POLARIZED LIGHT OBSERVATIONS ON STRIATED MUSCLE CONTRACTION IN A MITE

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ABSTRACT

Contraction of individual sarcomeres within the living mite *Tarsonemus* sp. was observed by polarized light microscopy. In unflattened animals the usual range of contraction was such that the minimum sarcomere length approximated the length of the A region, and the maximum sarcomere length was about twice the length of the A region. The central sarcomeres of the dorsal metapodosomal muscles were observed in detail. The A band length increased slightly with increasing sarcomere length since the regression of I region length on sarcomere length had an average slope of 0.91. When the A band length in a sarcomere which was shortening was compared with the length when the same sarcomere lengthened, no significant difference was seen. The A band of each sarcomere seemed to act as a not too rigid limit to further shortening; this agreed with the reversible shortening of a muscle in which the A band had been experimentally shortened. An H region was visible at long sarcomere lengths and was not visible at short sarcomere lengths, even when the muscle was actively shortening. The rate of change of H region length with sarcomere length suggested that I *filament* length may increase as sarcomere length increases. Despite this effect and the small increase in A length with sarcomere length, the results are considered to be consistent with a model in which shortening occurs by the relative movement of A and I filaments, with little or no change in length of either set of filaments. Sarcomere shortening was clearly associated with an increase in the retardation of the A region.

INTRODUCTION

The structurally well defined model proposed by A. F. Huxley and Niedergerke (1) and by H. Huxley and Hanson (2) to describe the light microscope–visible changes accompanying the shortening of striated muscle has been well supported for vertebrate muscle (3, 4). This paper will present evidence for the applicability of this model to the normally functioning striated muscle of a tarsonemid mite (Arthropoda, Arachnida, Acarina). The long sarcomeres and thin muscle fibers of this animal studied in conjunction with an optical system employing "rectified" polarization optics (5) permitted observations on the intact living animal which were of greater reliability than is usual.

MATERIALS AND METHODS

The animals were from the same stock used in a previous study of muscle development and have been tentatively identified as *Tarsonemus randsi* (6). For observation, larvae were mounted in mineral oil and lightly compressed by withdrawing part of the oil.

Observations were made with a polarizing microscope (model P-42 American Optical Company, Southbridge, Mass.) equipped with polaroid film "polars." A rectified (5) 97 ×, NA 1.25 objective was used for a condenser as well as for an objective,

and observations were usually made employing condenser numerical apertures of 0.8 and objective apertures of 1.25. The compensator was a thin sheet of mica ($\lambda/20$ maximum retardation) mounted beneath the condenser where it could be rotated and the degree of rotation measured.

The light source was a water-cooled mercury arc (AH-6, General Electric Company, Nela Park, Cleveland, O.) used with a filter to isolate the green region of the spectrum.

Most photographs were taken at a magnification of × 290 on Kodak Tri-X film (Eastman Kodak Co., Rochester, N.Y.) using a 1 sec exposure, and measurements were routinely made from the projected negative.

OBSERVATIONS

Description of Contraction

The dorsal metapodosomal muscles were most suitable for study but contraction was also examined in the two-sarcomere extensors of the first and second pairs of legs and in the four-sarcomere muscles which originate in the dorsal midline and insert on the gnathosoma. Observations were usually made on animals which had been lightly compressed between the slide and cover slip to keep them from moving out of the field too rapidly and to improve the image quality.

A dorsal metapodosomal muscle is composed of two fibers with three sarcomeres each. These fibers lie side by side and insert anteriorly through tonofilaments to the transverse apodeme separating the propodosoma from the metapodosoma. Within each fiber there appear to be about ten thin fibrils whose register is usually good but which can at times give a jagged appearance to the AI junction and to the Z line. Mitochondria were not visible with polarized light nor was there any indication of granules near the Z line. An occasional preparation suggested that an "N" line was detectable.

In polarized light a strongly positive birefringent A region was easily seen and at long sarcomere lengths a less birefringent central region was also visible. This less birefringent central region will be called the "H" region, but position in the middle of the A region only is implied. A Z line was visible (with additive compensation) as a dark or isotropic line flanked by thin bright lines and was centered in a weakly positive birefringent I region. These bands all reversed with negative compensation. The Z line was easily seen by phase contrast microscopy but the contrast of the A region was low.

The dorsal metapodosomal muscles, which are not directly opposed by other muscles, gave a graded amount of shortening with a wide variety of speeds. The impression gained was that they were contracting against a load which was presumably the internal pressure arising from the elasticity of the exoskeleton.

Shortening of the extensors of the first and second legs was usually more rapid and complete than shortening of the dorsal metapodosomal muscles and was estimated to occur at a maximum rate of about 15 μm/sec/half sarcomere at 22°C. Restraining forces on the extensor muscles were (*a*) the drag of the media through which the leg moved and (*b*) any resistance due to opposing muscles and to the exoskeleton.

When an animal was very active, the dorsal metapodosomal muscles contracted strongly about once every 2 sec and shortening occurred until the sarcomere length was close to that of the A band length. Relaxation was rapid and the muscle was usually extended as soon as it had stopped shortening. Extremes of both shortening and elongation were seen in such an animal. When an animal was less active, contraction was less frequent and the strength of contraction was apparently diminished in that shortening usually occurred in a range where the I band length was 0.5–0.8 times the A band length.

As a muscle shortened, the I bands decreased in length with the Z bands remaining well centered; no obvious change in A band length was ever seen. An H gap was first visible at sarcomere lengths, where the I band was a little shorter than the A band, the gap became more obvious with increasing sarcomere length. There was about a 20% increase in fiber width as a sarcomere shortened from a long stretch to near its shortest length. This did not appear to be accompanied by separation of the fibrils and may reflect a change in filament spacing, as has been shown for vertebrate muscle (7).

Seen under the microscope, the elongation of a sarcomere was smooth; the I region opened up without obvious jerking or tugging and without much unevenness at the AI junction or at the Z line. The Z line sat close to the center of the I region although there were instances, particularly in animals observed for a long time, in which the half I bands were not quite equal. Instances

in which one sarcomere shortened markedly, while others, in the same muscle, elongated, were not normally seen. Shortening also seemed smooth even when the rate of shortening was changing abruptly.

Relaxation in animals whose muscles had been contracting repeatedly for several hours, or in some initially sluggish animals, appeared less smooth. The AI junction tended to be irregular and the Z lines were often jagged and not perfectly centered. The usual sarcomere length was shorter in these animals, and the muscles only infrequently "relaxed" to sarcomere lengths in which an H region was visible.

Contractile Range in Unflattened Animals

The normal contractile range was determined by observing animals suspended without flattening in a viscous solution of 5% methyl cellulose (4000 centipoises). Sarcomere length has been expressed in terms of units in which *A* is the length of the A region when a good I band is visible. *A* was a convenient visual reference for estimating sarcomere length since its length did not vary appreciably. Table I gives some idea of the maximum sarcomere lengths observed during normal functioning.

Visual observations using an ocular micrometer as a reference showed the minimum sarcomere length in all muscles to be close to *A*. In several instances there appeared to be shortening such that the sarcomere length was 20% shorter than the initial *A* length. Flattening did not obviously affect the range over which contraction could occur, except for the four-sarcomere muscles inserting dorsally on the gnathosoma. Both long stretches and strong shortening were seen in flattened and unflattened mites.

TABLE I

Estimates of the Maximum Sarcomere Length Observed in Active Unflattened Mites

Muscle	Sarcomere length
	A
Dorsomedial metapodosomal muscles	2.5 (usually about 2.2)
Dorsolateral metapodosomal muscles	2.2
Extensor of the second leg	2.0 (usually about 1.8)
Dorsal muscles inserting on the gnathosoma	2.2

Sarcomere length was measured relative to A band length (*A*).

The animals observed were initially active and were responding to the additional stimuli of being compressed and suspended in fluid. They responded to light but only when the propodosoma was illuminated, and this was usually masked by an adjustable field aperture used to reduce the scattered light.

A and I Band Lengths in Single Sarcomeres at Varying Lengths

Individual sarcomeres in the dorsal metapodosomal muscles of slightly flattened mites were photographed at a variety of sarcomere lengths, with no consideration given to whether they were shortening or lengthening. The photographs were of variable quality owing mainly to movement, and about one-third were used for measurements.

Most usable photographs of sarcomeres having lengths below 1.5 *A* were obtained after a muscle had been contracting for several hours but no indication of time-dependent change in length was seen.

Figs. 1 and 2 show some of the photographs used in determining the graphs shown in Figs. 3 and 4. As shown in Fig. 3, the major change in sarcomere length was associated with a change in the length of the I band, with the exception of sarcomere lengths which began to approach the length of the A band. This deviation at short sarcomere lengths was considered to be at least partially due to optical properties of the Z line which obscure the birefringence of the A band. Deformation of the A filaments may also be a contributing factor. In view of these considerations a sarcomere length of 1.2 *A* was used as a lower limit in selecting data to determine the change in A or I length (1.2 *A* corresponds to an I region of about 0.8 μm). The least squares regression of I band length on sarcomere length was calculated for the central sarcomere of the two dorsomedial metapodosomal muscles. Sarcomere length could usually be determined independently of the A and I bands by measuring the distance between Z bands. Measurements of A band length were not considered statistically because of possible bias entering from directly

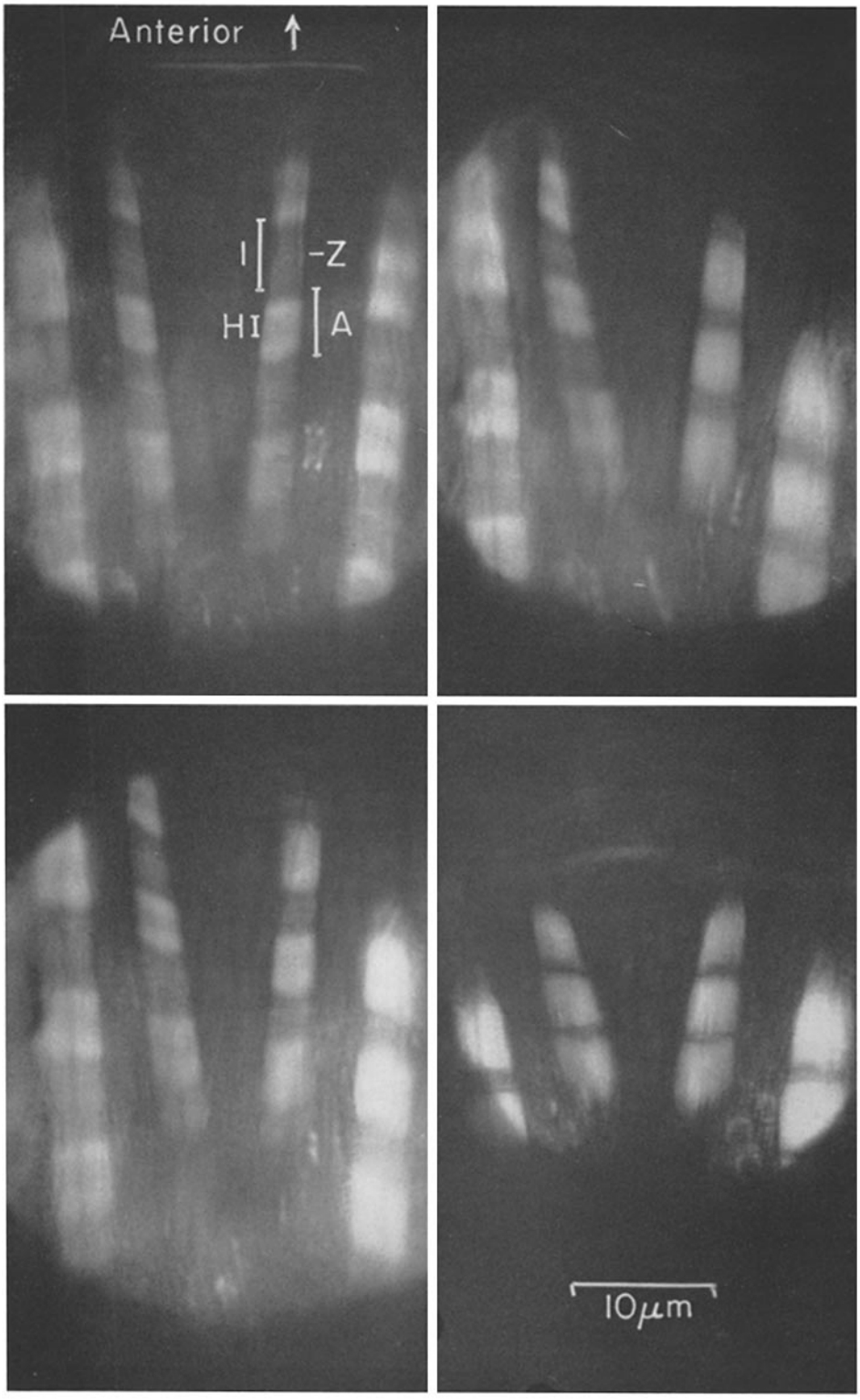

FIGURE 1 Polarized light photographs of dorsal metapodosomal muscles in a single individual. Measurements of A, I and H lengths for the labeled sarcomere from these and similar photographs are plotted in Fig. 3.

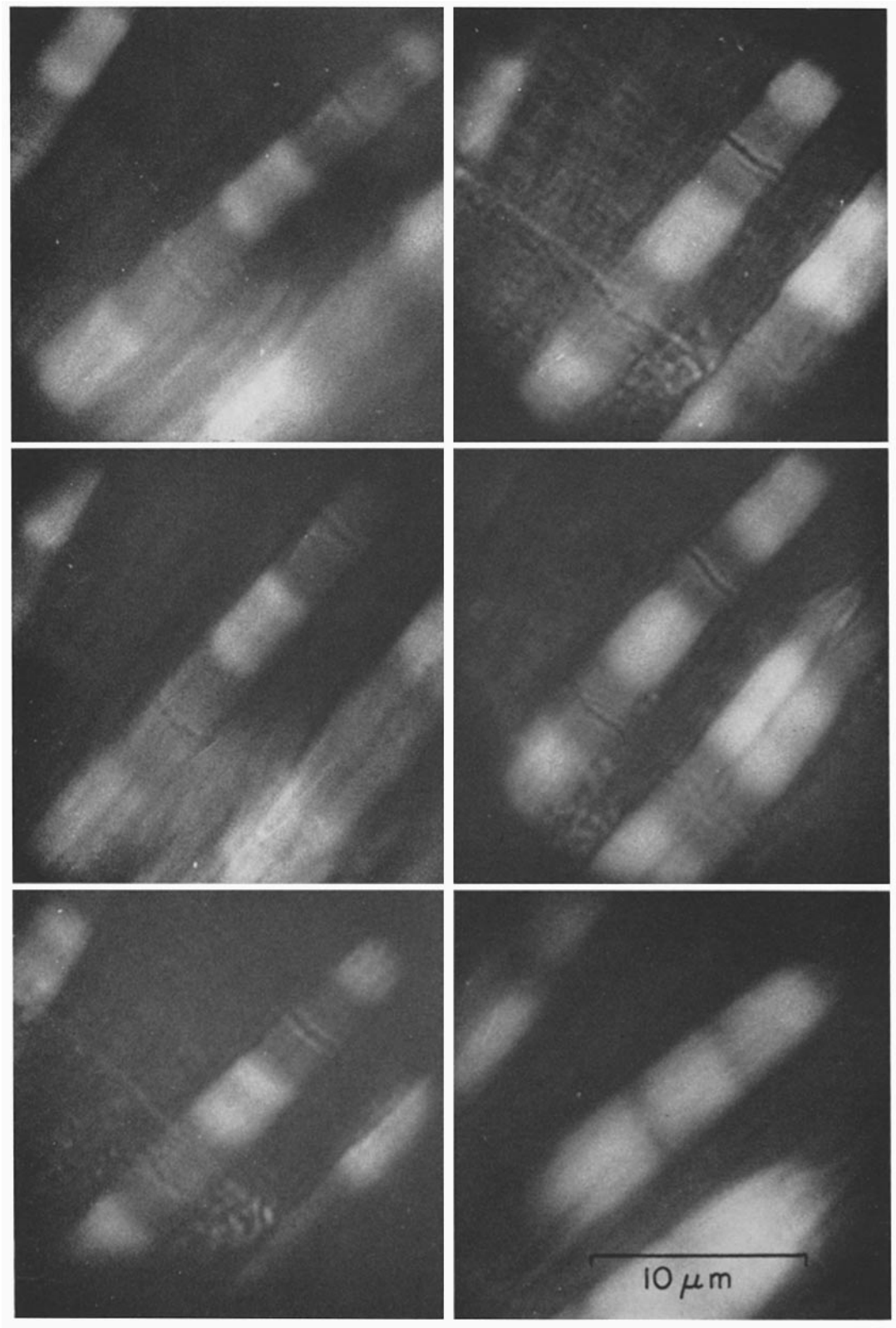

FIGURE 2 Polarized light photographs of the central sarcomere of one of the dorsomedial metapodosomal muscles in a single individual. Measurements of A, I, and H lengths are plotted in Fig. 4 for this sarcomere.

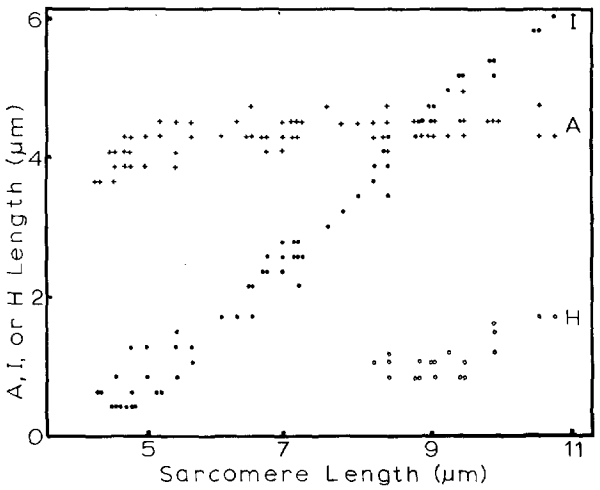

FIGURE 3 Measurements of A, I, and H lengths for the labeled sarcomere in Fig. 1 are plotted against sarcomere length determined from the distance between Z lines.

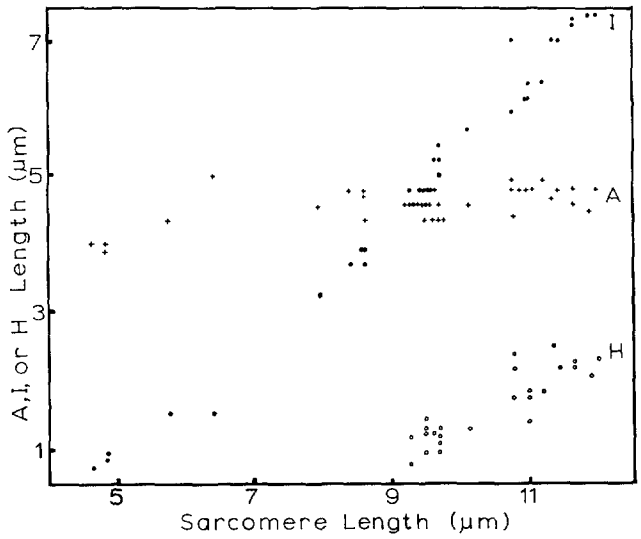

FIGURE 4 Measurements of A, I, and H lengths have been plotted against sarcomere length for the sarcomere shown in Fig. 2. These data are shown because they include the most extensive measurements on the H region.

measuring a length which tends to remain constant.

Table II summarizes the data available. The mean slope was 0.91, and all but one of the sarcomeres differed significantly from 1.00, the value expected if the A region maintained a constant length. Whether this change in A band length reflects a change in A filament length is uncertain since it could arise from differences in register of either the A filaments or of the fibrils as a function of sarcomere length. It may also be related to movement and sharpness of focus.

In general, the length of the A region was close to the minimum sarcomere length observed during contraction, and there is some reason to think that A regions abutting on each other or on the Z band may act as a not too rigid limit to further shortening, as appears to be the case for frog muscle (1, 3). An example of an experimentally modified mite muscle fiber supporting this view is shown in Fig. 5.

The anterior sarcomere of a dorsolateral metapodosomal muscle was irradiated for several seconds with heterogeneous ultraviolet light from a G.E. AH-4 mercury lamp. When this muscle contracted, some of the I filaments in the irradiated region (near the *X* in Fig. 5) appeared to separate from the A region. After a number of

contractions, all the A regions in the fibrils which had torn apart were shorter than those in adjacent fibrils although apparently still contractile. When these sarcomeres with shortened A regions contracted, reversibly, the I regions completely disappeared, supporting the view given above.

Sequential changes in an actively contracting sarcomere were recorded with a movie camera. By measuring[1] the A region at the "same" sarcomere length (in this case 1.5–2.0 *A*), and determining from the movie whether the sarcomere was shortening or lengthening, it was possible to compare the A region under different contractile conditions without considering systematic optical effects.

The data summarized in Table III suggest that the occurrence of an average difference of more than 0.07 μm in the length of the A band, when a sarcomere was shortening compared to when it was lengthening, was improbable, and, of course, that there may well have been no difference. The point-to-point resolution of the optical system used was calculated to be about 0.25 μm, but it is considered that differences somewhat below this limit should be detectable by repetitive measurement. Since there was no measure of the tension, one may question whether lengthening reflects relaxation as much as a decrease in the developed tension.

TABLE II

Regression of I Band Length on Sarcomere Length with 95% Confidence Interval

Seven sarcomeres in six individuals

N	Slope	
1	0.92	±0.04 (Fig. 3)
2	0.84	±0.10
3	1.07	±0.06 (Fig. 4)
4	0.90	±0.12
5	0.94	±0.04
6	0.90	±0.05
7	0.84	±0.06

The less birefringent H region seen by polarized light at long sarcomere lengths probably corresponds to the H region determined by the ends of the I filaments, since the birefringence of the I region is about one-third as strong as the birefringence of the A region. A clearly defined birefringent line corresponding to an orderly interdigitation of I filaments at the level of the M line was not seen at short sarcomere lengths (Figs. 1 and 2).

Visual observations showed that the H region opened and became longer at long sarcomere lengths (Figs. 3 and 4), and that at comparable sarcomere lengths sarcomeres which were shortening did not appear to differ from those which were lengthening. It is certain that H regions comparable to those occurring at long sarcomere lengths were not formed when muscles with sarcomere lengths below about 1.8 *A* were actively shortening. The length of the H region did not appear to change as rapidly as the I region (Fig. 2), and measurements made from the random photographs used to analyze the A region supported this for all individuals. Measurements, such as those shown in Figs. 3 and 4, indicate that the H region opens about 65% as fast as the I region and suggest that I filament length may increase with increasing sarcomere length. How accurately the H region reflects I filament length is uncertain, particularly in view of the possible variation in register suggested as an explanation for the increase in A length with sarcomere length. The proportional change in H length with sarcomere length should be considerably more sensitive to register than the length of the A region because of its shorter over-all length.

TABLE III

Summary of Measurements of A Band Length in a Single Sarcomere Which Was Actively Shortening and Lengthening

Appearance	State	N	Mean A length and standard error
			$m\mu$
Shortening	Contractile	96	5.21 ± .02
Lengthening	Relaxed ??	59	5.22 ± .03

A just significant difference between means is 0.07 μm.

[1] The procedure used in measuring the length of the A band was to trace the AI boundary as accurately as possible from the projected image, draw a straight line representing an average AI boundary perpendicular to the sarcomere axis, measure the distance between these parallel lines, and then finally determine whether the sarcomere was shortening or lengthening.

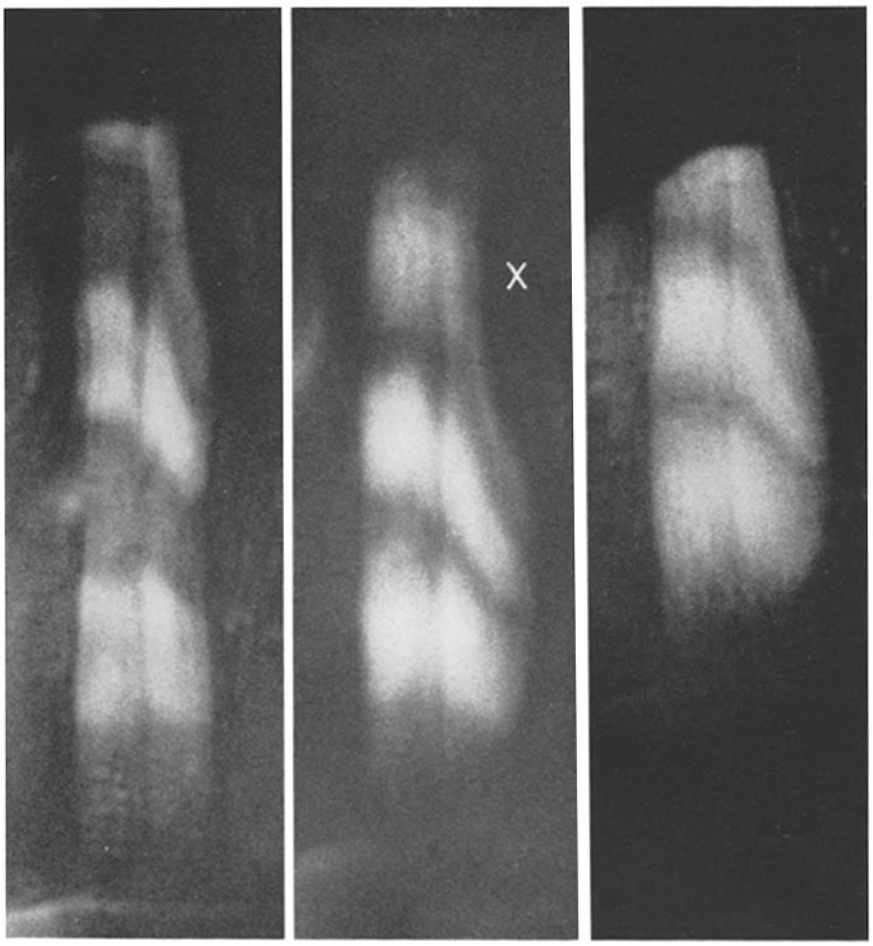

FIGURE 5 Polarized light photograph showing shortening of a fiber whose anterior-most sarcomere had been damaged by ultraviolet irradiation *X*. Shortening of the A region in the middle sarcomere and in the posterior sarcomere was probably not a direct effect of this irradiation. Both fibers were shortening and lengthening in an apparently normal manner when these photographs were taken. Dorsolateral metapodosomal muscles. × 3,500.

An effort was made to determine the length of the H region as a function of sarcomere length in anesthetized animals but a different optical system was employed and the results were not clear enough to compare.

Anesthetized Animals

Muscles of active animals tore when they were stretched until the H region was equal to or greater than half the length of the A region. Tearing appeared to result from several sarcomeres shortening at the expense of another sarcomere to produce irreversible changes. Muscles in mites, which were anesthetized with carbon dioxide or with ether, could be strongly stretched to an extent where the I filaments appeared to be pulled from the A region without causing irreversible changes. The sarcomeres generally remained equivalent while being stretched, as they do in normally functioning muscle, suggesting that there was a structural element maintaining their relation. However, in at least three instances carbon dioxide–anesthetized mites showed dorsal metapodosomal muscles with sarcomeres markedly differing in length. Fig. 6 *a* shows an example in which the anterior sarcomeres of the two medial

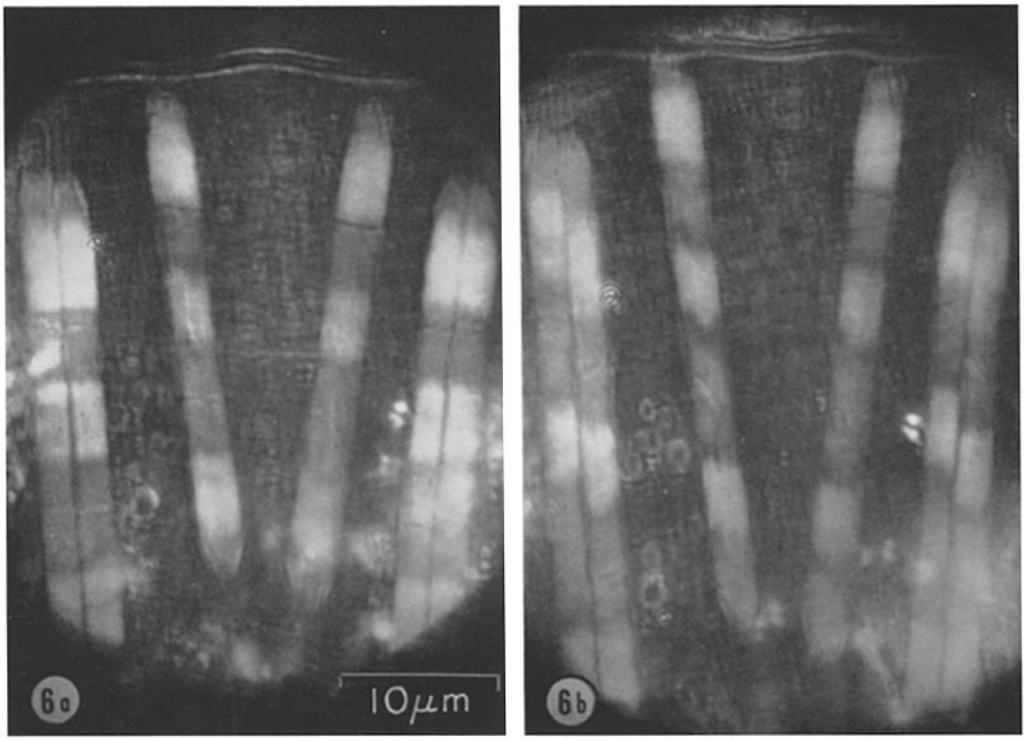

FIGURE 6 Dorsal metapodosomal muscles in a mite which had been anesthetized with carbon dioxide. This individual was exceptional in that the sarcomeres within a given fiber were not equivalent. *a* When first observed *b* After compression to stretch the fibers farther. Polarized light. × 2,100.

muscles were fully shortened, the central sarcomeres were long, and the posterior sarcomeres were half short and half long. The muscles shown in Fig. 6 *a* could be stretched farther (Fig. 6 *b*) and it was possible to pull the I filaments from the A region of shortened sarcomeres, but again in a non-equilibrium way. On the basis of the equivalence of sarcomeres in actively contracting muscles and in muscles of anesthetized animals, one anticipates some kind of coupling between sarcomeres. Why several different muscles in the same anesthetized individual occasionally behave as if the sarcomeres were not linked, except at very long sarcomere lengths, is not yet clear but is probably less puzzling than the mechanism which usually maintains sarcomere equivalence.

Birefringence Changes

The retardation of the A region of mite muscle clearly increased on shortening (Figs. 1, 2 and 6 *a*). Such a result might be expected on the basis of the interdigitating filament model of the sarcomere if the birefringence of the I region is characteristic of the I filaments. Estimates of the change in total retardation on shortening can be made, based on a comparison of the maximum retardation of the I and H regions with the retardation of presumed A and I filament overlap regions. In both actively contracting and anesthetized (Fig. 6) muscle fibers, the estimates indicate that the total retardation of a sarcomere does not decrease on shortening. It remains constant or increases. More accurate measurements of total retardation, based on summing the retardation over the projected area of the sarcomere, are necessary for a precise comparison. However, any difference observed will be small and correspondingly more difficult to interpret in terms of structural change.

Function-dependent changes in the birefringence of the I region were not noted visually, and densitometric measurements of the I region during two sequences of shortening in the movie referred to earlier also gave no indication of such changes.

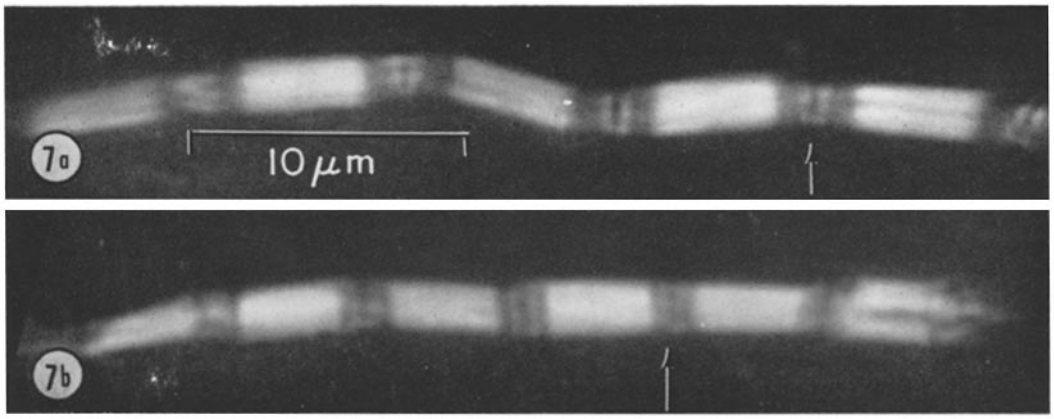

FIGURE 7 Glycerinated *Limulus* fibril bundle (*a*) before and (*b*) after shortening induced by adenosine triphosphate. Arrows indicate the same z line in (*a*) and in (*b*). Polarized light. × 3,700.

DISCUSSION

The observations presented have shown (*a*) that there was a little change in the length of the A region which could be considered characteristic either of the sarcomere length, or of the direction of movement, (*b*) that the H zone increased in length with increasing sarcomere length, and (*c*) that the retardation of the A region increased as the sarcomere shortened. All of these are in gross agreement with expectations of the interdigitating filament model. The measurements clearly show significant deviations from these expectations. That they represent second order effects on muscle structure or error in determining the length of the A and of the I filaments from the length of the A region and of the H gap was not established.

A. F. Huxley (8) has recently discussed many of the older cytological observations dealing with contraction of arthropod muscle and concluded that they generally support the interdigitating filament model. One observation on glycerinated arthropod muscle does not appear to fit and is not easily dismissed. De Villafranca (9) measured A band length as a function of sarcomere length in a population of fibrils from glycerinated *Limulus* muscle and suggested that the A band shortened during contraction. More importantly, he was able to demonstrate shortening of the A region in response to ATP (10). These observations were made by phase contrast microscopy. Using polarized light microscopy it was possible to obtain similar results, and Fig. 7 shows an example of ATP-induced shortening of the A region at a time when a good I gap was still present.

Surviving *Limulus* fibers do not appear to behave in this way. Speidel (11) observed waves of contraction in isolated *Limulus* muscle fibers by polarized light and noted that most of the shortening occurred in the I region. Using a similar preparation from the caudal spine extensor of *Limulus*, it was possible to observe sarcomere shortening almost as clearly as in mite muscle. There was no visual evidence of shortening of the A region in fibers which contracted reversibly and apparently completely. Since sarcomere shortening in living mite and in surviving *Limulus* muscle does not involve appreciable shortening of the A region, the results obtained with glycerinated *Limulus* muscle would appear to be most clearly explained on the basis of variation in the size of sarcomeres (12) and of undefined effects of the glycerination procedure (13) or of ATP-induced shortening.

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