Published May 1, 1963

ELECTRON MICROSCOPIC STUDIES ON THE INDIRECT FLIGHT MUSCLES OF *DROSOPHILA MELANOGASTER*

I. Structure of the Myofibrils

S. AHMAD SHAFIQ, D.Phil.

From the Department of Anatomy, University of Washington, Seattle, and the Institute for Muscle Disease, Inc., New York

ABSTRACT

The myofibrils in *Drosophila* have thick and thin types of myofilaments arranged in the hexagonal pattern described for *Calliphora* by Huxley and Hanson (15). The thick filaments, along most of their length in the A band, seem to be binary in structure, consisting of a dense cortex and a lighter medulla. In the H zone, however, they show more uniform density; lateral projections (bridges) also appear to be absent in this region. The M band has a varying number of granules (probably of glycogen) distributed between the myofilaments. The myofilaments on reaching the Z region appear to change their hexagonal arrangement and become connected to one another by Z filaments. The regular arrangement of the filaments found in most regions of the fibrils is not seen in the terminal sarcomeres of some flight muscles; the two types of filaments appear to be intermingled in an irregular pattern in these parts of the fibrils. The attachment of myofibrils to the cuticle through the epidermal cells is described.

INTRODUCTION

Flight muscles with oscillation frequencies of several hundreds per second are found in many insects, especially among the representatives of Diptera. Such muscles have usually been called "fibrillar" following von Siebold's (28) discovery that their fibrils are very easily dissociable and are exceptionally large in diameter. A great deal of work has been done on the physiology and metabolism of these muscles (2, 24, 26) and they have also been used for morphological and developmental studies by light microscopy (*e.g.*, 33). It appeared that they might also be very suitable material for developmental studies by electron microscopy. However, in order to study their differentiation, it was necessary first to ascertain their fine structure in the adult stage. Fibrils of the indirect flight muscles of adult fruit flies were, therefore, studied.

MATERIAL AND METHODS

Wild type adults of *Drosophila melanogaster*, Meigen, from a laboratory stock breeding on the usual cornmeal-agar medium were used. Flight muscles from chilled flies were fixed for 1 hour in an ice cold fixative consisting of equal parts of 5 per cent osmium tetroxide and *s*-collidine buffer (pH 7.4) (1) and embedded in an Epon (epoxy) resin mixture according to the procedure of Luft (18). Sectioning was done on a Porter-Blum microtome using glass knives. Sections

Published May 1, 1963

showing gold or silver interference colors were selected and stained in saturated solutions of uranyl acetate or lead hydroxide for 4 to 6 hours (35). They were then examined in a Siemens Elmiskop I or an RCA EMU 2C microscope operated on a specially designed power supply.

For light microscopy, Epon or methacrylate sections 1 to 2 μ in thickness were prepared on the Porter-Blum microtome and stained with toluidine blue (pH 7–8) or by the methylene blue technique (25).

OBSERVATIONS

There are four pairs of fibrillar muscles in *Drosophilia*, namely, the dorsal longitudinal, tergosternal, oblique dorsal, and tergocoxal. The dorsal longitudinal muscle consists of six fibers which run longitudinally through the thorax of the fly and are attached to the curved dorsal wall of the thorax at both ends. The tergosternal muscle has three fibers, and the oblique dorsal and tergocoxal have two each; these three muscles run in a general vertical direction in the thorax. The gross morphology and general histology of the flight muscles have already been described (*e.g.*, 36, 33). Some of the histological features may be seen in Figs. 1 and 2, which are photomicrographs of a tergosternal muscle. The muscle fibers appear to be composed mainly of myofibrils and mitochondria. The myofibrils in Fig. 1 are in the contracted state and therefore show only the A, Z, and M bands of their sarcomeres. They are about 1.5 μ in diameter and stain lightly with toluidine blue. The mitochondria are distributed in large numbers between the myofibrils (Figs. 1 and 2). They are globular in shape (diameter about 1 to 1.5 μ) and stain strongly with toluidine blue. Their fine structure appears to be of the usual type (Figs. 3 and 4).

The fibrils of both the dorsal longitudinal and the vertical groups of muscles were examined by electron microscopy. Most sarcomeres of the fibrils appear to have a similar structure, except those at the terminal parts of the fibrils. The structure of the various bands of the typical sarcomeres will be described first.

In the typical sarcomeres the A band is about 2.8 μ in length (Figs. 4 and 5). It appears to be composed of two types of filaments, namely, thick filaments (diameter about 120 A) and thin filaments (diameter about 30 A). The thick filaments form a hexagonal pattern and each of them is surrounded by six thin ones. Further, the thin filaments are so arranged that there is a thin one between every two of the thick type. Lateral projections (bridges) connecting the two types of filaments are also seen in the picture (Fig. 5). The thick filaments usually appear as binary structures consisting of a dense cortex and a lighter inner portion (Fig. 5). The cortex sometimes appears as two lamellae cut in different planes (*e.g.*, in Fig. 6), possibly owing to slight obliqueness of sections. The thick filaments also seem to taper in the terminal regions of the A bands (Fig. 7). In contracted sarcomeres, however, where the I bands are not evident, the tapering of the thick filaments is not seen (Fig. 8).

The structure of the H band is shown in Figs. 3 and 9. The former picture is of an oblique section through a myofibril, so that the H and A bands of the same fibril are seen, whereas the latter shows H and A bands of adjacent fibrils at higher magnification. It will be noted that the H band shows only the thick filaments; the thin filaments and the bridges seem to be absent. The thick filaments also apparently do not have the binary structure in the H band, but seem to be more uniformly

FIGURE 1

Photomicrograph of longitudinal section of a tergosternal muscle stained with toluidine blue. The myofibrils show A, Z, and M bands and large numbers of globular mitochondria between the fibrils. Their attachments to the cuticle (*c*) are also seen. × 1,750.

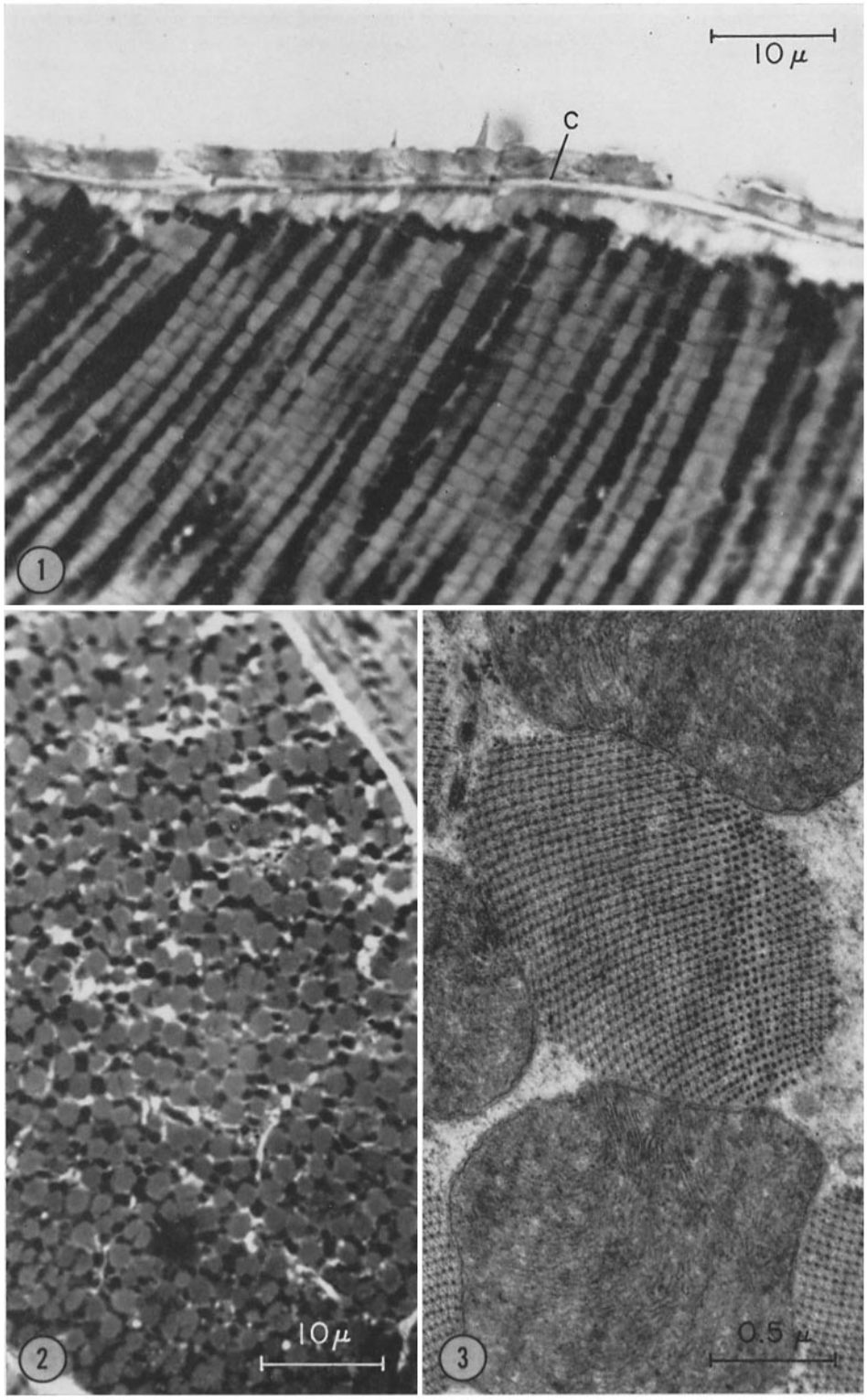
FIGURE 2

Photomicrograph of transverse section of the same muscle as in Fig. 1, stained with toluidine blue. The mitochondria stain more strongly than the fibrils. × 1,750.

FIGURE 3

Electron micrograph of a muscle fiber showing mitochondria with the usual cristae and limiting membranes. A fibril is cut obliquely so that both the H and the A bands are seen in the picture. × 36,000.

Published May 1, 1963



Published May 1, 1963

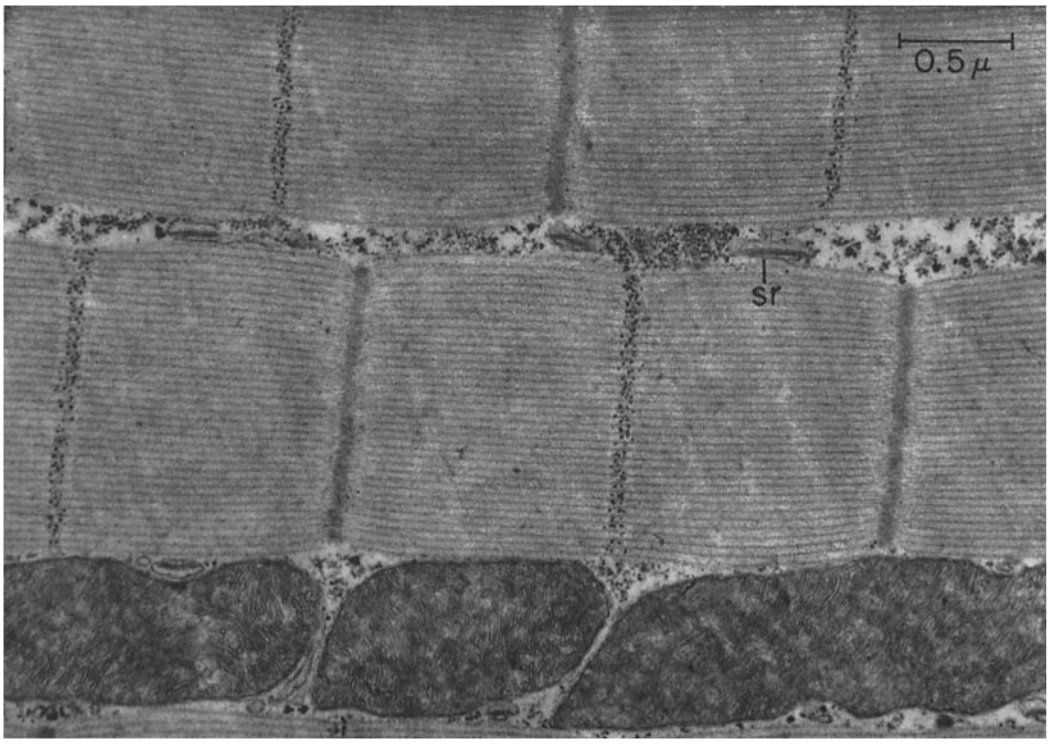


FIGURE 4

Longitudinal section of two contracted myofibrils showing glycogen granules in their M bands densely stained by lead hydroxide. Elements of sarcoplasmic reticulum (*sr*) are also seen around the myofibrils. × 24,000.

FIGURE 5

Transverse section of a myofibril showing its structure at the A band level. Thick and thin types of filaments arranged in hexagonal patterns are seen. The thick filaments appear to have a binary structure consisting of a dense cortex and a lighter medulla. × 187,000.

FIGURE 6

Transverse section of a myofibril from a freshly emerged fly. The cortex of many of the thick filaments does not appear as a uniform circular coat in this picture, but as two lamellae cut in different planes. × 187,000.

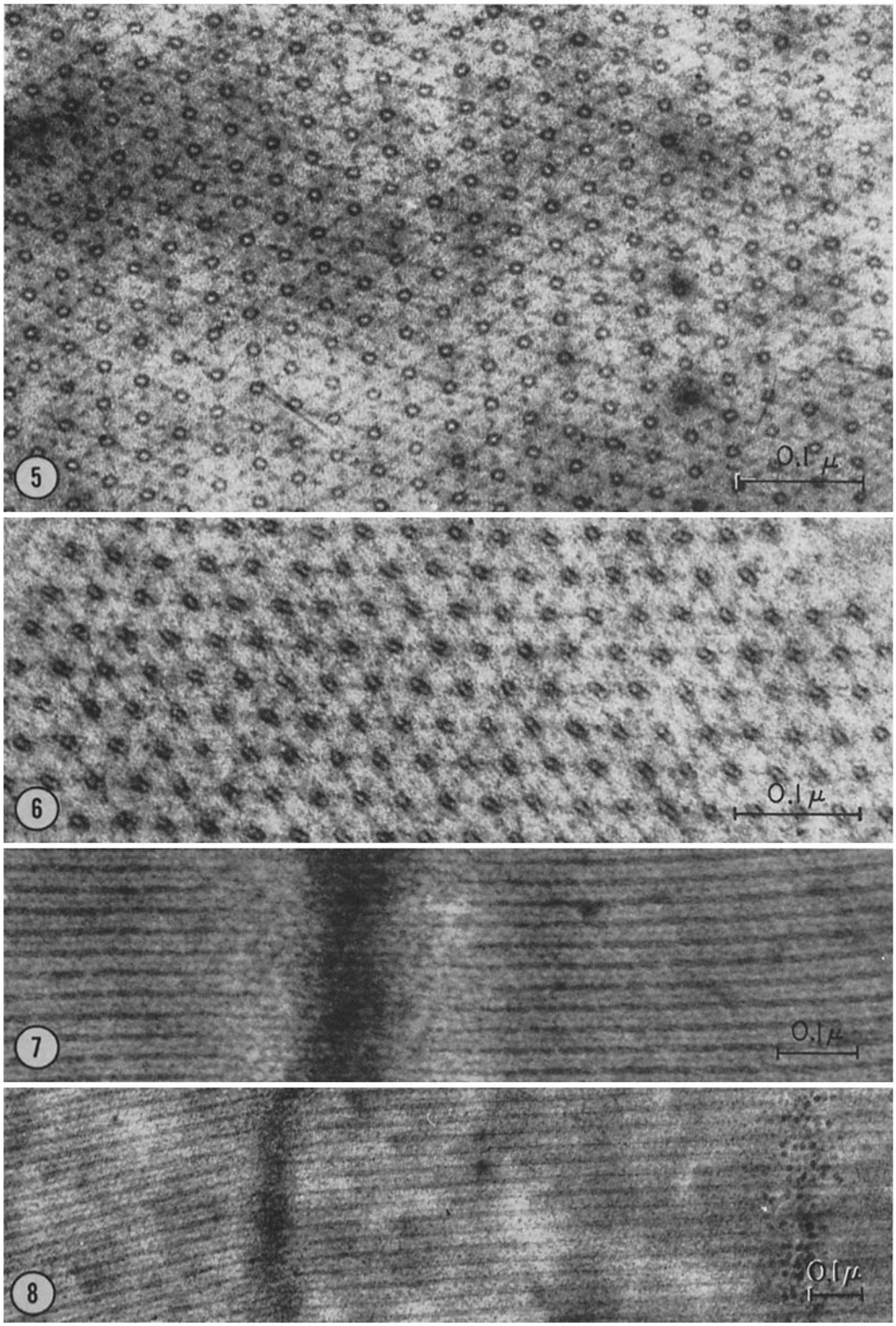
FIGURE 7

Longitudinal section of a fiber showing A, I, and Z bands. In the A band the thick and the thin filaments are seen. The thick filaments appear to have tapering ends which extend into the I band in this picture. The Z band appears as a dense region bisecting the I band. × 112,000.

FIGURE 8

Longitudinal section of a contracted myofibril. The tapering ends of thick filaments are not seen. × 69,600.

Published May 1, 1963



dense (Fig. 9). These special features of the H bands were seen in sections which had not been stained, as well as in those which were stained with uranyl acetate or lead hydroxide.

The M band is seen as a line bisecting the A band by light microscopy (Fig. 1). Electron microscopy showed only a varying number of dense granules about 150 A in diameter distributed between the myofilaments in this region (Fig. 4). The granules of the M band are seen particularly clearly in sections stained with lead hydroxide. Histochemical studies, which will be reported elsewhere, indicate that the granules are composed of glycogen.

The I band of the fibrillar muscle could be examined only rarely, as myofibrils in the fixed tissues were usually found in a contracted state. When the I band could be studied (*e.g.*, in Fig. 7) it showed the thin filaments, as well as the tapering parts of the thick ones.

The Z band was usually seen as a dense region limiting the sarcomeres and bisecting the I bands when the latter were present (Fig. 7). In thinner sections it was noted that the filaments come into the Z band and are connected to each other by filamentous bridges. The exact arrangement of the myofilaments extending into the Z band remains to be studied, but it appears that the hexagonal arrangement does not hold in this region (Fig. 10).

The structure of the sarcomeres of the terminal parts of the fibrils was examined in the dorsal longitudinal muscle only. The terminal parts of the fibers of this muscle have fewer mitochondria than does the remainder of their length, so that in sections of this region, the fibrils, when examined with the light microscope, appear to be separated by comparatively large "empty spaces." This can be seen in Fig. 11, which is a picture of a transverse section through two fibers of the dorsal longitudinal muscle. The upper fiber is cut nearer its attachment to the thoracic wall (terminal part) than the lower one and shows more of the "empty spaces" than the lower one.

The fine structure of the terminal part of the fibrils shows that the regular hexagonal arrangement of the thick and thin myofilaments is not present in this region, and the two types of filaments seem to be distributed in no regular pattern (Figs. 12 and 13). Some pictures also show what appears to be the transitional border between the irregular part and the hexagonal arrangement (Figs. 12 and 14). Another noteworthy feature of the terminal parts of the fibrils is that the thick filaments do not show the binary structure of a dense cortex and a lighter medulla as clearly as in other parts of the fibrils (Fig. 13).

The mode of attachment of myofibrils to the cuticle was studied in the tergosternal muscle. Fig. 1 shows the myotendinal region of this muscle as seen by light microscopy, whereas Figs. 15 and 16 are electron micrographs of the same region. It will be noted that the myofibrils do not attach directly to the cuticle, but that the epidermal cells are involved in the process. The myofibrils end in broad, dense bands (about 0.4 μ in thickness) which seem to correspond in position to terminal Z lines. The dense bands attach the individual myofibrils to the sarcolemma; the regions of the sarcolemma where the myofibrils are received appear to be specially bound to the overlying epidermal cells by well developed desmosomes (Fig. 15). Tendinal fibers about 230 A in thickness, extending from these desmosomes to the outer surface of the epithelial cells, will be noted in Figs. 15 and 16. It seems that the outer surface of the epithelial cells (which is closely applied to the cuticle) bears small dense plaques on which the tendinal fibers terminate. It may also be mentioned that the epidermal cells themselves are attached to one another by septate desmosomes (Fig. 16) described by Wood (37).

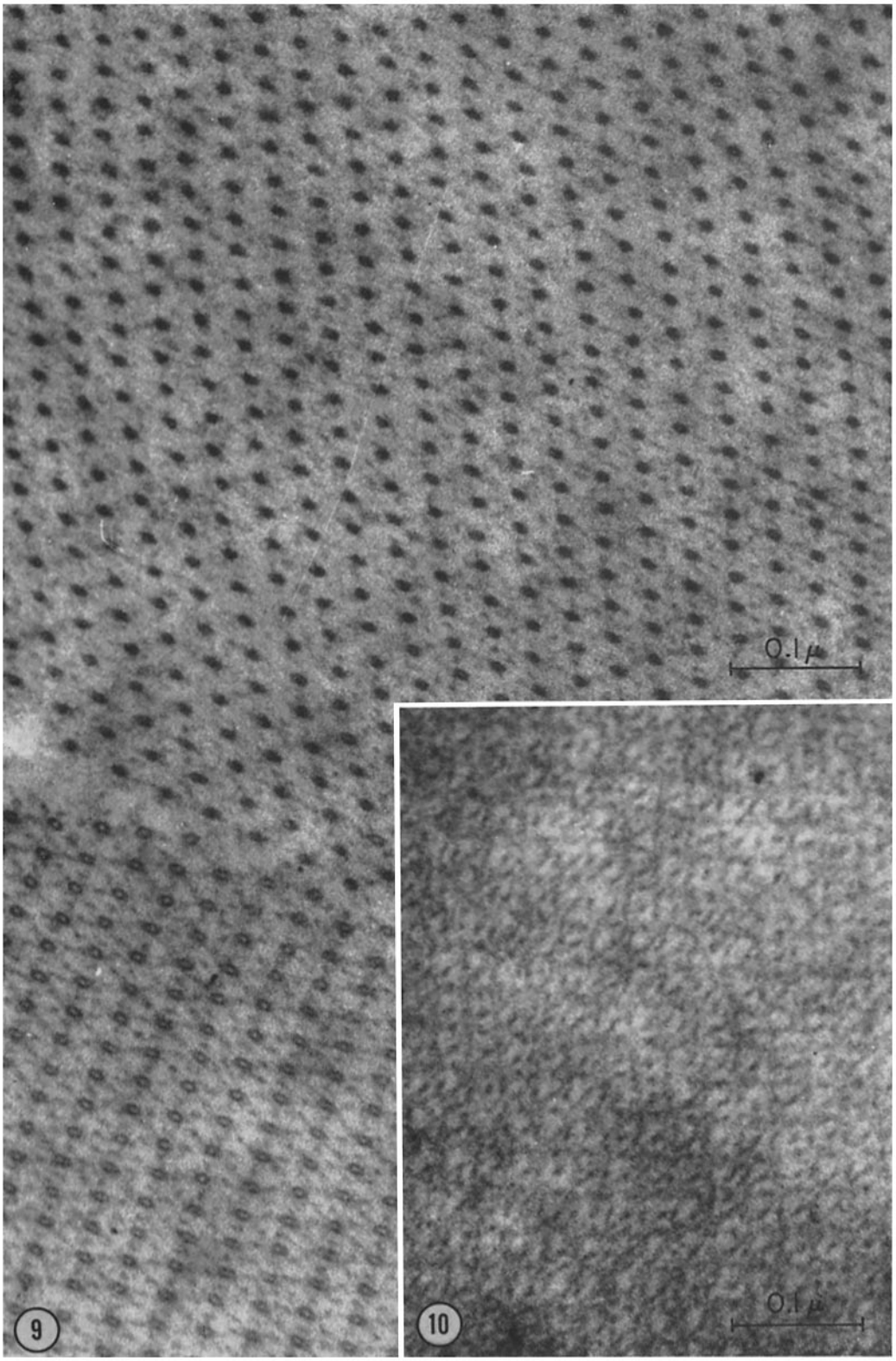
FIGURE 9

Transverse section of two adjacent myofibrils. The upper one in the picture is cut at the H level, the lower one at the A level. No bridges or thin filaments are seen in the H region. The thick filaments in the H region appear to be more uniformly dense than those in the A region. There is evidence of fine structures in some filaments at the H level, but this is not clearly resolved. × 187,000.

FIGURE 10

Transverse section of a fibril at the Z level showing that the myofilaments are not arranged in hexagonal patterns in this region. The Z filaments are also seen. × 187,000.

Published May 1, 1963



DISCUSSION

The fine structure of insect muscles has been studied by Chapman (6), Edwards *et al.* (9), Philpott and Szent-Györgyi (22), Hodge (11), Huxley and Hanson (15), and Smith (31), among others.

Huxley and Hanson's (15) studies on the flight muscles of the fly *Calliphora* showed that the structure of this muscle corresponds to their model for vertebrate striated muscle (16, 14) with the difference that the thin filaments are more numerous in the *Calliphora* muscle and are so arranged that each thin filament is shared by two of the thick type. Hodge (11) and Philpott and Szent-Györgyi (22), however, described only one type of filament in insect muscle. The view that the Huxley-Hanson model may be of general application to all striated muscles has also recently been contested by several authors (*e.g.*, 29, 30, 12, 34). In the present work on *Drosophila* flight muscle, sections of the A band clearly show that there are two types of filaments arranged in the same pattern as described for *Calliphora* by Huxley and Hanson (15). This hexagonal arrangement apparently does not characterize the sarcomeres of the terminal regions of the fibrils of the dorsal longitudinal muscle, where the two types of filaments are intermingled in an irregular manner. The significance of this is not clear.

The H band is usually regarded as the part of the sarcomere from which the thin filaments are withdrawn when the fiber is in relaxation. Thus, this band shows only the thick filaments. The structure of the thick filaments in the H band of vertebrate skeletal muscles appears to be essentially similar to that seen in the A bands (16). Huxley (13), however, had noted earlier that the thick filaments in the rabbit psoas increase in diameter as they pass through H zones and appear "hollow," "possibly due to incomplete penetration of the stain." It may also be mentioned that Draper and Hodge (8) and Perry and Horne (21) have described H sublines in skeletal muscles of toad and rabbit, respectively.

In the *Drosophila* muscles studied here, H sublines are not seen. Further, the thick filaments appear to be differentiated into a distinct middle zone (H region) where the filaments do not appear "hollow" and are more uniformly dense. The lateral projections (bridges) also seem to be absent in the H band of *Drosophila*.

The M line has been regarded as a well defined band of myofibrils (21), acting as a spacer to maintain the alignment of the filaments (8). Huxley and Hanson (16), however, believe that the M region appears as a band due in stretched fibrils to median thickening of the thick filaments, and in contracted fibrils to crumpling of the I filaments at their ends. The M line in mammalian cardiac muscle has been described as a "zone of segmental thickening of the thick filaments" (32).

In the present study the M line appears as a distinct region by light microscopy, but examination by electron microscopy shows only a varying

FIGURE 11

Photomicrograph of transverse section through two fibers of a dorsal longitudinal muscle. The upper fiber is cut nearer its attachment to the cuticle than the lower one. The upper fiber appears to have fewer mitochondria. × 1,800.

FIGURE 12

Electron micrograph showing transverse sections of two fibers of a dorsal longitudinal muscle. The fiber on the left is cut nearer its attachment to the cuticle than is the one on right. In the former fiber the filaments are not arranged in hexagonal patterns; in the latter the two myofibrils show the loss of hexagonal patterns to different extents. × 32,400.

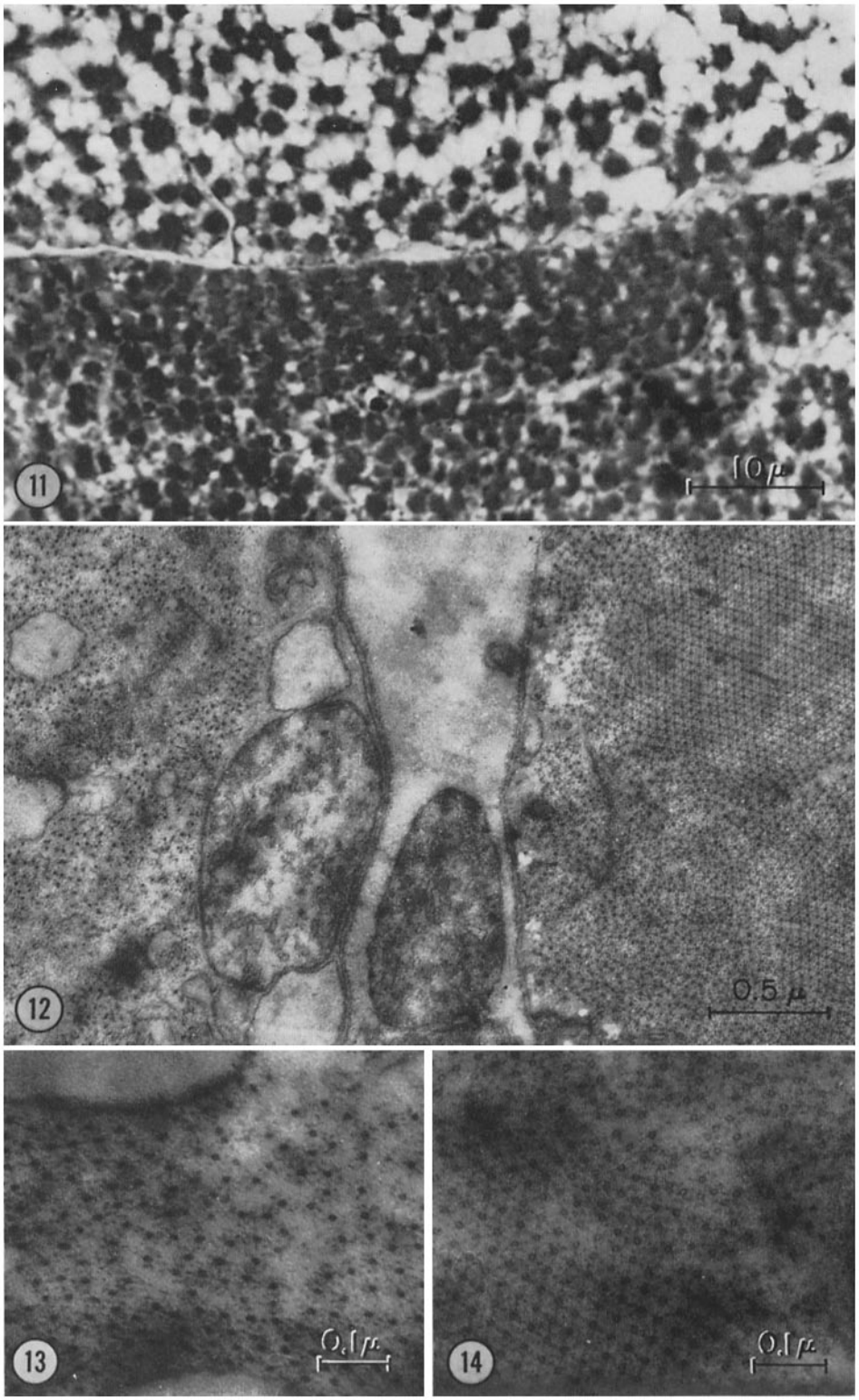
FIGURE 13

Higher magnification picture of the myofilaments from a region corresponding to that of the fiber on the left in Fig. 12. The thick myofilaments do not appear to have the binary structure seen in the A bands of the typical sarcomeres. × 93,700.

FIGURE 14

Higher magnification picture of the myofilaments from a region corresponding to that of the fiber on the right in Fig. 12. × 93,700.

Published May 1, 1963



number of granules, probably of glycogen, occurring irregularly between the filaments in this region. The filaments themselves in M and H bands appear to be identical in structure and thickness.

The Z band is usually described as a dense band composed of an amorphous material (16). Draper and Hodge (8), however, thought that it consists of a series of fine subbands running across the myofibril. Guba *et al.* (10) isolated Z bands from honeybee muscle and describe it as a tissue of woven fine threads. Recently, Knappeis and Carlsen (17) have done a detailed study of the Z band in amphibian skeletal muscle and show that the thin filaments of a sarcomere terminate at the Z band and are arranged in a rectangular pattern in this region. In the present material also, it appears that the hexagonal pattern of the myofilaments is changed in the Z band and that they are connected to one another by fine Z filaments. The precise structure of the Z band in this material remains to be studied.

Earlier accounts of the myotendinal junction of insect and vertebrate muscles (*e.g.*, 3, 19, 33, 5, 4) favor the view that the myofibrils become continuous with the tendinous elements in this region. Electron microscopy of vertebrate muscles (23, 7, 27, 20) has, however, shown that the sarcolemma, though thrown into numerous invaginations, remains a continuous structure in the myotendinal region. The myofibrils attach to the sides of the invaginations and the tendinous elements are simply spliced with them. In *Drosophila* also, it seems that the myofibrils are not continuous with tendinous fibers, and that there are several structural adaptations to transmit the tension generated in the muscle to the cuticle. Thus, the myofibrils terminate in special dense bands which are attached individually to the sarcolemma. Well developed desmosomes then bind these regions of the sarcolemma to the overlying epidermal cells, and many intracellular tendinal fibers (tonofibrillae) extend from the desmosomes to the cuticle. All together, the myotendinal junction of flight muscle seems to be more elaborate than that of vertebrate skeletal muscle.

The author is most grateful to Professor H. S. Bennett, Dr. J. H. Luft, and Dr. R. L. Wood for their kind help and advice during the course of this work.

Received for publication, August 14, 1962.

BIBLIOGRAPHY

1. BENNETT, H. S., and LUFT, J. H., *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 113.
2. BOETTIGER, E. J., *in* Recent Advances in Invertebrate Physiology, (B. T. Scheer, editor), Eugene, Oregon, University of Oregon Publication, 1957, 117.
3. VON BOGA, L., *Z. Zellforsch. u. mikr. Anat.*, 1938, **27**, 568.
4. BUTCHER, E. O., *Am. J. Anat.*, 1933, **53**, 177.
5. CARR, R. W., *Am. J. Anat.*, 1931, **49**, 1
6. CHAPMAN, G. B., *J. Morphol.*, 1954, **95**, 237.
7. COUTEAUX, R., *Compt. rend. Acad. sc.*, 1958, **246**, 307.
8. DRAPER, M. H., and HODGE, A. J., *Australian J. J. Exp. Biol. and Med. Sc.*, 1949, **27**, 465.
9. EDWARDS, G. A., RUSKA, H., and DE HARVEN, É., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 107.
10. GUBA, F., GRAMVÖLGYI, M., and ERNST, E., *in Proc. 4th Internat. Kongr. Elektronenmikr.*, (W. Bargmann *et al.*, editors), Berlin, Springer-Verlag, 1960, **2**, 324.
11. HODGE, A. J., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 361.
12. HODGE, A. J., *in* Neuromuscular Disorders, *Proc. Assoc. Research Nervous and Mental Diseases*,

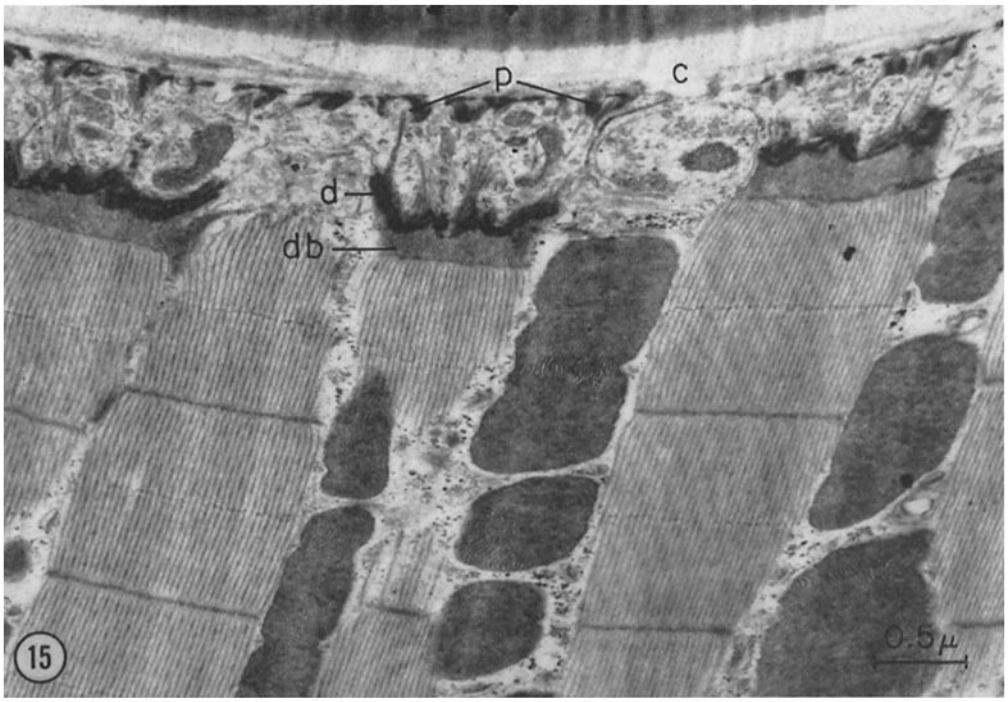
FIGURE 15

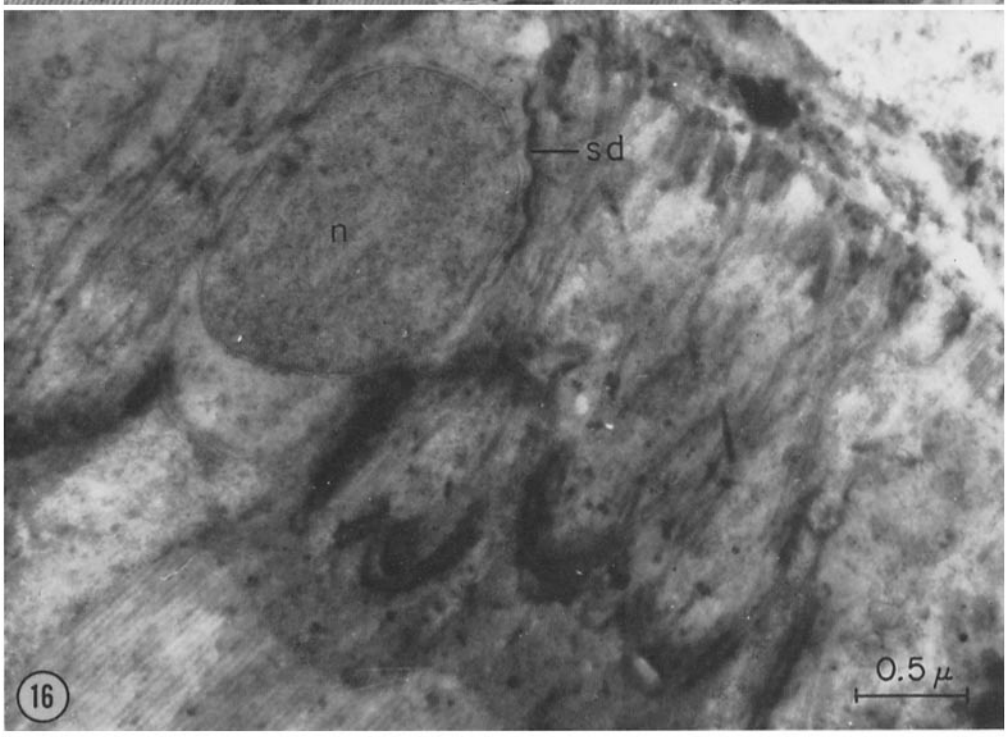
Electron micrograph showing the myotendinal region of a tergosternal muscle. The dense bands (*db*) which form the terminal parts of myofibrils, the overlying desmosomes (*d*) and the tendinal fibers running from the desmosomes to the dense plaques (*p*) are seen. Part of the cuticle (*c*) also appears in the section. × 24,000.

FIGURE 16

Section of the myotendinal region of a tergosternal muscle showing parts of two epidermal cells attached to each other by septate desmosomes (*sd*). The nucleus (*n*) of one of the epidermal cells is also seen. × 27,900.

Published May 1, 1963





(R. D. Adams *et al.*, editors), Baltimore, Williams and Wilkins Co., 1960, **38,** 31.
13. HUXLEY, H. E., *Biochim. et Biophysica Acta*, 1953, **12,** 387.
14. HUXLEY, H. E., *in* The Cell, Biochemistry, Physiology, Morphology, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., 1960, **4,** 365.
15. HUXLEY, H. E., and HANSON, J., *in Proc. 1st European Regional Conf. Electron Micr.*, Stockholm, Almqvist and Wiksells, 1956, 202.
16. HUXLEY, H. E., and HANSON, J., *in* The Structure and Function of Muscle, (G. H. Bourne, editor), New York, Academic Press, Inc., 1960, **1,** 183.
17. KNAPPEIS, G. G., and CARLSEN, F., *J. Cell Biol.*, 1962, **13,** 323.
18. LUFT, J. H., *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 409.
19. MORISON, G. D., *Quart. J. Micr. Sc.*, 1928, **71,** 563.
20. MUIR, A. R., *in* Electron Microscopy in Anatomy, (J. D. Boyd *et al.*, editors), Baltimore, Williams and Wilkins Co., 1961, 267.
21. PERRY, S. V., and HORNE, R. W., *Biochim. et Biophysica Acta*, 1952, **8,** 483.
22. PHILPOTT, D. E., and SZENT-GYÖRGYI, A., *Biochim. et Biophysica Acta*, 1955, **18,** 177.
23. PORTER, K. R., *Anat. Rec.*, 1954, **118,** 342.
24. PRINGLE, J. W. S., Insect Flight, London, Cambridge University Press, 1957.
25. RICHARDSON, K. C., JARETT, L., and FINKE, E. H., *Stain Technol.*, 1960, **35,** 313.
26. SACKTOR, B., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 29.
27. SCHWARZACHER, H. G., *Acta Anat.*, 1960, **43,** 144.
28. VON SIEBOLD, C. T., Vergleichende Anatomie der wirbellosen Tiere, 1848, 562.
29. SJÖSTRAND, F. S., and ANDERSSON-CEDERGREN, E., *J. Ultrastruct. Research*, 1957, **1,** 74.
30. SJÖSTRAND, F. S., and ANDERSSON-CEDERGREN, E., *J. Ultrastruct. Research*, 1959, **3,** 234.
31. SMITH, D. S., *J, Biophysic. and Biochem. Cytol.*, 1961, **10,** No. 4, suppl., 123.
32. STENGER, R. J., and SPIRO, D., *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 325.
33. TIEGS, O. W., *Phil. Tr. Roy. Soc. London, series B*, 1955, **238,** 221.
34. DE VILLAFRANCA, G. W., and PHILPOTT, D. E., *J. Ultrastruct. Research*, 1961, **5,** 151.
35. WATSON, M. L., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 727.
36. WILLIAMS, C. M., and WILLIAMS, M. V., *J. Morphol.*, 1943, **72,** 589.
37. WOOD, R. L., *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 343.