J. Physiol. (1972), **227**, *pp.* 1–17
With 4 plates and 3 text-figures
Printed in Great Britain

CHANGES IN SARCOMERE LENGTH DURING ISOMETRIC TENSION DEVELOPMENT IN FROG SKELETAL MUSCLE

BY D. R. CLEWORTH* AND K. A. P. EDMAN†
From the Department of Physiology, University of California, Los Angeles, California, U.S.A.

(*Received 13 September 1971*)

SUMMARY

1. Changes in sarcomere length during isometric contraction of isolated semitendinosus muscle fibres from the frog were studied using laser diffraction techniques. Movements of the first-order diffraction line relative to the zero-order reference were recorded from a screen on continuously moving film. Sarcomere length changes of 50 Å could be resolved in this way.

2. Following a latent period of approximately 12 msec after the stimulus of a single skeletal muscle fibre at 1–2° C, there appeared to be a simultaneous onset of tension development and sarcomere shortening. Provided that the fibre was uniformly excited along its length, different regions shortened together by approximately the same amount. The extent of the shortening was a function of the total compliance of the tendons and tension measuring device.

3. During the plateau of a smooth tetanus no fluctuations of first-order line width or zero- to first-order line spacing were detectable at any point examined along the preparation. This finding provides evidence that, in a functionally intact fibre, no synchronous oscillations of the sarcomeres, at least no length changes exceeding 50 Å, occur during a fused tetanus. Furthermore, the fact that the first-order line did not increase in width as the preparation went from rest to full activity indicates that contraction proceeds without appreciable change in distribution of sarcomere lengths.

4. The sarcomere movements during *relaxation* differed along the length of the fibre. As the tension declined smoothly, sarcomeres in some parts of the fibre underwent further shortening, while the end sarcomeres near the tendons and in one or two regions in the middle segment of the fibre were further extended. These data indicate that the duration of the

* USPHS Post-doctoral Research Fellow.
† Visiting Professor from the Department of Pharmacology, University of Lund, Lund, Sweden.

mechanical activity differs in different regions along the length of the fibre. The pattern of relaxation, i.e. the behaviour of the sarcomeres in different fibre segments, is unique to any particular fibre.

INTRODUCTION

There is ample evidence (e.g. A. F. Huxley & Niedergerke, 1954; H. E. Huxley & Hanson, 1954; H. E. Huxley, 1957, 1960, 1963) that the striated pattern of skeletal muscle fibres seen upon microscopical examination is resolvable as an interdigitating array of thick (A, myosin) and thin (I, actin) filaments within the sarcomere, or basic contractile unit of muscle. On the basis of these and other findings A. F. Huxley (1957) proposed a sliding-filament model to explain muscular contraction. According to this model, contraction results from the summation of forces acting at multiple sites between the two sets of filaments. Individual sites are thought to go through repeated cycles of activity, with each cycle contributing to the maintenance of position or relative motion of the A and I filaments. Thus, for example, during an isometric tetanus, filament overlap, i.e. interdigitation, increases as long as the contractile force developed can extend compliant structures in series with the regions of filament overlap. It would follow, therefore, that at the plateau tension of an iosmetric contraction when a maximum number of force producing sites are active (Huxley, 1957), the A–I filament overlap and thus the sarcomere spacing should remain constant.

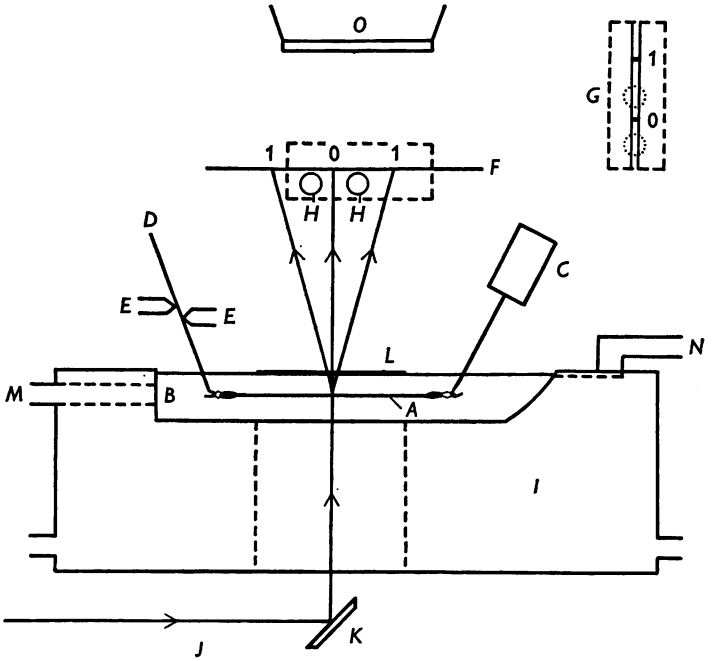
Recently, however, Larson, Kushmerick, Haynes & Davies (1968), using laser diffraction techniques on whole muscle reported that low amplitude sarcomere length changes ('dither') occurred during the plateau of a fused tetanus at a rate unrelated to the stimulation frequency. This rather unexpected finding contrasted with the preliminary results we had obtained at that time using the much simpler single skeletal muscle fibre preparation. The present study was conducted in order to clearly establish whether 'dither' could be observed in a single fibre preparation. Furthermore, as sarcomere length changes in the range of 50 Å are resolvable using the laser diffraction technique, we felt that much critical information could be obtained about the uniformity of sarcomere length in the fibre during contraction and relaxation. A brief account of these results has been published previously (Cleworth & Edman, 1969).

METHODS

Preparation. Single fibres were dissected from the dorsal head of the semitendinosus muscle of *Rana pipiens*. The essentials of the techniques of mounting and recording tension from the fibres have been described (Edman & Kiessling, 1971). As good optical conditions were crucial to the success of these experiments, care was taken to

obtain a very clean fibre from origin to insertion. All fibres had a non-circular cross-section, with the smallest and largest diameters in the range of 60–100 μm respectively. A schematic drawing of the experimental set-up is illustrated in Text-fig. 1. The dissected fibre (*A*) was mounted horizontally in a thermostated chamber (*B*) between a tension transducer (*C*) and a lever (*D*). The rest length of the fibre was set to the desired value by adjustment of opposing micrometer screws (*E*) operating on the lever. A glass cover slip (0·1 mm thickness) was placed on the surface of the Ringer fluid in the bath to prevent surface vibration.

The path of the laser beam (*J*) is illustrated in Text-fig. 1. The diffracted light was displayed on a partially transmitting horizontal screen (*F*) for photography. The distance between the fibre and the screen was generally within the range 10–14 cm. Three orders of diffraction lines could ordinarily be obtained. Normally a slit (1·5 mm width) was placed across the diffraction pattern on the screen (see *G*, camera view of screen) so that only a central segment of one first-order line (1) and the zero-order reference (0) was photographically recorded. Two neon bulbs (*H*) mounted on the under surface of the screen beneath the slit were used for time marking (60 c/s) and for the display of the stimulation signals.



Text-fig. 1. *A*, muscle fibre. *B*, muscle fibre chamber. *C*, tension transducer (RCA 5734). *D*, isotonic lever. *E*, micrometer screws. *F*, horizontal screen for display of diffraction pattern. *G*, camera view of slit placed across diffraction pattern on screen (*F*) illustrating segments of first- and zero-order diffraction lines. *H*, neon bulbs for time marking and display of stimulation signals. *I*, jacket for circulation of thermostatically controlled water-glycol mixture. *J*, laser beam. *K*, front surfaced mirror. *L*, glass slide placed on top of Ringer solution. *M*, inlet for bath solution. *N*, suction drain. *O*, camera.

Stimulation. The fibre was stimulated by passing current through a multi-electrode assembly consisting of six platinum wire electrodes arranged as alternate anodes and cathodes. Care was taken to ensure that each pair of electrodes produced a supra-maximal stimulus. Square pulses of 1 msec duration were used. One second trains of pulses of appropriate frequency were given at 3 min intervals to produce the desired complete or incomplete tetani. During a 4 hr period following completion of the dissection, the fibres were tetanized periodically and only those fibres which maintained a constant tension output were used.

Bathing solution. The Ringer solution used had the following composition (mM): NaCl, 115·5; KCl, 2·0; $CaCl_2$, 1·8; Na phosphate buffer, 2·0; pH 7·0. The bathing fluid (8 ml.) was replaced at approximately 1 hr intervals. The temperature of the bath varied between 1 and 2° C from experiment to experiment but was maintained with an accuracy of $\pm 0{\cdot}2°$ C throughout any particular experiment. Glass distilled water was used for the washing of glassware and for the preparation of solutions. All chemicals used were of analytical grade.

Tension recording. Isometric tension was recorded with an RCA 5734 mechano-electric transducer fitted with a glass extension (compliance 15 μm/g). The tension signal was displayed on a Tektronix 502 A oscilloscope and photographed with a Tektronix C 12 polaroid camera. The frequency response of the transducer with the glass tube attached to the anode pin was approximately 500 Hz.

Sarcomere length recording. The sarcomere length of the fibre at rest was measured at 800 × magnification within the middle segment (approximately 90 % of the fibre length, Edman, 1966) with a light microscope fitted with an ocular micrometer and a water immersion objective. Measurements were made for many different settings of the lever. At sarcomere lengths beyond 2·2 μm, a rectilinear relationship existed between sarcomere spacing and setting of the lever. It was possible to estimate sarcomere length in any selected region of the fibre to 0·02 μm with this method. In agreement with previous reports (Huxley & Peachey, 1961; Edman, 1966) the striation spacing at rest varied by less than $\pm 1{\cdot}5$ % of the mean along the middle segment of the fibre.

Marker studies. A large number of black nylon markers (13 μm diameter, approximately 125 μm in length) were spaced along the fibre and tendons, perpendicular to the long axis of the preparation. Regional length changes of the fibre were recorded photographically (Kodak 4X, 16 mm movie film) from the movements of these markers with a Bolex camera fitted to a Zeiss Stereo II microscope. The film speed used was 64 frames per second (fps) with an exposure per frame of 2·5 msec. The magnification on the film was 2·5 ×. Overlapping segments of the preparation were photographed for a series of tetani. Tracings of this photographic material were obtained at 15 × enlargement using a Kodak analysing projector. From the projected negatives, a reconstruction of the entire preparation could be traced from which the relative movements of the markers could be followed throughout a contraction cycle. Although any particular inter-marker distance could be read with an accuracy of $\pm 0{\cdot}1$ % of the fibre length, the reconstructed picture of the entire preparation was only accurate to $\pm 0{\cdot}75$ %. The reason for this discrepancy in accuracy lies in the difficulty in alignment of overlapping regions of the photographic recordings.

Laser diffraction. A helium-neon continuous-wave laser (Spectra-Physics, Stabilite Model 123) was used with the output adjusted to approximately 10 mW by means of a polarizer (beam diameter: 1·5 mm). This light intensity provided sufficient illumination for photographic recording and did not affect the mechanical performance of the fibre. The temperature difference between the illuminated area of the bath and the surrounding fluid was less than 1/50° C as measured by a thermocouple device. Movements of the first-order beam relative to the zero-order reference were recorded

on continuously moving film (streak photography on Kodak Tri-X 35 mm film at 50 and 100 mm/sec) by means of a Grass oscilloscope camera equipped with f/2 objective lens. For this purpose a slit (1·5 mm width) was placed across the diffraction pattern on the screen (see above).

The sarcomere spacing of the fibre may be calculated from the following relationship:

$$d = n\lambda/\sin\theta_n,$$

where n is the order of the diffraction line, λ the wave-length of the light, d the sarcomere spacing, and θ_n the angle between the nth and zero-order lines.

A theoretical discussion of the diffraction patterns obtained from multilayered gratings of the type present in whole muscle has been presented by Sandow (1936*a*, *b*). In these papers, Sandow concludes that the diffraction patterns obtained from a single grating and from the super-position of many such gratings are the same. In a single fibre, therefore, where the sarcomere I to I band distance along each myofibril represents the grating spacing, the superposition of the gratings results from the numerous myofibrils in the cross-section of the fibre arranged perpendicularly to the path of the incident beam. The diffraction angle θ_n, produced by such a composite structure is a function of the mean grating element distance within the fibre column illuminated by the laser beam. In the photographic image of the single fibre diffraction pattern obtained with the present experimental set-up, the meridional width of the order lines is dependent upon the diameter of the laser beam, the variation in sarcomere length within the fibre segment illuminated and the relative light intensities of the diffracted and non-diffracted light. As the majority of the incident light does not pass through the preparation, it contributes only to the zero-order reference with the result that this line is wider than the other diffraction lines. In order to prevent photographic over-exposure of the diffraction patterns, the zero-order intensity was reduced either by placing the appropriate neutral density filter on the upper surface of the screen (Text-fig. 1*F*) or by using an opaque mask with a pinhole (Pl. 1*A*). As the beam diameter, myofibril population sampled and the ratio of diffracted to non-diffracted light remain constant throughout a contraction, any change in width of the first-order line results from variations in sarcomere length in the illuminated region. Thus, in the present studies, the meridional width was used only to assess *changes* in distribution of sarcomere length within the fibre volume sampled.

As an independent check that the zero- to first-order diffraction spacing is solely a function of sarcomere length, optical measurements of sarcomere length (at 800 × magnification, see above) were made in parallel with diffraction measurements on the same fibre for a range of lever settings. The sarcomere lengths derived by the diffraction technique agreed with the microscopical data within the limits of accuracy of the latter measurement.

In some experiments the complete first- and zero-order diffraction pattern was photographed on 16 mm film (Kodak 4X) at 64 fps and 2·5 msec exposure using a Bolex camera. All photographic recordings were viewed in a Nikon Comparator at 10 and 20 × magnification.

Diffraction pattern of resting muscle fibre during length oscillations. A few experiments were designed to establish the lower limit of sarcomere length changes that could be resolved by means of the laser diffraction technique. For this purpose laser diffraction measurements were carried out while the fibre was subjected to passive oscillatory length changes. The fibre was mounted as illustrated in Text-fig. 1, but the lever used in these experiments was attached to an electromagnetic vibrator (Ling Shaker model 101) and could be made to oscillate in the longitudinal direction of the fibre. The wave form, amplitude and frequency of the

lever movements were controlled by means of a function generator which operated the vibrator. The movements of the lever were recorded with a capacitance type transducer. The output of the transducer was displayed on a Tektronix 502 A oscilloscope and photographed with a Cossor oscilloscope camera. A detailed description of the vibrator-lever system and the displacement transducer will be given later (Edman, manuscript in preparation). The output of the displacement transducer was calibrated by measuring the amplitude of the oscillatory movements (at a frequency of 0·4 c/s) at 80 × magnification using a Zeiss Stereo II microscope fitted with an attachment objective and an ocular micrometer. Such measurements could be made to 2 μm and were carried out for all amplitudes (10–100 μm) of lever oscillations studied in these experiments. The total fibre length (the distance between the insertions of the fibre to the tendons) was measured to 0·1 mm using a Zeiss Stereo II microscope at 6 × magnification. The sarcomere length was measured microscopically as described above.

The rationale of these experiments was to impose a length oscillation upon a resting fibre and successively reduce the amplitude of the oscillatory length changes until the cyclic movements of the first-order diffraction line were barely visible on the screen. The laser beam was moved in 0·5–1 mm steps along the entire length of the fibre, and the diffraction pattern was carefully examined at each new place while the same length oscillation was applied to the fibre. For visual examination of the diffraction pattern on the screen a Zeiss Stereo II microscope was used at 6 × magnification. Streak photographic recordings of the zero- to first-order spacing (see above) were also carried out at several different places along the length of the fibre. Knowing the sarcomere spacing, the total fibre length and the amplitude of the imposed length oscillation it was possible to estimate the changes in sarcomere spacing in Ångström units.

RESULTS

Diffraction patterns of resting single fibres and whole muscle

Pl. 1*A* illustrates a typical diffraction pattern obtained from a single, skeletal muscle fibre, consisting of three orders symmetrically spaced about the zero-order line. Pl. 1*B* is another example illustrating the first- and second-order diffraction lines on one side of the zero-order reference. It can be seen that the order lines have a distinct although irregular border. Furthermore, each order line is composed of a number of finer structures distributed in both the equatorial and meridional planes (Pl. 1*C*). When such photographic recordings were viewed in a Nikon comparator at 20 × magnification, changes in over-all line width and zero- to first-order spacing could be read to ± 0·2 mm. Typically at this magnification, the zero- to first-order spacing was 14–15 cm. This means that within the range of sarcomere lengths used in this study (2·2–2·6 μm), changes in sarcomere length could be resolved to 25–50 Å. This is illustrated in Pl. 1*D*, *E*, *F*, which consists of streak recordings of the zero- to first-order diffraction pattern of a resting muscle fibre that was subjected to sinusoidal length oscillations (frequency 12·2 c/s). The amplitude of these oscillations was 48 μm (*D*), 24 μm (*E*) and 12 μm (*F*), respectively. The total length of the fibre (between the insertions to the tendons) was 11·5

mm and the sarcomere spacing 2·48 μm. The imposed length oscillations would thus correspond to a fluctuation in sarcomere spacing of 104 Å (*D*), 52 Å (*E*) and 26 Å (*F*), respectively. The sarcomere movements are visualized as cyclic changes of the zero- to first-order spacing in the streak records. As can be seen on scrutinizing Pl. 1*F* the first-order streak line exhibits a fine waviness even for sarcomere changes as small as 26 Å. It was ensured (see Methods) that similar changes of the diffraction pattern were produced along the entire length of the fibre. The results would thus seem to make clear that it is possible to resolve changes resulting from 25 to 50 Å variations in sarcomere length.

The order lines in the diffraction patterns from single fibres (Pl. 1*A–C*) contain microstructural components and generally have a meridional width which does not exceed the diameter of the incident beam. In contrast with these distinct order lines, Pl. 1*G, H* shows, at equivalent magnification, a typical example of a diffraction pattern obtained from a whole frog sartorius muscle. The diffuseness of the whole-muscle pattern clearly does not allow for the high precision measurements of sarcomere length changes required in the present study.

Diffraction patterns of active muscle fibres

Recordings of zero- to first-order diffraction patterns (streak photography) and tension during isometric tetani of a single fibre are shown in Pls. 2–4. Pl. 2*A, B* shows length and tension records respectively, for the complete time course of a fused tetanic contraction. Pl. 2*C, D* shows the onset and plateau phases of Pl. 2*A* at 10·2 × magnification. Note that the first order during activity is recorded as a number of separate lines which result from the photographic streaking of the microstructure seen at rest. The sharpness of the edges of these streak lines and the fact that a given line could be followed, particularly during the plateau phase of tension, enables precise length measurements (to 50 Å) to be made throughout this period.

Following a latent period of approximately 12 msec after the first stimulus, there appeared to be a simultaneous onset of tension development and sarcomere shortening. The velocity of shortening measured as a slope value on Pl. 2*C* using a Nikon comparator increased smoothly to a maximum of 1·5 sarcomere lengths/sec reached approximately 17 msec after stimulation. Provided that the fibre was simultaneously and uniformly stimulated along its length, then different regions shortened together by approximately the same amount. This finding is illustrated in Pl. 3*A, B, C* which shows the onset of sarcomere shortening in three different regions of the same fibre. The microstructural pattern of the order line, as revealed by the non-parallel course of the streaking of the microstructural

components, often varied from region to region (Pl. 3*A*, *B*, *C*) during the initial, rapid sarcomere shortening phase associated with the rise of tension. However, during the plateau of a smooth tetanus (Pl. 2*A*, *D*) no fluctuations of first-order line width or zero- to first-order line spacing were detectable at any point of measurement. Furthermore, the microstructural lines ran a very nearly parallel course during this phase of the contraction. In two experiments streak photographic recordings like that illustrated in Pl. 2*A* were made at six to seven different places along the fibre. In these measurements the laser beam was moved in 1–2 mm steps along the entire middle segment of the fibre (approximately 90% of fibre length). Sixteen experiments were performed in which recordings were made at one to three places in the same fibre. In no case were any fluctuations in sarcomere length detectable during the plateau phase of an isometric tetanus.

From the magnified records of tension and length *vs.* time, a load-extension curve for the external series compliance of the preparation (tendons and connexions) and tension measuring device was obtained (Text-fig. 2). The points plotted were obtained from measurements made on the tension and diffraction records at specific intervals during the rising phase of a fused tetanic contraction. They have been empirically fitted with the equation:

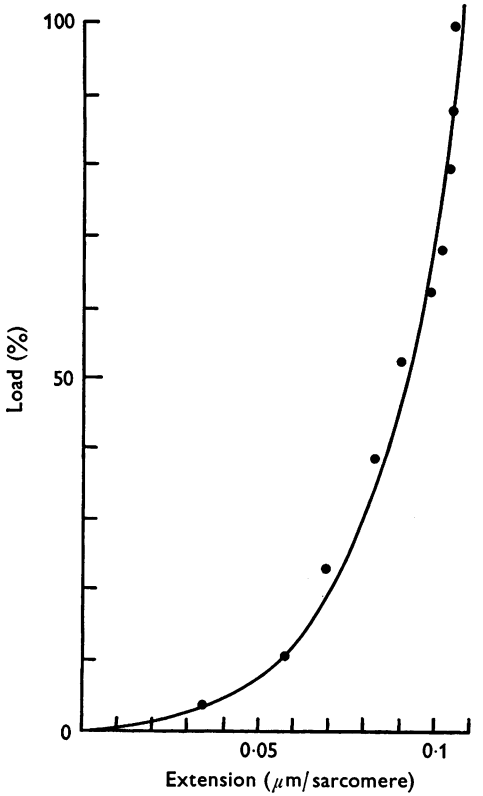
$$P = 1{\cdot}2(e^{\Delta s \cdot 41} - 1),$$

where P = load of the series elastic component in % maximal tetanic tension and Δs = extension in microns per sarcomere. Note that the curve becomes very steep as peak tension is approached with the result that a small length change is associated with a much greater change in tension. For this reason then, the length changes during an incomplete tetanus (Pl. 4*A*, *B*, *C*) were found to be less pronounced with each successive stimulus. This point is more clearly illustrated in Pl. 4*D*, *E*, *F* which shows at 4·0 × magnification the initial portion of length changes following each stimulus seen in Pl. 4*A*, *B*, *C*. At this frequency of stimulation (approx. 2·5 Hz) where the fibre was allowed to almost completely relax between successive stimuli, the diffraction pattern became more diffuse with each stimulus as is shown by the increased width and loss of intensity of the first-order line on repeated stimulation. The explanation for this increasing disorder lies with the complex sarcomere length changes occurring during relaxation which will be discussed in detail in the following section. It should be pointed out, however, that at frequencies of stimulation closer to fusion frequency (5–6 Hz), the diffraction pattern does not show any increased dispersion on successive stimulation.

The length changes occurring during relaxation were entirely unexpected.

For example, in Pl. 2*A*, during the latter part of the relaxation phase when tension is decaying smoothly, a widening and outward deflexion of the first-order diffraction line occurs indicating that the sarcomere population contributing to the diffraction pattern shortens further but is heterogeneous with respect to length at this time.

If some sarcomeres are shortening during relaxation then necessarily other sarcomeres must be stretched proportionally more at the same time in order for the tension to decay smoothly. Preliminary experiments indicated that sarcomeres at the ends were stretched during relaxation, but the extent of this yielding was insufficient to account for all of the shortening observed in parts of the middle segment of the fibre. Experiments were



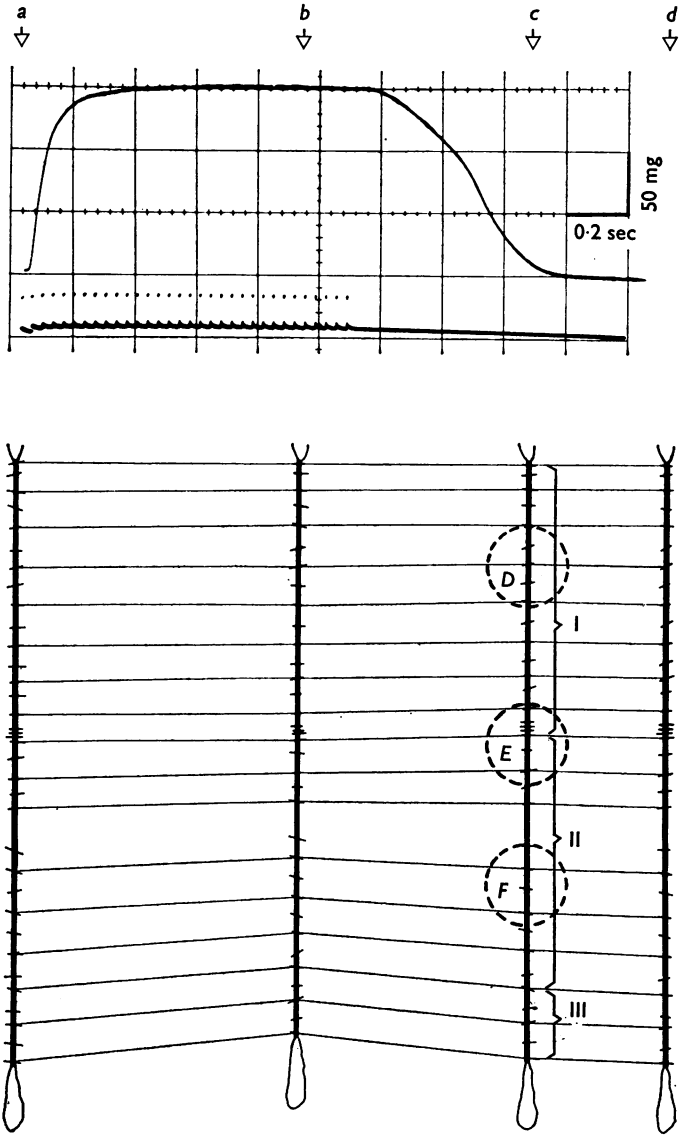
Text-fig. 2. Load-extension relationship of total series compliance of tendons and tension measuring device for single skeletal muscle fibre. Filled circles represent measured values (using a Nikon comparator) from tension/time and sarcomere-length/time records shown in Pl. 2*B*, *C*. Curve drawn from the empirical equation $P = 1{\cdot}2\ (e^{\Delta s \cdot 41} - 1)$ in which P denotes load (% maximal tetanic tension) and Δs the corresponding length change (μm/sarcomere).

therefore conducted to characterize the relaxation phase in detail along the entire length of the fibre.

Three typical diffraction patterns seen during relaxation are illustrated in Pl. 3*D*, *E*, *F*. These diffraction patterns are of portions of the first-order line obtained from three positions along the same single fibre preparation. It is evident that the sarcomere length changes occurring along the fibre are not synchronized during relaxation. In Pl. 3*D* the sarcomere population sampled clearly shortens further during the relaxation phase before returning to rest length. On the other hand, the diffraction pattern illustrated in Pl. 3*E* indicates that part of the sarcomere population is contracting and part is being stretched considerably beyond its rest length. In Pl. 3*F* the diffraction pattern indicates that almost all the sarcomeres are being stretched to some extent before returning to rest length once again.

In order to further elucidate the length changes seen during relaxation, and as an independent methodological check, diffraction measurements were combined with marker studies on the same fibre. Text-fig. 3 shows a tracing from a photographic montage of the movements of markers throughout the tetanic contraction-relaxation cycle shown in the upper part of the Figure. It can be seen that at the plateau of tension (*b*) the inter-marker intervals have shortened by the same proportion (5% of resting fibre length) in agreement with the uniform shortening of the sarcomeres seen from the laser diffraction recordings in Pl. 3*A*, *B*, *C*. The increase in length going from *b* to *c* in Text-fig. 3 corresponding to the relaxation phase of the isometric myogram, is 4% of the length existing at rest. However, the regional differences in length during relaxation observed in the diffraction studies (cf. above) are clearly demonstrated with the marker technique. It can be seen that region III stays virtually the same while region I undergoes a further shortening. On the other hand, region II is stretched beyond its rest length. The encircled areas of regions I and II in Text-fig. 3 correspond respectively to those sections of the fibre from which the diffraction patterns shown in Pl. 3*D*, *F* have been obtained. Pl. 3*E* corresponds to the encircled area *E* shown in Text-fig. 3.

Such differential length changes occurring during relaxation were found in all fibres examined. The pattern of relaxation, i.e. the localization within the fibre of the parts that shortened and were stretched respectively, differed from one fibre to another. However, the pattern exhibited by any particular fibre remained constant throughout the experiment, provided that the stimulus conditions were kept constant.



Text-fig. 3. Upper Figure: tension–time course of a fused isometric tetanus of a single skeletal muscle fibre. Lower Figure: tracings *a*, *b*, *c* and *d* from photographic montages of an entire fibre-tendon preparation showing the position of nylon markers at the times indicated by arrows in the upper Figure. In the construction of each montage, the position of each nylon marker was referenced to the marker closest to the upper tendon. The lines drawn between the fibre tracings link the same marker throughout the contraction-relaxation cycle. The dashed circles have approximately the same diameter (1·5 mm) as the laser beam used in these experiments. Diffraction patterns from the encircled regions (*D*, *E* and *F*) of the fibre obtained by streak photography are shown in Pl. 3 *D*, *E*, *F*. Resting sarcomere length, 2·53 μm.

DISCUSSION

In the present investigation a laser diffraction technique was used to record sarcomere movements in isolated skeletal muscle fibres during the time course of a contraction-relaxation cycle. The diffraction patterns obtained were found to have both meridional and equatorial components. A comparison of the meridional order line spacing in the diffraction pattern with high power light microscopical measurements enabled us to conclude that the zero- to first-order spacing used in this study is a very accurate measure of sarcomere length. Furthermore, as the width of the first-order line obtained from a fibre at rest was generally no greater than the diameter of the incident laser beam, it was concluded that there is little variation in the length of the sarcomeres in the fibre segment (i.e. approximately 90 % of fibre length) that gave sharp patterns using the present technique. An equivalent narrow distribution of sarcomere lengths was also seen throughout the plateau phase of an isometric tetanic contraction. These results are in marked contrast to the relatively diffuse patterns obtained for whole muscle where the distribution of sarcomere lengths from fibre to fibre is evidently greater than that for the single fibre preparation.

The equatorial and meridional components of the diffraction lines consisted of many, sharply defined microstructural elements. This microstructure proved to be an important factor for the purposes of the present investigation. By following the movements of these distinct structures throughout a contraction, it was possible to resolve sarcomere length changes with an accuracy of 50 Å.

Although the precise origins of the microstructure in the diffraction patterns have not been definitely established, attempts were made to evaluate them by obtaining diffraction patterns from various test gratings and, according to the method described by Klug & Berger (1964), from low power electron micrographs of single skeletal muscle fibres (D. Cleworth & K. A. P. Edman, unpublished). For example, the diffraction pattern obtained from a test grating consisting of a number of wavy slits in register in which the peak to peak interval of the wave along the slits was variable, gave a diffraction pattern complete with microstructure similar to that obtained from single fibre preparations. Furthermore, the diffraction patterns obtained from single or stacked electron micrographs of single skeletal muscle fibres were also similar to these from living fibres. Thus from an evaluation of the common features in these two test situations it appears very likely that the microstructure is derived in part from the slightly wavy A–I boundaries within a myofibril and the waviness due to

the staggering of sarcomeres along the long axis of the fibre in adjacent myofibrils across the intact fibre preparation. The equatorial spreading of the diffraction patterns also has a component which results from the placement of the recording screen beyond the back focal point of the lens-like circular cross-section of the fibre.

The distinct onset of deflexion of the first-order line (minimum resolution time 2 msec) upon uniform stimulation of the fibre is consistent with the findings of Gonzáles-Serratos (1971), who demonstrated by means of high speed cine-microphotography, that the inward spread of activation of a 100 μm thick fibre is complete within a time interval of 1–2 msec at 5° C. However, our observations do not exclude the possibility that there is a gradient of activation of the myofibrils from the periphery to the centre of the fibre. Even in the situation where the central myofibrils are barely activated they can shorten along with the more fully activated peripheral myofibrils which bear the load.

The findings of the present study on the single muscle fibre preparation have clearly demonstrated that no length oscillations occur during the plateau of isometric tension within the limits of resolution of the method used here. In contrast, experiments conducted by Nicolai (1936) on whole frog sartorius muscles at 20° C stimulated by means of a single anode and cathode pair seemed to indicate that length oscillations (approx. 5% of rest length) synchronized with the stimulation did occur during a tetanic contraction. (It was pointed out by Nicolai, however, that some of his experiments did not show any evidence of sarcomere oscillations during a fused isometric tetanus.) Davies and collaborators using similar techniques to those employed by Nicolai described oscillatory sarcomere length changes asynchronous with the stimulation in both frog (Larson *et al.* 1968) and mammalian (Goldspink, Larson & Davies, 1970) whole skeletal muscle preparations. As details of the stimulus parameters and electrode placement are not sufficiently discussed by these authors to allow us to make a direct comparison of their results with those presented here for single fibres, a series of experiments were conducted in which frog sartorius muscles were tetanized under carefully controlled stimulus and isometric conditions (D. Cleworth, unpublished). The results of these experiments, like those presented above for single fibres, indicate that no length oscillations are evident during the plateau of an isometric tetanus, though it must be remembered that the resolution of the method is not nearly as good with whole muscle as it is with single fibres.

The question of length oscillations during the plateau phase of tension in an isometric contraction has interesting implications in the evaluation of muscle energetics. It is well established that there is adenosine triphosphate break-down and heat production during the maintenance of isometric

tension (e.g. Mommaerts, 1969) indicating cycling of cross-bridge activity. The present results have shown that this cyclical cross-bridge activity does not involve sarcomere movements in excess of 50 Å.

The uniform behaviour of the sarcomeres in different parts of the fibre during the onset and plateau of an isometric contraction is not maintained during relaxation. It was shown (also see Cleworth & Edman, 1969) that the end sarcomeres and one or more regions in the middle segment of the fibre had shorter duration of activity and were therefore being stretched beyond rest length during relaxation by those parts which had a longer lasting activity. A similar phase shift between tension and sarcomere length near to one or both ends of the fibre has been observed by Huxley & Simmons (1970) using a different technique. A practical implication of this phenomenon is that a measurement of the duration of active state of the entire single fibre preparation is actually a measure of the longest duration of activity in the sarcomere population in the fibre. Furthermore these non-uniform length changes along the length and through the fibre cross-section during relaxation will seriously complicate attempts to length-clamp a fibre during this phase of a contraction.

The reason for this difference in duration of the mechanical activity along the fibre is still unclear. The shorter duration of the activity in the sarcomeres at the ends of the fibre is in agreement with the findings of Edman & Kiessling (1971) that the duration of the active state is directly related to sarcomere length. However, these latter observations cannot explain the difference in duration of contraction observed in the central segment of the fibre, where the sarcomeres were all of the same length at rest. It should be re-emphasized at this point that the regional relaxation pattern remains perfectly constant over a whole day of experimentation, which seems to rule out the possibility that it results from a progressively developing fibre damage. We can only speculate at the present time that variations in duration of contractile activity result from differences in the amount of activator-calcium being released and/or the rate at which the activator is resequestered. The interesting possibility exists that the amount of sarcoplasmic reticulum varies significantly along the length of the fibre.

The authors wish to thank Professor W. F. H. M. Mommaerts for his encouragement and facilities. Supported in part by PHS Grant HE-11351 to Professor Mommaerts and by grant No. 14X-184 (to K.A.P.E.) from the Swedish Medical Research Council.

REFERENCES

CLEWORTH, D. & EDMAN, K. A. P. (1969). Laser diffraction studies on single skeletal muscle fibers. *Science, N.Y.* **163**, 296–298.

EDMAN, K. A. P. (1966). The relation between sarcomere length and active tension in isolated semitendinosus fibres of the frog. *J. Physiol.* **183**, 407–417.

EDMAN, K. A. P. & KIESSLING, A. (1971). The time course of the active state in relation to sarcomere length and movement studied in single skeletal muscle fibres of the frog. *Acta physiol. scand.* **81**, 182–196.

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.

GONZÁLES-SERRATOS, H. (1971). Inward spread of activation in vertebrate muscle fibres. *J. Physiol.* **212**, 777–799.

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

HUXLEY, A. F. & NIEDERGERKE, R. (1954). Structural changes in muscle during contraction: Interference microscopy of living muscle fibres. *Nature, Lond.* **173**, 971–973.

HUXLEY, A. F. & PEACHEY, L. D. (1961). The maximum length for contraction in vertebrate striated muscle. *J. Physiol.* **156**, 150–165.

HUXLEY, A. F. & SIMMONS, R. M. (1970). Rapid 'give' and the tension 'shoulder' in the relaxation of frog muscle fibres. *J. Physiol.* **210**, 32–33*P*.

HUXLEY, H. E. (1957). The double array of filaments in cross-striated muscle. *J. biophys. biochem. Cytol.* **3**, 631–648.

HUXLEY, H. E. (1960). Muscle cells. In *The Cell*, vol. 4, ed. BRACHET, J. & MIRSKY, A. E. New York and London: Academic Press.

HUXLEY, H. E. (1963). Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. molec. Biol.* **7**, 281–308.

HUXLEY, H. E. & HANSON, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their natural interpretation. *Nature, Lond.* **173**, 973–977.

KLUG, A. & BERGER, J. E. (1964). An optical method for the analysis of periodicities in electron micrographs and some observations on the mechanism of negative staining. *J. molec. Biol.* **10**, 565–569.

LARSON, R. E., KUSHMERICK, M. J., HAYNES, D. H. & DAVIES, R. E. (1968). Internal work during maintained tension of isometric tetanus. *Biophys. J.* **8**, MA 4.

MOMMAERTS, W. F. H. M. (1969). Energetics of muscular contraction. *Physiol. Rev.* **49**, 427–508.

NICOLAI, L. (1936). Über das Beugungsspectrum der Querstreifung des Skeletmuskels und einen direkten Beweis der Diskontinuität der tetanischen Kontraktion. *Pflügers Arch. ges. Physiol.* **237**, 399–410.

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

SANDOW, A. (1936*b*). Diffraction patterns of the frog sartorius and sarcomere behaviour during contraction. *J. cell. comp. Physiol.* **9**, 55–75.

EXPLANATION OF PLATES

PLATE 1

A, an example of a diffraction pattern from a single muscle fibre of the frog. Three order lines symmetrically spaced about the incompletely masked zero-order reference are shown. The centre of the zero-order beam is visible as a spot (through a pinhole) in the masked area. Length scale (as also in *B*, *C* and *G*) refers to the original screen from which the diffraction pattern was photographed. Sarcomere spacing 2·42 μm. Distance between fibre and screen 7·1 cm.

B, first- and second-order diffraction lines on one side of partially masked zero-order reference illustrating microstructural detail of the order lines. Sarcomere spacing 2·42 μm. Distance between fibre and screen 14·0 cm.

C, the second-order diffraction line seen in *B* at 2× magnification.

D, *E* and *F*, streak photographs of zero- to first-order diffraction pattern of resting single fibre subjected to sinusoidal length oscillations. Inserts: oscilloscope records of changes in over-all fibre length. Calibrated value of transducer output given for each record. Mean fibre length and sarcomere spacing during length oscillations 11·50 mm and 2·48 μm, respectively.

G, diffraction pattern obtained from whole frog sartorius muscle at a magnification equivalent to that in *A*. Sarcomere spacing 2·53 μm. *H*, enlargement (3·3×) of the zero- to second-order pattern illustrated in *G*.

PLATE 2

Streak photographs of diffraction pattern and the corresponding isometric myogram of active single skeletal muscle fibre.

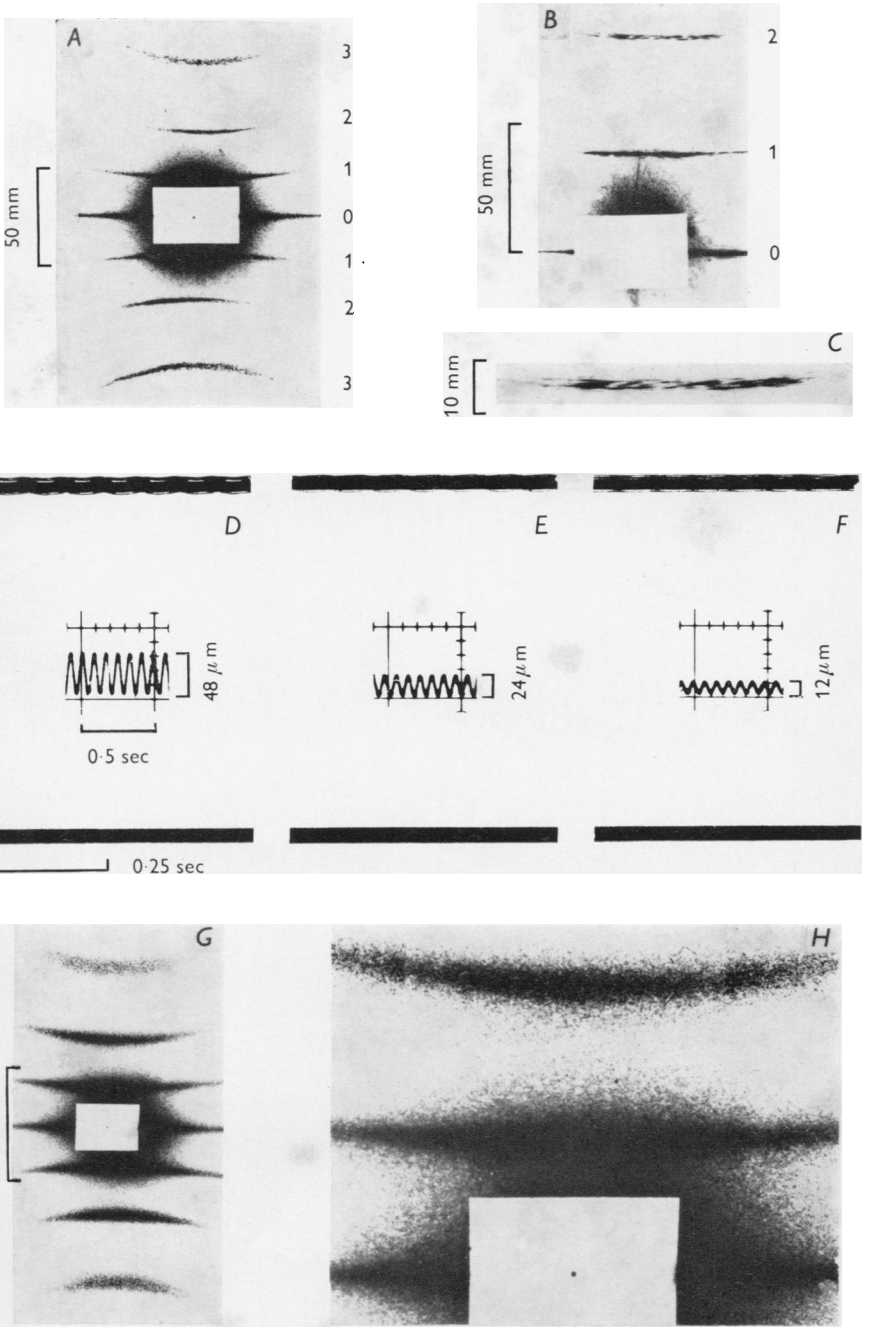
A, relative movements of the first-order line throughout the contraction-relaxation cycle of a fused tetanus. Stimulus and 60 Hz markers are shown respectively, above and below zero-order reference. First stimulus marker retouched.
B, isometric myogram and stimulus markers replotted from the original oscilloscope record to coincide with the time base of the diffraction record.

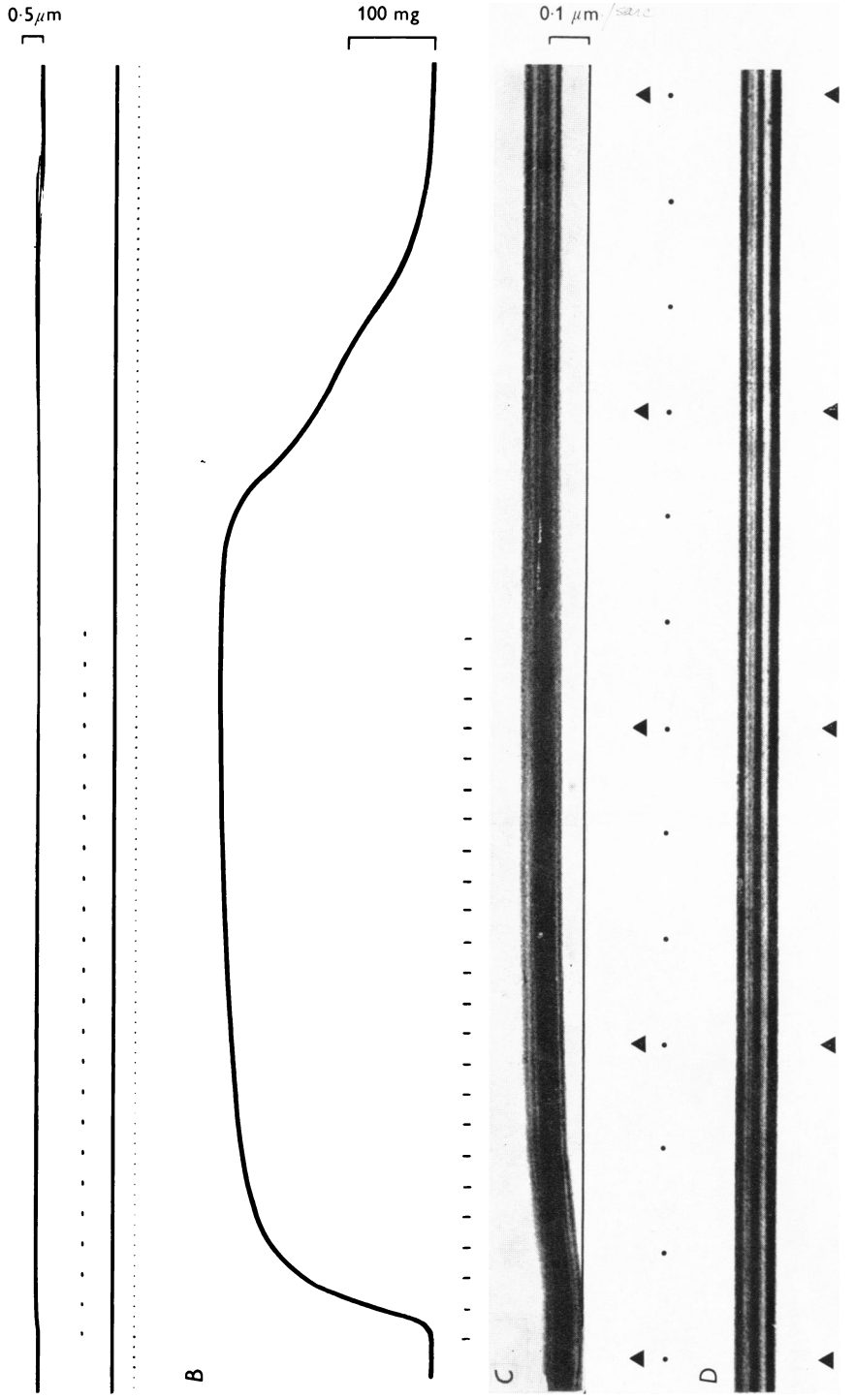
C and *D* show respectively, the first-order diffraction pattern at the outset and during the plateau of the fused tetanic contraction (10·2× magnification of *A*). The solid line drawn as a continuation of the first-order line in *C* serves as a base line to illustrate the position of the diffraction pattern if the fibre had remained inactive. Stimulus (triangles) and 60 Hz (dots) signals have been replotted and displaced towards the first-order line. Resting sarcomere length 2·56 μm.

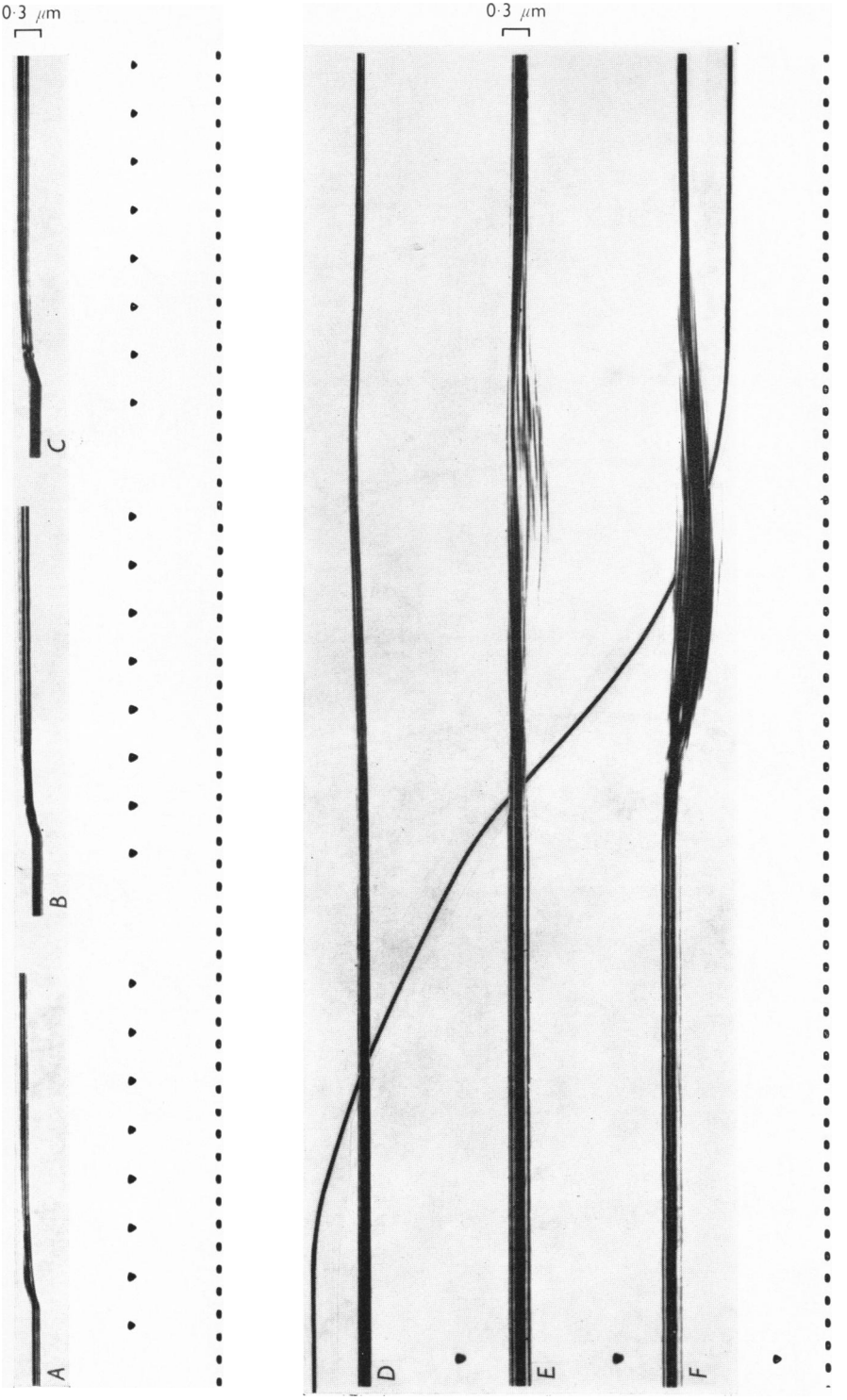
PLATE 3

A, *B* and *C*, streak photographic recordings of first-order diffraction patterns obtained from three different regions of the same fibre as it passed from rest to fused isometric tetanic contraction. Stimulus and 60 Hz event markers are shown below.

D, *E* and *F* are streak photographic recordings of movements of the first-order line from three regions of the same fibre obtained over the same time period during relaxation. The corresponding tension-time course of relaxation is superimposed. Data from the same experiment are illustrated in Text-fig. 3. The fibre segments investigated in *D*, *E* and *F* are indicated by the encircled areas (lettered correspondingly) in Text-fig. 3. Sarcomere spacing at rest: 2·53 μm.


A
3
2
1
0
1
2
3
50 mm
B
2
1
0
50 mm
C
10 mm
D
E
F
48 μm
24 μm
12 μm
0·5 sec
0·25 sec
G
H
50 mm


0·5 μm
100 mg
0·1 μm
A
B
C
D


0·3 μm
A
B
C
0·3 μm
D
E
F

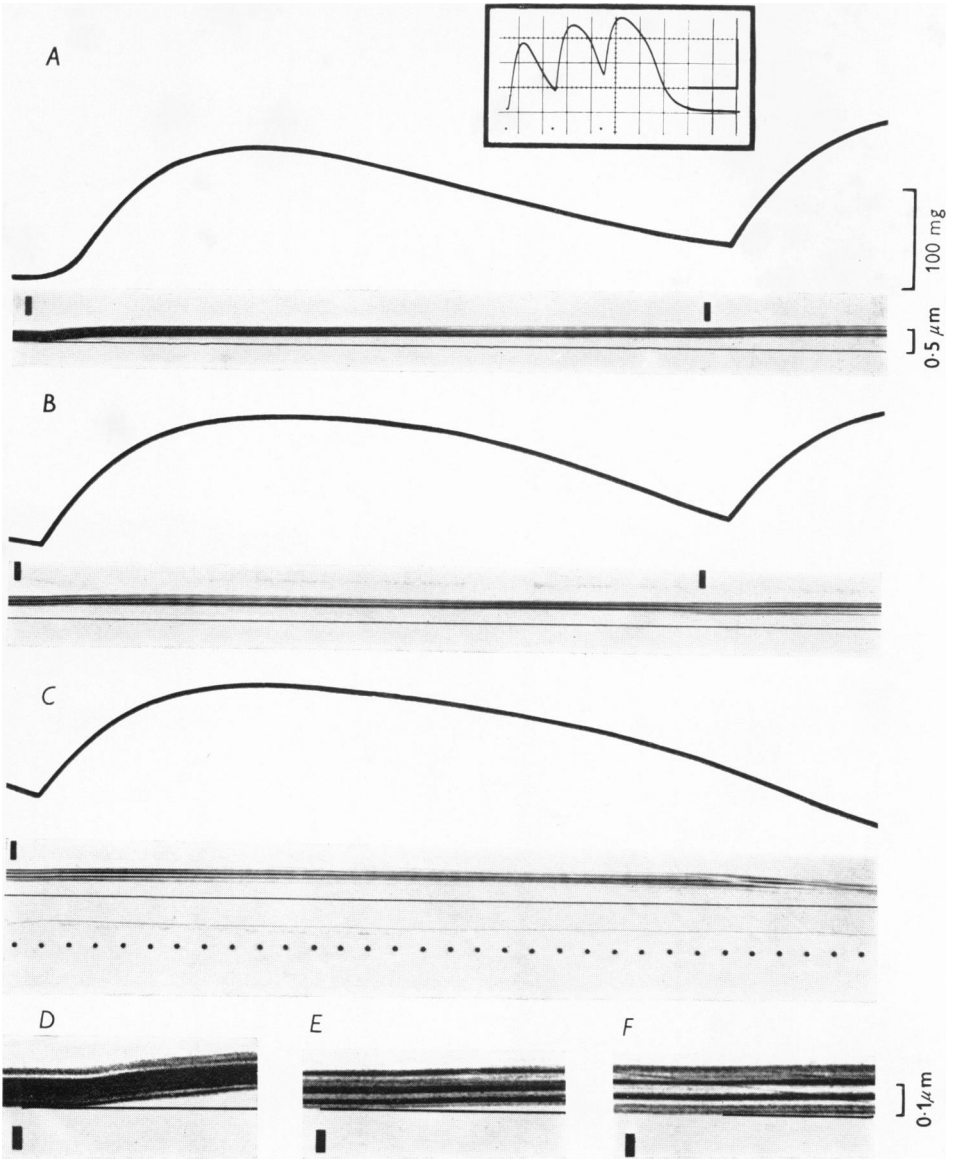

A
100 mg
0·5 μm
B
C
D
E
F
0·1 μm

PLATE 4

Streak photographs of first-order diffraction line and the corresponding isometric myogram during incompletely fused tetanus. Insert (top) illustrates original oscilloscope record of isometric myogram. Calibrations: vertical, 100 mg; horizontal, 425 msec.

A, *B* and *C* shows streak record of the first-order diffraction line and a replotting of the isometric myogram during the first, second and third contraction cycles, respectively. A thin continuous line is drawn through *A*–*C* to indicate as a reference the resting position of the first-order line. *D*, *E* and *F* illustrate at 4·0 × enlargement the first-order diffraction line following the first, second and third stimuli, respectively. Stimulus signals (vertical bars) replotted and displaced towards diffraction line. Time intervals between dots below *C* 16·7 msec. Resting sarcomere length 2·56 μm.