

Series Elasticity in Frog Muscle as Revealed by Optical Diffraction

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Abstract

Using an optical diffraction technique, the series elasticity of frog striated muscle fibres was investigated. One source of series elasticity was located in the cross-bridges during the application of either quick stretches or releases of muscle fibres. Evidence is presented here for a second component attributable to a small population of slowly activated sarcomeres. The size of the second component was progressively reduced until it virtually disappeared at a sarcomere length of $3\ \mu\text{m}$. A third component appears to reside in the thick filaments. Calculation of the elastic energy in the muscle fibres enabled an identification of the source of the energy to be made in terms of the components of the series elasticity. Evidence is presented of a short-range elastic component present in resting fibres.

Introduction

A knowledge of the origin of the series elasticity (SE) in striated muscle is required for the development of theories of muscle contraction. Several studies have aimed at identifying the source of the SE by employing techniques which have utilized fast, constant-velocity releases to determine the force–extension relation (e.g. Hill 1950; Jewell and Wilkie 1958; Huxley and Simmons 1971*a*). An alternative method (Cleworth and Edman 1972) involves simultaneous measurement of sarcomere shortening using an optical diffraction technique and developing tetanic tension.

The SE has been described in terms of two components. The first was identified by Hill (1938) who described it as an undamped element in series with the contractile element (CE) whose length changes much more slowly. The second component was described by Huxley and Simmons (1971*b*). This is an essentially undamped elasticity which possesses a stiffness that increases roughly in proportion to the rising tetanic tension and which responds to a quick release at speeds about 100 times faster than the SE component of Hill (1938). This elasticity appears to be a property of the CE.

Controlled shortening ramps have been applied to frog or toad muscles during tetanic tension (Hill 1950, 1953; Jewell and Wilkie 1958; Bressler and Clinch 1974 and Ford *et al.* 1977, 1981). The shortening amplitude required to reduce tension to zero ranged between 1.0 and $1.5\% l_0$, where l_0 is the normal rest length of the muscle.

This lower value of $1\% l_0$ was confirmed (Barden and Mason 1978) using optical diffraction to measure the working range of cross-bridges. For release amplitudes in excess of $1\% l_0$ or 12 nm per half-sarcomere, the sarcomeres were observed to undergo further shortening at V_{max} (the maximum active shortening velocity) which indicated that active cross-bridge cycling occurred in taking up the extra length.

Sarcomere length shortens in a nominally isometric contraction by an amount considerably larger than the contribution of transducer and tendon compliances. X-ray diffraction measurements (Huxley 1979) show that the sarcomeres are shortening (Barden and Mason 1979) as the cross-bridges are moving out from the thick to the thin filaments. Both these events are much faster than the development of tension (Barden and Mason 1979).

The magnitude of the sarcomere length shortening resulting from tetanic stimulation is reduced in stretched muscle fibres. The results in this paper shed light on the source of elastic energy not detected by quick-release techniques (Hill and Howarth 1959). Measurements of the magnitude of the SE in resting muscle fibres are also obtained.

Methods

Mounting of Specimens

Whole sartorius muscles or semitendinosus fibre bundles of the frog *Hyla caerulea* were suspended in frog Ringer's solution (115 mM NaCl; 2.5 mM KCl; 1.8 mM CaCl_2 ; 2.15 mM Na_2HPO_4 ; 0.8 mM NaH_2PO_4 ; corresponding to an ionic strength of 130 mM) at 5°C between rigid tungsten-carbide coupling pins. The solution was perfused with oxygen and replaced regularly. The connections to the tendons were supplemented with silk thread to reduce the coupling compliance. About 0.5 mm of tendon remained between muscle and connecting pins. Care was taken to ensure that the long axis of the fibres passed directly through the pin connections without any twisting. A microscope was used to measure the stretch in the tendons and transducer couplings during the application of static loads of up to 100 g. The total stretch in tendons and couplings of the muscle fibres was also measured during isometric tetanic stimulation. This involved placing a ruled grating against the sections of tendon between the myotendinous junctions and transducer coupling pins. In agreement with Jewell and Wilkie (1958), the proximal tendinous sheath of whole sartorius muscles was found to stretch up to 0.7% l_0 . As an example the sartorius muscle in Fig. 1 from which the data was obtained had a rest length of 2.4 cm. The proximal tendinous sheath was stretched by 0.16 mm while the muscle was generating peak tetanic tension. However, the distal tendon between transducer coupling and myotendon junctions was observed to stretch by at most 0.2% l_0 , a value equal to that obtained in all the semitendinosus fibre bundle samples. It is possible that extra stretch occurred in distal intrafibre tendon strands connecting internal muscle fibres. Dissections have revealed that the lengths of these strands range from 0 to 0.12 l_0 . It is clear that a maximum stretch of 4% can be made in the tendons from their *in situ* lengths. If one assumed the average strand length was even as high as 0.1% then the maximum stretch could be as much as 0.4% l_0 . Much of the observed stretch in the distal tendon actually includes stretch in the intrafibre strands; however, it is possible that a further stretch of 0.1% may be sited in the internal strands. This degree of stretch in the tendons was also observed to occur by adding small loads equal to about 10% of the peak tetanic tension. The total compliance of all connections (excluding tendon) to the muscle fibres was determined to be $1.9 \times 10^{-4} \text{ mN}^{-1}$ which was constant for all preparations.

Stimulation

To induce supramaximal tetanus, the specimens were stimulated directly with 40 V negative pulses of 0.2 ms duration and 30–35 Hz for a period of 850 ms at 5°C. These pulses were 8–10 V higher than the threshold voltage required to produce a supramaximal stimulus. The voltage pulses were delivered through two platinum wire electrodes placed in parallel with, and 1 mm above, the muscle fibres and separated by 6 mm. This geometry produced uniform stimulation and in no way interfered with the incident or diffracted laser beams.

The criterion by which stimulation was judged to be uniform was that the amplitude of sarcomere length shortening which occurred at all points along the length of the specimens had to be constant. Many specimens underwent about 30 tetanic stimulations spaced 5 min apart. Provided tetanic tension remained within about 5% of the original level the data were not discarded. Bundles of

3–5 fibres could be readily kept for 1–2 days at 2°C. At the end of each experiment, the specimens were weighed after removal of tendon. In general 1 g of tension was elicited for 1 mg of wet muscle fibre.

Sarcomere Length Measurements

An He–Ne laser was directed normally on a muscle specimen to produce a diffraction pattern resulting from the regular spacing of the isotopic–anisotropic (I–A) bands. The angular spacing of the first-order diffraction lines was measured dynamically at a fixed distance behind the specimen. This was achieved by measuring the time interval between sequential photocurrent pulses produced by the two first-order beams which passed through peripheral slits in a rotating disc and alternatively illuminated photodiodes (see Fig. 1). The measured time interval between pulses is a function of the diffraction angle which is itself dependent on sarcomere length (Borejdo and Mason 1976).

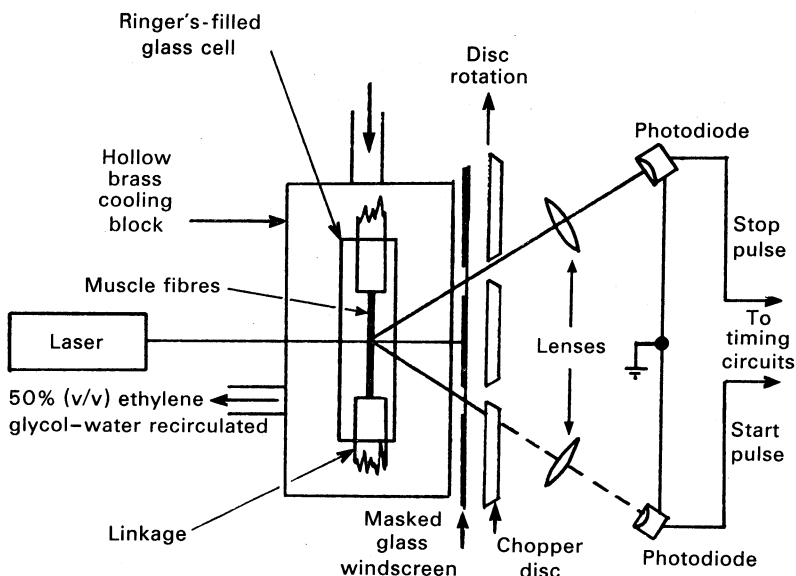


Fig. 1. The essence of the scanning system organization involves passing the two first-order diffraction lines, produced by passing a laser beam through a mounted muscle preparation, through a chopper disc. The geometry is such that only one beam can pass through the radially spaced slits on the disc's periphery at any one time. Pulses from the two photodiodes produce timing pulses. A change in muscle length causes a change in diffraction angle and, consequently, a change in the time between sequential pulses.

The sampling frequency was 2.5 kHz. Each photocurrent pulse was amplified and then integrated in turn. Sample and hold amplifiers detected the end of the pulses prior to each integrated waveform being fed through separate attenuators, the outputs of which were connected to voltage comparators together with the corresponding integrator outputs. The comparator outputs changed sign when the integrator outputs reached half their maximum level which corresponded to the mean of the photocurrent waveform (see Fig. 2). The system was thus insensitive to diffraction artefacts and to fluctuations in the spatial intensity profile of changes in length, including the line broadening and peak intensity fluctuations commonly observed during stimulation. The time interval between the appearance of sequential pulse-shaped comparator outputs arising from the two first-order diffraction lines was then processed by a Hewlett Packard 2100S control computer. Synchronous noise generated by the rotating disc was removed from the plots of sarcomere length by the computer. Software data files were collected from as many as 30 different 1 mm wide segments of the muscle preparations. These files were summed and averaged in the computer to determine the average

response from the preparations as a whole. A variation of at most 0.2 ms occurred because of uncertainty in the position of the first timing pulse.

Tension Measurements

Tension was recorded with a Harvard type 373 isometric capacitance force transducer with a sensitivity of 3.06 VN^{-1} . Tension was recorded on either a Tektronix 564B storage oscilloscope or else channelled through an analogue-to-digital converter to the HP2100S.

Length-step Application

Displacements of up to 30 nm per half-sarcomere could be applied to preparations in a time of 0.1 ms (Barden and Mason, 1978). The amplitude was pre-set with a micrometer gauge which limited the range of movement of the lever connecting the muscle preparation.

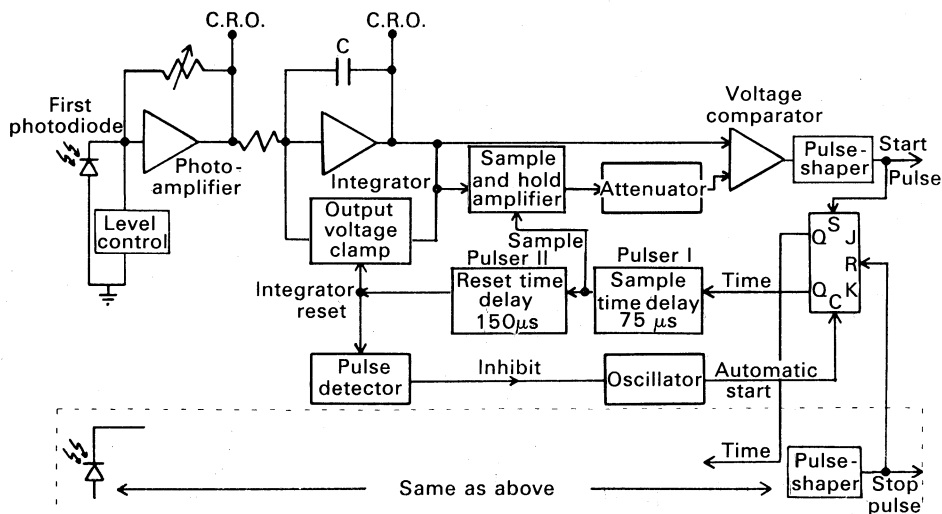


Fig. 2. A simplified block diagram of the detector circuit. Raw photoamplifier pulses are received and the spatial intensity profiles integrated and eventually shaped into appropriate timing pulses coinciding with the mean first-order diffraction line spacing.

Results

Stretch of the SE during Isometric Tetanus

An intact frog sartorius muscle was held isometrically and then tetanically stimulated. Fig. 3a shows the summed and averaged response of segments covering the complete length of the muscle rising towards a plateau more quickly than tension is generated. A total shortening of $3.8\% l_0$ is observed at a maximum tension (P_0) of 0.36 N. The tension-extension curve for the SE (Fig. 4a, ●) was derived from the data in Fig. 3a. It is substantially linear for tensions in excess of $0.5 P_0$ as observed by others (Hill 1970; Bressler and Clinch 1974).

Following the cessation of tetanic stimulation, the average sarcomere length increased much more gradually than the decrease in tension. Approximately 200 ms separated the half-maximum levels of sarcomere length and tension (Fig. 3b). The corresponding tension-extension curve, plotted during the relaxation phase, is shown in Fig. 4a, ▲.

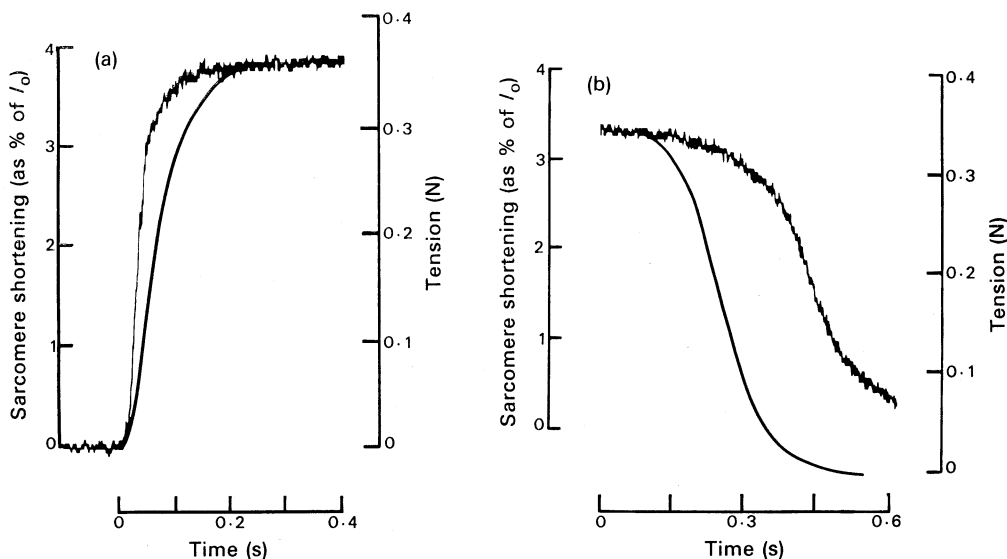


Fig. 3. (a) Mean sarcomere shortening and tension (smooth curve) generated in an intact frog sartorius muscle at 5°C during the development of isometric tetanus. Resting sarcomere length was 2.2 μm . Stimulation commenced at time zero. (b) Mean sarcomere shortening and residual tension (smooth curve) following cessation of tetanic stimulation in frog sartorius. Conditions as in (a).

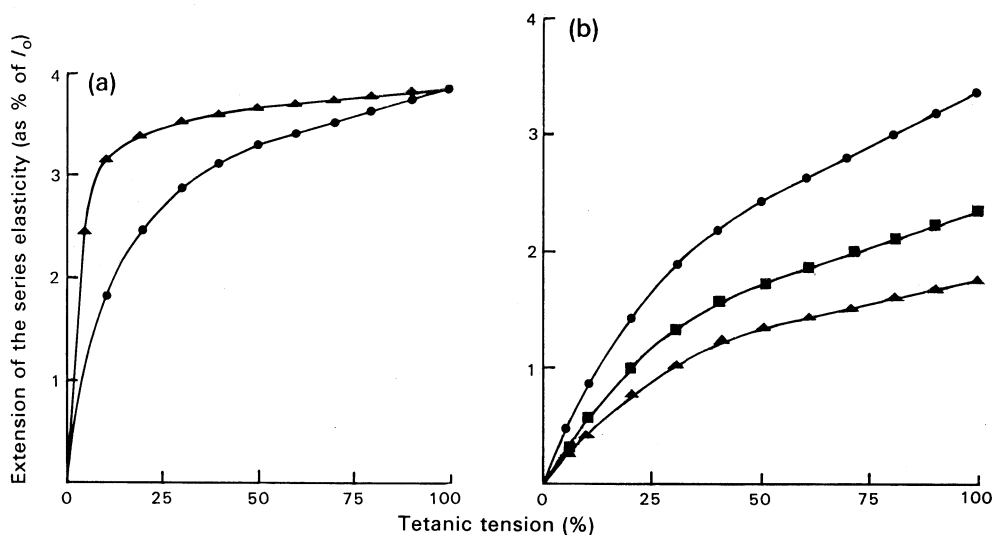


Fig. 4. (a) Curves of tension-extension measured from the data in Fig. 3a (●) and Fig. 3b (▲). The ordinate corresponds to the percentage of sarcomere shortening in Figs 3a and 3b. (b) Representative tension-extension relations exhibited by frog sartorius muscle held at resting sarcomere lengths of 2.2 μm (●), 2.6 μm (■) and 2.9 μm (▲) at 5°C. At 2.2 μm tetanic tension was estimated to be $2.6 \times 10^5 \text{ Nm}^{-2}$. As the resting sarcomere length increased, the stretch in the SE component fell. The ordinate corresponds to the percentage sarcomere shortening measured against developing tension.

The mean sarcomere length change measured during the tetanic plateau for six frog sartorius muscles was 3.8, 4.0, 3.3, 3.4, 3.5, and 3.4% l_0 (mean $\pm$ s.d. 3.6 ± 0.3) and six bundles of 3–5 frog semitendinosus fibres was 1.7, 1.8, 1.2, 1.5, 2.7 and 1.7% l_0 (mean $\pm$ s.d. 1.8 ± 0.5). In each preparation the resting sarcomere length was 2.2 μm . Tetanic tension was estimated to lie in the range 2×10^5 – 3×10^5 Nm^{-2} for each preparation.

An examination of the effect of muscle fibre length on the tension–extension curves was made. A frog sartorius muscle was held at sarcomere lengths ranging from 2.2 to 3.6 μm . The mean sarcomere length shortening decreased from 3.4% l_0 at 2.2 μm to 2.4% l_0 at 2.6 μm and 1.6% l_0 at 2.9 μm (see Fig. 4b). The peak tension developed at a sarcomere length of 2.2 μm was 0.32 N. Wet weight of the muscle after tendon was removed amounted to 26 mg and rest length was 2.0 cm. Tetanic tension was thus estimated to be 2.6×10^5 Nm^{-2} for this preparation.

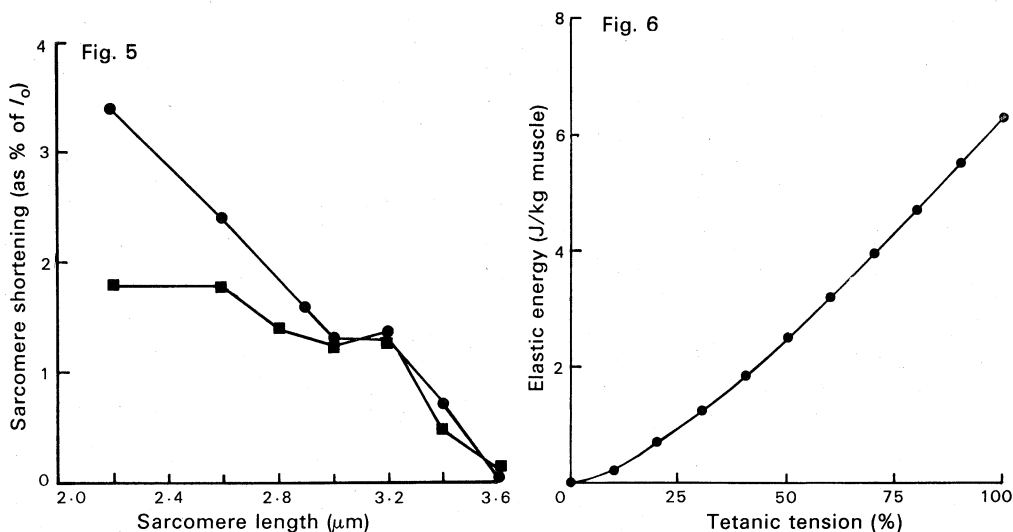


Fig. 5. Maximum sarcomere shortening elicited from the whole sartorius muscle in Fig. 4b during tetanus as a function of sarcomere length (●). A bundle of three frog semitendinosus fibres was similarly used to obtain the data marked with squares. In both sets of data a plateau is observed around a sarcomere length of 3.0–3.2 μm .

Fig. 6. Elastic energy in an intact frog sartorius muscle obtained by integrating the area under the tension–extension curve (Fig. 4a, ●). At peak tetanic tension, the muscle was generating 6.3 J/kg.

A small plateau in sarcomere length shortening was observed to occur for sarcomere lengths from 2.9 to 3.2 μm while for larger lengths the sarcomere length shortening reduced towards zero at *c.* 3.6 μm (Fig. 5, ●). Apart from a smaller sarcomere length shortening at the shorter lengths an almost identical trend emerged using a bundle of semitendinosus fibres (Fig. 5, ■).

The elastic energy given by the area under the curve in Fig. 4a is plotted in Fig. 6 against developing tension. It represents the internal work done by the CE in shortening and thereby stretching the SE component. At P_0 the elastic energy is equal to 6.3 J/kg muscle. This is equal to the relaxation heat following an isometric contraction as measured by Hill (1961) using an intact sartorius muscle under conditions comparable to those in Fig. 3b.

Effect of Applied Length Steps on Sarcomere Length

An alternative frame of reference for observing the behaviour of the SE was employed. Quick release or stretch steps were applied to both resting and active frog semitendinosus fibres. At a sarcomere length of $2.5\ \mu\text{m}$ a quick-release step

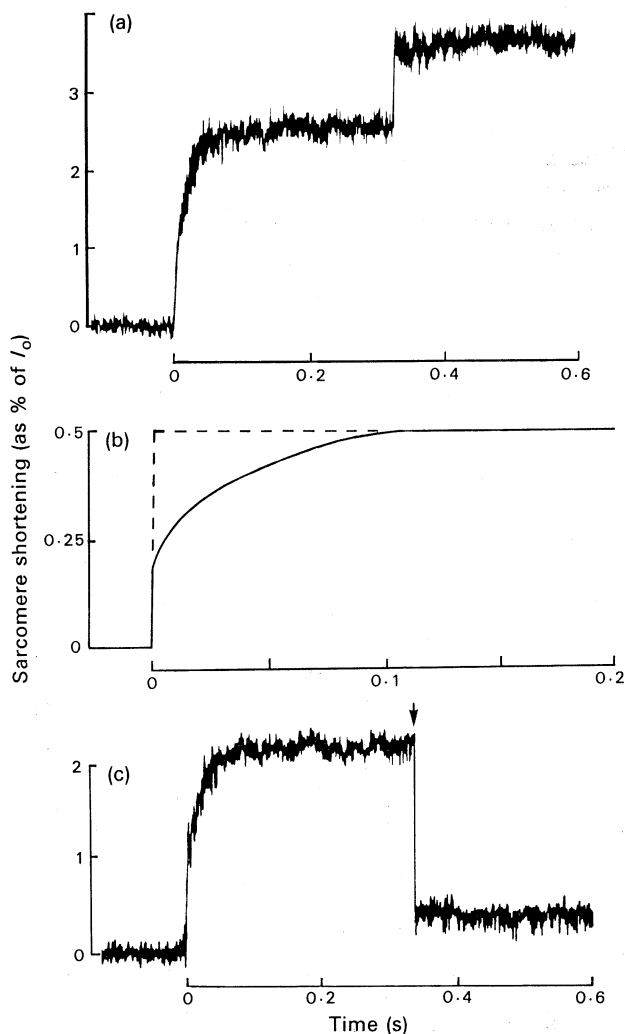


Fig. 7. (a) Following the onset of tetanic stimulation (*A*), the sarcomeres in a bundle of five frog semitendinosus fibres shortened by $2.5\% l_0$. At the point marked B a quick release of $1\% l_0$ was applied. This resulted in an identical amount of sarcomere shortening. Resting sarcomere length was $2.5\ \mu\text{m}$ and the temperature was 10°C . (b) A bundle of three resting frog semitendinosus fibres held at a sarcomere length of $2.5\ \mu\text{m}$ and subjected to three separate releases of $0.5\% l_0$ (---). The average response is revealed by the solid line. About 2–3 nm per half-sarcomere of shortening was virtually instantaneous with the remainder of the release being taken up in about 0.1 s. (c) A bundle of three frog semitendinosus fibres held at a resting sarcomere length of $2.5\ \mu\text{m}$. At the onset of tetanic stimulation the sarcomere length decreased by $2.2\% l_0$. The arrow marks the application of a quick stretch of $1.8\% l_0$. An instantaneous response of the sarcomeres by the same amplitude was elicited.

equal to $1\% l_0$ or 12.5 nm per half-sarcomere was applied on the tetanus plateau (Fig. 7a). The sarcomeres had initially shortened by $2.5\% l_0$ as tetanic tension developed. During the steady-state tetanic plateau the quick release was exactly matched by a $1\% l_0$ or 12.5 nm per half-sarcomere contraction within the 0.4 ms resolution of the technique. This apparently represented the elastic limit of the muscle fibres as observed using this technique since release amplitudes in excess of $1\% l_0$ were taken up by the sarcomeres at a much slower rate due to cross-bridge turnover (Barden and Mason 1978).

A similar experiment was performed with resting fibres. Fig. 7b reveals the results of applying a $0.5\% l_0$ or 6.5 nm per half-sarcomere release step to a bundle of three semitendinosus fibres held at a sarcomere length of $2.5 \mu\text{m}$. The applied release step is shown together with the response of the sarcomere length averaged over three releases. Approximately 2.3 nm per half-sarcomere of shortening was taken up very quickly with the remainder of the release taken up by sarcomeres shortening for about 0.1 s.

Application of a quick stretch to either resting or active muscle fibres produced identical results. Sarcomere length increased simultaneously with the applied stretch. Fig. 7c shows the result of applying a large amplitude stretch of $1.8\% l_0$ or 22.5 nm per half-sarcomere to a bundle of three semitendinosus fibres held at an initial sarcomere length of $2.5 \mu\text{m}$. The sarcomeres stretched by the amount imposed.

Discussion

There appear to be three distinct sources of SE in muscle fibres. Length step measurements indicate that cross-bridges themselves constitute one source equal to $1\% l_0$ (Barden and Mason 1978). Ford *et al.* (1977, 1981) have shown that of this 1% probably only 0.3% or 4 nm per half-sarcomere is instantaneous (undamped) elasticity, the remainder being a slightly damped component associated with cross-bridge movement.

Due to the highly non-linear stress-strain curve exhibited by tendons, a quick release applied to either a whole muscle or fibre preparation must abolish active muscle tension almost completely before the stretched tendons shorten. Thus the cross-bridge elasticity alone accounts for an elastic energy of close to 2.1 J/kg muscle. This value is in complete agreement with the results of Hill and Howarth (1959) and Hill (1970). However, Fig. 6 indicates that as a whole the muscle generates a total elastic energy including the contribution of thermoelastic heat of 6.3 J/kg muscle. The extra 4.2 J/kg muscle is exactly equal to the difference between the relaxation heat as measured by Hill (1961) and the elastic energy measured by Hill and Howarth (1959).

Fig. 3a and data mentioned in the Results section show that whole sartorius muscles at normal rest lengths exhibit a sarcomere shortening on average equal to $3.6\% l_0$ or 80 nm per sarcomere during tetanus. A maximum of $1.2\% l_0$ can be attributed to stretch in the tendons and transducer couplings although this value may increase to $1.3\% l_0$ if internal distal intrafibre tendon strands are responsible for a hidden $0.1\% l_0$ (see Methods). Fig. 5 shows that as sarcomere length increases to about $3.0 \mu\text{m}$ sarcomere shortening declines towards a plateau of about $1.3\% l_0$ or 28 nm per sarcomere. Furthermore, similar results were obtained with fibre bundle preparations in agreement with Kawai and Kuntz (1973). At $2.2 \mu\text{m}$, total

sarcomere shortening was about $1.8\% l_0$ declining to 1.3% at $3.0 \mu\text{m}$. A total of $0.5\% l_0$ can be assigned to tendons and linkages in these preparations at $2.2 \mu\text{m}$. As tendons become prestretched in the fibre bundle preparations the extent of sarcomere shortening is also reduced to $1.3\% l_0$ at $3.0 \mu\text{m}$.

Thus, at a sarcomere length of $2.2 \mu\text{m}$ whole muscles exhibit a total of only $1.1\% l_0$ or 25 nm per sarcomere in excess of the sarcomere shortening in fibre bundle preparations.

We suggest that in whole muscles the extra $2.4\% l_0$ or 53 nm per sarcomere consists of 28 nm per sarcomere of thick filament stretch which appears largely constant in fibre preparations over a wide range of lengths. A similar result was found using X-ray diffraction of contracting muscles by Huxley and Brown (1967). They concluded that there was approximately a 1% increase in the spacing of myosin molecules in the thick filaments during contraction. The remaining 25 nm per sarcomere of shortening in whole sartorius muscles at $2.2 \mu\text{m}$ sarcomere length during tetanus appears to be due to a small population of slowly activated sarcomeres that are stretched by the majority. ter Keurs *et al.* (1978) showed that the passive tension developed by a muscle increases from about zero at $2.8 \mu\text{m}$ sarcomere length to 100% of the maximum tetanic tension at $3.2\text{--}3.3 \mu\text{m}$. Thus passive sarcomeres could be stretched from 2.2 to $3.2 \mu\text{m}$ after which they develop almost as much tension as actively contracting sarcomeres and would thus not be stretched further. To account for the 25 nm sarcomere of extra apparent shortening only 2.4% of sarcomeres would have to be stretched by this amount. The intensity of diffracted light from this small population would go unnoticed for two reasons. Firstly, the intensity of diffracted light during muscle stimulation decreases with the maximum decrease occurring at a sarcomere length of $2.9 \mu\text{m}$ (Fujime 1975). Thus the contribution of the small passive sarcomere population is disproportionately reduced to about 1% of the diffraction intensity originating from most of the sarcomeres which are contracting. Secondly, a small component of the first-order diffraction lines, which moves in towards the zero order by the equivalent of a $45\% l_0$ expansion, is largely lost in the background scatter. The background must be eliminated by the detector level controls (Fig. 2). This heterogeneous behaviour has often been observed in uniformly stimulated muscle fibres (Borejdo and Mason 1976; ter Keurs *et al.* 1978; Barden and Mason 1979).

During the period of relaxation immediately following the cessation of tetanic stimulation (Fig. 3b) the sarcomere length remains relatively unchanged. This produces a significant hysteresis in the stress-strain curve of the SE component (Fig. 4a) which is further indicative of the presence of a somewhat viscous group of passive or, at least, slowly activated sarcomeres.

Sarcomeres readily stretch precisely by the amount of stretch applied (Fig. 7c) indicating that, as expected, they are the weakest structures situated between transducer linkages during isometric tetanus. During an applied release on the plateau of tetanus (Fig. 7a) cross-bridges alone take up the first 24 nm per sarcomere of shortening. This value has been shown to be independent of overlap (Barden and Mason 1978). Thus several structures which stretch in order that the major force generating sarcomeres can contract during tetanus react in a much more viscous way to an applied release. Of an average $3.6\% l_0$ or 80 nm per sarcomere of shortening displayed by a whole frog sartorius muscle in tetanus, $2.4\% l_0$ is a viscous component within the muscle structure itself. As the structures comprising this component

stretch during the development of tetanus they accumulate mechanical potential energy. This results in an increase in muscle temperature following a quick release (Hill 1961). Elastic energy is therefore derived from cross-bridges alone and thermo-elastic heat from thick filaments and passive sarcomeres. Tendons and transducer couplings have no effect on the muscle temperature.

Hill (1968), Lännergren (1970) and Flitney (1975) observed a 0.2% l_0 short-range elastic component in resting fibres. In Fig. 7*b* an elastic change of this size was elicited by applying a quick release. The resulting elastic change in overlap was 2–3 nm per half-sarcomere. As suggested by Hill, the source of this elastic component could be due to a few residually attached cross-bridges. Since rapid reattachment of cross-bridges subjected to a quick release in resting fibres is unlikely, then the value of 2–3 nm per half-sarcomere may represent the instantaneous elastic limit of cross-bridges as evidenced by the value of T_1 (Ford *et al.* 1977, 1981).

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