THE FINE STRUCTURE OF STRIATED MUSCLE

A Comparison of Insect Flight Muscle with Vertebrate and Invertebrate Skeletal Muscle*, ‡

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Plates 46 to 48

Much of the recent work on muscle fine structure has been discussed in a recent review by Bennett (1), who also covered the structure of various sarcoplasmic components and the sarcolemma. The present paper will be limited to myofibrillar fine structure.

In the main, the current controversy in relation to muscle structure centers around two models, (*a*) what might be termed the "classical" model, in which a continuous skeletal framework of myofilaments traverses all bands of the sarcomere, the band pattern arising from interstitial materials, and (*b*) the interdigitating model advocated by Huxley and Hanson in various forms (13, 19, 27). In the latter model, the primary array of filaments in the A band of vertebrate muscle is identified as myosin[1] and the secondary array seen in transverse sections as actin filaments. The bulk of evidence appears to favour the classical type of model.

The results to be discussed here support the concept that striated muscles from various sources have a similar basic structure, with variations adapted to the diversity of function found in nature. This structure appears to be of the classical type in that thin filaments (probably of actin) are found to traverse all bands in continuous array. Myosin is localized in the A bands proper, and there is a good deal of evidence to support the presence in the A bands of another protein of low molecular weight and viscosity, which appears to be involved in the formation of contraction bands. In addition, the myofilaments are apparently linked together by a system of transverse filamentous bridges which are spaced 250 to 400 A apart along the fiber axis, the magnitude of the spacing depending on the state of contraction of the myofibril.

* Much of the pictorial evidence presented here is from the work of Draper and Hodge (2–4), some from more recent work (5–7), and the remainder represents unpublished work of the author.

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[1] The term myosin denotes L-myosin and not actomyosin.

Materials and Methods

Myofibril suspensions from skeletal muscles of the toad, *Bufo marinus*, were obtained as already described (3). Myofibrils were shadow-cast with platinum, stained with phosphomolybdic acid, or incinerated by electron bombardment (2, 4). The preparation of material for thin-sectioning was essentially as described earlier (6, 7). Sections were obtained with a rotary cantilever microtome (8) and examined in a modified RCA EMU-1 electron microscope.

RESULTS

Since most of the material has already been described elsewhere (2–7), the results will be presented as briefly as possible, and confined mainly to vertebrate skeletal muscle (rabbit and toad) and to the specialized flight muscles of Diptera. Vertebrate muscle serves as a convenient reference base since most biochemical work has been carried out on this material. Dipteran flight muscle, on the other hand, provides an interesting contrast in that it is specialized for high frequency contraction (several hundred cycles per second) under essentially isometric conditions.

1. Vertebrate Skeletal Muscle

The myofibrils exhibit a considerable range of size and cross-sectional shape. They comprise a large number of myofilaments which are evident in shadowed fragmented material (Figs. 1, 5, 9), in myofibrils stained with phosphomolybdic acid (Figs. 2, 18), and in longitudinal sections of muscle (Figs. 6, 8, 11, 19). Many of the myofibrils examined give the strong impression that the myofilaments pass continuously through all bands of the sarcomere. Rozsa *et al.* (10) were able to show this continuity after removal of the A substance by suitable washing. Furthermore, they were able to isolate filaments 3 to 4 μ long, this being more than sufficient to run through all bands of a sarcomere. That the myofilaments represent continuous structural units on which A band material is superimposed is further supported by the appearance of longitudinal sections (Figs. 11 and 19), and also by micrographs such as Fig. 18, in which the myofilaments in the lower part of the figure are clearly long enough to reach the Z band at top right. The myofibrils and myofilaments are commonly observed to break at the A–I junction (those marked (*b*) in Fig. 18) and at the level of the Z band. (Fig. 15 shows a myofibril of dipteran flight muscle in which both types of break are clearly seen.)

In transverse section (Fig. 21) the myofilaments appear as a regular hexagonal array (*e.g.*, 7, 11, 12, 13) of circular dots (this is the primary array of Huxley (13)). It is this primary array which is observed to be continuous with the filaments of the I and H bands. Although only faintly suggested in Fig. 21, the myofilaments usually exhibit a compound or "hollow" appearance in the A band (a dense cortex surrounding a less dense core). Such an appearance has already been shown for the H band (13). In the trigonal posi-

tions between these compound myofilaments is seen a secondary array of smaller and less dense dots, interpreted by Huxley and Hanson (13, 19, 27) as representing actin filaments which extend through the I band to the Z. However, as will become clear later, the available evidence suggests that these secondary dots represent merely one particular configuration of interstitial material localized in the A bands (compare Figs. 21–23).

The Fine Axial Period

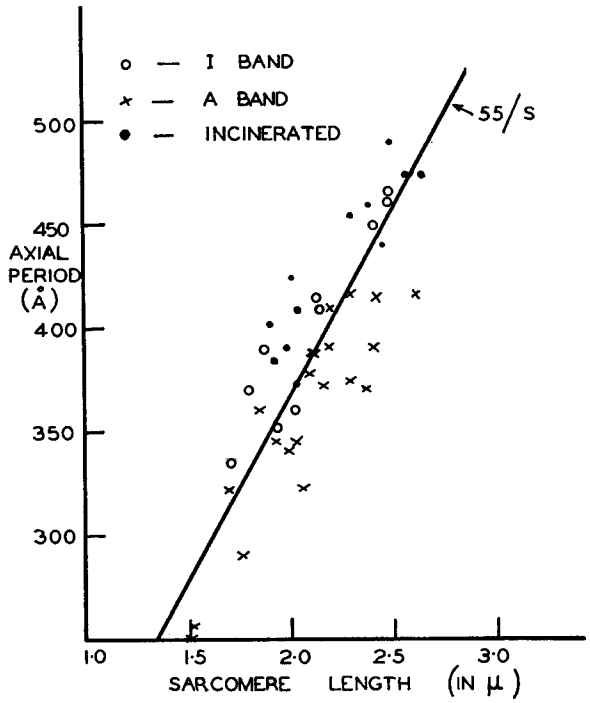
Hall, Jakus, and Schmitt (9) first described what appeared to be an axial periodicity of about 400 A in fragmented muscle stained with phosphotungstic acid (PTA). Draper and Hodge (2, 3) demonstrated this to be a regular fine striation and stated that the period appeared to vary with the state of contraction. The presence of such an axial period in muscle is also shown by x-ray diffraction data (24, 14).

The fine axial period shows up in stained myofibrils (whether isolated by fragmentation, or in thin sections) as a series of thin dark lines which traverse the space between the myofilaments (Figs. 2, 4, 6–8). This transverse continuity is also demonstrable in shadowed myofibrils (Figs. 1, 3, 5, 9). The myofibril surface is often reminiscent of woven fabric, but the actual appearance depends greatly on the shadowing direction. In Figs. 1 to 5 this is more or less transverse to the myofibril axis, and the cross-striation is not emphasized. When shadowed along the fiber axis, the lateral continuity is strongly accentuated (Fig. 3). In Fig. 9, it can be seen that the axial periodicity is associated with the presence of fine transverse bridges linking adjacent myofilaments and regularly spaced along the fiber axis.

When unstained myofibrils are subjected to intense electron bombardment in the electron microscope, the bulk of the myofibrillar material vanishes, the over-all effect being essentially that of microincineration, and the very faint residue of inorganic material is often observed as fine cross-striations (Draper and Hodge (2, 3)). Fig. 10 illustrates the continuity of the fine cross-striations obtained by this method and the close correspondence with the axial period seen in stained or shadowed muscle. The residue comprises mainly Mg, Ca, and P (3). Draper and Hodge (2) found preliminary evidence suggesting that the axial period varied with contraction. These data have been extended by the present author and the results are illustrated in Text-fig. 1, which shows the axial period as a function of sarcomere length. Although the data must still be regarded as preliminary, there does appear to be a significant variation of the axial period with sarcomere length and hence with contraction. It will be apparent also from the figures, particularly Figs. 1, 3, 5, and 8, that the axial spacing is usually somewhat greater in the I band and in the H band (when present) than in the A bands proper (see also Text-fig. 1).

Changes in Band Pattern

Myofibrils exhibiting a wide variety of sarcomere band patterns are found in most preparations of fragmented formalin-fixed muscle. These include typical relaxed patterns with or without H bands (see Figs. 1–8), semicontracted patterns (*e.g.* Fig. 9) in which the A band occupies the whole sarco-



TEXT-FIG. 1. Diagram to illustrate the relation between the fine axial period and the sarcomere length in vertebrate skeletal muscle. The straight line is drawn through the origin and indicates the variation in axial period to be expected if 55 such periods occupied one sarcomere. All the points, with the exception of those labelled "incinerated," represent the measured axial spacing in myofibrils of formalin-fixed fragmented toad muscle (3). The points for "incinerated" muscle (2, 4) were derived from both rabbit and toad muscle. Note that at all values of sarcomere length, the values for the I band are consistently higher than those for the A band.

mere, and myofibrils exhibiting typical "reversal of striation," *i.e.*, in which the A band material has migrated to the Z bands, piling up to give contraction bands (Fig. 12). It is convenient to designate the latter as C_z bands (see (6)). Note that in Fig. 12 the density distribution in the two myofibrils of comparable size, one relaxed, the other heavily contracted, could only have come about by actual migration of interstitial material from the A band into the Z region.

2. Blowfly Flight Muscle

The results on this material have been described in detail elsewhere by the author (5, 6) and a number of other papers on insect muscle have appeared recently (15, 16, 32, 33). A brief description will therefore suffice.

Phase Contrast and Polarization Optical

The highly specialized flight muscles of the Diptera and Hymenoptera appear to operate in the lower region of the contraction range as judged by band pattern (6). Thus freshly isolated myofibrils (which are of exceptionally uniform diameter) exhibit a band pattern (Fig. 13) comparable with that of semicontracted vertebrate muscle (*e.g.*, Fig. 9). Application of ATP results in the formation of typical contraction bands (Fig. 14) and a diminution in density of the rest of the sarcomere. The myofibrils shorten only moderately. If the flight muscle is glycerinated prior to isolation of myofibrils, many of them show a typical relaxed pattern (Fig. 15) comparable with vertebrate muscle (*e.g.*, Figs. 3 or 4). Application of ATP to such myofibrils results in very rapid and typical striation reversal, the final pattern being as in Fig. 14. However, if restrained from shortening, it is sometimes possible (as in Fig. 16) to obtain migration of the A substance to the Z bands without the disappearance of the I bands. This observation seems completely incompatible with the Huxley-Hanson type of model.

Electron Microscopy

In longitudinal sections of fresh flight muscle (Fig. 17) the myofilaments run continuously through all bands of the sarcomere (5, 6). This has also been shown for wasp flight muscle (16). The continuity of the myofilaments can also be demonstrated in glycerinated flight muscle (Fig. 20). Since transverse sections through the A band (Figs. 23 and 25) show only a single array of compound filaments, it is clear that this primary array is continuous with the I band filaments. This has also been demonstrated by means of slightly oblique transverse sections in the region of the A-I junction (6).

The myofilaments of dipteran flight muscle are arranged in an exceptionally uniform hexagonal array about 300 A apart (Figs. 23 and 25). They are about 120 A in diameter in the A band and comprise a central lightly staining core about 40 A in diameter surrounded by a dense cortex. The variations in the structure of the myofilaments as they traverse the various bands have been described (6). Each myofilament is linked to its six nearest neighbours by thin dense filamentous bridges which are spaced uniformly along the fiber axis, the repeat distance usually being about 250 A (Fig. 24). That the dense lines seen in the micrographs do in fact represent bridges and not fixation artifacts is supported by the observation that the outermost myofilaments of the myofibril often show oriented "bridges" which project blindly into the surrounding space (upper part of Fig. 23). The relatively wide separation of

the myofilaments (*ca.* 300 A) may be partly responsible for the fact that the bridge system has not yet been observed as a well defined series of cross-striations such as seen in vertebrate muscle. It has been shown (6) that the bridges are present in all bands of the sarcomere with the possible exception of the narrow light band (presumably the H band) bisected by the M band.

3. Cardiac Muscle

When observed in longitudinal section the myofibrils of vertebrate cardiac muscle show a structure resembling that of skeletal muscle. The band pattern is generally either of the semicontracted or contracted type. A strong impression of myofilament continuity is gained from the micrographs and a definite axial period (usually 250 to 350 A) is present (unpublished data). This has also been observed by Kisch (28) in bat ventricle, and the presence of lateral bridges has been described by Weinstein (29). In transverse section, the myofibrils are seen to comprise myofilaments packed in a relatively poorly ordered array. Cardiac myofibrils thus appear to be basically similar to those of skeletal muscle.

4. Insect Skeletal Muscle

Recent electron microscopical work on this material has been reported by Edwards *et al.* (33) and other authors (*e.g.* 15, 32). The structure is broadly comparable with vertebrate striated muscle, but little is known of the finer details of myofibril structure.

In transverse sections through the A band, the myofilaments appear as a poorly ordered array of compound structures (Fig. 22). Of particular interest is the distribution of part of the interstitial material in dense lines which surround each myofilament. In longitudinal sections this gives the false impression that a secondary set of filaments is present in the A band. This distribution of interstitial material contrasts with the secondary array of dots (Fig. 21) in rabbit muscle and with the apparently homogeneous distribution in dipteran flight muscle (Fig. 23).

DISCUSSION

Migration of A Substance.—It is a common observation that "reversal of striation" occurs during strong contraction and the results presented here support this concept. Harman and Osborne (30) showed that spontaneous reversible contraction may occur in isolated myofibrils. This involves a splitting of the A band and movement of the halves towards the Z bands. On further shortening, typical contraction bands are formed. The results discussed here and elsewhere (6, 3, 17, 9) indicate that there is an actual mass transfer from the A band during the formation of contraction bands.

The work of a number of authors (*e.g.* 18, 19) indicates that L-myosin is located only in the A bands and this view finds strong support in the results

of Finck *et al.* (20) who used fluorescein-labelled antibody. On moderate contraction, the fluorescent band (A band) splits into two, and the halves move towards the Z bands. Unfortunately, the results are incomplete in that no observations have yet been made by this technique on myofibrils showing definite "reversal of striation" (*i.e.* with contraction bands). It is therefore not possible as yet to identify the dense material in the C_z bands as myosin. If it is myosin, then the protein must be in a highly coiled state, for polarization optical data show that the birefringence of the Z region is not enhanced by the formation of C_z bands. The issue is further complicated by the recent observation (21) that selective extraction of myofibrils for myosin also removes considerable amounts of a low molecular weight protein (X protein), which is apparently located together with myosin in the A bands. It is pertinent then to enquire whether the antibody preparations of Finck *et al.* might not also be active for this X protein. Further work with this technique seems urgently necessary in order to determine whether or not some of the changes in band pattern (particularly the formation of C_z bands) might be due to a movement of X protein and/or other substances independently of the myosin during later stages of contraction. If so, the X protein may be regarded as the classical A substance. Certainly, the X protein could be expected to migrate more readily than myosin, which in any case must presumably, in part at least, remain in the central regions of the sarcomere if contraction results from an interaction between actin and myosin. The composition of the A band remains far from settled, however. The migratory material may in fact comprise a fairly complex mixture of substances, possibly including salts and small organic molecules. One may cite for instance the unexplained formation of dense bands (C_m bands, see Fig. 14) at the level of the M band during strong contraction. These appear to be analogous to the C_z bands and to arise similarly by migration of some A band component.

The Axial Period.—This feature of the myofibril was first described by Hall, Jakus, and Schmitt (9) and shown to be a regular repeating pattern by Draper and Hodge (2, 3). Hoffmann-Berling and Kausche (23) described the axial period as fine transverse lines spaced 225 to 250 A apart along the fiber axis, but were undecided as to whether these represented discoidal or filamentous elements. The presence of bridges has also been described by Bennett and Porter (12) and others (7), and previous results on dipteran flight muscle (5, 6) together with the evidence presented above leave little doubt that the regularly repeating system of transverse filamentous bridges represents an integral part of the structure of both vertebrate skeletal and dipteran flight muscle. The composition of these bridges remains a mystery, except that they can scarcely represent myosin or actin, since these are known to be oriented longitudinally. One speculation (6) is that they may be tropomyosin.

The fact that the bridges are spaced about 400 A apart in relaxed myofibrils

is of interest in relation to the frequency with which this spacing is encountered in muscle proteins. Bear (24) interpreted the small angle x-ray pattern of muscle[2] in terms of a period of 360 or 420 A and Huxley (14) has shown that the axial period is 420 A in fresh muscle. Recently it has been shown that actin itself exhibits a period of about 400 A (25). Yet another instance is the beautiful 420 A period observed in crystals of L-meromyosin (22). It seems probable, therefore, that the bridge system contributes to the axial x-ray pattern, and that the interactions of myosin and actin with each other and with the bridges are in some way related to these repeating units of about 400 A. The periodic structure in unstained L-meromyosin crystals consists of a series of fine transverse lines 30 to 50 A wide (22). The most likely explanation of the very high density of these lines is that they represent salts accumulated between the ends of molecules or "bound" to them. This is of great interest since it has been found (4) that formalin-fixed muscle preparations retain considerable amounts of Ca, Mg, and P, and that this mineral residue is localized in fine transverse striations, the spacing being about 400 A (see Fig. 10) in relaxed myofibrils.

The axial spacing appears to decrease as the band pattern changes and the sarcomeres shorten (Text-fig. 1). The value of the spacing also varies from band to band. Thus, in myofibrils which, to judge from their band pattern, were fixed while under tension, the spacing is higher in the H and I bands than in the A bands. These findings are clearly most compatible with a structure in which continuous filaments (actin) extending through all bands are linked together by a regularly spaced system of transverse bridges, and in which interstitial materials are present between the filaments. The myosin and perhaps other materials in the A band would be expected to modify the extensibility of the basic framework by being at least partly combined with it. Such a model would behave in a manner consistent with the results of Carlsen and Knappeis (26) on the relative lengths of A and I bands as a function of stretching.

Unfortunately, no evidence concerning variation of the axial period with contraction has yet been obtained by x-ray diffraction. It has been found (14) that there is no apparent change in the axial pattern when fresh muscle is stretched. This is a surprising result at first sight, but a possible explanation is that the major portions of the diffracted beams represent x-ray scattering by the A bands (the mass density ratio of A:I is about 3:1), which extend but little on stretching, most of the increase in sarcomere length being due to an increase in width of the H and I bands.

Birefringence of Striated Muscle.—It is well known that in all stages of con-

[2] It is of interest to note that there appears to be a fine axial period of 50 to 60 A associated with the myofilaments in muscle treated with PTA (Fig. 11). This does not appear to arise from lens asymmetry or specimen drift and the spacing is in good agreement with x-ray diffraction data (*e.g.* reference 35). Bennett (34) described a periodic structure of this magnitude in electron micrographs of isolated myofilaments.

traction and relaxation, the region of maximum birefringence remains unchanged. In myofibrils with A, I, and H bands, only the A bands are significantly birefringent.[3] In strongly contracted muscle (with contraction bands) the birefringence is less but is still confined essentially to the same region. The Z regions remain isotropic despite the heavy accumulation of material forming the C_z bands. The myofibrils of dipteran flight muscle exhibit similar birefringence properties (6).

The above properties and the relative isotropy of the I band are readily explained in terms of the modified classical model already discussed provided myosin is strictly localized in the A bands proper (not including the H band) and that a second mobile component is also present in the A bands, but is capable of migration to the Z bands. As already discussed, there is good evidence (20, 21) in favour of these two provisos. The qualitative argument can be outlined briefly as follows. Quantitative aspects must await much more work.

The skeletal framework of longitudinal filaments (composed of actin and possibly including some of the insoluble stroma proteins) would exhibit strong positive birefringence. This would then be compensated by the system of transversely oriented filamentous bridges, yielding an essentially isotropic structure. The strong positive birefringence of longitudinally oriented myosin localized in the A bands would then be primarily responsible for the anisotropy of the A bands. The presence of an additional component (the low molecular weight, low viscosity X protein of Szent-Györgyi *et al.* (21)) in the A band would not alter the birefringence pattern. A possible role for this substance is that of a "modifier" of the interaction between actin and myosin.

The type of structure proposed here finds support in the work of Aloisi *et al.* (31). They found that muscle fibers became essentially isotropic when treated with myosin solvents, and the band pattern disappeared. If the residue was then extracted for actin, the ghost fibers became negatively birefringent. A possible explanation is that the birefringence of the transverse bridge system predominates when the actin is partly extracted.

Contraction in Terms of the Modified Classical Model.—In the above schema, the actin filaments shorten by interaction with myosin, so that as contraction proceeds, those parts of the myofilaments in the I bands are progressively incorporated into the A band until the I bands have disappeared (*e.g.* Fig. 9). Further shortening, which is accompanied by a decrease in density through most of the sarcomere, a decrease in birefringence, and accumulation of material in the C_z bands, could be achieved by migration of the X protein to the Z regions. Conceivably a lowering of the X protein concentration could allow a more intense interaction between myosin and actin, resulting in further shortening of the actomyosin system.

The Huxley-Hanson Model.—These authors have recently advanced an in-

[3] The relatively low birefringence of the Z line itself is neglected for the purposes of the discussion.

genious interdigitating model (13, 19, 27) in which thin actin filaments extend from the Z band to the H band and thick filaments run through the A and H bands. They identify the primary array in the A band (see Fig. 21) as myosin filaments and the secondary array as actin filaments. S filaments are postulated to exist in the H band, joining up with the ends of the actin filaments.

This concept is attractive in many ways but is open to a number of criticisms. If correct, the primary array of filaments in the A band should not be continuous with the I band filaments. This has not been directly demonstrated, nor has it been shown that the secondary array of dots seen in transverse sections actually represents filaments, or that such filaments are continuous with those in the I bands. Hanson and Huxley (19) regard the appearance of myosin-extracted myofibrils as supporting their model, but as already pointed out (6), the finding of a single set of thin filaments traversing the sarcomere after myosin extraction is equally compatible with the classical model. Another difficulty is that the postulated S filaments have not so far been observed.

A serious defect of the interdigitating model is that it fails to take account of the transverse bridge system,[4] and offers no explanation for the decrease in density of the central regions of the sarcomere during formation of contraction bands. Huxley and Hanson consider that contraction bands result when the free ends of the myosin filaments are crushed or folded against the Z bands. However, as we have seen, the evidence is against this interpretation. Further objections to this model are (*a*) that the secondary array of dots in transverse sections of rabbit muscle represents only one type of distribution of the interstitial material (compare Figs. 21–23) and (*b*) that it does not offer an adequate explanation of the birefringence distribution in the relaxed myofibril. (F actin shows strong positive birefringence and the I bands are very nearly isotropic.)

It seems clear that the evidence available at the present time is insufficient to allow the formulation of any very precise model of striated muscle or exact theory of contraction.

SUMMARY

The available evidence from phase contrast, polarization optical, and electron microscopic studies on vertebrate skeletal muscle, insect skeletal muscle, and dipteran flight muscle is interpreted as favoring the following general structure of striated muscle.

A continuous array of filaments (actin) runs through all bands of the sarcomere. These are linked by an axially periodic system of transverse filamentous bridges. Myosin (and probably other substances) are localized in the A bands. The system of transverse bridges compensates the birefringence of actin and is

[4] In a recent paper, Hanson and Huxley (36) suggest that the bridges may represent links between actin and myosin filaments. However, it is difficult to see how this can be reconciled with the probability that contraction involves a much more intimate and direct molecular interaction between actin and myosin than their interdigitating model would allow.

thus responsible for the isotropy of the I band. Myosin is responsible for the birefringence of the A bands. On strong contraction, A band material migrates to the Z bands to form contraction bands. It is not yet certain whether this migration involves myosin or another A band component.

BIBLIOGRAPHY

1. Bennett, H. S., *Am. J. Physic. Med.*, 1955, **34,** 46.
2. Draper, M. H., and Hodge, A. J., *Nature*, 1949, **163,** 576.
3. Draper, M. H., and Hodge, A. J., *Australian J. Exp. Biol. and Med. Sc.*, 1949, **27,** 465.
4. Draper, M. H., and Hodge, A. J., *Australian J. Exp. Biol. and Med. Sc.*, 1950, **28,** 549.
5. Hodge, A. J., *Proc. Internat. Congr. Electron Microscopy, Roy. Microscop. Soc., London*, London, 1954, in press.
6. Hodge, A. J., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 361.
7. Hodge, A. J., Huxley, H. E., and Spiro, D., *J. Exp. Med.*, 1954, **99,** 201.
8. Hodge, A. J., Huxley, H. E., and Spiro, D., *J. Histochem. and Cytochem.*, 1954, **2,** 54.
9. Hall, C. E., Jakus, M. A., and Schmitt, F. O., *Biol. Bull.*, 1946, **90,** 32.
10. Rozsa, G., Szent-Györgyi, A., and Wyckoff, R. W. G., *Exp. Cell Research*, 1950, **1,** 194.
11. Morgan, C., Rozsa, G., Szent-Györgyi, A., and Wyckoff, R. W. G., *Science*, 1950, **111,** 201.
12. Bennett, H. S., and Porter, K. R., *Am. J. Anat.*, 1953, **93,** 61.
13. Huxley, H. E., *Biochim. et Biophysic. Acta*, 1953, **12,** 387.
14. Huxley, H. E., *Proc. Roy. Soc. London, Series B*, 1953, **141,** 59.
15. Philpott, D. E., and Szent-Györgyi, A., *Biochim. et Biophysic. Acta*, 1955, **18,** 177.
16. Chapman, G. B., *J. Morphol.*, 1954, **95,** 237.
17. Ashley, C. A., Porter, K. R., Philpott, D. E., and Hass, G. M., *J. Exp. Med.*, 1951, **94,** 9.
18. Hasselbach, W., *Z. Naturforsch.*, 1953, **8 b,** 450.
19. Hanson, J., and Huxley, H. E., *Nature*, 1953, **172,** 530.
20. Finck, H., Holtzer, H., and Marshall, J. M., Jr., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 175.
21. Szent-Györgyi, A. G., Mazia, D., and Szent-Györgyi, A., *Biochim. et Biophysic. Acta*, 1955, **16,** 339.
22. Philpott, D. E., and Szent-Györgyi, A. G., *Biochim. et Biophysic. Acta*, 1954, **15,** 165.
23. Hoffmann-Berling, H., and Kausche, G. A., *Z. Naturforsch.*, 1950, **5 b,** 139.
24. Bear, R. S., *J. Am. Chem. Soc.*, 1945, **67,** 1625.
25. Selby, C. C., and Bear, R. S., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 71.
26. Carlsen, F., and Knappeis, G. G., *Exp. Cell Research*, 1955, **8,** 329.
27. Huxley, H. E., and Hanson, J., *Nature*, 1954, **173,** 973.
28. Kisch, B., *Exp. Med. Surg.*, 1954, **12,** 335.
29. Weinstein, H. J., *Exp. Cell Research*, 1954, **7,** 130.
30. Harman, J. W., and Osborne, U. H., *J. Exp. Med.*, 1953, **98,** 81.
31. Aloisi, M., Ascenzi, A., and Bonetti, E., *J. Path. and Bact.*, 1954, **67,** 475.

32. Farrant, J. L., and Mercer, E. H., *Exp. Cell Research*, 1952, **3**, 553.
33. Edwards, G. A., and collaborators. Various papers are cited in (6).
34. Bennett, H. S., *Anat. Rec.*, 1949, **103**, 423.
35. Astbury, W. T., *Nature*, 1947, **160**, 388.
36. Hanson, J., and Huxley, H. E., Fibrous proteins and their biological significance, *Symp. Soc. Exp. Biol. (Great Britain)*, 1955, No. 9, 228.

EXPLANATION OF PLATES

All figures except Figs. 13–16 are electron micrographs. When the figures have been taken from already published work, this is indicated in the appropriate figure legend.

PLATE 46

FIG. 1. Relaxed myofibril from fragmented formalin-fixed toad muscle, shadowed with platinum. The direction of shadowing is indicated by the arrow. Note the fine transverse striation in the A and I bands (Draper and Hodge (3), Fig. 3). × 35,400.

FIG. 2. Myofibril from fragmented formalin-fixed toad muscle, stained with phosphomolybdic acid. The fine axial period is evident, particularly in the A band, as a series of transversely continuous dense lines (45 to 60 A wide) which traverse the spaces between the myofilaments. The band pattern is comparable with that in Fig. 1 (Draper and Hodge (3), Fig. 7). × 73,500.

FIG. 3. Myofibril from fragmented formalin-fixed toad muscle, shadowed with platinum. The direction of shadowing (arrow) was along the fiber axis. Note that under these conditions, the transverse continuity of the fine cross-striation is emphasized, also that the spacing in the I band (*ca.* 410 A) is greater than is that in the A band (*ca.* 380 A). × 29,400.

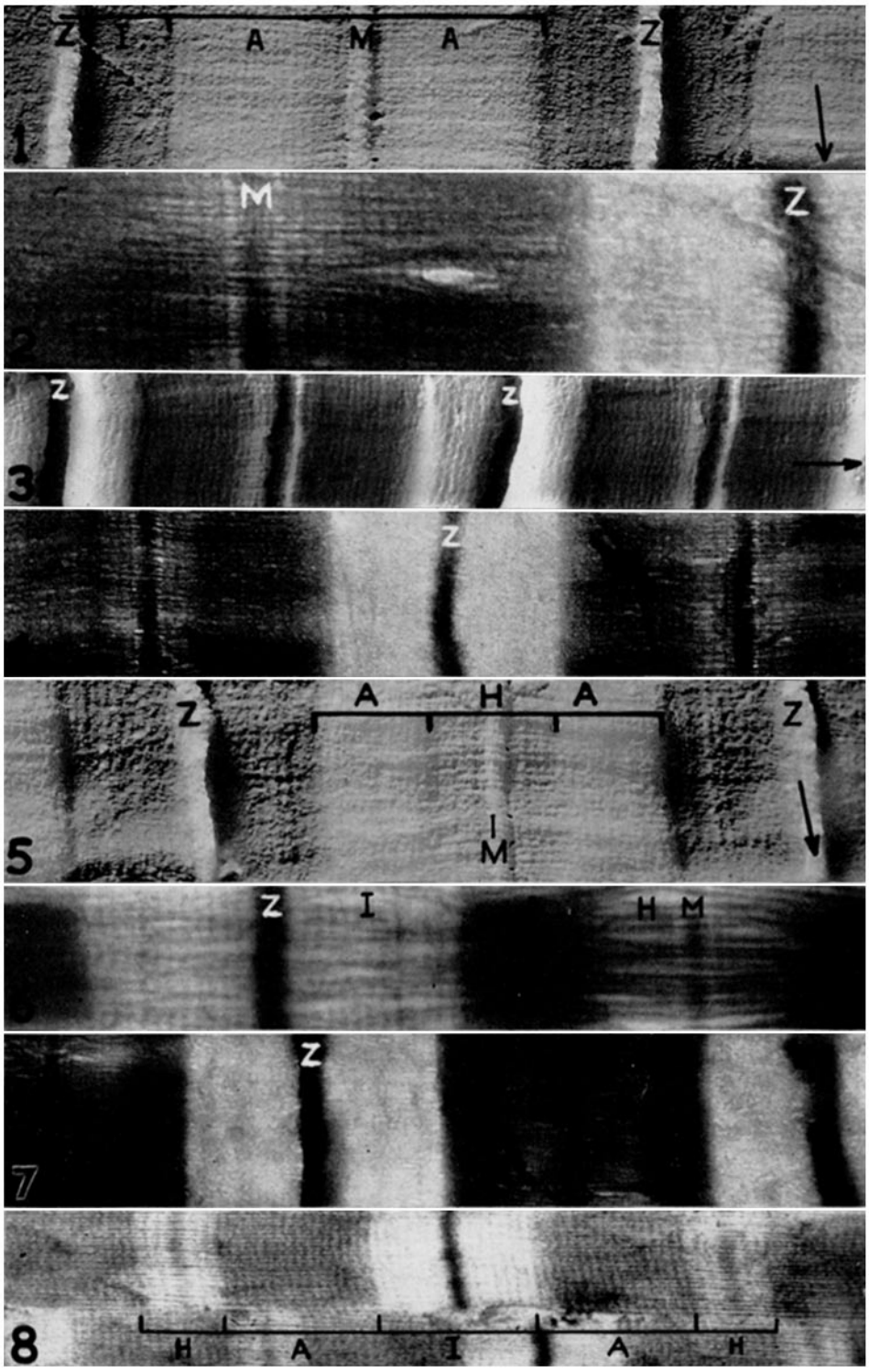
FIG. 4. Myofibril from fragmented formalin-fixed toad muscle, stained with phosphomolybdic acid. Note the sharply defined A-I junction and that the spacing between the fine cross-striations is greater in the H band than in the A band (Draper and Hodge (3), Fig. 8). × 40,000.

FIG. 5. Myofibril from fragmented formalin-fixed toad muscle, shadowed with platinum, showing a relaxed pattern with a relatively wide H band. Shadowing direction indicated by arrow. The fine cross-striation is present in the A, H, and I bands (Draper and Hodge (3), Fig. 4). × 35,200.

FIG. 6. Longitudinal section of toad muscle, fixed in formalin as in (3) and stained with phosphotungstic acid. Some degree of swelling is present (due to omission of osmium fixation), but the lateral continuity of the axial period is evident in the I and H bands. × 55,000.

FIG. 7. Myofibril from fragmented formalin-fixed toad muscle, stained with phosphomolybdic acid, showing a band pattern with wide H band. Note the sharpness of the A-I junction as compared with that of the A-H junction (Draper and Hodge (3), Fig. 9). × 29,400.

FIG. 8. Rabbit psoas muscle in thin longitudinal section. Fixation in buffered osmium tetroxide followed by treatment with PTA (7). Note the obviously larger spacing in the H and I bands as compared with the A band (Hodge, Huxley, and Spiro (7), Fig. 7). × 40,000.



(Hodge: Fine structure of striated muscle)

PLATE 47

FIG. 9. Semicontracted myofibril from fragmented formalin-fixed toad muscle, shadowed with platinum. Note the regular fine cross-striation which appears as fine transverse bridges (especially in the region indicated by the small arrow). The large arrow indicates the direction of shadow-casting (Draper and Hodge (3), Fig. 5). × 44,500.

FIG. 10. Formalin-fixed rabbit muscle after electron-induced microincineration as described by Draper and Hodge (4). The mineral residue (mainly Ca, Mg, and P (4)) is arranged in fine striations showing a high degree of continuity (compare with Fig. 2). The Z and M bands appear to be deficient in these elements. × 29,400.

FIG. 11. Longitudinal section of fresh rabbit psoas fixed in buffered osmium tetroxide and stained with PTA, showing a fine axial period (spacing *ca.* 55 A) in the myofilaments. This appearance does not seem to be due to either specimen movement or lens asymmetry since the direction of the fine transverse lines varies from place to place. This period may correspond to that shown by x-ray diffraction in F actin. Part of the A band is seen at the right of the micrograph. The A-I junction is in this case poorly defined, as is the 400 A axial period. The latter feature is, however, observable when the micrograph is printed at lower magnification. × 270,000.

FIG. 12. Formalin-fixed toad muscle showing at top, a myofibril with a relaxed band pattern, and beneath this, a strongly contracted myofibril. Since the myofibrils are of comparable size, the picture strongly suggests that the C_r bands are formed by migration of dense material from the A band (Draper and Hodge (3), Fig. 12). × 21,000.

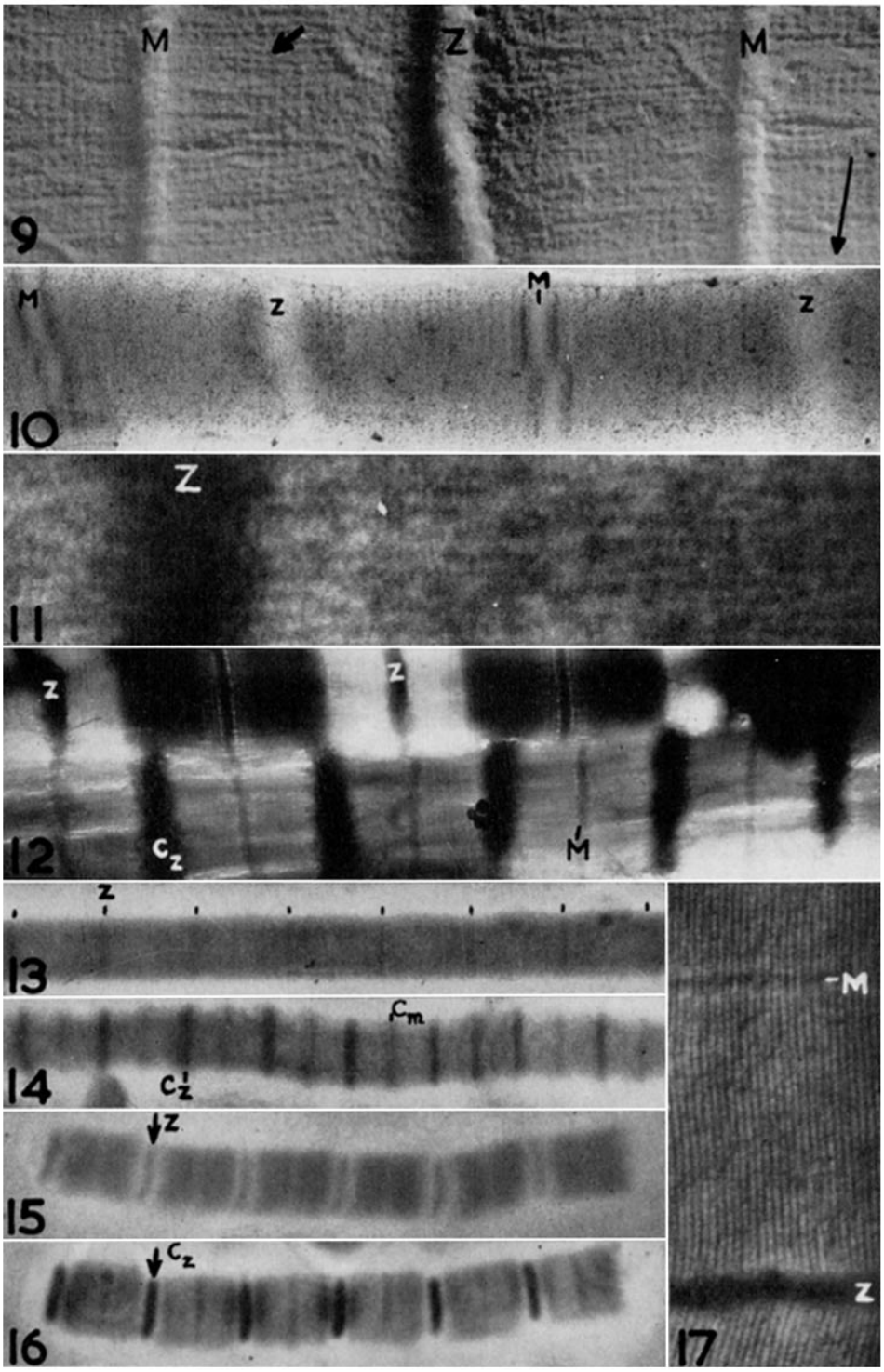
FIG. 13. Freshly isolated myofibril of blowfly flight muscle, illustrating the typical semicontracted band pattern found in this muscle (Hodge (6), Fig. 3 *a*), phase contrast. × 3,500.

FIG. 14. Same preparation as Fig. 13, showing a myofibril after ATP addition. Photographic conditions are identical with those in Fig. 13. Note the decrease in density of the sarcomere associated with the formation of C_z and C_m bands (Hodge (6), Fig. 3 *c*), phase contrast. × 3,500.

FIG. 15. Myofibril from glycerinated blowfly flight muscle, showing typical relaxed band pattern with A, I, Z, and H bands (Hodge (6), Fig. 2 *a*), phase contrast, × 3,750.

FIG. 16. The same myofibril illustrating the effect of ATP addition. The myofibril was unable to shorten appreciably because of its adhesion to the coverslip. Note that typical contraction bands (C_z) have formed (presumably by migration of A substance) despite the persistence of the I bands (Hodge (6), Fig. 2 *b*), phase contrast. × 3,750.

FIG. 17. Longitudinal section of blowfly flight muscle fixed in buffered osmium tetroxide and stained with PTA (6). The continuity of the myofilaments through all bands is evident. × 34,000.



(Hodge: Fine structure of striated muscle)

PLATE 48

FIG. 18. Myofibril from fragmented formalin-fixed toad muscle, stained with phosphomolybdic acid, illustrating continuity of the myofilaments through all bands of the sarcomere. The frayed myofilaments in the central part of the picture (*b*) have broken at the A-I junction, while the lengths of the myofilaments in the lower part of the figure (*a*) suggest that they have broken at the level of the Z band. × 28,000.

FIG. 19. Longitudinal section of rabbit psoas fixed in buffered osmium tetroxide (pH 7.4) followed by staining with 1 per cent aqueous PTA. Note the apparent continuity of the I band filaments with those in the A band. The A band filaments are represented in transverse sections as the primary array (*p*) in Fig. 21. The relatively good preservation of order in the I band is, in this case, probably due to the narrowness of this band. × 100,000.

FIG. 20. Longitudinal section of glycerinated blowfly flight muscle fixed as in Fig. 19 (OsO_4, PTA) showing continuity of the A band filaments with those in the narrow I and H bands. Note the N bands close to the Z band (Hodge (6), Fig. 16). × 32,000.

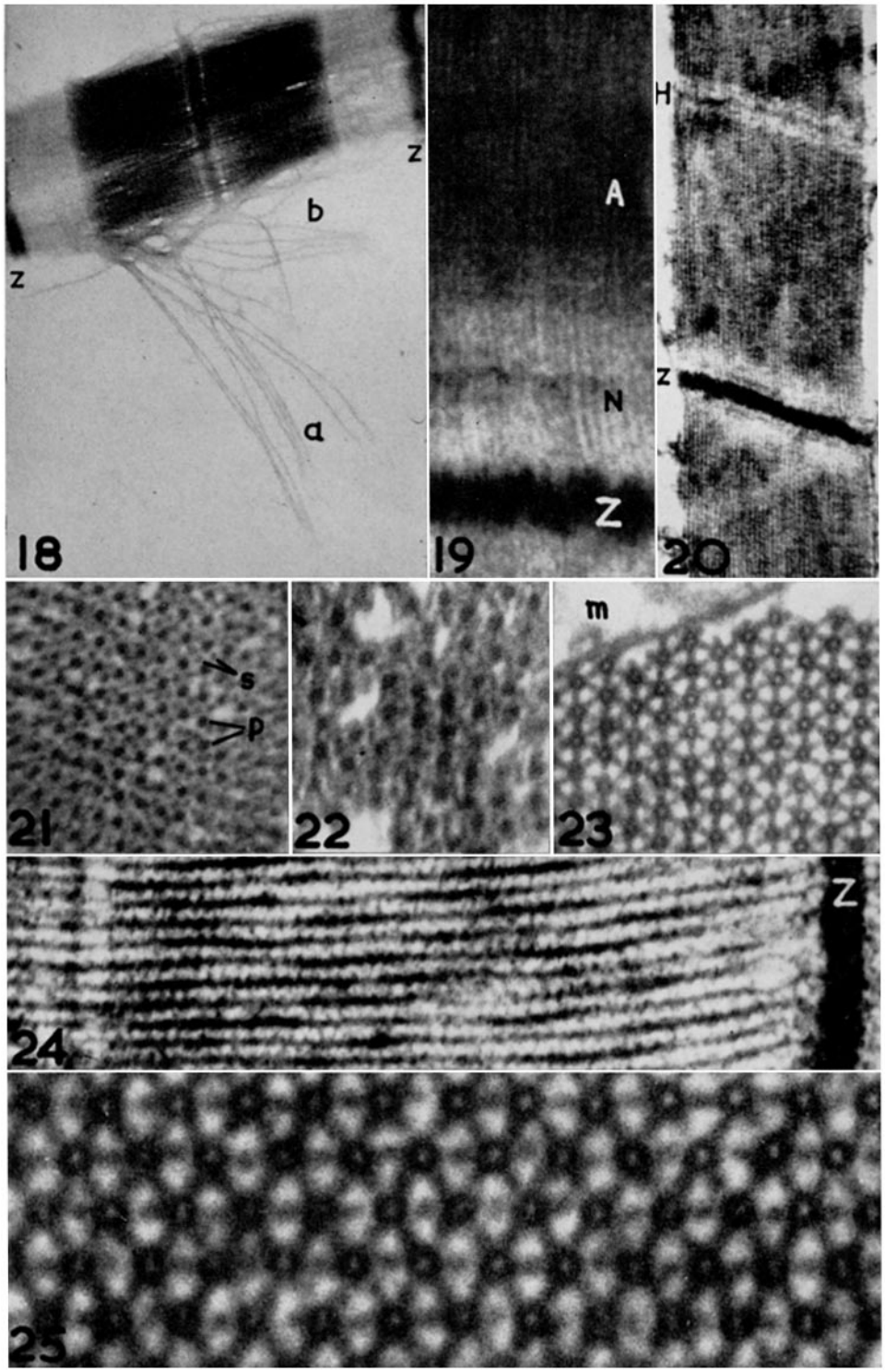
FIG. 21. Transverse section through the A band of fresh rabbit psoas muscle fixed as in Fig. 19 (OsO_4, PTA), showing the primary array of dots (*p*), corresponding to the A band filaments seen in longitudinal sections (Figs. 8 and 19) and in fragmented muscle (*e.g.* Fig. 2) and a secondary array of smaller and less well defined dots (*s*) representing one particular configuration of the interstitial material of the A band. There does not appear to be any necessity from the appearance of longitudinal sections for interpreting this secondary array as a set of filaments. The dots of the primary array often show a compound structure similar to that in Figs. 22 and 23. This is observed only when the A band filaments are accurately normal to the plane of the section. × 134,000.

FIG. 22. Transverse section through the A band of relaxed grasshopper leg muscle fixed as in Fig. 19 (OsO_4, PTA), showing the poorly ordered array of myofilaments, which appear as compound or "tubular" structures. The interstitial material in this case appears not as a secondary array of dots, but as a series of dense lines "surrounding" each myofilament. Further interstitial material is also present in a homogeneously dispersed form (compare the density of the interstitial spaces with that of the background (*e.g.* to the right of the figure number)). × 140,000.

FIG. 23. Transverse section through the A band of blowfly flight muscle showing the regular hexagonal array of compound myofilaments (300 to 350 A apart) linked together by a system of transverse bridges. Comparison with longitudinal sections (*e.g.* Fig. 24) indicates that these bridges represent filamentous rather than lamellar structures. Note the disorganized structure of the mitochondrion (*m*) and the presence of freely projecting bridges on the myofilaments at the periphery of the myofibril. No secondary array is present, and the relatively small amount of interstitial material is apparently homogeneously dispersed. Fixation as in Fig. 19. × 150,000.

FIG. 24. Longitudinal section of glycerinated blowfly flight muscle contracted with ATP, prior to fixation in buffered OsO_4 followed by staining with alcoholic PTA. The treatment has resulted in considerable disorganization, but sufficient order is left to illustrate the axially periodic arrangement of thin filamentous bridges spaced about 250 A apart. × 85,000.

FIG. 25. Transverse section through the A band of blowfly flight muscle fixed as in Fig. 19 (OsO_4, PTA) showing the regular hexagonal array of compound myofilaments and the system of transverse filamentous bridges Hodge (6). × 335,000.



(Hodge: Fine structure of striated muscle)