function of the muscle length. The experimental evidence reported in this paper leads also to the conclusion that the state of activation depends on the muscle length. However, it could be shown that the mechanism of the activation is not based on an increased attachment or detachment rate of cross bridges in the stretched muscle but rather to consist in a recruitment of a larger number of acting cross bridges with unchanged kinetic properties (cf. also Wray 1979).

Methods

Preparations

All investigations reported in this paper were done on glycerol-extracted fibres of the dorsal longitudinal muscle of Lethocerus maximus. The muscle was

extracted in 50% glycerol solution at pH 7 (cf. Jewell and Rüegg 1966) while still attached in the thorax. After extraction for 24 h the muscle was stored in 50% glyerol solution containing 2 mM EGTA at pH 7. From this preparation single fibres were prepared and fixed between a length change generator and a force transducer. To prevent disturbance of the measurement by the limited transmission time of the fibre (cf. Güth and Kuhn 1978) the distance between length change generator and force transducer was about 3–5 mm. For information about fibre mounting see Güth et al. (1979).

Solutions

In order to remove the glycerol of the storage solution from the fibre it was washed for ca. 5 min after attachment between the length change generator and the force transducer in rigor solution containing 60 mM KCl, 1 mM NaN_3, 20 mM imidazole, 4 mM EGTA at pH 6.7. After washing, the fibre was transferred into relaxing solution containing 7.5 mM ATP, 8.5 mM $MgCl_2$, 18 mM KCl, 1 mM NaN_3, 20 mM imidazole, 4 mM EGTA at pH 6.7. In the relaxed state the fibre was prestretched until it attained ca. 20 μN passive tension. Then the fibre was transferred into contracting solution containing 7.5 mM ATP, 8.5 mM $MgCl_2$, 10 mM KCl, 1 mM NaN_3, 20 mM imidazole, 4 mM CaEGTA at pH 6.7. All solutions contained 5 mM Phosphoenolpyruvate and 50 U/ml Pyruvate Kinase as an ATP regenerating system.

Mechanical Equipment

The cross bridges between actin and myosin are believed to change their mechanical state very rapidly if the length of the muscle fibre is changed. Under certain conditions this change can take place within fractions of 1 ms (Huxley and Simmons 1971; Kuhn et al. 1979). The measurement of muscle stiffness in order to determine the total number of attached cross bridges (cf. Huxley and Simmons 1973) consequently requires very rapid length changes and a force determination with a corresponding high temporal resolution.

Length Change Generator: The basic instrument of the length change generator is a Ling Dynamics 101 vibrator. The vibrator is fed by a feedback circuit controlling a power amplifier. As a consequence of the feedback circuit ramp shaped length changes could be applied within 0.5 ms (see Fig. 1). The length is measured by an integrated fieldplate arrangement supplied by Siemens (Type FP 210), which detects the displacement of a small iron plate fixed at the moving part of the length change generator.

Force Transducer: The force was measured by a semiconductor transducer supplied by Aksjeselskapet Mikro-Elektronikk (Type 801). The silicon pin of the transducer was carefully pointed and reduced in length in order to remove mass thereby enhancing the resonance frequency. The resonance frequency

achieved was higher than 25 kHz. The electrical noise superimposed on the force signal was equivalent to ca. 3 μN. The force transducer was protected from water by covering the sensitive parts of it with grease.

Stiffness Measurement

The changes of stiffness after a rapid length change administered to the muscle fibre had to be measured as a function of time. For this purpose a sinusoidal length change with a frequency of 2.5 kHz and an amplitude up to 10 μm was superimposed to the ramp shaped length change. The duration of the ramp was increased to ca. 0.8 ms for technical reasons (saturation of the feedback), if a

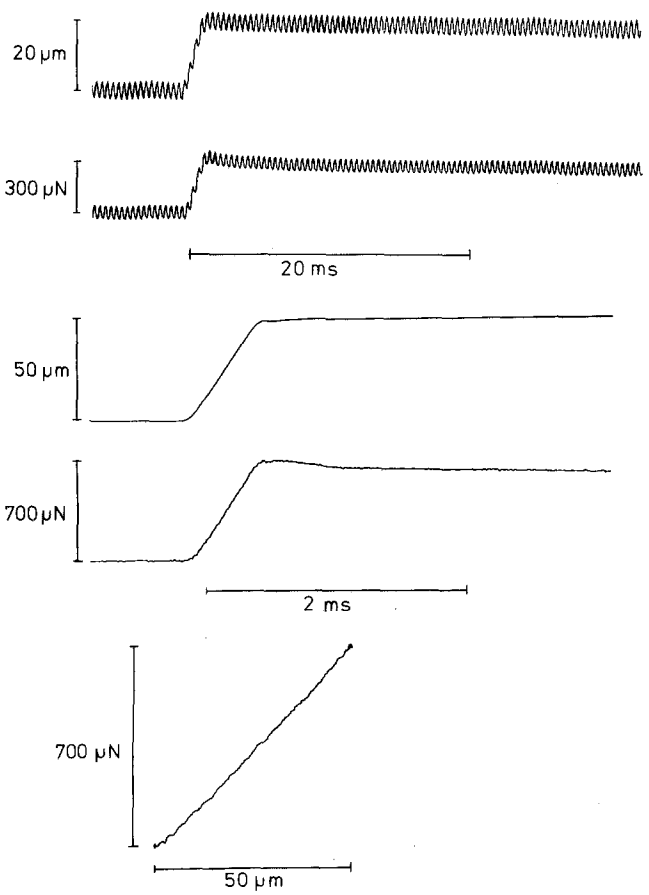

Fig. 1. Measurement of stiffness. The measurements are obtained from a rubber string fixed between the length change generator and the force transducer. *Upper trace:* Length signal with a sinusoidal length change superimposed. *Second trace:* Corresponding force transient. *Third trace:* Length signal with a higher time resolution and without sinusoidal superimposition. *Fourth trace:* Corresponding force signal. *Lowest part of the figure:* Length-tension diagram obtained plotting the length signal of trace three versus the force of trace four

sinusoidal length change was superimposed. Figure 1 shows such an experiment performed with a rubber string fixed between length change generator and force transducer. The upper trace shows the length, the second trace the corresponding force. The stiffness can be calculated from the ratio of the sinusoidal force amplitude and the sinusoidal length amplitude.

In order to estimate the number of attached cross bridges the sinusoidal superimposition technique might present a problem if the release amplitude is larger than that necessary to unload the cross bridge tension completely. Therefore another technique was applied for release amplitudes of 1% L_i and more: The length-tension diagram was determined during the length change. Using a rubber string in order to check the linearity of the system, such a relationship obtained during a stretch is shown in the lowest part of Fig. 1. The corresponding length and force transients are presented in the middle part of the figure. It can be seen that the length-tension diagram is linear except for a short period at its beginning, where the slope is lower. This very early phase in the curve is due to the limited resonance frequency of the force transducer, or – if the distance between length change generator and force transducer is large – to the transmission time[1] caused by the limited velocity of the force signal moving along the fibre (cf. Güth and Kuhn 1978). For the determination of the stiffness the slope of the length-tension diagram must consequently be taken after the kink, i.e., in the linear part of the curve.

To obtain the stiffness an adjustable time after the release a restretch was performed in order to get a length-tension diagram starting from the isometrically contracting state and to get another one starting an adjustable time after the release. Such diagrams are shown in the lower part of Figs. 3 and 4. The curves were obtained during the length changes of the release-restretch cycles shown in the upper parts of the figures. The numbers indicate the curves corresponding to the release (1) and the restretch (2). The stiffness was taken from the slope of the steepest part of the curves (this procedure will be justified in the "Results" section).

The Problem of Zero Tension

The zero tension of the force transducer, which is immersed into the incubation solution, is rather strongly affected by the surface tension of the liquid. This influence on the zero tension can in general not be eliminated by calibration of the system before the experiment. This is because the correction is too sensitive to the shape of the liquid surface, which can change during the experiment for several reasons. Therefore zero tension is detected in all experiments (except those shown in Figs. 3 and 4), where absolute tensions are given, by releasing the fibre until it becomes slack. The corresponding tension signal was taken to be zero tension. In order to ensure that the fibre was really slack, the amplitude of the release was increased until no further change in the tension after release was

1 The order of magnitude of the transmission time in a 0.5 cm long fully activated muscle fibre is several 10 μs

observed. To eliminate thermal drifts, too, zero tension was determined immediately after or before the fibre was activated (see below).

Activation of the Fibre

The insect flight muscle incubated in contracting solution (containing Ca^{2+}) is only partly activated. Full activation is achieved if the Ca^{2+}-activated fibre is stretched by an amount of 1–2% L_i. Such an additional stretch induced activation is demonstrated in Fig. 2. The upper trace shows the length signal, the lower trace the corresponding force. Zero tension is measured as described above.

The Passive Stiffness and Tension

Passive Stiffness: An insect flight muscle which is relaxed in the absence of Ca^{2+} holds its stiffness to a certain degree. This passive stiffness has to be subtracted from the measured stiffness in the activated state (White et al. 1977) in order to determine the contribution from straining the C-filament. The resulting difference which is assumed to reflect the actin-myosin cross-links, will be called "active stiffness". To obtain the passive stiffness the experiment done in the active state is repeated in the relaxed state.

Passive Tension: Because of the existence of a passive stiffness there exists in general a passive force. Analogous to the "active stiffness" an "active tension" is defined as the difference of the tension measured in the relaxed and in the activated state.

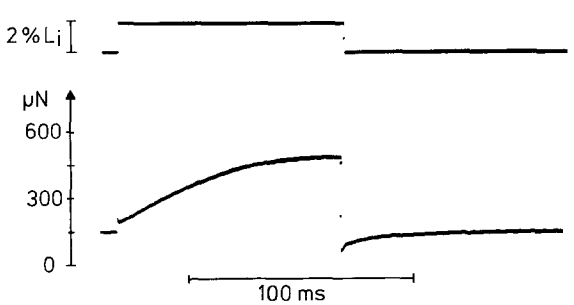

Fig 2. Stretch activation and release deactivation. A single glyerinated insect flight muscle fibre was stretched in order to activate it and released to the initial length in order to deactivate it. The upper trace shows the length change, the lower trace the corresponding force transient. Temperature: 24° C

Results

Activation and Deactivation[2] *by Stretch and Release*

Figure 2 shows the force transient of a single insect flight muscle fibre which is stretched with an amplitude of ca. 2% of its initial length (L_i). A delayed tension increase induced by the stretch is observed. The tension level reached exceeds markedly the isometric tension of the unstretched fibre (cf. also Thorson and White 1969; White and Thorson 1972; Pringle 1977). When the tension had nearly reached its maximum value the fibre was released to its initial length. The

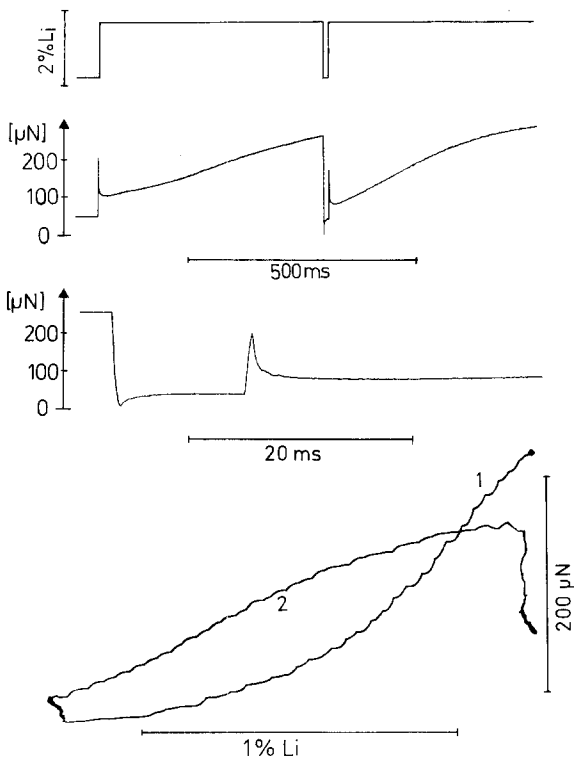

Fig. 3. Complete deactivation 12 ms after fibre release. The upper trace shows the length change, the second trace the corresponding force transient. The third trace shows the section of the force transient which corresponds to the release restretch cycle at a higher time resolution. The lowest part of the figure shows the length-tension diagram corresponding to the release (1) and the restretch (2) shown in trace three. Zero tension was extrapolated by extrapolating the length-tension graph. T = 10° C

2 In the following the term "activation" of the muscle fibre will synonymously be used for an increase in stiffness *or* tension. The term "deactivation" will be used analogously. This simplification in the presentation may be allowed since the processes discussed in this paper have the special property that force and stiffness always changes in the same direction (without being exactly in parallel)

activation which is induced by the stretch seems to be cancelled by the release.

In the upper part of Fig. 3 it is demonstrated that the activation which is cancelled by the release, can be restored by another stretch to the length before the release.

Time Requirement of Deactivation

Although the force drops immediately after the release to a nearly constant low level (cf. third trace in Fig. 3) the fibre is not completely deactivated immediately after the release. This is demonstrated in Figs. 3 and 4. Whereas in the experiment reported in Fig. 3 the delay between the release and the restretch of the fully activated fibre was 12 ms, in Fig. 4 the delay in the analogous experiment was only 1 ms. The time interval of 1 ms, during which the fibre was in the released state, seems not to be sufficient to abolish the activation

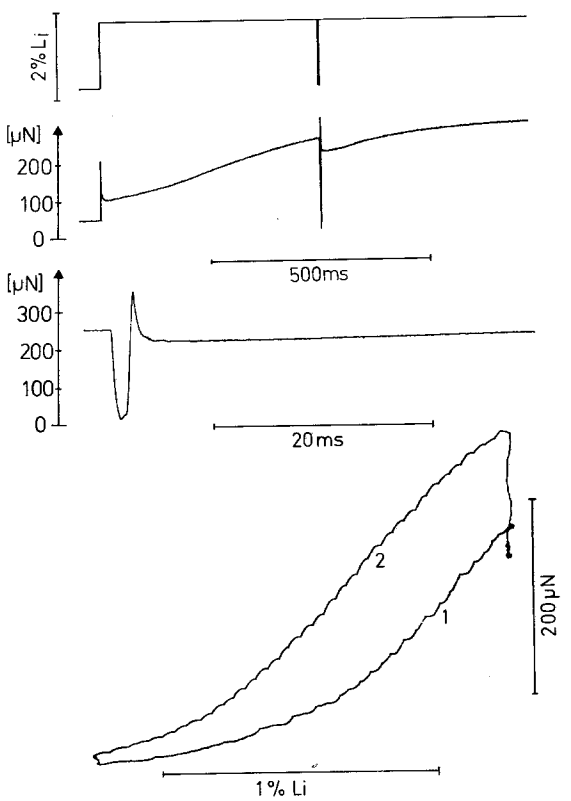

Fig. 4. Incomplete deactivation 1 ms after fibre release. The upper trace shows the length change, the second trace the corresponding force transient and the third trace the section of the force transient which corresponds to the release-restretch cycle at a higher time resolution. The lowest part of the figure shows the length-tension diagram corresponding to the release (1) and to the restretch (2) shown in trace three. Temperature: 10° C

completely: The force after the restretch is nearly as high as it was before the release (compare also the third traces in Figs. 3 and 4).

Deactivation by Release Depends on the Amount of Activation by the Preceding Stretch

In order to improve the evidence that the deactivation after release matches quantitatively the activation after stretch, we first stretched a fibre thereby inducing a delayed tension rise and subsequently deactivated it at different times, i.e., at different levels of the activation. The result is shown in Fig. 5. It can be seen that the force, which increased after stretch, decreased again after the subsequent release nearly to the level before the stretch. (Note the small stretch and release amplitudes which cause a slower delayed decrease in tension than caused by the larger releases shown in Figs. 3 and 4.) Obviously the degree of deactivation depends on the extent of the stretch induced activation: The more the tension is increased after the stretch, the larger is the amplitude of the delayed decrease in tension after the release.

To get more information on the nature of the deactivation induced by fibre release, we investigated stiffness and tension development after releases of different amplitudes.

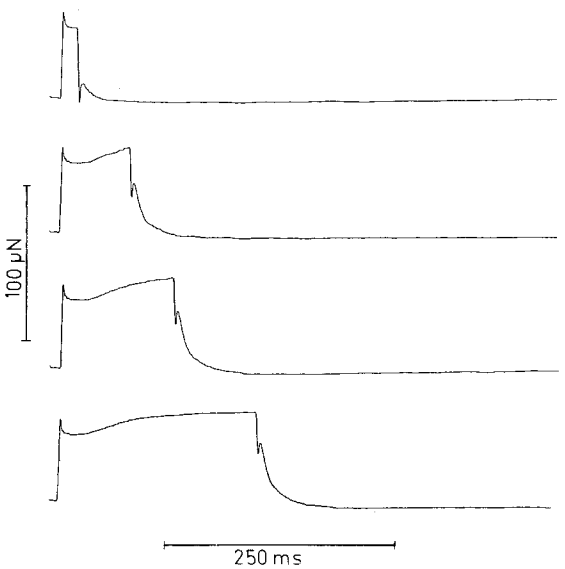

Fig. 5. Deactivation induced at different extents of activation. A single glycerinated insect flight muscle fibre was activated by a 0.25% stretch and at different extents of activation deactivated by a release to the initial length. $T = 24°$ C. *Note:* The amplitude of the immediate tension fall during release becomes smaller with increasing delay between stretch and release. This does *not* indicate a decreasing stiffness but rather a more and more dominant truncation effect. The stiffness obtained from length-tension plots or sinusoidal superimposition *increases* with increasing delay between stretch and release

Delayed Tension Decrease After Release of Different Amplitudes

Figure 6 shows force transients obtained from a fibre which was activated by a 1.5% L_i stretch and which was subsequently released from the peak tension with different release amplitudes. (The release amplitudes are noted on the corresponding force transients.) Three phases of the force transient can be clearly distinguished:

1. The elastic phase during which the tension drops in phase with the applied length change.

2. The quick recovery phase during which the force partly recovers towards the level before the release.

3. The delayed deactivation phase during which the force again decreases.

The amplitude of the deactivation phase depends largely on the release amplitude and has a maximum in the range of 0.4% L_i release amplitudes. Since the force transients obtained from the relaxed muscle only show rather small changes in force after the length change is finished (cf. Fig. 17), the active force behaves qualitatively the same. The rate constants of the tension decrease during the deactivation seem to increase with increasing release amplitudes.

Stiffness During Deactivation

In order to get information about the number of attached cross bridges during the release induced deactivation phase, we measured the stiffness transient after releases of different amplitudes.

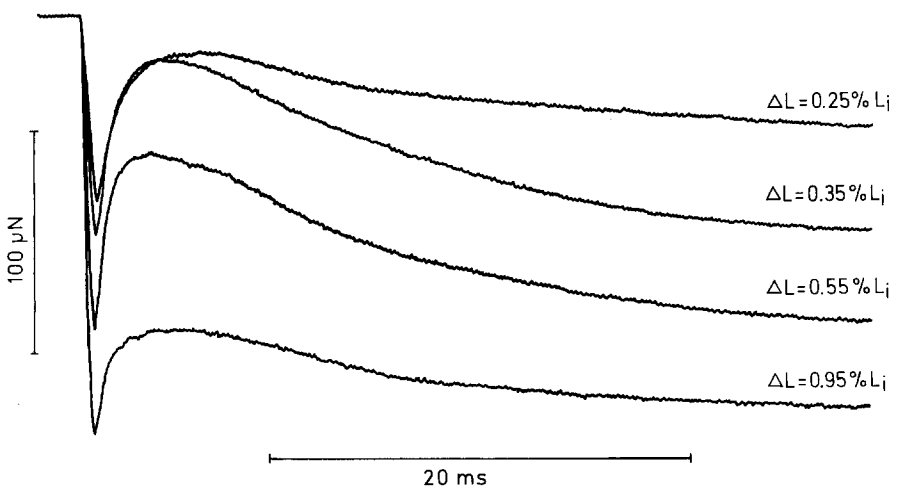

Fig. 6. Deactivation after releases of different amplitudes. A single glycerinated insect flight muscle fibre was activated by a 1.5% L_i stretch. After full activation was achieved, releases of different amplitudes were applied. The induced force transients of the releases are plotted. The corresponding length changes are noted at the transients. T = 10° C. Note that these force transients are obtained from the same fibre as the stiffness transients in Fig. 8

Stiffness by Sinusoidal Superimposition: It proved to be most satisfactory to measure stiffness *transients* during the deactivation after release by a sinusoidal length change superimposed on the stepwise length change of the deactivation inducing release (see also "Methods"). The advantage of this method is that a complete stiffness transient is obtained from one release experiment. The force transient of such an experiment is shown in Fig. 7 trace "c". The stiffness transient of the fibre is calculated by dividing the amplitude of the sinusoidal component of the length signal by the sinusoidal component of the force signal. To get the "active stiffness" (see "Methods") the stiffness of the relaxed muscle (from trace "a" in Fig. 7) was subtracted. The result – the active stiffness

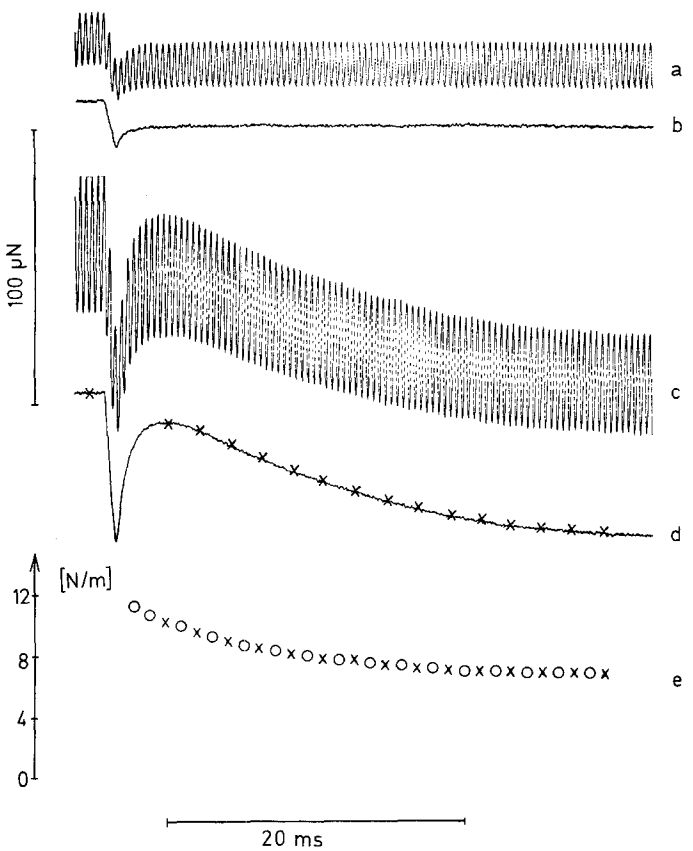

Fig. 7a–e. Stiffness transient during deactivation. A single glycerol-extracted muscle fibre of the DLM of Lethocerus maximus was – after activation by an 1.5% L_i stretch – rapidly released with an amplitude of 0.35% L_i. The force transient induced by the release is shown in trace **b** for the fibre in the relaxed state and in trace **d** for the fibre in contracted state. The traces **a** and **c** show the corresponding transients with sinusoidal superimposition on the length signal of an amplitude of 0.2% L_i (frequency 2.5 kHz). The crosses in trace **d** correspond to the mean of the upper and lower force peaks of trace **c**. Trace **e** shows the "active stiffness" transient calculated from the amplitude of the sinusoidal component in the traces **a** and **c** (see "Methods"). The crosses correspond to the measured values, the circles to the best exponential fit. T = 10° C

transient – is plotted in trace "e" of Fig. 7 (the crosses give the measured values, the open circles the best exponential fit).

Disturbance of the Fibre State by Sinusoidal Superimposition: The amplitude of the superimposed sinusoidal length change (0.1%–0.2% L_i) is of the same order of magnitude as that of the ramp-shaped release of the fibre (0.2%–1.0% L_i). Therefore it must be shown that the state of the fibre is unaffected by the superimposed length changes:

1. In some experiments the amplitude of the sinusoidal length change was reduced from 0.2% to 0.1% L_i. Neither time constant nor amplitude of the obtained stiffness transient was thereby changed.
2. The fibre was released with and without superimposition of a sinusoidal length change. The force transient without superimposition did not differ from the force transient obtained from the mean of the upper and lower force peaks of the force transient with superimposition: The force transient without superimposition is shown in trace "d" of Fig. 7. The crosses in the transient represent the mean of the upper and lower peaks of the transient shown in trace "c". This finding indicates that the process effecting the deactivation is not disturbed by the sinusoidal superimposition.
3. For large releases the signal to noise ratio was sufficiently high to determine the stiffness transient from the analysis of the length tension diagrams of a restretch following an adjustable time after the release (see "Methods"). The stiffness transient obtained by this method was very similar to that obtained from the sinusoidal superimposition method (see also Fig. 10 where rate constants of the stiffness transient of both methods are plotted).

Stiffness by Length-Tension Diagrams: The stiffness measurement by sinusoidal superimposition presents problems after large releases during which the cross bridges are completely unloaded. Therefore after large releases the stiffness transient was determined from the length-tension diagrams obtained during the releases itself and during a subsequent restretch which was performed at a variable time after the release. Such length-tension diagrams are shown in the lower part of Figs. 3 and 4. The curves obtained are not straight lines and consequently the question arises as to which part of the curve should be analysed for the determination of the stiffness as a measure of the number of attached cross bridges. Since the stiffness of the muscle is assumed to be mainly located in the cross bridge elasticities (Ford et al. 1977), the muscle becomes slack when the cross bridges become slack. This is probably the reason for the drastically decreasing slope of the curve at the end of the release as is shown in Figs. 3 and 4. Consequently, the slope stiffness[3] detected during a release must be taken from the early part of the curve. On the other hand at the very beginning of the curve the measurement will be disturbed by the transmission time as discussed in "Methods". Therefore the slope stiffness was taken from the steepest part of the curve where it is not yet affected by slack cross bridges and no longer falsified by the effect of transmission time.

3 Stiffness detected during the length change by plotting length versus tension

The curve of the length-tension diagram (Fig. 4) which corresponds to the restretch 1 ms after the release is rather similar in shape to the curve of the release. The similarity and the parallel shift may indicate that the cross bridges which were unloaded during the release changed the state of strain of their elastic elements and are then restrained by the subsequent restretch. The slightly decreasing slope of the curve at the end of the restretch may be caused by the onset of slippage of cross bridges at these lengths (cf. Güth et al. 1979 and see also below "The State of Muscle After Deactivation"). Consequently the slope stiffness obtained during the restretch 1 ms after the release was also taken from the steepest part of the curve.

The length-tension diagram obtained during the restretch 10 ms after the release (curve 2 in Fig. 3) shows at its beginning the typical kink brought about by the transmission time. In the later part of the curve the slope starts to drop beginning at a relative length of ca. 1% L_i. It will be concluded in "The State of Muscle After Deactivation" that this decrease in the slope is probably effected by cross bridge slippage. Therefore the slope stiffness was taken from the part of the curve not yet affected by cross bridge slippage and not any longer by the transmission time, i.e., again from the steepest part of the curve.

A stiffness transient obtained as described above after the release of 2.4% L_i is shown in Fig. 9. Note that stiffness, not "active stiffness" is plotted (cf. "Methods")[4].

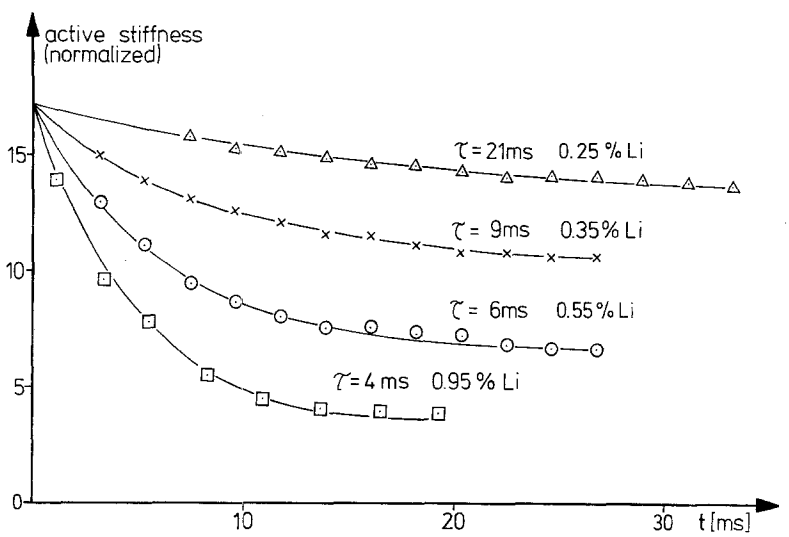

Fig. 8. Stiffness transients induced by rapid release. Single glycerinated insect flight muscle fibres were activated by a 1.5% L_i stretch. When they were fully activated they were rapidly released. The stiffness transients, after the release are plotted. The release amplitude was 0.25% L_i (△), 0.35% L_i (×), 0.55% L_i (○), and 0.95% L_i (□). The solid line represents the best exponential fit. The time constants are noted at the curves. T = 10° C. Note that these stiffness transients are obtained from the same fibre as the force transients shown in Fig. 6

4 The corresponding force transient is not shown since very large release amplitudes result in a more or less rectangular character of the corresponding force transient (cf. Figs. 2 and 3)

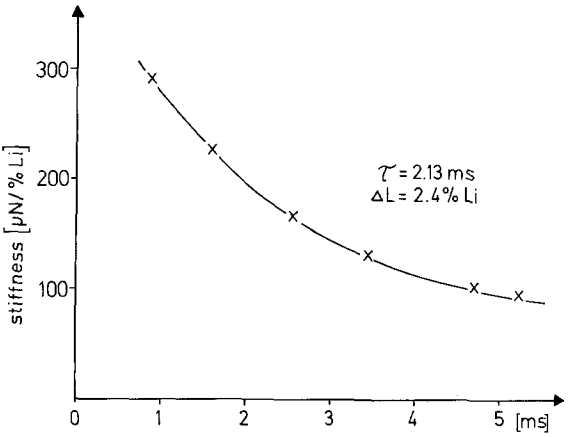

Fig. 9. Stiffness transient after large release. The release amplitude was 2.4% L_i. The stiffness was obtained from the analysis of length-tension diagrams (cf. "Methods"). The solid line corresponds to the best exponential fit. T = 10° C

Time Course[5] of Stiffness After Release

Figure 8 shows the stiffness transient after release for four different release amplitudes. The solid line corresponds to the best single exponential fit.

As can be seen from the figure a single exponential fits the data obtained rather well. The time constants and the amplitude of the exponentials are distinctly different for the four release amplitudes, indicating that the "process of deactivation" is faster and more complete after a large than after a small release.

In Fig. 10 the rate constants of the stiffness loss after release obtained from different fibres (of the same animal) and with different methods (sinusoidal superimposition and length-tension diagrams, see "Methods") are plotted[6]. It can be seen from the figure that – except for the smallest release amplitude – neither different methods of stiffness detection nor different fibres (from the same animal) result in a markedly different time constant for a given release amplitude and a given temperature.

The Difference Between Force and Stiffness Transient

In Fig. 7 the force transient induced by a release is shown in trace "d" and the corresponding stiffness transient in trace "e". Obviously the force does not decrease in parallel with the stiffness: This is a typical observation[7]. In many

5 It has to be noted that all the time courses given in this paper depend rather strongly on the animal from which the muscle was taken. Consequently all comparisons between time constants at different conditions are in this paper based on results obtained from muscle fibres of the same animal

6 The corresponding rate of the force decay could not be given because of the often observed not single exponential character of the force transients (see next chapter)

7 This feature is much more distinct if the contraction solution contains ADP (~ 2 mM): the force transient shows then very often a plateau phase before it decreases, whereas the stiffness transient can again be fitted by a single exponential (unpublished results)

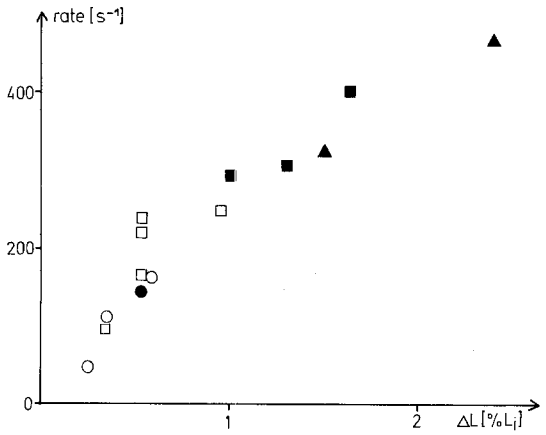

Fig. 10. Rate constant of stiffness decay after release. Single glycerol-extracted muscle fibres were activated by a 1.5% L_i stretch and subsequently deactivated by releases of different amplitudes. In the diagram the rates of the stiffness loss induced by different release amplitudes (ΔL) are plotted. The symbols (○, □, ●) show results obtained from three different fibres by sinusoidal superimposition (see "Methods"). The symbols (■, ▲) show the rates of stiffness loss evaluated from length-tension diagrams (see "Methods"). Temperature: 10° C

cases the force transient was furthermore clearly composed of two or even more exponentials whereas the corresponding stiffness could be rather accurately fitted by a single exponential. Therefore a direct comparison of the tension decay and the stiffness decay rate is difficult.

Whereas the amplitude of the stiffness transient increases continuously with increasing release amplitudes (see Fig. 8), the amplitude of the delayed tension decrease rather seems to have a maximum in the range of 0.4% L_i release amplitudes (see Fig. 6).

The Similar Character of Activation and Deactivation for Small Amplitudes of the Length Change

It is shown in Fig. 3 that a fibre which is deactivated by a release to the length before the initial activating stretch can be reactivated by a restretch. This is also demonstrated in Fig. 11: The fibre which was activated by a 1% L_i stretch immediately before the experiment was partly released by a 0.5% L_i length change which brought about the delayed tension decrease already described. The restretch to the length before the release restores the force within a moderate delayed phase of tension increase. The stiffness measured immediately before the release decreases – as already described – during the deactivation phase but increases to the level before the release during the reactivation induced by the restretch.

To summarize, it appears that the change in the state of activation induced by a length change can be quantitatively reversed by a length change which restores the initial muscle length (see also Fig. 5). However, in the examples mentioned

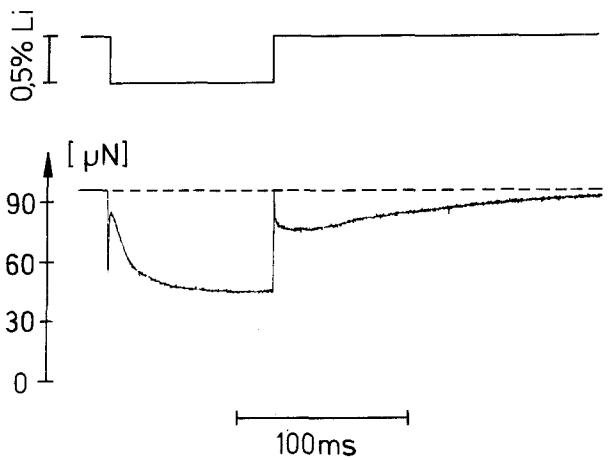

Fig. 11. Reversibility of deactivation. A single glycerol-extracted insect flight muscle fibre was at first activated by a 1.5% L_i stretch. The force transient shown is induced by a subsequent release of the fully activated fibre by a 0.5% L_i release. After the deactivation was completed the fibre was reactivated by a restretch to the length before the release. The upper trace shows the length change, the lower trace the corresponding force transient. The dashed line may indicate that the force recovers during the reactivation nearly to the level before the release induced deactivation. T = 10° C

above the time needed for the deactivation is obviously much shorter than the time needed for the activation. But this distinct difference in the deactivation and activation process becomes less pronounced the more the release-restretch amplitude decreases: We lowered the amplitude down to ca. 0.035% L_i. Since the amplitude of the corresponding force transient became extremely small it had to be averaged to improve the signal to noise ratio. Figure 12 shows the force transient induced by a "high" release-restretch amplitude (trace "a", 0.23% L_i) and by a "low" amplitude (trace "b", 0.035% L_i). A comparison of the time needed for deactivation and reactivation effected by large length changes is often difficult because the deactivation and reactivation phase are frequently rather different in shape: The upper trace in Fig. 12 shows a deactivation phase which is clearly composed of at least two exponentials (the crosses correspond to the best fit with time constants of 6 ms and 27 ms, the dashed line corresponds to the slower component only). On the other hand, in this particular case the reactivation phase could be fitted by a single exponential with a time constant of 80 ms (crosses in trace "a" of Fig. 12).

When the release-restretch amplitude is reduced the deactivation phase becomes in general more similar to the activation phase and in many cases more similar to a single exponential (cf. also Abbott and Steiger 1977). This tendency can be seen from the comparison of trace "a" and "b" in Fig. 12. (Note that the similarity of the activation and deactivation for small length changes is typical, while the single exponential character in the reported experiment may be accidental: It seems to be rather a particular property of the individual fibre under the special conditions used.) Figure 13 shows the time constants of tension change following release (×) and those following stretch (○) in an analogous

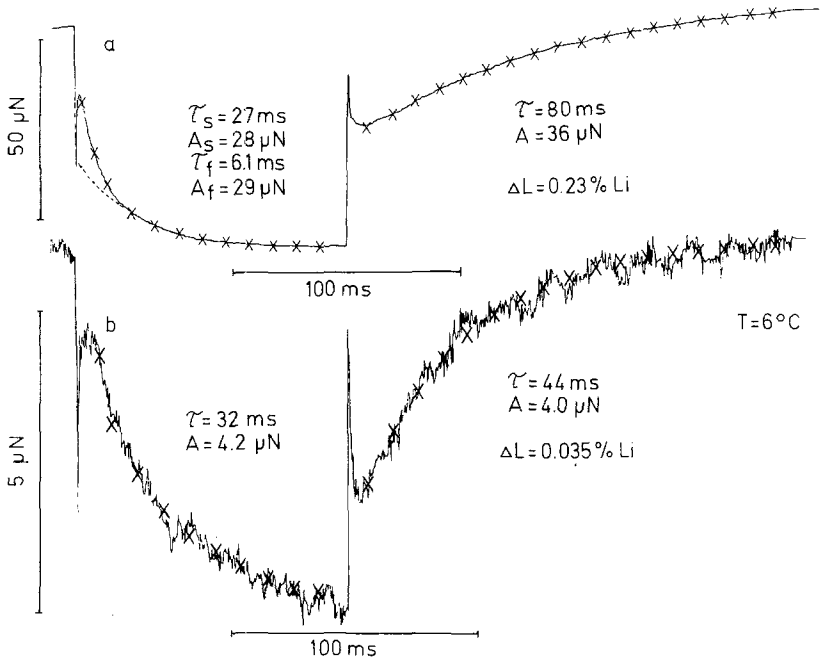

Fig. 12a and b. Similar time constant of de- and reactivation at low stretch-release amplitudes. A single fibre was first activated by a 1.5% L_i stretch and then released by a 0.23% L_i (trace **a**) or 0.035% L_i (trace **b**). The force transients shown start with the release. After the deactivation is completed the fibre was reactivated by a stretch to the length before the release. The crosses in the curve correspond to the best exponential fit for two exponentials (trace **a**) or one exponential (trace **b**) in case of the release and for a single exponential for the restretch. The amplitudes (A_s, A_f, A) and time constants (τ_s, τ_f, τ) are noted at the transient. The dashed line indicates the shape of the slower exponential after the release. In order to improve the signal to noise ratio the transients were averaged over 32 periods. $T = 6°$ C. Note that the transients shown in Figure 14 are from the same fibre

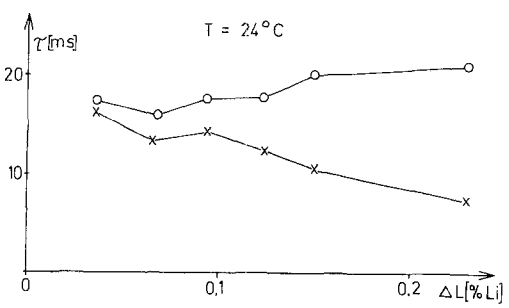

Fig. 13. Time constants of the deactivation (delayed tension fall) and the reactivation (delayed tension rise) in dependence on the amplitude of length change. Single glycerol-extracted fibres of insect flight muscle were deactivated by release and reactivated by restretch with different amplitudes of the lenght changes. The time constants of the deactivation (×) and the reactivation (○) are plotted versus the amplitudes of the length changes. $T = 24°$ C. The corresponding force transient of the lowest (trace b) and the highest (trace a) amplitude of the length changes is shown in Fig. 14

experiment to that shown in Fig. 12 performed at higher temperature (24° C). In case of the higher temperature the force transients due to all release-restretch amplitudes could in this particular case be fitted by single exponentials which had similar amplitudes but different time constants for deactivation and reactivation. As can be seen from Fig. 13 the difference between the time constants of deactivation and reactivation becomes smaller and smaller with decreasing release-restretch amplitudes.

The Effect of Temperature on Deactivation and Reactivation

Both the deactivation and the reactivation phase following a length change is accelerated with increasing temperature. As already noted the measured rates are occasionally different from animal to animal. Therefore only the data obtained from the same fibre are compared quantitatively.

The Q_{10} of the time constant of the stiffness transient after release is ca. 2.5. The force transient very often changed not only its velocity but also its shape, indicating that the force transient is effected by several processes with different

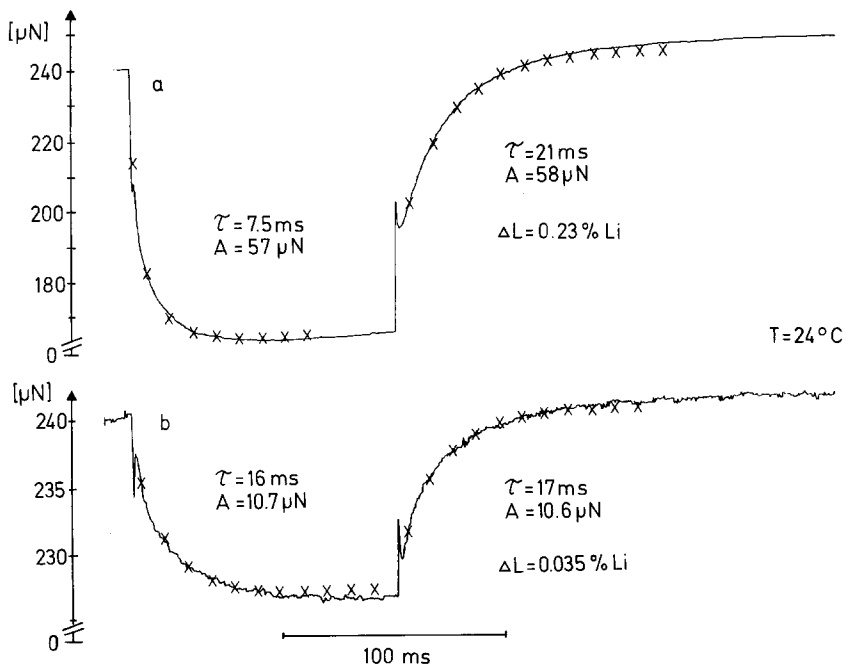

Fig. 14a and b. Similar time constant of de- and reactivation at low stretch-release amplitudes. A single fibre was first activated by a 1.5% L_i stretch and then released by 0.23% L_i (trace **a**) or 0.035% L_i (trace **b**). The force transients shown start with the release. After the deactivation is complete the fibre was reactivated by a stretch to the length before the release. The crosses in the curve correspond to the best exponential fit for two exponentials in case of the release and for a single exponential for the restretch. The amplitude (A) and time constant (τ) are noted at the transient. T = 24° C. Note that the transients shown in Fig. 12 are from the same fibre

Q_{10}. This can be seen from the comparison of the force transients shown in Figs. 12 and 14: Whereas the release and stretch induced transient corresponding to the higher temperature could be fitted fairly well by a single exponential (crosses in Fig. 14), the transient obtained after the larger release in Fig. 12 at lower temperature is obviously composed of more than one exponential (crosses: the best fit with two exponentials).

The State of the Muscle After Deactivation

It was recently shown (Güth and Kuhn 1978; Güth et al. 1979) that the crosslinks between actin and myosin rapidly detach and reattach, if the muscle is stretched with an amplitude larger than 0.5% L_i. The velocity of these detachment and reattachment processes depends largely on the amplitude of the stretch: At large stretch amplitudes the processes are so fast that they take place already during the length change. The cross bridge slippage occurs already *during* the stretch at stretch amplitudes of 1% L_i if the stretch velocity is ca. 30 muscle lengths per second. The stretches reported in the following to induce slippage have this velocity. Consequently, if slippage is observed during a stretch it is expected to occur at amplitudes of 1% L_i. Cross bridge slippage during the length change causes the length-tension diagram to reach a plateau or even to decrease at $\Delta L \sim 1\%\ L_i$. This phenomenon – interpreted to be caused by rapid detachment and reattachment of overstrained cross bridges – can consequently be taken to indicate the state of the cross bridge strain at the moment of stretch. This phenomenon was used to investigate the state of strain of the cross bridges after the release induced deactivation phase. The fibre was firstly activated by a 1.7% L_i stretch and was then – after the delayed stretch activation reached its maximum – deactivated by releases of different amplitudes. 20 ms after the release, i.e., at a time when the deactivation was certainly completed for all release amplitudes, the fibre was restretched with a 1.5% L_i stretch performed within 0.5 ms (corresponding to a stretch velocity of 30 muscle lengths per second). The procedure is demonstrated in Fig. 15. The upper trace shows the length signal with the activating initial stretch ΔL_1 of 1.7% L_i, the *variable* release ΔL_2 and the restretch ΔL_3 of *constant* amplitude (ΔL_3 = 1.5% L_i). The corresponding force transient is plotted in the lower trace. The time between release ΔL_2 and restretch ΔL_3 was 20 ms. The length-tension diagrams obtained during the restretches (ΔL_3) are shown in Fig. 16A in the contracted and in Fig. 16B in the relaxed state of the muscle. The different curves correspond to the different amplitudes of the release ΔL_2. In Fig. 16C the difference curves between Fig. 16A and B are plotted in order to get the "active" length-tension diagram. All length-tension diagrams (starting from different lengths corresponding to the different release amplitudes ΔL_2 and also starting from different forces) are shifted so that the starting points of all curves coincide.

It can be seen from the "difference" length-tension diagrams (part C in Fig. 16) that independent of the amplitude of the preceding release ΔL_2, all length-tension plots obtained during ΔL_3 are bent to a plateau at the same relative length change of ca. 1% L_i. This is the same length at which the

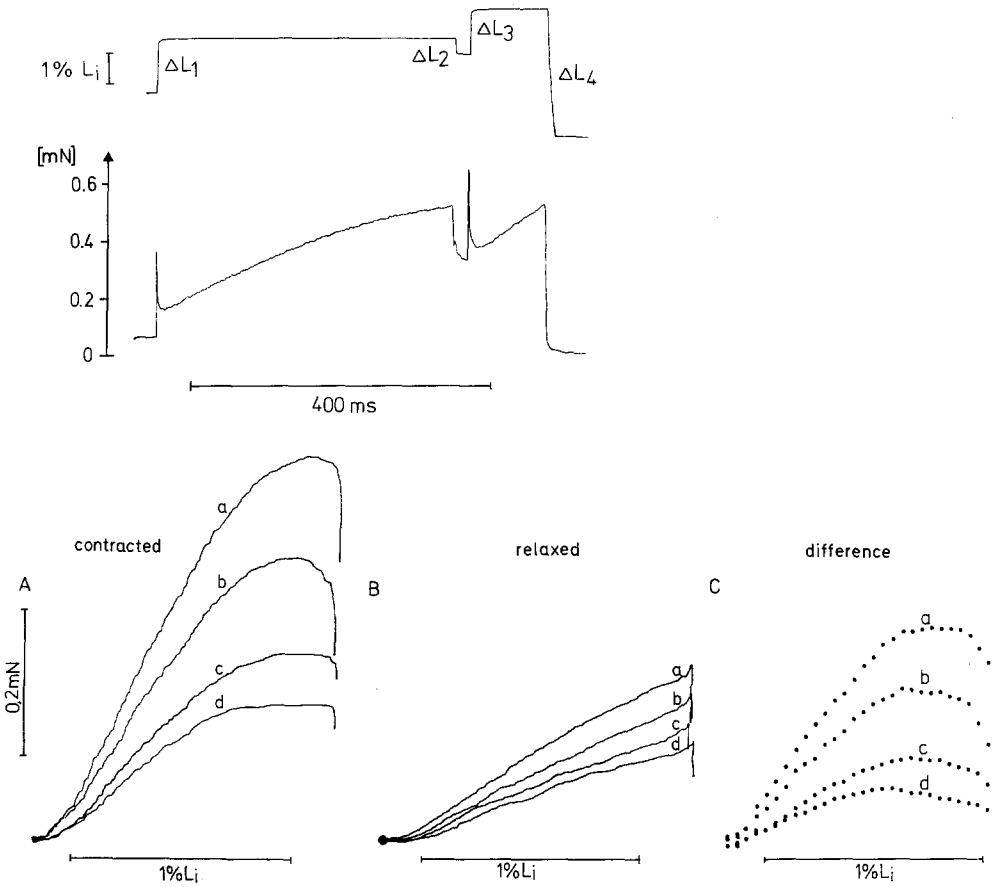

Fig. 15 and 16A–C. The state of the muscle after deactivation. A single fibre of glycerinated DLM of *Lethocerus maximus* was subjected to a sequence of length changes as shown in the upper trace of Fig. 15. In the lower trace the corresponding force transient is shown. In Fig. 16 the length-tension diagrams obtained during the stretch ΔL_3 are plotted. The curves of diagram **A** correspond to the contracted, the curves of diagram **B** to the relaxed muscle. In diagram **C** the difference between diagram **A** and **B** is shown. The letters *a–d* correspond to different release amplitudes ΔL_2: *a* 0.05% L_i; *b* 0.5% L_i; *c* 1% L_i; *d* 1.4% L_i. The starting points of the curves are shifted till all curves started at the same point. T = 12° C. In all curves tension *change* is plotted versus length *change*

length-tension diagram of the non released but isometrically contracting muscle fibre is bent to a plateau.

As mentioned above, the length change, at which the kink in the length-tension diagram occurs is interpreted to be correlated with the state of cross bridge strain from which on the cross bridges start to slip along the actin filament. Therefore the experiment indicates that the state of strain of attached cross bridges is the same, for all release amplitudes after the completed deactivation, as it is in the isometrically contracting state. This is expected if the cross bridges attached prior to the release are at the time of the restretch already completely replaced by cross bridges which attached after the release.

The experiment shown in Fig. 16 does not answer the question as to whether the adjustment of the cross bridge strain to the new muscle length after release takes place already during or immediately after the release, or whether this process takes time as reported for the deactivation process. Therefore we measured in addition to the force transients shown in Figs. 3 and 4 the corresponding length-tension diagrams during the length changes of the release-restretch cycles. They are shown in the lower part of the Figs. 3 and 4 (number 1 indicates the release, number 2 the restretch). It can be seen that the curve of the restretch 1 ms after the release is – apart from the parallel shift and the slightly decreasing slope at the very end of the curve – rather similar in shape to the curve of the release. On the contrary the curve corresponding to the restretch 10 ms after the release shows a distinct decrease of its slope at a length of ca. 1% L_i indicating the onset of cross bridge slippage. Since this is not observed in the length-tension diagram plotted 1 ms after the release it must be concluded that the re-establishment of the original state of the cross bridge strain after release takes time, as does the deactivation process.

Effect of the State of Activation on the Time Constants of Deactivation and Reactivation Induced by Length Changes

To check whether the state of activation of the muscle influences the time constants of deactivation or reactivation, the muscle fibre was released and restretched by multiple steps with small amplitude length changes. The resulting force transient is shown in Fig. 17 (upper trace: relaxed, second trace: activated state). The single length change was ca. 0.08% L_i. Consequently, the total length change of the "triple step" was ca. 0.25% L_i. As can be seen from the figure the rate constants of the deactivation phases are rather similar from step to step and are, furthermore, similar to the rate constants of the activation phases following the restretches. The similarity of the deactivation phases induced by the three small steps in Fig. 17 (second trace) is shown more distinctly in Fig. 18: From the transients shown in Fig. 17 the corresponding transients obtained in therelaxed state of the fibre are subtracted in order to obtain the "active" force transients. Then the transients are scaled in such a way that the overall amplitude, i.e., the loss in force from the state just before the release to the state at the end of the deactivation phase, became the same for all plotted transients. It can be seen from the figure that all three transients corresponding to the small releases shown in the second trace of Fig. 17 (symbols ×, ○, □ in Fig. 18) are very similar. The similar rate of tension decay indicates that the velocity of the deactivation process is the same for all the small steps in length, having the same amplitude, but starting from different total lengths. This indicates that the time constant of deactivation is not affected by the total length of the muscle or by the force generated.

The lower part of Fig. 17 (c: relaxed, d: activated state) shows the force transient, which is obtained if the total length change of 0.25% L_i is performed within one step. The force transient of the deactivation induced by the larger release is – after correction and scaling as described above – also plotted in

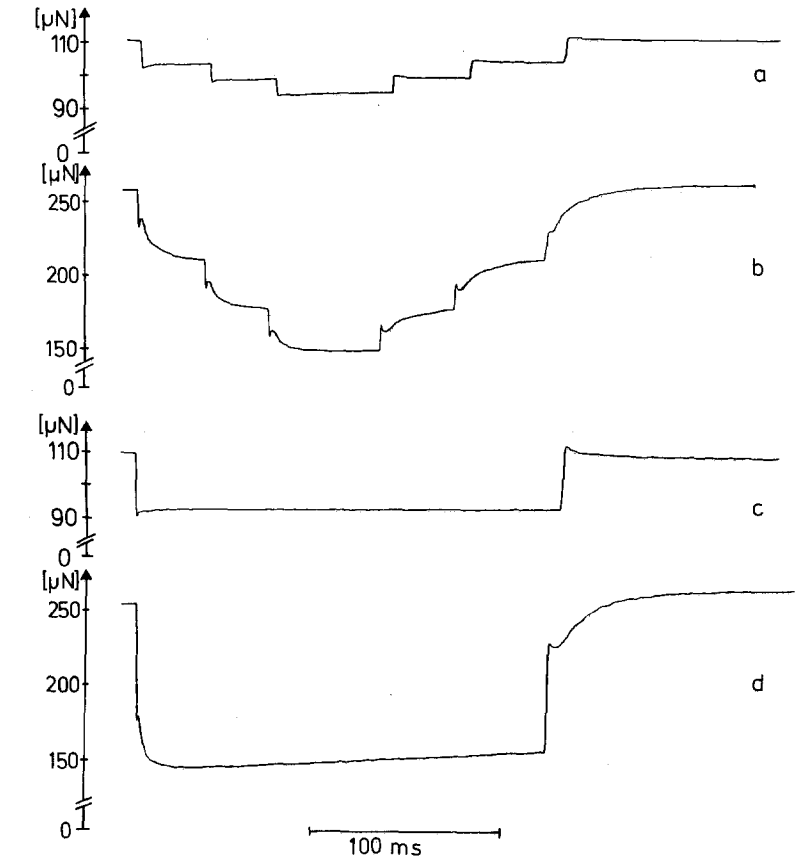

Fig. 17a–d. Effect of muscle force and length on the time constants of deactivation and reactivation. A single glycerol-extracted fibre of DLM of Lethocerus maximus was activated by a 1.5% L_i stretch. After maximum activation was achieved the fibre was stepwise released and then restretched (**a, b**). Each length change had an amplitude of 0.08% L_i (not shown). The corresponding force transients are shown in the relaxed state (**a**) and the contracted state (**b**). Trace (**c**) and (**d**): The fibre was after activation released and then restretched to the initial length within one step. The amplitude of the length change was 0.25% L_i. The corresponding force transients are shown for the relaxed state (**c**) and the contracted state (**d**). After the procedure the fibre was released. The experiment was repeated after ca. 2 s in order to average the force transient (number of repetitions was 64. T = 24° C

Fig. 18 (symbols △). As can be seen the deactivation phase induced by the larger length change differs markedly from those induced by the smaller releases. This finding is in principle not surprising, since the cross bridge elasticities are much more discharged by the large than by the small release amplitudes. However, not only the shape or amplitudes of the curves are different but also the time constant (cf. τ_1 and τ_2 in Fig. 18) of the later part of the transients, i.e., of the deactivation phase. This indicates that the velocity of the deactivation process after release is sensitive to the change of the amplitude of the length change rather than to the change in the total length of the muscle. Consequently the

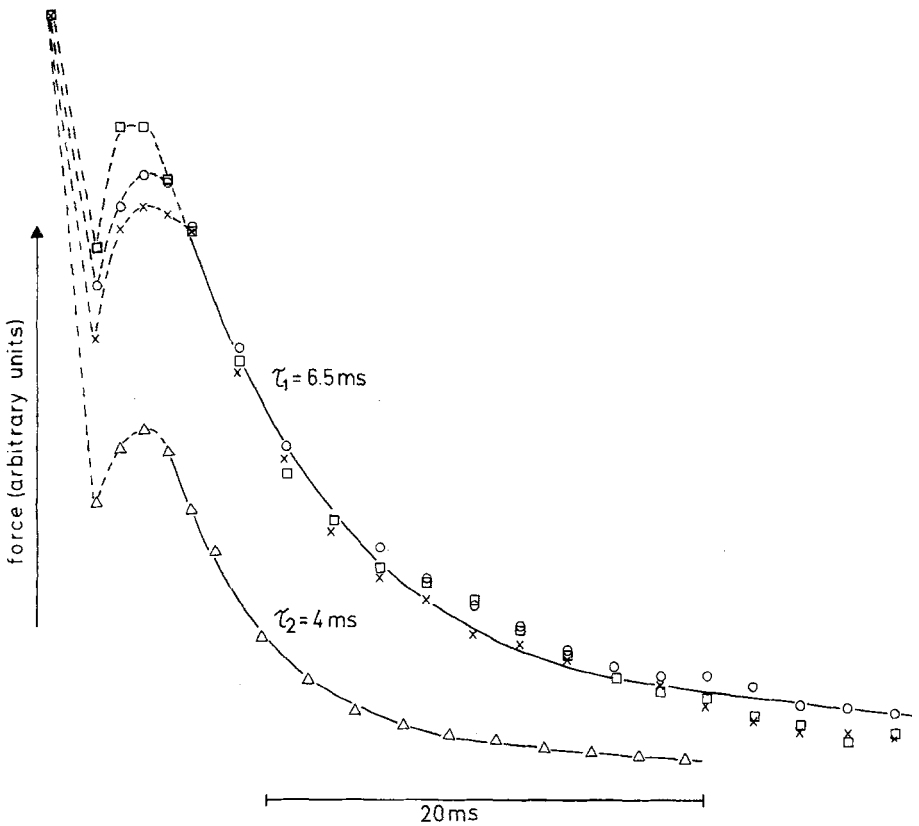

Fig. 18. Scaled active force transients of Fig. 17. The release induced force transients of Fig. 17 are scaled to have all the same over all amplitudes. The (×) belong to the first, the (○) to the second and the (□) to the third release of the triple release shown in the upper part of Fig. 17. The (△) correspond to the one large release shown in the lower part of Fig. 17

different states of activation corresponding to the different muscle lengths do not affect the time course of the cross bridge processes responsible for the delayed fall in tension after a muscle release.

Also, the time course of the net cross bridge detachment after a muscle release was independent on the state of activation. In order to prove this statement we measured the stiffness transient after a 0.5% L_i release performed in the state of the muscle in which it was only Ca-activated and in the state in which it was Ca- and stretch activated. The amplitude which induced the stretch activation was 2% L_i. The result is shown in Fig. 19a. The (○) belong to the stiffness transient without, the (×) to the transient with the additional stretch activation. Figure 19b shows the corresponding force transients. In order to make it easier to compare the transients they are normalized to the same amplitude. (The stretch activated fibre was 1.7 times stiffer than the non stretch activated fibre.) As one can see from the figure neither the time course of the stiffness transients nor the time courses of the force transients are different in the two activation states.

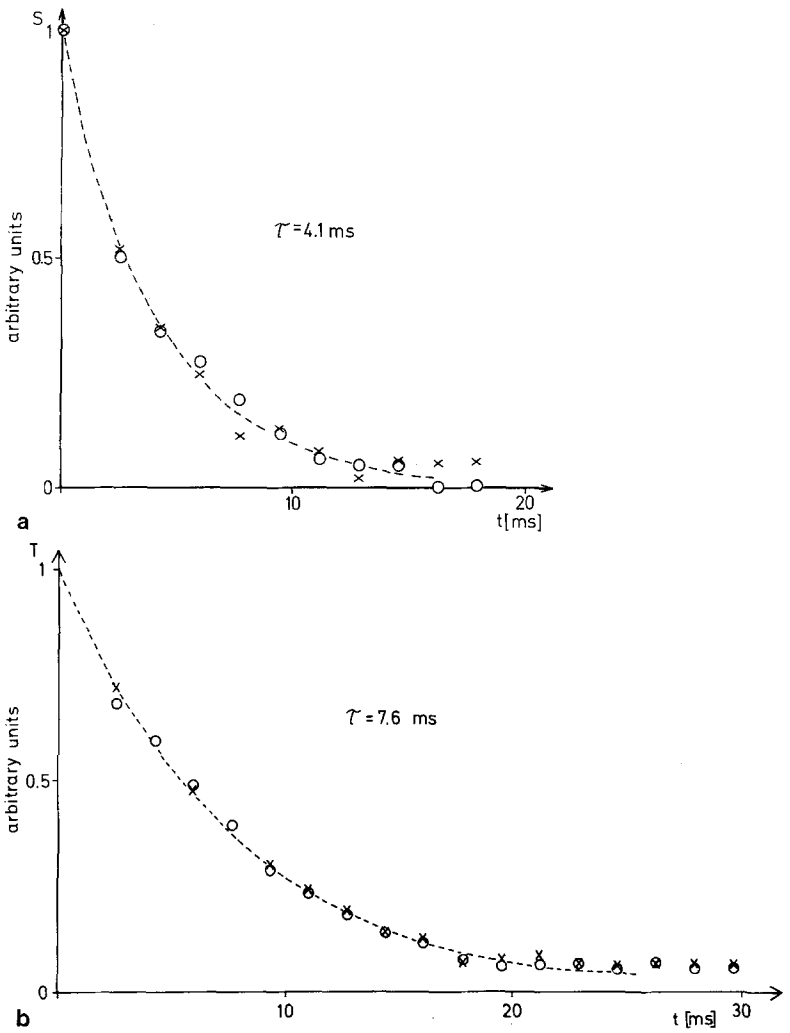

Fig. 19a and b. Stiffness and force transients with and without activating prestretch. A single fibre was released by a 0.5% L_i length change. The release was started in the only Ca-activated and in the Ca- and stretch activated state. (The amplitude of the activating stretch was 2% L_i.) The corresponding stiffness transients after the 0.5% L_i releases (obtained by sinusoidal superimposition) are plotted in Fig. 19a, the corresponding force transients are shown in Fig. 19b. In order to make it easier to compare the time courses of the transients they are scaled to have the same amplitude. The (×) correspond to the release with, the (○) to the release without preceding activating stretch. The temperature was 10° C

Discussion

A. Stiffness as a Measure of the Number of Attached Cross Bridges

From variation of immediate stiffness as a function of overlap Huxley and Simmons (c.f. also Ford et al. 1977), concluded that most, if not all, of the

elasticity in muscle fibres is located in the cross bridges. Hence stiffness at the given time may be taken as a measure for the number of cross bridges attached to actin. However, the validity of this conclusion clearly depends on the assumption that stiffness can be measured so rapidly that rapid attachment and detachment processes do not occur during the fraction of a ms required for the stiffness measurement. However, recent kinetic studies (Stein et al. 1979; Taylor 1979) on the actin-myosin interaction in vitro indicate very rapid establishment of equilibria between the associated actomyosin complexes and corresponding dissociated states. The rates of association and dissociation cannot be straightforwardly compared to the rates of the corresponding reactions of the myosin cross bridges with the actin filament in the contracting muscle. Nevertheless, it must be considered that very rapid detachment and attachment processes with rates of several thousands per second and more may also occur in intact muscle. The influence of such rapid processes on mechanical measurements has consequently to be discussed.

From the kinetic studies in vitro it is not convincingly proved that the different association-dissociation reactions in the kinetic scheme are all very fast (fast compared to the duration of the length changes administered to muscle fibres for mechanical measurements). This uncertainty, in addition to the unknown relationship between the in vitro and the in vivo rates, makes it impossible to treat the problem on the basis of detailed kinetic rates. Therefore only some general statements can be made:

1. It may be assumed that the detachment-attachment rates of a particular actomyosin state are fast compared to the duration of the length-change administered for stiffness measurement. Then the cross bridges in this state would not contribute to the measured stiffness value: Before the cross bridge elasticity is displaced to a considerable amount by the length change, the cross bridge detaches, discharges its elastic element and reattaches. Consequently the average increment in force during the length change and thereby also the measured stiffness ($\sim dF/dL$) would be nearly zero.

2. If the time constant of the detachment-attachment equilibrium in question is comparable to the duration of the length change, the length-tension diagram is expected to have a viscous character, i.e., its shape should be different for different velocities of the length change. In order to rule out this possibility for the stiffness measurements shown in this paper, the release velocity in the experiments of the type shown in Figs. 3 and 4 was changed from 30 muscle lengths per second to 15 muscle lengths per second. The slope of the length-tension diagrams obtained was in its early part (where it is taken for the stiffness measurement) not different for the two different velocities of the length change. (The temperature was ca. 10° C as for all stiffness measurements.) Since no viscous effects are observed, it can be concluded that the measured stiffness reported in this paper reflects the number of attached cross bridges, being not in a rapid equilibrium ($\tau \ll 0.5$ ms) with a detached state. (On the other hand this result does not rule out the possibility that in addition attached cross bridges may exist in other states which are in a sufficiently rapid equilibrium to be completely undetected by the stiffness measurement because of the mechanism described under point 1.)

B. The Nature of Activation and Deactivation

It is shown in Fig. 5 that the activation which is induced by a stretch can be cancelled out completely by the subsequent release to the initial length independent of the extent of activation achieved. Conversely it is demonstrated in Fig. 11 that the activation which is reduced after a release is quantitatively restored by the restretch to the initial length.

On the basis of the sliding filament theory of muscle contraction (Hanson and Huxley 1953; Gordon et al. 1966) several mechanisms can be thought of which result in a release induced delayed tension decrease. In the following some of the possible processes are discussed. The most probable process which explains most of the experimental data will be discussed under point 3 concept c.

1. Change of Cross Bridges into a Lower Force Generating State During the Deactivation Phase: It has been proposed firstly by Huxley and Simmons (1971) that the cross bridges linking the myosin with the actin filament can be attached in at least two different force generating states. More recently Kuhn et al. (1979) showed that two different force generating states, having the same stiffness, account for an accurate description of the mechanical response in tension within the very first period after and/or within a rapid length change administered to a single insect flight muscle fibre. Consequently the delayed tension decrease after release could in principle be thought to be effected exclusively by a shift in the distribution of cross bridges attached in the two different states. However, the number of attached cross bridges and consequently the fibre stiffness would then be expected to remain constant during the delayed tension drop. On the contrary, the stiffness decreases after release (Figs. 7 and 8), a finding which at least indicates an additional net detachment of cross bridges induced by the fibre release. Nevertheless the decreasing stiffness does not exclude an additional shift in the distribution of populations of different force generating cross bridges. This possibility would be disproved by the finding of a constant stiffness to tension ratio detected before and after the deactivation phase. (The certainly not constant stiffness to tension ratio *during* the deactivation phase – different time constant of stiffness and tension decay in Fig. 7 – is due rather to the non steady state condition of the system during the time of establishing the deactivated state – cf. Sect. C –.) Unfortunately the active tension of a fibre in insect flight muscle is difficult to determine, since the passive tension has to be subtracted; the tension generated in the active and the passive state has to be measured in different incubation vessels with different shape of the liquid surface. The surface tension of the liquid acts on the force transducer disturbing the force measurement. (This effect does not affect the relative force measurement during a force transient and also does not affect any kind of stiffness measurement.) It is therefore difficult to get the stiffness to tension ratio sufficiently accurate. Nevertheless, the stiffness to tension ratios obtained seem to be rather constant before and after the deactivation phase, indicating that there is no shift in the distribution of cross bridge in different force generating states.

2. The Effect of the Release is Exclusively an Increased Detachment Rate Constant of the Discharged Cross Bridges: In this section will be discussed whether an increased detachment rate of discharged cross bridges will be sufficient to account for the observed phenomena or whether an additional process must be postulated to take place after muscle release. If in the cross bridge cycle one forward rate constant is enhanced whereas the others remain constant, the turnover time for the cycle is expected to be shortened rather than increased. For isometrically contracted insect flight muscle, Breull et al. (1973) calculated a turnover time of ca. 120 ms from ATPase activity measurements at 20° C. During the turnover time most of the attached cross bridges are replaced by new ones. After a fibre release it is consequently expected that during the time of one turnover or during an even shorter time the state and thereby also the force of the muscle is nearly restored. This is not the case even at 24° C as can be seen from Fig. 14, where 120 ms after the release no appreciable tendency for force to recover to the state before the release is observed.

The estimation of the turnover time is based on the assumption that all cross bridges are acting within the fully activated muscle. This condition may not be fulfilled. However, if only a lower fraction of the cross bridges is acting the turnover time must be shorter than assumed above. Consequently the muscle would be expected to return to the state before the release even faster after the release.

3. The Actin-Myosin Interaction is Controlled by the Fibre Length: Many of the observed phenomena can be explained assuming that the actomyosin system in the muscle is activated, when the muscle is strained and it is deactivated, when it is released (cf. also Pringle 1977). If so, the rate limiting process responsible for the time needed to establish the new steady state could be the activation and deactivation process of the actin-myosin system itself (discussed in concept a) or the reaction of the cross bridges on an altered activation or deactivation state of the system (discussed in concept b and c).

Concept a: *The activation or deactivation of the actin-myosin interaction requires time, which is manifested in the delay of the tension or stiffness response to a length change (Julian 1969).* (I) The state of activation may be controlled by the muscle length: In all reported stretch-release or release-restretch cycles the delayed decrease in tension after the release was found to be faster than the corresponding stretch induced delayed increase in tension, if the amplitudes of the length changes were sufficiently large. Under the preassumption above this finding indicates that not only the extent of the activation but also its time constants must be assumed to be a function of the muscle length. However, this prediction is disproved by the experiment reported in Fig. 17: It is shown that the deactivation induced by all length changes in the sequence of multiple small releases (second trace) are slower than the deactivation induced by the large release shown in the fourth trace. Since the latest small release of the sequence corresponds to the same muscle length as the large release of the fourth trace, this finding indicates that it is not the absolute muscle length which controls the velocity of the deactivation but rather the amplitude of the individual length change. This can be also concluded from the experiment reported in Fig. 19. There it is shown that independent of the state of activation, i.e., with and without a preceding activating stretch of 2% L_i, no difference in the rate of net cross bridge detachment is observed at constant amplitude of the length change. (II) The state of activation may be controlled by the *change* of length or strain of the myosin filament: With this preassumption the concept can explain the experiments mentioned above. On the other hand the cross bridge cycle would then be expected to remain in a steady state all the time during the process of deactivation. The latter since the deactivation was assumed to be rate limiting, i.e., the transitions from one cross bridge state to another are for all states assumed to be fast compared to the time needed for the deactivation of the whole system. As a consequence one would expect a constant stiffness to tension ratio to be maintained during the deactivation phase. In contrast there is some evidence (Figs. 7 and 19) that this is not the case.

Concept b: *The activation-deactivation of the actomyosin system is rapid and it is the attachment and/or the detachment of the cross bridges (controlled by the muscle length) which is rate limiting.* Thorson and White (1969) proposed an enhanced attachment rate of the cross bridges in the stretched muscle. They propose this increase of the attachment rate to be proportional to the local strain of the myosin filament or to its displacement with respect to the actin filament. Both of these concepts explain the observed stretch-induced activation and the release-induced deactivation as well as the observation that the activation or deactivation is maintained as long as the muscle is in the stretched or released state. However, the model of Thorson and White predicts a slower rate of the deactivation than of the activation, whereas the reverse is observed. The model also fits this observation if the detachment rate is *decreased* by a muscle *stretch*. However, it is reported by Rüegg and Tregear (1966) and Breull et al. (1973) that in the stretched insect flight muscle the ATPase activity is enhanced. In the Thorson and White model the ATPase activity is given by $fg/(f+g)$, where f is the attachment rate and g the detachment rate. Consequently an exclusively decreased detachment rate after stretch would be predicted to cause a decreased ATPase activity in the stretched muscle. Nevertheless it can be shown that the model is at least qualitatively able to describe all these phenomena (including the ATPase activity increase induced by stretch) if both the detachment and the attachment rate is properly chosen as a function of length. However, the Thorson and White model assumes in any case that the rate of activation and deactivation is a function of the muscle length. On the other hand, it is indicated by the experiment reported in Figs. 17 and 19 that it is not the muscle length per se but rather the amplitude of the length change which

controls the rate of deactivation. To be able to account also for this phenomenon the Thorson and White model has to be modified: It would be necessary to introduce a dependence of the rate constants on the state of cross bridge strain, i.e., on the amplitude of the length change administered to the muscle in addition to the dependence of the rate constants on the muscle length. The experimental evidence reported in this paper is not sufficient to rule this possibility out. However, a less complex concept can be postulated which covers the experimental findings as well:

Concept c: *As discussed in "b" the activation and deactivation process of the actin-myosin system is rapid and it is the cross bridge reactions which are rate limiting: However, in contrast to "b", the attachment and detachment rates of the cross bridges are here a function of their internal state of strain only. The number of cross bridges which can interact with the I-filament is a function of muscle length.* The state of the cross bridge strain is exclusively a function of the sudden length *change* administered to the muscle fibre. Consequently the velocity of the deactivation and activation processes induced by a length change are also exclusively controlled by the amplitude of the initiating length change as indicated by the experiment reported in Figs. 17, 18, and 19. On the other hand the state of activation of the actin-myosin system, i.e., under steady state conditions the number of acting cross bridges, is exclusively a function of muscle length (Wray 1979). Therefore the force generated is also a function of the muscle length. The force, which decreased during the deactivation phase after release, is consequently expected to remain low even after replacement of the discharged cross bridges by new ones during the turnover time (cf. Fig. 14). The restretch to the initial length restores the activation state to the level before the release and – after the new steady state is established – the force is as high as it was before the release (see Figs. 11 and 14). Analogously the activation induced by a stretch is expected to be cancelled out by the release to the intitial length (Figs. 2 and 5).

The ATPase in the steady state is predicted to increase in proportion to the number of acting cross bridges. The tension developed by the muscle is – under steady state conditions – also predicted to increase proportionally to the number of acting cross bridges. Consequently tension and ATPase activity is expected to be proportional. This prediction is confirmed by the experimental evidence reported by Rüegg and Stumpf (1969), Breull et al. (1973), and Pybus and Tregear (1973). If the muscle is repetitively stretched and released, Breull et al. (1973) reported the ATPase activity not to be exclusively a function of the averaged muscle force but also to be correlated to the frequency of the stretch-release cycles. Each stretch and each release of the muscle influences the kinetics of cross bridge interaction with the actin filament, since the state of strain of the cross bridges is changed by the length changes. The kinetics is only changed for the assembly of cross bridges attached at the moment of the length change, i.e., for a certain number of cross bridges per length change. Therefore a linear dependence between the repetition frequency of the length changes and the ATPase activity is predicted if the repetition frequency is slow compared to the time constant of the stretch activation. This coincides with the experimental results reported by Breull et al. (1973). Since concept "c" explains in the most simple way all the data presented in this paper and in addition some features of ATPase activity measurements of other investigators, it will form the basis for all the following considerations.

C. The Different Shape of Force and Stiffness Transients After Release

Since exclusively the number of acting cross bridges is controlled by the muscle length and not their kinetics one could expect a strictly constant tension to stiffness ratio during the deactivation phase after release. This is not observed, as indicated by the different time constants for stiffness and tension decrease during the deactivation (Fig. 7).

However, within the time needed to establish the new steady state after the release discharged "old" cross bridges left from the time before the release are mixed up with "new" cross bridges which attached after the release: Whereas the stiffness is proportional to the total number of attached cross bridges, the stiffness to tension ratio identical to that before release cannot occur before the new steady state is established. This does not contradict the findings of White et al. (1977), who reported a constant active stiffness to tension ratio after stretch during the activation phase: After stretch "new" cross bridges form with a comparatively slow rate (rate of activation cf. Fig. 3). The "old" cross bridges, which are strained by the stretch, thereby lowering the stiffness to tension ratio, may detach and reattach in a discharged position relatively quickly. In addition for stretch amplitudes larger than 0.5% L_i very fast detachment-reattachment processes are reported by Güth et al. (1979).

D. Relevance for Other Muscle Types

The phenomenon of a delayed reaction in force of a muscle induced by a length change is not observed exclusively in fibrillar insect flight muscle. Steiger (1971, 1977) and others reported a similar phenomenon, but less distinct, in rabbit papillary heart muscle and, to a rather small extent also in skeletal muscle (Rüegg et al. 1970; Edman et al. 1978a and b). However, the insect flight muscle – elongated or released by an amount of 1.5% L_i – is nearly completely activated or deactivated respectively. This must be different (and is different) in the muscle types mentioned; these muscles are able to shorten themselves actively to a much larger extent than 1.5% L_i.

This and some other difficulties arise from the straight forward extension of the molecular interpretations given here to other muscle types. Nevertheless it may be helpful to consider the possibility of activation and deactivation as a general property of shortening or elongated muscles.

In particular the reported dependency of the kinetics of the cross bridges on their state of strain may be a general feature of all muscle types (see chapter E).

E. Rate of Net Cross Bridge Detachment Affected by Release

It is concluded straightforwardly from the experimental results that the apparent rate of net detachment of acting cross bridges is greatly enhanced when their elastic elements are discharged, i.e., cross bridge detachment (and/or cross

bridge attachment) kinetics depends on the state of strain of their elastic elements. This behaviour of the cross bridges is postulated by most of the molecular-mechanical contraction models as for instance the models proposed by Huxley (1957), by Julian et al. (1973), and by Podolsky et al. (1969). All these models predict a strongly increasing detachment rate of discharged cross bridges in order to account for Hill's force-velocity relationship and the increased amount of total energy liberated in the shortening muscle. While the attachment-detachment kinetics seem to be exclusively a function of the cross bridge strain we propose in *addition* that in the isometrically contracted *insect flight* muscle the number of recruited cross bridges is a function of the muscle length or force. This mechanism gives a new aspect of the length or force dependent activation state of muscle in general and of insect flight muscle in particular.

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