THE FINE STRUCTURE OF MOSQUITO MIDGUT MUSCLE

CARL W. SCHAEFER, JEROME P. VANDERBERG, and JOHANNES RHODIN. From the Department of Zoology and Entomology, University of Connecticut, Storrs, Connecticut 06268; and the Department of Preventive Medicine, New York University School of Medicine, New York

INTRODUCTION

Smith et al. (1966) have recently described in detail the fine structure of several insect visceral muscles. The purpose of the present note is to extend their observations to the muscle of the *Anopheles quadrimaculatus* midgut by making available some electron micrographs obtained in the course of other studies.

MATERIALS AND METHODS

The midguts and associated muscle fibers of *A. quadrimaculatus* were dissected out in saline solution and immediately fixed in a solution of 1% osmium tetroxide in 0.1 M potassium phosphate buffer, pH 7.2, for 5 min at room temperature. The specimens were subsequently dehydrated in ethyl alcohol for 2 hr and embedded in Epon 812 (Luft, 1961). Sections were cut on an LKB ultra microtome with glass knives. After mounting on Formvar film, we stained the sections with uranyl acetate (Watson, 1958) and/or with lead hydroxide (Millonig, 1961). Subsequent to the staining, the sections were covered with carbon. Micrographs were taken with a Siemens Elmiskop I.

RESULTS AND DISCUSSION

The muscle of the mosquito midgut occurs as isolated fibers, often in the form of a network (Jones, 1960; O'Brien, 1965). The basement membrane of the gut is not confluent with that of the muscle fiber (Figs. 1 and 2), and the muscle is held to the midgut by a narrow connective tissue layer (Fig. 2). This attachment of muscle to midgut appears to be a loose one, judging by the ease with which the muscle is pulled away in our sections. A

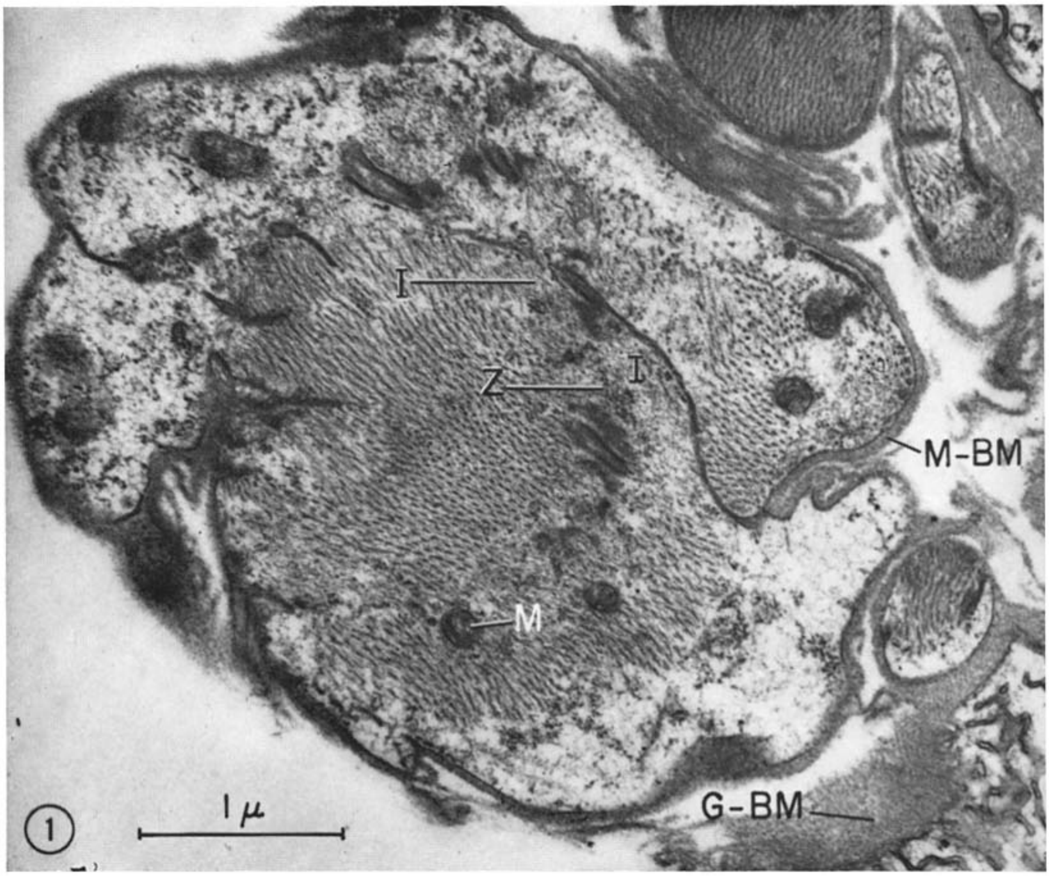

FIGURE 1 Cross-section (slightly oblique) of *Anopheles* midgut muscle, showing part of the gut wall (lower right corner) and the muscle fiber. *G-BM* and *M-BM* are the basement membrane of the gut and muscle fiber respectively. Mitochondria (*M*) are few. The dense region at *Z* may represent Z-band material; and the less electron-opaque areas at *I*, associated with cisternae of the sarcoplasmic reticulum, are portions of the I-band. × 26,500.

similar loose attachment is seen in the larval midgut muscle of the beetle, *Malacosoma* (Beams and Anderson, 1957).

The plasma membrane (= sarcolemma, see Bennett, 1960) of the fiber is a well defined layer which averages about 8 mμ in thickness (Fig. 3). It is surrounded by a granular and more amorphous layer (35–50 mμ), the basement membrane. Collagen-like fibrils appear to be absent in the basement membrane of the muscle fiber, although they are present and very regularly arranged in the basement membrane of the gut (Fig. 2); Baccetti (1963) notes their presence in other tissues of other insects. Infoldings of the plasma membrane form the T-system tubules (Figs. 3 and 5). The basement membrane passes across the gap of the infolded plasma membrane and occasionally enters it to some slight distance (Fig. 3).

The T-system is scant and very little convoluted, and bears little resemblance to that of insect fibrillar muscle (Smith, 1961, 1962; Shafiq, 1964). In Figs. 3 and 5, a T-system tubule is seen passing from one side of the fiber to the other with very few twists or turns. Occasionally, the tubules appear to fold back on themselves to enclose part of the ground substance in a double-membraned vesicle (Figs. 4 and 5). Cisternae of the sarcoplasmic reticulum are generally absent in a given section, which indicates that they are sparse; they may be

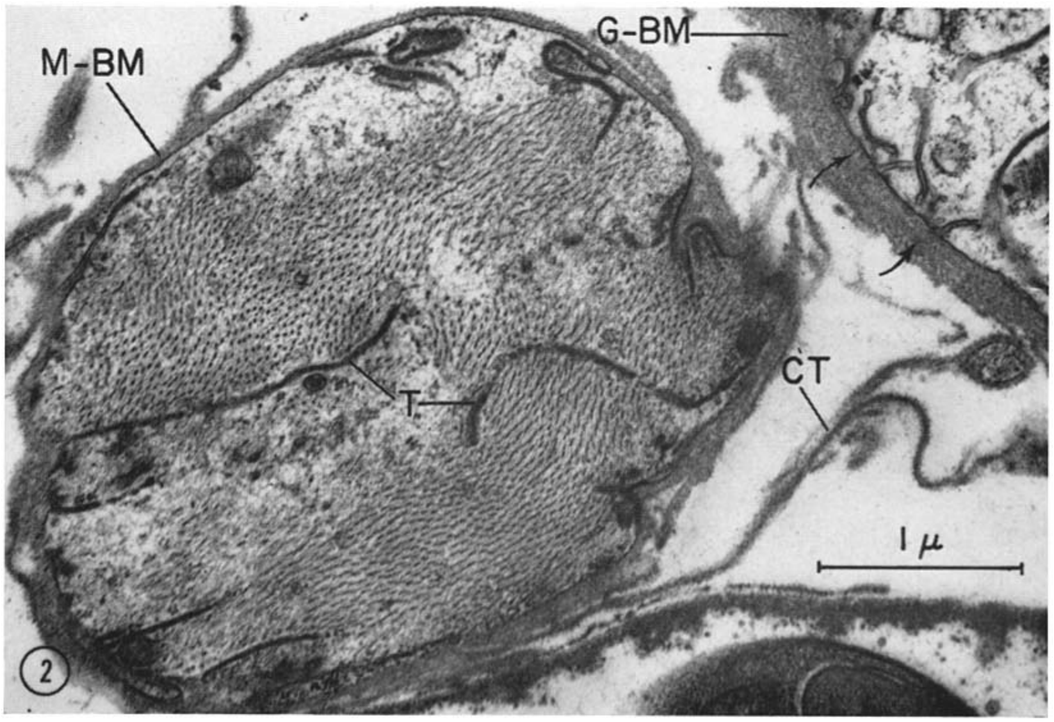

Figure 2 Cross-section of *Anopheles* midgut muscle, with muscle and part of gut in view. The two structures are loosely bound by a strand of connective tissue (*CT*). Note that T-system tubules (*T*) divide the fiber incompletely into fields of contractile material. Collagen fibrils are visible (curved arrows) in the basement membrane of the gut (*G-BM*) and are absent from the muscle basement membrane (*M-BM*). × 26,500.

represented in Fig. 3 (*cis*). Such cisternae are more abundant in the visceral muscles of other insects (Smith, Gupta, and Smith, 1966).

Mitochondria (Figs. 1 and 2) are nearly spherical with few and poorly developed cristae (Fig. 4). The space between the cristae contains some granular material. In all these features, the mitochondria resemble the developing sarcosomes of late-pupal honeybee flight muscle (Herold, 1965) and the degenerating sarcosomes of flight muscle in diapausing *Leptinotarsa* (Stegwee, Kimmel, de Boer, and Henstra, 1963). Mitochondria are small and few also in the visceral muscles (gut and reproductive system) studied by Smith, Gupta, and Smith (1966). However, they are more plentiful and their cristae are better developed in the heart muscle of the cockroach *Blattella* (Edwards and Challice, 1960). Heart muscle contracts continuously, whereas muscle of the gut and the reproductive system does not, and this functional difference appears to be reflected in the number and development of the mitochondria. The mitochondria of the mosquito midgut muscle are scattered about the periphery of the contractile fields, like those of the rectal musculature of the orthopteran *Ailopus* (Baccetti, 1962).

No tracheoles were seen in our sections, indicating that they are absent from this muscle (unlikely) or sparse. This scant tracheolation, and the lack of well developed mitochondria, suggest that this muscle is not particularly active; such is also the case in the insect visceral muscles studied by Smith, Gupta, and Smith (1966), and in the (nonfunctional) developing flight muscle of the honeybee (Herold, 1965).

Contractile material, consisting of an array of thick (myosin) and thin (actin) myofilaments, occurs throughout most of the fiber. The regions of this material are only incompletely separated from one another by tubules of the T-system (e.g., Fig.

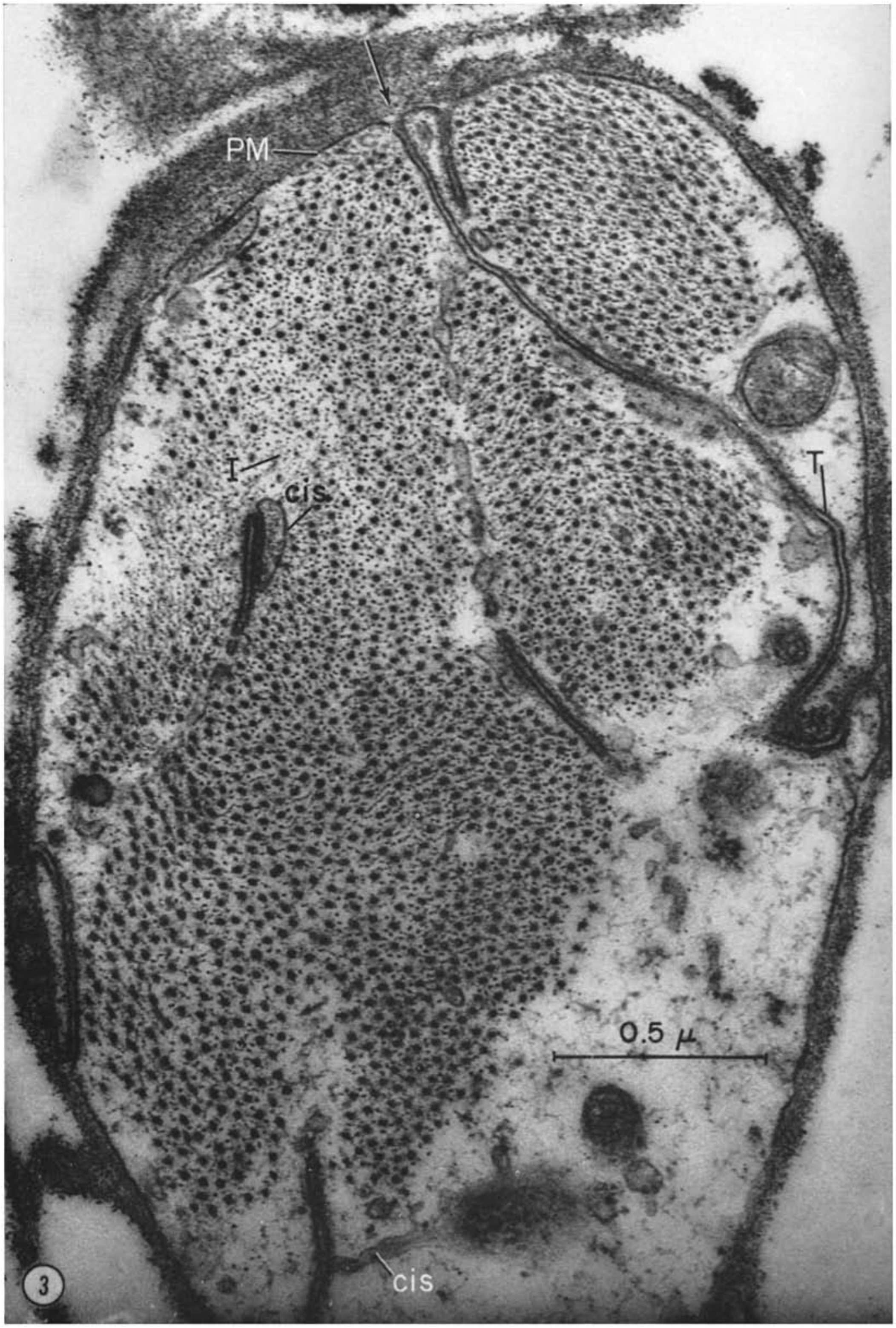

Figure 3 Cross-section of *Anopheles* midgut muscle. Basement membrane material is carried partway in (arrow) with the invagination of the plasma membrane (*PM*) which forms a T-system tubule (*T*). Note that the tubule is continuous from one side of the fiber to the other. Elsewhere, cisternae (*cis*) of the sarcoplasmic reticulum appear to be present. I-band material is evident at *I*. × 66,000.

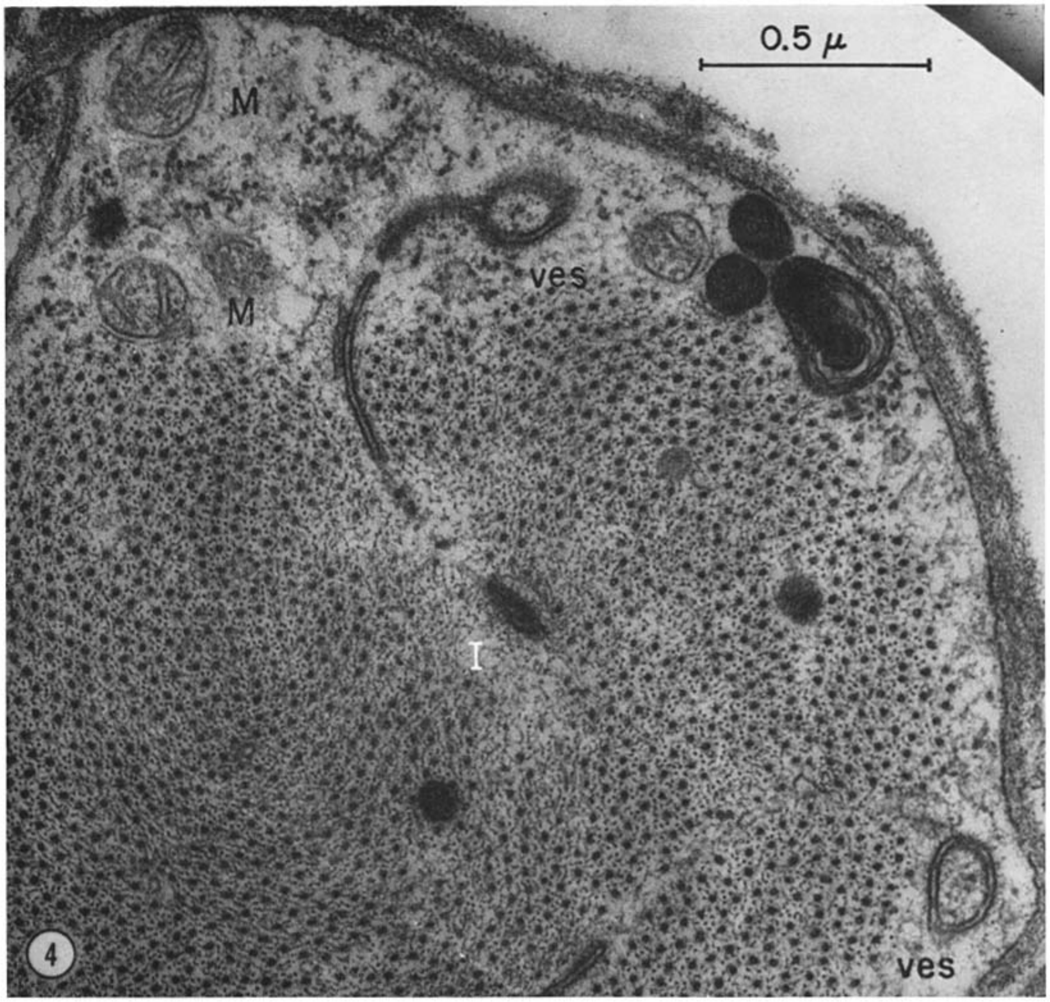

FIGURE 4 Cross-section of *Anopheles* midgut muscle. Note the vesicles (*ves*) formed by the T-system tubules folding-back on themselves. Cristae of the mitochondria (*M*) are few and poorly developed. *I*, I-band material. × 60,000.

2), and, in this respect, insect visceral muscle appears to differ from other types of insect muscle.

The thick (200-A) and thin (40-A) myofilaments are not arranged in the hexagonal pattern typical of vertebrate striated muscle and of insect flight muscle (Smith, 1962, 1964; Shafiq, 1963). Rather, each myosin (thick) filament is surrounded by 12 actin (thin) filaments, whose distances from the myosin and from each other range from 10 to 30 mμ. The 12-membered lattice is not easily seen in these sections; actin filaments are easily distorted, however, and the lack of pattern frequently observed, and the varying distances between filaments, may be more artifact than real This arrangement of the filaments occurs in other insect visceral muscle, in which it has been well described by Smith et al. (1966); a 6:1 actin:myosin ratio is characteristic of insect skeletal muscle, although Hagopian (1966) has described a 12:1 ratio in cockroach femoral muscle. Other examples of a 12-membered lattice occur in several other invertebrate phyla (references in Hagopian, 1966), and a similar arrangement may occur in heart muscle of the snail, *Helix* (see Fig. 2 of North, 1963).

There is little evidence of banding in these sec-

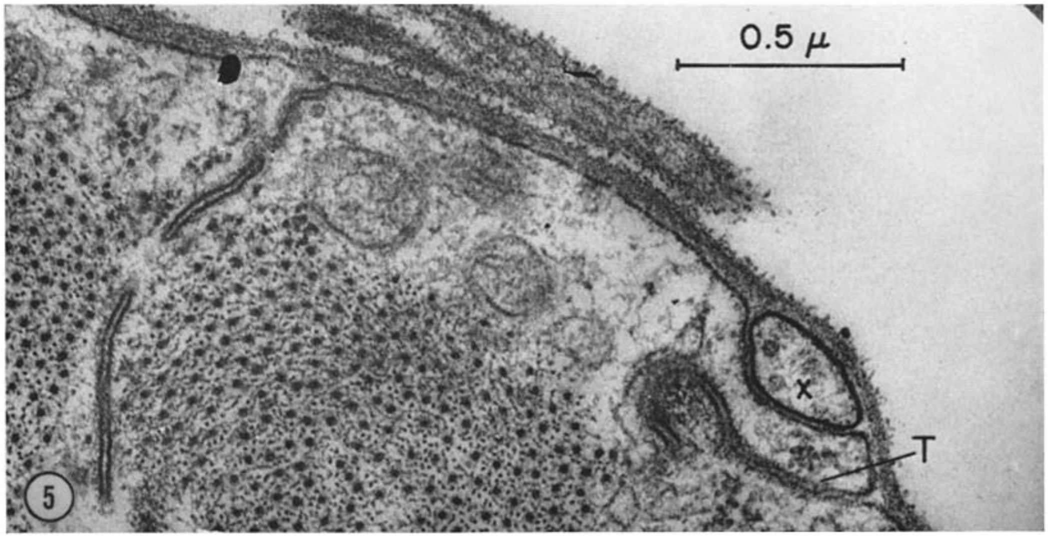

FIGURE 5 Cross-section of *Anopheles* midgut muscle. Note region (*x*) cut off by continuity of a tubule (*T*) of the T-system from one side of the fiber to the other. × 70,000.

tions, although dense Z-band material is suggested in Fig. 1; and I-band aggregates, with disorganized actin filaments, are seen (Figs. 1, 3, and 4) associated with cisternae of the sarcoplasmic reticulum. Unfortunately, no longitudinal sections were available and the details of the banding are obscure.

The muscle of the *Anopheles quadrimaculatus* midgut resembles that of the male reproductive system in the orthopterans *Carausius morosus* and *Periplaneta americana* and the midgut of the lepidopteran *Ephestia kühniella* (Smith et al., 1966). The cisternae of the sarcoplasmic reticulum are fewer in *Anopheles*, and the double-membraned T-system vesicles described here seem not to occur in the other insects. Nevertheless, the visceral muscles of insects in these phylogenetically distant orders are very similar.

The chief ways in which visceral muscle differs from other types of insect muscle are in the few and poorly developed mitochondria and tracheoles; the relatively random arrangement of the T-system; the incomplete separation of the fiber into discrete fields of contractile material (Edwards, 1960); and the doubling of the number of actin filaments relative to the myosin (Smith et al., 1966). The first three differences doubtless reflect the slower, more independent, and more random contractions of visceral muscle; the significance of the fourth difference is obscure.

SUMMARY

This muscle is very similar to other described insect visceral muscles. Tubules of the T-system and cisternae of the sarcoplasmic reticulum are sparse, mitochondria are few and their cristae are poorly developed, and the muscle is poorly tracheolated. These features are correlated with the muscle's low activity. Myosin myofilaments appear to be surrounded by 12 actin filaments, and there is little clear evidence of banding.

We are very grateful to Dr. David S. Smith (Papanicolaou Research Institute, University of Miami School of Medicine) for a critical reading of this paper.

Received for publication 27 February 1967.

REFERENCES

BACCETTI, B. 1962. *Redia.* **47**:105.

BACCETTI, B. 1963. *Monitor Zool. Ital.* 70–71:361.

BEAMS, H. W., and E. ANDERSON. 1957. *J. Morphol.* **100**:601.

BENNETT, H. S. 1960. Structure and Function of Muscle. G. H. Bourne, editor. Academic Press Inc., New York. **1**:137.

EDWARDS, A. E. 1960. *Ann. Rev. Entomol.* **5**:17.

EDWARDS, A. E., and C. E. CHALLICE. 1960. *Ann. Entomol. Soc. Am.* **53**:369.

HAGOPIAN, M. 1966. *J. Cell Biol.* **28**:545.

Herold, R. C. 1965. *Develop. Biol.* **12**:269.
Jones, J. C. 1960. *Ann. Entomol. Soc. Am.* **53**:459.
Luft, J. H. 1961. *J. Biophys. Biochem. Cytol.* **9**:409.
Millonig, G. A. 1961. *J. Biophys. Biochem. Cytol.* **11**:736.
North, R. J. 1963. *J. Ultrastruct. Res.* **8**:206.
O'Brien, J. F. 1965. *J. N. Y. Entomol. Soc.* **73**:226.
Shafiq, S. A. 1963. *J. Cell Biol.* **17**:351.
Shafiq, S. A. 1964. *Quart. J. Microscop. Sci.* **105**:1.
Smith, D. S. 1961. *J. Biophys. Biochem. Cytol.* **10**:123.
Smith, D. S. 1962. *Rev. Can. Biol.* **21**:279.
Smith, D. S. 1964. *J. Morphol.* **114**:107.
Smith, D. S., B. L. Gupta, and J. Smith. 1966. *J. Cell Sci.* **1**:49.
Stegwee, D., E. C. Kimmel, J. A. de Boer, and S. Henstra. 1963. *J. Cell Biol.* **19**:519.
Watson, M. L. 1958. *J. Biophys. Biochem. Cytol.* **4**:475.