OBSERVATIONS ON THE VARIATIONS IN SIZE OF THE A REGION OF ARTHROPOD MUSCLE

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ABSTRACT

The muscles of three different arthropods, a mite, a fly, and an ostracod, show variations in the length of the A region within a given individual. There is no indication that the observed differences in A band length are related to the functional state of the muscle since little, if any, decrease in the length of the A bands was noted when sarcomeres shortened. The length of the A region was determined by polarized light microscopy and in the case of the mite and the ostracod this measurement was made on intact muscles. It is concluded that the size of the A filaments in an individual can vary in a manner unrelated to immediate functional changes. The I filaments may vary in size, but this could not be clearly demonstrated.

INTRODUCTION

The length of the striated muscle sarcomere reflects both the functional state of the muscle and the underlying structure. Where it is possible to determine the dimensions of this underlying structure, independent of functional changes, one can speak of its *size*. The sarcomere as seen with the light microscope has only one significant dimension, that of length along the muscle axis. This length is determined by how far the muscle is stretched or contracted and by the number, arrangement, and size of the molecules which make up the basic sarcomere structure. This structure is mainly filamentous and these filaments, due to their sharp register within a fibril, determine part of the sarcomere pattern. Because of this, it is possible to measure their length. When changes in the lengths of filaments due to stretching or contraction are small or can be adequately evaluated, then length becomes a measure of their *size*.

Little attention has been paid to the size of sarcomeres, although it is well known that differences exist between muscles in different species. Variations in the size of vertebrate muscle sarcomeres have not been noted, and it is among invertebrate muscles, particularly those of the arthropods, that the most striking variations are seen. Arthropod muscle also may show differences in the size of sarcomeres between different muscles in the same individual (1, page 505, 2) as well as differences among fibrils in the same muscle (3).

Although the size of the A region seems constant in striated muscle from frog (4, 5) and rabbit (6) and probably from fish (7), birds (8), and rodents (9), this constancy has not been determined for individual sarcomeres. One would like to know whether or not the length of the A filament is sharply distributed around a mean of about 1.5 μ. Probably the most direct approach to demonstrating that there is no variation among individual sarcomeres has been commented on by Huxley (6, page 642), who noted that the number of

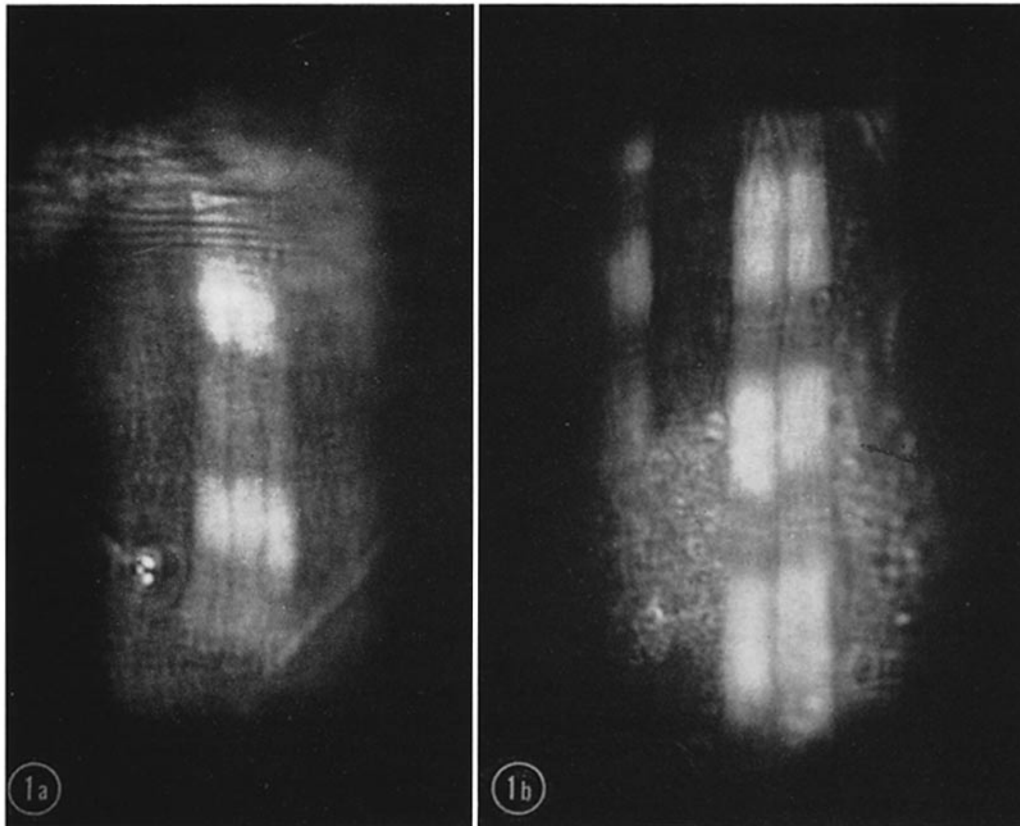

FIGURE 1 Two muscles seen in a live mite which had been anesthetized with carbon dioxide. The muscles are in the same individual. Note that the A regions in the muscle shown in Fig. 1 *b* are considerably longer than those shown in the muscle in Fig. 1 *a*, despite the longer I region of the muscle in Fig. 1 *a*. Fig. 1 *a* shows a two-sarcomere muscle of the propodosoma which inserts on the posterior edge of the coxa of the second leg, and Fig. 1 *b* demonstrates a three-sarcomere muscle of the dorsal metapodosoma. Polarized light with slight positive compensation. About × 2900.

400-A periods in the A filaments of rabbit muscle seems constant (at about 38) for each A region.

The concept that the length of the A region is invariant (4, 6) or changes only slightly (5) with shortening in the physiological range is commonly accepted for vertebrate muscle, but there is little direct evidence supporting this view for arthropod muscle (1, 10). Published observations on a variety of arthropod muscles indicate that the length of the A region decreases as the sarcomere shortens (11–13), in some cases very markedly.

This paper is concerned with demonstrating that A bands, and presumably sarcomeres, of different sizes can occur in different muscles of a single individual. The problems raised by this are pertinent to the mechanisms by which sarcomeres develop.

METHODS

The observations by polarized light were made with the equipment described previously (14). For most of the work the objective and condenser (American Optical Company) were 97 ×, NA 1.25 strain-free achromats which had been rectified (15).

Preparations of fibrils from the indirect flight muscle of 10-day-old *Drosophila melanogaster* were made by teasing pieces of muscle in a solution containing 0.1 M KCl, 2 mM EDTA,[1] 4 mM $MgCl_2$, 5 mM KH_2PO_4, and 2.5 to 5 mM ATP.[2] The final pH

[1] EDTA, ethylenediaminetetraacetate.

[2] ATP, adenosine triphosphate.

was adjusted to 7.0 with KOH. The fibrils were washed by passing this same solution under the coverslip to remove free sarcosomes and during the course of the observations to keep the fibrils from contracting.

The mites were from a strain which had been kept in culture since an earlier study (14), and the ostracods were collected from a roadside ditch in the vicinity of Union Village, Vermont.

TARSONEMUS SP.

Muscles in this mite have been studied previously and a change in A band length with development described (14). For the observations which follow, active larval mites were mounted in mineral oil with flattening and were observed by polarized light. All muscles at this stage function actively and neither the muscles nor the animals were damaged by flattening. In most instances, animals were anesthetized with carbon dioxide so that muscles could be stretched farther without damage and so that measurements could be made more easily.

Anatomically different muscles in the same individual can have A bands of varying lengths, as shown in Figs. 1 *a* and *b*. In readily visible muscles the shortest A band observed was about 3.5 μ and the longest about 5 μ, with the most extreme differences among the muscles of an individual being found in late larval stages. Muscles which have long A bands in one individual are always found to have long A bands in other individuals, although the absolute lengths may vary among animals. The dorsal metapodosomal muscles each have three sarcomeres. Frequently, the length of the A region of the anterior sarcomere appears 10 per cent longer than that of the central

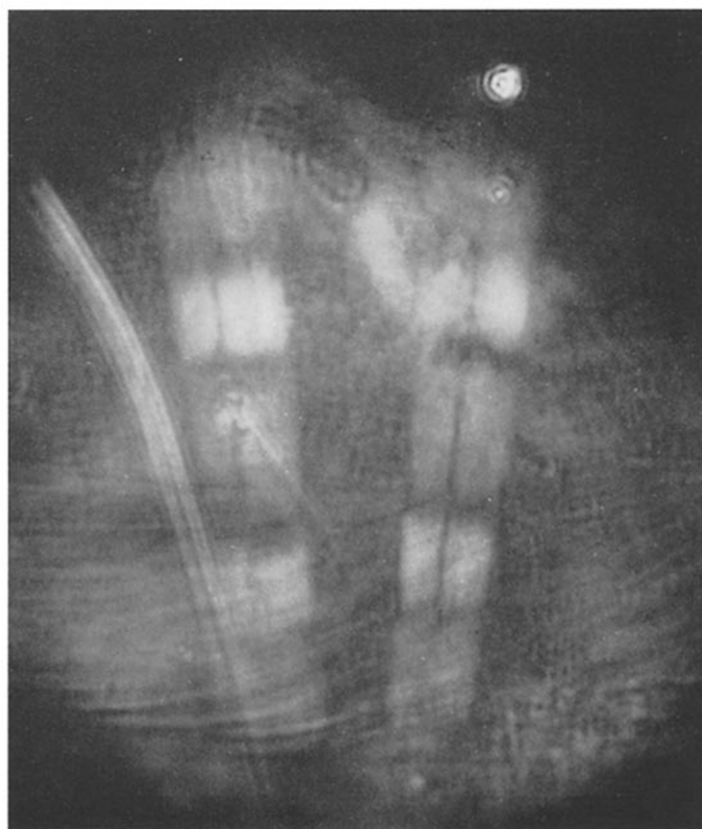

FIGURE 2 These two-sarcomere mite muscles have been stretched by strongly flattening an animal which had been anesthetized with carbon dioxide. The animal was undamaged. Note the almost complete absence of birefringence at the edge of the A band. The conditions are extreme, but such a muscle can recover and function normally when released. Polarized light with slight positive compensation. About × 2900.

sarcomere, suggesting that all of the A regions in a fibril need not have the same length. Since the AI junctions of the anterior sarcomere are slightly jagged due to the insertion, the significance of this observation is questionable.

When a muscle is highly stretched, one can see a birefringent region which centers on the Z line and which is separated from the A band by an isotropic gap, as in Fig. 2. From the available measurements, which are not very satisfactory, it appears that the length of this region is less variable than that of the corresponding A band but probably is not constant. Muscles which have been strongly stretched in this way can recover and function normally. The birefringence of the I band is seen during the early development of the muscle, well before it shortens (14), and is not associated with the degree of stretching.

TABLE I

Maximum and Minimum Lengths of Ten-Sarcomere Segments

Maximum	Minimum
μ	μ
40.5	34.2
39.8	34.0
39.6	32.1
41.1	31.3
36.9	30.8
38.0	33.8

These values were obtained by the measurement of ten-sarcomere segments in each of ten randomly selected fibrils from the thoracic muscles of individual *Drosophila*. Values of the extremes are given for only six of the thirty individuals measured.

The birefringent part of the I region of stretched mite muscle appears similar to the I filament region of stretched vertebrate muscle (4, 5) but it cannot be assumed that the birefringence is necessarily an intrinsic property of the I filaments and diagnostic of their presence. Positive intrinsic birefringence in the I region of arthropod muscle has been seen previously (16), but in dipteran indirect flight muscle, the one type of arthropod muscle in which I filaments have been clearly demonstrated (17), no intrinsic birefringence is apparent (Fig. 3). The I region of glycerinated vertebrate muscle is at best very weakly birefringent.

The changes in A band length which occur when the sarcomere contracts and relaxes have been studied in detail by polarized light in the living animal and will be presented separately. From this study it is clear that the A region, defined by its strong positive birefringence, shows little or no change in length over a range of sarcomere lengths where an appreciable I gap is present. If there is any change, it is a decrease in A band length with decreasing sarcomere length. The two muscles shown in Fig. 1, both of which are in the same individual, demonstrate that A regions can vary independently of the degree of stretching. The muscle with the shorter A bands (Fig. 1 *a*) has the longer I bands and is, therefore, more highly stretched, but despite being stretched more its A bands are still shorter than those found in the muscle in Fig. 1 *b*.

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Fibrils from the indirect flight muscle of *Drosophila* can be isolated in a state in which all the fibrils have a similar striation pattern, in which the I gap is about 10 per cent of the sarcomere length, and in which no sign of contraction bands can be seen (18). These fibrils are potentially able to shorten, but this does not occur in the adenosine triphosphate–containing solution in which they are isolated. Since the indirect flight muscle normally shortens by only a few per cent, and *in vitro* contraction by 5 per cent involves the formation of cytologically visible alterations (3, 18, 19), it is likely that changes in sarcomere length greater than 5 per cent would be detected. This is true only if the *in vitro* changes are similar to those which occur *in vivo*. Most observations and measurements were made with phase contrast microscopy. The phase contrast image does not give an accurate view of the I region, but is sensitive to changes which occur on shortening.

Ten-sarcomere periods were measured in ten fibrils in different fields in thirty preparations from well aged flies. All of the sarcomeres measured showed good I gaps and no sign of contraction bands. Table I illustrates the variation observed. The within animal distribution of ten-sarcomere segments has a mean length of about 35.5 μ with a standard deviation of 2.2 μ. Fibrils teased from single fibers show considerably less variation.

That these variations in sarcomere length are not due to changes in the length of the I region can be appreciated if we consider the A region, which is more than 90 per cent of the relaxed

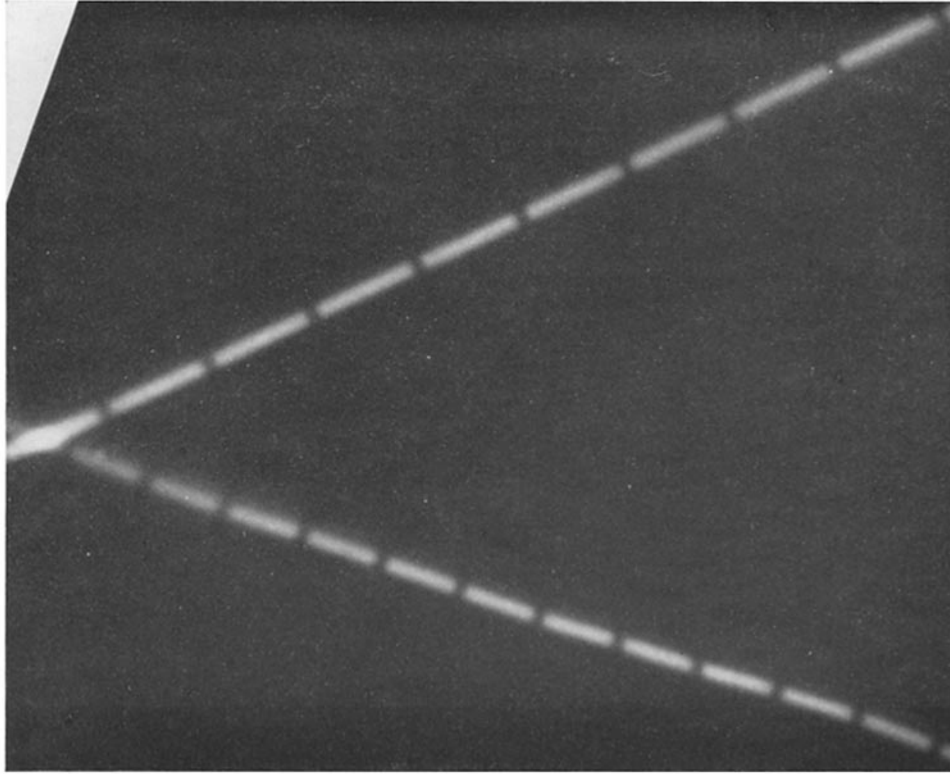

FIGURE 3 These two fibrils were teased from the indirect flight muscle of a single *Drosophila*, as described in the text, and then dehydrated in acetone and immersed in nitro-benzene. Despite the difference in sarcomere length between the two fibrils, the I regions have about the same lengths. Polarized light with slight positive compensation. About × 3500.

sarcomere length, to have a constant length in all fibrils of an individual. The relative I band length will then be very sensitive to small changes in sarcomere length. Fig. 3 shows two fibrils from a single animal photographed by polarized light after dehydration and immersion in nitro-benzene. These fibrils show clearly that the I band length in short and in long sarcomeres is about the same and that, therefore, most of the change in sarcomere length is associated with a change in A band length. For this reason, data such as those in Table I are considered to show differences in A band length as well as in sarcomere length.

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The most extreme differences in A band lengths were noted in this ostracod. Muscles in the legs and antennae of animals mounted in mineral oil after their valves had been removed were easily seen and specific muscle fibers in each individual easily identified. The bodies were badly distorted and torn when the valves were taken off, but the extremities retained a good appearance.

The changes occuring during contraction have not been well defined. In a few animals spontaneous contraction and relaxation was observed soon after a preparation was made. These visual observations showed clearly that most of the shortening in a sarcomere occurs in the I region and relatively little if any in the A region. It was estimated that a change in A band length of 25 per cent would have been detected. Some of the muscles which spontaneously contracted eventually formed fixed contraction nodes, whereas others relaxed, but the contractile cycle was similar in both.

The most striking difference in A band lengths was found in the second antenna where the A regions in two adjacent muscles differ in length by a factor of about 2.5. This difference was visible

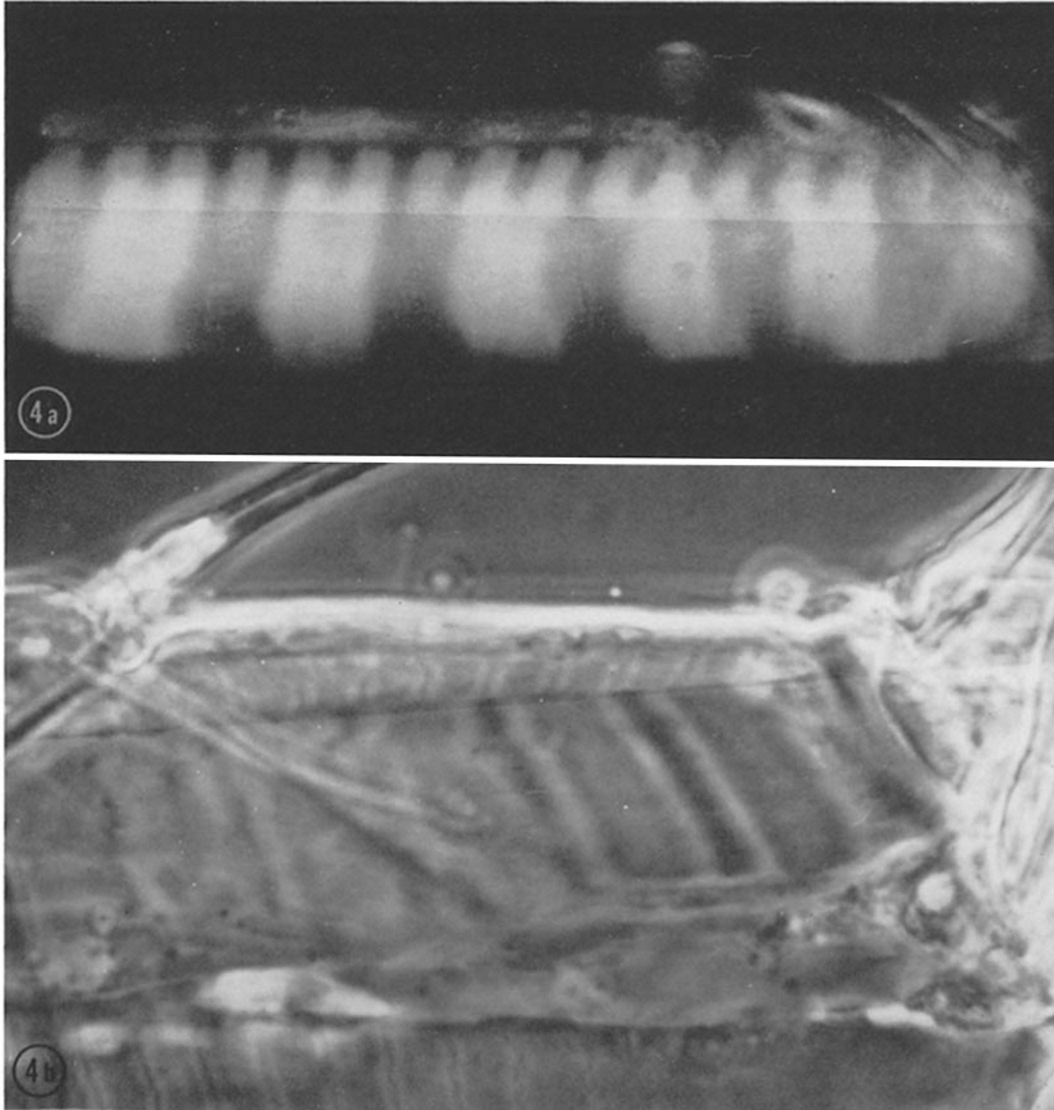

FIGURE 4 Muscles having two very different sarcomere sizes can be seen in the second antennae of this ostracod (Fig. 4 *a*). Neither muscle is sharply focused, but the difference in A band lengths is clear. Polarized light with positive compensation. About × 2800.
4 *b*, The two uppermost muscles are the same ones shown in Fig. 4 *a* but in a different individual. Note the general similarity in appearance and proportion of sarcomeres in all three muscles. Phase contrast, about × 2300. In both Figs. 4 *a* and *b* it was necessary to print the muscle with small sarcomeres separately.

by phase contrast microscopy as well as by polarized light microscopy, as shown in Figs. 4 *a* and *b*.

In Fig. 4 *b* it is possible to see an "H" gap in the muscle with large sarcomeres and in one of the muscles with short sarcomeres. It has been proposed that the edge of the H gap represents the ends of the I filaments (10, 20), and for vertebrate striated muscle there is good evidence supporting this view (4, 5). If this concept is valid for ostracod muscle and the H gap observed is comparable, then there is a twofold difference in the lengths of the I filaments between the two muscles. The

I filament/A band ratio of a large sarcomere in Fig. 4 *b* is about 1.5, and for a small sarcomere about 1.4. The ratio for frog muscle is near 1.2 (4, 5) and for the mite muscle shown in Fig. 2 it is 1.9. The I filament length of frog muscle seems independent of sarcomere length (4, 5), but this need not be true for arthropod muscle.

Muscle fibers having a region of mismatch because some fibrils have an extra sarcomere were common. These regions which are known as verniers can show variations in A band lengths both within a fiber and within a fibril. Short A regions such as those shown in Fig. 5 are found in fibers in which fibrils with the extra sarcomere are in a minority. The lengths of the A regions in fibrils with the extra sarcomere are not all equal, and as one moves away from the center of the vernier the A bands get longer until at a distance of about six sarcomeres the A regions appear equivalent to those in adjacent fibrils. In one instance a fiber, with a vernier in which fibrils with the extra sarcomere were in a minority, as in Fig. 5, spontaneously shortened and relaxed, and it could be seen that all the sarcomeres were functional.

A fibril with an extra sarcomere may shorten farther relative to sarcomeres in adjacent fibrils before developing much tension, and it is suggested that this additional shortening leads to a decrease in the length of the A band. Evidence related to this view can be adduced from observations showing shortening of the A region where the sarcomere length is less than the usual A band length, as in localized contraction nodes (21–23).

Heidenhain (24) has suggested that verniers arise by the intercalation of new sarcomeres into a fibril. This ostracod may be favorable material to demonstrate the validity of this view and possibly to discover how it occurs.

DISCUSSION

These observations have been presented to demonstrate that the A region of the striated muscle sarcomere can differ in size within individuals in at least three classes of arthropods and in a manner which is not related to the immediate functioning of the muscle.

The A band, defined as the strongly anisotropic band, has been investigated by electron microscopy in recent years and shown to contain a characteristic set of filaments (6). In fixed vertebrate muscle these are 110 A thick, have a periodicity of about 400 A, and are 1.5 micra long with tapering ends. These filaments are removed by salt solutions which selectively extract myosin, and their loss is associated with a loss in the birefringence of the A region (20).

Comparable evidence about the structure of the A region in the arthropod muscles studied here is not available, but in the arthropod muscles which have been studied by electron microscopy one can see thick filaments which are restricted to the A region (17, 25–27). The length, width, and periodicity of these filaments may differ in respect to vertebrate muscle (27).

Actomyosins having properties similar to those of vertebrate actomyosin have been extracted from a variety of arthropod muscles (28). Since

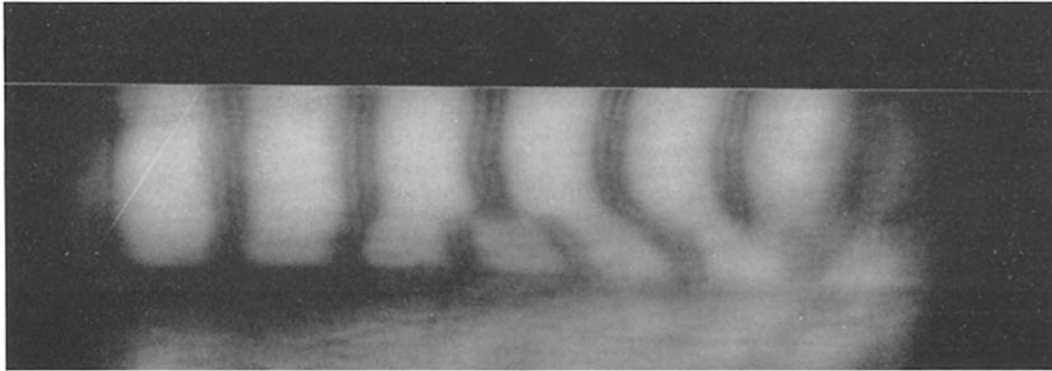

FIGURE 5 This vernier in an ostracod muscle is thought to be within a single fiber because of the continuity of Z membranes. The difference in A band lengths in different fibrils of a single fiber is most obvious at the surface between the out-of-register fibrils. Polarized light with positive compensation. About ×2800.

actomyosin is extracted at high ionic strengths, the proteins which compose the A filaments of arthropod muscle are probably held together by forces similar to those of vertebrate muscle.

For the three animals studied we can suggest that the strongly birefringent A region contains A filaments and that these filaments contain myosin with or without other proteins held together in a non-covalently linked structure. It is unlikely that the length of the A filaments in these animals is determined by the physical dimensions of a single molecule, a possibility which has been suggested for vertebrate muscle (29), but rather the length must be regulated by factors which affect the protein aggregate which makes up the A filament. If the length of an A filament is controlled internally by some self-terminating property which, in turn, reflects a molecular property, then we can suggest that in the arthropod muscles described this mechanism is either not present or is inactive, does not function sharply, or is modified by variations in environment found in different cells. By contrast, the A filament length of the vertebrate sarcomere appears to be sharply determined not only within an individual but among a variety of species. If it were certain that the number of molecules of myosin in the A filament, or, at least, the number of molecules in the length-determining structure, was constant, then a self-terminating mechanism would be an important property to incorporate into molecular models for the A filament.

Since a self-terminating concept either does not apply as rigidly to arthropod muscles as to vertebrate muscle or is not active in the size range studied, it is possible that the length of the A region in arthropod muscle can be determined by factors external to the A filament. The size of a sarcomere in the mite studied here reflects the number of sarcomeres present in a given muscle, which is, with one exception, fixed, and the distance between the insertions. From observation of a given sarcomere in both small and large animals, it is difficult to envisage that differences in A band length are due to genetically determined fluctuations in environmental conditions but rather that there is some mechanism for sensing the necessary size.

I filaments are another element in the structure of the vertebrate sarcomere which have been demonstrated by electron microscopy. In vertebrate muscle these center on the Z line, are about 1.8 μ long, and are connected to I filaments in adjacent half sarcomeres (6). They have been demonstrated most clearly in dipteran indirect flight muscle (17), but there appears no particular reason to doubt their presence in other arthropod muscle.

Both mite muscle and ostracod muscle showed some indication of an element comparable to the I filaments and this element was longer in sarcomeres with long A bands than in those with short. Since the A filaments and the I filaments are the main structural components of the sarcomere as it is presently envisaged, it seems possible to conclude that striated muscle sarcomeres of different sizes can exist within a given animal.

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