SARCOMERE SIZE IN DEVELOPING MUSCLES OF A TARSONEMID MITE

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ABSTRACT

The embryo of a tarsonemid mite was found to be suitable for *in vivo* observations of muscle development by polarization microscopy. The four dorsal muscles of the metapodosoma each contain three sarcomeres, the anterior two of which can be seen clearly. These sarcomeres can be identified and followed during much of their development. Sarcomeres are about 2.5 micra long when first detected and increase in length until they are about 10 micra long. The change in length is associated with a slow, approximately constant rate of increase in the length of the A region, and an initially slow then much more rapid increase in the length of the I band. Preceding the period when the I band elongates rapidly there is an increase in the diameter of the muscle fibers and an increase in the retardation of the A band. A, I, Z, and H bands are visible during most of these changes. The change in A band length has been interpreted in terms of the growth of the A filaments which have been observed by electron microscopy in muscles of other animals. It is suggested that the exceptionally long sarcomeres in this mite result from the early fixing of the number of sarcomeres in a given muscle fiber.

The development of the striated muscle sarcomere is poorly understood despite many investigations of muscle differentiation. With light microscopy a uniform filamentous structure has been seen before striations are observable (1–4), but investigations by electron microscopy (5, 6) indicate that the sarcomere period may be present before A bands are detectable. Details of A, I, and H band formation have not been described.

Polarization microscopy is especially suitable for the study of some aspects of muscle structure *in vivo*. Muscles thin enough for good resolution by polarization microscopy, using a rectified (7) lens system, were observed in a tarsonemid mite. During a period of about 36 hours preceding the first contractions, changes in sarcomere length and in A and I band length could be followed in the living animal.

METHODS

Mites collected and identified by Heinemann[1] as *Tarsonemus randsi* were kept in culture (8) on *Fusarium oxyporum* at 25°C. Embryos selected when muscles are first apparent may be flattened, and most will develop and hatch when mounted in mineral oil which has been saturated with water.

Observations were made with a × 97, N.A. 1.25, strain-free immersion objective,[2] using a model P-42 American Optical Company polarizing microscope.[3] The substage of this microscope was modified to

[1] I am indebted to Mr. Richard Heinemann for a culture of *Tarsonemus randsi* and for advice on maintaining the culture.

[2] American Optical Company. These lenses were specially selected for their strain-free qualities.

[3] This microscope was considerately loaned by the American Optical Company to Dr. Shinya Inoué.

carry a × 97, N.A. 1.25, "rectified" objective as condenser, and a rotatable mica plate compensator. The extinction factor of the system without the mite was about 5×10^3 at full aperture, and condenser numerical apertures of the order of 0.8 could be routinely used.

Measurements of the length and width of developing mite muscle were made in triplicate with a × 10 filar micrometer using additive compensation. Measurements of contractile muscle were made with an ocular micrometer. A water-cooled high pressure mercury arc lamp (General Electric A-H6) was used as a light source in conjunction with a Wratten 58 filter.

OBSERVATIONS

1. *Description of the Muscles*

The dorsal muscles of the metapodosoma (9) have been studied most intensively because they have the longest sarcomeres and can be clearly seen. In the larvae these consist of six symmetrically arranged muscle fibers which for convenience have been labeled 1 to 6 as in Fig. 1. The two lateral fibers on each side (1, 2; 5, 6) lie close to each other and develop in register. The medial two (3 and 4) differ from these in being broader, having shorter sarcomeres, and being slightly curved. Each of the six muscle fibers has three sarcomeres, of which the anterior two are most clearly observable. The A band of the middle sarcomere in each muscle usually appears to be about 10 per cent shorter than the anterior one.

Muscle fibers 1, 2, 5, and 6 insert at their posterior ends beneath the heavy ridge of cuticle behind the third set of legs. Muscles 3 and 4 insert anterior to this ridge. The anterior insertions of all six fibers are near the cuticular ridge separating the propodosoma from the metapodosoma. Fibers 1, 2, 5, and 6 attach by tonofibrillae which are about 3 micra long, while muscles 3 and 4 insert more directly, the I band appearing to reach the insertion.

2. *Description of the Sarcomeres*

The following description is based on the continued observation of single sarcomeres for periods which in some cases covered the entire range of development described here. Detailed measurements of sarcomere length and of A and I band length were made in five mites. Fig. 2 shows the changes in sarcomere length observed in the animal in Fig. 1. In this animal, as Fig. 3 shows, there was a threefold increase in the length of the A band and a fivefold increase in the length of the I band during the course of the observations. The A band length increases quite regularly, whereas most of the change in I band length occurs in a period of 6 to 8 hours. The temperature at which most observations were made was held close to 25°C, as the rate of sarcomere and of A band elongation is increased at higher and decreased at lower temperatures.

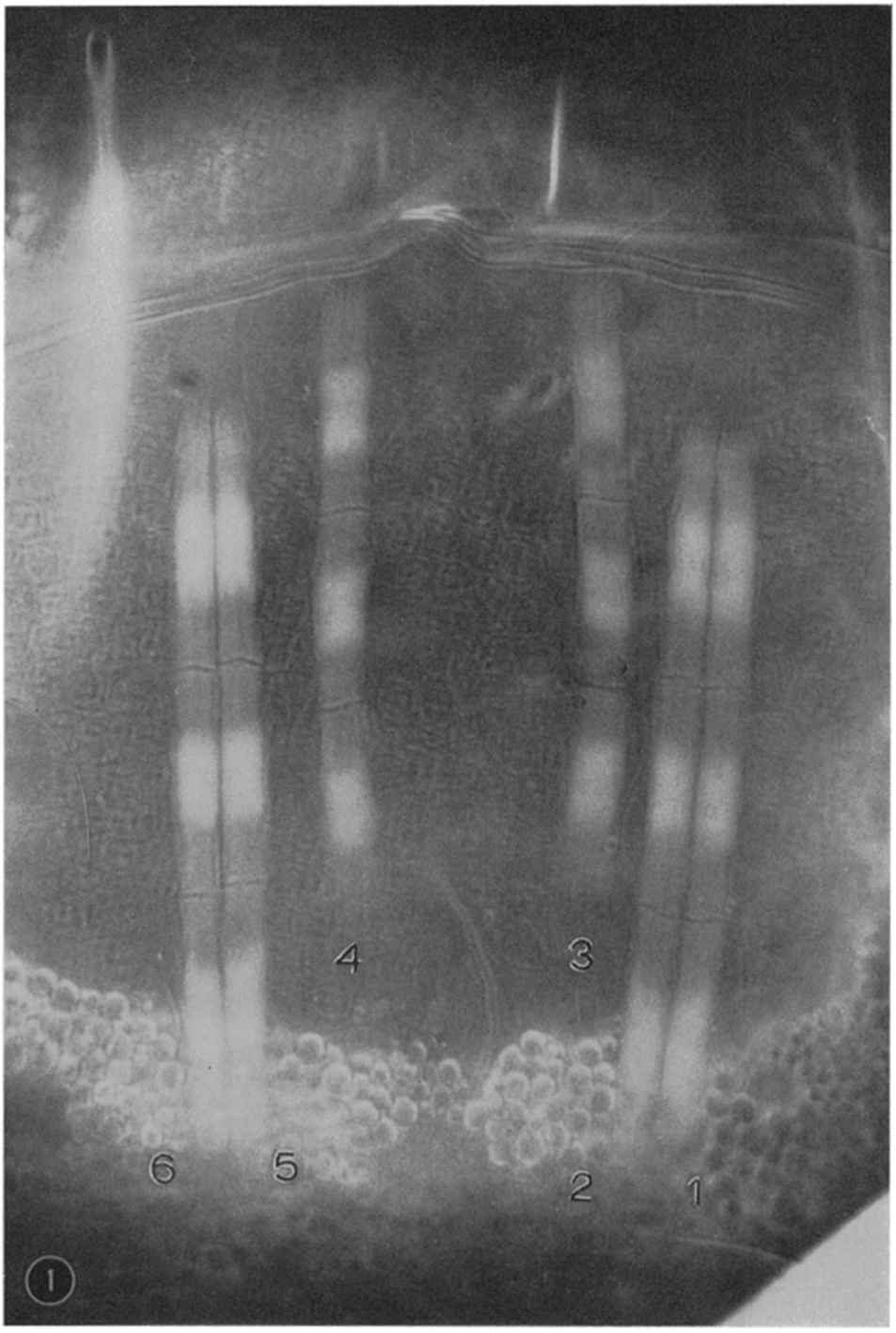
When first seen each dorsal metapodosomal muscle had three distinct sarcomeres which were 2.0 to 2.5 micra long, and the A, I, and Z bands could be resolved. Overlying and adjacent refractile and birefringent materials obscure the muscles, making observation and measurement of birefringence difficult at this time. Fig. 5 *a* is an example of this stage in the animal shown in Fig. 1. It was not possible to make earlier observations on these muscles owing to poor optical conditions.

Over the next 8 hours a slow increase in the length of the A and of the I bands can be detected. During this same period there is an increase in muscle diameter (Fig. 4) and also an increase in the total retardation of the A region. The insensitivity of birefringence measurements due to the adjacent refractile and birefringent material, as well as an inability to obtain a direct measure of fiber thickness, has not permitted me to determine how nearly constant the strength of birefringence remains as the diameter increases.

By the time the sarcomeres are 4 micra long the

FIGURE 1

Photomicrograph of the dorsal metapodosomal muscles (labeled 1 to 6) of a living tarsonemid mite taken with polarized light. Slight additive compensation was used to improve the contrast, and positively birefringent regions appear bright. The muscles are close to their maximum length and will become actively contractile within the next 10 hours. × about 2900.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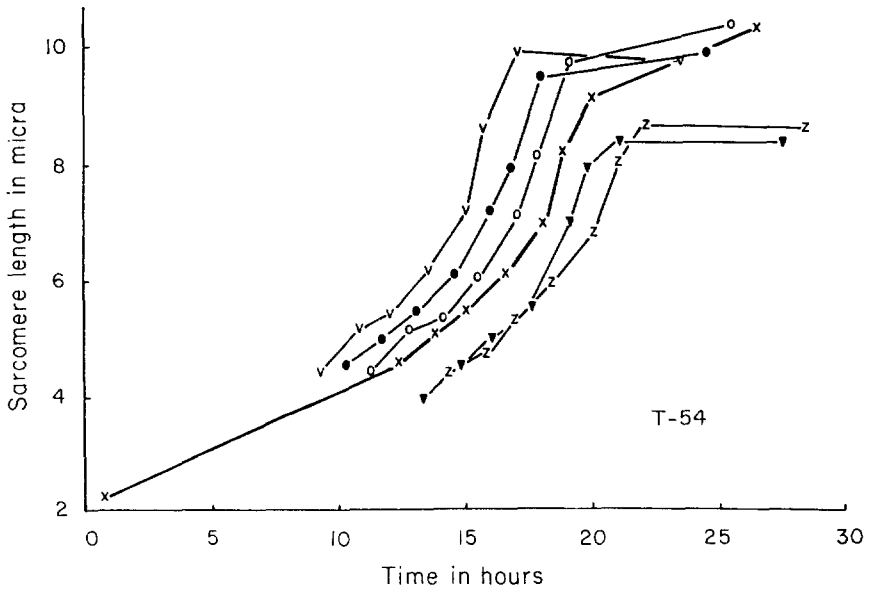


FIGURE 2

The length of the central sarcomere in the six dorsal metapodosomal muscles of a single mite has been plotted against time. For clarity each curve has been shifted by 1 hour from the one to its left. Symbols: -x-, muscle 1; -o-, muscle 2; -▼-, muscle 3; -z-, muscle 4; -●-, muscle 5; -v-, muscle 6. The six sarcomeres measured were in the animal shown in Fig. 1.

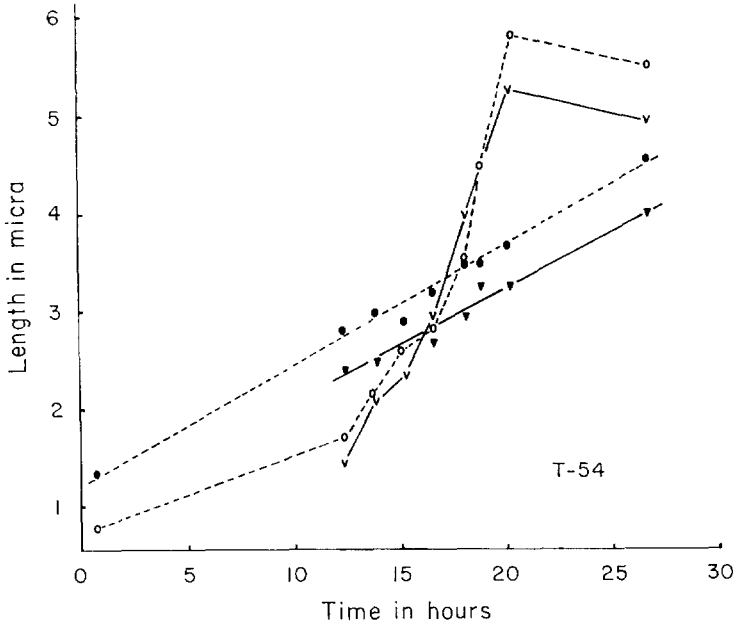


FIGURE 3

The change in length of the A and I bands during development can be seen. The central sarcomeres of muscles 1 and 3 were measured in the animal shown in Fig. 1. Symbols: -o-, I band muscle 1; -v-, I band muscle 3; -●-, A band muscle 1; -▼-, A band muscle 3.

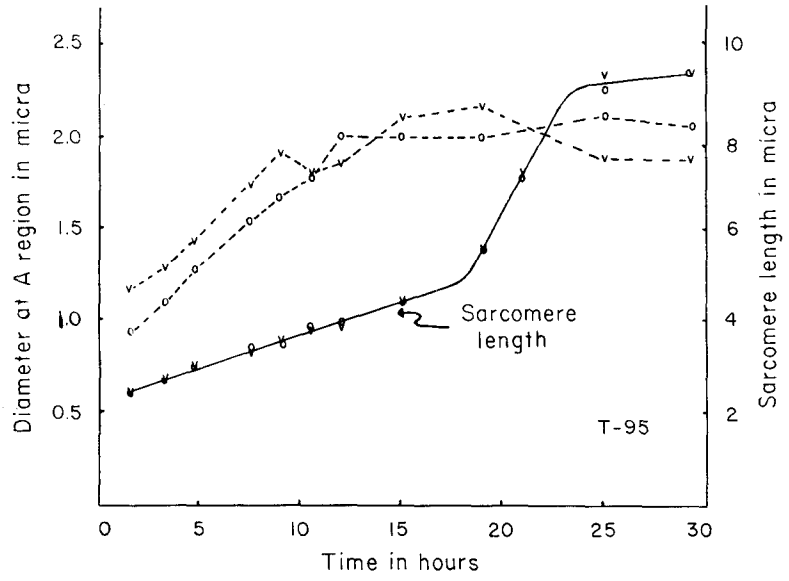


FIGURE 4

The width of the A region in the central sarcomere of muscles 1 and 2 is plotted against time. Sarcomere length is included as a reference. Note that the diameter stops increasing before the sarcomere length begins to increase rapidly. These data are not from the animal shown in Fig. 1.

muscles have begun to migrate out of the refractile material and the image improves greatly. The H regions as well as the A, I, and Z bands are visible as in Fig. 5 *b*. The Z band is seen at this time (additive compensation) as a dark band flanked by two bright bands. The A band has a strong positive birefringence, and the I band exhibits a positive birefringence about one-fourth that of the A band. An H region can be seen in the center of the A band and with positive compensation appears as a less bright region with a bright M band in its center.

For the next 6 to 10 hours the A band continues to lengthen slowly while the I band length increases more rapidly (Fig. 3). There is little if any increase in diameter during this time (Fig. 4). This stage is followed by a period of about 5 hours during which the I band lengthens rapidly until the anterior insertion is formed. The A band continues to lengthen slowly during this time, but the sharpness of the A-I junction decreases considerably, making the measurements less reliable. The decrease in register at the A-I junction is correlated with an increase in the visibility of the individual longitudinal fibrillar elements of which each fiber is composed.

After the muscle insertion has formed, the A band continues to lengthen at the expense of the I band. The sarcomere length may decrease during this time also.

Contracting muscles are seen within the next 24 hours and lead to hatching from the egg chorion. After hatching, the larvae walk away, making it necessary to flatten the animals strongly before making further observations. From the data presented in Fig. 6 it can be seen that the A band stops lengthening at *about* the time that contractility is first seen, and there is no indication that a further increase in length occurs after hatching.

Changes in the H region can be followed in particularly favorable sarcomeres. The H region is first seen definitely when the muscles emerge from the obscuring material, at which time the H region is about 0.6 micron long (Fig. 5 *b*) By the time the sarcomeres have obtained their maximum length the H region is about 1.5 microns long. This increase may be seen by comparing Figs. 5 *b* and 5 *c* with Figs. 5 *d* and 5 *e*. It is certain that the H region does not increase in length as much as the I region during the period of rapid increase in I band length.

DISCUSSION

Observations by polarized light on the abdominal muscles of a trombidiform mite fixed with osmium tetroxide have shown most of the details of muscle structure described here (10). Sarcomeres with lengths up to 10 microns, a positively birefringent A band containing a less strongly birefringent H region, an isotropic I band, and a positively birefringent Z band were noted by Flogel in 1872.

Our observations on the living animal add little to this description of muscle structure despite our having described anisotropy of the I band, the appearance of a more complex Z band than Flogel observed, and the presence of an "M" band. In view of the work of Inoué (11), in which he showed that spurious bands can appear at refractive interfaces, the three components of the Z band and the "M" band may or may not directly reflect birefringent structures.

Muscle development in the mite can be considered from our observations as having five definable aspects: the initial differentiation of a myofibril, the elongation of the birefringent A region, the increase in width of the fibers, the elongation of the I region, and the onset of visible spontaneous contraction.

No change in the number of sarcomeres was observed, and the A, I, Z, and possibly H bands were present when muscles were first seen. Since the sarcomeres and the A, I, and Z bands are the major differentiations of the adult myofibril, most of the observations described here relate to changes in structures which have already been determined.

Elongation of the A Region

The A region was always seen to increase in length in an approximately linear fashion and gave no indication of maintaining a consistent relation either to sarcomere length or to I band length. A possible mechanism for this increase in length, based on the aggregation of protein molecules into filaments, can be suggested from electron microscopical and biochemical observations on vertebrate muscle and muscle proteins.

Parallel filaments which are restricted to the A region have been observed by electron microscopy in rabbit psoas muscle by Huxley (12). These "A band filaments" are about 1.5 micra long and 110 A thick. They taper to a blunt point at each end, suggest further differentiation in the middle near the H region, and show "interfilament" bridges at regular intervals along their length. Similar filaments have been observed by electron microscopy in muscles of other animals (12, 13).

It has been suggested (see 14, 15) that most of the myosin, and by implication the birefringence, is localized in these "A band filaments." This view is based on the differential extraction of myosin by salt solutions. These solutions remove most of the material from the A band of glycerinated rabbit psoas muscle and greatly reduce its birefringence (14, 16). Further support for the localization of myosin in the A band is presented in a study (1) of the binding of fluorescein-labeled antimyosin to glycerinated chick muscle.

This suggested correlation between A band filaments, myosin, and the birefringence is im-

FIGURE 5

These pictures show the central A band and the I region anterior to it in muscle fibers 5 and 6 of the animal shown in Fig. 1. All pictures are of the same individual. *a* to *e*, × about 3900; *f*, × about 2500.

a. Shows muscle fibers 5 and 6 when first observed. Three sarcomeres can be seen and A, I, and Z bands are identifiable.

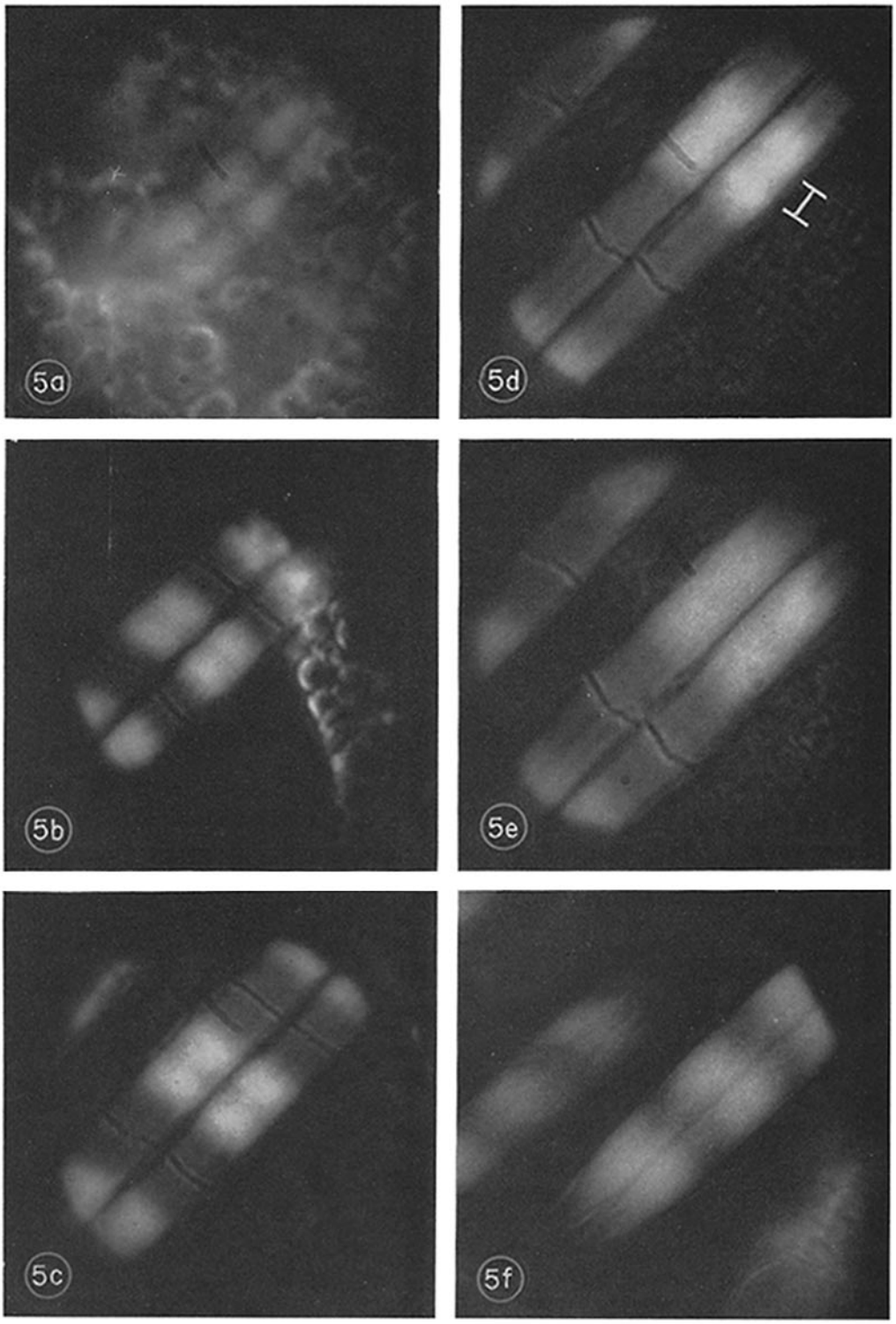
b. 13 hours after *a* both the anterior and center sarcomeres are free of obscuring material. The diameter of the muscles has increased and the A and I bands are longer. An H region is visible in the middle of the A band.

c. 18 hours after *a*.

d. 20 hours after *a*. Note the increased length of the *H* region. The marker indicates the extent of the H region.

e. 27 hours after *a*.

f. 37 hours after *a*. The muscle is contractile and the picture was taken as the muscle began to relax after having contracted. The animal had hatched during the preceding 10 hours and had walked away from the chorion.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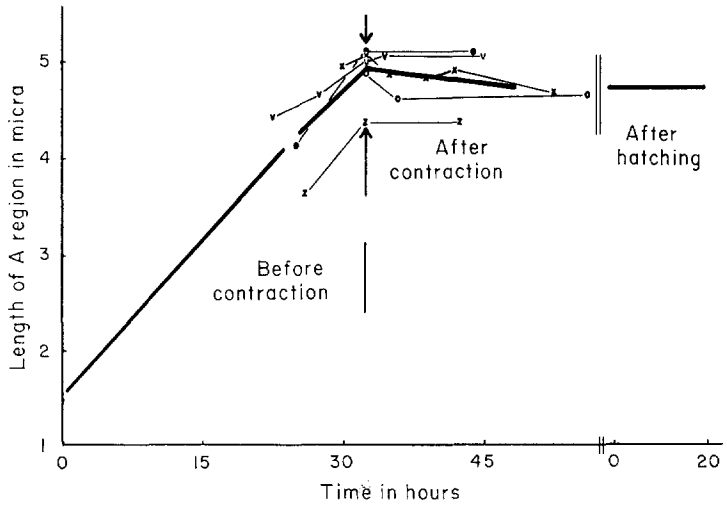

FIGURE 6

The heavy dark lines show the average A band lengths which were observed. For A band lengths of 1.5 microns to about 4 microns the average rate of increase was 0.1 μ/hr at 25°C, measured in four mites. The heavy line indicating this has been included and positioned to give continuity to the chart. The heavy line which includes the time when the muscles were observed to contract was calculated from the points which are plotted in the same region. It shows the average change observed before contraction and the average change after contraction. The after hatching data are from six active larval mites which were measured after mounting and again after 20 hours. Symbols: v, o, ●, x, and z are average values for A band lengths in mites which were measured before and after contraction could be seen; z is the mite shown in Fig. 1.

portant, as some *in vitro* properties of muscle proteins have been studied and may serve as models for A band growth. In particular, filaments several micra long have been formed from actin and from myosin which contained some actin (17). These large elongated aggregates of protein molecules which are produced non-enzymatically by changes in pH or in salt concentration are strongly birefringent.

Experiments using fluorescein-labeled antibodies to myosin and to the meromyosins (18) indicate that the distribution of myosin and the meromyosins is not uniform throughout the A region, and suggest that filament formation may be considerably more complex than pictured above.

Elongation of the I Band

The elongation of the I band is less easily discussed than that of the A region because this band lengthens irregularly, is detected more by position than by amount of birefringence, and changes in length during contraction. A model based on the growth of actin filaments is possible, but the argument seems much weaker than that proposed for myosin and the A band filaments, at least in terms of causing an increase in I band length.

Evidence obtained from vertebrate muscle by differential extraction of actin and myosin (14, 16) and by staining with fluorescent antibodies (18) suggests that the filaments seen by electron microscopy in the I region are composed of actin. Filaments formed from actin *in vitro* have dimensions, when seen by electron microscopy (17, 19), which are close to those found in muscle.

Electron microscopic evidence for animals other than mites shows that the filaments present in the I region ("I filaments") extend well into (12) and possibly through (20) the A region. If, during development, these I filaments were to be withdrawn from the A region, one would expect to see a longer I band. The final length of the I band is greater than the initial length of the sarcomere (Fig. 3), therefore withdrawal of the filaments will not explain the observed changes completely unless the I filaments are also folded. The lack of a large change in H band length

also supports the conclusion that the increase in I band length cannot be explained completely on the basis of the withdrawal of I filaments from the A region.

In developing muscle the increase in I band length may reflect an increase in the amount of material present, but passive stretching, unfolding, and elastic stretching are other possible explanations. A complete explanation based on the elastic stretching of I filaments is unlikely since a muscle in which a fiber has been bent, either in mounting or in later stages by contraction of an adjacent muscle, will frequently retain long I bands. These observations suggest that elongation of the I region might occur by the growth or lengthening of actin filaments. However, it is also believed that this lengthening or growth may result from an impressed strain rather than as an expression of an intrinsic growth process.

By comparison with other muscles in this mite, the sarcomeres and in particular the I regions of muscles 1 to 6 are exceptionally long. Since a large part of this I band lengthening occurs over a period of about five hours, it may be separable from the slower lengthening of the I region which is observed during the earlier stages of muscle development.

A cell process extending from near the myofibrillar material to the anterior insertion can be observed in muscles 1 to 6 soon after the I region begins to elongate rapidly, and may be present even earlier. This suggests that the myofibrils are pulled to the insertion, although, as mentioned previously, there is little indication of elastic stretching. It is felt that, regardless of the mechanism, the rapid lengthening of the I region reflects the means by which these muscles reach their insertions before becoming contractile, and is not a reflection of a growth rate intrinsic to the myofibrils.

Increase in Muscle Width

Changes in muscle width during development could result from changes in filament number, spacing, or diameter, or from some combination of these. From the very limited electron microscopic evidence for chick muscle (5) and for honeybee muscle (21), an increase in both filament number and diameter seems reasonable. Although the diameter of the mite fibers appears to stop increasing during development, a further increase does occur, since fibers in adult animals are considerably thicker.

Increases of Sarcomere Length in Other Animals

An increase in sarcomere length during development has been suggested on the basis of electron micrographs of muscle from embryonic mouse heart (22), muscle from the latissimus dorsi of the thrush (22), and muscle of the metatarsal rudiment of the chick (5). However, it has been pointed out (1, 5) that such studies are uncontrolled for possible shortening during and after fixation.

An electron microscopic study of honeybee flight muscle (21) indicates that sarcomere length remains fairly constant during a period when a large increase in the diameter of the sarcostyles (myofibrils) occurs. The sarcomere length and the A band length of sarcostyles isolated from the indirect flight muscle of *Drosophila* increase by about 20 per cent during a similar period of development (23).

Change in A Band Length and Description of Development in Other Muscles

A recent study (1), utilizing immunological techniques coupled with fluorescence microscopy to demonstrate the localization of myosin, suggests that little if any change in the length of the A bands of chick somite muscle occurs once striations are apparent. At an early stage of development this muscle has striated fibrils in which the fluorescent A band is about as long as the A band in mature muscles. At even earlier stages the fibrils appear unstriated and uniformly fluorescent.

Descriptions of developing vertebrate (4, 24, 25) and arthropod (3) muscle studied by polarization microscopy show that a uniformly birefringent fiber is present initially and that this later appears striated. Speidel (2), who followed the development and regeneration of frog muscle *in vivo* by ordinary light and by polarized light microscopy, concluded that the appearance of the anisotropic band was correlated with the appearance of histologically demonstrable A bands. His conclusions differ from those of Schmidt (25), who observed fixed and sectioned larval frog muscle which appeared to have uniformly birefringent regions preceding the appearance of striations. Since uniform weakly birefringent regions of early

muscle fibers should be difficult to detect *in vivo*, Speidel's observations do not necessarily disagree with those of Schmidt on this point.

Although these observations suggest that there are differences in the development of sarcomeres in mites and in other animals, these differences may be related to differences in the length of the sarcomeres or to poor register of adjacent fibrils, as well as to technically limited observations. The apparent differences might also be explained by considering that it is the length of the A band or of the sarcomere which is comparable. In this case, mite sarcomeres which are 2.0 to 2.5 microns long when first seen would be comparable to adult vertebrate sarcomeres. If this were so, vertebrate muscle would not have a process corresponding to the later increase in sarcomere length of the mite muscle.

Electron microscopic observations have demonstrated the presence of "interdigitating filaments" in muscle from frog (12), rabbit (12), and blowfly (13); and biochemical studies of muscle proteins from crayfish and honeybee (26) show them to be similar to proteins from vertebrate muscles. These observations give little reason to expect basic differences between vertebrate and arthropod muscle. The most obvious difference between the muscle followed in this study and those of vertebrates is in the length of the sarcomeres. Possibly this results from the early fixing of the number of sarcomeres followed by the continued formation of muscle material.

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