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CROSS-BRIDGE DETACHMENT AND SARCOMERE 'GIVE' DURING STRETCH OF ACTIVE FROG'S MUSCLE

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SUMMARY

1. A study has been made of the tension responses and sarcomere length changes produced by servo-controlled stretches applied to isometrically contracting frog muscle. Sarcomere lengths were monitored by cine-photography of diffraction spectra obtained by illuminating a small area of muscle with a laser.

2. The tension increment produced by a ramp-and-hold stretch of approximately 1 mm (*ca.* 4% of the muscle length) comprises three phases whose limits are defined by two points, S_1 and S_2, where the slope of the response decreases abruptly. S_1 and S_2 correspond to extensions of 0·13 and 1·2% of the muscle length.

3. Movements of the first order spectra relative to the zero order recorded during stretch reveal that S_2 coincides with an abrupt elongation of the sarcomeres. This is termed sarcomere 'give' and it occurs when the filaments are displaced by 11–12 nm from their steady-state (isometric) position.

4. The stiffness of the sarcomeres, E_s, up to S_2 decreases with increasing sarcomere length. The maximum force sustained by the muscle at S_2, P_{S_2}, also shows an inverse dependence on sarcomere length. Both E_s and P_{S_2} fall to zero at an extrapolated sarcomere spacing of 3·6–3·7 μm, coinciding with the length at which the actin and myosin filaments no longer overlap.

5. The ratio P_{S_2}/P_0 (where P_0 = maximum isometric tension) varies with temperature and speed of stretch. It increases with increasing speeds of stretch until a certain critical velocity, V_c, is reached, beyond which it is almost independent of any further increase. V_c has a positive temperature coefficient, increasing 5–6 in the range 0–30 °C (Q_{10} = 1·8). There is a positive correlation between the maximum speed of isotonic shortening ($V_{max.}$) and V_c in different muscles.

6. Sarcomere 'give' during stretch is considered to be due to forcible detachment of cross-bridges between the actin and myosin filaments. This results in recoil of the extended series elastic elements in the muscle at the expense of the sarcomeres. The amount of filament displacement required to induce sarcomere 'give' (11–12 nm) is thought to represent the range of movement over which a cross-bridge can remain attached to actin during a stretch.

INTRODUCTION

It is generally agreed that the force for a muscle contraction is generated between the sliding filaments, in the region of the sarcomere where they overlap (Huxley, 1957; Gordon, Huxley & Julian, 1966), and that the cross-projections on the myosin

filaments play an essential role in developing and transmitting the force. The work to be described in this and the subsequent paper was undertaken initially to obtain more information concerning the nature of the molecular changes involved in the cyclical interaction of a myosin cross-projection with its attachment site on the actin filament, about which there is far less agreement (Podolsky & Nolan, 1971; Huxley & Simmons, 1971*a*, *b*, 1973; Shear, 1970; Elliot, Rome & Spencer, 1970). The experiments involved subjecting muscles to controlled length changes while recording simultaneously the changes in sarcomere spacing and tension which result. Sarcomere lengths were sampled throughout the period of stretch by filming the diffraction spectra produced by illuminating a small area of muscle with a laser. The difference in refractive index between the A and I bands within its sarcomeres (Huxley, 1957) causes a muscle to act like a multi-layered, one-dimensional grating, so that a parallel beam of light incident upon it is split into a series of diffracted rays (Sandow, 1936; Hill, 1953). The spatial separation of the peak intensities of the various spectral orders in the diffraction pattern is inversely related to the statistical mode of the sarcomere length distribution in the population sampled and by monitoring the angle subtended by the first and zero (undiffracted) orders it is possible to detect changes in length of about 5 nm, even when using a whole muscle like the frog's sartorius.

The change in sarcomere length resulting from stretch is seen to be strikingly non-linear. It is found that when the actin and mysoin filaments are displaced by 11–12 nm from their steady-state (isometric) position, there is an abrupt increase in the length of the sarcomeres which coincides with a large reduction in the slope stiffness of the muscle. This phenomenon is termed sarcomere 'give'. It marks the transition between two steady-states: first, when the muscle is exerting its maximum isometric force at constant length, *before* stretch; and second, *during* stretch when it maintains a force greater than the isometric level appropriate to the new length, at a time when its sarcomeres are extending at a uniform rate. The degree of extension required to induce yielding of the sarcomeres is shown to represent the maximum range of relative sliding movement between the actin and myosin filaments that a cross-bridge can accommodate before it becomes forcibly detached.

Preliminary accounts of some aspects of this work have been presented to the Physiological Society (Flitney & Hirst, 1975*a*, *b*; Flitney, Hirst & Stephens, 1975; Flitney, Hirst & Jones, 1976).

METHODS

The majority of the experiments were made using the sartorius muscle of the frog, *Rana temporaria*. Muscles were dissected free and immersed in a solution of the following composition (mM): NaCl, 115; KCl, 2·5; $CaCl_2$, 1·8; $NaHCO_3$, 5·0; pH, 7·3. The solution was oxygenated continuously and except where stated otherwise, the experiments were performed at a temperature of 0–2 °C.

Apparatus

Stimulation. Stimulation was achieved by passing current through two parallel, platinum electrodes (4 cm × 0·5 cm) located on either side of the muscle. Square pulses (intensity: 12 V/cm; 0·1 msec duration) were used at frequencies ranging from 15 to 100 Hz. The polarity of the pulses was reversed with successive shocks to minimize electrode polarization effects.

Muscle chamber. Muscles were suspended vertically in a narrow, glass-sided chamber. The tibial tendon was attached by a fine chain to a tension recorder and the pelvic bone was secured

by a loop formed at one end of a platinum rod. The rod was connected at its lower end to an electromagnetic displacement transducer (see below). The chamber was cooled by means of a thermoelectric module (type 12.15G, De La Rue Frigistors Ltd) clamped to one of its sides. The temperature of the solution was monitored continuously by means of a thermo-couple positioned close to the muscle. Temperature was maintained to within $\pm 0{\cdot}1$ °C of the required value.

Length changes. Changes of muscle length were produced by a moving coil electromagnetic unit (Ling Altec vibrator, model 201) controlled by a servo-amplifier incorporating positional and velocity feed-back. Displacement was monitored by a photo-electric sensor comprising a light source, a vane attached to the connecting rod and two silicon photodiodes (Ferranti, type MS 1AE). The system was driven by a waveform generator (Servomex, type LF 141) permitting ramp stretches of variable speed and amplitude to be applied to the muscle. Linear length changes of up to 1·5 mm could be made in less than 10 msec (equivalent to 150 $mm.s^{-1}$, or 6 muscle $lengths.s^{-1}$). We are most grateful to Dr W. G. S. Stephens for designing and constructing the servo system for the stretcher.

Tension recorder. A variable capacitance gauge similar to the one employed by Huxley & Simmons (1968) was used to record muscle tension. Its natural frequency was around 2 kHz and it had a compliance of $0{\cdot}1\ \mu m.mN^{-1}$. The output of the capacitance bridge amplifier was displayed on one beam of a dual channel oscilloscope and the applied length change was displayed on the other.

Optical system

The diffraction pattern produced by illuminating an area of muscle with a laser beam was recorded on cine film and used to measure the sarcomere spacing at intervals throughout the period of stretch. The sarcomere length, s, is given by

$$s = n\lambda/\sin\theta_n$$

where n is the order of the diffraction line; λ, the wave-length of light used to obtain the pattern; and θ_n, the angle subtended by the zero and nth order line. A helium–neon continuous wave laser (Metrologic Neon Laser) with a power output of 3 mW and a beam diameter of 1 mm was used to illuminate the muscles. The resulting diffraction patterns were projected on to a ground glass screen and photographed with a cine camera (Bolex H 16 M camera) operating at 64 frames/sec. Kodak 4X film was used. A system of mirrors enabled the oscilloscope display of tension and applied length change to be photographed simultaneously (Flitney, Hirst & Stephens, 1975).

The technique permitted muscle tension, over-all length and mean sarcomere length (within the area illuminated by the laser) to be recorded with a time resolution of approximately 15 msec. This meant that speeds of stretch in excess of about 6 $mm.s^{-1}$ could not be investigated satisfactorily, since too few data points could be gathered during the period of stretch. Fortunately, this was not a serious limitation because there is little change in the form of the tension records when working with speeds of stretch in excess of 4 $mm.s^{-1}$ (at 0 °C) and so most experiments were made at this velocity.

The diffraction spectra from individual frames of the film were later scanned with a Vickers scanning microdensitometer (M85) and the optical density profiles obtained were displayed on a chart recorder. Traces produced in this way showed sharp peaks corresponding with the zero and first orders and changes in their spacing of $\pm 0{\cdot}2\%$ could be detected, equivalent to a relative sliding movement of the filaments of $\sim 2{\cdot}5$ nm.

Two synchronized Devices 'Digitimers' (type 3290) were used to co-ordinate events.

RESULTS

Tension reponses

Characteristic form of the response to stretch. The tension responses produced by ramp-and-hold stretches fall into two categories, showing either *three* phases defined by two points, labelled S_1 and S_2, where the slope of the tension increment changes abruptly (Fig. 1*A*); or, more usually, *two* phases separated by a single point, S_2

(Fig. 1*B*, *C*). The point S_1 is reached for extensions of 40–60 μm (equivalent to 0·14–0·20% of the muscle length) and S_2 for extensions of 327–375 μm (equivalent to 1·16–1·34% muscle length). Tension rises steeply up to S_2, but thereafter the resistance to stretch is greatly diminished; in some cases tension actually falls during the remainder of the stretch (Fig. 1*B*) so that the muscle exhibits a negative slope stiffness. This paper is concerned primarily with the nature of the abrupt change of muscle stiffness which occurs at S_2.

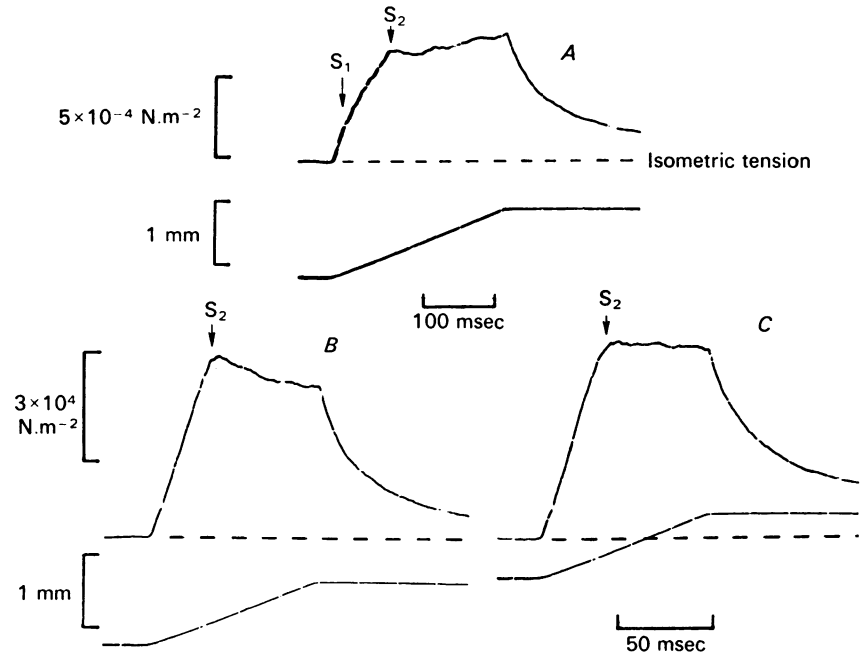

Fig. 1. Typical force records made during ramp-and-hold stretches applied to isometrically contracting muscles. *A*, tension record shows two points of inflexion, S_1 and S_2, with a small increase of tension after S_2. *B* and *C*, these records show only one point of inflexion, S_2, and tension either falling slightly (*B*) or remaining constant (*C*). *A*, stretch, 1060 μm; velocity, 4·24 mm.sec^{-1}; temperature, 0 °C. *B* and *C* stretch, 960 μm; velocity, 11·5 mm.sec^{-1}; temperature 0·5 °C. Sarcomere length before stretch = 2·34 μm (*B*), 2·48 μm (*C*) and 2·59 μm (*A*).

Rack & Westbury (1974) obtained qualitatively similar responses to stretch from cat soleus muscles. Again, the change in resistance to stretch at S_2 is the most striking feature of their records. They estimate that it occurs when the actin and myosin filaments are displaced by 25–35 nm. The question of the amount of filament sliding required to reach S_2 and its significance is considered later.

Effects of velocity of stretch and temperature on the tension supported by the muscle at S_2. The form of the tension response is affected by the velocity of stretch and by temperature. Several records showing responses obtained by stretching at velocities ranging from 0·25 to 24 mm.sec^{-1} are shown in Fig. 2. The muscle was stimulated tetanically at 0 °C and subjected to a stretch of 1060 μm. S_2 is visible on all the records, although for slower stretches the response is more rounded in form.

The results from two experiments in which the tension supported by the muscle at S_2 (P_{S_2}), expressed as a multiple of the maximum tetanic tension (P_0), is plotted against velocity of stretch are shown in Fig. 3. The ratio P_{S_2}/P_0 increases steeply with increasing speed of stretch, reaching a maximum value (1·55) at 3·8 mm.sec^{-1} (equivalent to 0·14 muscle length.sec^{-1}); thereafter, it remains nearly constant, up to 64 mm.sec^{-1} (2·35 muscle lengths.sec^{-1}). Thus, there is a range of speeds of stretch over which P_{S_2}/P_0 displays a marked dependence on velocity, and a range over which it shows little or no dependence. The particular velocity marking the transition between the two (3·8 mm.sec^{-1} in this example) is referred to as the *critical velocity*, or V_c.

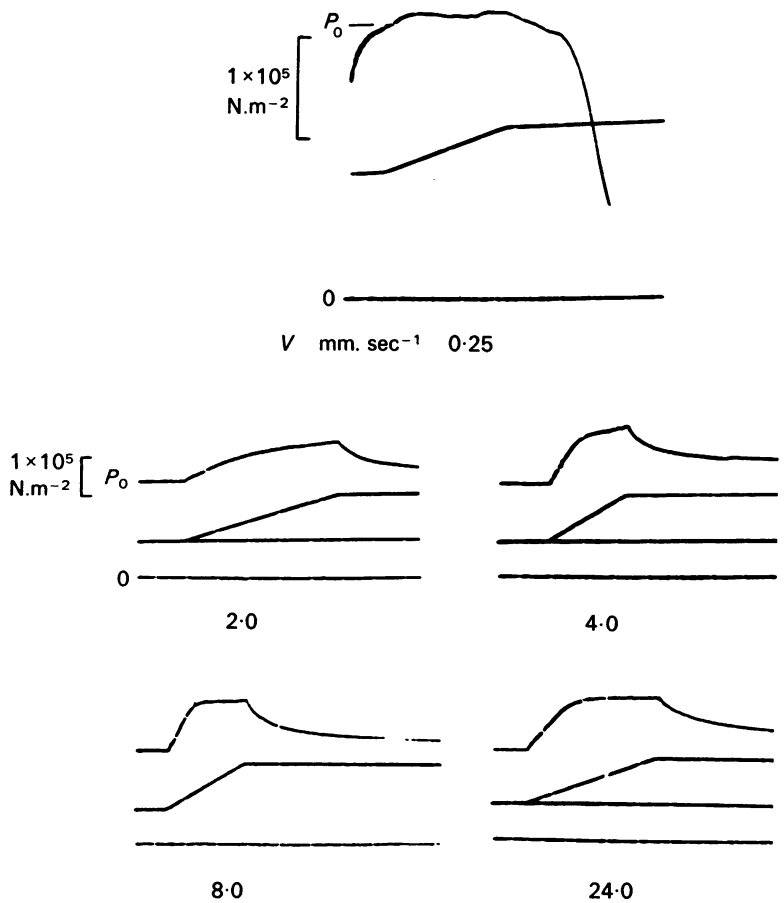

Fig. 2. Form of the tension responses produced by stretch (1060 μm) at different velocities. Numbers beneath traces, velocity in mm.sec^{-1}. Lower trace in each case represents base-line of zero tension. Note the more rounded form of the responses at slower speeds.

For a given velocity of stretch, P_{S_2}/P_0 decreases with increasing temperature. The discontinuity on the force–stretch velocity curve (representing the V_c) is displaced to the right, towards higher speeds of stretch, with increasing temperature. Table 1 lists V_cs for a muscle stretched at different velocities at 6 temperatures,

ranging from 0 to 30 °C, as well as 'plateau' values for P_{S_2}/P_0. The V_c has positive temperature coefficient (Q_{10}) of 1·78. Note the decrease ($\times$ 1·33) in P_{S_2}/P_0.

V_cs for muscles with different maximum velocities of shortening ($V_{max.}$). The V_c is found to vary with the intrinsic speed of shortening when different muscles are compared (Flitney *et al.* 1976*a*). The velocity dependence of P_{S_2}/P_0 for frog and toad

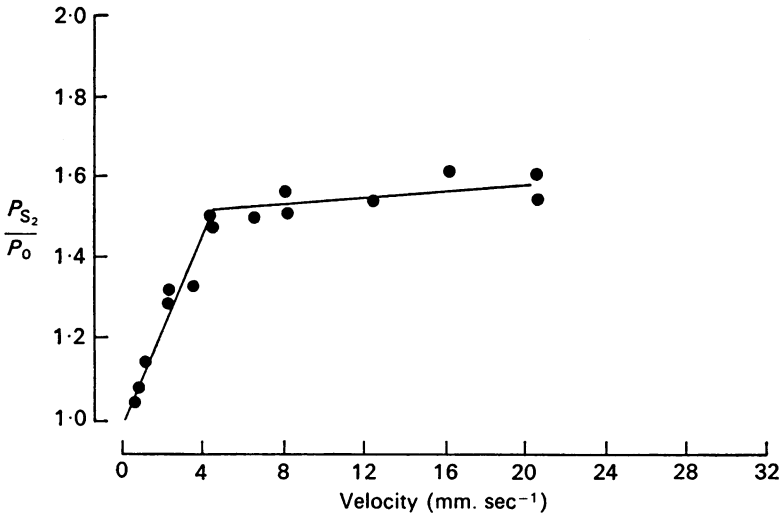

Fig. 3. Dependence of the ratio P_{S_2}/P_0 on velocity of stretch. Results for two frog sartorii at 0 °C. Note the angular form of the curve. The lines are fitted by eye and the point at which they intersect is taken to be the V_c. In this experiment, $V_c \cong 4$ mm.sec^{-1}.

TABLE 1. Critical velocities and peak tension at the point of sarcomere 'give' at different temperatures

Temp. (0 °C)	V_c mm.sec^{-1}	V_c Muscle lengths sec^{-1}	'Maximum'* P_{S_2}/P_0 values
0	3·8	0·136	1·58
6	5·0	0·179	1·47
12	7·5	0·268	1·41
18	10·5	0·375	1·29
23	14·5	0·518	1·25
30	21·8	0·779	1·19

* Measured at an arbitrary stretch velocity (greater than the V_c at all temperatures studied) of 24·4 mm.sec^{-1}. The critical velocity increases with increasing temperature (5·74 $\times$ in the range 0–30 °C) and the maximum P_{S_2}/P_0 value decreases with temperature (1·33 $\times$ over the same temperature range).

sartorii and for mouse soleus muscle are shown in Fig. 4. It is known that despite great similarities in their structure, toad and frog sartorii have different maximum speeds of unloaded shortening. At a given temperature, frog muscle is approximately 2 times faster than toad muscle (Abbott & Richie, 1951). The two curves of Fig. 4*A*, for an experiment made at 0 °C, shows that the V_c for toad muscle is

approximately one half of that for the frog. The values obtained were: frog, 3·8 mm.sec^{-1}; toad, 1·6 mm.sec^{-1}. The P_{S_2}/P_0–velocity curve for the mouse soleus muscle has a more rounded form (Fig. 4*B*), possibly due to the presence of fibres with different intrinsic speeds of shortening, with P_{S_2}/P_0 reaching 90 % of its maximum value at a velocity of 25 mm.sec^{-1} at 23 °C. It resembles the curves obtained for cat soleus muscle by Joyce, Rack & Westbury (1969; their Fig. 5) and by Joyce & Rack (1969; their Fig. 5).

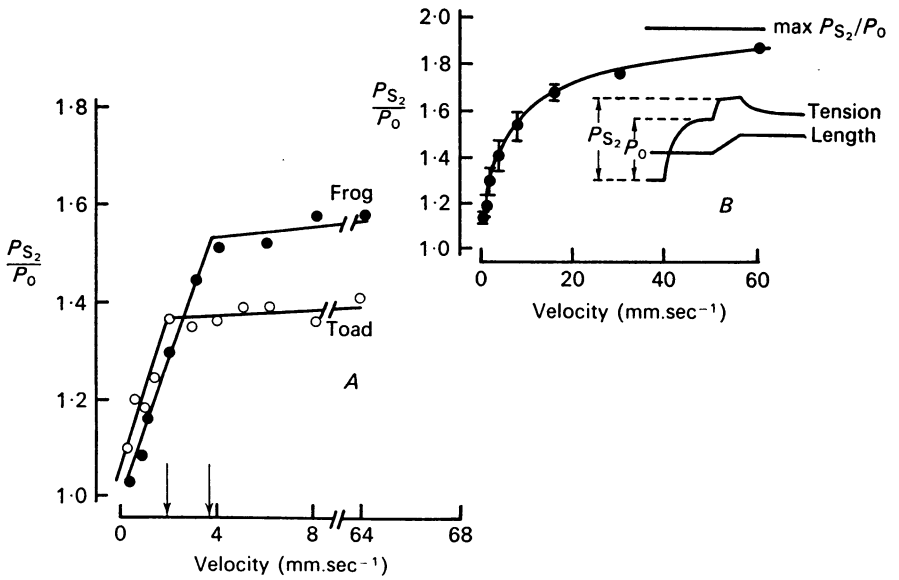

Fig. 4. *A*, the dependence of P_{S_2}/P_0 on velocity of stretch for the toad (open circles) and frog (filled circles) are shown for comparison. Temperature 0 °C. Stretch 960 μm. The V_cs obtained are: frog, 4 mm.sec^{-1}; toad, 2 mm.sec^{-1}. Note that maximum P_{S_2}/P_0 value is greater for frog than for toad (1·55 compared with 1·38). *B*, P_{S_2}/P_0–velocity relation for the mouse soleus muscle. Temperature 23 °C. P_{S_2}/P_0 reaches 90 % of its maximum value at a stretch velocity of 25 mm.sec^{-1}.

Sarcomere movements

Sarcomere lengths during development and maintenance of a tetanus

Length–tension diagram of the series elastic component. Cine recordings of the diffraction spectra made during the development of an isometric tetanus generally show a progressive increase in the spacing of the lines, caused by shortening of the sarcomeres at the expense of inert elastic elements arranged in series with them. The extent of the internal shortening in a muscle held at constant length is a function of the force generated by the sarcomeres and the length–tension characteristics of the series elastic elements. Measurements of the change of sarcomere spacing during tension development were made at four different levels in the muscle (4, 12, 16 and 20 mm from the pelvic end). Fig. 5 shows the isometric force plotted against the mean internal shortening (integrated over the length of the muscle) for the four positions sampled. The resulting curve is non-linear and, as with many biological materials, the stiffness increases with increasing amounts of extension. At peak

isometric tension the compliance of the 'lumped' series elastic elements, including 'stray' compliance in the apparatus, is estimated to be $6{\cdot}3 \times 10^{-4}$ m.N^{-1}.

Sarcomere 'dither'? Fluctuations of the length of the sarcomeres during the tension plateau of a fused tetanus have been reported previously (Goldspink, Larson & Davies, 1970) in chick anterior and posterior latissimus dorsi muscles. Oscillations

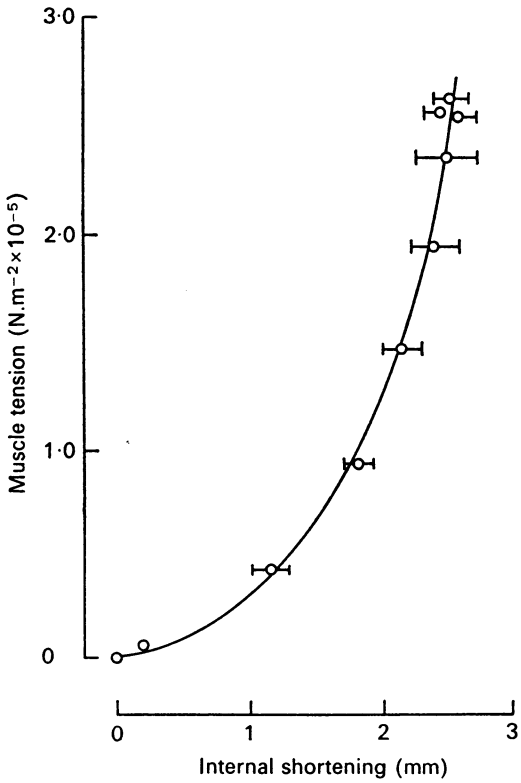

Fig. 5. Length–tension relation for the 'lumped' series elastic elements. Internal shortening is estimated from the change in sarcomere length during the development of isometric tension, integrated over the length of the muscle. Sarcomere spacings at three different levels in the muscle were sampled on successive runs. $P_0 = 2{\cdot}8 \times 10^5$ N.m^{-2}. $l_0 = 28$ mm.

of up to 90 nm with a period of 75 msec were described and referred to as sarcomere 'dither'. No fluctuations of this magnitude were observed with the system employed here, although the occurrence of much smaller oscillations, less than about ± 3 nm, cannot be ruled out; neither can the possibility of larger but asynchronous movements of the filaments be excluded (Fujime, 1975). This result is in agreement with the more recent work of Cleworth & Edman (1972), Kawai & Kuntz (1973) and of Borejdo, Mason & Unsworth (1974), all of whom experimented with single fibres.

Sarcomere movements during stretch

Rapid 'give' of sarcomeres. Fig. 6*C* shows two frames from a cine film showing muscle tension, external length change and diffraction patterns produced by the sarcomeres at the moment of exposure. The changes in sarcomere length, expressed as nm per half-sarcomere (phs), tension and muscle length during the period of stretch applied at the peak of a tetanus are depicted in Fig. 6*A*. The tension response is similar in form to the one shown in Fig. 1*B*, *C* with a well defined 'slip' point at S_2 (vertical arrow).

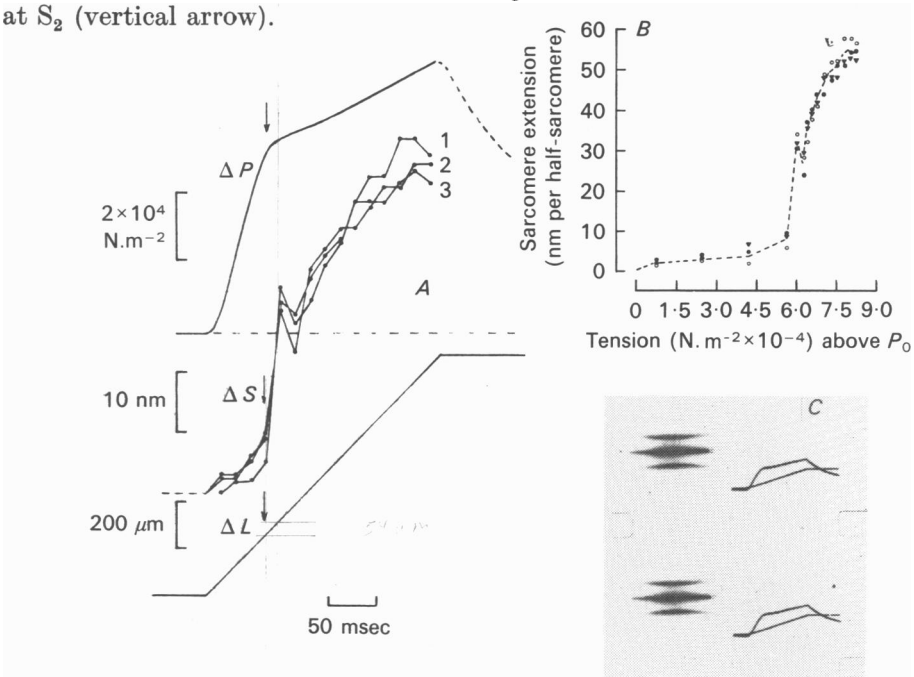

Fig. 6. *A*, values for muscle tension (ΔP), length (ΔL) and sarcomere length (ΔS) are shown during a ramp-and-hold stretch of 1080 μm, made at a velocity of 4·3 mm.sec^{-1}. Temperature 21 °C. Sarcomeres sampled at three different levels in the muscle: 8, 16, and 24 mm from the pelvic bone. Initial sarcomere length, 2·59 μm. The distance the filaments move to reach S_2 = 7·5–8·5 nm. This is less than the value obtained (11–12 nm) by stretching at speeds in excess of the V_c, which at this temperature is around 14 mm.sec^{-1} (see (Table 1).

There are three points of interest to note about the change in length of the sarcomeres.

First, in the period up to S_2 they elongate at a rate which is considerably less than that calculated from the rate of change of length of the muscle. The following figures serve to illustrate the point. The initial muscle length was 25 mm and steady state sarcomere length at the peak of the tetanus immediately prior to stretch was 2·49 μm. The *external* length change was 1·08 mm applied at a velocity of 4·3 mm.sec^{-1}. This would be expected to extend the sarcomeres at the rate of 0·43 μm.sec^{-1}, whereas the actual value, estimated from the first six frames of the film, was only 0·28 μm.sec^{-1}. It is concluded from this that a substantial fraction of the stretch

(in this example, about 0·35) is not taken up by the sarcomeres themselves, but instead by elastic elements arranged in series with them.

Secondly, when the sarcomeres are extended to the point S_2 their length increases abruptly. This sudden elongation is referred to as sarcomere 'give'. In the record of Fig. 6*A* it occurs for an increase of 7·5–8·5 nm phs. During the period of rapid

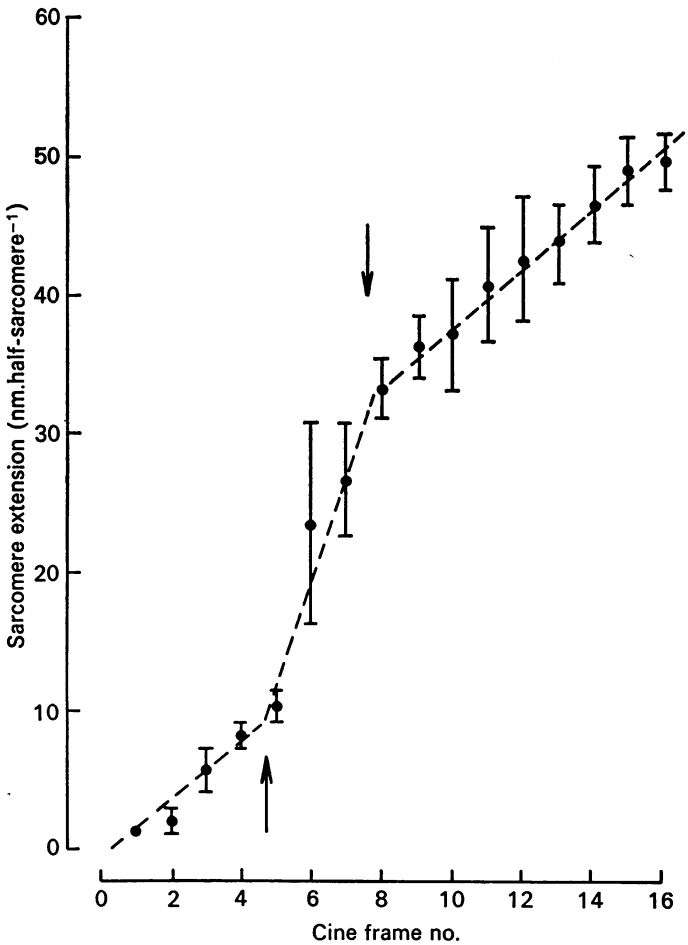

Fig. 7. Mean values (± 1 S.E.) for relative filament displacement for four muscles subjected to a stretch of 1020 μm at a velocity of 4·1 mm.sec^{-1}. Dashed line drawn by eye. Sarcomere 'give' commences at the upward-pointing arrow and ends at the downward arrow. Small differences in the time at which sarcomere 'give' commences produce large differences in the displacement of the filaments *during* 'give', hence the markedly larger error bars in this region of the diagram.

'give' their length increases by a further 22–24 nm.half-sarcomere, at an estimated (minimum) velocity of 1·42 nm.half-sarcomere^{-1}.msec^{-1}, bringing the total longitudinal displacement to 29·5–32·9 nm.half-sarcomere^{-1}.

Thirdly, elongation of the sarcomeres after rapid 'give' follows the external length change closely (velocity 0·4 μm.sec^{-1}, compared with the predicted value of

0·43 μm.sec^{-1}), although in nearly all of the records there is some overshoot initially. A heavily damped oscillation is clearly visible in Fig. 6*A*. During this phase of the response the tension supported by the muscle is substantially greater than the maximum isometric level, and is held more or less constant at this elevated level throughout the remainder of the stretch; there is sometimes a slight increase (at longer initial muscle lengths the parallel elastic elements in the muscle contribute to this) or a small decrease (Fig. 1*A*, *B*).

The change in sarcomere length for four different muscles subjected to a stretch of 1020 μm at a velocity of 4 mm.sec^{-1} are depicted in Fig. 7. Each point is the mean elongation (nm.half-sarcomere^{-1}) at the time corresponding with the cine-frame number shown on the abscissa. Sarcomere 'give' occurs for an increase of 10–11 nm.half-sarcomere^{-1} and the length increases by a further 21–23 nm.half-sarcomere^{-1} during the rapid phase of the movement.

Sarcomere stiffness and its dependence on filament overlap

The simplest explanation for the pattern of sarcomere length changes observed here is that a relative sliding movement of the actin and mysoin filaments occurs with stretch and that this movement results initially in deformation of the cross-bridges between the filaments, and ultimately in their detachment. An alternative explanation is that the change in sarcomere length occurs as the result of extension of the A and/or I bands without a relative sliding movement occurring. In order to distinguish between these possibilities experiments were made in which stretches were applied to muscles at different initial (peak tetanic) sarcomere lengths. If the increase in sarcomere length observed during the stretch is caused by sliding of the filaments, rather than to a change in their length, then the resistance of the sarcomeres to stretch should vary inversely with sarcomere length, decreasing to zero at a length of $\sim$ 3·6 μm, since the number of cross-bridges potentially able to contribute to the stiffness is proportional to the amount of filament overlap. Experiments were therefore made in which stretches were applied to muscles at starting lengths from 0·93 to 1·38 $\times$ l_0. The method of analysing the records was as follows. At lengths greater than about 1·1 $\times$ l_0 there is an appreciable contribution to the tension response from extension of passive elastic elements arranged in parallel with the contractile component, and to compensate the tension developed during stretch of a *resting* muscle was subtracted from that produced by an identical stretch applied to the muscle during an isometric tetanus. The slope of the line relating 'corrected' muscle tension to sarcomere extension ($\Delta P/\Delta S$) gives the required measure of sarcomere stiffness (see Fig. 6*B*). It is expressed as the force per unit area of muscle required to elongate each half-sarcomere by one metre (N.m^{-2} per m extension). The values are all related to the sarcomere length at the peak of the tetanus, immediately before stretching, rather than to the length at rest, to allow for the increase in the amount of filament overlap which occurs during the development of the tetanus.

Fig. 8 shows maximum isometric tension (P_0) and sarcomere stiffness (E_s) plotted against sarcomere length. The extrapolated region of the regression line fitted to the eight points falling on the descending limb of the sarcomere length–isometric tension diagram intercepts the abscissa at a value of 3·61 μm. This is very close to the

'theoretical' value of ~ 3·60 μm, at which sarcomere length the actin and mysoin filaments no longer overlap (Page, 1964; Huxley & Peachey, 1959; Guld & Sten-Knudsen, 1960), so that there is no possibility for cross-bridge formation. The regression line fitted to the values for E_s corresponding with the same eight points on the length–tension diagram intercepts the abscissa at a sarcomere length of 3·70 μm, within 3% of the expected value.

It is concluded from this that sarcomere stiffness is directly proportional to filament overlap, which is consistent with the view that the cross-projections linking the myosin filaments to the actin filaments provide the *major* source of the resistance to stretch.

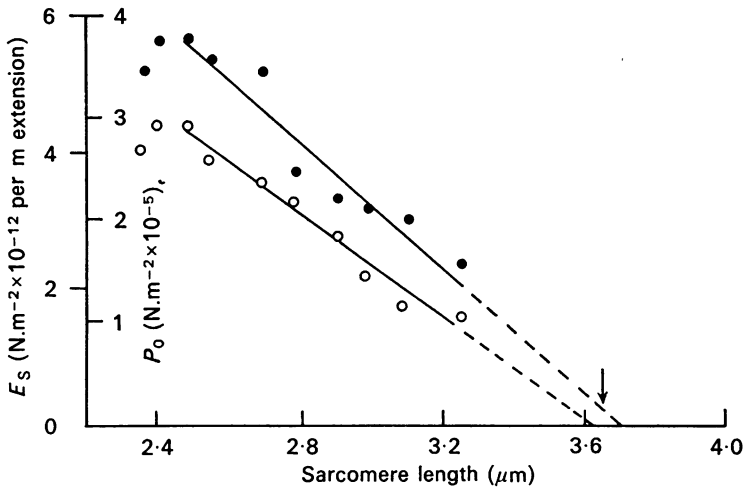

Fig. 8. Peak isometric tension (P_0, open circles) and sarcomere stiffness ($E_s = \Delta P/\Delta S$, filled circles) plotted against sarcomere length immediately prior to stretching. E_s and P_0 are maximal at a sarcomere length of 2·40–2·45 μm and both decrease progressively with increasing muscle length. The extrapolated portion of the isometric tension–sarcomere length curve intercepts the abscissa at a value of 3·61 μm and the E_s curve at 3·70 μm. The vertical arrow marks the length at which the actin and myosin filaments are disengaged. Stretches, 960 μm; velocity, 11·5 mm.sec^{-1}. Temperature 0 °C.

Bressler & Clinch (1974) used controlled releases and also showed that muscle stiffness decreased with increasing muscle length in direct proportion to the decrease in maximum tetanic tension. When extrapolated to zero stiffness, the line in their Fig. 7*A*, relating relative stiffness to muscle length, intercepts the abscissa at about 1·72 l_0, equivalent to a sarcomere spacing of 4·13 μm (taking the sarcomere length at l_0 as 2·4 μm). When muscle stiffness in the experiment of Fig. 8*A* is expressed as the change of tension per unit extension *of the whole muscle* ($\Delta P/\Delta L$), it too declines to zero at a length equivalent to a sarcomere spacing of 4·2 μm. This demonstrates clearly the need to record the movement of the filaments themselves, rather than to rely on calculations based on the degree of extension of the whole muscle.

The amount of filament sliding required to reach S_2 was found to be reasonably constant (to within 8%) over the range of sarcomere spacings investigated (2·34–3·27 μm). The mean value was 12·3 ± 0·9 nm (mean S.D.; n = 10). This figure represents the extent of the axial sliding movement required to generate maximum tension in the cross-bridges and to induce sarcomere 'give'.

Dependence of P_{S_2} on filament overlap. The maximum force the sarcomeres are able to sustain at the point S_2 displays a similar, inverse dependence on sarcomere length. The values obtained for P_{S_2}, taken from the same set of records used to obtain the data for Fig. 8, are plotted against sarcomere length in Fig. 9*A*. At lengths greater than 2·5 μm, P_{S_2} decreases with increasing sarcomere spacing, and the regression line intercepts the abscissa at a value of 3·6 μm. The linear relationship between P_0 and P_{S_2} (Fig. 9*B*) shows that the *ratio* P_{S_2}/P_0 is constant at all sarcomere lengths and it can therefore be concluded that the strength of each myosin-actin link is independent of the surface to surface distance separating the filaments. This is an interesting observation because the ability of each cross-bridge to *generate* force is likewise independent of the interfilamentary spacing (Gordon *et al.* 1966).

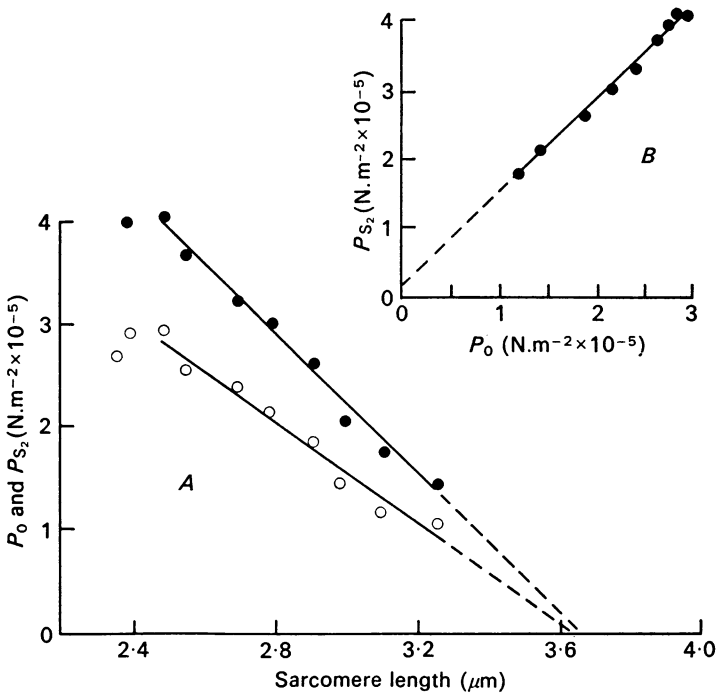

Fig. 9. *A*, P_0 (open circles) and P_{S_2} plotted against sarcomere length. Both parameters decrease with increasing sarcomere length (above 2·4–2·45 μm). The regression line for P_0 intercepts the abscissa at 3·61 μm and that for P_{S_2} at 3·66 μm. *B*, P_{S_2} plotted against P_0 over the range of sarcomere lengths 2·4–3·25 μm. The extrapolated regression line intercepts close to the origin.

DISCUSSION

The physiological significance of sarcomere 'give' is that it marks the transition between two steady states, caused by the deformation and eventual breakage of cross-linkages between the sliding filaments. It was seen (p. 458) that both the sarcomeres and the series elastic elements are extended by about the same amount during the initial part of a stretch and since muscle tension rises steeply, it follows that both components must offer a high resistance to the movement. It is postulated

that when displacement of the filaments in the axial direction exceeds 11–12 nm, the myosin cross-bridges linking the filaments together are forcibly detached and as a consequence the stiffness of the sarcomeres falls abruptly so that they are no longer able to resist the tension stored in the series elastic elements. The latter recoil and shorten at the expense of the weakened sarcomeres, causing them to lengthen rapidly.

The importance of the series elastic element cannot be over-emphasized. Edman, Elzinga & Noble (1976) monitored sarcomere movements during stretch of single frog fibres and found that their extension was uniform, even for length changes sufficiently great to displace the filaments by more than 12 nm from their starting position. There was no indication of 'give' and the sarcomeres were extended at a rate determined only by the speed of stretch. Significantly, the amount of series elasticity is much less in single fibres; their Fig. 1 shows that it could not have been more than 20% of that in a whole muscle.

If the above explanation is correct, then the stiffness of the sarcomeres during a contraction should decrease progressively with decreasing filament overlap, falling to zero at the length where the actin and myosin arrays no longer interdigitate. We have seen (p. 460) that this is so. Moreover, the tension supported by the muscle at the point of rapid 'give' displays a similar inverse dependence on sarcomere length. These observations taken together provide compelling evidence that *both the resistance to stretch, and the maximum tension developed in the muscle at the point of rapid 'give' are colligative properties of the myosin cross-bridges that interact with actin to generate the force for contraction.* Other results substantiate this view. It will be recalled that P_{S_2}/P_0 increases with increasing speed of stretch up to a critical velocity, V_c beyond which it is relatively independent of any further increase; and that the V_c has a large, positive temperature coefficient. These observations reinforce the idea that the force sustained by the muscle during sarcomere 'give' represents the sum of the tensions in the individual linkages, since P_{S_2} is clearly influenced by the rate at which cross-bridges cycle. In this connexion, it is worth emphasizing that under comparable experimental conditions muscles with greater intrinsic speeds of shortening ($V_{max.}$) have the greater V_c (Fig. 4).

It follows from what has been said that measurements of the movement of the sarcomeres in muscles subjected to stretch and the accompanying tension responses may offer a means of investigating the mechanical properties of the myosin cross-bridges and this possibility is exploited more fully in the following paper (Flitney & Hirst, 1978). Below, attention is drawn to two features of the response which appear anomalous at first sight and which require some explanation.

What limits filament sliding during sarcomere 'give'? It has been argued that at S_2 the series elastic elements shorten abruptly at the expense of the sarcomeres. It is evident from the records that the over-all change in length of the series elasticity during 'give' is small by comparison with the total amount by which it has been extended during the onset of the tetanus and the subsequent stretch. Clearly, something must limit the lengthening of the sarcomeres. We consider it unlikely that they are restrained by a few cross-bridges *remaining* attached to the actin throughout the stretch; in Fig. 7, for example, the total amount of filament displacement up to the end of 'give' is 33 nm, and this figure is greatly in excess of most

estimates for the maximum working range of a cross-bridge (see e.g. Curtin, Gilbert, Kretzschmar & Wilkie, 1974). It seems reasonable to argue instead that the resistance to continued movement arises either from the *re*-attachment of cross-bridges previously broken, or from the attachment of additional cross-bridges to sites on the actin filament that are not available initially, but which become accessible as a result of the movement, or to a combination of the two. It is not difficult to envisage that the attachment of a few cross-bridges will have the effect of decreasing the rate of filament sliding and, in doing so, increase the probability for the formation of more linkages, until the rapid phase of the movement is curtailed. The speed of sliding will be determined thereafter by the rate at which the muscle length is changing.

How is the high level of force maintained after sarcomere 'give'? The second, apparent anomaly is that in the period following sarcomere give the muscle maintains a force which is substantially *greater* than the maximum isometric level appropriate to the longer length. Délèze (1961) commented on this phenomenon and the matter has since been re-investigated by Edman *et al.* (1976). It is worth noting briefly that in many records there is a *transient* drop in tension immediately after sarcomere 'give', but this is generally quite small and it is followed either by a gradual increase, a small but continuing decrease, or else the tension remains constant. The question is, then, how is this high level of tension maintained? Although the speed at which the filaments move during sarcomere 'give' is high (in Fig. 6*A*, for example, the minimum velocity is estimated to be 1·42 nm . msec sec^{-1}, which is comparable with the maximum speed of shortening in an unloaded contraction, around 1·7 nm . msec sec^{-1} at 0 °C) it is improbable that viscous forces contribute much to the maintenance of this tension. The reason is simply that the resistance to stretch of a *resting* muscle at the same sarcomere length (i.e. with the same amount of filament overlap) is very small; furthermore, during stretch of active muscle at speeds in excess of the V_c (up to $15 \times V_c$ have been studied) the steady-state force after S_2 is virtually independent of velocity. Neither of these results would be anticipated if viscous effects were largely responsible for supporting tension after rapid 'give'.

A more likely explanation is that throughout the remainder of the stretch a new steady-state is established, during which cross-bridges attach to the actin filaments as and when sites become available, are extended until they develop maximum tension and break, then re-attach at a new position further along the filament. We should then expect tension to remain more or less constant beyond S_2 (or, for longer stretches where the amount of filament overlap decreases appreciably, show a small decrease); moreover, if the rate constant for cross-bridge formation is high, the maintained force should vary little with speed of stretch over a wide range of velocities. Both expectations are confirmed by the experimental results. Of course, if the speed of stretch were *greatly* increased then it might be anticipated that tension would fall steeply beyond S_2 (see e.g. Sugi, 1972) since there would then be insufficient time for re-attachment to occur.

Finally, some reference must be made of the point S_1 mentioned earlier, where it was pointed out (p. 451) that it is not a consistent feature of the response. Even when present, it is much less striking than the change of stiffness which occurs at S_2. Its significance is uncertain, but the length change required to reach S_1 corresponds closely with the amount of stretch required to fully extend the so-called

'short-range elastic component' (or SREC) of resting (Hill, 1968; Lannergren, 1971; Flitney, 1975) and contracting (Flitney & Hirst, 1974) frog's muscle and of 'skinned' frog fibres exposed to solutions containing calcium ions (Moss, Sollins & Julian, 1976). It should be noted that Rack & Westbury (1974) observed a qualitatively similar short-range elastic response in cat soleus muscle.

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REFERENCES

ABBOTT, B. C. & RICHIE, J. M. (1951). The onset of shortening in striated muscle. *J. Physiol.* **113**, 336–345.

BOREJDO, J., MASON, I. & UNSWORTH, J. (1974). Optical diffraction studies on stimulated single fibres of frog muscle. (*Hyla caerulea*). *Experientia* **30**, 373–374.

BRESSLER, B. H. & CLINCH, N. F. (1974). The compliance of contracting skeletal muscle. *J. Physiol.* **237**, 477–493.

CLEWORTH, D. R. & EDMAN, K. A. P. (1972). Changes in sarcomere length during isometric tension development in frog skeletal muscle. *J. Physiol.* **227**, 1–17.

CURTIN, N. A., GILBERT, C., KRETZSCHMAR, K. M. & WILKIE, D. R. (1974). The effect of the performance of work on the total energy output and metabolism during muscular contraction. *J. Physiol.* **238**, 455–472.

DELEZE, J. B. (1961). The mechanical properties of the semi-tendinosus muscle at lengths greater than its length in the body. *J. Physiol.* **158**, 154–164.

EDMAN, K. A. P., ELZINGA, G. & NOBLE, M. (1976). Force enhancement induced by stretch of contracting single isolated muscle fibres of the frog. *J. Physiol.* **258**, 95–96*P*.

ELLIOT, G. F., ROME, E. M. & SPENCER, M. (1970). A type of contraction hypothesis applicable to all muscles. *Nature, Lond.* **226**, 417–420.

FLITNEY, F. W. (1975). Light scattering associated with tension changes in the short range elastic component of resting frog's muscle. *J. Physiol.* **244**, 1–14.

FLITNEY, F. W. & HIRST, D. G. (1974). Short-range elastic properties of contracting frog's muscle. *J. Physiol.* **239**, 119–120*P*.

FLITNEY, F. W. & HIRST, D. G. (1975*a*). Rapid 'give' of sarcomeres and tension changes during servo-controlled stretches applied to contracting frog's muscle. *J. Physiol.* **244**, 68–69*P*.

FLITNEY, F. W. & HIRST, D. G. (1975*b*). Tension responses and sarcomere movements during length changes applied to contracting frog's muscle. *J. Physiol.* **251**, 66–68*P*.

FLITNEY, F. W. & HIRST, D. G. (1978). Filament sliding and energy absorbed by the cross-bridges in active muscle subjected to cyclical length changes. *J. Physiol.* **276**, 467–479.

FLITNEY, F. W., HIRST, D. G. & JONES, D. A. (1976). Effects of temperature and velocity of stretch on the maximum tension borne by the sarcomeres in contracting muscle. *J. Physiol.* **256**, 127–128*P*.

FLITNEY, F. W., HIRST, D. G. & STEPHENS, W. G. S. (1975). Apparatus for investigating sarcomere movements in contracting frog's muscle during servo-controlled length changes. *J. Physiol.* **251**, 8–9*P*.

FUJIME, S. (1975). Optical diffraction study of muscle fibres. *Biochim. biophys. Acta* **379**, 227–238.

GOLDSPINK, G., LARSON, R. E. & DAVIES, R. E. (1970). Fluctuations in sarcomere length in the chick anterior and posterior latissimus dorsi muscles during isometric contraction. *Experientia* **26**, 16–18.

GORDON, A. M., HUXLEY, A. F. & JULIAN, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol.* **184**, 170–192.

GULD, C. & STEN-KNUDSEN, O. (1960). Correlation of isometric twitch tension and latency relaxation to sarcomere length in frog muscle fibres. *Acta physiol. scand.* **50**, suppl. 63–65.

HILL, D. K. (1953). The optical properties of resting striated muscle. The effect of rapid stretch on the scattering and diffraction of light. *J. Physiol.* **119**, 489–500.

HILL, D. K. (1968). Tension due to interaction between the sliding filaments in resting striated muscle. The effect of stimulation. *J. Physiol.* **199**, 637–684.

HUXLEY, A. F. (1957). Muscle structure and theories of contraction. *Prog. Biophys. biophys. Chem.* **7**, 255–318.

HUXLEY, A. F. & PEACHEY, L. D. (1959). The maximum length for contraction in striated muscle. *J. Physiol.* **146**, 55–56*P*.

HUXLEY, A. F. & SIMMONS, R. M. (1968). A capacitance gauge tension transducer. *J. Physiol.* **197**, 12*P*.

HUXLEY, A. F. & SIMMONS, R. M. (1971*a*). Mechanical properties of the cross-bridges of frog striated muscle. *J. Physiol.* **218**, 59–60*P*.

HUXLEY, A. F. & SIMMONS, R. M. (1971*b*). Proposed mechanism of force generation in striated muscle. *Nature, Lond.* **233**, 533–538.

HUXLEY, A. F. & SIMMONS, R. M. (1973). Mechanical transients and origin of muscular force. *Cold Spring Harb. Symp. quant. Biol.* **37**, 669–680.

JOYCE, G. C. & RACK, P. M. H. (1969). Isotonic lengthening and shortening movements of cat soleus muscle. *J. Physiol.* **204**, 475–491.

JOYCE, G. C., RACK, P. M. H. & WESTBURY, D. (1969). The mechanical properties of cat soleus muscles during controlled lengthening and shortening movements. *J. Physiol.* **204**, 461–474

KAWAI, A. & KUNTZ, D. (1973). Optical diffraction studies of muscle fibres. *Biophys. J.* **13**, 857–876.

LANNERGREN, J. (1971). The effect of low level activation on the mechanical properties of isolated frog muscle fibres. *J. gen. Physiol.* **58**, 145–162.

MOSS, R. L., SOLLINS, M. R. & JULIAN, F. (1976). Calcium activation produces a characteristic response to stretch in both skeletal and cardiac muscle. *Nature, Lond.* **260**, 619–621.

PAGE, S. G. (1974). Filament lengths in resting and excited muscle. *Proc. R. Soc.* B **160**, 460–466.

PODOLSKY, R. J. & NOLAN, A. C. (1971). Cross-bridge properties derived from physiological studies of frog muscle fibres. *Contractility of Muscle Cells.* New Jersey: Prentice-Hall.

RACK, P. M. H. & WESTBURY, D. R. (1974). The short-range stiffness of active mammalian muscle and its effect on mechanical properties. *J. Physiol.* **240**, 331–350.

SANDOW, A. (1936). Diffraction patterns of the frog sartorius and sarcomere behaviour under stretch. *J. cell. comp. Physiol.* **9**, 37–54.

SHEAR, D. B. (1970). Electrostatic forces in muscle contraction. *J. theor. Biol.* **28**, 531–546.

SUGI, H. (1972). Tension changes during and after stretch in frog muscle fibres. *J. Physiol.* **225**, 237–253.