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# THE ELASTICITY OF RELAXED INSECT FIBRILLAR FLIGHT MUSCLE

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SUMMARY

1. The mechanical properties of glycerol-extracted fibres from the dorsal longitudinal muscle of *Lethocerus* have been determined by sinusoidal and transient analysis in the time range 1 ms–1000 s, and from rest length to 10% strain for fibres in relaxing and rigor solutions. The fibres behave reversibly up to strains of about 5%, but reach an elastic limit in the range 5–9% strain, depending upon the rate of strain.

2. Electron micrographs of fibres at different degrees of stretch, and after partial extraction of the contractile proteins, suggest that a connexion between the end of the A filament and the Z line, named a C filament, is responsible for the high stiffness of the relaxed muscle.

3. Estimates are made of the compliance of the A, I and C filaments. The mechanical response of the relaxed muscle, over the entire frequency range studied, is assignable to the C filaments.

4. An analysis of the stiffness of the fibres at different tensions in activating and relaxing solutions, and in fibres relaxed by orthovanadate, shows that the C filaments still exert their mechanical effect in the active muscle. That is, the response of the active muscle consists of the contribution from the cross-bridges plus that of the C filaments, acting mechanically in parallel. This situation is incompatible with earlier explanations of the fully activated mechanical dynamics of fibrillar muscle. Alternative explanations at the cross-bridge level are described in the paper that follows this one.

## INTRODUCTION

The asynchrony between the nervous input and the mechanical output of insect fibrillar flight muscle was first described by Pringle (1949), and the properties of this muscle have been the subject of considerable study since that time (see reviews by Pringle, 1967, 1978; Tregear, 1975; White & Thorson, 1973).

A characteristic property of fibrillar flight muscle is that even when fully relaxed and at normal body length the muscle is mechanically stiff (Machin & Pringle, 1959; Boettiger, 1960). One important effect of this high stiffness is that it contributes considerably to the stiffness of the mechanically resonant system determining wing-beat frequency.

The stiffness of the relaxed muscle is comparable to that observed in the active muscle (Machin & Pringle, 1959). This is in marked contrast to the stiffness of relaxed

vertebrate striated muscle, which is negligible by comparison with that of the insect (Hill, 1968). There is no connective tissue around fibres of the insect muscle like that found in vertebrate muscle (Tiegs, 1955); further, the high stiffness is still present in the glycerol-extracted fibres, in which the membrane systems have been largely disrupted (Jewell & Ruegg, 1966), and small bundles of myofibrils, dissected from a single fibre from the giant water bug *Lethocerus*, likewise still show the high stiffness when relaxed (Thorson & White, 1975). We can conclude therefore that the structure responsible for the high stiffness is within the myofibril; as discussed by White (1967) and Pringle (1974, 1977) there are several possible locations for it.

(i) *A structure within the myofibrils in parallel to the filaments.* Maruyama, Matsubara, Natori, Nonomura, Kimura, Ohashi, Murakami, Handa & Eguchi (1977) described a structure, which they named connectin, which remains after the A and I filaments have been extracted; they isolated connectin from a variety of sources both vertebrate and invertebrate. *Lethocerus* had the least amount of connectin of any of the muscle studied (Maruyama, Cage & Bell, 1978), yet this muscle has the highest stiffness.

(ii) *A structure linking the ends of the I filaments.* Such a structure was first proposed by Huxley & Hanson (1954) to account for the integrity of the sarcomere after A-filament extraction, and was termed S filaments. However, no corresponding structure has been described following electron microscopy.

(iii) *Cross-bridges linking the A and I filaments.* The small viscoelastic properties of vertebrate striated muscle have been ascribed to a special form of cross-bridge activity in the relaxed muscle (Hill, 1968; Lannergren, 1971), and the finding that the myofibrils of insect flight muscle have a significant concentration of paramyosin (Bullard, Hammond & Luke, 1977), which was first described in the 'catch' muscles of molluscs, suggests a possible correlation between paramyosin and the ability to form long-lived cross-bridges. Both Reedy & Garrett (1977) from their electron microscope studies, and Barrington-Leigh, Goody, Hoffman, Holmes, Mannherz, Rosenbaum & Tregear (1977) from their X-ray observations, suggest that there may be residual cross-bridge attachment in relaxed muscle, and imply that this might be responsible for the mechanical properties of the relaxed muscle.

(iv) *A structure linking the A filaments to the Z line.* Such a structure was first described by Auber & Couteaux (1963) from electron micrographs of blowfly muscle, and there have been a number of papers since then purporting to show the existence of these connexions (Garamvolgyi, 1965; Trombitas & Tigyi-Sebes, 1974, 1975, 1979). However, there has been no unequivocal demonstration in the relaxed insect flight muscle that such a structure, which has been called a C filament (White, 1967), exists. The evidence has been well summarized by Ashhurst (1977).

(v) *Steric impedance to stretch without permanent connexions.* Wray (1979) has shown that if the cross-bridges of the A filaments of insect fibrillar flight muscle rise on a four-start helix, then the periodicity of the cross-bridge helix on the A-filament lattice is the same as the helical repeat of the I filament. If the I filament contains a molecule which projects away from the filament at this same repeat (for example the troponin), then there may be steric hindrance between the A and I filaments opposing longitudinal displacement. This would show up as a viscoelasticity. If the steric hindrance were sufficiently severe then tension changes resulting from small displacements could be long lasting.

The purpose of this paper is to characterize the mechanical properties of the resting elasticity of the dorsal longitudinal muscles of bugs of the genus *Lethocerus*, to provide further evidence that the structure responsible is a connexion between the ends of the A filament and the Z line, and to discuss the role of this stiffness in the response of the active muscle.

Some of this work formed part of my D.Phil. thesis (White, 1967) and some other parts have been published in short notes (Donaldson & White, 1977; White, Donaldson, Pearce & Wilson, 1977; White, Wilson & Thorson, 1978).

## METHODS

### *Muscle preparation*

*Lethocerus cordofanus* was obtained from Uganda, *L. maximus* from Trinidad and *L. indicus* from India. The dorsal half of the thorax was removed, and the dorsal longitudinal muscles, still attached to the rigid thoracic box, were immersed in glycerol solution (50% glycerol (v/v), 20 mM-phosphate buffer pH 7·0, 3 mM-sodium azide plus, in the experiments indicated, 2% Tween 80) at 0 °C and evacuated with a filter pump for 10 min to remove air from the trachea. The fibres were kept in glycerol solution at 0 °C for 24–48 h (changing the solution after 1 and 6 h) and were then stored in fresh solution at −16 to −20 °C until used.

### *Mechanical apparatus*

The mechanical experiments were carried out on apparatus similar to that described elsewhere (White, 1970). Bundles of between one and ten fibres were stripped from the main body of the muscle and mounted between two arms using cellulose acetate dissolved in acetone as glue. One arm was connected to the moving coil of a Ling Dynamics electromagnetic vibrator (type 101) whose output was directly monitored and servo-controlled. The stiffness of the vibrator was 25 N $mm^{-1}$. The other arm was connected to a tension transducer. This was either an RCA valve (type 5734) with a glass extension on the anode to increase sensitivity, or a silicon strain gauge (Akers, type 801). Two lengths of glass extension arm were used with the RCA valve. With the longer, used for the experiments in which the time course of tension decay was measured for periods of 1000 s, the stiffness was about 0·2 N $mm^{-1}$ and the resonant frequency about 250 Hz. With the shorter arm the stiffness was about 0·45 N $mm^{-1}$ and the resonant frequency 480 Hz. The stiffness of the Akers gauge was 3·2 N $mm^{-1}$, and the resonant frequency, loaded with fibres, was 2·7 kHz. The RCA valve was underdamped; the Akers gauge was critically damped.

### *Procedure*

The fibres were immersed in the required solution in a temperature-controlled bath. A 3 or 5 mm length of fibre was used. The compositions of the three types of solution, relaxed, activating and rigor, are shown in Table 1. Variations from these solutions are noted, when relevant, in the Figure legends.

The following procedure was used for the mechanical experiments on the relaxed muscle. The fibres were immersed in rigor solution, after attachment to the apparatus, to wash out the glycerol, and then transferred to relaxing solution. Slack length was found by applying a small (0·2%) periodic length change to the fibre and slowly varying the mean length of the fibres using the fine control on the micromanipulator holding the tension transducer, until tension was generated over about half the cycle of length change. Slack length could be set to an accuracy of about 0·2% of the muscle length. The fibre was then usually extended by 1% above slack length. When measurements were made on the muscles in rigor, the fibres were transferred to the rigor solution at 1% above slack length in relaxing solution, and rigor tension allowed to develop whilst the muscle was held isometrically (White, 1970). The active properties of the muscle were always recorded to ensure that the fibres were behaving normally.

Length and tension records were monitored on a cathode ray oscilloscope, and the traces photographed with a 35 mm camera. A continuous paper chart recording was made of all experiments using a Devices pen-recorder (band width 70 Hz) type M4 or MX2. Sinusoidal analysis was performed with a Solatron Resolved Component Indicator (type VP 253·3) or a Solatron

Transfer Function Analyser (type JM 1600). The servo-loop was driven by a minicomputer (Hewlett–Packard model 2114) for the step-response experiments, and the resulting 35 mm film record of the oscilloscope records measured by projecting the traces onto the table of a Hewlett–Packard 9864A Digitizer linked to a 9830 calculator.

*Electron microscopy*

For the electron microscopy the fibres were fixed, still attached to the mechanical apparatus, in the relevant solution containing 2·5% glutaraldehyde for 1 hr. They were then washed for 1 h in 50 mM-KCl plus 20 mM-phosphate buffer at pH 7·0 and post-fixed in 1% osmium tetroxide. The fibres were dehydrated with acetone, which also served to release them from the apparatus by dissolving the glue, and embedded in epoxy resin (Araldite, CIBA). Sections were cut with a glass knife, stained with 2% potassium permanganate for 15 min and further stained with lead citrate for 5 min. The micrographs were taken on an A.E.I. EM6B electron microscope.

TABLE 1. Solutions

All solutions, pH 7·0 and 20 °C.

| Solution | $MgCl_2$ (mM) | ATP (mM) | EGTA (mM) | $CaCl_2$ (mM) | KCl (mM) | Buffer (mM) | CP (mM) | CPK (mg/ml) |
|---|---|---|---|---|---|---|---|---|
| Relaxing | 5 | 5 | 5 | — | 50 | 20 | 10 | 2 |
| Activating | 5 | 5 | 5 | 5 | 50 | 20 | 10 | 2 |
| Rigor | 5 | — | 5 | — | 50 | 20 | — | — |

*Chemicals and solutions*

With the exceptions noted below chemicals were of Analar grade and supplied by either British Drug Houses or Fisons. ATP, creatine phosphate (CP) and creatine kinase (CPK) were obtained from Boehringer and Sigma. EGTA was supplied by Sigma.

The vanadate solutions were prepared less than 24 h before use. A stock solution of 10 mM-vanadate was prepared in the following way. The requisite amount of sodium orthovanadate was dissolved in water and boiled. The cooled solution was adjusted to pH 9 with HCl, and the solution boiled again. The pH was readjusted to pH 9. This procedure was continued until the cooled solution remained at pH 9 after boiling. This stock (colourless) was then used to make up the solutions (yellow) for muscle observations.

## RESULTS

### *Mechanical properties of the relaxed muscle*

(*i*) *Stress–strain relationship in the range 0–10% strain.* The glycerol-extracted fibres of *Lethocerus* have a well defined rest length in relaxing solution. Extension above this length results in the generation of tension, and passive shortening below this length in buckling of the fibres. The sarcomere length at this rest length is about 2·4 $\mu$m (White, 1967).

If a relaxed fibre is shortened up to a few percent below rest length and then transferred to activating solution, it straightens out and then generates tension. Presumably the A and I filaments slide past one another to produce these effects. When the fibre is returned to relaxing solution it buckles once again, and the rest length is unchanged.

When the fibre is extended above the rest length the extra tension exerted decays with time, as illustrated in Fig. 1, in which the tension is recorded for about 1000 s, following a 2% extension applied by rotating a micro-manipulator by hand. Note

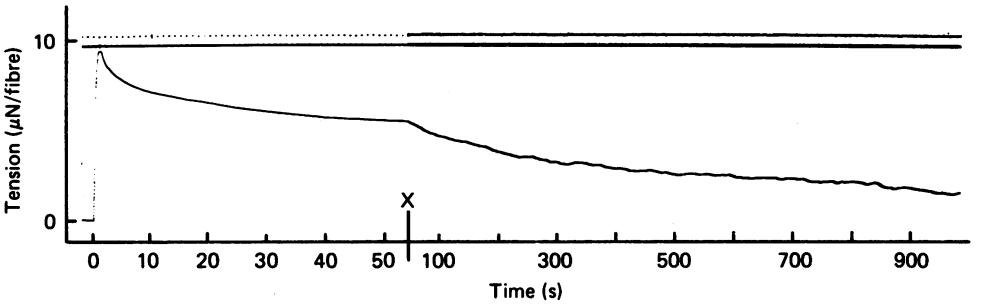


Fig. 1. Pen-recorder record of tension *vs.* time for a bundle of eight fibres of *L. cordofanus* stretched by 2% at time zero. The fibre had previously been stretched a total of 5% in steps of 1, 2 and 2% starting from rest length, allowing 1000 s stress relaxation at each length. The time marker shows seconds. The chart speed was slowed by a factor of 10 at X, illustrating the logarithmic nature of the relaxation.

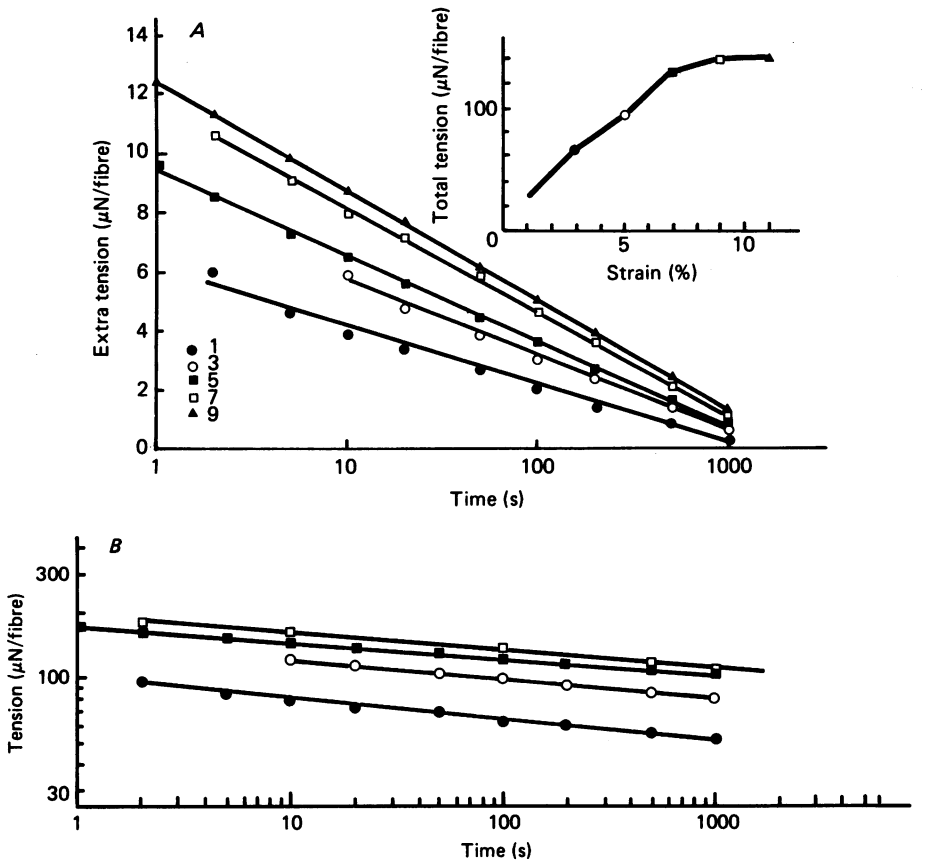


Fig. 2. *A*, stress relaxation plotted as a function of log (time) for a succession of 2% stretches of relaxed fibres, starting from 1%, as described in the text. The absolute position of tension of each trace is arbitrary; the inset shows the final tensions reached at each length. The figures indicate the percentage strain in the muscle during each relaxation. *B*, the stress relaxation of A plotted as log (tension) *vs.* log (time). The absolute values of tension are plotted.

that the speed of the chart-recorder is changed at the point marked X. In Fig. 2*A* the changes in tension are plotted against log (time) for a series of stretches of 2% in which the stress relaxation was followed for 1000 s between the successive stretches. The inset shows the tension–length curve derived from the final tension reached at each length. In Fig. 2*B* the data are plotted as log (tension) against log (time). Over the time range 1–1000 s the relaxation is well fitted by the relationship:

$$\text{Tension} = \text{constant} \,.\, \text{time}^{-k},$$

in which the value of $k$ is approximately 0·1.

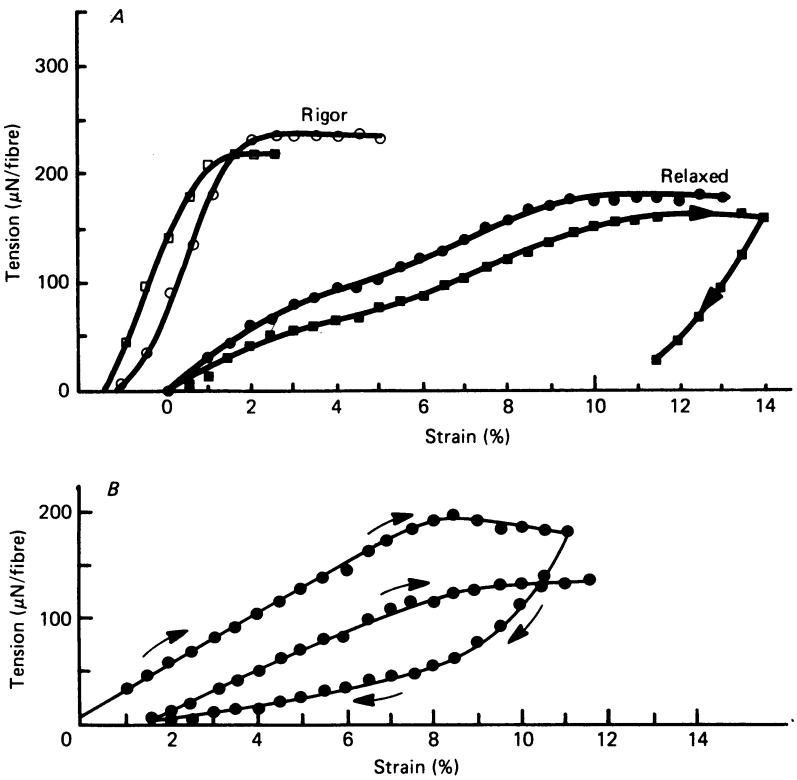


Fig. 3. Tension–length curves of relaxed and rigor fibres obtained as described in the text. *A*, comparison of the responses of relaxed and rigor fibres. The four traces are from different bundles of eight to ten fibres from *L. cordofanus*. *B*, response of a bundle of relaxed fibres to stretching by 11%, releasing and restretching.

Since no proper steady-state tension was obtainable over the time range of the experiments, it follows that any stress–strain relationship will be time-dependent. In the following determinations of the stiffness of the muscle the fibres were extended by 0·5% every 4 min and the tension at the end of this period measured. Fig. 3*A* shows the form of the tension–length curves obtained. The tension reaches a plateau after about 8% extension (8·2% ± 1·7%) with a tension of 15·8 ± 2·3 μN/fibre. Fig. 3*A* also shows two curves from fibres in rigor. If the relaxed fibres are extended to lengths

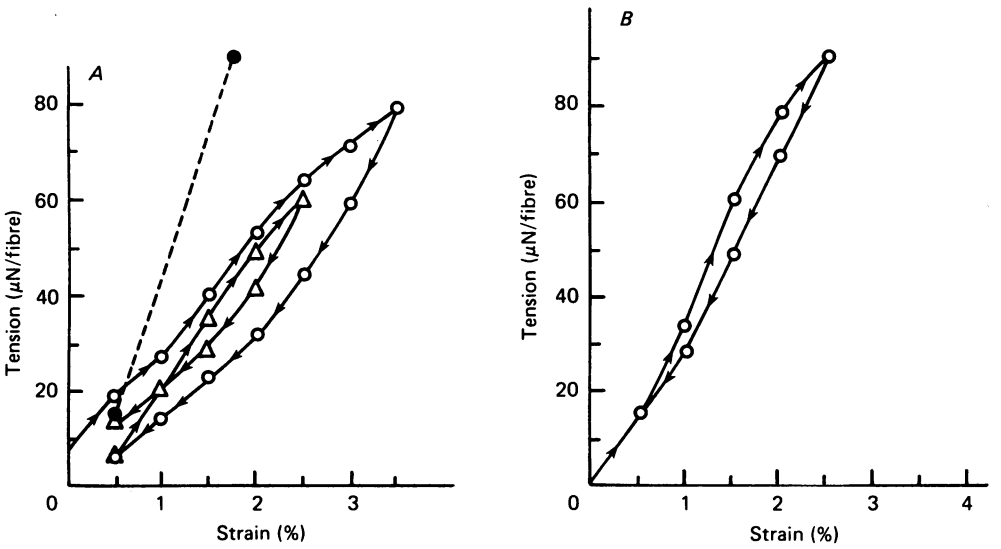


Fig. 4. Tension–length curves from bundles of relaxed fibres from *L. cordofanus* obtained as described in the text. *A*, successive stretch–release cycles from a bundle of seven fibres. ○: first and △: second cycle of response to successive 0·5% length changes at 120 s intervals. ●: response to a 1% ramp applied in 1 s. *B*, single stretch–release cycle from a bundle of nine fibres.

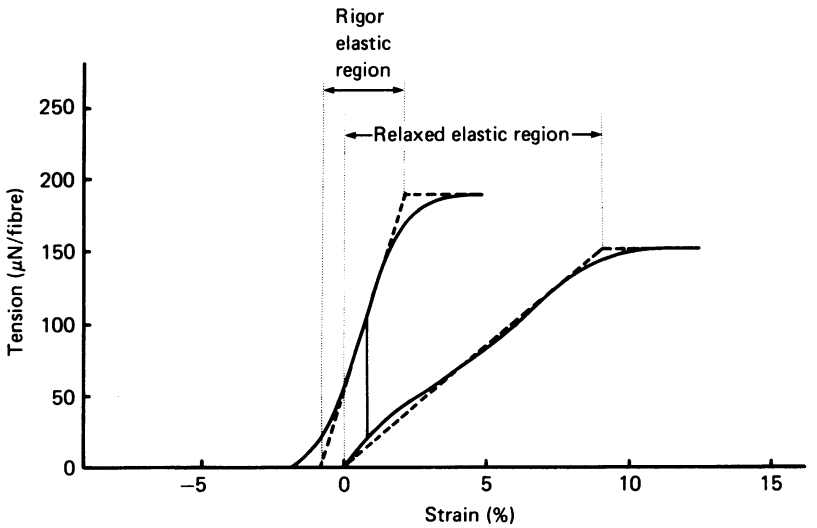


Fig. 5. Schematic tension–length curves of relaxed and rigor muscle, defining the elastic regions, for fibres stretched 0·5% every 4 min and allowed to stress–relax between stretches.

greater than the point at which the plateau is reached and then shortened, a considerable amount of hysteresis is obtained and the fibres do not regain the same rest length (Fig. 3*B*).

However, for strains up to about 5% the fibres behave in a reversible manner, in that when the fibre length is reduced again the rest length is unchanged. Furthermore,

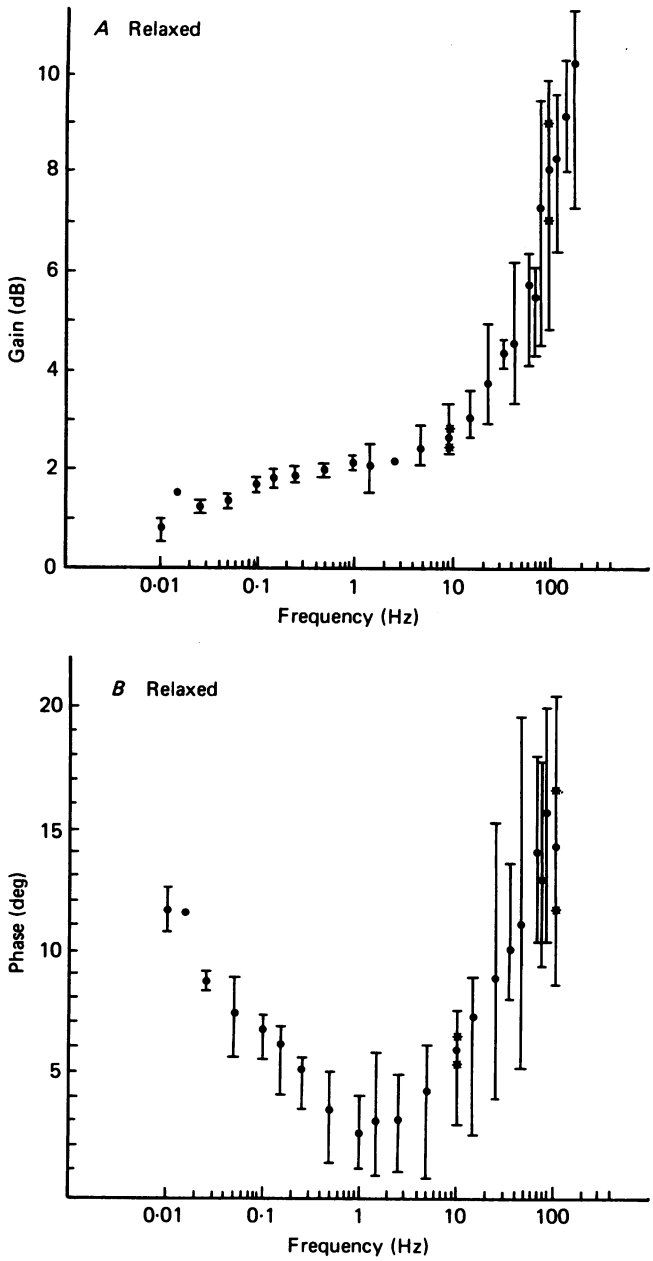


Fig. 6. For legend see opposite page.

the amount of hysteresis is small. Fig. 4 shows experiments in which fibres have been extended and shortened by amounts less than 4 %. The fibres were stretched by 0·5 % every 2 min up to the required degree of stretch and then released by 0·5 % every 2 min. Fig. 4*A* shows successive cycles on the same fibre bundle. The symbols represent the tensions measured after 100 s stress relaxation. The dashed line in Fig. 4*A* shows the tension–length relationship obtained on this same fibre bundle in response to a ramp increase of length at the rate of 1 % strain per second.

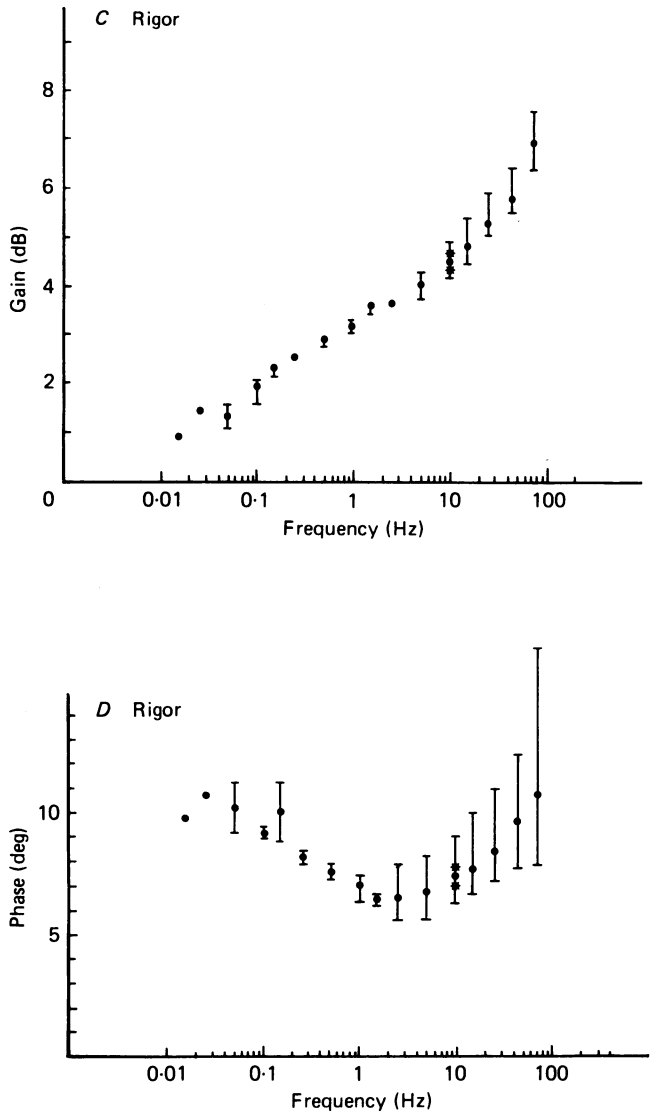


Fig. 6. Frequency response of relaxed and rigor muscle, plotted as Bode plots as described in the text. *A*, gain (in dB) *vs.* log (frequency) of relaxed muscle. *B*, phase *vs.* log (frequency) of relaxed muscle. *C* and *D* are the equivalent curves of rigor muscle. The absolute values of the gain in these diagrams are arbitrary, but are given in Fig. 7. The points indicate the mean, and the bars the range, of values obtained.

The stress–strain relationship of relaxed and rigor insect flight muscle is summarized in Fig. 5, which indicates the elastic regions of the muscle in these two states.

R. H. Abbott (see Pringle, 1974, 1977) measured the dynamic stiffness of relaxed fibres during the application of large strains by applying a small 5 Hz sinusoidal length change to the fibres and measuring the magnitude of the oscillatory change in tension. He found that the dynamic stiffness increased with strains up to 5–10 %, but when the strains exceeded 5–10 % the dynamic stiffness started falling. Abbott's

results, together with the findings of irreversible effects of strain above the plateau tension, indicate that irreversible effects occur at such strains, but that for moderate strains (less than about 5 %) the fibres behave in a reversible manner.

(*ii*) *Small-signal dynamic response.* The dynamic response of the relaxed muscle was determined by sinusoidal analysis in the frequency range 0·01–100 Hz. In these experiments the muscle length was driven sinusoidally at an amplitude $L_0$. For the experiments on relaxed muscle, $L_0$ was 0·1–0·5 %; for those on rigor muscle the

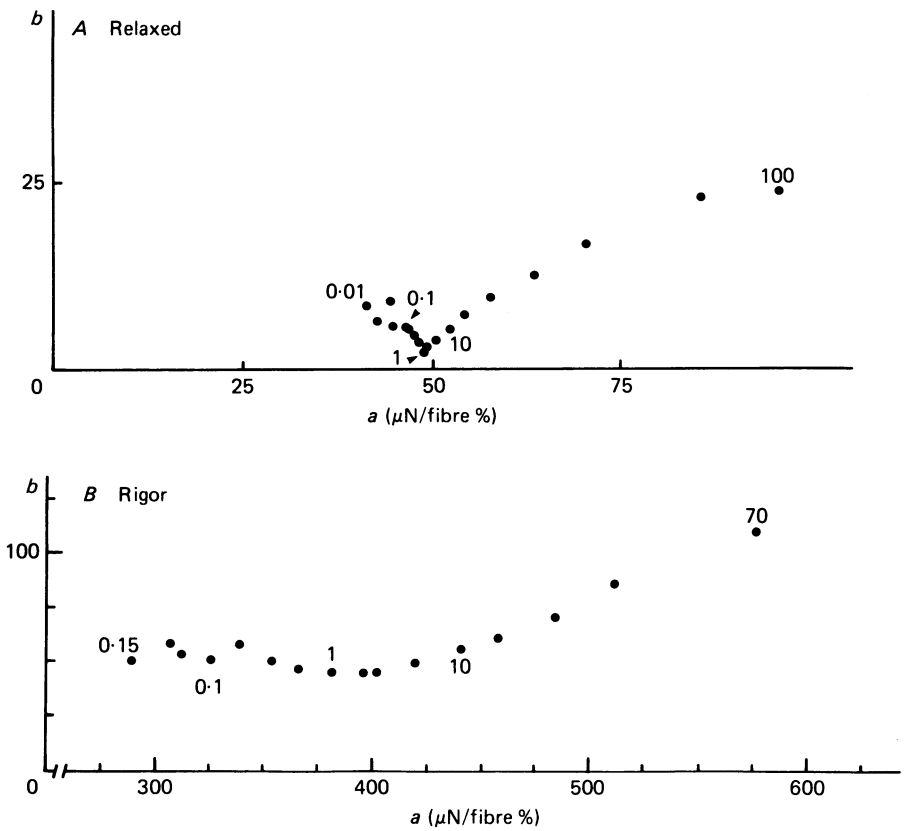


Fig. 7. Frequency responses of: *A*, relaxed and *B*, rigor muscle plotted as Nyquist plots.

amplitude was 0·025–0·1 %. The resulting tension was a sinusoidal signal at the same frequency as the driving frequency but shifted in phase with respect to the length by a phase angle $\theta_f$, and of amplitude $T_f$. For a constant amplitude of length change at different frequencies the amplitude of the tension varied with frequency. The dynamic stiffness $S_f$ of the muscle is $T_f/L_0$. The component of this stiffness in phase with the applied length change, $a_f$, and that 90° out-of-phase, $b_f$, can be determined from $a_f = S_f \cos(\theta_f)$ and $b_f = S_f \sin(\theta_f)$.

The responses obtained from the relaxed and rigor muscle are shown in Figs. 6 and 7. In Fig. 6 the results are plotted as Bode plots, in which the phase $\theta_f$ and the gain (here plotted in decibels as $20 \times \log(S_f/S_{2·5})$) are plotted against the logarithm of the

frequency. In Fig. 7 the results are plotted in Nyquist plot form, in which $a_f$ is plotted against $b_f$, the different points representing the different frequencies. Note the marked dip in the Nyquist plot of the relaxed fibres (and the corresponding minimum in the phase of the Bode plot) at 1 Hz.

*Structural studies*

The following experiments were attempts to distinguish between the above possible locations for the structure responsible for the resting elasticity. The first four of the possibilities discussed in the Introduction involve mechanical continuity between the Z lines. If this continuity is broken, then the high stiffness should disappear. It is possible to extract the A filaments from muscle with solutions of high ionic strength (Huxley & Hanson, 1957). If this procedure leaves the I filament intact, and does not extract S or T filaments, then this experiment distinguishes between possibilities (i) or (ii) and (iii) or (iv).

Fibres, attached to the mechanical apparatus, were irrigated with relaxing solution containing a total of 0·6 M-KCl. After 30 min the mechanical stiffness was reduced to an undetectable level. The fibres were fixed for electron microscopy; Pl. 1 *A* and *B* shows the appearance of the fibres. The A filaments have been dissolved in their central region. The I filaments appear intact. Thus it would appear that the A filaments are required for the high stiffness to be observed.

Irrigation with 1 M-KI (Gilmour & Robinson, 1964) produced fibres with the appearance shown in Pl. 1 *C*. The Z lines remain in approximately their normal positions, but there is no evidence for any filamentous material between them. The mechanical behaviour of fibres treated in this way is interesting. They could be extended to approximately double their rest length without eliciting detectable tension. Upon release, the fibres shortened back to their original length again (i.e. they did not simply buckle, as might be expected). Furthermore, despite there being considerable remains of mitochondria adjacent to the myofibrils, little entered the region between the Z lines, suggesting that some material remained between the Z lines of a non-filamentous nature preventing penetration of the vesicular remains. This structure must be very compliant mechanically, and could possibly be the connectin of Maruyama *et al.* (1978)

Evidence for possibility (iv), C filaments, comes from preparations of rigor muscle extended above their elastic limit. Plates 2 and 3 show micrographs from a fibre which was allowed to develop rigor from relaxing solution in which it was extended 1 % above rest length. The fibre was stretched a further 20 % after rigor had developed, and was then prepared for electron microscopy. Micrographs of longitudinal sections show 'clear zones' between the ends of the A–I filament array and the Z line. The I filaments have broken away from the Z line. These clear zones are irregular in occurrence and they never seem to exceed 1 $\mu$m in width. The Z line is frequently bordered by a clear zone on each side.

Longitudinal sections of the clear zones (Pl. 2) show a filamentous, dark-staining material, smaller in diameter than the I filaments. Transverse sections (Pl. 3) show clearly that this material is linked to the A filaments; the points of this material can be seen to lie on the A-filament lattice by viewing the micrograph obliquely.

If relaxed muscle is stretched by large amounts (greater than 30 %) then the regular

alignment of the A filament is lost (White, 1967; Pringle, 1977). Plate 4 shows the appearance of fibres stretched by 50 % in relaxing solution. The I filaments are clearly still aligned with respect to the Z line. Note that the I filaments appear to be buckled in these micrographs, although there is a straight and more electron-dense region immediately adjacent to the Z line.

The above evidence strongly supports the conclusion that the high stiffness of the muscle is due to C filaments linking the A filaments with the Z line. There must be some mechanically strong structures linking the A filaments to the Z line to cause the appearance of the overstretched rigor muscle; with no such structure the Z lines would not separate from the A–I filament array on *both* sides and the clear zone, once formed, would pull out indefinitely, yet it never exceeds about 1 $\mu$m. The filamentous structure in the clear zone is clearly linked to the A filaments. If the micrographs in Pl. 3 are viewed obliquely the filaments in the clear zone line up with the A filaments, and there is a one-to-one correspondence between them. If these structures were the pulled out portions of I filaments attached to A filaments this correspondence in number would not be expected.

The appearance of the highly stretched relaxed muscle (Pl. 4) also suggests a mechanical link between the A filaments and another structure. The A filaments are linked in *Lethocerus* flight muscle by a well structured M line. The misalignment of the A filaments seen in Pl. 4 requires these links to be broken. Such misalignment does not prove the existence of C filaments rather than residual cross-bridges, but C filaments provide a satisfactory explanation of this finding.

*The mechanical contribution of the structure responsible for properties of relaxed muscle to those of active muscle*

The steady-state tension in active *Lethocerus* muscle can be controlled by changing the calcium ion concentration or by changing the length of the muscle. In vertebrate muscle, under steady-state conditions, it is commonly held that the tension in the muscle is proportional to the number of cross-bridges that are attached at any instant; it has also been shown that a measure of this fraction of attached bridges in frog muscle can be obtained by measuring the stiffness of the muscle at such high frequencies that the biochemical/mechanical state of the cross-bridges does not have time to alter (Ford, Huxley & Simmons, 1981). Here we shall examine stiffness at different tensions under a variety of conditions, and compare the results with what one expects if the structure responsible for the high stiffness of the relaxed insect flight muscle also contributes mechanically to the active muscle. The relationship to be expected between stiffness and tension is derived in Appendix 1.

In earlier studies we have shown: (1) that during the delayed change of tension following a step change of length the relationship between stiffness and tension, measured by applying a small test step at varying times during the delayed tension change, is linear; the line, extrapolated to zero tension passes through the stiffness axis at a value approximately equal to the stiffness of the relaxed muscle (Donaldson & White, 1977; White, Wilson & Thorson, 1979); (2) that the same relationship measured during the rise of tension following calcium activation is identical to that measured during the rise of tension following a step change of length (White *et al.* 1979).

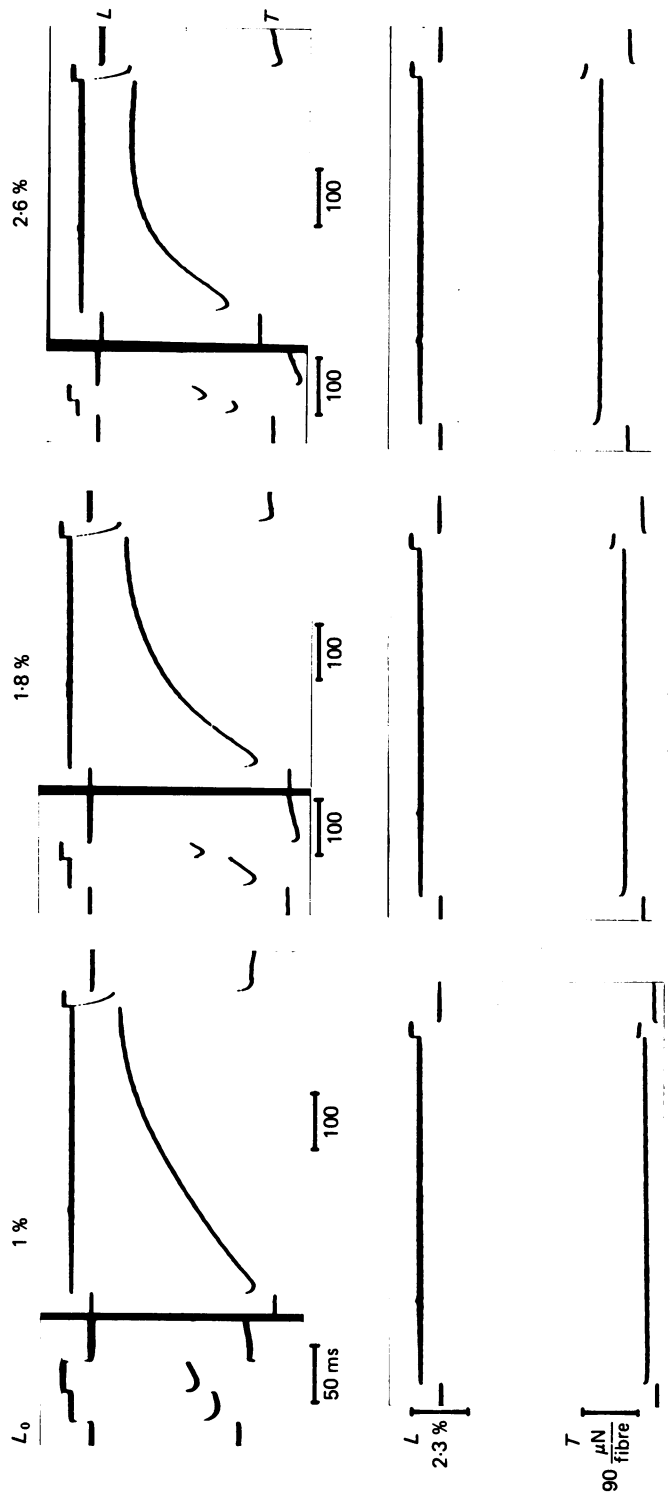


Fig. 8. The response of a bundle of two fibres of *L. maximus* to a conditioning step of 0·8 % followed by a test step of 0·3 % as described in the text. The top row are responses in activating solution, and the bottom row responses in relaxing solution. The three columns are responses starting from 1, 1·8 and 2·6 % above the rest length respectively. Zero tension was determined by applying large releases to the fibre. Two traces at each starting length are shown for the active response: one with a short delay between the two steps, and one with a longer delay showing the response at approximately the peak of the delayed tension.

The analysis of Appendix 1 shows that if the structure responsible for the stiffness of the relaxed muscle exerts its full effect in the active muscle, then the relationship between tension and stiffness measured at different starting lengths of the muscle will be displaced from one another unless the stiffness of the relaxed muscle increases with tension in a similar way.

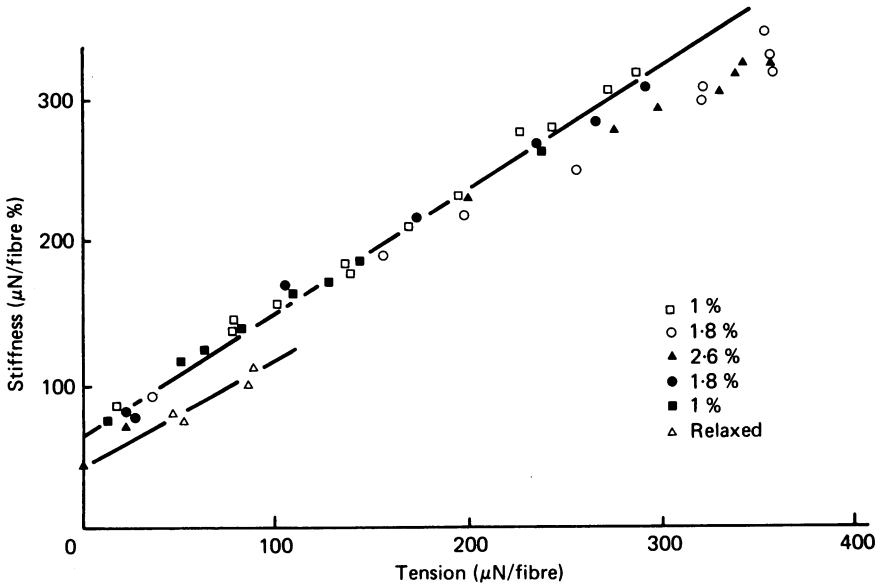


Fig. 9. Stiffness plotted against tension for both active and relaxed muscle during the change in tension after a 0·8 % step change of length for the experiment shown in Fig. 8. The experiment was repeated at three different starting lengths as described in the text. The figures against the points indicate the strain in the muscle immediately before the application of the 0·8 % step.

The results of an experiment in which the relationship between stiffness and tension was measured at different starting lengths is shown in Figs. 8 and 9. In this experiment the muscle was initially stretched 1 % above rest length in relaxing solution, and its stiffness measured by applying a 0·3 % 1 ms ramp length change and measuring the resulting instantaneous change in tension. The muscle was given a 0·8 % rapid stretch, and its stiffness measured during the stress relaxation with a further 0·3 % length change. The fibre was then activated, and its stiffness measured during the delayed change in tension following a 0·8 % increase in length, by applying a 0·3 % length change. In these experiments the stiffness was determined from the magnitude of the initial tension change occurring when the length was changed, divided by the magnitude of the length change.

The fibre length was then increased by 0·8 % and the whole procedure repeated at this new length. This was repeated after a further 0·8 % stretch, and then following each of two 0·8 % releases to ensure that the results were reproducible, and that there was no deterioration in the fibre. This sequence of length changes was repeated with the fibres in relaxing solution.

The relationship between stiffness and tension is shown in Fig. 9. The results from

all three lengths of the active fibre are linear, and are not significantly different from each other. The relationship between tension and stiffness of the relaxed fibre shows a similar slope to that of the active fibre. As shown in Appendix 1, if this same slope is shown by the relaxed muscle, then the relationship between stiffness and tension found for the active muscle will be obtained only if the structure responsible for the high stiffness of the relaxed muscle acts mechanically in parallel to the cross-bridges, and is unchanged in its contribution by the activation of the muscle.

*Effect of calcium ions on the resting elasticity.* In the experiments described above, the stiffness of the muscle was measured as rapidly as possible. The conclusion from these experiments, that the full mechanical effect of the relaxed muscle contributes to the response of the active muscle, is thus strictly valid only for these very short times. It is possible that calcium ions affect the structure responsible for the passive elasticity in such a way that the high frequency stiffness is unaffected, but the low frequency stiffness is much reduced.

We require a method of investigating the effect of calcium ions upon the mechanical properties of the relaxed muscle in the absence of any activation of the cross-bridge cycle. The inhibition of actin–myosin interaction by orthovanadate enables this test to be done. Goodno (1979) demonstrated that vanadate acted as a potent inhibitor of myosin ATPase by forming an extremely long lasting state: M . ADP . $V_i$, and Goodno & Taylor (1982) showed that the association constant of the M . ADP . $V_i$ state for actin is smaller than that of M . ADP . $P_i$. Goody, Hoffman, Reedy, Magid & Goodno (1980) showed that glycerol-extracted insect flight muscle became relaxed when 5 mM-vanadate was added to the activating solution, presumably because of the formation of the M . ADP . $V_i$ state which is dissociated from the actin filament. Goody *et al.* (1980) state that the muscle stiffness is also reduced to the value obtained in the relaxed muscle, but they do not state over what range of frequencies they measured the stiffness.

Muscle fibres inhibited with orthovanadate can thus be used to test the effect of calcium ions upon the structures responsible for the high stiffness of the relaxed muscle. Figs. 10 and 11 illustrate an experiment in which the mechanical properties of the muscle were tested before and after inhibition by 5 mM-vanadate, and in the presence of low and high concentrations of calcium ions. The concentrations chosen were those that would give fully relaxed muscle and fully calcium-activated muscle.

As can be seen from Fig. 11 there is no significant difference in the mechanical properties of the vanadate-relaxed muscle and the normal relaxed muscle, and moreover, calcium ions have no detectable effect on the properties of the relaxed muscle under conditions in which cross-bridge activation is inhibited. Goodno & Taylor (1982) observed a 10–20-fold change in regulated actin association between low and high calcium-ion concentrations. The lack of effect of calcium on the mechanical response of vanadate-treated muscle is further evidence that the cross-bridges are essentially detached in relaxed muscle.

We can draw two conclusions from this experiment: (1) the high stiffness of the relaxed muscle is not due to cross-bridges and (2) calcium ions, at the concentrations required to activate the muscle, produce no change in the mechanical properties of the structure responsible for the high relaxed stiffness over the considerable frequency range sampled by the step responses.

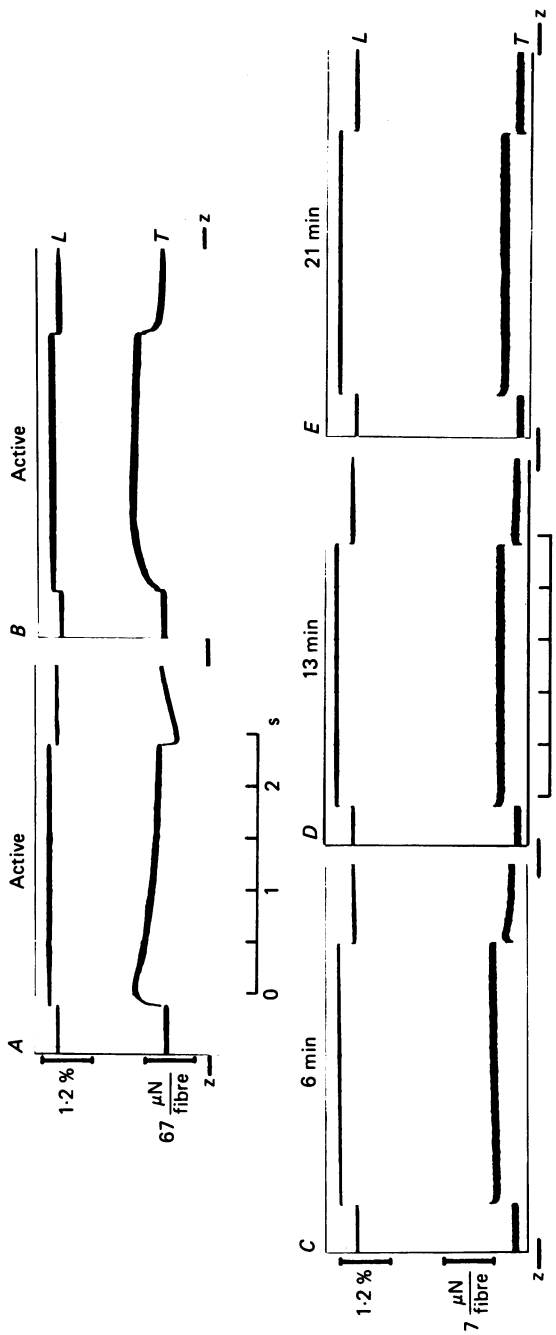


Fig. 10. *A* and *B*, the response of a bundle of three fibres of *L. indicus* in activating solution at two different sweep speeds, to show the normal response before the addition of vanadate. *C*, *D* and *E*, the response of the fibres after 6, 13 and 21 min in vanadate solutions. The fibres were transferred to a vanadate relaxing solution for 4·5 min and then to vanadate activating solution. The fibres still show traces of activity at 6 and 13 min. Solutions (mM): activating – $MgCl_2$, 5; Ca EGTA, 5; KCl, 55; histidine, 20; ATP, 10; pH 7·0; 20 °C. Vanadate relaxing – $MgCl_2$, 10; EGTA, 5; KCl, 48; vanadate, 5; histidine, 20; ATP, 10; pH 7·0. Vanadate activating solution is the same as vanadate relaxing solution but with 5 mM-Ca EGTA in place of EGTA.

## DISCUSSION

### *The structural basis of the resting stiffness of fibrillar muscle*

Five possibilities were proposed above for the localization of the structure responsible for the resting elasticity: (i) T filaments or connectin, (ii) S filaments linking the ends of the I filaments, (iii) cross-bridges linking the A and I filaments, (iv) C filaments linking the A filaments to the Z line and (v) steric hindrance between repeating structures along the length of the A and I filaments

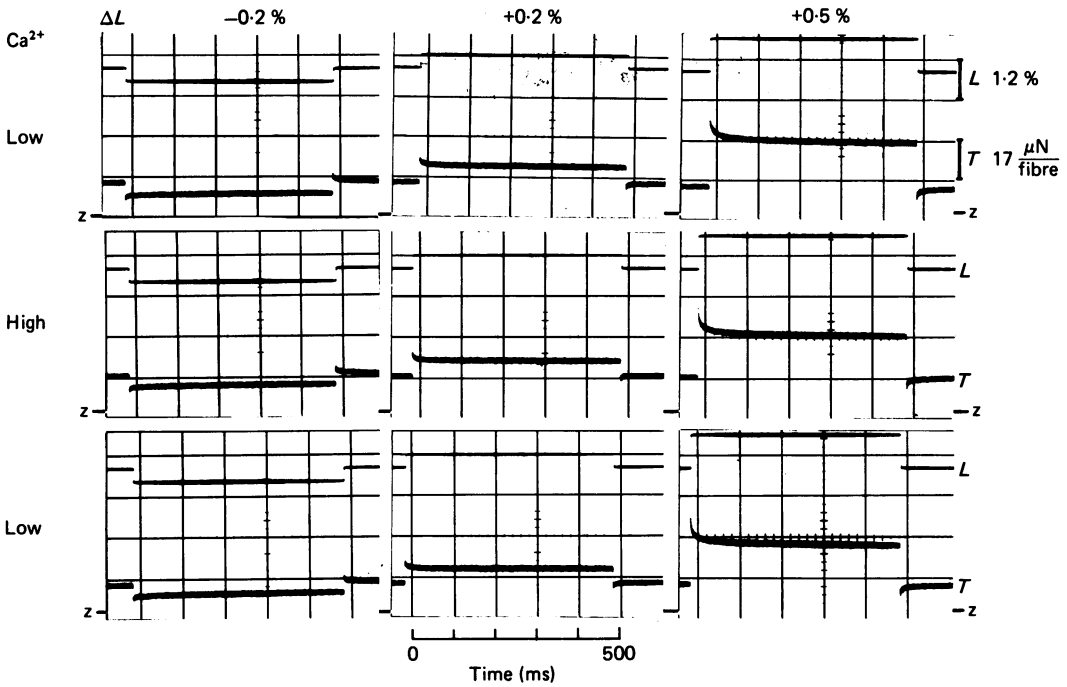


Fig. 11. The effect of $Ca^{2+}$ upon the step response of fibres in 1 mM-vanadate solutions. The rows of records were obtained in the order laid out. Calcium ions have no detectable effect upon the response. The low and high $Ca^{2+}$ concentration solutions are the vanadate relaxing and vanadate activating solutions, respectively, of Fig. 10, giving $Ca^{2+}$ concentrations that in the absence of vanadate would produce relaxed and maximally activated muscle, respectively. z indicates zero tension.

We can dismiss (i) on the basis that the fibrillar flight muscle has less connectin than any other muscle, and that apart from one brief note (Hoyle, 1966), filaments parallel to the A and I filaments running from Z line to Z line have not been reported. We shall dismiss (ii) on the basis that S filaments have never been observed. Their existence was proposed to explain the integrity of the structures remaining after extraction of the thick filaments (Huxley & Hanson, 1954), and this role would equally well be performed by connectin.

For proposal (iii) there would have to be two types of cross-bridge: those that operate normally (i.e. producing the active response) and those that are responsible for the resting elasticity. The evidence for this is that vanadate, which complexes

with myosin to produce a long-lasting detached cross-bridge, does not affect the resting stiffness. If some form of cross-bridge is responsible for the resting stiffness, then it would have to be unaffected by vanadate. However, the cross-bridge responsible for the relaxed state on this hypothesis could not be a permanently attached structure, since the extensibility of the muscle (about 9 %) is too great to be obtainable from a cross-bridge. Until such time as two types of myosin can be demonstrated from fibrillar muscle, this remains a very doubtful possibility.

It is more difficult to exclude the steric hindrance proposal (v). The 9 % extensibility is difficult to account for, given that the relevant periodicities on the A and I filaments are approximately 3 % of the half-sarcomere length (38·5 nm in a half-sarcomere about 1·2 $\mu$m long). Although the tension–length curve of the relaxed muscle does show periodic changes in slope (with two maxima in the elastic region (see Fig. 3*A*), this periodicity also is much greater than that of the structures, and for this reason we exclude this proposal.

There is now a considerable body of evidence that the structure responsible is the C filament. It would appear that in relaxed muscle at rest length, any material in the I region stains rather poorly, but that if the sarcomere is extended a stainable material is seen. This procedure will have taken the myofibril beyond its elastic limit, and it would appear that the structure in this region takes up stain only when internal bonds are broken by strain. Several authors have shown material in the correct location in highly stretched muscle, (Pls. 2 and 3; Garamvolgyi, 1965; Trombitas & Tigyi-Sebes, 1974) and although it is difficult to be sure that this material is not I filaments in relaxed muscle, it is more difficult to explain away the structures seen in this paper in the clear zones of overstretched rigor muscle, or in the micrographs of Trombitas & Tigyi-Sebes in which the actin has been disrupted.

Furthermore, Bullard *et al.* (1977) have demonstrated an extra protein which lies between the Z line and the A filaments, by staining with antibody, and Sainsbury & Bullard (1980) tentatively attribute the 180 kDalton and possibly the 105 kDalton proteins which they extract from Z disks to this connecting material.

Our conclusion therefore is that the structure responsible for the high resting stiffness of *Lethocerus* muscle is a protein linking the A filaments with the Z line. Moreover, as described in the Results section, we can conclude further that this structure is unchanged in active muscle by the process of activation, and that when discussing the properties of the cross-bridges it is essential to take the mechanical properties of the C filaments into account. This is not always done in modelling cross-bridge action (e.g. Abbott, 1973; Steiger & Abbott, 1981). In the following paper (Thorson & White, 1983) we discuss the implications of this conclusion.

*Estimates of the stiffness of the filaments and cross-bridges*

The following dimensions have been used for those of a half-sarcomere (Reedy, 1968): half-sarcomere length = 1·2 $\mu$m; C-filament length = 0·05 $\mu$m; $\frac{1}{2}$ A-filament length = 1·1 $\mu$m; $\frac{1}{2}$ H band = 0·1 $\mu$m; width of Z line = 0·05 $\mu$m; I-filament length = 1·05 $\mu$m.

Chaplain & Tregear (1966) estimated that in *Lethocerus cordofanus* there are $6{\cdot}34 \times 10^5$ A filaments per cross-section of fibre. The cross-bridge spacing is 14·5 nm

(Reedy, Holmes & Tregear, 1965). If we assume, with Wray (1979), that there are four cross-bridges arising at every crown (Reedy, 1968), then in the 1·0 $\mu$m of overlap between A and I filaments in one half-sarcomere there are 276 cross-bridges. It is assumed that a cross-bridge is equivalent to a heavy meromyosin molecule rather than to an S1 head. Offer & Elliott (1978) estimate that only 50 % of cross-bridges are attached in rigor. If this estimate implies that only half the cross-bridges are sterically capable of attachment at any instant, then at maximum A–I-filament overlap there are about 145 cross-bridges per half A filament available for attachment. In the following paper a figure of 150 is used, for conversion from thermodynamic to mechanical analyses.

We shall use a model consisting of one A filament and one 'associated I filament'. In the intact muscle the ratio of I filaments to A filaments is 3 to 1, so the mechanical properties of the I filament in our single-filaments model are equivalent to those of three real filaments. The Z line will be assumed to be rigid.

(*a*) *A-filament stiffness.* Armitage, Tregear & Miller (1975) determined that the longitudinal A-filament spacing in active muscle was independent of tension in the muscle, with a maximum error of 0·2 % for tension changes of about 150 $\mu$N/fibre. If the tension were uniform along the length of the A filament, this would give a minimum stiffness for the A filament of

$$\frac{150\ \mu\text{N/fibre}}{0{\cdot}002 \times 1100\ \text{nm}} \times \frac{1\ \text{fibre}}{6{\cdot}34 \times 10^5\ \text{filaments}} = 108\ \text{pN/nm}.$$

This gives an A-filament stiffness times unit length of 119 nN.

Ford *et al.* (1981) give a lower estimate of frog filament stiffnesses based upon mechanical measurements at different sarcomere lengths in which the degree of overlap of the filaments changes. They estimate that at least 90 % of the compliance of the sarcomere is within the cross-bridges, leaving at most 10 % elsewhere. If the A and I filaments contribute equally to the remaining 10 % compliance this gives an individual I-filament stiffness times unit length of 500 nN and an individual A-filament stiffness times unit length of 1000 nN (there are two I filaments per A filament in vertebrate striated muscle). Note that the length changes in the experiments of Ford *et al.* (1981) were applied, and the resulting tension changes measured, in about 100 $\mu$s, whereas in the experiments of Armitage *et al.* (1975) the muscle was held at a particular length for many minutes. Estimates based on these two kinds of experiment may disagree because of stress relaxation.

(*b*) *C-filament stiffness.* That these estimates of the A- and I-filament compliances are so much smaller than the measured compliance of the intact sarcomere means that, on our interpretation of the mechanical properties of the relaxed muscle being due to the continuous structure formed by the C and A filaments, the entire form of the dynamics of the relaxed muscle is ascribed to the properties of the C filament.

The stiffness of the relaxed muscle, measured quasi-statically, is about 21 $\mu$N (fibre %)$^{-1}$, and that measured at 70 Hz is about 100 $\mu$N (fibre %)$^{-1}$. On our single-filaments model these values are equivalent to C-filament stiffnesses of 2·8 pN nm$^{-1}$ and 13·1 pN nm$^{-1}$ respectively. In the following paper (Thorson & White, 1983) we have used a figure of 10 pN nm$^{-1}$ for modelling purposes.

(*c*) *Cross-bridge force.* The maximum force exerted by a single fibre is about 400 $\mu$N; this force corresponds to an average force per half A filament of 630 pN. In the following paper the value 600 pN is used.

(*d*) *Cross-bridge stiffness.* The above estimates of the A- and I-filament stiffness are also much greater than the stiffness of the rigor muscle. If the only difference between relaxed and rigor muscle is that cross-bridges are detached in relaxed muscle and attached in rigor muscle, then estimates of cross-bridge stiffness can be obtained from the difference between the stiffness of the relaxed and rigor muscle fibres.

The stiffness of rigor muscle, measured quasi-statically, is about 90 $\mu$N (fibre %)$^{-1}$ (Fig. 3*A*). Kuhn (1978) gives a figure of 100 $\mu$N (fibre %)$^{-1}$. If both the A and I filaments can be considered rigid, then the total stiffness due to the cross-bridges is equal to the difference in stiffness between the rigor and relaxed muscle (since C filaments and cross-bridges act in parallel). Thus the total cross-bridge stiffness = 90–21 = 69 $\mu$N (fibre %)$^{-1}$, equivalent on our single-filaments model to 9·6 pN nm$^{-1}$. This is the combined stiffness of all contributing cross-bridges between one A filament and its surrounding I filaments. If all cross-bridges are attached in rigor, then the individual cross-bridge stiffness is 0·035 pN nm$^{-1}$. If we use Offer & Elliott's (1978) estimate that 50 % of cross-bridges are attached in rigor *Lethocerus* muscle then the cross-bridge stiffness is 0·07 pN nm$^{-1}$.

The stiffness of rigor muscle, measured at 70 Hz (Fig. 7*B*), is about 575 $\mu$N (fibre %)$^{-1}$, equivalent to 80 pN nm$^{-1}$ on our single-filaments model. This value, measured at a relatively low ionic strength, can be compared with other reported values (e.g. 200 $\mu$N (fibre %)$^{-1}$, Kuhn, 1978; 650 $\mu$N (fibre %)$^{-1}$, White *et al.* 1979). The high-frequency stiffness of the relaxed fibres (Fig. 7*A*) is about 100 $\mu$N (fibre %)$^{-1}$. The contribution of the cross-bridges to the stiffness of the muscle at these frequencies is thus 575–100 = 475 $\mu$N (fibre %)$^{-1}$, equivalent to 65·9 pN nm$^{-1}$ on the single-filaments model. This results in an individual cross-bridge stiffness of 0·24 pN nm$^{-1}$ if all cross-bridges are attached in rigor, or 0·48 pN nm$^{-1}$ if 50 % are attached.

Since the stiffness of the rigor muscle shows a large frequency dependence when measured over several decades of frequency, it follows that the above estimates of cross-bridge stiffness demonstrate a similar dependence upon frequency. In all cases, however, the contribution to rigor muscle stiffness from the cross-bridges is several times greater than the contribution from the C filaments. For this reason the pronounced dip seen in the Nyquist plot of the relaxed muscle (Fig. 7*A*) is masked in that of the rigor muscle (Fig. 7*B*).

We do not know the basis of this frequency dependence. In the model of attached cross-bridges of active muscle of Huxley & Simmons (1971), there is such a marked dependence due to the availability to a cross-bridge of two or more distinct mechanical states, and it is possible that distinct states are available to a rigor cross-bridge.

For the qualitative considerations of the following paper (Thorson & White, 1983) we have assumed that an attached cross-bridge in a single state has a frequency-independent stiffness, and we have arbitrarily chosen a value of 22 pN nm$^{-1}$ for the contribution of the cross-bridges of a half-sarcomere to the stiffness of the single-filaments model for the situation in which all cross-bridges are attached. This

compares with the above estimate of 9·6 pN nm$^{-1}$ estimated from the quasi-static measurements and 65·9 pN nm$^{-1}$ estimated at 70 Hz.

The above calculations of cross-bridge stiffness have assumed that the A and I filaments are rigid. One can determine mathematically the stiffness expected of a half-sarcomere in which filaments and cross-bridges are compliant. The over-all stiffness of a model of a half-sarcomere in rigor with compliant A, I and C filaments, and with cross-bridges distributed uniformly along the overlap region between A and

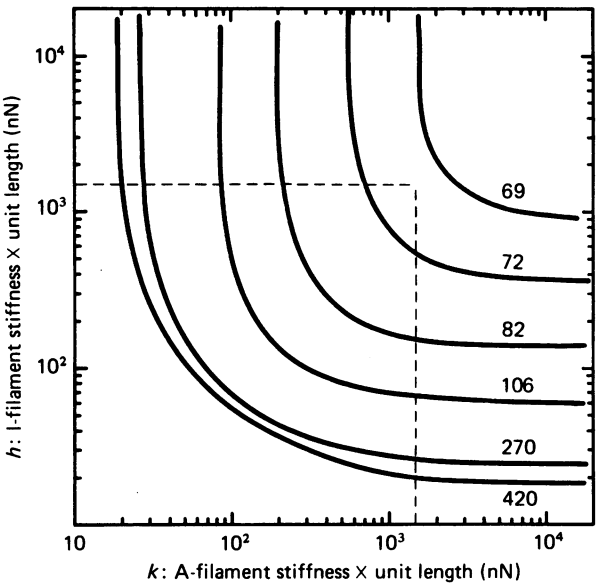


Fig. 12. Lines of constant rigor stiffness at the stated value of distributed cross-bridge stiffness per unit length (in units of nN $\mu$m$^{-2}$) on a plot of A-filament *vs.* I-filament stiffness times unit length. The dashed lines are drawn at $h = k = 1{\cdot}5$ $\mu$N.

I filaments, is derived in Appendix 2. In this model the cross-bridges are assumed to generate a shear force proportional to the relative displacement between the A and I filaments. This derivation enables us to determine the combinations of stiffness of the various components which give an over-all stiffness equal to that of the rigor muscle. These combinations are plotted in Fig. 12 for a rigor stiffness of 80 pN nm$^{-1}$ (i.e. for the rigor stiffness at 70 Hz). We have assumed that the C-filament stiffness is 13·1 pN nm$^{-1}$. The lines on the graph are drawn at equal values of distributed cross-bridge stiffness in units of nN $\mu$m$^{-2}$; for conversion to values of individual cross-bridge stiffness (given 1 $\mu$m of overlap), these values should be divided by the number of cross-bridges attached.

With rigid A and I filaments we obtain a lower bound to the distributed cross-bridge stiffness of 66·9 nN $\mu$m$^{-2}$. With A and I filament stiffnesses times unit length of 1500 nN the required distributed cross-bridge stiffness per unit length is 70·1 nN $\mu$m$^{-2}$.

## APPENDIX 1

### *The lumped-bridge model*

If we assume that the A and the I filaments are rigid, and the elastic properties of the relaxed fibrillar muscle are due to C filaments, then a satisfactory model of the muscle is one in which we consider the cross-bridges of an A filament in one half-sarcomere to be lumped together. The model is then as shown in Fig. 13.

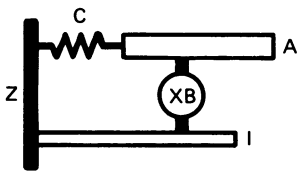


Fig. 13. The lumped-bridge model of insect flight muscle. The C filaments are compliant; the A and I filaments are rigid and the cross-bridges are lumped into a single element.

If we further assume for the moment that we are working under steady-state conditions, such that the tension exerted by the muscle is proportional to the fraction of cross-bridges attached, then the total tension $T$ in the muscle at any given extension $L$ is given by

$$T = q_s . L + F . n, \tag{1}$$

in which $n$ is the fraction of bridges attached to the $I$ filament, $F$ is the tension exerted when $n = 1$ and $q_s$ is the static (or low-frequency) stiffness of the C filament.

If we now apply a rapid change of length $\Delta L$, then the change in tension obtained is

$$\Delta T = q_d . \Delta L + K . n . \Delta L, \tag{2}$$

where $q_d$ is the dynamic (or high-frequency) stiffness of the C filament, and $K$ is the (lumped) stiffness of the cross-bridges for $n = 1$. Substituting for $n$ from eqn. (1) we obtain

$$\frac{\Delta T}{\Delta L} = \frac{1}{x_0} . T + q_d . \left(1 - \alpha . \frac{L}{x_0}\right), \tag{3}$$

where $x_0 = F/K$ and $\alpha = q_s/q_d$.

Thus a plot of stiffness against tension has, on the model, a slope of $1/x_0$ and an intercept of $q_d . (1 - \alpha L/x_0)$.

In general therefore we expect the intercept to change with the steady-state length of the model. However, if $\alpha L/x_0$ is constant then the intercept will remain constant.

From Fig. 9 we see that the dynamic stiffness of the relaxed muscle is linearly related to the tension, the slope being approximately that obtained for the active muscle, i.e. $1/x_0$. That is, for the relaxed muscle, the tension $T_r$ and stiffness $q_d$ are

$$T_r = q_s . L, \tag{4}$$

$$q_d = q_{0d} + \frac{T_r}{x_0} = q_{0d} + \frac{q_s . L}{x_0}, \tag{5}$$

where $q_{0d}$ is the intercept of the relaxed stiffness at zero tension.

The stiffness-axis intercept for the active muscle thus becomes

$$\text{Stiffness-axis intercept} = q_d(1-\alpha L/x_0)$$
$$= q_d(1-q_s L/(q_d x_0)).$$

Substituting for $q_s$ from eqn. (5) gives

$$\text{Stiffness-axis intercept} = q_d(1-(q_d-q_{0d})/q_d) = q_{0d}.$$

Thus, since the relaxed muscle has a stiffness/tension relationship with the same slope as that obtained for the active muscle, the intercept of the stiffness/tension relationship of the active muscle will be independent of the initial length of the active muscle.

## APPENDIX 2

BY D. C. S. WHITE AND JOHN THORSON

### *The distributed cross-bridge model of rigor muscle*

In the previous Appendix we assume that the A and I filaments are rigid. In this Appendix we do not make this simplifying assumption, and determine the stiffness of a half-sarcomere in which the A and I filaments have stiffness times unit length of $k$ and $h$ respectively. We assume that the resting elasticity is due to C filaments (of stiffness times unit length $q$), that the cross-bridges are evenly distributed along the A-filament region and that rigor bridges contribute per unit length stiffness $K$.

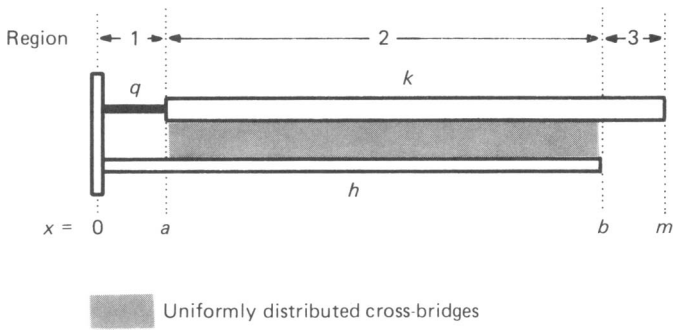


Fig. 14. The distributed model of a half-sarcomere. Region 1 is the C-filament region, region 2 has A–I filament overlap and region 3 is the H band.

The model is shown in Fig. 14. We consider the interaction between one A filament and one I filament. In the muscle the ratio of thin to thick filaments is 3; our model I filament is thus equivalent to three muscle I filaments. The treatment of this problem is similar to that of Thorson & White (1969), except that time dependence is not included.

In the analysis the Z line is considered to be fixed rigidly, and a length input $L$ is applied to the A filament at the middle of the M line. Let $x$ be a variable that specifices the distance between the Z line and any point along the sarcomere. The A filament is connected to the C filament at $x = a$, the end of the I filament is at $x = b$

and the middle of the M line is at $x = m$. The three regions of the muscle between the boundaries at $x = 0, a, b, m$ will be labelled regions 1, 2, 3 as shown in Fig. 14.

The required variables are as follows.

$y(x)$ = the (small) displacement in the direction of a point on the A or C filament.
$z(x)$ = the (small) displacement in the $x$ direction of a point on the I filament.
$T_A(x)$ = the local tension in the A filament or C filament.
$T_I(x)$ = the local tension in the I filament.
$T(x)$ = the total tension in the two-filament system = $T_A(b) = T_A(m)$.

Physical considerations show that (the subscript denoting differentiation with respect to $x$)

$y_x(x)$ = the local A- or C-filament strain.
$z_x(x)$ = the local I-filament strain.

*For region 1*

$$q \cdot y_x(x) = T_A. \tag{1}$$

$$h \cdot z_x(x) = T_I. \tag{2}$$

$$T_A + T_I = T. \tag{3}$$

*For region 2*

$$k \cdot y_x(x) = T_A. \tag{4}$$

$$h \cdot z_x(x) = T_I. \tag{5}$$

$$T_{A_x} = K \cdot (y - z). \tag{6}$$

$$T_A + T_I = T. \tag{7}$$

Differentiating twice and substituting (see Thorson & White, 1969) yields

$$y_{xxx}(x) - K \cdot \left(\frac{1}{k} + \frac{1}{h}\right) \cdot y_x + KT/kh = 0. \tag{8}$$

Since region 3 is a simple spring, the relationship between the length input, $L$, and tension $T$, is solved for regions 1 and 2 first. The required boundary conditions are as follows.

1. At $x = 0$, $y(0) = z(0) = 0$.
2. At $x = a$, the displacements on either side of the boundary are equal.

Using subscripts 1 and 2 to refer to regions 1 and 2:

$$y(a)_1 = y(a)_2.$$

$$y(a)_1 = z(a)_2.$$

3. At $x = a$ tension in the C filament equals the tension in the A filament

$$q \cdot y_x(a)_1 = k \cdot y_x(a)_2.$$

4. The displacement at $x = b$ is the forcing function $L$

$$y(b) = L.$$

5. At $x = b$ the tension in the A filament is the total tension $T$

$$k \cdot y_x(b) = T.$$

The solution to these equations, for the DC response of regions 1 and 2, is

$$S_{1,2} = \frac{T}{L} = \frac{B_0 + (B_1/\beta)\tanh[\beta(b-a)]}{A_0 + A_1 \operatorname{sech}[\beta(b-a)] + (A_2/\beta)\tanh[\beta(b-a)]},$$

where

$$B_0 = \frac{q(h+k)}{h}, \quad B_1 = \frac{a(h+q)(h+k)K}{h^2},$$

$$A_0 = \frac{ah(h+q)+bq(h+k)+ak(k-q)}{h(h+k)}, \quad A_1 = \frac{2a(k-q)}{h+k},$$

$$A_2 = \frac{q}{k} + \frac{K[a^2(k-q)+ab(h+q)]}{h^2}, \quad \beta = \sqrt{K\left(\frac{1}{k}+\frac{1}{h}\right)}.$$

The full response of the rigor muscle is found by solving for the stiffness of regions 1 and 2 given above, with the addition of the stiffness of region 3 in series. Region 3 is a length $(m-b)$ of A filaments of stiffness times unit length $k$ nN, giving a stiffness $S_3$ of $k/(m-b)$.

The over-all stiffness of the rigor muscle is thus

$$S = 1/(1/S_{1,2} + 1/S_3).$$

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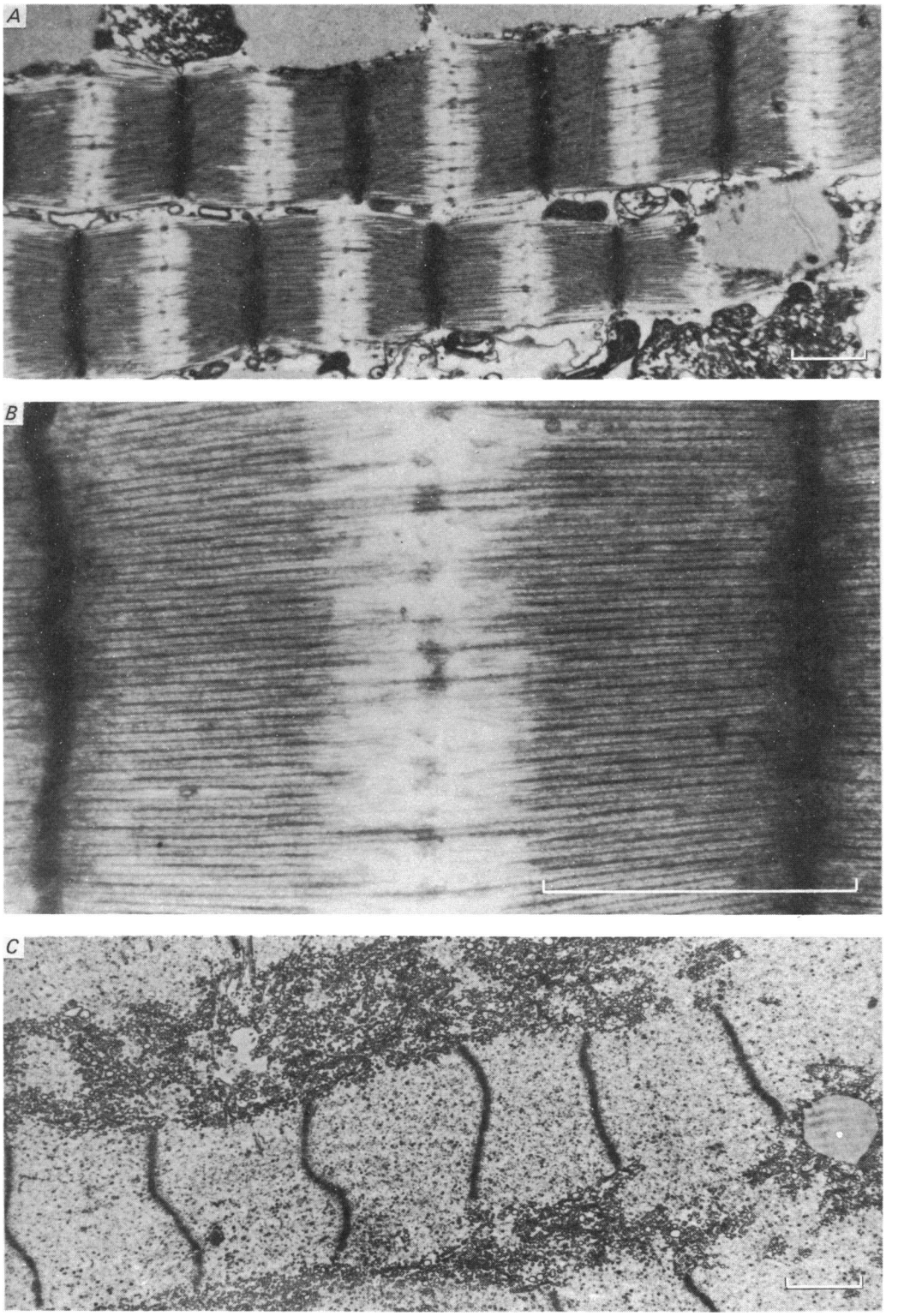


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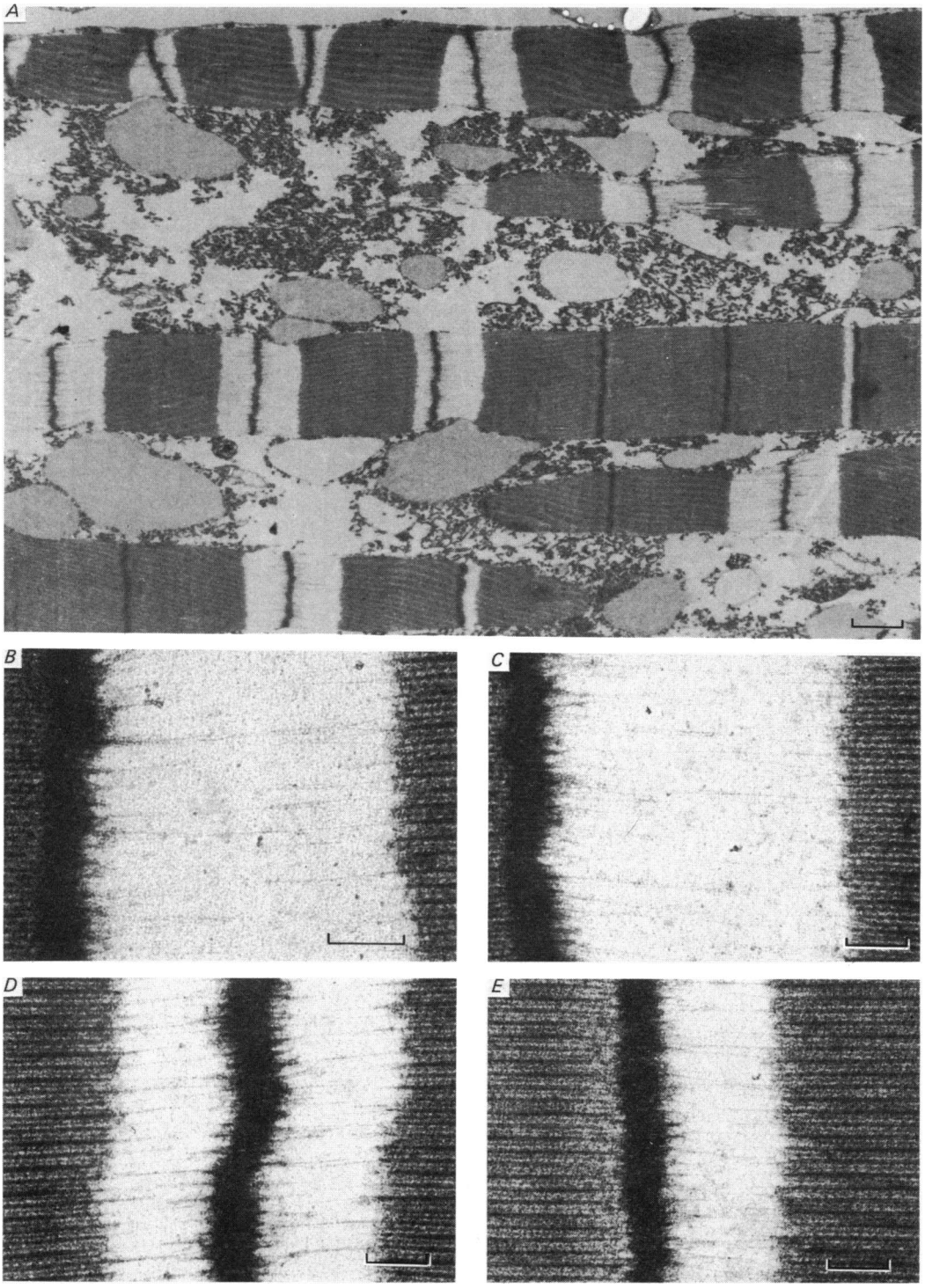


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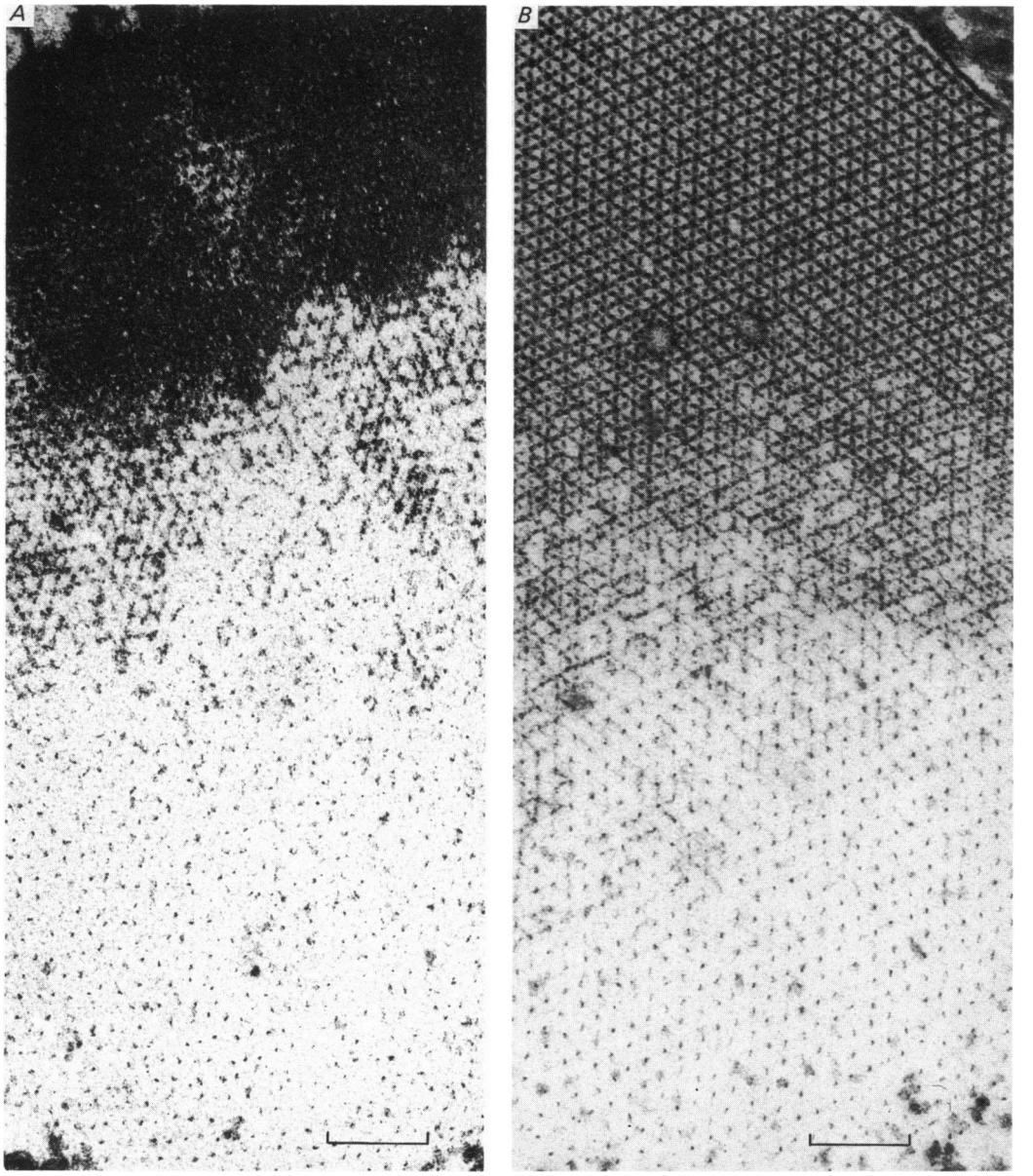
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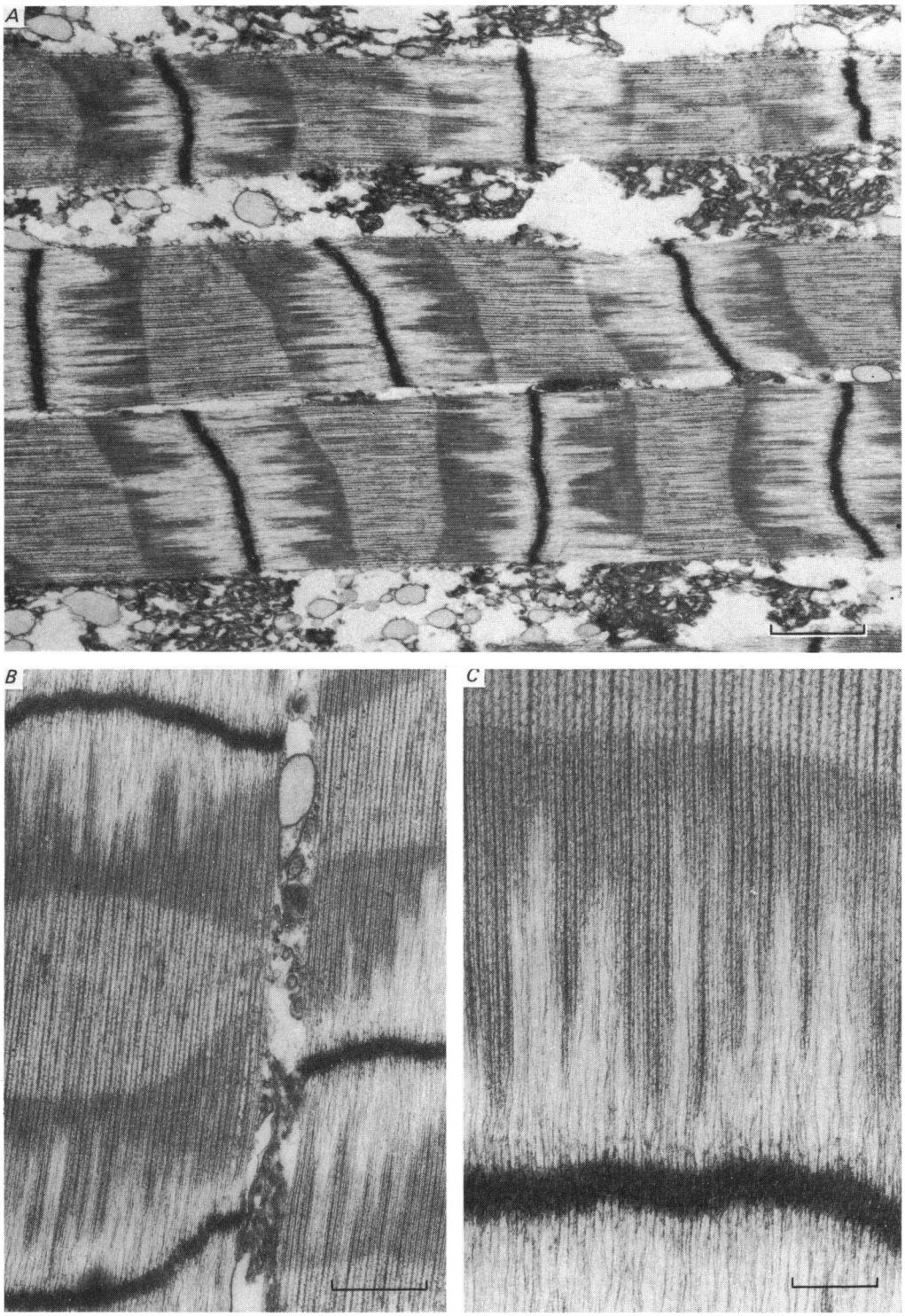


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## EXPLANATION OF PLATES

### PLATE 1

*A* and *B*, electron micrographs of *L. cordofanus* muscle fixed after 30 min irrigation with relaxing solution containing 0·6 M-KCl, showing the loss of A filaments from the central region of the sarcomere. *A* ×11500. *B* ×48000. Calibration bar, 1 μm. *C*, *L. cordofanus* muscle after 10 min irrigation in relaxing solution containing 1 M-KI. ×11500. Calibration bar 1 μm.

### PLATE 2

Longitudinal sections of *L. cordofanus* fibres stretched in rigor by 20%. *A*: ×8000. *B* ×60000. *C*, *D* and *E*: ×50000. Calibration bars: *A*, 1 μm; *B–E*, 0·2 μm.

### PLATE 3

Transverse sections from the same preparations as Pl. 2. *A*, the top of the micrograph is cut through a Z disk. ×75000. *B*, the top of the micrograph is cut through the region of overlap. ×75000. Calibration bars, 0·2 μm.

### PLATE 4

*L. cordofanus* fibres stretched by 50% in relaxing solution. *A*: ×15000. *B*: ×30000. *C*: 63000. Calibration bars: *A*, 1 μm; *B*, 0·5 μm; *C*, 0·2 μm.