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THE TENSION IN A LOCUST FLIGHT MUSCLE AT VARIED MUSCLE LENGTHS

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Summary

1. Tension in a resting muscle increases when the muscle is stretched. If the stretch is maintained, the tension decays (stress relaxation). The time course of stress relaxation in the metathoracic second tergocoxal muscle (Tcx_2) of the locust *Schistocerca americana* was found to be adequately described by a multi-exponential function with four or more time constants. These constants were independent of strain, and the slowest had a value of more than 60 min. Tension continued to diminish even when stretch was maintained for 2–4 h.

2. Tension in a stretched, unstimulated muscle increased with increased length of stretch. Resting tension in the locust Tcx_2 at the *in vivo* length was estimated to be $1\,N\,cm^{-2}$ or less. At 10% strain, resting tension was about $2\,N\,cm^{-2}$. The stiffness of the unstimulated locust muscle was similar to that of unstimulated frog muscles.

3. The active tetanic tension was maximal (average = $32{\cdot}4\,N\,cm^{-2}$) at slightly less than the *in vivo* muscle length. Tetanic tension was 50% or more of its maximum value over a range of 80–130% of the *in vivo* muscle length.

4. The active twitch tension was maximal at slightly greater than the *in vivo* muscle length. The ratio twitch/tetanic force increased with muscle length.

5. Twitch relaxation time increased with muscle length, but the time to peak for twitch force was nearly independent of muscle length in a stretched muscle.

Introduction

Vertebrate skeletal muscle is cross-striated, owing to the aligned sarcomeres of the myofibrils. Arthropod muscle is also cross-striated, and is similar to vertebrate skeletal muscle in many other aspects of form and function. Insect flight muscle, however, is often described as being unusual, differing from other striated muscles mechanically and physiologically (Podolsky & Schoenberg, 1983; Tregear, 1983; McMahon, 1984; Aidley, 1985). Some insect flight muscles differ from vertebrate muscles in that the timing of their cyclic contractions is determined largely by mechanical events and not by phasic input from motor neurons. Such muscles

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were called asynchronous by Pringle (1949) when he found that the frequency of muscle contraction was not directly correlated with the frequency of activity in the motor nerve (for discussions of asynchronous muscles see Pringle, 1981; Tregear, 1983; Aidley, 1985). The asynchronous muscles are unusual in several respects, such as their ability to oscillate. They have also been reported to be extremely stiff and inextensible (Machin & Pringle, 1959).

Although asynchronous flight muscles are unusual, the flight muscles of many insects are neither asynchronous nor peculiar. Dragonflies, locusts, moths and insects in several other orders have wing muscles whose contraction frequency is directly controlled by motor-nerve firing patterns, as are those of other arthropod and vertebrate skeletal muscles.

The length–tension relationships of one major locust flight muscle, the dorsal longitudinal muscle (DLM), have been studied. This muscle has been described as being almost as stiff and inextensible as are the few asynchronous muscles which have been examined (Weis-Fogh, 1956). These DLM studies were included in an early review of muscle structure and function (Hanson & Lowy, 1960), and have been extensively reported. It has become generally accepted that insect flight muscles, both synchronous and asynchronous, are very stiff even when relaxed (Buchthal & Weis-Fogh, 1956; Hanson & Lowy, 1960; Tregear, 1983; McMahon, 1984; Aidley, 1985), and that the length–tension relationships are unlike those of conventional skeletal muscle (i.e. frog leg muscle).

The studies presented in this paper were undertaken to elucidate the length–tension relationships in a locust wing muscle. The resistance to stretch that was examined was the low-frequency stiffness, similar to that measured in other muscles to establish passive length–tension curves. Surprisingly, the muscle was found to be not unusually inextensible, and its length–tension properties, both when stimulated and at rest, were not greatly different from frequently studied vertebrate muscles.

In doing these studies, it quickly became apparent that passive tension in a stretched muscle is a function of time after stretch as well as of the distance stretched. The force in a resting, stretched muscle falls continuously from the value reached at the end of stretch. The decline in stress is called stress relaxation. The extent and time course of stress relaxation were investigated to determine an appropriate time after the end of stretch at which to measure the passive length–tension relationships, as well as to obtain some insight into the components that are responsible for the stiffness of the resting muscle.

Materials and methods

Adult, male locusts, *Schistocerca americana* (Drury), were used in all experiments. They were maintained at 29°C, on a 16 h :8 h L: D cycle. Animals were fed growing wheat seedlings and a commercial dog food, based on soya bean and beef.

All experiments were done on *in vivo* preparations of the metathoracic second tergocoxal muscle (Tcx_2), which is an indirect wing levator and a coxal remoter

extending from the tergum to the posterior coxal rim. It is composed of parallel fibers, all of which are nearly the same length. The muscle is approximately 8 mm long and weighs about 4 mg. Locust flight muscle is very sensitive to oxygen lack (Weis-Fogh, 1956), so care was taken during muscle preparation to keep intact the tracheae that form the pathway of oxygen delivery to the Tcx_2.

To prepare the muscle for recording, the locust's wings and legs were detached, after which the metathoracic ganglion was cut free and removed to avoid spontaneous Tcx_2 activation or extraneous movement. In some stress relaxation experiments, the mesothoracic ganglion was also removed to further reduce movement artefacts. All muscle attachments except those of the Tcx_2 were cut from one metathoracic coxa, and the coxal cuticle was trimmed to a small piece of the proximal rim containing the attachment site of the Tcx_2 apodeme. Silver wire electrodes (50 μm diameter) were inserted through holes in the tergum on either side of the origin of the Tcx_2 and waxed in place. The animal was then fastened on its back with epoxy glue to a flat holder.

Muscle force and position changes were measured with an ergometer (Cambridge model 300H), to which the muscle was coupled by an insect pin (about 15 mg) that was bent into a hook at each end. The animal on its holder was mounted at 45° on a plastic platform, with the abdomen higher than the head. At this angle the Tcx_2 was vertical, and the muscle force developed was normal to the horizontal transducer arm. The plastic platform was mounted on a micromanipulator so that it could be positioned with respect to the ergometer. At the start of each experiment, the remnant of coxal cuticle was slipped over the insect-pin hook, linking the Tcx_2 muscle and the transducer. The Tcx_2 was stretched to approximately its normal *in vivo* length, as judged by the position of the coxal remnant relative to the sternum when viewed through a dissecting microscope. This experimental length was used as reference length (L_{ref}) in all experiments.

The thoracic temperature was monitored by a thermistor inserted in the thorax through the contralateral coxa. The output of the temperature monitor also controlled the intensity of a microscope lamp shining onto the animal's sternum. A decrease in temperature increased the light intensity. In this way the temperature of the thorax was maintained at 25°C by feedback control. The muscle was moistened throughout the experiments with locust saline (Usherwood & Grundfest, 1965) or with saline to which 90 mmol l^{-1} sucrose had been added (Evans, 1981).

The stimuli for muscle contractions were 0·5 ms electrical shocks through the implanted electrodes. Three distinct twitch-tension increments were obtained with sequential stimuli of slowly increasing intensity – corresponding to the three motor units which form the muscle (Kutsch & Usherwood, 1970; see Josephson, 1973, for a discussion of relative stimulation thresholds for nerve and muscle). During measurements of twitch or tetanic contractions, the shocks were applied at 1·5 times the minimum voltage needed to activate all three motor units. Tetani were induced with multiple stimuli at a frequency of 250 Hz. Tetanizing stimulus bursts lasted 50 ms. A single stimulus given after tens of seconds of rest sometimes

evoked multiple firing of motor units, as evidenced by multiple peaks in the tension response. Multiple firing was rare with interstimulus intervals of a second or less. In twitch measurements, errors due to multiple firing were largely avoided by using a pair of stimuli with a 0·5 s interval, and measuring the twitch evoked by the second shock of the pair.

In stress relaxation experiments, a command voltage to the ergometer position control caused a quick change in muscle length. Length changes were at 50–80 mm s^{-1}. After reaching the new length, the muscle remained stretched for 15 min. Force and position values were monitored with an oscilloscope, and collected with A to D converters at 2 ms per sample for the first 1–2 s and at 0·13 s per sample for 15 min. At the end of a stretch period, the muscle length was returned to L_{ref}. At the beginning of an experiment, and after the return to L_{ref} following a stretch, the muscle was stimulated several times with stimulus pairs (0·5 s interval) at 30 s intervals to take up the slack and to monitor potential deterioration of the preparation. This stimulation was followed by more than 2 min of rest before the next elongation.

In experiments on the effect of muscle length on passive, twitch and tetanic tension, the muscle length changes were accomplished in 3–5 s by manually adjusting the voltage controlling the position of the ergometer arm. One minute after the change in length, the muscle was tetanically stimulated. One minute after the tetanic stimulation, a twitch pair was evoked, after which the muscle length was returned to L_{ref}. The same stimulation sequence was repeated at L_{ref}. Using this protocol each experimental value was obtained approximately 2 min after a corresponding reference value and was followed, after another 2 min, by another determination of a reference value.

All experiments started with 10–20 twitch pairs at approximately 30 s intervals at L_{ref} to allow the muscle to reach a steady state. After equilibration and testing at L_{ref}, the muscle length was adjusted to the shortest length to be investigated. After testing at this and each subsequent experimental length, the muscle length was again returned to L_{ref}. At L_{ref}, the twitch and tetanic tension were characterized as described above. The experimental lengths examined were progressively greater, and the largest strains were examined last. An increasing length series was used rather than randomized length changes so that long extensions, which can damage the muscle, would not affect the results at shorter lengths. It has often been observed that the force of a twitch following a tetanus is greater than that preceding it, a phenomenon called post-tetanic potentiation. In the locust muscle post-tetanic potentiation persists for some time, 3–5 min at 25 °C (J. G. Malamud, unpublished observation). To obtain a constant influence of any post-tetanic potentiation, in investigations including both twitch and tetanus, all twitches were 1 min after a tetanic contraction.

At the end of each experiment, the muscle was held at L_{ref} and fixed by injecting the thorax with 70 % ethanol. After 20 min of fixation, the Tcx_2 was removed from the transducer coupling, and the preparation was stored in 70 % ethanol. Several days to several weeks after the experiment, the preparation was dissected and the

lengths of the experimental Tcx_2 (L_{ref}) and of the contralateral control Tcx_2 were measured with an ocular micrometer. The data presented are only from preparations in which L_{ref} was between 95 % and 105 % of the length of the control muscle. The mass of experimental muscles was determined after rehydration in saline. *Schistocerca* flight muscles lose 18 % of their fresh mass when rehydrated in saline from 70 % ethanol (J. G. Malamud, unpublished observation). All masses were corrected for this loss. The mean length of the control muscle was 8·1 mm (S.D. = 0·29, $N = 17$), and the mean cross-sectional area, calculated as the ratio of mass to length, was 0·58 mm^2 (S.D. = 0·077).

Results

Passive tension and stress relaxation

When a resting muscle is stretched beyond the normal *in vivo* length, its resting force increases. If the stretched muscle is held at its new, longer length, there is a decay in the observed force (Fig. 1). This decay in force is called stress relaxation. Stress relaxation was measured at strains ranging from 5 to 30 % of the muscle length. The peak force, measured at the end of stretch, ranged from 40 to 125 mN (mean = 78 mN, $N = 4$) following a 10 % stretch from L_{ref}, and from 109 mN to 172 mN (mean = 148 mN, $N = 3$) following a 30 % stretch. By 2 min after the stretch, forces had declined to about 17 % of the peak values, and by 15 min after stretch the forces had declined to about 13 % of their peak values [to 7·8 mN (S.D. = 0·9 mN) following the 10 % elongation; to 31·2 mN (S.D. = 0·9 mN) after

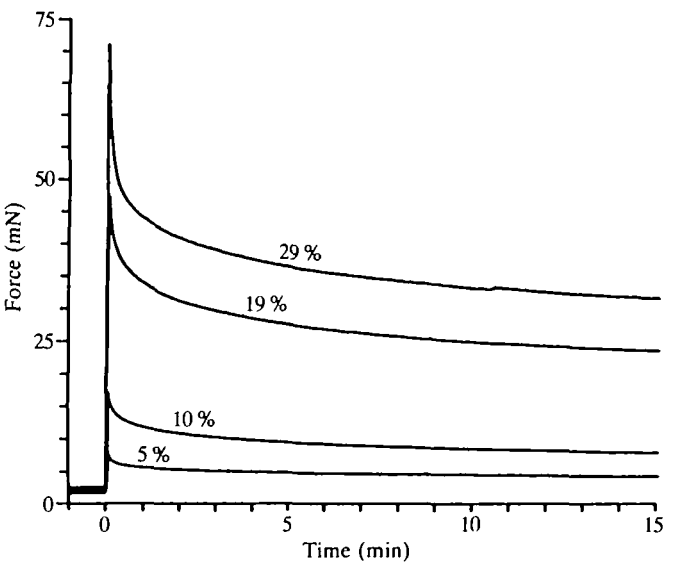

Fig. 1. Decline of passive muscle force following stretch. The curves are all from the same muscle, which was stretched to, and held at, a length above the normal *in vivo* length. The amplitude of stretch is indicated above each trace. The muscle was returned to normal length for more than 4 min between stretches.

the 30 % stretches]. In a few trials, a muscle was held stretched for 2–4 h. In these trials, the force continued to decline throughout the observation period.

Both the peak force immediately following stretch and the residual force after a period of time increased as muscle strain was increased (Fig. 1). Responses to similar stretches were similar on sequential trials.

The time course of force decay was fitted to a multiple exponential function of the form:

$$F = A_1 e^{-t/K_1} + A_2 e^{-t/K_2} + A_3 e^{-t/K_3} \ldots + A_n e^{-t/K_n},$$

where F is force, t is time, $A_1 \ldots A_n$ are force constants and $K_1 \ldots K_n$ are time constants.

The logarithm of the force was plotted against time after stretch, and each term ($A_n e^{-t/K_n}$) found by successive exponential curve 'peelings' of the straight-line portion of the curve at long elapsed time (see Riggs, 1963, for a discussion of the technique). Some of these experimental data were also fitted to multiple exponential functions by nonlinear least-squares computer routines. The various least-squares curve-fitting routines that were tried suffered from problems caused by the non-convergence of the program or by reaching local minima. Results from these routines were found to depend greatly on the starting values estimated for the parameters. In fact, these routines worked best when the starting values chosen were those determined by curve peeling. Use of a computer curve-fitting routine may be a useful means of quantifying the goodness of fit, or of 'fine tuning' parameter estimates found by exponential curve peeling, but it was not found to be a practical first-approach procedure.

With curve-peeling techniques, two or three exponential terms (Fig. 2A) were inadequate to fit data points over the full 15 min time course of stress relaxation, but four time constants did give a reasonably accurate representation of the declining force (Fig. 2B). The time constants, K, and force constants, A, of decay were calculated (Table 1) for preparations held stretched for 15 min. In these preparations, the slowest time constant, K_1, was found to be more than an hour, with additional slow to moderate time constants of a few minutes and of tens of seconds. The fastest time constant detected by our protocol was a few seconds (in one preparation movement in the early record prevented calculation of the fastest exponential component). Although there was a great deal of variation between the time constants of the preparations tested, for any particular preparation the time constants did not change substantially at strains of 5–30 %. However, each of the force constants for a single muscle did increase with increased strain.

It is clear from the muscle performance during stress relaxation that there is no stable rest force in a stretched muscle, at least not for the first several hours following stretch. The values obtained for passive tension depend on when they are measured. In the following, passive force was arbitrarily defined as the force 2 min after the cessation of stretch. By that time, the rate of force change was 5–7 % min^{-1}. Had longer intervals between stretch and measurement of passive force been used, the rate of force decay at the time of measurement would have

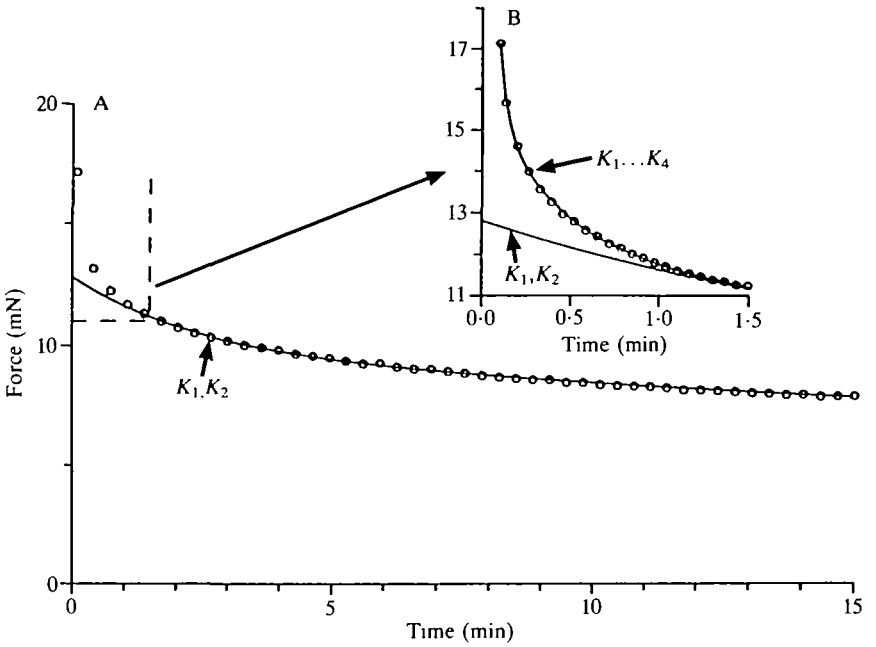

Fig. 2. Stress relaxation for a muscle stretched to, and held at, 110% of the normal *in vivo* length. (A) The data at times longer than 1–2 min can be fitted to the sum of two exponential components (the solid line, $y = A_1 e^{-t/K_1} + A_2 e^{-t/K_2}$), but this function does not fit the data points well at shorter times. (B) A function including four exponential components fits the data reasonably well over the entire time range examined. The time constants, $K_1 \ldots K_4$, were 69, 2·4, 0·31 and 0·04 min. These data are from the preparation shown in Fig. 1. For clearer presentation, only three points per minute are shown in A and 15 points per minute in B.

been smaller, as would have been the values of passive force. Practically, there are problems of morbidity and decay with very long intervals. Two minute intervals seemed a reasonable compromise between the competing demands of allowing enough time to minimize measurement variability, collecting enough data points to characterize a curve of the resting muscle force at different muscle lengths, and avoiding pathological changes associated with approaching death.

Force in the unstimulated Tcx_2 muscle at the *in vivo* length averaged only 3·4 mN [0·6 N cm^{-2} (S.E = 0·1 N cm^{-2}, $N = 7$)]. Passive force in stretched muscles increased approximately exponentially with muscle length (Fig. 3). There was a change of slope at stretches greater than about 25% of the muscle length, which might have been due to damage to the muscle.

Active force

The force exerted by a stimulated muscle is the sum of the passive force described above and the active force (sometimes called the active increment) produced by a muscle contraction. The tetanic force (P_o) increased with muscle

length to a maximum, and declined with increasing length thereafter (Figs 4 and 5). That length at which P_o is highest will be called the optimal length (L_o). L_o averaged slightly less than the length of the muscle *in vivo* ($L_o = 97\% L_{ref}$,

Table 1. *Time constants (K_n) and force constants (A_n) found by fitting stress relaxation data to summed exponential functions*

	F1		F2		F3		F4	
	A_1 (mN)	K_1 (min)	A_2 (mN)	K_2 (min)	A_3 (mN)	K_3 (min)	A_4 (mN)	K_4 (min)
A Values from four preparations at 10% strain								
Mean	8·8	113	2·5	2·3	2·9	0·32	14·0	0·08
S.D.	0·73	60	1·3	0·28	0·74	0·07	11·1	0·07
N	4		4		4		3	
B Values from one preparation at varied strains								
5% strain	5·0	91	1·0	2·0	0·71	0·30	2·3	0·08
10% strain	9·7	69	3·1	2·4	3·43	0·31	21·5	0·04
19% strain	28·1	82	8·6	2·5	6·35	0·41	14·0	0·09*
29% strain	37·3	87	9·4	2·5	6·69	0·48	11·0	0·15*

The expression used to describe stress relaxation was $F = A_1 e^{-t/K_1} + A_2 e^{-t/K_2} + A_3 e^{-t/K_3} \ldots A_n e^{-t/K_n}$.

The stress relaxation curves for this preparation are shown in Fig. 1.

Note that the time constants are essentially independent of the amount of strain.

* In these two curves, systematic errors between observed and predicted forces suggested that an even better fit would be achieved with a fifth exponential term containing an even shorter time constant.

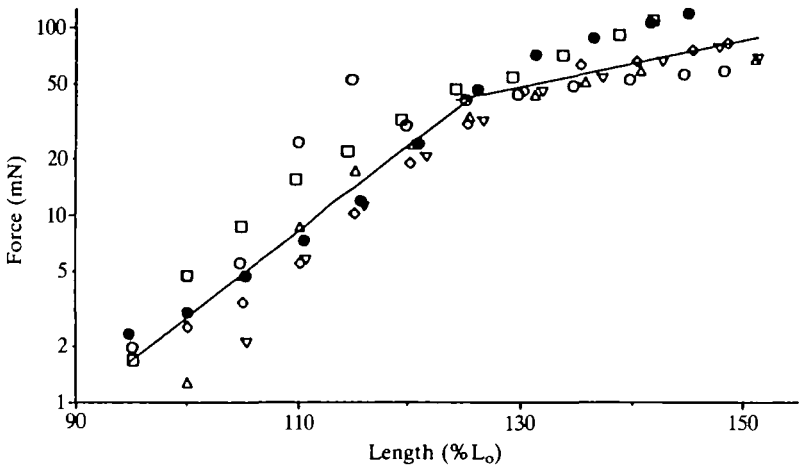

Fig. 3. Passive force in the Tcx_2 as a function of muscle length. L_o is the muscle length at which tetanic tension is maximal. Separate straight lines have been fitted to the data in the regions below and above 125% L_o.

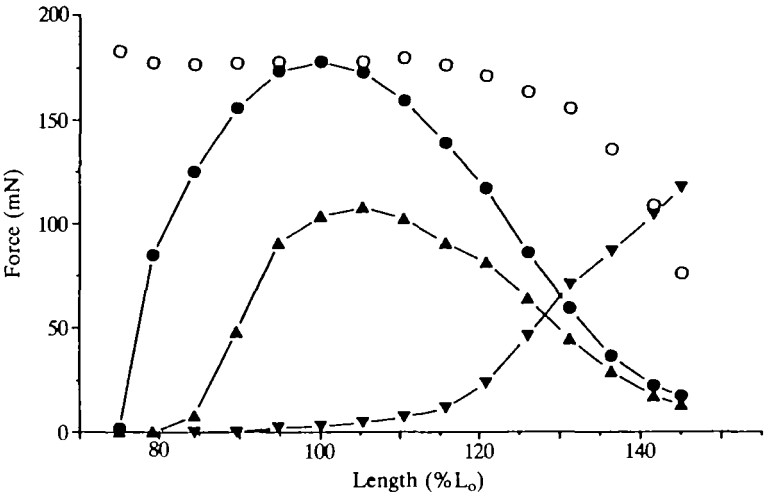

Fig. 4. Passive and active force in the Tcx_2 as a function of muscle length. In this preparation L_o was 7·7 mm. Inverse triangles (▼) show the passive force at each muscle length. The active increment of force (that is, the increase in force above the passive force at that length) is shown at each experimental length (▲, twitch; ●, tetanic). The open circles show the active tetanic tension in the trial at reference length preceding the trial at the corresponding experimental length. The active force at reference length dropped rapidly after stretch to, and contractions at, lengths above about 130 %L_o.

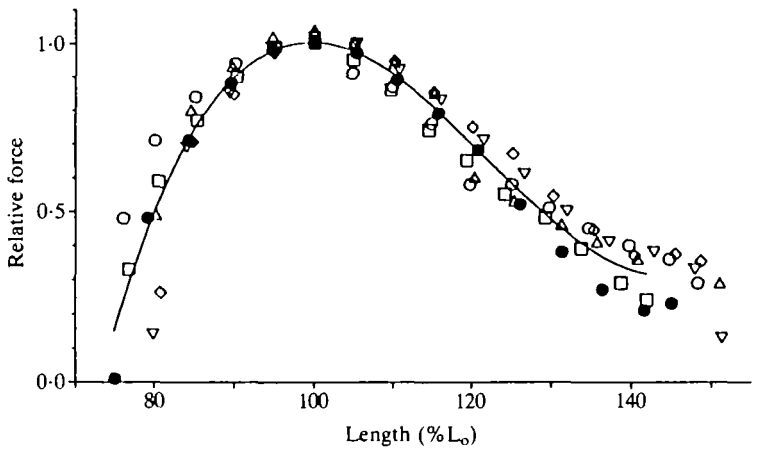

Fig. 5. The active tetanic force in six preparations as a function of muscle length. Each preparation is represented by a different symbol. The solid circle is the same preparation as that shown in Fig. 4. Force is shown as a fraction of the preceding tetanic tension (P_o) at reference length. The average P_o at reference length at the beginning of each experiment was 32·4 N cm^{-2} (S.E. = 1·7 N cm^{-2}, $N = 7$). Since the reference forces were reduced after long extensions, this presentation could exaggerate the relative forces at long lengths. The solid line was fitted by least-squares regression to a third-order polynomial.

Table 2. *Twitch tension and twitch/tetanic force ratio in the Tcx_2 at 25°C*

Muscle length ($\%L_o$)	Twitch tension (mN)	Twitch/tetanic ratio
90	67·6 (±8·0)	0·42 (±0·05)
100	123·9 (±4·6)	0·67 (±0·02)
110	114·2 (±5·2)	0·69 (±0·02)
120	85·9 (±5·9)	0·74 (±0·02)

L_o, optimal muscle length.
L_o was 7·9 mm (S.E. = 0·09 mm), twitch force at L_o was 21·7 N cm^{-2} (S.E. = 1·09 N cm^{-2}).
Values are given as mean (±S.E.), $N = 6$–7.

S.E. = 1·9 %, $N = 7$). The relationship between the active force and muscle length is a fairly broad curve, with the 50 % point of the rising and falling limbs occurring at 80 $\%L_o$ and 130 $\%L_o$, respectively (Fig. 5). The active force in Fig. 5 is shown relative to the preceding contraction at L_{ref}, to compare the forces at different muscle lengths without interference from the effects of fatigue or injury. This curve may be artificially elevated at lengths above 125 $\%L_o$ because the tetanic and twitch tension at return to reference lengths was depressed (Fig. 4), and reduction of the control tetanic tension increases relative forces which are based on them as a reference value. The loss of active force following long stretch may have been a result of tearing of muscle membranes (sarcolemma or sarcoplasmic reticulum), or of disrupting part of the tracheal or nerve supply to the muscle. The depression of active force was partly reversible. After 4–10 min at L_{ref}, twitch force typically increased by 15–20 % over that immediately following the return from stretch.

The maximum twitch tension (P_{tw}) occurred at a muscle length that was slightly longer than that at which the tetanic tension was maximum (mean difference = 0·33 mm, S.E. = 0·07, $N = 6$). The ratio of twitch to tetanic tension (P_{tw}/P_o) in the Tcx_2 was moderately high, averaging 0·67 (S.E. = 0·02, $N = 7$) at the *in vivo* length. There was a gradual decrease in the control P_{tw}/P_o over the course of most experiments, possibly reflecting gradual muscle fatigue. The active twitch force curve dropped more steeply at lengths below L_o than did that of tetanic force (Fig. 4, Table 2), but less rapidly as muscle length was increased above L_o. The different shapes of the twitch and tetanic curves resulted in an increase in P_{tw}/P_o as muscle length was increased (Table 2).

Twitch time course

Twitch amplitude and time course are both functions of muscle length (Fig. 6). Peak twitch force was lower and relaxation time was longer in the stretched muscle than at L_{ref} (Fig. 7). There was little change in twitch rise time except at long muscle lengths, when there was a small (not statistically significant) increase in twitch rise time.

Measured latency between stimulation and force onset depends on such things as electrode placement and stimulation intensity. Although the value for latency

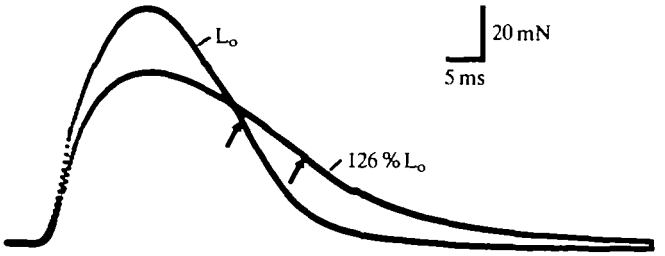

Fig. 6. Twitches at L_o and 126 % L_o. The twitch at L_o was recorded 2 min after that at the longer length. The resting tension of the muscle at the two lengths has been made to coincide to facilitate comparison of the two twitches. The arrows are at half maximum force.

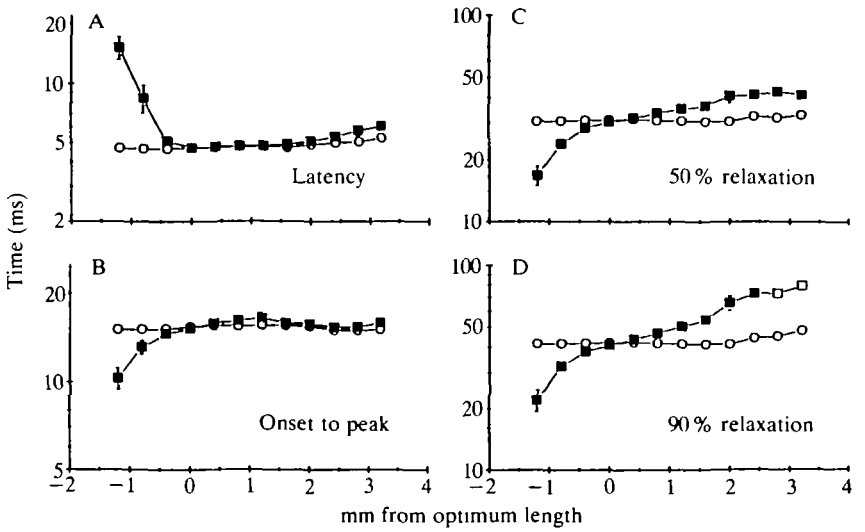

Fig. 7. Twitch contraction and relaxation times in the Tcx_2 as a function of muscle length (25 °C). Solid squares are average values from six animals at the muscle lengths shown on the abscissa. The circles are the preceding reference-length values. In two preparations at L_o+2·8 mm and one at L_o+3·2 mm the twitch duration to 0·9 relaxation was longer than the data sampling period (about 96 ms). The estimated 0·9 relaxation time was close to 98 ms, so that value was arbitrarily used for these preparations. Therefore, the mean 0·9 relaxation time at the longest lengths (open squares) are underestimates. Standard errors are shown in the cases where they are larger than the symbols. Time is plotted logarithmically so that similar relative changes in the parameters will result in similar displacement from the reference values. At very short lengths, the onset of measured force is delayed until existing slack is taken up by the contracting muscle. Therefore, the latency (stimulus to force onset) is overestimated and the contraction time from force onset to twitch peak is underestimated at these lengths.

varied from preparation to preparation, in a single preparation at a given length it was reasonably constant. The stimulus-to-response latency increased slightly at longer muscle lengths (Fig. 7).

Discussion

Stress relaxation in a stretched resting muscle is initially rapid but becomes progressively slower. The equivalent, but reversed, changes can be observed after a stretched muscle is returned to rest length. The shortened muscle is slack at first, and several minutes elapse before the usual resting force is re-established. Stress relaxation in a stretched muscle, as well as in a muscle returned to rest length after the stretch, is hastened by muscle contractions (Ullrick, 1970). The tension decay in the stretched Tcx_2 muscle was continuous. As was noted in early mechanical studies of frog muscle (Buchthal *et al.* 1944), the passive length–tension curve for a muscle depends on when, after the end of stretch, the measurements were made. The relaxation processes which became dominant late in the relaxation of the Tcx_2 had long time constants, and there was no evidence for a non-zero asymptote – implying that the resting tension in the stretched muscle would eventually decay almost completely. Tension in a stretched, unstimulated muscle is classically described as being due to stretching a 'parallel elastic component' (see Hill, 1949; Jewell & Wilkie, 1958). Since the passive muscle tension showed viscoelastic components, with none that were strictly elastic, a better term for the parallel elastic component would be the parallel stiffness component.

It has not yet been determined which components bear the passive tension of a stretched muscle. Ramsey & Street (1940) suggested that the equilibrium resting tension of stretched muscle was due to the sarcolemma, and that the 'muscle substance itself behaves plastically'. This early view has been displaced, because of evidence suggesting that most of the resting tension resides in the myofibrils (Buchthal & Weis-Fogh, 1956; Podolsky, 1964; Ullrick, 1970; Magid & Law, 1985). However, the source of the stiffness in the myofibril has yet to be firmly identified. A very thin filament was seen connecting the A-filament to the Z-line in insect fibrillar flight muscle (Auber & Couteaux, 1963, cited by White, 1983) and was thought to be the basis for the high resting tension of that muscle (White, 1983). Connecting filaments have now also been seen in vertebrate skeletal muscle (Magid *et al.* 1984). It has been proposed that C-filaments, containing the very large proteins nebulin and titin (Horowits *et al.* 1986), are responsible for much of the passive tension, as well as for keeping the thick filament centered in each sarcomere.

Stress relaxation in vertebrate and arthropod skeletal muscle has been described as being a time-dependent process, fitted by a fast and a slow exponential term (Hoyle, 1983; Chapple, 1983, 1987; Magid & Law, 1985). In the locust Tcx_2, however, more than two time-dependent terms were required to fit the data points. The difficulty of adequately fitting data with multiple exponential terms either by curve peeling or by nonlinear least-squares regression analysis is well

known (Riggs, 1963; Landaw & DiStefano, 1984) but it is clear that two exponential terms, found either by exponential curve peeling or by nonlinear regression analysis, failed to fit my data adequately (see Fig. 2). At least four exponential terms are needed to fit stress relaxation over a 15 min period, and it is possible that more terms would be needed to fit force decay over longer periods.

Many biomaterials such as collagen, and even crystalline polymers such as polyethylene, show multiple-component relaxation spectra rather than simple one- or two-exponential relaxation curves (Wainwright *et al.* 1976). It is proposed that the different domains of these large molecules relax at different rates, which leads to multiple-component relaxation spectra. The relaxation curve formed by multiple spectra will, under some circumstances, be fitted by a power function ($F = At^{-K}$). A power function is linear when both force and time are plotted on logarithmic axes. A power function was used to describe the stress relaxation of an insect fibrillar flight muscle (White, 1983). A power function did not, however, fit stress relaxation in the Tcx_2. When plotted logarithmically, the data diverged from a straight line at both short (less than about 30 s) and long (greater than 2–3 h) times.

The maximum active tension in the Tcx_2 is about 32 N cm^{-2}, which is somewhat greater than most values reported for frog muscle [e.g. for frog sartorius: 20 N cm^{-2} at 2°C (Jewell & Wilkie, 1958); 33 N cm^{-2} at 20°C (Close, 1972); 24 N cm^{-2} at 25°C (Renaud & Stevens, 1981); 27 N cm^{-2} at 25°C (Rome, 1983)]. The relationship between active force and muscle length (Figs 4 and 5) was not as broad as for some frog muscles, but the active force curve was less sharply peaked than that reported for the locust DLM (Weis-Fogh, 1956). The muscle force was $0{\cdot}5P_o$ or more over a range equal to 50 % of the optimal muscle length in the Tcx_2. The comparable range in the DLM is 40 % of the optimal muscle length (Weis-Fogh, 1956). Whole frog sartorius develops $0{\cdot}5P_o$ or more over 53–62 % of its optimal muscle length (Wilkie, 1956; Aubert *et al.* 1951).

The twitch tension was maximal at a muscle length that was slightly longer than the *in vivo* length. The length–tension curve for twitches was somewhat narrower than that for the tetanic contractions, but the whole length–tension curve for twitches was shifted to the right, so that at long muscle lengths the maximum twitch force more closely approached the tetanic force (Fig. 4). A pronounced increase in muscle relaxation time (Figs 6 and 7) occurred at long muscle lengths, and was evident in both the twitch and tetanic contractions. Similar increases in twitch relaxation times have been reported for the frog sartorius (Close, 1972) and a katydid wing muscle (Josephson, 1973).

An increase in the duration of the active state would increase P_{tw}/P_o in a muscle, and could also increase the relaxation time. The longer active period would allow more time for force development – hence the larger P_{tw}. An increase in the duration of the active state would not, of itself, affect the tetanic tension, since the muscle is already fully active at the plateau of tetanic force development. A direct correlation has been found between muscle length and the duration of the active state in the frog sartorius (Ritchie, 1954). However, if the active state were

to become more prolonged, the time to peak tension would be expected to lengthen, which did not happen in the locust Tcx_2 (nor in the katydid wing muscle, Josephson, 1973).

The lengthened period of relaxation and the higher twitch-to-tetanus ratio in the Tcx_2 can best be explained by an increased calcium sensitivity in the muscle. It has long been known that a stretched mammalian heart beats more strongly (the Frank–Starling mechanism). Myofilament calcium sensitivity has now been shown to be increased by stretch in cardiac muscle as well as in a number of skeletal muscles (Ruegg, 1987; Stephenson & Wendt, 1984), and may be modulated by troponin C (Babu *et al.* 1988). If the muscle were more sensitive to calcium, the force at equivalent calcium concentrations would be higher and the muscle would develop more force and relax more slowly even though there were no changes in the liberation and sequestration of calcium by the sarcoplasmic reticulum. The increase in calcium sensitivity at long lengths is much greater in partially activated skinned muscle fibers than in fully activated fibers (Stephenson & Wendt, 1984), which correlates well with the increased twitch-to-tetanus ratio and the longer muscle length of the maximal twitch tension than of the maximal tetanic tension that was seen in the Tcx_2.

Recently, considerable attention has been given to high-frequency stiffness of muscles (e.g. Farrow *et al.* 1988; Tsuchiya & Sugi, 1988; for comparisons of high-frequency stiffness and static tension see Haugen & Sten-Knudsen, 1981; Schoenberg & Wells, 1984). Since high-frequency muscle stiffness was not measured for the locust Tcx_2, it is not known if the Tcx_2 is similar or dissimilar to vertebrate striated muscle in the high-frequency domain. However, the static resting tension of the locust Tcx_2 is less than that reported for the locust DLM and

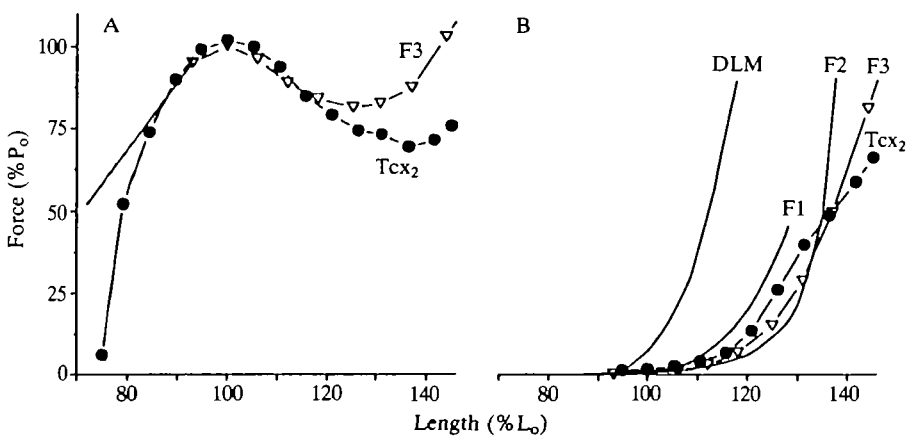

Fig. 8. Active and passive force as a function of muscle length. (A) Total tension during a tetanic contraction in a locust wing muscle (Tcx_2) and a frog sartorius muscle (F3). The frog values are from Aubert *et al.* (1951). (B) Passive tension in locust wing muscles and frog leg muscles. DLM, locust dorsal longitudinal muscle (after Weis-Fogh, 1956); F1, F2, F3, frog sartorius muscles (F1 from Wilkie, 1956; F2 from Hill, 1970; F3 from Aubert *et al.* 1951).

is not dissimilar to that found in whole frog muscle (Fig. 8B). It is thus incorrect to describe insect muscles as being unusually stiff and inextensible. Since the length–tension relationships for both active tension (Fig. 5) and passive tension (Fig. 8B) in the Tcx_2 are similar to that of a typical vertebrate skeletal muscle, it is hardly surprising that the total tension relationships (Fig. 8A) also are not substantially different in the frog leg muscle and the insect wing muscle.

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