

ELECTRON MICROSCOPICAL STUDY OF SKELETAL MUSCLE DURING ISOTONIC (AFTERLOAD) AND ISOMETRIC CONTRACTION

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Observations by light microscopy on contracted muscle have been made chiefly under isometric conditions: on striated muscle of frog (9, 14, 5, 2, 4, 15), of *Astracus fluviatilis* and *Carcinus maenas* (17), and guinea pig (12); and on frog cardiac muscle (18).

Studies dealing with isotonic contraction are those of Hürthle (13) who investigated the contraction waves of *Hydrophilus* muscle; of A. F. Huxley and Niedergerke (15) who investigated frog muscle by interference microscopy; the studies on glycerol-extracted muscle by H. Huxley and Hanson (16); and recently the investigations of Hodge (11) on the flight muscle of the blowfly.

For both isometric and isotonic contraction the published results are contradictory. In part this may result from the fact that during isotonic contraction the shortening of the fibre may bring the length of the anisotropic and isotropic bands below the resolving power of the light microscope. The changes in length that occur in the anisotropic and isotropic bands of skeletal muscle during stretch were described in a previous study (6). The present electron microscopic study was undertaken to determine the length of the anisotropic and isotropic bands during isotonic (afterload) and isometric contraction.

Method

Bundles (75 to 100 fibres) from the curarized semitendinosus muscle of *Rana temporaria* and *Rana esculenta* were isolated and the equilibrium length determined. They were stretched to 30 to 40 per cent above the equilibrium length ($L_0 = 100$) for about 30 minutes in Ringer's solution at 0-4°C. The fibre bundle was then placed in a myograph (3), adjusted to the desired length (25 per cent stretch for afterload and 0 to 50 per cent stretch for isometric contraction). The term "afterload contraction" denotes a contraction in a muscle initially stretched to a desired length by an external load. Upon stimulation the muscle first contracts isometrically until the developed tension equals the external force, and then it shortens against this "afterload." The muscle was maintained at the desired length until the myograph gave constant readings for at least 30 minutes. The Ringer's solution was then removed, and the muscle stimulated directly before and for some time during fixation of the muscle in 10 per cent ice cooled neutral formalin solution. The stimulating square wave pulses had a duration of 10

milliseconds, a frequency of 25 per second, and an intensity greater than that necessary to produce a maximal contraction. Only preparations were used which remained constant in length and tension after cessation of stimulation. The fixation time was 3 to 4 hours depending on the thickness of the fibre bundle. After fixation the fibre bundle was washed in distilled water, stained in 1 per cent neutral osmic acid for 30 minutes, washed in distilled water, cut in $1\frac{1}{2}$ to 2 mm. lengths and fractioned in a micro Waring blender in 15 ml. distilled water for 30 minutes.

The preparation of the material for electron microscopy and the measuring technique have been described (6). Whole fibrils, separated by fractioning, were found to be more suitable for the purpose of this study than ultrathin sections. The greater detail attainable with thin sections was not necessary for this study, and the mechanical distortion incident to preparing and cutting such sections was a great disadvantage. Some preparations were shadowed with palladium.

RESULTS

Isotonic (Afterload) Contraction.—

The two curves in Fig. 1 show the length of the anisotropic (A) and the isotropic (I) bands as a function of the length of the sarcomere during afterload contraction. Each curve represents measurements from three different groups of experiments: muscles with an average shortening of about 10, of 25, and of 45 per cent.

The electron micrographs in Fig. 2 show myofibrils with an approximate shortening of from 5 to 50 per cent. There is no doubt as to the differentiation of the A and I bands in fibrils A to D (fibril D sarcomere length 1.7μ). Below a sarcomere length of 1.7μ a marked change in the appearance of the striations occurs. There is less contrast and the I bands can no longer be identified with certainty; a broad dense zone (contraction band) appears in the fibril and two dark zones (A_3 in Fig. 3) with a thin light line (H) can be seen to the right and left of the M line (Fig. 2, E and F). As seen in Fig. 3 A the band pattern of the uncontracted sarcomere can be divided into the zones Z, I_1 , A_1 , A_2 , M, A_2 , A_1 , I_1 , Z. In addition around the dark M zone in the middle of the A band two light lines (H) and two dark zones (A_3) can be differentiated. Assuming the contraction band (C) to be at the Z line, the pattern in the strongly contracted sarcomere could be interpreted in two ways. The light zone on both sides of the contraction band could be considered as belonging to the I band (Fig. 3 B). This interpretation corresponds to that of Bennett and Porter (1). However, if A and I are defined in this way, the curves for the length of the A and I bands as a function of sarcomere length become discontinuous, because at a sarcomere length of 1.6μ the I band would measure 1.0μ while it would decrease to 0.4μ at a sarcomere length of 1.7μ . This interpretation is therefore rendered unlikely. Alternatively, as shown in Fig. 3 C, the light line (A_2 in Fig. 3 C) may be a part of the A band. The question in this case is what has happened to the line A_1 . The simplest interpretation would be that in strongly contracted fibrils, after I has disappeared, the A

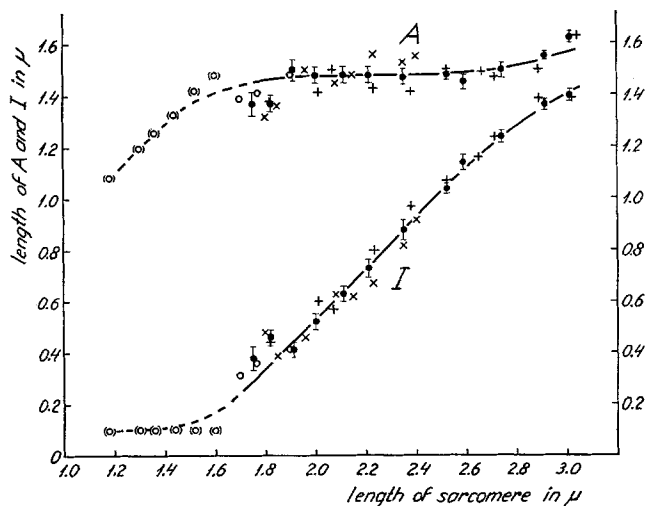


FIG. 1. Length of the anisotropic and isotropic bands during afterload contraction as a function of the sarcomere length. Abscissa: sarcomere length in μ . Ordinate: length of the A and I band in μ .

Experiments with different average shortening.

+ — + — +, about 10 per cent shortening.

× — × — ×, about 25 per cent shortening.

○ — ○ — ○, about 45 per cent shortening.

● — ● — ●, average from all three groups.

The vertical lines indicate the mean error. When the I band cannot be distinguished in strongly contracted myofibrils (○) its length is plotted as the thickness of the Z line in the resting fibril = 0.1μ .

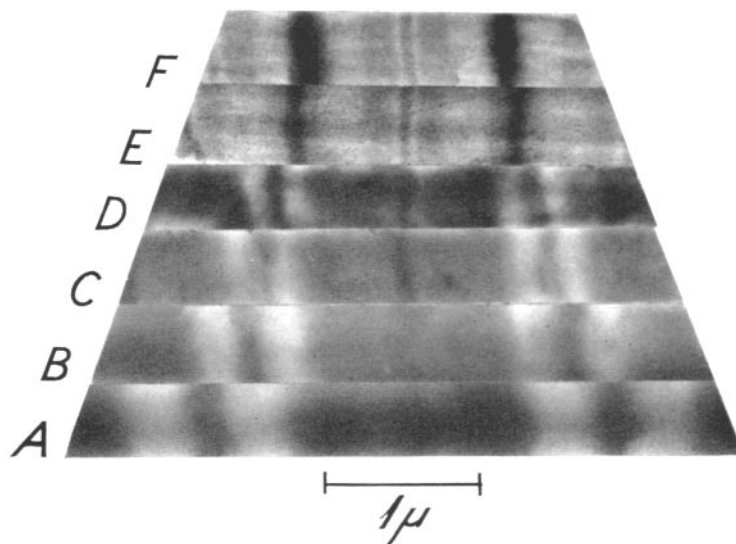


FIG. 2. Electron micrographs of cross-striated myofibrils during afterload contraction with a shortening of about 5 (A), 20 (B), 30 (C), 35 (D), 45 (E) and 50 (F) per cent.

band is drawn to the Z line (*cf.* Fig. 2 E where on both sides of Z is a darker zone which might be A_1), forming together with Z the contraction band (C).

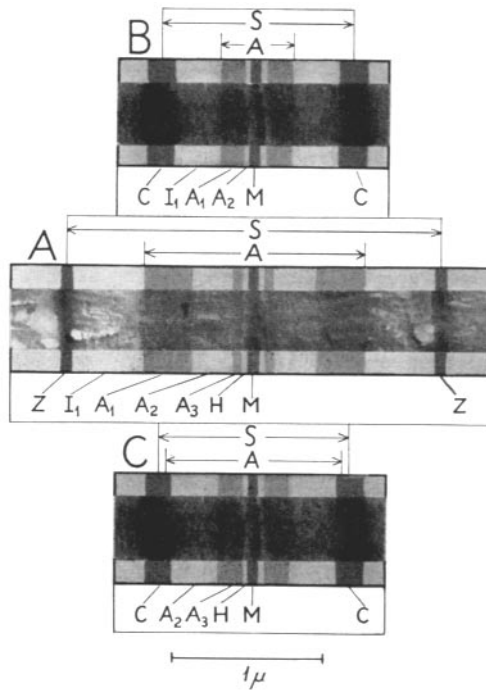


FIG. 3. Electron micrographs and schematic representation of the band pattern of resting and strongly contracted myofibrils. A, myofibril at rest, sarcomere length 2.5μ ; B and C, myofibril contracted to a sarcomere length of 1.35μ ; in B the light line (I_1 and I_2) on both sides of the contraction band (C) is shown as the I band, in C as belonging to the A band.

TABLE I

Distribution of Sarcomere Lengths in a 25 Per Cent Stretched Resting Muscle (180 Fibrils)

Length of sarcomere	No. of fibrils	Difference in length
μ	<i>per cent</i>	<i>per cent</i>
2.50	4	-9
2.60	25	-5.5
2.75	43	0
2.90	20	+5.5
3.00	8	+9

The contraction band would then consist of: A_1 , I_1 , Z, I_1 and A_1 of the next sarcomere (*cf.* Fig. 3 C). The measurements presented (Figs. 1, 5, and 6) have been based on this assumption (8, 19, 10, 7, 11).

Hodge (11) defined the length of the sarcomere in strongly contracted fibrils from the middle of the contraction band in one sarcomere to the middle of the contraction band of the next sarcomere, assuming the length of the A band

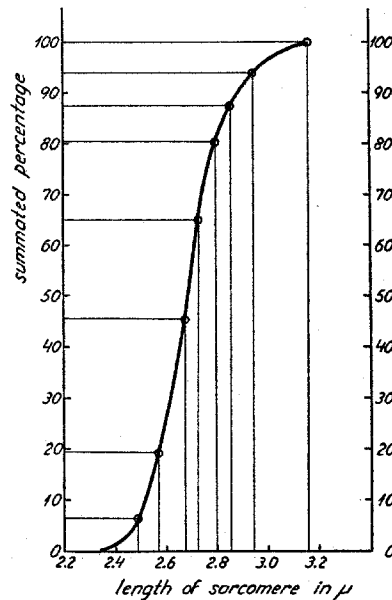


FIG. 4. To show the method of extrapolating the initial length from the shortened length (average shortening about 25 per cent). Ordinate: sumamated percentage of sarcomeres. Abscissa: initial sarcomere length in μ (description in text).

TABLE II

Distribution of Sarcomere Lengths during Afterload Contraction Initiated at 25 Per Cent Stretch

Average shortening per cent	Percentage of fibrils according to sarcomere length								
	1.20- 1.39 μ	1.40- 1.59 μ	1.60-1.79 μ	1.80-1.99 μ	2.00-2.19 μ	2.20-2.39 μ	2.40-2.59 μ	2.60-2.79 μ	2.80-2.99 μ
10			2.5	5	10.5	21	24	15.5	21
25			6.5	13	27	37	6.5	10.5	
45	22	69	9						

to be equal to the length of sarcomere. However, in measurements of the length of the I band the thickness of the Z line is usually included. From ultrathin sections of uncontracted muscle, and muscle with an isotonic shortening of about 30 per cent, the width of the Z line was determined and found to be on an average 0.1μ . There is no indication that the width of the Z line, as long as Z can be identified, increases during contraction. Therefore, we have

defined the length of the strongly contracted sarcomere as the length of the A band plus 0.1μ , and in Figs. 1 and 5 the minimum length of the I band is not plotted as 0 but as 0.1μ .

The length of the contracted sarcomere ranged between 3.0 and 1.2μ . To evaluate the degree of shortening of the different fibres of the muscle the ini-

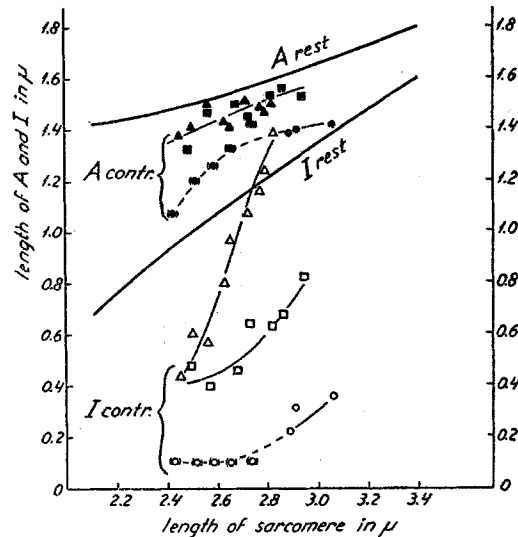


FIG. 5. Length of the A and I bands in experiments with different average shortening as a function of their extrapolated initial length.

A = \blacktriangle — \blacktriangle — \blacktriangle } about 10 per cent shortening.
 I = \triangle — \triangle — \triangle }
 A = \blacksquare — \blacksquare — \blacksquare } about 25 per cent shortening.
 I = \square — \square — \square }
 A = \bullet — \bullet — \bullet } about 45 per cent shortening.
 I = \circ — \circ — \circ }

— — — — — Curves for the A and I bands at rest.

Abscissa: resting sarcomere length in μ . Ordinate: length of the A and I bands in μ . In strongly contracted myofibrils (A = \bullet), I = \circ) the length of the I band is plotted as the thickness of the Z membrane in the resting fibril = 0.1μ .

tial sarcomere length has to be known. Owing to the different degrees of stretch of the fibres of a resting muscle, the sarcomere length varies by about ± 10 per cent around a mean value (Table I). The initial sarcomere length was graphically extrapolated from the distribution of sarcomeres in the resting and in the contracted muscle as described below. In a 25 per cent stretched muscle the length of sarcomere in the individual fibres varied between 2.5 and 3.0μ . The figures for the average distribution of sarcomere length of 180 fibrils from five resting muscles are given in Table I.

Owing to the variation in length and stretch different fibres, at a given

afterload during contraction, shorten to different lengths. The distribution of contracted sarcomere lengths in the three groups of experiments is given in Table II.

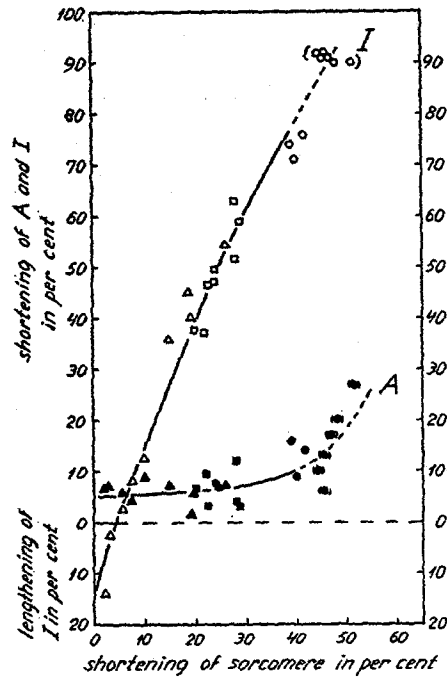


FIG. 6. Shortening of the anisotropic and isotropic bands during afterload contraction in per cent as a function of the shortening of sarcomere in per cent (calculated from the extrapolated initial length).

A = ▲ — ▲ — ▲ } about 10 per cent shortening.
 I = △ — △ — △ }
 A = ■ — ■ — ■ } about 25 per cent shortening.
 I = □ — □ — □ }
 A = ● — ● — ● } about 45 per cent shortening.
 I = ○ — ○ — ○ }

Abscissa: shortening of the sarcomere in per cent. Ordinate: shortening of the A and I bands in per cent. In strongly contracted myofibrils (A = (●), I = (○)) the thickness of the Z line of the resting fibril was taken as the length of the I band.

The length of the contracted fibril depends on its length in the resting state and on the afterload applied. Therefore, it may be assumed that the distribution of sarcomere lengths in contracted fibrils corresponds directly to the distribution of sarcomere lengths of the resting fibrils. On this assumption the resting sarcomere length of a given contracted fibril was estimated by extrapolation from the length distribution spectrum of contracted fibrils to the cor-

responding point of the length distribution spectrum of resting fibrils. An example for this procedure is given in Fig. 4. The solid curve represents the distribution spectrum (*cf.* Table I) of sarcomere lengths of the resting fibres, *i.e.* the percentage incidence of fibrils with a sarcomere length below a given value. To this curve one may now refer the corresponding summated percentage from the length distribution spectrum (*cf.* Table II) of the shortened fibres. The points (Fig. 4) shown are from the group of measurements with 25

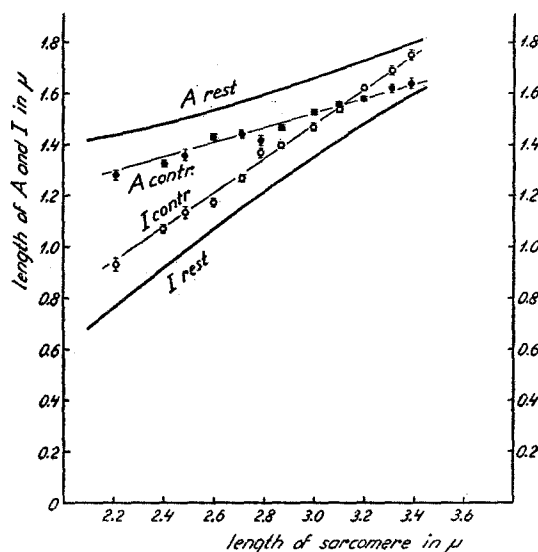


FIG. 7. Length of the anisotropic and isotropic bands at rest and during isometric contraction as a function of the length of the sarcomere (fixed muscle). Abscissa: sarcomere length in μ . Ordinate: length of the A and I bands in μ .

— : A and I bands at rest.

A = ● — ● — ● } A and I bands during isometric contraction.
I = ○ — ○ — ○ }

The vertical lines indicate the mean error.

per cent shortening and with a 2.1μ average length of the shortened sarcomere (Table II). Projection from the points to the abscissa gives the corresponding sarcomere length of the resting fibre, *i.e.* the initial sarcomere length. The length of the A and I bands as a function of the extrapolated sarcomere length at rest is shown in Fig. 5. From this curve the percentage shortening was calculated (Fig. 6). For a shortening of the sarcomere to 70 per cent, a length at which the I band could still be measured with certainty, the I band shortened 60 per cent. Within the shortening range of the sarcomere of about 5 to 30 per cent, the shortening of the A band was practically constant, amounting to 6 to 8 per cent. With further shortening the A bands contracted up to 25 per cent at a shortening of the sarcomere of about 50 per cent.

Isometric Contraction.—

Isometric contraction was initiated from resting lengths varying between equilibrium length and a 50 per cent stretch. The curves in Fig. 7 show the length of the A and I bands during isometric contraction as a function of the sarcomere length. The vertical lines indicate the mean error. The solid curves represent the length of the A and I bands of the resting muscle (6). During isometric contraction the A band shortened by about 8 to 10 per cent and the I band was elongated over the whole range of stretch investigated. The shortening of the A bands and the elongation of the I bands are statistically significant, the difference between rest and contraction being on the average larger than 6 times the mean error.

DISCUSSION

There is general agreement that strong isotonic contraction is characterized by the occurrence of a contraction band around the Z line. Opinions differ as to the interpretation of the light zone (in Fig. 3 B denoted as I_1 , in Fig. 3 C denoted as A_2) in the strongly contracted sarcomere. Bennett and Porter (1) considered it to be the reduced isotropic substance while others consider it to be part of the A band (8, 19, 10, 7, 11). The smooth curves for the length of the A and I bands favor the latter interpretation. If this interpretation is accepted the densitometric tracings of Bennett and Porter (1) suggest that the A band changes very little in density during isotonic contraction, whereas the region of the H and M bands and the region around the Z line gain appreciably in density.

The present study indicates, furthermore, that during *isotonic* (afterload) *contraction* both the A and I bands shorten and that during *isometric contraction* the A bands shorten and the I bands are correspondingly elongated. The results from isometric contraction are in agreement with the findings on living frog (2, 5) and mammalian muscle (12). They do not agree with the findings on living fibres of A. F. Huxley and Niedergerke (15) nor with the findings of H. Huxley and Hanson (16) on myofibrils of glycerol-extracted muscle, that the length of the A and I bands remains constant during isometric contraction.

In view of the occurrence of fixation artifacts it can be questioned to what degree the present findings give a correct picture of the changes of the A and I bands during contraction of the living muscle. Carlsen and Knappeis (6) found a difference in the length of the A and I bands between living and fixed muscle, the difference depending on the degree of stretch applied before fixation. However, no matter what the degree of difference, both the A and I bands increased in length with stretch, whether the muscle was alive or fixed.

Fixed and living muscle during isometric contraction were compared, assuming fixation artifacts to be of the same order of magnitude for resting and contracted muscle. From measurements on living and fixed muscle (6) a defor-

mation factor D due to fixation was calculated for both the A and I bands:—

$$D = \frac{\text{A or I living fibre}}{\text{A or I fixed fibre}}$$

The results from the present experiments during isometric contraction were corrected by this deformation factor. The curves of the corrected A and I bands are in fairly good agreement with the findings on living fibres (Fig. 8).

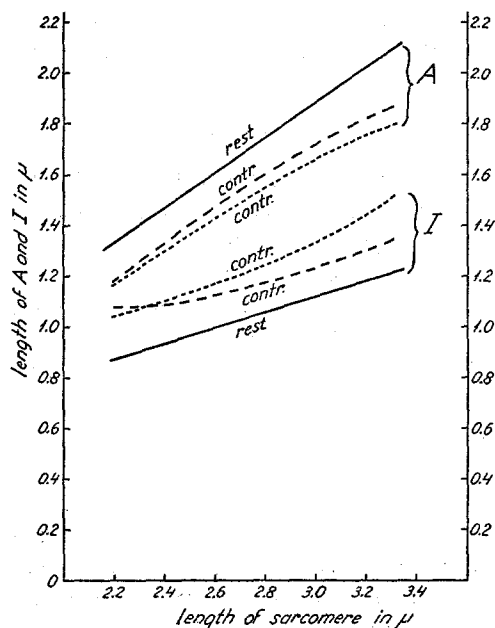


FIG. 8. Length of the anisotropic and isotropic bands at rest and during isometric contraction as function of the length of sarcomere. Abscissa: sarcomere length in μ . Ordinate length of the A and I bands in μ .

- , length of the A and I bands in the *living* muscle fibre at rest (Carlsen and Knappeis (6)).
- - -, length of the A and I bands in the *living* muscle fibre during isometric contraction (Buchthal (2)).
- · - ·, length of the A and I bands of *fixed* myofibrils during isometric contraction, corrected by a deformation factor.

The results with electron microscopy during isotonic (afterload) contraction were in essential agreement with the findings of A. F. Huxley and Niedergerke (15) on living muscle and H. Huxley and Hanson (16) on glycerol-extracted muscle: the main shortening took place in the I band and with marked shortening of the sarcomere there was a material shortening of the A bands as well. We differ, however, from these authors in finding a shortening

of the A band over the whole range of afterload contraction and during isometric contraction, and an elongation of the A band during passive stretch.

SUMMARY

Bundles of the curarized semitendinosus muscle of the frog were fixed during isotonic (afterload) and isometric contraction and the length of the A and I bands investigated by electron microscopy.

The sarcomere length, during afterload contraction initiated at 25 per cent stretch, varied depending on the afterload applied between 3.0 and 1.2 μ , *i.e.* the shortening amounted to 5 to 50 per cent. The shortening involved both the A and I bands. Between a sarcomere length of 3.0 to 1.7 μ (shortening 5 to 35 per cent) the A bands remained practically constant at about 1.5 μ (6 to 8 per cent shortening); the length of the I bands decreased from 1.4 to 0.3 μ (80 per cent shortening). Below a sarcomere length of 1.7 to 1.2 μ the A bands shortened from 1.5 to 1.0 μ (from 6 to 8 to 25 per cent). At sarcomere lengths 1.6 to 1.2 μ the I band was replaced by a contraction band.

During isometric contraction the A bands shortened by about 8 to 10 per cent; the I bands were correspondingly elongated.

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