ELECTRON MICROSCOPE STUDIES ON ULTRATHIN SECTIONS OF MUSCLE

By ALAN J. HODGE,* Ph.D., HUGH E. HUXLEY,‡ Ph.D., and DAVID SPIRO,§ M.D.

(From the Biology Department, Massachusetts Institute of Technology, Cambridge)

Plates 1 to 5

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Much of the previous electron microscope work on muscle has been carried out using various types of fragmentation techniques to produce specimens sufficiently thin for satisfactory inspection, and a great deal of information about the fine structure has been found by this method. In such a process, however, a great deal of the organization of the original muscle fiber is necessarily lost. Furthermore, it is not possible to examine cross-sections of muscle by such methods, for fragmentation takes place principally by cleavage in a direction parallel to the fiber axis.

The technique of thin sectioning appears in many ways to be a more promising approach, and has already been applied to muscle by Pease and Baker (1) and by Morgan, Rozsa, Szent-Györgyi, and Wyckoff (2); recently, this technique has undergone substantial development, and we have considered it worth while to carry out further studies on muscle by this method. Some preliminary results of this investigation can now be described.

Techniques

A microtome of rather simple design, capable of cutting sections down to 200 A in thickness has been constructed, and is described in detail elsewhere (Hodge, Huxley, and Spiro (3)). The principles of this microtome are briefly as follows: The advance of the specimen towards the knife-edge is produced by the expansion of an electrically heated steel rod. One end of this rod is attached *via* a flexible steel wire to a rigid support, the other end carrying the specimen passes through a rubber gasket in a hole near the periphery of a circular disc. This disc is mounted on a shaft and can be rotated at the desired speed either by a belt drive from an induction motor or by hand. The rod then moves in a path lying on a surface of a cone, and the specimen moves in a circle, passing the knife-edge once per revolution. The use of the flexible steel wire support obviates the necessity for close-tolerance bearings at this point, and hence is superior. A glass knife is employed, backed by a trough containing a 10 to 20 per cent solution of acetone in water.

Specimens were fixed in buffered 1 per cent osmic acid (Palade (4)) and embedded in

* Present address: The Division of Industrial Chemistry, C.S.I.R.O., Melbourne, Australia.

‡ Fellow of the Commonwealth Fund.

§ Fellow of the Damon Runyon Memorial Fund for Cancer Research Inc.

methacrylate in the usual way (Newman, Borysko, and Swerdlow (5)); additional staining by phosphotungstic acid was also used in many cases. Some specimens were previously fixed in buffered formalin. The most satisfactory sections were 200 to 400 A in thickness. An RCA microscope type EMU was employed, with an externally centerable objective aperture (Farrant and Hodge (6)).

RESULTS

Transverse Sections.—

Electron micrographs of transverse sections of muscle from rabbit (psoas), frog (sartorius), fly (flight muscle), and clam (adductor) are shown in Figs. 1 to 5. In fly flight muscle (Fig. 3), the myofibrils are distinct, well separated, and of fairly uniform size and shape, being roughly circular in section and 2 to 3μ in diameter: occasionally (see Fig. 4) their shape approaches that of a hexagon. Large numbers of mitochondria can be observed lying between them. In frog and rabbit skeletal muscle, however, the myofibrils are generally quite closely packed, except where nuclei and the relatively few mitochondria lie between them: in some cases (*e.g.* Fig. 1), the myofibrils appear separated by large empty spaces, but we believe that this is an artefact of fixation. The myofibrils in frog (Fig. 2) and rabbit have rather irregular shapes and their diameter is generally of the order of a few microns. We have not observed any membranes surrounding the myofibrils in any type of muscle.

Within these myofibrils, and also within those from rat heart muscle, filaments are visible, ranged in a very regular array; the basic packing is hexagonal, but the apparent type of lattice varies somewhat, being affected by the angle of cut with respect to the fiber axis, and by the fixation conditions. The lattice extends continuously across the myofibrils; the division between the myofibrils in frog and rabbit muscle often takes place along fault-lines in the lattice. In sections of frog or rabbit muscle where the direction of cut is not exactly perpendicular to the fiber axis, sections through both A and I bands can be observed lying side by side, *e.g.* Fig. 1 in which it can be seen that under the fixation conditions used, the regular array is only maintained in the A band, which also appears much denser than the I band.

In mammalian and amphibian muscle the filaments are 100 to 130 A in diameter and 200 to 300 A apart. In fly flight muscle, the filaments are 200 to 250 A in diameter and 500 to 600 A apart. In clam adductor muscle, the filaments are of much larger size (600 to 1000 A in diameter) and do not appear to form a regular array (Fig. 5).

Longitudinal Sections.—

Electron micrographs of such sections are shown in Figs. 6 to 10. In the case of frog and rabbit skeletal muscle the A and I bands, M bands, H bands, and Z bands are well marked and in many sections can be seen lined up across a complete fiber: the tendency to divide into myofibrils is often only faintly evi-

dent. In sections in which more shrinkage or mechanical disruption has occurred, breaks are seen in the Z band (*e.g.* Fig. 8) and the fibers cleave in an axial direction into smaller units of rather constant size, 0.5 to 2 microns in width. In fly flight muscle (Figs. 9 and 10), the individual myofibrils are well defined and the Z bands are not continuous; occasionally, one observes pairs of myofibrils in close contact, with their striations in register. The cross-striated appearance is well marked and the pattern appears to be a contracted one, the I bands being largely absent (*cf.* Hanson (7)).

In rat heart muscle, the myofibrils generally show a contracted band pattern also. An interesting feature of this muscle is the continuity of the Z bands across the columns of mitochondria between the fibrils (Fig. 6). The figure also shows the attachment of the Z bands to the sarcolemma. The sarcolemma itself is thrown into folds by the contraction of the underlying muscle.

Rat heart muscle seems to occupy an intermediate position between skeletal muscle and insect flight muscle. The myofibrils vary considerably in size and shape. In many areas myofibrils of 1 or 2 micron diameter appear as individual units, separated by large numbers of mitochondria, but in some areas several myofibrils appear to fuse together.

A characteristic feature of many of these longitudinal sections is the appearance of pseudostriations (*e.g.* Figs. 9 and 10). The effect is produced when the direction of cutting is not quite parallel to the fiber axis: under these circumstances, the cut passes through successive layers of the regular array of filaments, giving rise to a series of dark and light bands in the section. If the knife-edge is parallel to a layer of the lattice (although the plane of cutting is not) then the striations are parallel to the fiber axis: otherwise they are inclined to the fiber axis. When the fibrils have been distorted during fixation or embedding, the striations are curved. The spacing of the striations varies with the angle between the plane of cutting and the layers of the lattice of filaments being cut; the greater this angle, the closer together the striations will be, until the condition of transverse sectioning is reached.

In fly muscle, in which the individual myofibrils are well defined, cases are frequently observed in which an oblique cut through an entire fibril is visible on one square of the grid. In such cases, the number of striations, *i.e.* the number of layers in the lattice, can be counted and is always found to be approximately the same as the number of filaments visible in the fibril at its widest point (generally 40 to 50). This is the result we should expect from fibrils of roughly circular cross-section.

Very occasionally, sections are seen in which the direction of cutting is accurately parallel to a plane of the lattice (Fig. 10, right). In the instance pictured one whole layer of the lattice shows up clearly, and, in this rather thick section, the adjacent layer also can be observed, with poorer definition, lying in between the filaments of the front layer.

In sections of frog and rabbit skeletal muscle and rat heart muscle, a 400 A

axial period is sometimes observed when phosphotungstic acid is used as a stain. This period has the same value in both A and I bands. The darkly staining region responsible for the periodicity is rather sharply defined in an axial direction, but extends sufficiently far radially on each filament to produce the appearance of a cross-striation extending continuously across the fibril. This periodicity has not been observed in muscle stained with osmic acid alone.

DISCUSSION

Previous electron microscope studies have directed considerable attention to the nature of the myofibrils in mammalian and amphibian muscle, and to the manner in which the filaments are arranged within them. Many workers (*e.g.* Hall, Jakus, and Schmitt (8), Draper and Hodge (9), Perry and Horne (10)), using fragmentation techniques, observed myofibrils whose width was 0.5 to 2 microns, and which had the appearance of flat sheets or collapsed tubes. It is now evident that such an appearance arises during the preparative processes, either as a result of a tendency of the lattice of filaments to cleave into sheets, or as a result of an extensive surface tension flattening during the final drying on the electron microscope grid. In the intact muscle, the filaments are undoubtedly arranged continuously across the myofibrils in a regular 2-dimensional array. Indeed, such an array was observed by Morgan, Rozsa, Szent-Györgyi, and Wyckoff (2) in their thin sections, though with less clarity than present techniques now permit. It was also predicted by Huxley (11, 12) from low angle x-ray diffraction studies on living muscle. The x-ray spacing between filaments in living muscle was found to be 440 A, in contrast to the value of 200 to 300 A now observed. It thus appears that the specimens examined in the electron microscope have undergone considerable lateral shrinkage.

The nature of the division of the muscle fiber into myofibrils is less clear. In insect flight muscle, there can be no doubt as to the genuine existence of myofibrils as morphological units. In frog and rabbit muscle, however, some specimens show filaments arranged in a continuous lattice for considerable distances across the muscle fiber, with little evidence of a genuine myofibrillar subunit; the filaments appear to be held in register at the Z bands, and in fibers which have split up longitudinally, portions of the ruptured Z band can be observed protruding on either side of the myofibrils now visible. It is not apparent why the fibers should break up into subunits in this fashion, but observations on transverse sections suggest that it is correlated to some extent with "faults" in the lattice of filaments. We would also draw attention to the possibility that the effects are a consequence of the nature of the embryonic formation of muscle, when developing myoblasts give rise to fibrils which grow until the whole fiber is filled: electron microscope studies on muscle during embryogenesis might provide useful information on this question.

The width of the filaments seen in sections of frog and rabbit skeletal muscle

and rat heart muscle (100 to 130 A) is significantly less than that reported on fragmented, shadowed material: Draper and Hodge (9), for example, observed filaments whose widths were around 160 A. However, the measurements agree well with the widths of the densely staining cores of the filaments seen in fragmented material stained with phosphotungstic acid (Hodge, personal observation). Furthermore, the density between filaments, as seen in both longitudinal and transverse sections, is definitely considerably greater than the background density. It seems likely, therefore, that the "filaments" seen in fragmented material consist of a central core (corresponding to the filaments seen in sections) onto which interstitial material has been deposited during drying. Such a division of the structural substance of muscle into filaments and interstitial material naturally suggests some correlation with the two principal structural proteins in muscle, actin and myosin. However, the assessment in the electron microscope of the relative quantities of material present in the two forms is not yet sufficiently precise to permit of a more specific identification.

The 400 A axial period observed in frog, rabbit, and rat muscle stained with phosphotungstic acid arises from a preferential affinity for this stain, which is localized over regions about 50 A long in an axial direction, and which extends transversely across the interstitial material, giving the appearance of "bridges" between the filaments. It is of interest to compare the resulting sharp cross-striations with those seen in incinerated muscle by Draper and Hodge (13), which were ascribed to local high concentrations of Ca, Mg, and/or phosphate. Since the 400 A period is present in the interstitial material (myosin?), one must consider the possibility that the cross-striation is aligned across the fibril because that material is held "in register" by cross-linkage to the filaments: the period might then be an indication of the manner in which actin and myosin complex with each other.

SUMMARY

Thin sections of striated muscle from frog (sartorius), rabbit (psoas), rat (heart), and fly (flight muscle), and of smooth muscle from clam (adductor) have been obtained using a new microtome. Electron micrographs of them are presented. The sections are sufficiently thin to achieve a resolution of 30 to 40 A. In fly flight muscle, the myofibrils are distinct and well separated morphological units. In frog and rabbit muscle the myofibrils appear to be so closely packed under normal conditions that their identity as separate units is almost lost. In all types of striated muscle examined, it was found that the filaments are arranged within the myofibrils in a continuous and highly regular hexagonal array. The diameter of the filaments in embedded, sectioned muscle appears to be significantly less than that observed in dried shadowed material. The 400 A axial period, observed in frog and rabbit muscle, and in rat heart muscle, was found to extend across the interstitial material between the filaments.

The significance of these findings is discussed.

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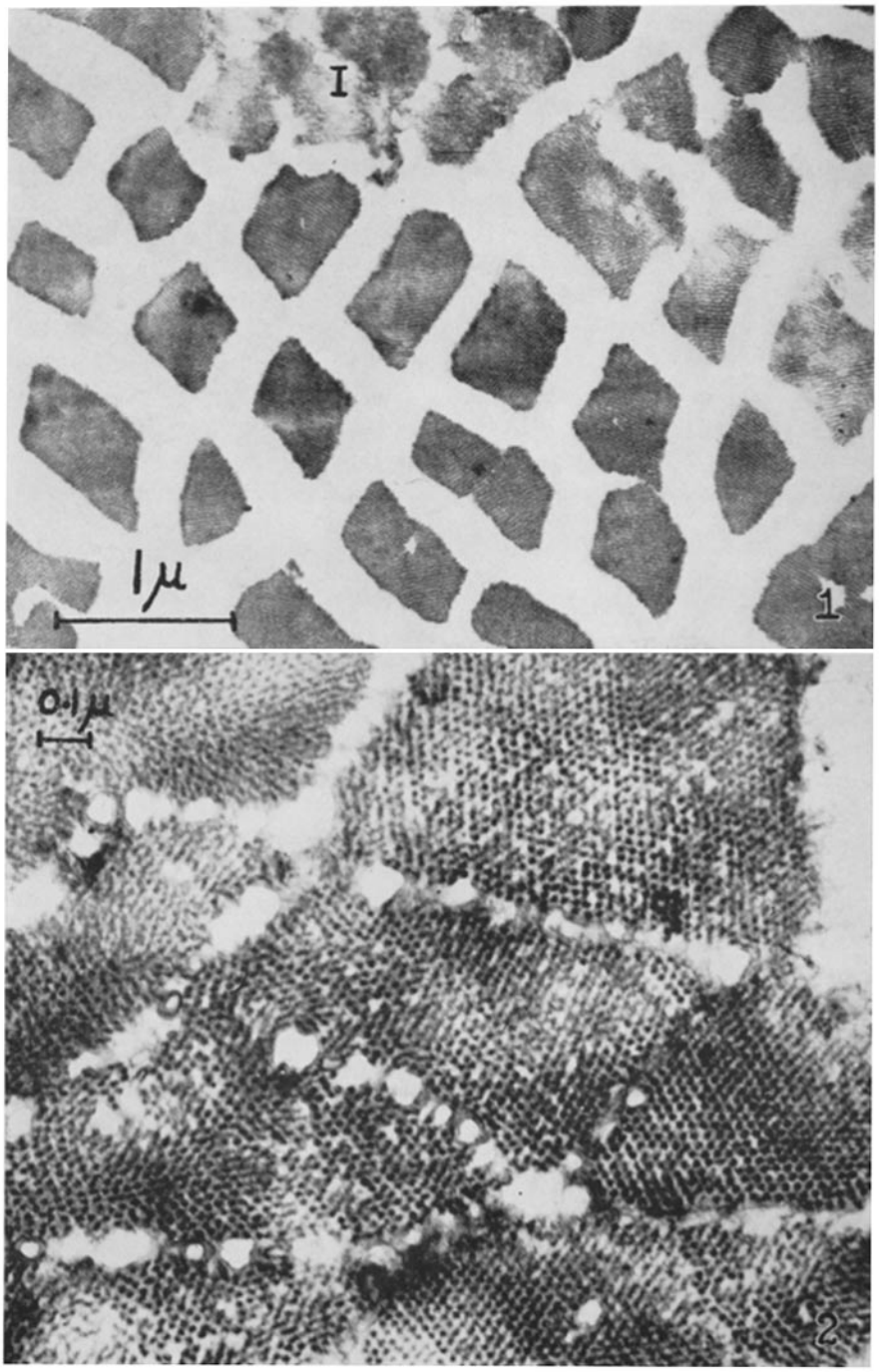
EXPLANATION OF PLATES

PLATE 1

FIG. 1. Transverse section of rabbit psoas muscle; fixed in osmium tetroxide pH 7.7 followed by phosphotungstic acid pH 5.4. The myofibrils appear very irregular in shape and are separated here by large empty spaces, which have probably formed during fixation. The hexagonal array of filaments within the myofibrils is barely perceptible at the magnification given. In the upper center of this picture, the disorganized appearance of an I band is visible. ×23,600.

FIG. 2. Transverse section of frog sartorius muscle; fixed in osmium tetroxide pH 7.7. The filaments (cut end-on) form a regular hexagonal array. A separation into myofibrils has occurred to a lesser extent than in Fig. 1. ×65,000.

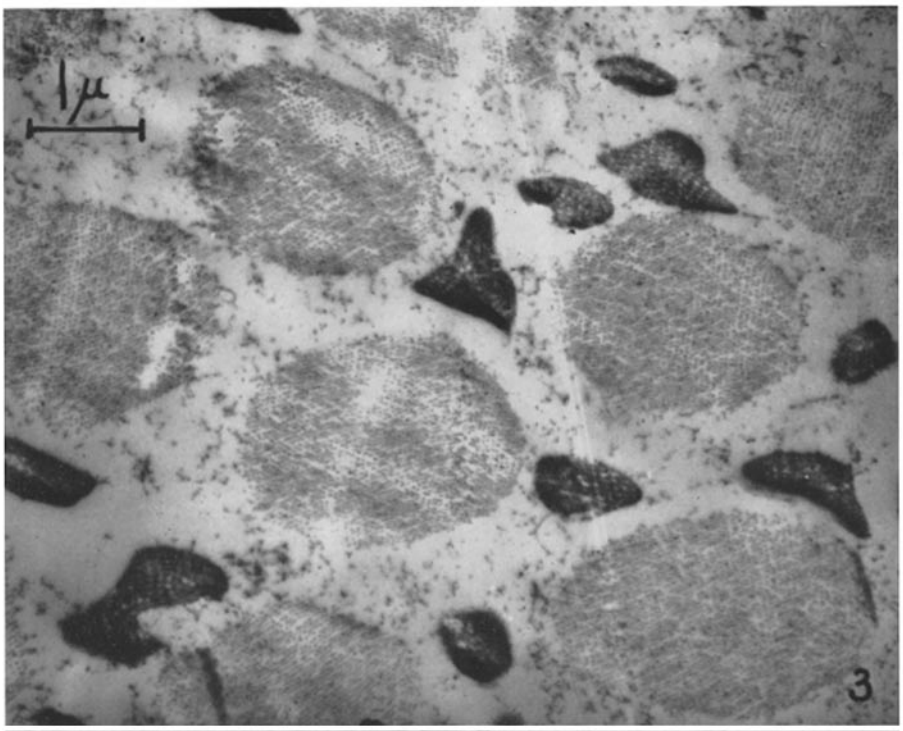


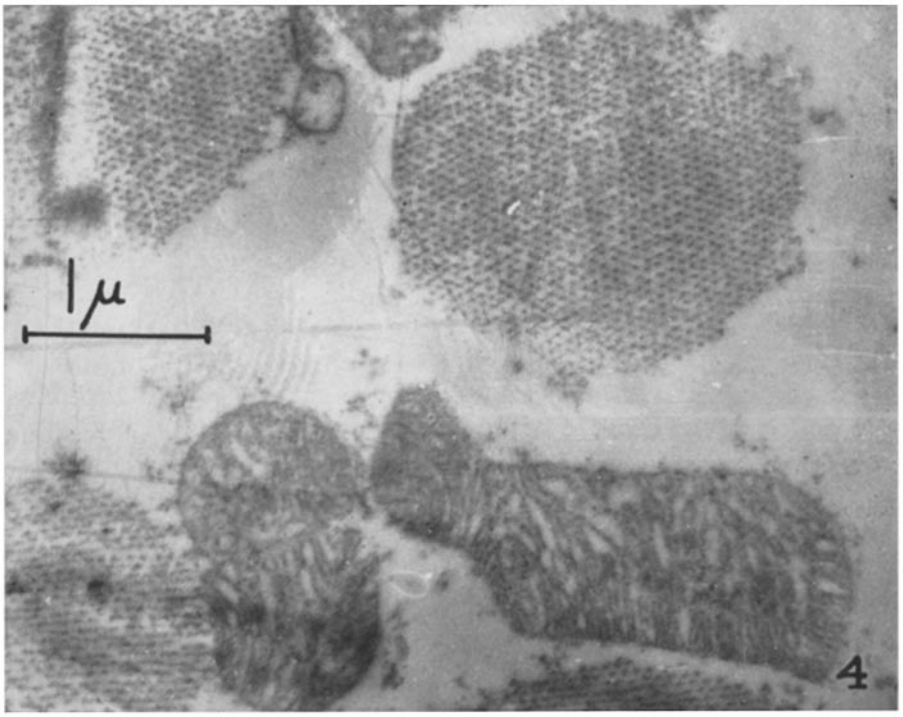
(Hodge *et al.*: Ultrathin sections of muscle)

PLATE 2

FIG. 3. Transverse section of fly wing muscle; fixed in osmium tetroxide pH 7.4. Large numbers of mitochondria can be observed lying between the myofibrils, which here appear roughly circular in section. ×15,500.

FIG. 4. As in Fig. 3, but higher magnification; showing hexagonal array of filaments within the myofibrils: the roughly hexagonal outline of the fibril in the top right of the picture may be noted. ×24,200.



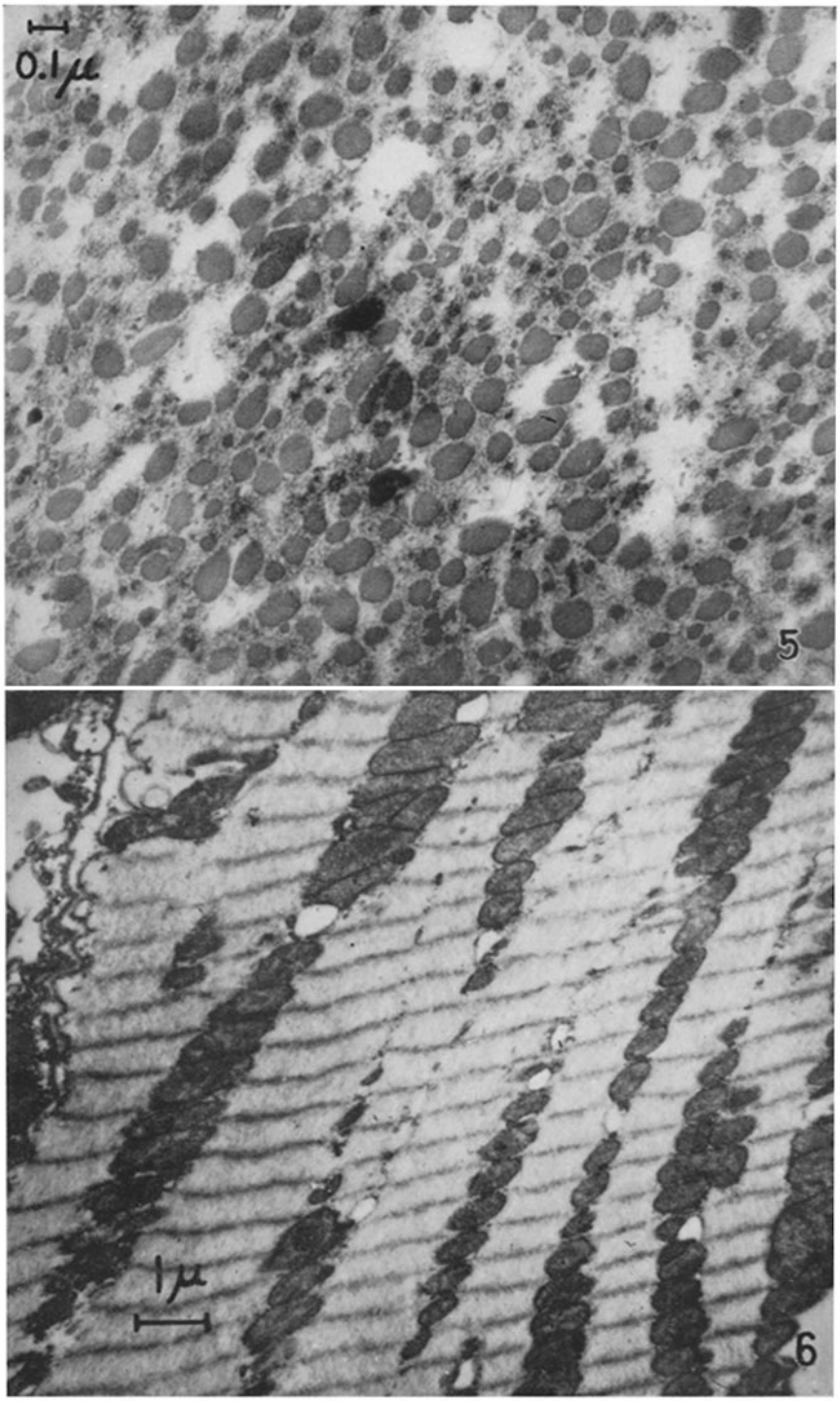


(Hodge *et al.*: Ultrathin sections of muscle)

PLATE 3

FIG. 5. Transverse section of clam (*Venus anodonta*) adductor muscle; fixed in formalin, stained in phosphotungstic acid pH 5.4. The filaments, cut end-on here, are roughly circular in section and their diameters range from 500 to 1000 A. They are apparently arranged at random. ×51,000.

FIG. 6. Longitudinal section of rat heart muscle; fixed in osmium tetroxide pH 7.5 followed by phosphotungstic acid pH 5.4. The muscle is greatly shortened and strong contraction bands have formed. Mitochondria can be seen lying in columns between areas of muscle. Very often one mitochondrion is associated with each sarcomere. The Z band and its attachment to the sarcolemma can be seen in the top left of the picture. ×10,000.

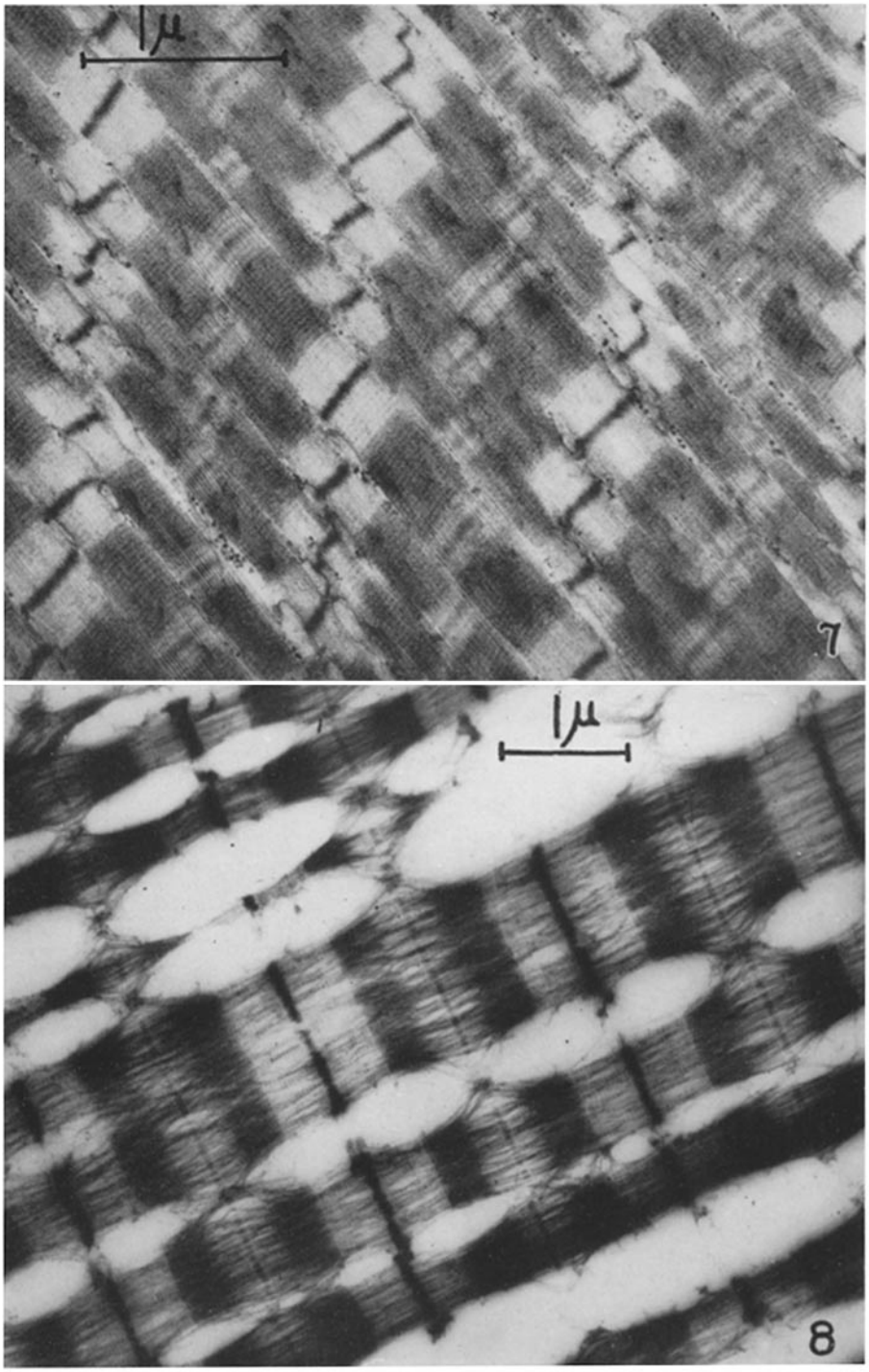


(Hodge *et al.*: Ultrathin sections of muscle)

PLATE 4

FIG. 7. Longitudinal section of rabbit psoas muscle; fixed in osmium tetroxide pH 7.7 followed by phosphotungstic acid pH 5.4. An axial period of about 350 A can be seen in both A and I bands. (It is not considered that the difference between this value and the usual one of about 400 A is significant, for shrinkage of the whole specimen may have occurred during preparation.) The darkly staining region which forms this period can be seen to extend across the space between adjacent filaments. ×27,600.

FIG. 8. Longitudinal section of frog sartorius muscle; fixed in formalin and stained in phosphotungstic acid. Portions of the ruptured Z band may be seen protruding on either side of the myofibrils, which have become separated during preparation: the 400 A axial period is again visible in both A and I bands. ×17,400.

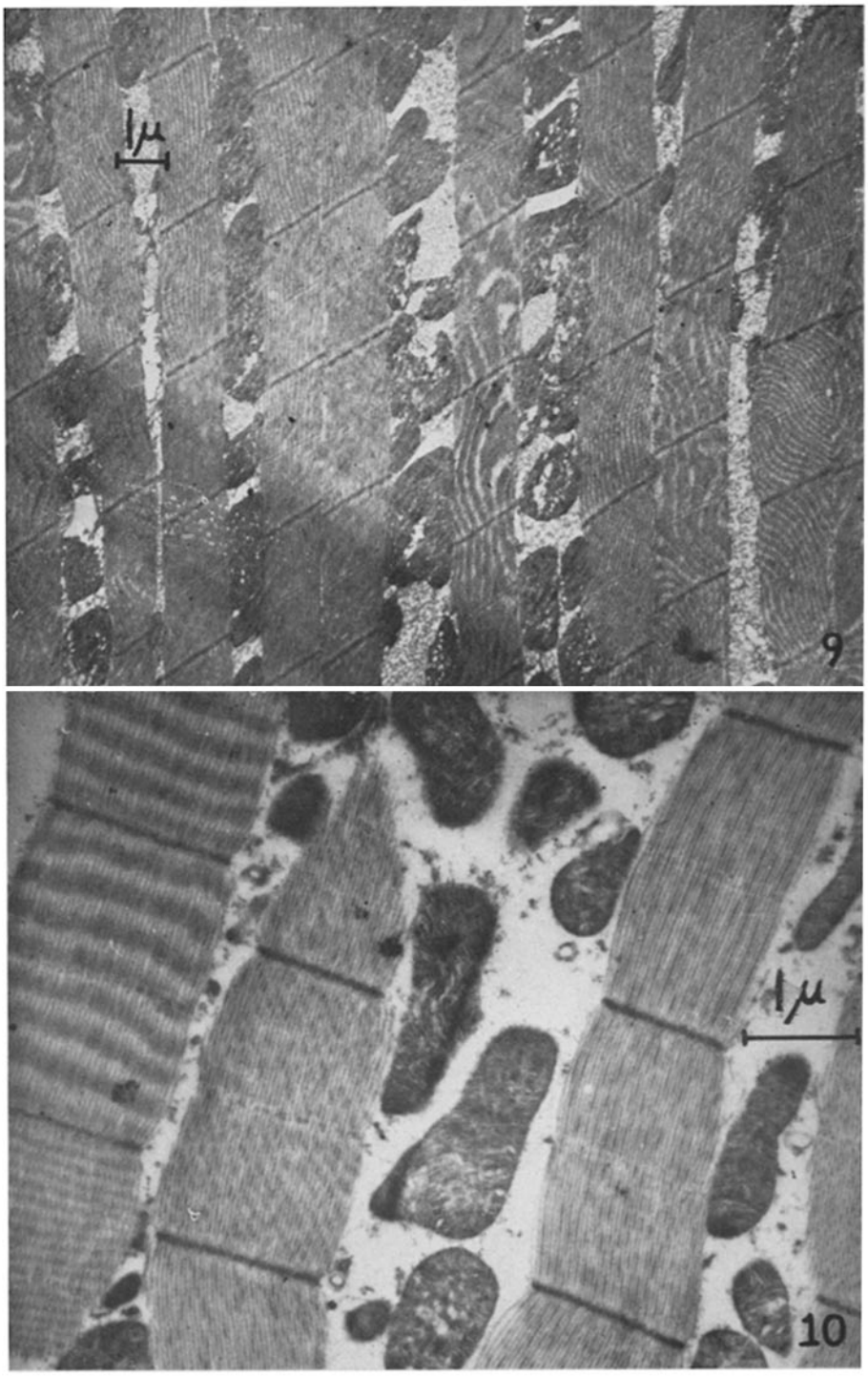


(Hodge *et al.*: Ultrathin sections of muscle)

PLATE 5

FIG. 9. Longitudinal section of fly flight muscle; fixed in osmium tetroxide pH 7.4. The individual myofibrils appear as distinct structural units, with large numbers of mitochondria between them. Pseudostriations, caused by the section passing through successive layers of the lattice of myofibrils, are visible in many areas. ×7,100.

FIG. 10. As in Fig. 9, but higher magnification; again showing arrangement of mitochondria and the occurrence of pseudostriations. In the fibril on the right hand side of the picture, the section is almost exactly parallel to one set of planes of the lattice; the filaments in one layer show up clearly, with those of the next layer lying in between them. ×16,100.



(Hodge *et al.*: Ultrathin sections of muscle)