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THE MAXIMUM LENGTH FOR CONTRACTION IN VERTEBRATE STRIATED MUSCLE

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In their well-known experiments on isolated muscle fibres, Ramsey & Street (1940) showed that the rise of tension during an isometric tetanus decreases linearly as the length is increased beyond a certain value l_0 near to the resting length, reaching zero when the fibre is stretched to about twice this length. A. F. Huxley & Niedergerke (1954) pointed out that a simple explanation for this result was given by the sliding-filament theory of muscular contraction put forward by them and by H. E. Huxley (1953; H. E. Huxley & Hanson, 1954), if it is supposed that a relative force between filaments of the two sets is generated at each of a number of sites in the zone where the two sets of filaments overlap. If these sites are distributed evenly, the total tension generated should be proportional to the length of the overlap zone, which should decrease linearly as the sarcomere length is increased from about 2·0 μ (the length of the secondary filaments), reaching zero at a sarcomere length of about 3·5 μ (the sum of the lengths of the primary (about 1·5 μ) and of the secondary filaments).

There were, however, several uncertainties about this explanation, as follows.

(1) Ramsey & Street had not measured striation spacing in their fibres, so it was not clear exactly what the sarcomere length was when the developed tension just fell to zero. From their definition of l_0 it did nevertheless seem likely that the tension reached zero only if the fibre was stretched to a sarcomere length appreciably greater than 3·5 μ.

(2) Ramsey & Street had found that in these highly stretched fibres the tension during an isometric tetanus rose in two stages, the first with about the same speed as in less stretched fibres, the second being very much slower. The quick phase formed a progressively smaller proportion of the total tension rise as the fibre was stretched, and reached zero at a considerably lesser degree of stretch than the slow phase. It was not clear which of these end-points ought to be used.

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(3) No accurate determination of filament lengths had been made, so it was not possible to say beyond what sarcomere length there was no overlap between the two sets of filaments.

The slow component in the rise of tension in stretched muscle was also observed by Hill (1953) in whole sartorius muscles of frogs and toads. He suggested that the explanation might be that those parts of the muscle which happened to be less extended than others would generate more tension, and would shorten, and stretch the more extended parts, so increasing any initial inequality in the degree of extension. The tension would rise slowly as this redistribution of length took place. Fischer (1926) showed that the passive extensibility of the sartorius muscle is less near its ends than in the middle, so that the redistribution of length in an isometric tetanus of a highly stretched muscle might take place by shortening of the ends, with corresponding elongation of the middle.

Preliminary experiments showed that the slow phase of tension increase in isolated fibres during an isometric tetanus does arise in this way and that isometric recording did not give a sharp end-point. We therefore measured the maximum sarcomere length at which contraction occurs, by mounting a fibre isotonically and detecting shortening by microscopic observation of the striation spacing in its middle part. In these experiments the ends of the fibre were able to shorten without raising the tension in the middle of the fibre, and we were thus able to distinguish between shortening of the ends of the fibre and that of the middle. The filament lengths in the muscle used were determined in two ways by electron microscopy.

The results (excluding the measurements by electron microscopy) have already been reported briefly (Huxley & Peachey, 1959).

METHODS

Dissection

Twitch fibres were isolated from the dorsal head of the semitendinosus muscle (and in a few cases from the iliofibularis muscle) of the frog *Rana temporaria*. The muscle was initially divided with fine scissors, and the fibres subsequently separated by cutting with a knife the collagen fibrils which unite them. The muscles were immersed during the dissection in Ringer's solution with the addition of tubocurarine (10^{-5} g/ml.) at room temperature.

Solutions

The Ringer's solution used both in the dissection and for the experiments themselves had the following composition (mM): NaCl, 115; KCl, 2·5; $CaCl_2$, 1·8; Na_2HPO_4, 2·15; NaH_2PO_4, 0·85 (pH 7·2).

Stimulation and recording

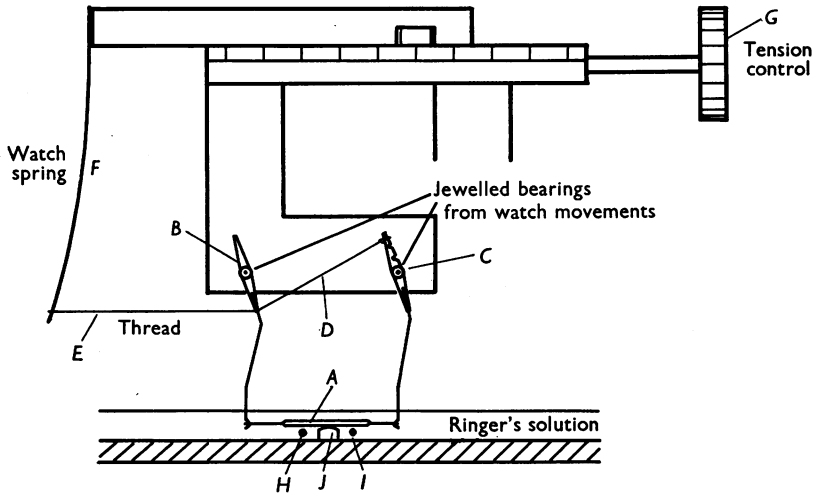
The fibre was immersed in Ringer's solution in a channel 3 mm broad and 3 mm deep. In the isotonic experiments a piece of glass rod 1·5 mm diameter was laid across the bottom of this channel, and the fibre was so placed that it rested lightly on the surface of this rod

at a point which did not move appreciably on stimulation. The stimulating electrodes were Pt wires which ran across the bottom of the channel, 4 or 6 mm apart, one each side of the glass rod. Since the fibres were 10–20 mm long, the stimulating electrodes were well within the length of the fibres. For tetanic stimulation alternating condenser discharges of about 1·7 msec time constant at either 40 or 50/sec were employed.

An RCA 5734 electromechanical transducer valve was used for isometric recording; it was mounted on a screw-driven slide with a vernier scale for adjusting the length of the fibre.

An isotonic device was constructed from the balance wheels of two watches, with their jewelled bearings. Their movements were linked by a thread, as shown in Text-fig. 1, so that a point near the middle of the fibre would remain stationary during shortening of the fibre. The over-all shortening was not recorded, but changes of striation spacing in the part of the fibre which lay on the glass rod were recorded by cine micrography.

All experiments were carried out at room temperature (17–20° C approx.).



Text-fig. 1. Diagram of isotonic lever system. The fibre *A* is held between two levers of stainless-steel wire attached to the cut-down balance wheels *B* and *C* of two watch movements. These are linked by a thread *D* which ensures that they turn in opposite directions, so that some point within the length of the fibre stays stationary during shortening. The ratio of the amounts of movement permitted at the two ends can be altered by hooking *D* on to different notches on *C*. Tension is applied through the thread *E* from a piece of watch mainspring *F*, and is adjusted by moving the fixed end of the spring by the knob *G*. The levers lie in an oblique plane so that the microscope objective (not shown) can come down vertically above the fibre. *H*, *I*, stimulating electrodes; *J*, glass rod supporting the fibre.

Microscopy

The trough was mounted on the mechanical stage of a microscope so that any point on the fibre could be brought into the field. The fibre was observed by direct microscopy, using a Zeiss D* water-immersion objective, focal length 4·4 mm, n.a. 0·75, which dipped directly into the Ringer's solution. The metal surrounding the front lens was coated before each experiment with 'paraffin and rubber lubricant' (British Drug Houses Ltd.) to prevent metallic ions from entering the solution and damaging the fibre. The n.a. of the illuminating cone was about 0·25. Still photographs were taken with a 35 mm reflex camera, using Ilford

Pan F film and an over-all magnification of 138× or 360×. Cine photographs were taken, generally at 55 frames/sec, on Ilford 16 mm FP3 film, with an over-all magnification of 63 ×. A low-voltage tungsten lamp was used, with a green filter.

Determination of filament lengths

Bundles of 30–50 fibres were dissected from semitendinosus muscles of frogs, with the same procedure as in the early stages of preparing a single fibre (the procedure eliminates the region of the nerve entry, where slow fibres are found).

Sections. Four bundles were used; they were stretched to give striation spacings of 3·0–3·8 μ in the middle parts of the fibres. The length of each bundle was measured between two threads tied around the tendons as near as possible to the ends of the muscle fibres. The bundles were fixed while held at these lengths in the trough by running in a fixative with the following composition: 1 % OsO_4, 2 % sucrose, 0·01 M-$CaCl_2$ buffered to pH 7·4 with 0·035 M sodium acetate and 0·035 M sodium veronal. After 40 min fixation at room temperature, the ends of the bundles were detached and their lengths measured again. The bundles were then dehydrated through a series of ethanol–water mixtures and lengths were measured again in absolute ethanol. Each bundle was cut transversely into four pieces and the length of each piece measured, still in absolute ethanol. These pieces were then divided into two groups, the two proximal pieces of each bundle for methacrylate embedding and the two distal pieces for embedding in Vestopal-W (Ryter & Kellenberger, 1958). All pieces were stained overnight in 1 % phosphotungstic acid in ethanol and then transferred through the various solutions for impregnation with the respective embedding media. The length of each piece was measured in its final liquid embedding medium and again after the blocks had been hardened for approximately 15 hr in a 60° C oven.

One shrinkage factor was determined from the above measurements for each bundle from fixation to absolute ethanol, and another for each piece from absolute ethanol to the final embedded block. These two factors were combined to give an over-all shrinkage factor for each piece.

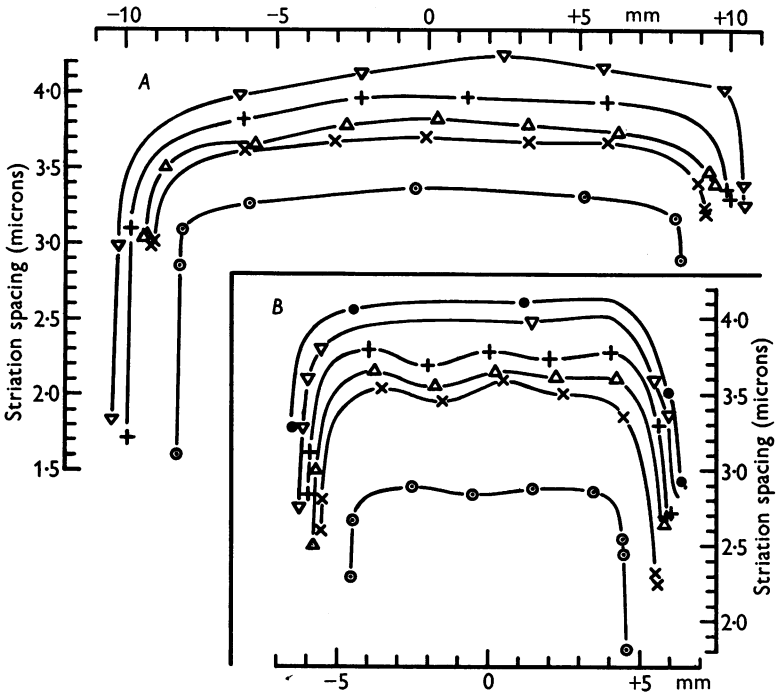
Longitudinal sections were cut on either a Porter–Blum or an A. F. Huxley (1957*a*) microtome, with the long axis of the fibres perpendicular to the direction of movement in order to avoid alteration of filament lengths from compression of the sections. The sections were mounted on grids covered with carbon films, and low power electron micrographs were taken in either a Siemens Elmiskop I or an RCA EMU-2B electron microscope. The magnification of each electron micrograph was determined accurately by photographing the same area of the grid under the light microscope and measuring the distance between features that could be identified in both images. Measurements for magnification determination and measurements of filament length in the electron micrographs were made on the negatives with a travelling microscope.

Fibrils. Each bundle was stretched to give a striation spacing of 2·7–2·8 μ, flooded with formol-calcium (1 vol. formalin, 1 vol. 10 % $CaCl_2$, 8 vol. water) and left several hours in the fixative. It was then cut up into small pieces, suspended in 50 ml. water and treated for 1 min in a homogenizer (Measuring and Scientific Instruments Ltd.). The suspension was centrifuged (1600 *g*) for 1 min, the supernatant discarded, the precipitate resuspended in 50 ml. water and treated in the homogenizer for three further periods of 10 min each, with cooling in between. It was centrifuged again to remove large pieces, the supernatant transferred to fresh tubes and centrifuged for 1 hr. The sediment was resuspended in a total volume of 1 ml. A drop of this suspension was placed on an electron microscope specimen grid with a formvar film on the underside of which a layer of carbon had been deposited. Thin fibrils were selected under the electron microscope (Siemens Elmiskop I) and micrographs were made at a magnification of about 2000. The exact magnification was determined in the same way as with the sections. Measurements were made on the negatives with a travelling microscope.

RESULTS

Experiments on living muscle

Non-uniformity of striation spacing in stretched fibres. The striation spacing was measured by photography with the 35 mm camera, at a series of positions along two isolated muscle fibres. In each case it was found that the spacing near the ends of the fibre was substantially smaller than in the middle; this is illustrated by the micrographs reproduced in Pl. 1, figs. 1 and 2. The variation of striation spacing along the fibres at several degrees of stretch is shown graphically in Text-fig. 2. It is seen that the spacing varies little except within about a millimetre of each end of the fibre, where it drops sharply. Isolated measurements made visually with a



Text-fig. 2. Variation of striation spacing along isolated muscle fibres at rest. *A*, fibre from semitendinosus muscle (15. xi. 58); *B*, fibre from iliofibularis muscle (8. xi. 58). Abscissa: distance from centre of fibre; ordinate: striation spacing, measured on photomicrographs. The various curves for each fibre were obtained at different degrees of elongation. Each point gives the mean value of the striation spacing found by measuring several (usually three or four) rows of 15–30 striations at different parts of the same negative, except in a few cases near the ends of the fibres where the striations were partly obscured by overlying fibrous tissue, and could only be measured in one or two small fields.

micrometer eyepiece showed a similar drop at the ends of several other fibres, all of which were considerably stretched. In no case was the drop absent.

Electron micrographs of sections taken near to one end of an isolated fibre are reproduced in Pl. 3, figs. 1 and 2. The sarcomere lengths are 2·7 and 3·1 μ and the two sets of filaments overlap considerably, although in the middle part of the same fibre the sarcomere length was about 3·8 μ.

At the extreme left-hand end of Text-fig. 2*A* three of the graphs drop to spacings below 2 μ while the other two only reach down to about 3·0 μ. All these measurements were made at the extreme end of the fibre, so that the difference cannot be due to observing slightly different parts of the fibre. In the cases where the spacing is short, the striations are very irregular, and the fibre had been stimulated tetanically just before it was photographed. It is thus probable that this end of the fibre had entered the 'delta state' (Ramsey & Street, 1940), and had not yet stretched out when these micrographs were taken.

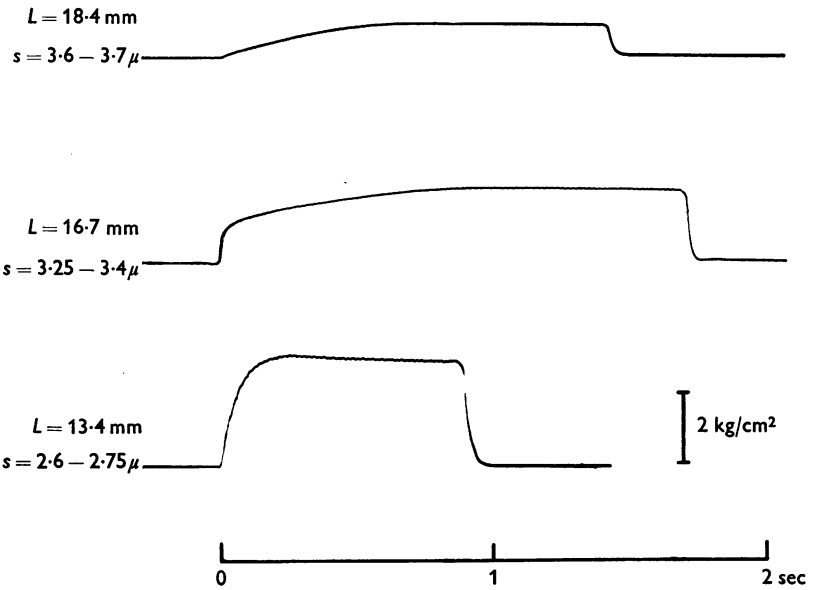
A single complete toe muscle (extensor longus digiti IV) was also examined visually; the striation spacing was 2·6 μ at the ends of the fibres when it was 3·6 μ near the middle of the muscle.

Isometric contractions. A series of records of tension during isometric tetani at varying degrees of stretch is shown in Text-fig. 3. Over the range investigated, the rise of tension during the tetanus becomes smaller as the striation spacing is increased, but has not reached zero even when the striation spacing, measured in the middle of the fibre, is 4 μ.

At this sarcomere length one would expect that the two sets of filaments would no longer overlap, suggesting at first sight that overlap is not necessary for the development of tension. An alternative explanation is, however, provided by the observation described in the preceding section, since the uppermost curves in Text-fig. 2*A* and 2*B* show that even when the striation spacing over the main part of a fibre is 4 μ, at the ends it may be well under 3·5 μ, roughly the maximum value at which overlap could be expected. The tension rise may therefore be due to shortening of the ends of the fibre without any active contribution from those parts where the spacing exceeds 3·5 μ, as was suggested in the introduction. This explanation is further suggested by the time course of the tension rise. In agreement with the observations of Ramsey & Street (1940) and Hill (1953), this contains a slow component which becomes progressively more conspicuous as the fibre is stretched to greater lengths. As Hill pointed out, this is what would be expected if one part of the fibre were shortening at the expense of other parts.

It was found by microscopic observation of the ends of the fibre that such a shortening does take place. Plate 1, figs. 3 and 4, show micrographs of the end of a fibre stretched so that the striation spacing in the middle was 3·9–4·2 μ, taken before (fig. 3) and during (fig. 4) tetanic stimulation.

A high degree of shortening is seen although the fibre is held at both ends so that its total length remained constant to within a few microns. Both ends were found to shorten in this way, so that the middle part of the fibre must have been stretched. Cine-micrographs of the middle of the fibre showed little change apart from slight longitudinal movement; the increase of sarcomere length expected is rather small, as only a small fraction



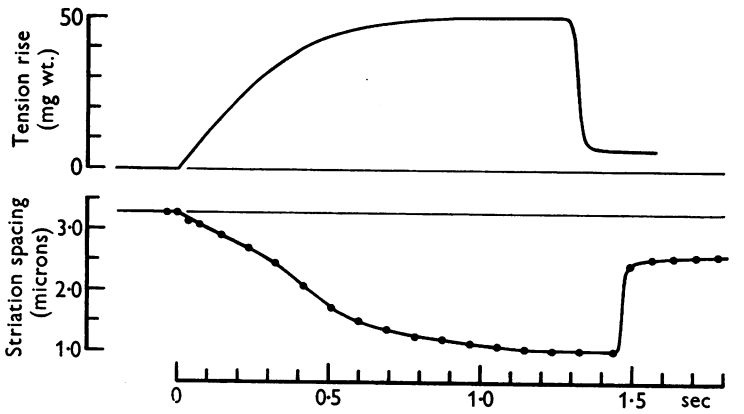
Text-fig. 3. Records of tension during isometric tetani at three lengths. Semitendinosus fibre; same experiment as Text-fig. 2*A*; top record corresponds to points +, and middle record to points ⊙, in that diagram. The values for the sarcomere length *s* refer to the main part of the length of the fibre, the value being substantially lower at the ends. The scale for tension per unit area is calculated from the cross-section at a striation spacing of 2·5 μ.

of the fibre length at each end shortens. The fact that only the ends shortened cannot be attributed to a failure of propagation, since the two stimulating electrodes were fairly close together near the middle of the fibre, and the action potentials must have propagated over a distance of several millimetres in order to reach the ends.

Text-figure 4, lower curve, shows the time course of the shortening, found by measurement of the cine-micrographs. It is seen to agree fairly well with the time course of the tension rise, which is plotted in the upper curve.

If the slow component is attributed on this evidence to shortening at the ends, the fast component should be due to active tension developed along the whole length of the fibre, and should disappear if the sarcomere length

in the main part of the fibre reaches a value at which no tension is developed. Text-figure 3 shows that the fast component is not recognizable when the sarcomere length in the middle is above about 3·6 μ, but its disappearance is not sharp enough to allow a precise determination of the limit to be made. In the same way the isometric twitch became very small when the main part of the fibre was stretched to a sarcomere length above about 3·8 μ, but again a sharp end-point was not found, presumably because of shortening at the ends.



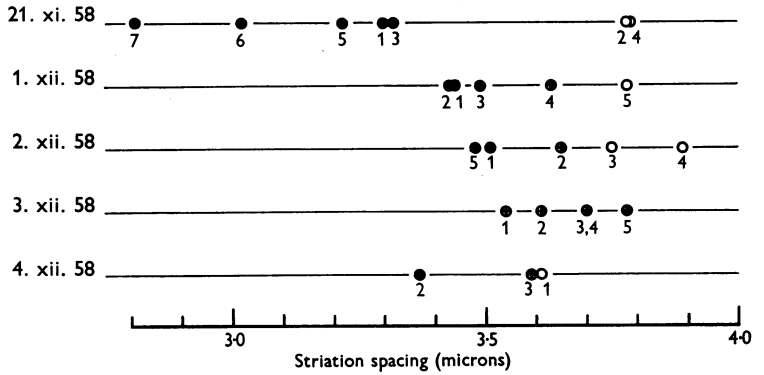
Text-fig. 4. Time course of shortening near one end (lower curve) and tension rise (upper curve), recorded during isometric tetani. Same fibre as Text-figs. 2*A* and 3; successive tetani at length corresponding to points marked △ in Text-fig. 2*A*. Shortening obtained from measurements on cine film.

Experiments on toe muscles. Two attempts were made to observe this shortening near the ends in a whole muscle stimulated at great length. The extensor longus digiti IV of the frog was used. Both experiments failed because the muscles broke. In one case this occurred when the striation spacing in the middle was about 3·3 μ; in the other it had reached 3·6 μ, but the muscle broke before a record could be obtained.

Isotonic experiments. To obtain a result unaffected by shortening at the ends, fibres were held under constant tension in the apparatus shown in Text-fig. 1 and shortening of the middle part of the fibre was recorded by cine-micrography. Changes in total length were not recorded and shortening of the ends was immaterial, since the isotonic lever prevented length changes from altering the tension to which the middle part of the fibre was subjected.

Three types of result were observed: (1) no change apart from slight longitudinal movement, (2) vigorous shortening, (3) slow, delayed, irregular shortening, occurring only in patches within the field of view.

These are illustrated in Pl. 2, and the conditions under which they were observed in the various experiments are shown in Text-fig. 5. It will be seen that vigorous shortening was always obtained if the striation spacing before stimulation was 3·5 μ or less, and that at 3·54 μ or more no shortening occurred apart from the delayed, patchy shortening which was seen in a few cases, chiefly in fibres which had already been stimulated at a length where vigorous shortening just occurred. In run 1 of the experiment of 3. xii. 58, where the initial value of the striation spacing was 3·54 μ, the spacing did not begin to fall until more than 0·2 sec after stimulation was begun.

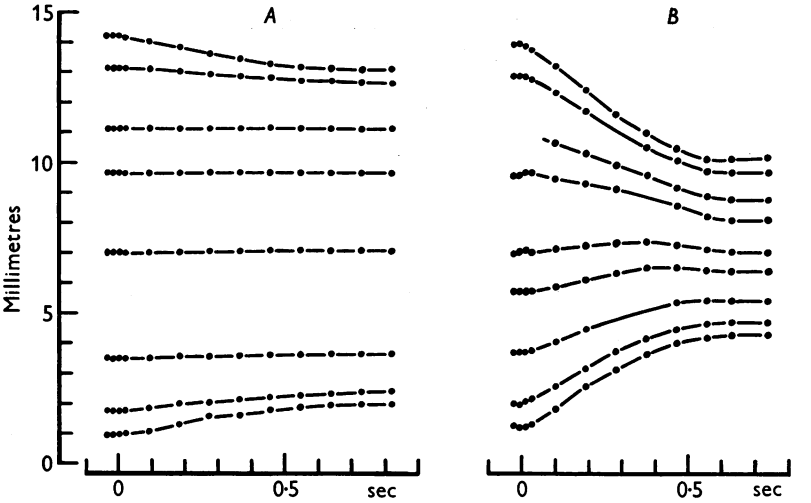


Text-fig. 5. Results of tetanic stimulation of fibres held isotonically. ●, vigorous shortening; ○, no shortening; ⊕, delayed, patchy shortening. Abscissa: striation spacing immediately before stimulation, measured on the cine film. Five experiments, all on fibres from the semitendinosus muscle. The numbers indicate the order in which the tetani at different initial lengths were carried out.

In a single experiment a different method of recording was used. The fibre was held by the same isotonic lever system, but its whole length was photographed with the cine camera (55 frames/sec; over-all magnification 0·5 ×). Short pieces of thread were placed on the fibre to act as markers. The striation spacing near the middle of the fibre was measured with the water-immersion objective and a micrometer eyepiece. The fibre was first stimulated (40/sec) at a striation spacing of 3·6 μ; both ends of the fibre shortened but at least the middle 60 % was unchanged. The tension was then reduced, giving a spacing of 3·4 μ; the whole of the fibre now shortened on stimulation, the middle part decreasing to a little below half its unstimulated length. The length changes of the various segments of the fibre in the two cases are plotted in Text-fig. 6.

Conclusions from experiments on living fibres. It seems fair to conclude from these experiments that no contraction occurred in parts of fibres where the striation spacing exceeded a critical value which we estimated at

3·52 μ (limits of error 3·50–3·54 μ). The irregular shortening sometimes seen when the measured spacing was slightly above this value may reasonably be attributed to contraction which began in small areas where the spacing was below the critical figure, and spread slowly as laterally adjacent areas became passively shortened. This process would greatly exaggerate any pre-existing differences of spacing; the resulting irregularities were clearly visible on relaxation and did not disappear at once, which would explain the fact that this type of response was seen more frequently in



Text-fig. 6. Shortening of different segments of a fibre during isotonic tetani. Fibre from iliofibularis muscle. Initial striation spacing near middle of fibre was 3·6 μ in *A*, 3·4 μ in *B*. Top and bottom lines represent the ends of the fibre; intermediate lines show positions of pieces of thread adhering to the fibre. Measurements made on selected frames of the low-magnification cine film, taken at 55 frames/sec.

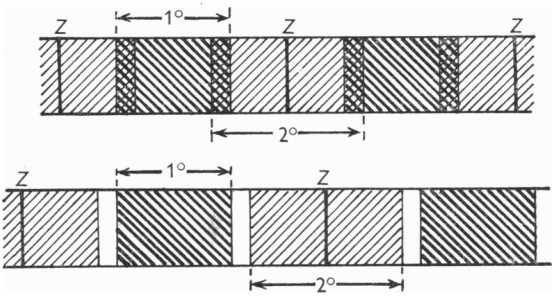
fibres which had already been stimulated at lengths close to the critical value. The only experiment where this type of behaviour was seen at spacings more than 3 % above our estimate of the critical value was that of 3. xii. 58; in and after run 3 of that date the cine film shows differences of spacing of that order before stimulation even within its rather limited field of view.

Measurement of filament lengths by electron microscopy

Method of measurement. The method of measurement of filament lengths was in accordance with the arrangement of primary and secondary filaments demonstrated by H. E. Huxley (1957). The length of the A band was taken, in each case, as the length of the primary filaments. In cases where

the sarcomere length was such that the two sets of filaments overlapped (Pl. 3, figs. 1, 2 and 4), the length of the secondary filaments was taken as the length of the I band plus the sum of the lengths of the adjacent dark overlap zones of the two neighbouring A bands (Text-fig. 7, upper diagram).

In cases where the sarcomeres were stretched so far that there was no overlap and the two sets of filaments were 'out of mesh' (Pl. 3, fig. 3), the A bands contain no outer dark zone, but the I bands contain an outer pale zone. This region was taken to be a gap between the two sets of filaments and the length of the secondary filaments was taken as the length of the darker portion of the I band (Text-fig. 7, lower diagram).



Text-fig. 7. Diagrams showing the dimensions taken as representing the lengths of the primary and secondary filaments. See text.

Embedded material. A sample field from one of the embedded preparations used for filament length determination is illustrated in Pl. 3, fig. 3. The measurements of filament lengths are summarized in Table 1. The final mean value for the sum of the lengths of the primary and secondary filaments is 3·52 μ, which is the same as the value found in our isotonic experiments for the maximum sarcomere length at which contraction occurs.

The final values in Table 1 have been corrected by shrinkage factors determined for each embedding medium as described under Methods; the distribution of the shrinkage between the various stages of the embedding procedures is given in Table 2. These factors are for shrinkage from the fixed bundle after release of tension to the final embedded pieces. Shortening during fixation and on release of tension has been neglected; it was small (0, 0, and 1·6 %) except in the case of one bundle which appeared to lose 10·8 % of its length in this step. Since this bundle closely paralleled the others in all subsequent stages and gave final results in good agreement with the others, it seems likely that the original measurement of length was incorrect in this one case.

These shrinkage factors have been applied to the sum of the measured lengths of the two kinds of filaments, a procedure which is precisely valid only if both filaments shrink by the same percentage of their lengths. It is not obviously true that this is the case; indeed it seems quite possible that A and I bands might shrink by different amounts, and for this

reason the correction for shrinkage has not been applied to the two types of filament separately. However, on any reasonable assumption about the mode of shrinkage, there is no error in the corrected value of the sum of the filament lengths if the sarcomere length is such that the two sets of filaments just do not overlap, and the error will be of opposite sign according as the sarcomere length is greater or less than this value. In our material the average sarcomere length was close to this value, so that the error of the final mean should be small. As an example, we recalculated the data on the assumption that all the shrinkage took place in the I bands, the A bands including the overlap zones remaining constant. The resulting value for the sum of the filament lengths for the methacrylate specimens was increased by 0·017 μ from that obtained by the procedure used in Table 1; that for the Vestopal-W specimens was decreased by 0·021 μ; and there was no change in the second decimal place for the mean of the two values. It does not seem likely that non-uniformity of shrinkage could introduce an error of more than a few hundredths of a micron.

TABLE 1. Measurements of filament lengths in sections from embedded preparations. Values in microns, without correction for shrinkage except in the last row. Each value for a filament length is the mean of five measurements on the negative. All calculations carried to at least one extra decimal place before rounding.

The striation spacings measured on the four bundles before fixation were 3·8, 3·5, 3·0 and 3·7 μ respectively

	Methacrylate					Vestopal-W				
Bundle no.	1	2	3	4	S.E. of means	1	2	3	4	S.E. of means
Striation spacing in area measured	3·18	3·09	2·41	3·71	—	3·48	2·57	2·77	3·14	—
Filaments lengths										
Primary	1·45	1·48	1·40	1·46	0·015*	1·48	1·44	1·42	1·49	0·025*
Secondary	1·73	1·73	1·73	1·72	0·023*	1·70	1·72	1·70	1·70	0·017*
Sum	3·19	3·21	3·13	3·18	0·028†	3·17	3·16	3·12	3·20	0·031†
Mean of sums		3·176			0·016‡		3·160			0·016‡
Mean over-all shrinkage (%)		9·05			—		10·78			—
Sum of filament lengths, corrected for shrinkage		3·492			0·017		3·542			0·018

* Standard error of mean from any one bundle, estimated from the combined variance between individual measurements on each bundle.

† Square root of sum of squares of values for primary and secondary filaments.

‡ Standard error of combined mean estimated from the differences between the 'sum' values for the four bundles.

TABLE 2. Longitudinal shrinkage (%) at various stages of embedding in methacrylate and in Vestopal-W. Each value is the mean of the shortening during the corresponding stage expressed as a percentage of the length of the material lying free in the fixative. The figures in brackets give the number of measurements on which the values are based

Stage		Longitudinal shrinkage	
From	To	Methacrylate	Vestopal-W
Free in fixative	Ethanol	5·91 (4)	
Ethanol	Embedding liquid	1·55 (7)	4·12 (8)
Embedding liquid	Block	1·59 (7)	0·75 (8)
Combined		9·05	10·78

There is no significant correlation between sarcomere length and the small variations in the measured filament lengths. This is illustrated both by the values in Table 1 and by the micrographs reproduced in Pl. 3.

Separated fibrils. Plate 3, fig. 4, shows an electron micrograph of a separated fibril, typical of the ones on which measurements were made. Fibrils were selected in the field of view solely on the criteria of having a straight stretch several sarcomeres long and of being thin enough for the band pattern to be well seen. Two preparations gave the following values for the filament lengths (in microns): primary filaments, $1{\cdot}516 \pm 0{\cdot}010$ (S.E. of mean from 7 fibrils) and $1{\cdot}492 \pm 0{\cdot}011$ (8); secondary filaments, $1{\cdot}918 \pm 0{\cdot}009$ (7) and $1{\cdot}923 \pm 0{\cdot}020$ (8). The pairs of results do not differ significantly from one another, and the combined value for the sum of the lengths of the two filaments is $3{\cdot}425 \pm 0{\cdot}013$ μ.

These preparations were in distilled water for about 4 hr, from the time when the disintegration was begun till the droplet was dried down on the grid. Two other preparations in which the fibril suspension in water was kept in one case for 2 and in the other for 3 days before making the grids gave lower values for both sets of filaments (means $1{\cdot}477$ μ for primary and $1{\cdot}858$ μ for secondary filaments, sum $3{\cdot}335$ μ). Presumably shrinkage had occurred, and these values are therefore discarded. This result does suggest the possibility that even the value obtained from the fibrils which had not spent a long period in water is slightly low on account of shrinkage.

As in the embedded preparations, there was no correlation between sarcomere length and filament lengths. However, only a rather narrow range was covered, most of the sarcomere lengths falling between $2{\cdot}5$ and $2{\cdot}8$ μ.

DISCUSSION

The electron microscope observations provide further evidence that passive length changes take place by a sliding motion of one set of filaments relative to the other, the changes in filament length being within experimental error and in any case not more than a small percentage of the changes in sarcomere length. Since most of our measurements on sections were made at striation spacings near the point where overlap ceases, any small change of filament length with stretch will alter only slightly our estimate of the critical length. Allowance for the amount of change apparently present would raise our estimate from $3{\cdot}52$ to $3{\cdot}54$ μ. The measurements on separated fibrils are less reliable since there is a suggestion that some shrinkage had occurred and there was no means of allowing for it; nevertheless, the value obtained for the sum of the filament lengths, $3{\cdot}43$ μ, is only 3 % below that derived from the sections, and this is probably as good an agreement as could be expected.

The experiments on living isolated fibres were carried out on the same type of muscle (twitch fibres of m. semitendinosus of the frog) as was used in the electron-microscope work, and showed that contraction does not occur when the sarcomere length exceeds 3·52 μ. The agreement between this figure and the electron-microscope value for the point where the filaments cease to overlap suggests strongly that contraction can occur only if there is an overlap between the two sets of filaments. This is probably the first actual evidence that contraction depends on an interaction between the filaments in the region where they overlap, as postulated in all forms of the sliding-filament theory. It does not, however, throw any light on the nature of this interaction, or help to eliminate any of the particular systems that have been proposed (A. F. Huxley, 1957*b*; Weber, 1958; Podolsky, 1959; Spencer & Worthington, 1960).

The observation that during tetanic stimulation a high degree of shortening can occur at the ends of a fibre whose total length is held constant is an example of the instability which, as Hill (1953) pointed out, results from the negative slope of the isometric length–tension diagram of highly stretched muscle. The delayed, irregular shortening that was sometimes seen in fibres stretched to a point where normal contraction had disappeared is probably another way in which this instability can show up. These observations emphasize the need, in experiments on stretched muscle, for checking with the microscope whether contraction is taking place uniformly along the length of the muscle.

SUMMARY

1. In isolated striated muscle fibres of the frog under stretch the striation spacing was found to be substantially lower near the ends than in the main part of their length.

2. The slow tension rise during an isometric tetanus of greatly stretched fibres was found to be due to shortening of the ends.

3. In isotonic tetani it was found by cine-micrography that no shortening occurs at points where the striation spacing exceeds a critical value which is close to 3·5 μ.

4. The filament lengths in the same muscle were determined by electron microscopy both on sections and on separated myofibrils. It was found that the two sets of filaments do not overlap if the sarcomere length exceeds 3·5 μ.

5. Contraction thus ceases to occur if a fibre is stretched to such an extent that there is no overlap between the two sets of filaments. This is taken as evidence that contraction depends on an interaction between filaments of the two types in the regions where they overlap, as postulated

in the various theories of contraction based on the idea that length changes take place by a relative sliding motion of the filaments.

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EXPLANATION OF PLATES

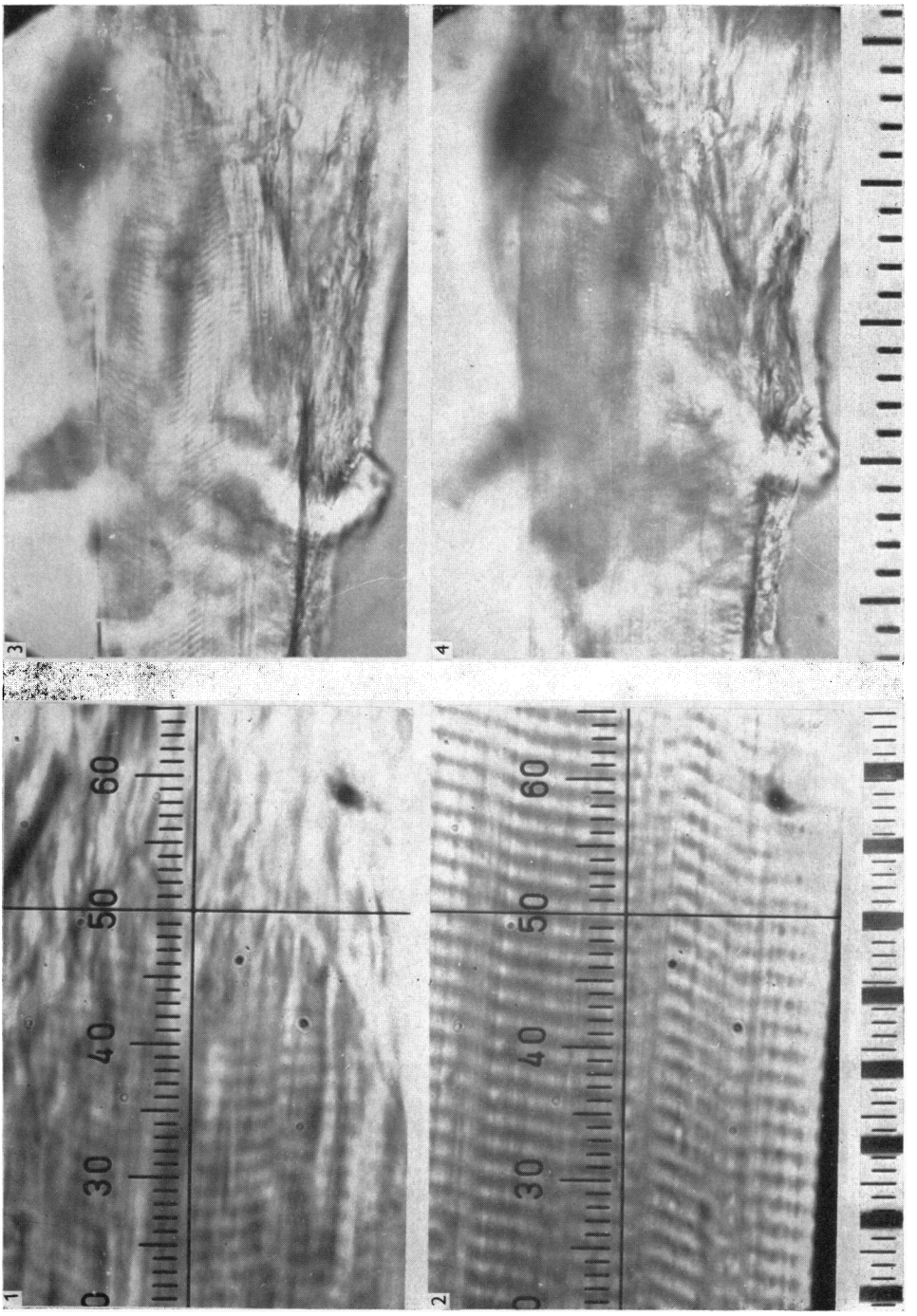
PLATE 1

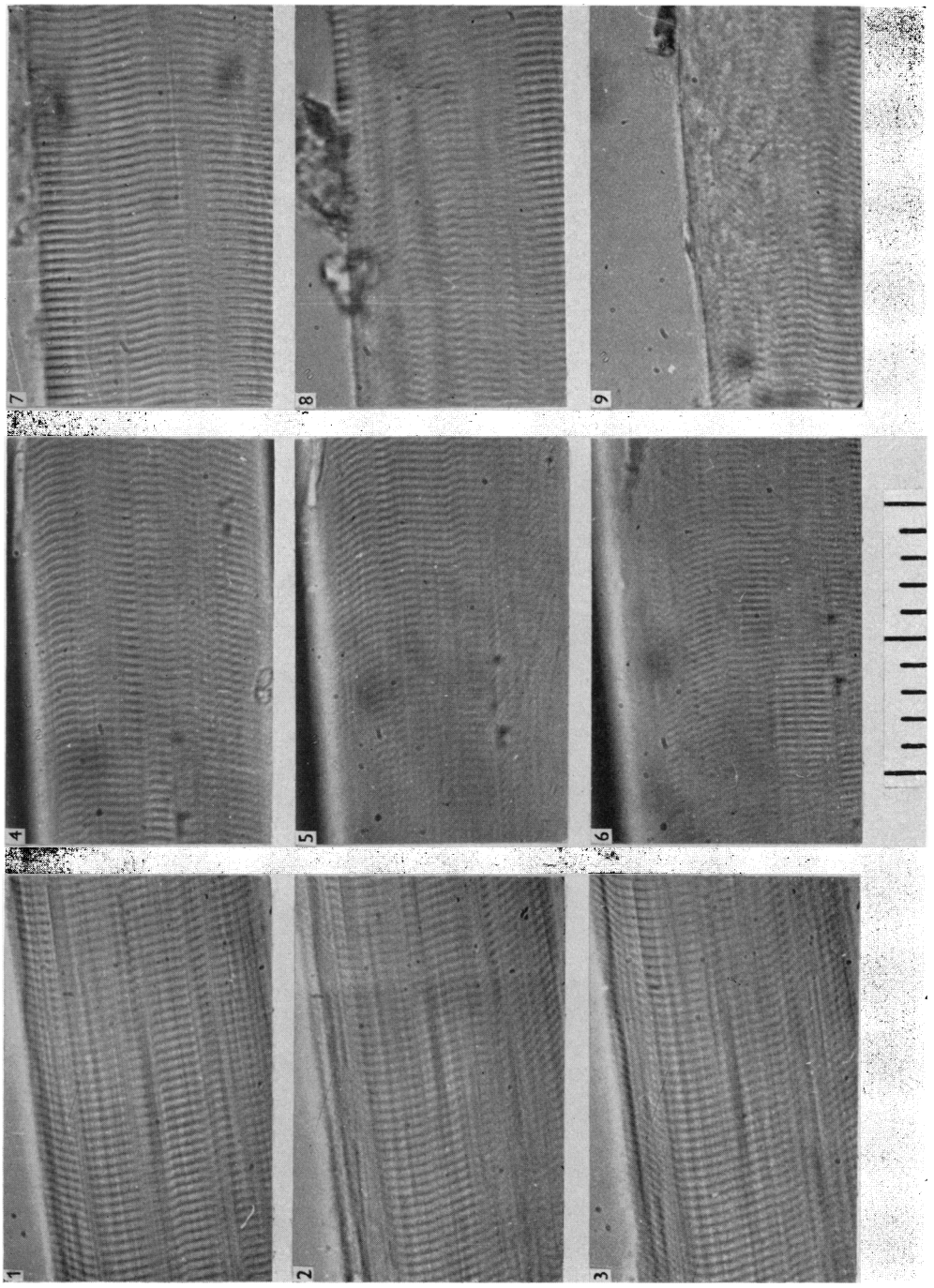
Figs. 1 and 2: still micrographs showing the difference in striation spacing between the end of a fibre (fig. 1) and a point close to the centre of the same fibre (fig. 2), the over-all length of the fibre being the same in both cases. Striation spacing 2·83 μ in fig. 1; 3·42 μ in fig. 2. Fibre from semitendinosus muscle; prints from same series of exposures as lowest points in Text-fig. 2*A*. Stage micrometer: 10 μ divisions.

Figs. 3 and 4. Shortening at end of a fibre during repetitive stimulation at fixed over-all length. Figure 3 before, fig. 4 about 1 sec after, beginning of stimulation. Sarcomere length in fig. 3, 3·1 μ; sarcomere length in middle region of the fibre was 4·0–4·2 μ. Same fibre as in figs. 1 and 2, but opposite end. Stage micrometer: 10 μ divisions.

PLATE 2

Fibres held under constant tension during tetanic stimulation. Selected frames from cine records, showing the three types of response obtained. In each column the top print is from the last frame before movement due to stimulation can be detected on the film.


3
4
1
2
0
30
40
50
60



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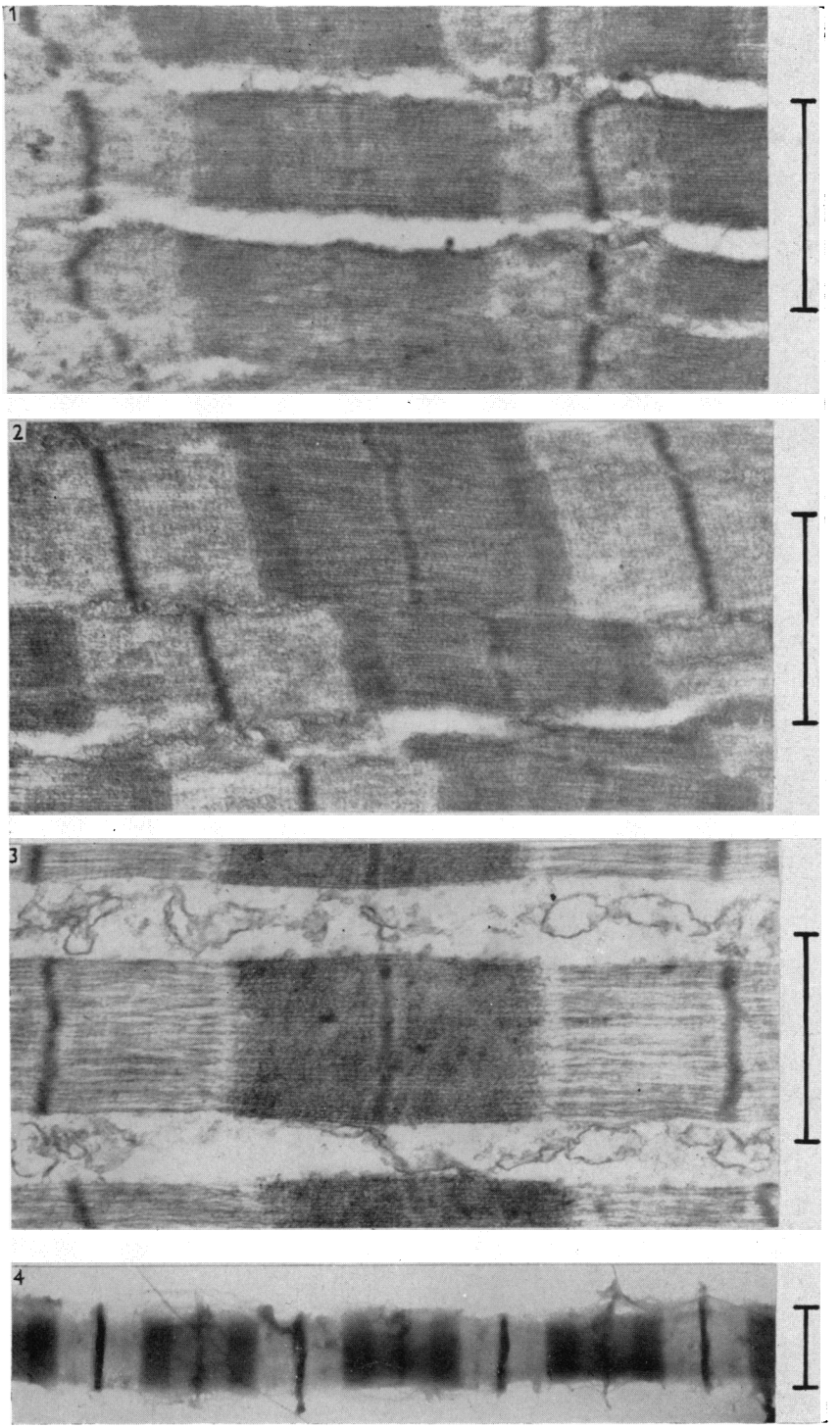
1
2
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4

Figs. 1–3. Run 1 of 4. xii. 58. No shortening at initial striation spacing of 3·61 μ. Figure 1, immediately before stimulation; fig. 2, 1·0 sec later; fig. 3, 2·2 sec after fig. 1. No detectable change in striation spacing.

Figs. 4–6. Run 1 of 2. xii. 58. Rapid shortening at initial striation spacing of 3·51 μ. Figure 4, immediately before stimulation; fig. 5, 0·13 sec later, striation spacing 3·28 μ; fig. 6, 0·24 sec after fig. 4, striation spacing 2·80 μ.

Figs. 7–9. Run 2 of 3. xii. 58. Delayed, irregular shortening at initial striation spacing of 3·61 μ. Figure 7, immediately before stimulation; fig. 8, 1·0 sec later; fig. 9, 2·2 sec after fig. 7. Shortening barely detectable in fig. 8 or in lower left-hand part of fig. 9, but upper right-hand part of fig. 9 shows irregular contraction.

Stage micrometer (10 μ divisions) at same magnification as figs. 1–3 and 7–9; figs. 4–6 are at 1·5 % lower magnification.

PLATE 3

Figs. 1–3. Electron micrographs of longitudinal sections of fast striated muscle fibres from the semitendinosus of *Rana temporaria* cut with the long axis of the fibres parallel to the knife edge. Section thickness, 60–80 mμ. Scale bars, 1 micron.

Fig. 1. Section about 40 μ from the end of the isolated fibre used in the experiment of 3. xii. 58, showing a sarcomere length of 2·7 μ and an overlap of about 0·5 μ (corrected values) even though the centre of the fibre was stretched to a sarcomere length of about 3·8 μ. Fibre fixed and embedded in methacrylate as described in the text, except that fixation was for only 15 min. Magnification, × 28000.

Fig. 2. Section about 400 μ from the end of the same fibre as in fig. 1, showing somewhat less overlap (about 0·2 μ) and a sarcomere length of 3·1 μ (corrected values). Magnification, × 28000.

Fig. 3. Section from a bundle of fibres embedded in Vestopal-W showing a sarcomere stretched to a length (3·7 μ, corrected value) slightly greater than the critical length, with the primary and secondary filaments 'out of mesh'. The suggestion of fine filaments connecting the ends of the two major sets of filaments may be a result of superposition in the section or may represent an additional component of the myofibril. Magnification, × 28000.

Fig. 4. Electron micrograph of a separated myofibril representative of those on which measurements of filament lengths were made. Magnification, × 11000.