

THE ULTRASTRUCTURE OF STRIATED MUSCLE AT VARIOUS SARCOMERE LENGTHS*· †

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PLATES 53 AND 54

It is generally believed that muscular contractility involves a reversible interaction of the two proteins, actin and myosin. The manner in which this interaction produces contraction or relaxation is not clear, but the fact that it is extremely difficult to demonstrate a change in the wide angle x-ray diffraction pattern when muscle shortens (2) suggests that contractility is associated with intermolecular events rather than intramolecular structural changes. Interactions on the macromolecular level may be observed with the electron microscope and a considerable literature concerning muscle fine structure already exists. The results of previous studies have led to two major concepts concerning the filamentous organization of striated muscle, which are in certain respects contradictory. According to one view (5, 6, 10, 21), only a single set of filaments extends continuously through all levels of the sarcomeres. On the other hand Huxley (13) and Hanson and Huxley (7) believe that two sets of filaments exist, a thick (myosin) set and a thinner (actin) set, and that these interdigitate in the A band.

It seems fairly apparent that in most of the previous work the muscle was not maintained at either rest or equilibrium length (26) during fixation (which in itself may cause shortening). Consequently few high resolution pictures of unshortened muscle have thus far been published. The work described here, of which a briefer report has been submitted elsewhere (24), was made possible by taking great care to hold the muscle fibers at known lengths throughout the preparative procedures. The results thus obtained differ importantly from those of other workers and reveal a clear cut structural change at the macromolecular level when muscle shortens. On the basis of these observations a mechanism for contractility based on the interaction of actin and myosin macromolecules is suggested.

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Materials and Methods

Narrow strips of fresh rabbit psoas muscle were fastened to specially modified calipers by means of Pearl cotton No. 5 thread. The muscle fibers, whose lengths could be carefully controlled by adjusting the calipers, were fixed at rest length, at equilibrium length, and in several stages of shortening induced by freezing and thawing. The fixative used was buffered isotonic osmium tetroxide solution (18). However, the muscle was frequently fixed first in buffered 10 per cent formalin and then stained with the osmium solution; this treatment tended to yield better preservation of I band structure. Rabbit psoas muscles which had been glycerinated and glycerinated followed by plasticizing (26, 27) were also fixed at rest length. In all cases the prefixation length was maintained through the dehydration process, and any specimens which revealed slippage or tearing when examined under the dissecting microscope were discarded.

Fresh young chick leg muscle, usually sartorius, was fixed at equilibrium length and in some cases was allowed to shorten as a result of fixation.

Dehydration and embedding were conventional. Most of the thin sections were obtained with the Hodge-Huxley-Spiro microtome (11, 12) and were examined in an RCA EMU-2A electron microscope using a 25 to 50 micron objective aperture.

RESULTS

Similar results were obtained using both rabbit psoas and chick leg muscle and no differences were noted between glycerinated muscle as compared with fresh or glycerinated-replasticized muscle.

The sarcomere length in rest length living rabbit psoas as measured with the interference microscope is 2.5 micra, a figure which agrees well with Sandow's (22). Sarcomere lengths in electron micrographs corresponded to within 10 per cent of what they should have been in the living state if the muscles had been sectioned with the knife parallel to the fiber axis. Inasmuch as sectioning in this plane often obscured the filamentous details due to compression, the specimens were generally cut with the knife at some angle to the fiber axis. Despite the angle of cutting, however, the A/I ratio remained constant for a given length and could thus be used as a measure of sarcomere length. However the actual lengths of the sarcomeres were usually verified by cutting some sections parallel to the fiber axis.

The structure of rest and equilibrium length muscle is in general similar except for differences in sarcomere length and A/I ratios (the latter being about 1.4 in rest length and about 2 in equilibrium length muscle). Longitudinal sections of myofibrils fixed at these lengths are characterized by the following features (see Figs. 1-3). The H band, in the center of the A band, is composed of a parallel array of thick filaments (*ca.* 120 A) which are spaced 200 to 300 A apart. In the A band proper (on either side of the H), the filaments are thinner (60 to 70 A) and are approximately twice as numerous (the ratio is actually 1.7) as the thick filaments in the H band. The density of the A band interfilamentous region is greater than in the H band. The multiple (1.7) ratio of thin to thick filaments in the A as opposed to the H bands may be explained by assuming

that several thin filaments aggregate to form one thick H band filament. Convergence of pairs of thin filaments to form thick ones has been frequently observed in favorable sections (Figs. 4 and 6). The I bands present a rather disorganized array of tenuous filaments and a low interfilamentous density. The Z membranes, however, are traversed by the same number and type of filaments as are present in the A band proper. Therefore one may assume continuity of thin filaments from the Z membranes through both I and A bands up to the H band where several of them associate to form the thicker filaments. Oblique sections (Figs. 4 and 5) demonstrate clearly the location of different types of filaments in different portions of the A band and reveal no evidence whatsoever of interdigitation of the two types. Sufficiently transverse sections necessary to disclose the packing of the thin filaments have so far not been obtained.

The filamentous structure of myofibrils which have been allowed or caused to shorten to about 65 per cent of rest length is strikingly different from that described above (Fig. 7). In such myofibrils the entire A band (A band proper as well as H band) contains only thick filaments. At this length, however, thin filaments are still observed penetrating the Z membranes in the center of the much shortened I bands. A further decrease in sarcomere length results in the disappearance of the I bands; thick filaments now traverse the entire sarcomere.

DISCUSSION

One may characterize the filamentous structure of muscle at various lengths as follows: In muscle fibers at rest length and at equilibrium length, thin filaments traverse the sarcomeres up to the H band where several combine to form single thick filaments, a high degree of interfilamentous density being present in the A band proper. Shortening of muscle beyond equilibrium length is accompanied by a progressive transformation of the thin filaments into thick filaments, which in muscle at 60 per cent of rest length (in which the I band has disappeared) are present throughout the lengths of the sarcomeres. It is clear therefore that, on shortening, some mechanism causes thin filaments to aggregate in the form of thick filaments.

As an interpretation of these phenomena the following is offered. There is now convincing evidence to support the view that myosin is localized in the A band and is largely responsible for the optical properties of that region (8, 9). It is reasonable to assume that the fibrous lattice of the myofibrils is due mainly to F actin, which may be identified with the thin filaments. If myosin *in vivo* exists in a form similar to that in solution after extraction, it would consist of a dispersion of macromolecules about 23×2300 Å long (20). We suggest that these macromolecules are located between the thin filaments in the A band and impart the high interfilamentous density to that area.

The observation that, on shortening, thin filaments are transformed into thick filaments suggests the following mechanism for the contractile process.

It is generally accepted that shortening involves an association of actin and myosin, and that this complex formation may be related to ATP splitting. We suggest that the manner in which these two proteins interact is by combination of the long thin myosin macromolecules with the F actin filaments. If these two protein types have sufficiently complementary structures they will form a tightly knit complex which may well assume the configuration of a compound supercoil or rod; this is seen in the conversion of thin filaments into a smaller number of thick filaments. As this process progresses more and more of the actin filaments will associate with myosin in the form of coiled coils until there is a maximal degree of actomyosin formation. The actin filaments are then drawn completely into the A band with consequent disappearance of the I band. Recent observations reveal that muscular contraction involves a shortening of the I band, the A band remaining approximately at constant length (14, 15). On relaxation a fresh supply of ATP becomes available and causes the two proteins to dissociate and thus uncoil.

The mechanism postulated above avoids invoking the action of long range forces which would appear to be necessary for the slippage of the actin relative to the myosin filaments in the Huxley-Hanson model. Nor does it require any drastic changes in the intramolecular configuration of the protein macromolecules themselves. Rather this model proposes a macromolecular complementarity of structure of actin and myosin as the source of spontaneous and rapid, reversible combination of these two molecular types when the chemical environment is suitably changed (splitting of ATP, etc.).

It is possible that the "hollow appearance" of thick filaments noted by Huxley (13) and Hodge (10) as well as others is not due to incomplete penetration of stain alone, but may reflect the compound helical coiling of the actomyosin complex.

Polarized light data are of interest here. The birefringence of the H band in unshortened muscle is lower than that of the A band proper, and Ströbel (25) has shown a decrease in total birefringence in muscle when it contracts. This decrease is thought to be due to a fall in the intrinsic birefringence alone, the form contribution remaining constant. These data suggest that the thick filament regions of the A band have lower intrinsic birefringence than the thin filament regions and offer support for the hypothesis that the thick filaments form by supercoiling as outlined above. It should be remembered, however, that the system is in fact a complex mixed body system and it is difficult without a detailed analysis to assess the contributions of the several components to the intrinsic and form birefringence of the system.

The nature of the thick filaments in the H band itself is not clear. Several workers have indicated ways in which they differ from their counterparts in the A band proper (7, 8, 13, 19).

In contrast, the conclusions reached by Huxley and Hanson (7, 13) may be

explained in part by the fact that muscle considered by them to be at rest length was actually moderately shortened. This is not surprising in view of the fact that even glycerinated muscle will shorten during washing procedures, etc. if not maintained at rest length (4). The structures seen by them in transverse sections to occur at trigonal points between the thick filaments, and which they chose to interpret as representing a second set of filaments, we would suggest are similar to the lateral or connecting bridges cut in a particular plane which have been so elegantly demonstrated by Hodge (10) as well as others (3, 23). Hodge's recent results (10) which disclosed only a single set of thick filaments throughout the entire sarcomere are in our opinion explained by the fact that the muscle shown in this work is all markedly contracted.

In earlier studies by Ashley *et al.* (1) on isolated myofibrils, it was mentioned that filaments in uncontracted muscle appear to be associated in pairs to produce a thicker longitudinal component, and that the filaments increase in diameter on contraction. These observations are consistent with the results obtained by us.

Finally it should be noted that Huxley's small-angle x-ray evidence for two sets of filaments (16, 17) does not require that the two sets occur at the same level in the sarcomere; it could reflect the presence of thick and thin filaments as observed in the present work rather than the interdigitating filaments hypothesized by Huxley and Hanson.

SUMMARY AND CONCLUSIONS

1. Rest and equilibrium length muscle sarcomeres are composed of thin filaments (actin) which traverse the sarcomeres from the Z membranes up to the H band; at this level the filaments are considerably thicker and less numerous.
2. Shortening of muscle is associated with a transformation of thin into thick filaments in the A band.
3. These observations are discussed in terms of interaction of actin and myosin to form a supercoiled structure as the basis of contraction.

Addendum.—A very brief report of some preliminary observations concerning the embryogenesis of chick heart and limb muscle as they bear on the structure of the mature myofibrils was given. The essential facts presented are:

1. Primitive myofibrils, *i.e.* those with or without Z membranes, but which are not differentiated with respect to A, I, or H bands, are composed of thin (60 to 70 A in diameter) filaments only, no thick filaments being present.
2. Evidence was presented for the origin of the Z membranes as ingrowths of the myoblast surface.

Further details will be presented in due course.

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EXPLANATION OF PLATES

PLATE 53

FIG. 1. Longitudinal section of rest length rabbit psoas cut with fiber axis parallel to knife edge. $\times 23,000$.

FIG. 2. Same as Fig. 1, but cut at an angle to the fiber axis. Note thick filaments in the H band and thin filaments in the A band proper. $\times 50,000$.

FIG. 3. Longitudinal section of chick leg muscle fixed at equilibrium length discloses thick H band filaments and thin A band filaments. Thin filaments are also observed traversing the Z membranes. $\times 50,000$.

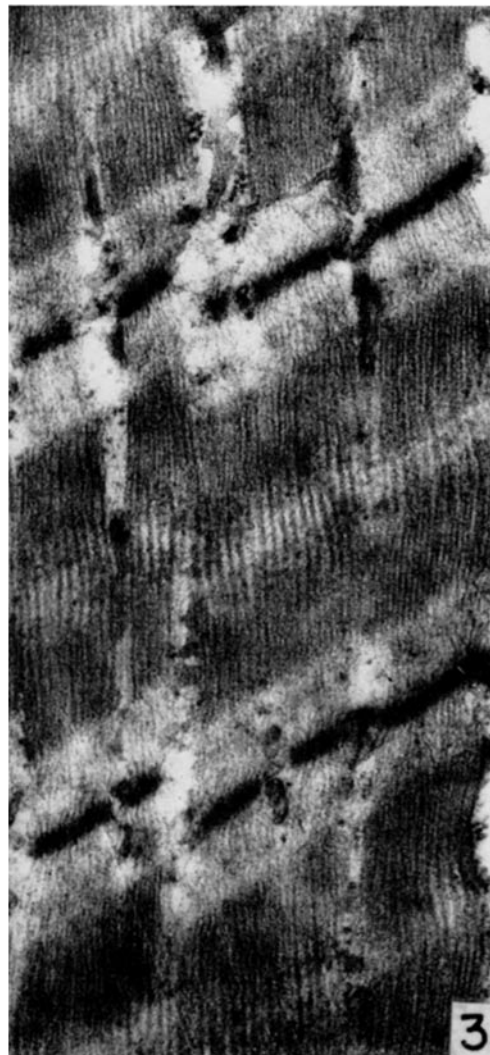
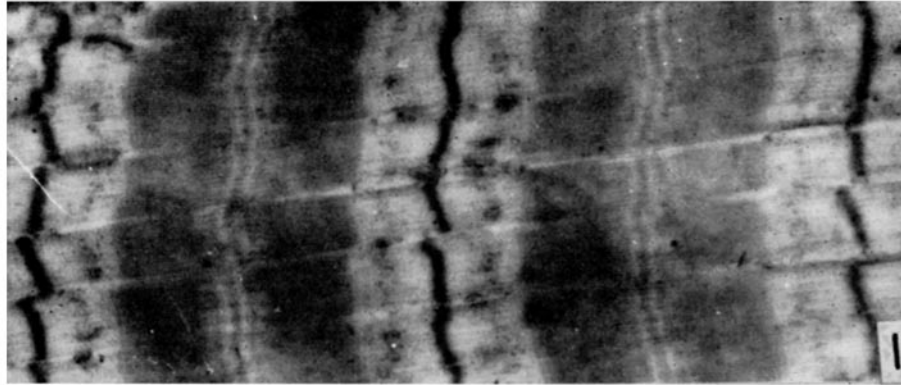


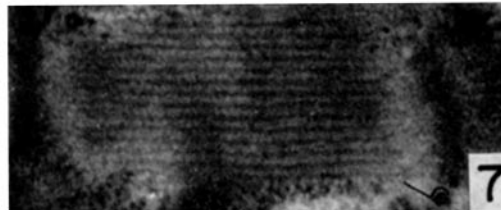
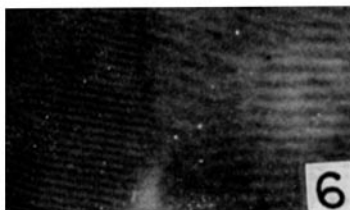
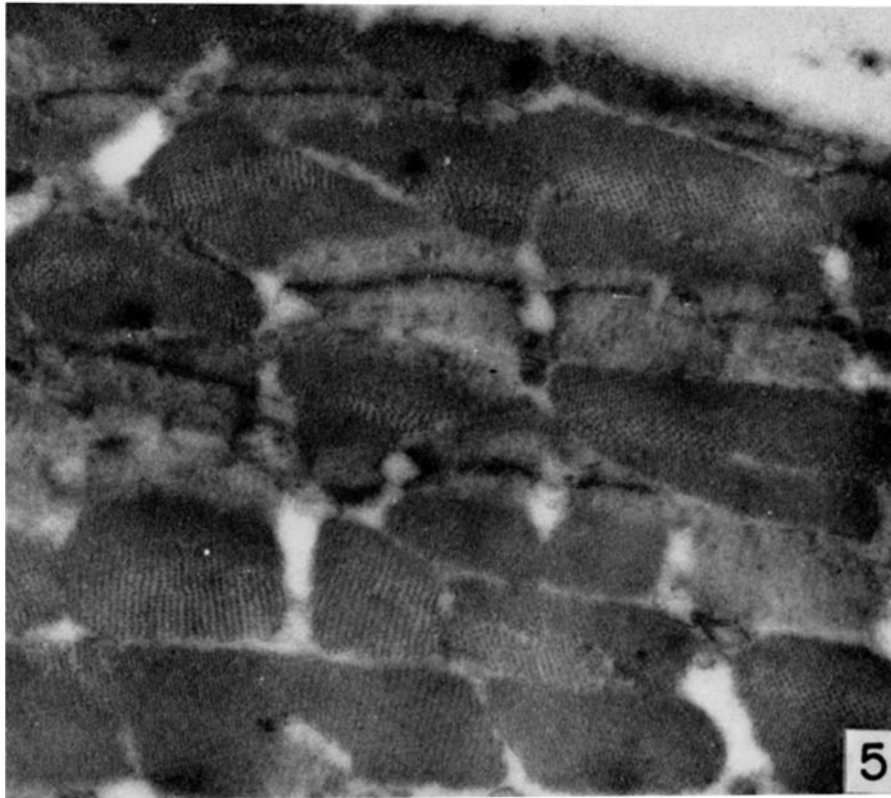
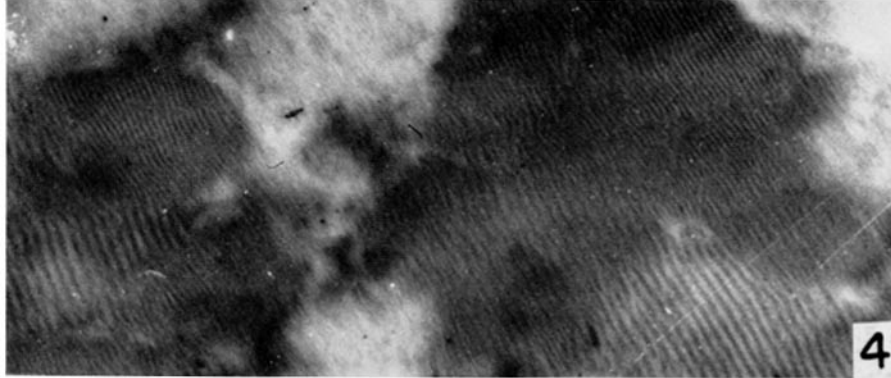
PLATE 54

FIG. 4. Oblique section of rest length rabbit psoas. Myofibrils are sectioned at various levels through the sarcomeres to reveal the different arrays of filaments present in H, A, and I bands. Convergence of pairs of thin filaments to form thick ones can be seen. $\times 46,000$.

FIG. 5. Near transverse section of rest length rabbit psoas to disclose again structural differences between various bands. In areas thick filaments are seen approximately end on, but section is not sufficiently well oriented to reveal the packing of the thin filaments. $\times 35,000$.

FIG. 6. A band-H band junction, of rest length rabbit psoas, to reveal convergence of thin filaments to form thick ones. $\times 75,000$.

FIG. 7. Longitudinal section of chick leg muscle shortened to about 65 per cent of rest length discloses the presence of thick filaments only in the A band. $\times 50,000$.



(Spiro: Ultrastructure of striated muscle)