# ULTRASTRUCTURE OF THE RESTING AND CONTRACTED STRIATED MUSCLE FIBER AT DIFFERENT DEGREES OF STRETCH

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## ABSTRACT

Passive stretch, isometric contraction, and shortening were studied in electron micrographs of striated, non-glycerinated frog muscle fibers. The artifacts due to the different steps of preparation were evaluated by comparing sarcomere length and fiber diameter before, during, and after fixation and after sectioning. Tension and length were recorded in the resting and contracted fiber before and during fixation. The I filaments could be traced to enter the A band between the A filaments on both sides of the I band, creating a zone of overlap which decreased linearly with stretch and increased with shortening. This is consistent with a sliding filament model. The decrease in the length of the A and I filaments during isometric contraction and the finding that fibers stretched to a sarcomere length of 3.7 $\mu$ still developed 30 per cent of the maximum tetanic tension could not be explained in terms of the sliding filament model. Shortening of the sarcomeres near the myotendinous junctions which still have overlap could account for only one-sixth of this tension, indicating that even those sarcomeres stretched to such a degree that there is a gap between A and I filaments are activated during isometric contraction (increase in stiffness). Shortening, too, was associated with changes in filament length. The diameter of A filaments remained unaltered with stretch and with isometric contraction. Shortening of 50 per cent was associated with a 13 per cent increase in A filament diameter. The area occupied by the fibrils and by the interfibrillar space increased with shortening, indicating a 20 per cent reduction in the volume of the fibrils when shortening amounted to 40 per cent.

## INTRODUCTION

The structural changes in striated muscle at the molecular level during stretch and contraction have been explained according to two different concepts: (*a*) unfolding and folding of molecular chains in myofilaments assumed to pass uninterrupted throughout the sarcomere, and (*b*) sliding of two arrays of filaments past each other.

Evidence from electron microscopy of ultrathin sections (1, 2), from phase contrast microscopy of glycerinated muscle (1), and from interference microscopy of living fibers (3) strongly favors the second concept, though recent electron microscopic studies have revived the folding model concept (4–6). Thus, Sjöstrand and Andersson-Cedergren (5, 6) describe a substantial increase in filament diameter with shortening, and attribute previous electron microscopic evidence, supporting the sliding model, in part to the effect of glycerol extraction, phosphotungstic acid (PTA) staining, and methacrylate embedding (7).

Undoubtedly, preparation of the sample for electron microscopy gives rise to distortions which

might limit the use of electron microscopy in quantitative studies. Almost all electron micrographs of striated muscle hitherto published show evidence of contraction. In the investigation presented in this paper we have determined the degree of distortion and ascertained the mechanical state in which the muscle fibers were fixed by measuring the sarcomere length and the mechanical tension in the resting and the stimulated fiber before and during fixation.

According to the interdigitating filament model, the zone in which the two arrays of myofilaments overlap should decrease linearly with passive stretch. Phase microscopy of glycerinated muscle fibrils (1) and interference microscopy of living muscle (3) indicate that the H zone (the lighter region in the center of the A band) is wider in stretched muscle The quantitative relationship could not be established since the width of the zone of overlap is near the limit of resolution. We have determined the width of the zone of overlap in electron micrographs of resting muscle fixed at different degrees of stretch.

According to H. E. Huxley (2), the number of filaments in the I band of glycerinated muscle fibrils is equal to the number of thin filaments in the adjoining A band, indicating that the thick filaments terminate at the end of the A band. We have confirmed these counts in ultrathin sections of non-glycerol-extracted fibrils, both stained with PTA and unstained.

So far, no studies have been published which deal in any detail with the change in the zone of overlap during isometric contraction. Hanson and H. E. Huxley (8) suggest that the actin filaments move a short distance into the A band during isometric contraction. This would result in an increase in the width of the zone of overlap. We have carried out a systematic investigation of the changes induced by isometric contraction at different degrees of stretch and found a decrease in the zone of overlap. In view of the importance attributed to the zone of overlap for the production of mechanical tension (2), we have furthermore studied the structural changes in resting and contracted fibers stretched to such a degree that the overlap between the two filaments disappeared. That these fibers are still capable of developing tension has been ascribed to shortening of the sarcomeres near the myotendinous junctions, the remainder of the fiber being merely inactive (9, 10). In living fibers and in electron micrographs we have investigated the validity of this assumption.

The increase in the zone of overlap with shortening has been demonstrated on ultrathin sections of glycerinated muscle (1, 2) and is evidenced by the disappearance of the I band with shortening in ATP-induced contractions of glycerinated muscle fibrils (1) and with physiological shortening of living muscle fibers (3). From electron micrographs of fibers fixed at different degrees of shortening we have ascertained whether there are changes in filament length in addition to sliding.

Finally, we have measured the diameter of and the distance between A filaments in ultrathin sections of resting and contracted muscle fibrils and determined the change in volume of the interfibrillar space during contraction.

## METHOD

*a) Dissection and Measurement of Sarcomere Length and Tension:* Single fibers, small bundles of 4 to 20 fibers, and, in a few instances, whole semitendinosus muscles of frogs were used. The fibers or small bundles were dissected in ice-cooled Ringer's solution from the semitendinosus muscle of *Rana temporaria* or *Rana esculenta*. The mechanical tension at rest, during stretch of the resting fiber, and during isometric contraction was measured by means of an electromechanical transducer (RCA 5734) and recorded with an ink writer at a speed of 0.3 mm/sec. The tension was recorded before and during fixation, and for each sample the maximum force ($P_0$) developed during a tetanic contraction at equilibrium length was determined. The samples could be stretched to the desired length with a micrometer screw. To determine the degree of stretch, the fibers were photomicrographed before fixation and the equilibrium length was determined (sarcomere length 2.2 $\mu$ (11)), the desired degree of stretch was applied, and time was allowed for adjustment to the new length. The muscle fibers were stimulated by 3- to 4-second trains of rectangular pulses. The pulse frequency was 40 to 60 per second and the pulse duration was 1.5 msec.

*b) Fixation:* Fixation was performed in the same chamber in which the muscle fibers were tested before fixation. The muscles were fixed in a solution composed of 1 per cent osmium tetroxide, buffered at pH 7.2 with veronal-acetate (12), and of sodium, potassium, and calcium chloride to increase the osmotic pressure (13, 14). Depending on the number of muscle fibers, the sample was kept from 1 to 4 hours in the $OsO_4$ solution at about 0°C.

*To obtain the muscle fibers in a resting state* the follow-

ing procedures were used: (*a*) the samples were kept at 0°C before and during fixation; (*b*) D-tubocurarine chloride (50 μg/ml) was added to the Ringer's solution; (*c*) two-thirds of the samples were immersed for 2 to 4 hours before fixation in isotonic Ringer's solution with 6 times the potassium content (13 mM K) of normal Ringer's solution, while one-third of the samples were immersed in a Ringer's solution to which a local anesthetic (lidocaine 1 mg/ml) was added; (*d*) the $OsO_4$ was added stepwise in the following concentrations: $1:10^4$, $1:10^3$, $1:10^2$. Replacement of 13 mM NaCl by 13 mM KCl did not cause a visible increase in the interfibrillar space as described (15) for an isotonic Ringer's solution containing 50 mM KCl.

To obtain *isometrically contracted* fibers at a given degree of stretch, the fibers were stimulated tetanically immediately before and several seconds after addition of the fixative. About a 1 per cent concentration of the fixating agent was ensured by withdrawing half of the Ringer's solution simultaneously with the addition of a 2 per cent $OsO_4$ solution. Samples were discarded when the isometric tension during fixation was less than the tension in a preceding tetanic contraction or when the tension after addition of the fixing solution decreased by more than 0.02 $P_0$.

To study the structural changes in *shortened fibers* the sample was allowed to shorten from equilibrium length or 25 per cent stretch. Different degrees of shortening were obtained when the fibers shortened against different loads. In two experiments the semitendinosus muscle or a bundle of its fibers was fixed *in situ* after it had shortened from its length at maximal extension to its length at maximal flexion of the leg.

The ratio of the length of the A band to the length of the I band as a function of sarcomere length differed systematically, depending upon whether the fiber was fixed at rest, during isometric contraction, or during shortening (Fig. 1). Hence, the A/I ratio can serve as a sensitive check of the state in which the fiber was fixed.

*c*) *Dehydration and Embedding:* After fixation the sample was washed for 1 hour in Ringer's solution, removed from the myograph, and stained in 1 per cent PTA dissolved in 70 per cent ethanol for 15 to 20 hours at 0° to 4°C. In a series of experiments the effect of PTA was evaluated by embedding samples of the same muscle with and without staining. To ensure that the removal of the sample did not cause changes in length, six experiments were performed in which the fiber (three experiments) or the muscle (three experiments) remained in the isometric myograph during staining and dehydration. There was only a negligible increase in tension (at most 0.03 $P_0$).

The solutions used for dehydration, impregnation and embedding are listed in Table I. The tissue was embedded in prepolymerized methacrylate of a honey-like viscosity and polymerized at 45° to 60°C for 24 to 48 hours. The methacrylate was vacuum-distilled after removal of the inhibitor.

*d*) *Sectioning:* Before sectioning the sample was adjusted under the microscope with its axis precisely in, or perpendicular to, the cutting plane. Both, longitudinal and transverse sections were made at a speed of 60 sections per minute with a glass knife in an LKB 3314A Sjöstrand ultramicrotome. To estimate the compression caused by sectioning, longitudinal sections of each sample were cut with the fiber axis both parallel and perpendicular to the knife edge.[1] The sections were usually mounted on carbon films; in some cases films with holes were used. To determine the length of the different parts of the sarcomere, sections were used in which the fiber axis was parallel to the knife edge and which were thick enough to contain several filament layers. To prevent compression of the structures transversely to the fiber axis, the longitudinal sections were cut with the fiber axis perpendicular to the knife edge and were thin enough to contain only one or at most two filament layers. The distances between filaments and the filament diameters were measured in cross-sections and the filament diameters found in cross-sections were compared with those found in longitudinal sections.

*e*) *Electron Microscopy:* The electron microscope used was an RCA type EMU-2B modified with a Canalco aperture system and an electrostatic pole piece compensator. To ascertain the magnification the following procedures were used: (*a*) The electron microscope was calibrated at five evenly spaced times during the series of experiments. For these calibrations parts of the same aluminum replica from an optical grating were used. The spacing of the replica was determined spectroscopically in sodium light. In four of five calibrations the magnifications were the same within 2 per cent; in one calibration the deviation was 5 per cent. (*b*) The lenses were demagnetized two or three times before an electron micrograph was taken. This reduces the effect of hysteresis. (*c*) The voltage of the reference batteries of the power supply was checked at least every two months. Finally, to avoid having a systematic change in magnification affect the measurements, the electron micrographs of samples fixed at different degrees of stretch or during contraction were taken in random order.

Samples with severe damage from preparation,

[1] The inset diagram in the figures indicates whether longitudinal sections were cut with the fiber axis parallel or perpendicular to the knife edge.

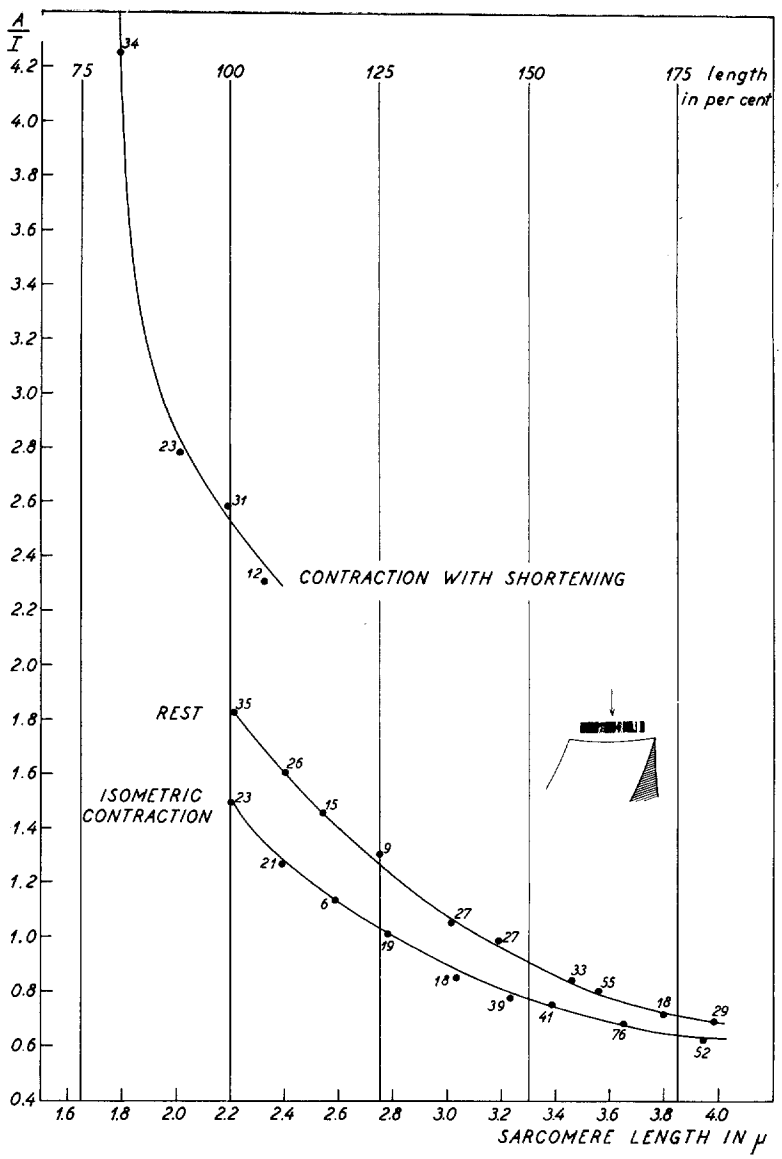



FIGURE 1

The ratio of the length of the A band to that of the I band at rest and in contraction, as a function of sarcomere length. The ratio varied with the state in which the fiber was fixed. The numbers at points on the curves indicate the number of fibrils measured at the different sarcomere lengths. The sections were cut with the fiber axis parallel to the knife edge.

including those with swelling during polymerization of methacrylate, were discarded. Electron micrographs were taken at random from regions with intact sarcomeres. The magnification used was such that slightly more than one sarcomere was in the field of view and hence non-linearity in magnification due to image distortion was negligible (less than 1 per cent). The diameter of the A filaments was measured on electron micrographs of cross-sections. The measurements were made in two directions perpendicular to each other, and the average of the two measurements was taken as the filament diameter. The distance between A filaments was determined in cross-sections at right angles to the cutting direction in order to avoid distortion due to compression. The distances between 8 to 10 consecutive

TABLE I

*Diameter of Muscle Fiber Bundles before and during the Different Stages of Preparation for Electron Microscopy*

| Stage of preparation | Medium | Experiment I | | Experiment II | |
|---|---|---|---|---|---|
| | | Measured after hours | Diameter in per cent of diameter before fixation | Measured after hours | Diameter in per cent of diameter before fixation |
| Before fixation | Ringer's solution | 0 | 100 | 0 | 100 |
| Fixation | Buffered 1% $OsO_4$ | 4 | 102 | 4 | 100 |
| After fixation | Ringer's solution | 5 | 98 | 5 | 103 |
| Dehydration | 33% ethanol | 5 | 112 | | |
| | | 5½ | 102 | | |
| | 66 or 70% ethanol | 5½ | | | 114 |
| | | 6 | 84 | 21½ | 89 |
| | 96% ethanol | 6½ | 82 | 22 | 86 |
| | Absolute ethanol | 7½ | 82 | 24½ | 83 |
| Impregnation | 50% absolute ethanol + 50% butyl methacrylate | 8 | 79 | 25 | 83 |
| | Butyl methacrylate + 1% benzoylperoxyd | 9½ | 76 | 27½ | 78 |
| Embedding | Butyl methacrylate partially polymerized | | | 28 | 74 |
| | After polymerization at 60°C before sectioning | | | 50 | 70 |

A filaments in a section were measured in 50 resting and 50 contracted fibrils.

*f) Living Fibers:* To study the distribution of sarcomere lengths in the stretched muscle fiber, with particular regard to the effect of contraction at the myotendinous junctions, two procedures were used: (*a*) Isolated living muscle fibers were stretched and photomicrographed at the myotendinous junctions and in other parts of the fiber at rest and during isometric contraction (water immersion objective, × 40, N.A. 0.75). (*b*) Isolated fibers were marked with graphite granules 300 to 400 $\mu$ apart near the myotendinous junctions, and 800 to 1500 $\mu$ apart in the remaining part of the fiber. In each fiber the maximum tension ($P_0$) was determined at equilibrium length. The fiber was then stretched and was photomicrographed to determine the sarcomere length, and the site of the graphite granules was determined at rest by photographing the fiber in its entire length (Leitz Microsummar, × 3.5, field of view 30 mm). Then the fiber was stimulated tetanically, and, about 4 seconds after the onset of stimulation, when the tension had become stationary, the site of the graphite granules was determined anew by photography.

## RESULTS

The electron micrographs presented in this study were all obtained from muscles fixed in $OsO_4$ without previous extraction by glycerol. They appear to have less contrast than those of glycerinated fibers from which an appreciable protein fraction has been removed.

### *Comparison between Living and Fixed Fibers*

1. The influence of the different steps of preparation of the muscle tissue for electron microscopy was evaluated by measuring the changes in fiber diameter and in sarcomere length. Fixation of isometrically held fiber bundles (three fibers) for 4 hours in $OsO_4$ and subsequent washing of the samples in Ringer's solution did not change the fiber diameter (Table I). Dehydration in ethanol caused 20 per cent decrease in diameter after a transient increase. Impregnation and embedding in methacrylate and polymerization of the methacrylate were accompanied by a 10 per cent decrease in diameter. Swelling of the sample due to the use of the methacrylate monomer for embedding was in most cases prevented by using partially polymerized methacrylate. The resulting transverse shrinkage was thus 30 per cent, similar to that observed after preparation of fibroblasts for electron microscopy (16).

The sarcomere length was determined in isolated fibers before and during the different steps of

TABLE II

*Sarcomere Length of the Same Fiber As Determined by Light Microscopy and by Electron Microscopy*

(Living fiber and during the different stages of preparation for electron microscopy)

| Stage of preparation | Fiber 347 isom.* | | | Fiber 359 isom.* | | | Fiber 357 rest‡ | | | Fiber 358 isom.* | | | Fiber 296§ rest | | Fiber 386 isot.‖ | | | Fiber 398 isom.* | | Fiber 399 isom.* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sarcom. length | n¶ | SD | Sarcom. length | n¶ | SD | Sarcom. length | n¶ | SD | Sarcom. length | n¶ | SD | Sarcom. length | n¶ | Sarcom. length | n¶ | SD | Sarcom. length | n¶ | Sarcom. length | n¶ |
| | μ | | | μ | | | μ | | | μ | | | μ | | μ | | | μ | | μ | |
| Before fixation | 2.8 | 120 | | 3.8 | 80 | | 4.0 | 225 | | 4.0 | 65 | | 2.7 | 45 | | | | | | | |
| After fixation in $OsO_4$, in Ringer's solution | | | | | | | | | | | | | 2.8 | 10 | | | | 3.3 | 100 | 3.0 | 375 |
| After dehydration in ethanol | | | | | | | | | | | | | | | | | | 3.0 | 159 | | |
| After impregnation in methacrylate | | | | | | | | | | | | | | | | | | 3.1 | 184 | 3.0 | 83 |
| After embedding and polymerization | | | | | | | | | | | | | 2.6 | 14 | 3.2 | 220 | | 3.0 | 156 | 2.9 | 156 |
| After sectioning, electron microscopy** | 2.77 | 11 | 0.10 | 3.73 | 40 | 0.13 | 3.85 | 58 | 0.12 | 4.10 | 21 | 0.05 | | | 3.11 | 20 | 0.13 | | | | |

* Isometrically contracted fiber, removed from the myograph after fixation.

‡ Resting fiber removed from the isometric myograph after fixation.

§ Resting fiber held isometrically throughout all stages of preparation.

‖ Isotonically contracted fiber, removed from the myograph after fixation.

¶ Number of sarcomeres measured.

** Longitudinal sections cut with the fiber axis parallel to the knife edge.

preparation for electron microscopy and after sectioning as follows (Table II): (*a*) in four fibers (nos. 347, 359, 357, 358) by light microscopy before fixation and by electron microscopy after sectioning, (*b*) in one fiber (no. 296) by light microscopy before and after fixation and after embedding, (*c*) at the same point in one fiber (no. 386) by light microscopy after embedding before sectioning and by electron microscopy after sectioning, with subsequent calibration of electron microscope magnification, (*d*) in two fibers (nos. 398, 399) by light microscopy after fixation and during the different steps of preparation. With one exception the changes in sarcomere length were less than 3 per cent; in fiber 398 the sarcomere length changed by 10 per cent.

In addition, the fiber length was determined in six single fibers, placed in an isotonic myograph, immediately after and 1 hour after addition of $OsO_4$. The longitudinal shrinkage in five of these fibers was less than 1 per cent and in one fiber it was 3 per cent. In three single fibers and in three muscles placed in an isometric myograph the tension was measured before and during fixation and dehydration (*cf.* p. 97). The small increase in tension during fixation and dehydration (0.01 to 0.03 $P_0$) found in these experiments is a further indication of the small longitudinal shrinkage.

It is surprising that the longitudinal shrinkage was so much less pronounced than the transversal. In their measurements of the changes in length of parts from four fixed fiber bundles, A. F. Huxley and Peachey (10) found a slightly greater longitudinal shrinkage than we have observed (6 per cent during dehydration and 3 per cent after impregnation and embedding in methacrylate).

2. Sectioning causes a compression of the tissue in the cutting direction (2, 4). In longitudinal sections cut with the fiber axis perpendicular to the knife edge, this compression caused a reduction in sarcomere length of 20 to 40 per cent. In longitudinal sections cut with the fiber axis parallel to the knife edge, the compression occurred in the transverse direction of the fibril, resulting in a reduction in filament diameter and in the spacing between filaments (compare Fig. 2 and Fig. 6 *a*). Therefore, to avoid dimensional changes introduced by compression artifacts, the length of the sarcomeres was determined in sections cut with the fiber axis parallel to the knife edge.

3. In addition to the comparison of sarcomere length in the living fiber and by electron microscopy, the influence of artifacts could be evaluated by comparing the degree of shortening in the living fiber, as measured in the isotonic myograph, with the reduction in sarcomere length in the fixed fiber determined by electron microscopy. Shortening was calculated from the increase in the cross-sectional area of the fibrils and in the volume of the interfibrillar space. Assuming fiber volume to remain unaltered during shortening, the calculated shortening was 43 per cent and corresponded to the observed 40 per cent shortening of the living fiber (*cf.* p. 110).

## A. *The Muscle Fiber at Rest*

1. *Equilibrium Length, Sarcomere Length 2.2 μ*: In *longitudinal* sections cut with the fiber axis parallel to the knife edge, the A band had a width of 1.43 μ, SD 0.03 μ. It contained a central region, the H zone, 0.49 μ wide, in which there were filaments about 100 A in diameter (A filaments). The H zone was limited on each side by a marginal zone of increased density due to the presence of a larger number of filaments (Fig. 2). The A filaments could be traced through both marginal zones of higher density (Fig. 3). In the center of the H zone the A filaments were slightly thicker, resulting in a dense zone 0.08 μ in width—the M line (Figs. 2 and 3).

At equilibrium length the I band had a width of 0.77 μ, SD 0.04 μ. It contained filaments about 50 A in diameter (I filaments). In the I band the I filaments frequently appeared poorly aligned except near the Z line, where the arrangement was more regular. In the stretched or contracted fibril the alignment was better than in the resting fibril at equilibrium length. The I filaments could be traced to within the A band between the A filaments on both sides of the I band (Fig. 3). The length of the I filaments from the edge of the H band in one sarcomere to the edge of the H band of the adjacent sarcomere was 1.72 μ ± 0.01. Hence the length of the I filaments exceeded that of the I band by 0.94 μ. The marginal zones of higher density in the A bands are termed the zones of overlap (1).

Both within and outside the zones of overlap the I filaments showed periodic changes in density, faint substriations, which can be seen better in thick than in thin sections. There were 24 of these periods in the I filaments on each side of the Z line. Hence, with the total length of the I filaments

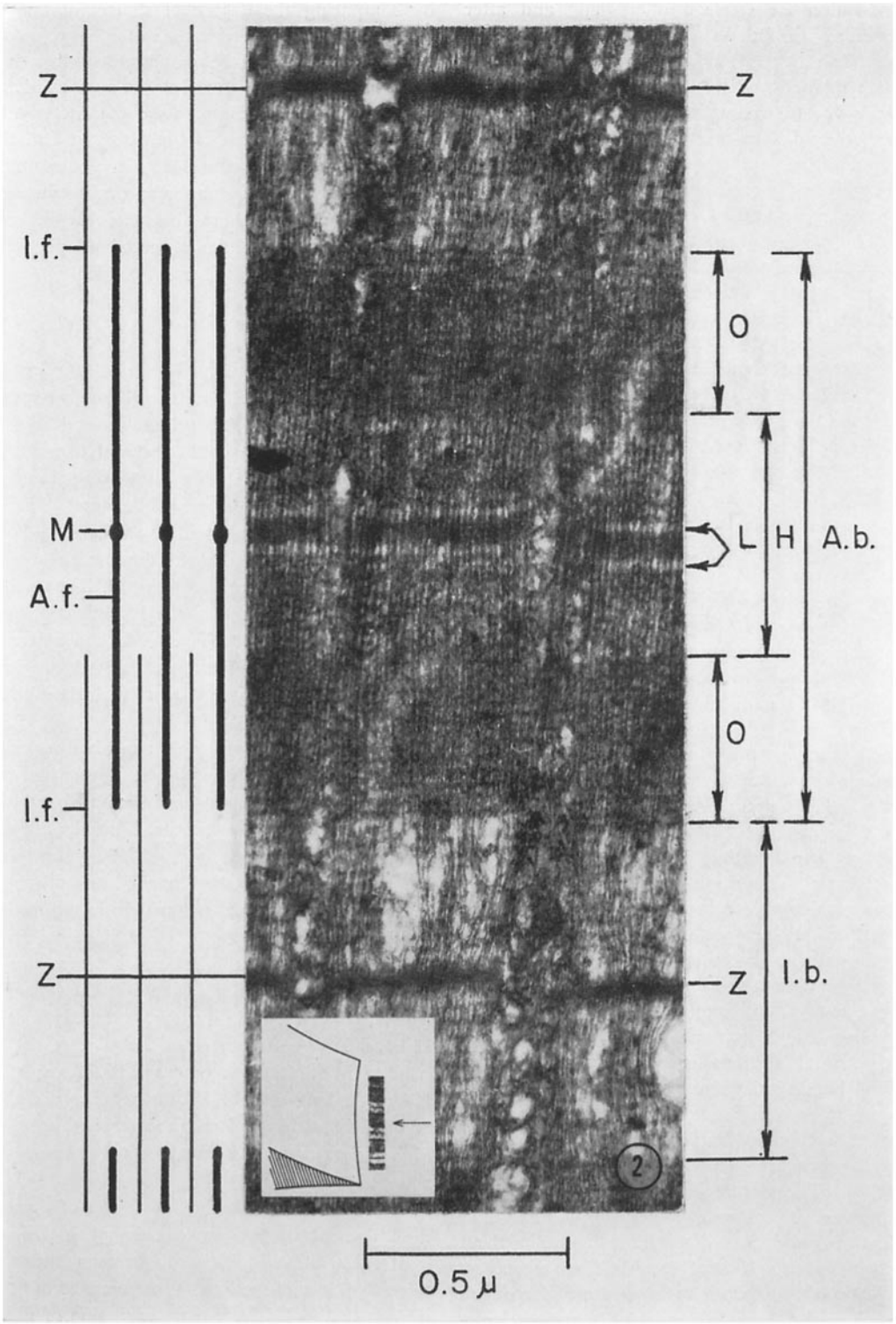



FIGURE 2

To show the different structural components in a longitudinal section of resting myofibrils at equilibrium length. The sarcomere length was the same as before fixation. *A.b.*, A band; *O*, zone of overlap; *H*, H zone; *L*, lines of reduced density adjacent to *M*; *I.b.*, I band; *Z*, Z line; *A.f.*, A filaments; *I.f.*, I filaments; *M*, M stripe.

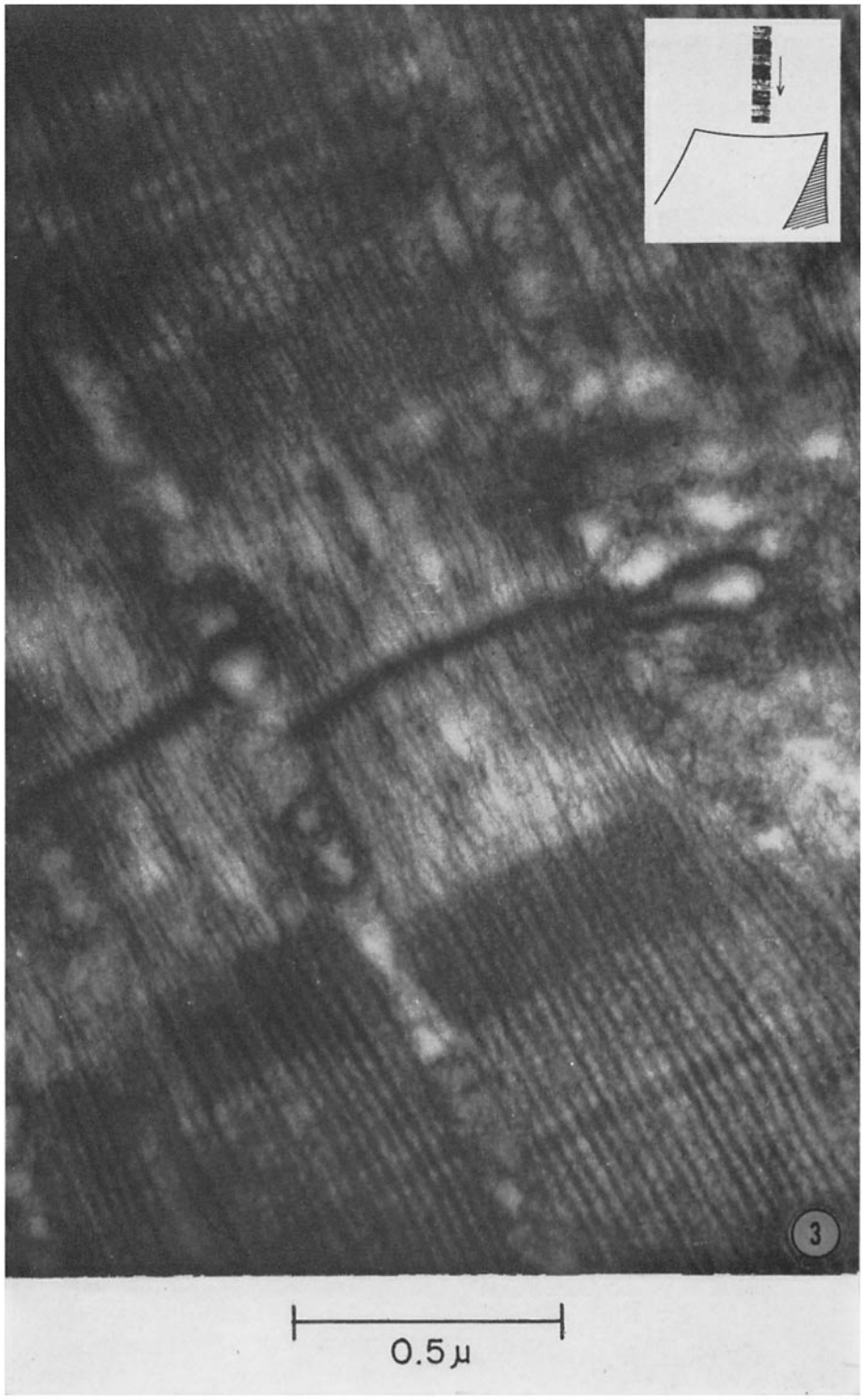



FIGURE 3

To show the larger number of filaments in the zone of overlap as compared with the H zone and the I band in the fibril without compression in the transverse direction. Longitudinal section of resting fiber at equilibrium length.

TABLE III

*Length of A and I Bands, and of Filaments at Rest at Different Sarcomere Lengths**

| No. of sarcomeres/fibers‡ | Sarcomere length | A band | I band | A band / I band | A filaments | I filaments | Overlap | "Gap" |
|---|---|---|---|---|---|---|---|---|
| | μ | μ | μ | | μ | μ | μ | μ |
| 35/6 | 2.21 (2.10–2.29) | 1.43 | 0.78 | 1.83 | 1.43 | 1.72 | 0.47 | |
| SD | | | | 0.10 | 0.03 | 0.06 | 0.04 | |
| 26/4 | 2.40 (2.30–2.49) | 1.48 | 0.92 | 1.61 | 1.48 | 1.72 | 0.40 | |
| SD | | | | 0.12 | 0.03 | 0.11 | 0.07 | |
| 15/3 | 2.54 (2.50–2.69) | 1.51 | 1.03 | 1.46 | 1.51 | 1.67 | 0.32 | |
| SD | | | | 0.08 | 0.05 | 0.09 | 0.05 | |
| 9/1 | 2.75 (2.70–2.89) | 1.56 | 1.19 | 1.31 | 1.56 | 1.73 | 0.27 | |
| SD | | | | 0.05 | 0.02 | 0.05 | 0.03 | |
| 27/4 | 3.01 (2.90–3.09) | 1.55 | 1.46 | 1.06 | 1.55 | 1.70 | 0.12 | |
| SD | | | | 0.05 | 0.04 | 0.08 | 0.04 | |
| 27/3 | 3.19 (3.10–3.29) | 1.59 | 1.60 | 0.99 | 1.59 | 1.76 | 0.08 | |
| SD | | | | 0.05 | 0.04 | 0.09 | 0.04 | |
| 33/5 | 3.46 (3.30–3.49) | 1.59 | 1.87 | 0.85 | 1.59 | 1.79 | | 0.04 |
| SD | | | | 0.03 | 0.05 | 0.11 | | 0.07 |
| 55/4 | 3.56 (3.50–3.69) | 1.59 | 1.97 | 0.81 | 1.59 | 1.87 | | 0.05 |
| SD | | | | 0.04 | 0.06 | 0.10 | | 0.05 |
| 18/3 | 3.80 (3.70–3.89) | 1.59 | 2.21 | 0.72 | 1.59 | 1.95 | | 0.13 |
| SD | | | | 0.03 | 0.05 | 0.07 | | 0.02 |
| 29/4 | 3.98 (3.90–4.09) | 1.64 | 2.34 | 0.70 | 1.64 | 1.94 | | 0.20 |
| SD | | | | 0.03 | 0.05 | 0.12 | | 0.07 |

*Length of A and I Bands, and of Filaments during Isometric Contraction at Different Sarcomere Lengths**

| No. of sarcomeres/fibers‡ | Sarcomere length | A band | I band | A band / I band | A filaments | I filaments | Overlap | "Gap" |
|---|---|---|---|---|---|---|---|---|
| | μ | μ | μ | | μ | μ | μ | μ |
| 23/5 | 2.20 (2.10–2.29) | 1.32 | 0.88 | 1.50 | 1.32 | 1.50 | 0.31 | |
| SD | | | | 0.09 | 0.03 | 0.06 | 0.03 | |
| 21/5 | 2.39 (2.30–2.49) | 1.34 | 1.05 | 1.27 | 1.34 | 1.61 | 0.28 | |
| SD | | | | 0.08 | 0.05 | 0.07 | 0.04 | |
| 6/3 | 2.59 (2.50–2.69) | 1.38 | 1.21 | 1.14 | 1.38 | 1.69 | 0.24 | |
| SD | | | | 0.09 | 0.05 | 0.12 | 0.07 | |
| 19/3 | 2.78 (2.70–2.89) | 1.40 | 1.38 | 1.01 | 1.40 | 1.76 | 0.19 | |
| SD | | | | 0.06 | 0.04 | 0.07 | 0.03 | |
| 18/2 | 3.03 (2.90–3.09) | 1.40 | 1.63 | 0.86 | 1.40 | 1.79 | 0.08 | |
| SD | | | | 0.06 | 0.06 | 0.20 | 0.13 | |
| 39/6 | 3.23 (3.10–3.29) | 1.42 | 1.81 | 0.78 | 1.42 | 1.75 | | 0.03 |
| SD | | | | 0.03 | 0.04 | 0.06 | | 0.04 |
| 41/5 | 3.39 (3.30–3.49) | 1.47 | 1.92 | 0.76 | 1.47 | 1.80 | | 0.06 |
| SD | | | | 0.04 | 0.04 | 0.10 | | 0.06 |
| 76/5 | 3.65 (3.50–3.79) | 1.49 | 2.16 | 0.69 | 1.49 | 1.80 | | 0.18 |
| SD | | | | 0.03 | 0.04 | 0.18 | | 0.10 |
| 52/5 | 3.93 (3.80–4.09) | 1.52 | 2.41 | 0.63 | 1.52 | 1.81 | | 0.30 |
| SD | | | | 0.01 | 0.01 | 0.11 | | 0.04 |

* Longitudinal sections cut with the fiber axis parallel to the knife edge.

‡ In all, 37 fibers from 10 muscles at rest and 39 fibers from 12 isometrically contracted muscles were examined.

1.72 $\mu$, the spacing of the periodicity was

$$\frac{17{,}200 \text{ A}}{2 \times 24} = 360 \text{ A}$$

Thirteen of the 24 periods on each side of the Z line were situated within the zone of overlap.

Corresponding to the findings in longitudinal sections, *cross-sections* showed regions with thin filaments (50 A), regions with thick filaments (100 A), and regions in which there were both thick and thin filaments. The number of filaments per 0.1 $\mu^2$ in cross-sections through the H zone, the zone of overlap, and the I band near the Z line was 185, 525, and 380, respectively, *i.e.*, 1:3:2. This ratio indicates that there were filaments in the A band which did not continue into the I band. In the zone of overlap the thick and the thin filaments were arranged in a double hexagonal pattern, as found by H. E. Huxley and Hanson (1) in glycerinated fibers. Because of the 20 per cent greater distances between the A filaments in the shortened state (*cf.* p. 110) this arrangement was more readily discernible in the shortened than in the resting muscle. We have seen this mixture of thick and thin filaments in the zone of overlap in resting fibers as well, both in those stained with PTA and in unstained ones (see, however, reference 7).

2. *Stretch:* The most conspicuous change with stretch was the increase in the width of the I band at the expense of the zone of overlap. Thus, 50 per cent stretch of the sarcomere was associated with a 90 per cent reduction in the zone of overlap, and three-quarters of the increase in the sarcomere length could be accounted for by withdrawal of the I filaments (Table III; Fig. 4). From equilibrium length to a sarcomere length of 3.3 $\mu$ the zone of overlap decreased linearly (17 per cent decrease per 10 per cent stretch). There was, however, a slight increase in filament length; the length of the A filaments increased by 8 per cent from sarcomere length 2.2 to 2.8 $\mu$ and remained at this length with further stretch. The I filaments remained unchanged from sarcomere length 2.2 to 3.3 $\mu$ and increased by about 8 per cent from sarcomere length 3.3 to 4.0 $\mu$. At 50 per cent stretch only 3 of the 24 periods in the I filaments on each side of the Z line were situated in the two zones of overlap. At still higher degrees of stretch the two sets of filaments were separated by a zone of reduced density which increased linearly with increasing stretch ("gap"; see Fig. 5). The width of the "gap" as a function of stretch represents a direct continuation of the linear decrease in overlap (Fig. 4). The "gap" contained a filamentary structure which possibly was continuous with the I filaments, but all the 24 periodic changes in density which characterize the I filaments were situated outside the "gap" (Fig. 5, below).

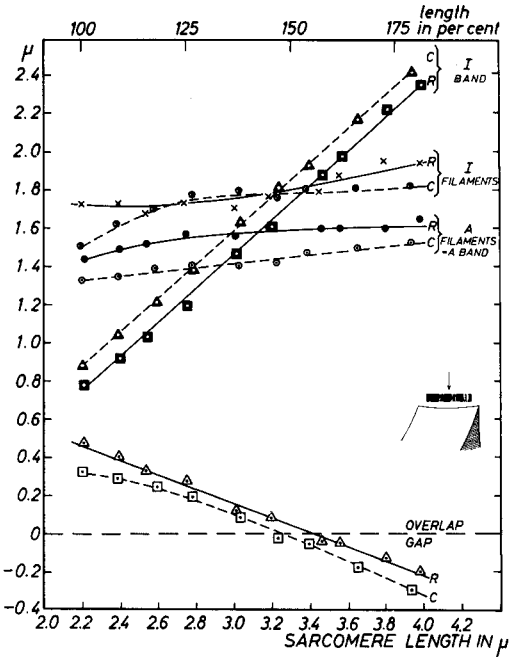



FIGURE 4

Length of A and I bands, A and I filaments, overlap, and "gap" at rest (*R*) and in isometrically contracted fibrils (*C*) as a function of sarcomere length. Sections cut with fiber axis parallel to knife edge.

The width of the Z and M lines and of the two lines with reduced density around M was not increased by stretch.

## B. *Isometric Contraction*

1. *Equilibrium Length:* When the fiber was fixed during isometric contraction at equilibrium length, the I band increased and the A band decreased by about 0.15 $\mu$ (Fig. 6 *a* and *b*). The width of the zone of overlap decreased from 0.5 to 0.3 $\mu$ and the H zone increased from 0.5 to 0.7 $\mu$. These changes correspond to a decrease in the length of the A and I filaments of 8 and 15 per cent, respectively. The width of the Z and M

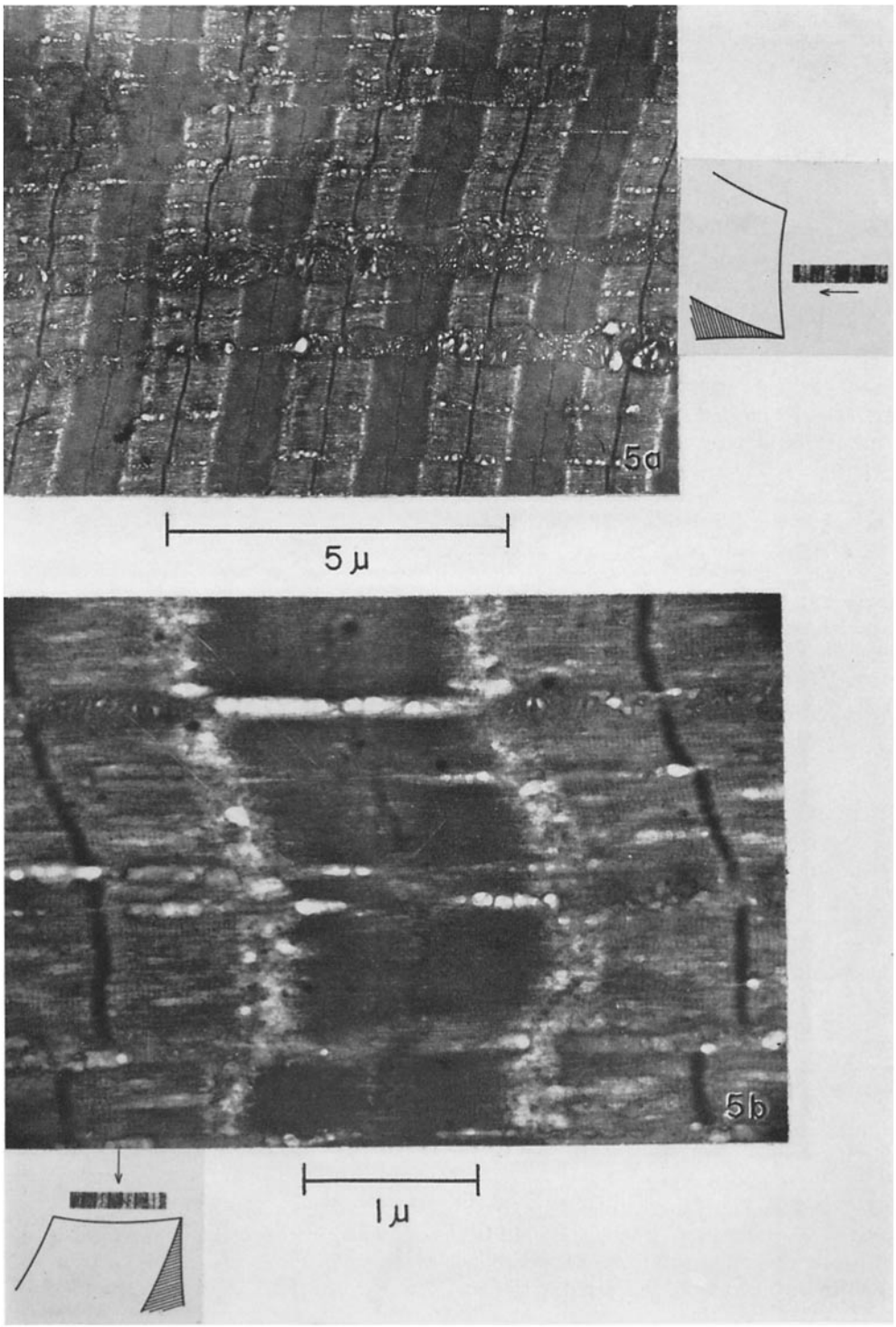



FIGURE 5

To show the zone of reduced density ("gap") between A and I. Longitudinal section of a 70 per cent stretched fiber. *Above*, "gap" in ten adjacent fibrils; *below*, the same in three fibrils at higher magnification.

lines and of the two lines with reduced density adjacent to M remained unaltered during isometric contraction.

2. *Stretch:* During isometric contraction of the stretched fiber the length of the A band decreased and that of the I band increased by 0.15 $\mu$ independently of the degree of stretch (10 to 80 per cent). The zone of overlap decreased linearly with stretch as found in the resting fiber, but the two zones of overlap were each reduced by about 0.1 $\mu$ (Table III; Fig. 4). The zone of reduced density at the edge of the I band ("gap") appeared during isometric contraction at a sarcomere length of 3.2 $\mu$ as compared with 3.4 $\mu$ in the resting fiber.

In terms of filament length, the I filaments shortened about 0.15 $\mu$ below 15 and above 55 per cent of stretch; the A filaments shortened about 0.15 $\mu$ at all degrees of stretch. The Z and M lines and the zone of reduced density adjacent to M did not change during isometric contraction of the stretched fibril.

3. *Does a Sarcomere with a "Gap" Develop Tension?* From the electron microscopic findings on fixed fibrils it must be assumed that when a living fiber is stretched 50 to 60 per cent above equilibrium length the A and I filaments no longer overlap but are separated by a "gap." At this degree of stretch an isolated fiber develops a tetanic tension of 0.3 to 0.5 $P_0$ (17, 18; Fig. 8; Table IV). The question is whether the tension is developed in these stretched sarcomeres or in parts of the fiber in which the A and I filaments still overlap, as near the myotendinous junctions (9, 10).

Photomicrographs of isolated living fibers from five muscles at rest showed that the sarcomere length near the myotendinous junctions was about 20 per cent less than in the center portion of the fibers (Fig. 7). The sarcomeres at the very end of the fiber may have been still shorter. In any case the region near the myotendinous junctions of the fiber in which overlap still occurred amounted to at most 1 per cent of the total fiber length.

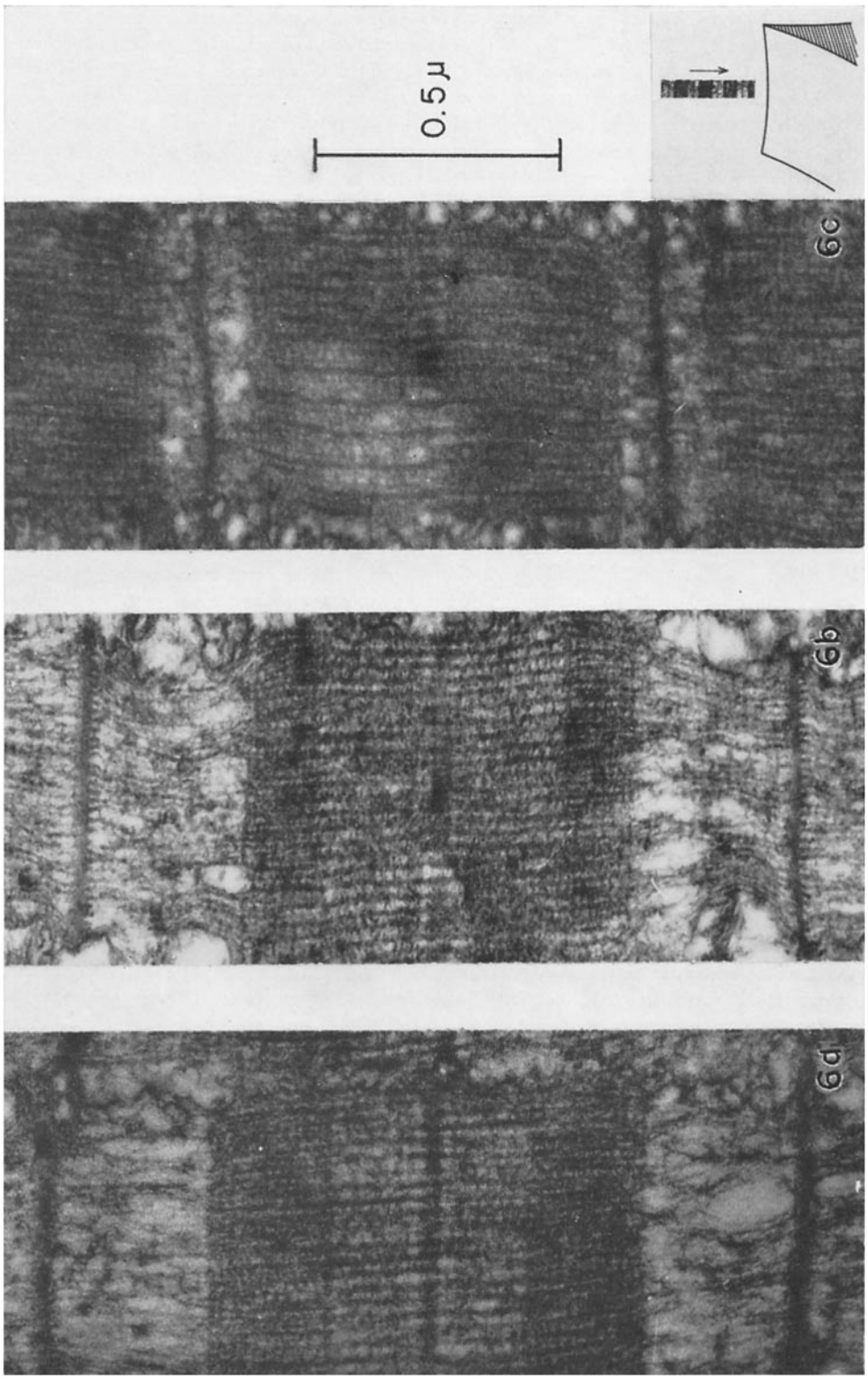
Investigations were done to determine whether the shortening of these fiber regions could account for the tension developed during isometric contraction. In this case the tension would be derived from passive stretch of the portion of the fiber without overlap.

For this purpose, (*a*) the length-tension diagram was determined in each resting fiber and the sarcomere length was measured on micrographs, (*b*) the isometric tetanic tension was recorded near equilibrium length and at different lengths above 60 per cent of stretch, (*c*) a comparison was made of the static and semidynamic elasticity of the resting fiber, and (*d*) the lengthening of the middle portion of the fiber which occurred during isometric contraction was determined by measuring the distance between graphite granules distributed along the fiber.

Outside the fiber regions near the myotendinous junctions there was only a 1 to 2 per cent lengthening during isometric contraction (Table IV). A similar increase was found by comparing the sarcomere lengths outside the regions of the fiber near the myotendinous junctions on micrographs of stretched isolated muscle fibers at rest and during isometric contraction. In electron micrographs we have found that the fiber portion near the myotendinous junctions containing sarcomeres with overlap extended over no more than 100 $\mu$ (7 fibers from two isometrically contracted muscles and 4 fibers from one resting muscle). Except near the myotendinous junctions, the A and I filaments were separated by a "gap" and the variation in the sarcomere length did not exceed 10 per cent.

It is seen from the length-tension diagram that a resting fiber stretched 60 to 70 per cent develops an increment in tension of 0.05 $P_0$ for an additional stretch of 1 to 2 per cent (Fig. 8). This is the tension which could be expected to develop in a similarly stretched fiber caused to contract if the whole tension were derived from passive stretch of the main portion of the fiber due to contraction of the sarcomeres near the myotendinous junctions.

Under semidynamic conditions the elasticity modulus is about twice that calculated from the static length-tension diagram of the resting fiber (19). In our experiments the ratio between the semidynamic tension recorded immediately after stretch and the adjusted tension measured about 5 minutes later was maximally 1:2 (Fig. 8). Hence, the increase in tension due to an additional passive stretch of 1 to 2 per cent in the 60 to 70 per cent stretched fibers would average $2 \times 0.025\ P_0$ (Table IV, column 5). In fact, the extra tension developed during tetanic contraction of the 60 to 70 per cent stretched fiber was on the average 0.3 $P_0$. Column 6 in Table IV indicates for each fiber the tension developed during contraction for 60 to 100 per cent stretched fibers. Passive


0.5 μ
6c
6b
6d

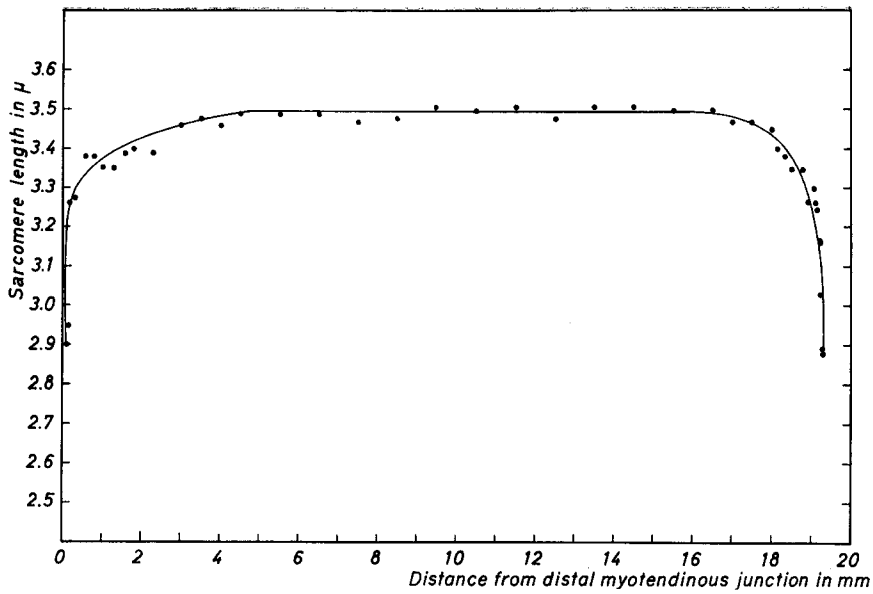



FIGURE 7

Sarcomere lengths along a living muscle fiber measured from one myotendinous junction to the other in a 60 per cent stretched muscle fiber at rest. Each point represents a mean from 10 to 20 adjacent sarcomeres.

stretch of the major part of the fiber could account for only about one-sixth of the tension actually measured during isometric contraction.

### C. *Contraction with Shortening*

When shortening was induced from equilibrium length (sarcomere length 2.1 to 2.3 $\mu$), the fiber shortened 20 to 40 per cent. The 25 per cent stretched bundles had a sarcomere length of 2.6 to 2.9 $\mu$ at rest and shortened by 25 to 30 per cent of their initial length (Table V; Fig. 9). The most prominent changes during shortening were the reduction in the length of the I band, the increase in the zone of overlap, and the corresponding decrease in the width of the H band (Fig. 6 *c*). This is in agreement with the findings of H. E. Huxley and Hanson (1) on glycerinated fibrils. When the unstretched fiber shortened by 26 per cent, the I band decreased 0.55 $\mu$, the zone of overlap increased 0.16 $\mu$, and the length of the A band remained practically unchanged. When 25 per cent stretched fibers were allowed to shorten to about the equilibrium length of the resting fiber, the I band decreased 0.58 $\mu$, the zone of overlap increased 0.45 $\mu$, and the length of the A band remained unchanged. Thus, the increase in width of the zones of overlap on both sides of the I band exceeded the decrease in the length of the I band by 0.32 $\mu$. These changes indicate that the I filaments shortened 0.24 $\mu$ when the unstretched fiber shortened 26 per cent, and lengthened 0.32 $\mu$ when a 20 per cent shortening was induced in the 25 per cent stretched fiber.

In a heavily shortened fiber it is difficult to decide whether the I filaments terminate at or within the M line. Therefore, in these fibrils the length of the I filaments and of the zone of overlap could only be determined to within about 10 per cent (Table V).

Both with a shortening of 20 per cent in a 25 per

FIGURE 6

To show the decrease in overlap in the isometrically contracted fibril and the increase in overlap in the shortened fibril. *a*, rest, equilibrium length; *b*, isometric contraction, equilibrium length; *c*, 20 per cent shortening, initiated from equilibrium length.

TABLE IV

*To Show That the Extra Tension Developed during Isometric Contraction in Fibers Stretched 60 per Cent or More Did Not Originate Solely from Shortening of Sarcomeres near the Myotendinous Junctions*

| Fiber no. | 1 $\frac{L}{L_0}$ | 2 $\frac{\Delta P_r}{\Delta L}$ | 3 $P_c$ | 4 $\Delta L_m$ | 5 $\Delta P_m$ | 6 $\frac{\Delta P_m}{P_c}$ |
|---|---|---|---|---|---|---|
| 2 | 1.59 | 0.7 | 0.44 | 0.011 | 0.025 | 0.06 |
| | 1.67 | 0.8 | 0.32 | 0.017 | 0.048 | 0.15 |
| 1 | 1.60 | 0.7 | 0.14 | 0.012 | 0.027 | 0.19 |
| 7 | 1.63 | 1.2 | 0.11 | 0.008 | 0.033 | 0.30 |
| 4 | 1.69 | 1.8 | 0.21 | 0.010 | 0.058 | 0.28 |
| 6 | 1.69 | 0.8 | 0.36 | 0.025 | 0.068 | 0.19 |
| | 1.75 | 1.0 | 0.24 | 0.014 | 0.049 | 0.20 |
| 3 | 1.69 | 0.8 | 0.46 | 0.031 | 0.085 | 0.19 |
| | 1.78 | 1.0 | 0.39 | 0.021 | 0.075 | 0.19 |
| 5 | 1.70 | 1.1 | 0.13 | 0 | | |
| 8 | 1.73 | 0.8 | 0.35 | 0.016 | 0.045 | 0.13 |
| | 1.85 | 0.9 | 0.25 | 0.013 | 0.045 | 0.18 |
| | 1.93 | 1.0 | 0.20 | 0.007 | 0.027 | 0.14 |
| | 1.97 | 1.3 | 0.14 | 0.005 | 0.035 | 0.25 |

*Column 1.* $\frac{L}{L_0}$ = length in units of equilibrium length $L_0$.

*Column 2.* $\frac{\Delta P_r}{\Delta L}$ = stiffness of the resting fiber in units of $P_0 L_0^{-1}$. $P_r$ = resting tension in units of $P_0$ ($P_0$ = maximum tetanic force).

*Column 3.* $P_c$ = tetanic extra tension in units of $P_0$.

*Column 4.* $\Delta L_m$ = relative stretch of middle part of fiber during contraction.

*Column 5.* $\Delta P_m = 2\left(\frac{\Delta P_r}{\Delta L} \cdot \Delta L_m \frac{L}{L_0}\right)$ = semidynamic increase in force in a resting fiber stretched by the same amount as the middle region of the fiber during contraction. Ratio between static and semidynamic increase in tension as 1:2.

*Column 6.* $\frac{\Delta P_m}{P_c}$ = fraction of the tension developed during tetanic contraction which can be accounted for by passive stretch of the middle part of the fiber.

cent stretched fiber and with a shortening of 40 per cent in an unstretched fiber, the I filaments were found at the edges of or within the zones of reduced density adjacent to the M line. At these shortenings the width of the M line and of the two zones of reduced density adjacent to it remained unaltered.

With a shortening to a sarcomere length below 1.6 $\mu$ the length of the A filaments decreased as well, the reduction being 10 per cent when the sarcomere shortened to 1.4 $\mu$.

### *Diameter of A Filaments*

Sjöstrand and Andersson-Cedergren (6) found an increase in A filament diameter in the shortened fibril and considered it to indicate a true shortening of these structures during contraction and to be at variance with a sliding model. Even with a shortening of 50 per cent we have found only a slight increase in A filament diameter (from 104 A, SD 9 A, at rest, to 117 A, SD 9 A, in the shortened fiber (Fig. 10 *a* and *c*). The A filament diameter did not change with 25 per cent shortening (Fig. 10 *b*), with 60 per cent stretch of the resting fiber, nor during isometric contraction.

### *Area Taken Up by the Myofibrils and the Interfibrillar Space*

The increase in cross-sectional area of the muscle fiber with shortening must be associated with changes in the distance between filaments, between myofibrils, or both. The distance between A filaments increased 20 per cent with a 40 per cent shortening, corresponding to a 44 per cent increase in the cross-sectional area of the fibrils. With 40 per cent shortening the interfibrillar space was increased by 250 per cent (Fig. 11; Table VI).

The increase in the interfibrillar space and in the distance between filaments in the shortened fiber was evenly distributed over the cross-section of the fiber, and in the main was localized outside the tubular network. It appeared consistently in all fibers from shortened muscle (9 muscles) and was absent in fibers from resting or isometrically contracted muscle (19 muscles). These findings were confirmed in 3 muscles embedded in a shortened state and 1 muscle embedded in an isometric condition in Epoxy resins (Vestopal and Epon).

## DISCUSSION

### 1. *Electron Micrographs and Living Fibers*

Whether or not a structure which can be distinguished solely by electron microscopy is produced or changed by a preparation artifact can

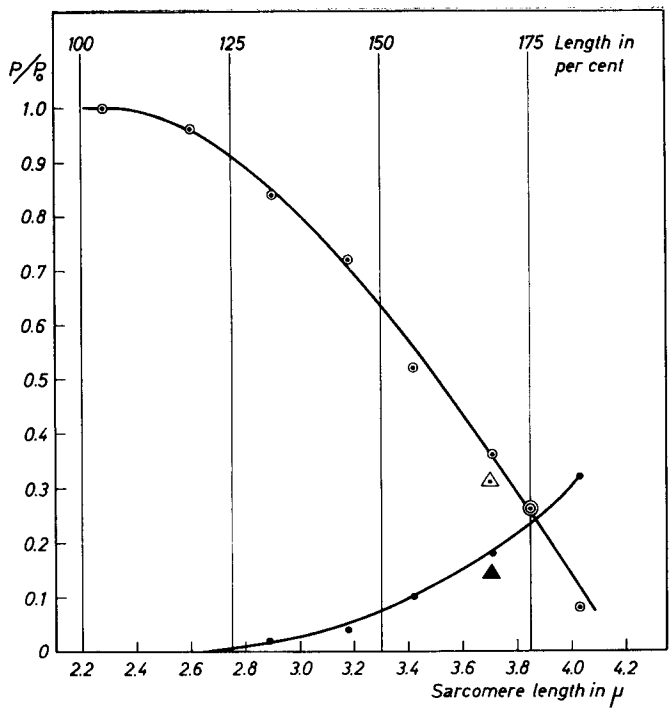



FIGURE 8

Static length-tension diagram of a single muscle fiber at rest (solid circles) and extra tension during isometric contraction (open circles with dot). For comparison are shown the tension of a fiber at rest before fixation (solid triangle) and the extra tension maintained during fixation (open triangle with dot) (sarcomere length 3.75 $\mu$).

only be deduced with a limited degree of certainty. The regularity with which it appears under given conditions indicates at any rate that it is a reproducible artifact. This applies to the filament length measured at different degrees of stretch and during contraction and to the zone of reduced density ("gap") which appeared only when a fiber was stretched more than 50 per cent. This "gap" cannot be due to fixation artifact: if it were due to filament rupture during fixation, this would have caused a decrease in tension. Furthermore, the "gap" appeared in all sarcomeres except those at the myotendinous junctions, and in the range of stretch between equilibrium length and 90 per cent stretch there was a gradual transition between the reduction in the zone of overlap, the occurrence of the "gap," and its increase.

Rupture of filaments in the living state is unlikely as the cause of the "gap," since the length-tension diagram at rest and during isometric contraction was reversible up to about 90 per cent of stretch.

When structural changes, as they appear in electron micrographs, are compared with the structure of the living fiber under given mechanical conditions, distortions can be expected to arise from fixation, dehydration, embedding, and sectioning and from lack of maintenance of the mechanical state during preparation of the sample for electron microscopy. Dehydration and embedding were associated with a shrinkage of 30 per cent in the diameter of the muscle fiber. That a similar shrinkage occurred within the fibril is indicated by a comparison between the distances of A filaments in electron micrographs of the resting fiber (310 A) and corresponding distances obtained from low angle x-ray diffraction in living muscle (455 A (20)).

It is remarkable that the shrinkage along the longitudinal axis of the fiber was much less than in the transverse direction; there was at most a 5 per cent difference between the sarcomere length as measured on electron micrographs and the sarcomere length as measured by light microscopy

TABLE V

*Length of Sarcomeres, of A and I Bands, and of Filaments in Resting and Shortened Fibrils**

| No. of sarcomeres/fibers‡ | Sarcomere length (μ) | A band (μ) | I band (μ) | A band / I band | A filaments (μ) | I filaments (μ) | Overlap (μ) |
|---|---|---|---|---|---|---|---|
| | | | Rest, equilibrium length | | | | |
| | 2.20 | 1.43 | 0.77 | 1.86 | 1.43 | 1.72 | 0.47 |
| | | | Contraction with shortening | | | | |
| 34/4 | 1.79 (1.70–1.89) | 1.45 | 0.34 | 4.26 | 1.45 | 1.66 | 0.66 |
| SD | | | | 0.55 | 0.02 | 0.06 | 0.02 |
| 13/2 | 1.62 (1.50–1.69) | 1.40 | 0.22 | 6.37 | 1.40 | 1.48 | 0.63 |
| SD | | | | 1.50 | 0.04 | 0.05 | 0.02 |
| 15/2 | 1.34 (1.30–1.49) | 1.31 | | | 1.31 | 1.2–1.3 | 0.6–0.7 |
| | | | Rest, 25 per cent stretch | | | | |
| | 2.75 | 1.56 | 1.19 | 1.31 | 1.56 | 1.73 | 0.27 |
| | | | Contraction with shortening | | | | |
| 12/3 | 2.32 (2.30–2.40) | 1.62 | 0.70 | 2.31 | 1.62 | 2.18 | 0.74 |
| SD | | | | 0.09 | 0.03 | 0.03 | 0.01 |
| 31/5 | 2.19 (2.10–2.29) | 1.58 | 0.61 | 2.59 | 1.58 | 2.05 | 0.72 |
| SD | | | | 0.28 | 0.06 | 0.06 | 0.03 |
| 23/4 | 2.00 (1.90–2.09) | 1.45 | 0.55 | 2.63 | 1.45 | 1.83 | 0.64 |
| SD | | | | 0.25 | 0.05 | 0.05 | 0.02 |

* Longitudinal sections cut with the fiber axis parallel to the knife edge.

‡ In all, 20 fibers from 6 muscles

in the same fiber before fixation. This applied to fibers fixed both at rest and in an isometrically contracted state. Similarly, the degree of shortening calculated from the increase in cross-sectional area and from the increase in distance between A filaments and between myofibrils agreed with the degree of shortening of the living fiber. There is therefore reason to assume that the filaments in electron micrographs had the same length as in the living fiber. With regard to the length of the A filaments (A bands), this conclusion agrees with observations on living fibers by interference microscopy (3) and is at variance with measurements of the band widths by ordinary light microscopy (11).

The distortion arising from compression during sectioning was estimated by sectioning each sample in two directions. A random use of sections obtained with undefined cutting directions may give rise to misinterpretations. The A filament diameter is smaller in sections cut with the fiber axis parallel to the knife edge than in sections cut with the fiber axis perpendicular to the knife edge. The sarcomeres are shorter in sections cut with the fiber axis perpendicular to the knife edge than in sections cut with the fiber axis parallel to the knife edge. This may possibly account for the large increase in A filament diameter and the decrease in A band length during shortening as described by Sjöstrand and Andersson-Cedergren (6).

Since maintenance of the mechanical conditions during fixation is not always possible, many fibers in which the mechanical conditions changed during fixation had to be discarded. However, by recording the tension and the length before and during fixation it could be ascertained whether a fiber was in fact fixed at rest or whether the fixative

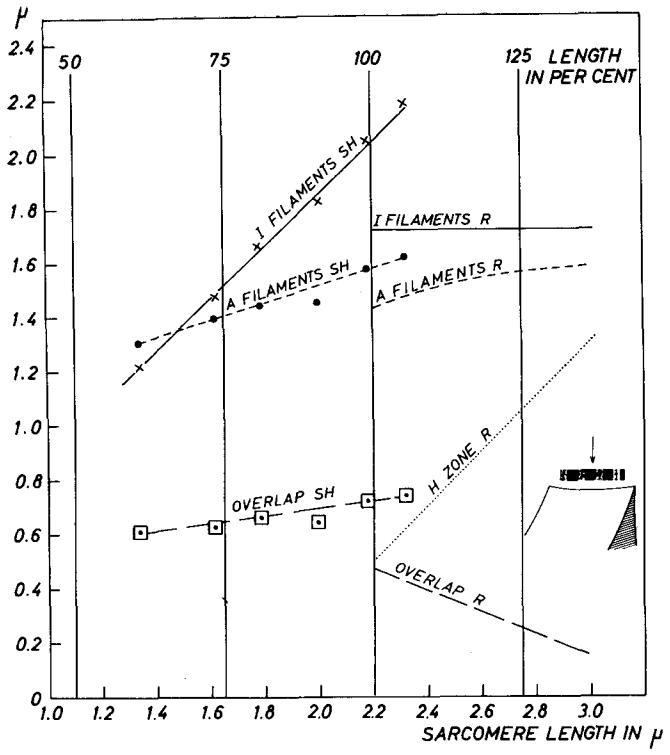



FIGURE 9

Length of A and I filaments, H zone, and overlap at rest (*R*) and in shortened fibrils (*SH*), as a function of sarcomere length. Sections cut with fiber axis parallel to knife edge.

had induced contraction; and, when stimulated, that the tension was maintained during fixation. Only these fibers were further prepared for electron microscopy.

Finally, when the lengths of the structural components are compared in fibers fixed in different degrees of stretch, at rest and during contraction, the accuracy of the measurements depends on the avoidance of systematic errors in magnification. The procedures used to ascertain a constant magnification have been described (see p. 97).

## 2. *Interdigitating Filaments or Filament Branching*

Our findings in non-glycerinated muscle fibers with and without staining by PTA confirm in several respects the results which H. E. Huxley and Hanson (1) and H. E. Huxley (2) obtained on glycerinated fibres: (*a*) there were two separate types of filament, (*b*) the I filaments passed in between the A filaments, (*c*) the zone of overlap decreased with passive stretch, and (*d*) the zone of overlap increased during shortening at the expense of the width of the I band. These findings as well as the insignificant changes in A filament diameter with stretch and shortening are consistent with the concept that the I filaments slide between the A filaments. Sjöstrand and Andersson-Cedergren (6) suggest a continuous filamentary structure, the thicker A filaments branching at the edge of the H zone. That cross-sections through the zone of overlap showed two thin filaments for each thick filament would be consistent both with a splitting of the A filaments and with interdigitating filaments. To obtain the observed ratio of filament numbers in the different zones of the sarcomere one would have to assume in the case of filament branching that one of the three branches of an A filament terminates at the edge of the A band. Filament branching would require the additional assumption that the sites of the points at which the filaments divide would move with stretch and shortening, since the length of the A band remained largely unaltered (Figs 4 and 9).

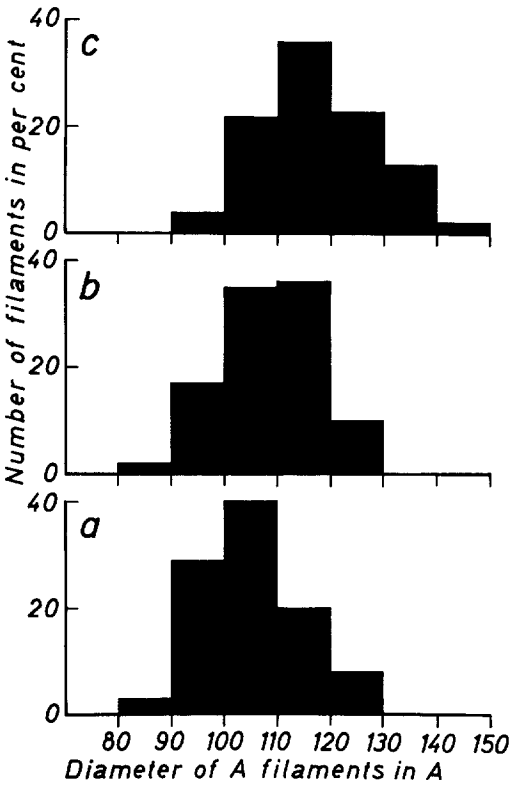



FIGURE 10

Histograms of diameters of 100 A filaments in resting and shortened fibrils measured on cross-sections through the H zone or the zone of overlap. *a*, rest, equilibrium length, mean diameter 104 A, SD 9 A; *b*, 25 per cent shortening, mean diameter 108 A, SD 9 A; *c*, 50 per cent shortening, mean diameter 117 A, SD 9 A. *b* and *c* are afterload contractions initiated from equilibrium length.

### 3. *Findings Which Require a Modification of the Sliding Model*

Sliding alone, in the sense of a mutual filament displacement without changes in filament length, cannot account for shortening to a sarcomere length below 1.6 $\mu$. Shorter sarcomere lengths have been explained by filament curling (1). Also, with lesser shortenings there were changes in filament length in addition to sliding: with shortening to a sarcomere length above 1.8 $\mu$ the I filaments were longer than in the resting fiber. This means that shortening to a sarcomere length of, for example, 2.3 $\mu$ would require an additional sliding of 0.25 $\mu$ to compensate for the 0.5 $\mu$ elongation of the I filaments. Both with small (20 per cent) and with greater degrees of shortening (40 per cent) the I filaments were drawn up to or into the light zone adjacent to the M stripe. That shortening of the stretched sarcomeres was only 20 per cent was due to the elongation of the I filaments. It seems paradoxical that shortening against a load below the maximum isometric tension caused lengthening of the I filaments, while isometric tetanic contraction was associated either with a shortening or with an unchanged length of the I filaments. The decrease in A and I filament length in the isometrically contracted fiber is evidenced by a smaller zone of overlap than in the fiber at rest. This is at variance with the increase of the overlap found in isometric contraction of glycerol-extracted fibrils after addition of adenosine triphosphate (1, 8).

According to the sliding model, the force developed in the active fiber originates from an interaction between the A and I filaments in the zone of overlap. There is, however, evidence that muscle fibers stretched to such a degree that the two types of filament no longer overlap can still develop tension during isometric contraction. In a 70 per cent stretched fiber this tension amounted to 30 per cent of the maximum tetanic tension. It has been reported that shortening in fibers stretched to a sarcomere length of more than 3.6 $\mu$ was due solely to shortening at the fiber ends (9, 10). In view of this finding the possibility existed that the extra tension developed by a stretched fiber during an isometric tetanus might be due solely to contraction of the sarcomeres near the myotendinous junctions where overlap persists even during stretch. However, if the tension were developed solely by the shortening of the sarcomeres near the myotendinous junctions, the remaining sarcomeres would be passively stretched by about 10 per cent (sarcomere length 3.6 $\mu$). In fact, there was only a 1 to 2 per cent elongation of the sarcomere length in the main part of the fiber, which could account for only one-sixth of the tension developed by the fiber in isometric contraction. Therefore, the stretched sarcomeres without overlap are active during contraction and there is at any rate an increase in elasticity modulus. This is in agreement with the three- to fourfold increase in longitudinal and torsional elasticity moduli observed during isometric, tetanic contraction at 60 per cent stretch (21, 22).

### 4. *Fluid Displacement during Shortening*

Early findings on sections of fibrillar muscle of insects have been interpreted to indicate a transfer

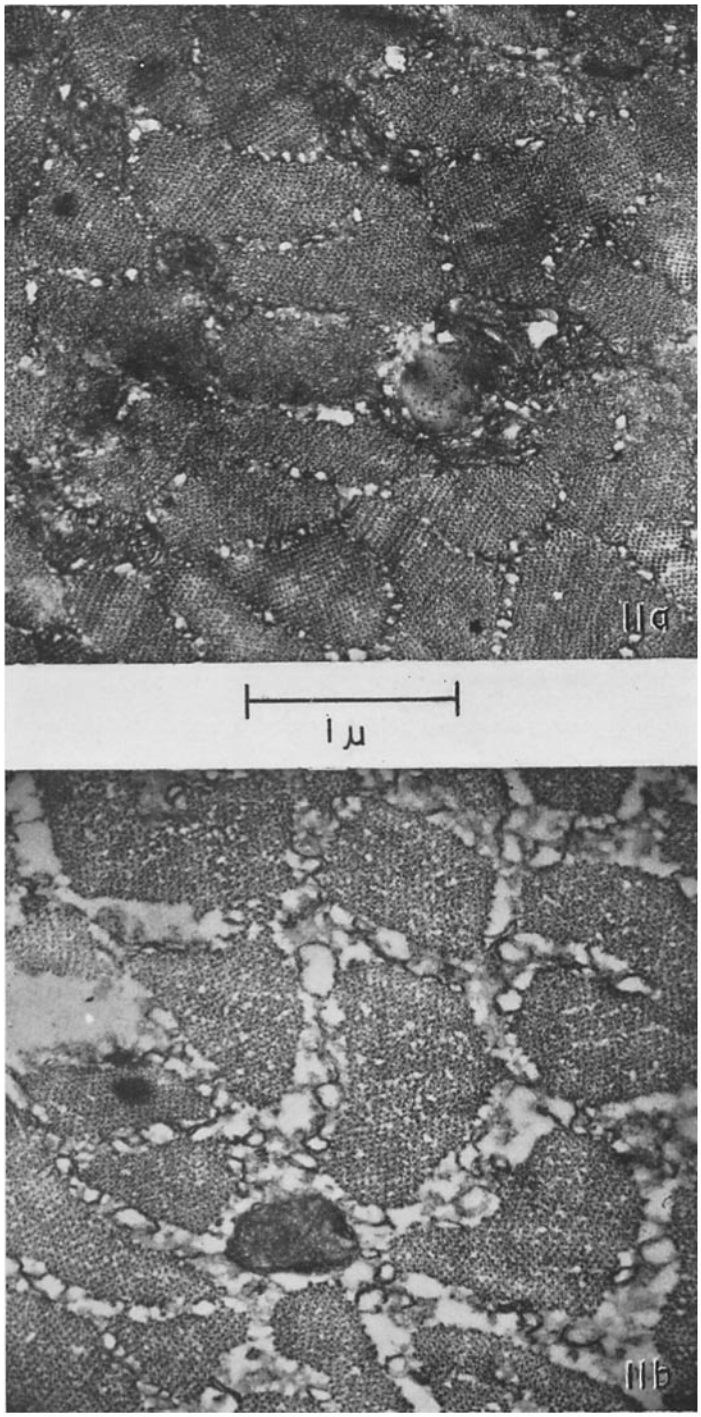



FIGURE 11

To show the increase in the volume of the interfibrillar spaces in cross-sections of a 40 per cent shortened fiber (below) as compared with the resting fiber (above).

TABLE VI

*Interfibrillar Space and A Filament Spacing in Resting and Contracted Fibers*
(Four resting, four isometrically contracted, and two shortened muscles)

| | Length of resting fiber* | Distance between A filaments | Space occupied by fibrils ‡ | Space occupied by fibrils § | Interfibrillar space ‡ | Interfibrillar space § | Total cross-sectional area§ | Fiber length* |
|---|---|---|---|---|---|---|---|---|
| Resting fiber, equilibrium length | 100 | 312 SD 18 | 85 | 85 | 15 | 15 | 100 | 100 |
| Shortened fiber | 100 | 377 SD 28 | 70 | 122‖ | 30 | 53¶ | 175 | 57 |
| Isometrically contracted fiber | 100 | 324 SD 20 | | | | | | |
| Isometrically contracted fiber | 140 | 280 SD 29 | | | | | | |

* Expressed in per cent of equilibrium length.
‡ Expressed in per cent of total cross-sectional area.
§ Expressed in per cent of the cross-sectional area of the resting fiber.
‖ Cross-sectional area occupied by shortened fibrils expressed in per cent of the total area of the resting fiber: $85 \times \left(\frac{377}{312}\right)^2 = 122$.
¶ Interfibrillar space in shortened fiber expressed in per cent of the total area of the resting fiber: $122 \times \frac{30}{70} = 53$.

of fluid from the isotropic into the anisotropic elements during contraction (23). In frog muscle we have found that shortening was associated with an increase in distance between fibrils (Fig. 11). A similar increase was never found in the fiber fixed at rest, even when it had been exposed to a Ringer's solution in which 13 mM NaCl was replaced by 13 mM KCl (15). Nor did fibers fixed in isometric contraction show an increase in the interfibrillar space. Only when the fibrils appeared obviously damaged by preparation artifact was an increase in interstitial space observed independent of the state in which the fiber was fixed.

The relative magnitude of the change in the distance between filaments and in the interfibrillar space may indicate whether the fibril loses fluid during contraction. The cross-sectional area occupied by the fibrils was 85 per cent at rest and 70 per cent in the shortened fiber. Expressing the area occupied by the fibrils in the shortened fiber in per cent of their area in the resting fiber, and assuming the total fiber volume to remain unchanged during shortening, the fibrillar volume had decreased by about 20 per cent in the 40 per cent shortened fiber, indicating a displacement of fluid to the interfibrillar space.

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