Fine Structure of the Fibrillar Flight Muscles in the Housefly, *Musca domestica* (Diptera)

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INTRODUCTION

Insects of the orders Hemiptera, Hymenoptera, Coleoptera and Diptera have developed an asynchronous flight mechanism. The frequency of the muscle contraction is not directly related to the rate of nervous stimulation (Pringle 1965). An initial nervous stimulus produces an active state in the flight muscle during which a variable number of oscillatory contractions take place. Consequently, insects like the house-fly can maintain a very high frequency of wing beat (180-200 per second). Asynchronous flight muscles differ from the vertebrate skeletal muscles and insect synchronous flight muscles; in the latter each nerve impulse produces a single contraction of the innervated fibres. The asynchronous flight muscles are usually referred to as "fibrillar" muscles because of the easy dissociability of their large myofibrils. The existing information on the cytology of the fibrillar muscles is quite meagre as compared to the vertebrate striated muscles. The present report is concerned with the ultrastructural organization of the fibrillar flight muscles of the common house-fly, *Musca domestica*.

MATERIAL AND METHODS

The house-flies were raised in the laboratory and were maintained on the nutritive medium containing sugar, dry milk and egg. Dorsal longitudual and tergosternal muscles were dissected and fixed in 4% phosphate buffered glutaraldehyde for two hours, washed in phosphate buffer and post-fixed in 1% osmium tetroxide for one hour. Following the buffer wash but prior to the osmium fixation some of the tissues were incubated in saliva at 37°C for 2 hours for the digestion of glycogen. Tissues were dehydrated in an ascending series of ethanol concentrations and embedded in Maraglas. Thin sections were cut with an LKB Ultrotome microtome and stained with uranyl acetate and lead citrate. A Hitachi HU11B2 electron microscope was used for observations.

Supported by NSF Institutional Grant GU-3093 awarded to Southern Methodist University.

OBSERVATIONS

The thoracic flight muscles are comprised of large elongated myofibres. Each myofibre is bounded by a sarcolemma which consists of an outer amorphous-looking basement membrane and an inner osmiophilic plasma membrane (Fig. 1).

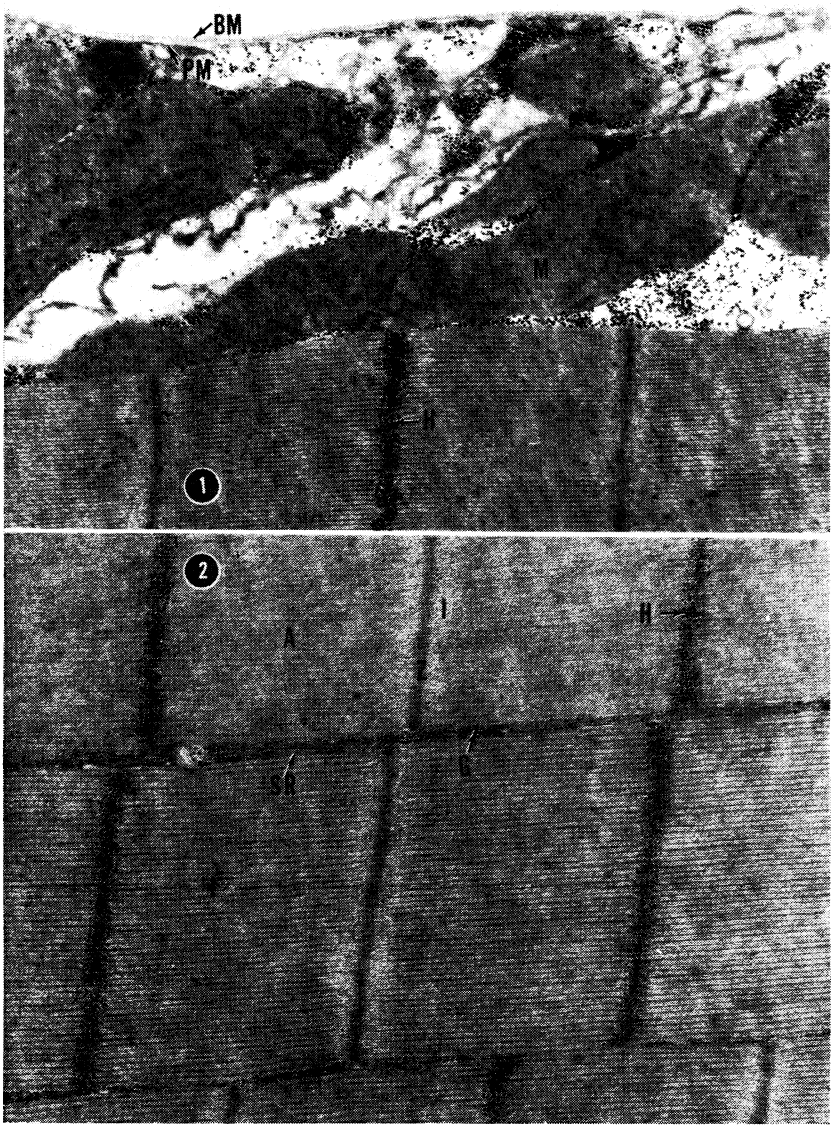
Contractile elements of the myofibre consist of numerous myofibrillar bundles which run across the longitudual plane of the myofibre. The myofibrils are approximately 1.5μ in diameter and are striated (Fig. 2). Each sarcomere (Z- to Z-band) consists of a Z-, 1-, A-, and H-band. The Z- band is a dense structure consisting of interconnecting filaments (Fig. 2). The detailed structure of the Z- band could not be adequately resolved. The Z- band is flanked on either side by a very narrow 1- band. The 1- band consists of thin filaments approximately 60A° in diameter which extend to the margin of the H- band.

The A- band is composed of thick filaments approximately 110A° in diameter and the thin filaments of the I- band which extend into the A- band region. The thick filaments have an electron luscent interior bonded by a dense cortex. In transverse sections the filaments of the A- band show a hexagonal pattern, each thick filament is surrounded by six thin filaments (Fig. 3). The thick and thin filaments are arranged in such a manner that there is one thin filament between every two thick filaments. The interconnecting bridges between thick and thin filaments can be identified in Figure 3. The thick filaments taper off towards their terminal portions.

The H- band is in the center of the A- band where the I- band filaments are absent. Glycogen particles approximately 220A° in diameter are seen in this region. The H- band can be seen advantageously in transverse sections of saliva incubated tissues where glycogen has been digested (Fig. 4). The thick filaments in H- band region are uniformly dense and are not surrounded by a ring of thin filaments

FIG. 1. A longitudinal section through the peripheral region of a myofibre from transverse thoracic muscle. The myofibre is bounded by a sarcolemma consisting of an outer basement membrane (BM) and an inner plasma membrane (PM). Large mitochondria (M) are seen in the subsarcolemmal region. Glycogen (G) particles are seen in the subsarcolemmal and interfibrillar regions and also in the H— band (H) region × 40,000.

FIG. 2. A longitudinal section through the transverse thoracic muscle showing the Z—, I—, A—, and H— bands of the myofibril. Glycogen (G) particles are aligned along the H— band. Narrow cisternae of sarcoplasmic reticulum (SR) are seen in the interfibrillar regions × 39,000.


BM
PM
M
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1
2
A
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H
SR

(Fig. 4). M- line resulting from the local thickenings of the thick filaments is seen in the center of the H- band (Fig. 5).

The myofibre is richly populated by the mitochondria which occur in the subsarcolemmal and interfibrillar spaces closely aligned with the myofibrils (Figs. 1, 5). Mitochondria are large and elongated. They are densely packed with cristae and have a dense matrix. In several mitochondria cristae are arranged in stacks.

Sarcoplasmic reticulum (SR) in the fibrillar muscles of *M. domestica* is poorly developed. The cisternae of SR are narrow and have an electron dense lumen (Fig. 2). They are most frequently seen in the interfibrillar spaces. There are numerous vesicular structures of variable sizes within the sarcoplasm (Fig. 6) which may be comparable to the transverse tubules in other muscles or may represent the finer branches of trachaeoles. Several of these vesicular structures have a rather thick, diffused, and irregular limiting membrane which makes it difficult to establish their true identity. The T- tubules are formed by the invaginations of the surface plasma membrane into the sarcoplasm and their lumen is in continuity with the external milieu (Smith, 1966). Consequently it is possible to identify the T- tubules by immersing the myofibres in a physiological solution containing ferritin or lanthanum. Such studies are currently in progress in our laboratory. In this study only the plasma membrane extensions from the peritracheolar lining were considered as T- tubules.

Dyadic configurations are formed by the close approximation of the cisternae of SR and the T- tubules (Fig. 7). The dyads do not show any definite orientation in relation to the striation pattern of the myofibrils. Frequently, they are seen at the level of the H- band. Sarcoplasm is very richly populated with glycogen particles. These particles are approximately 220A° in diameter and stain intensely with lead stains. That these particles are glycogen in nature was confirmed

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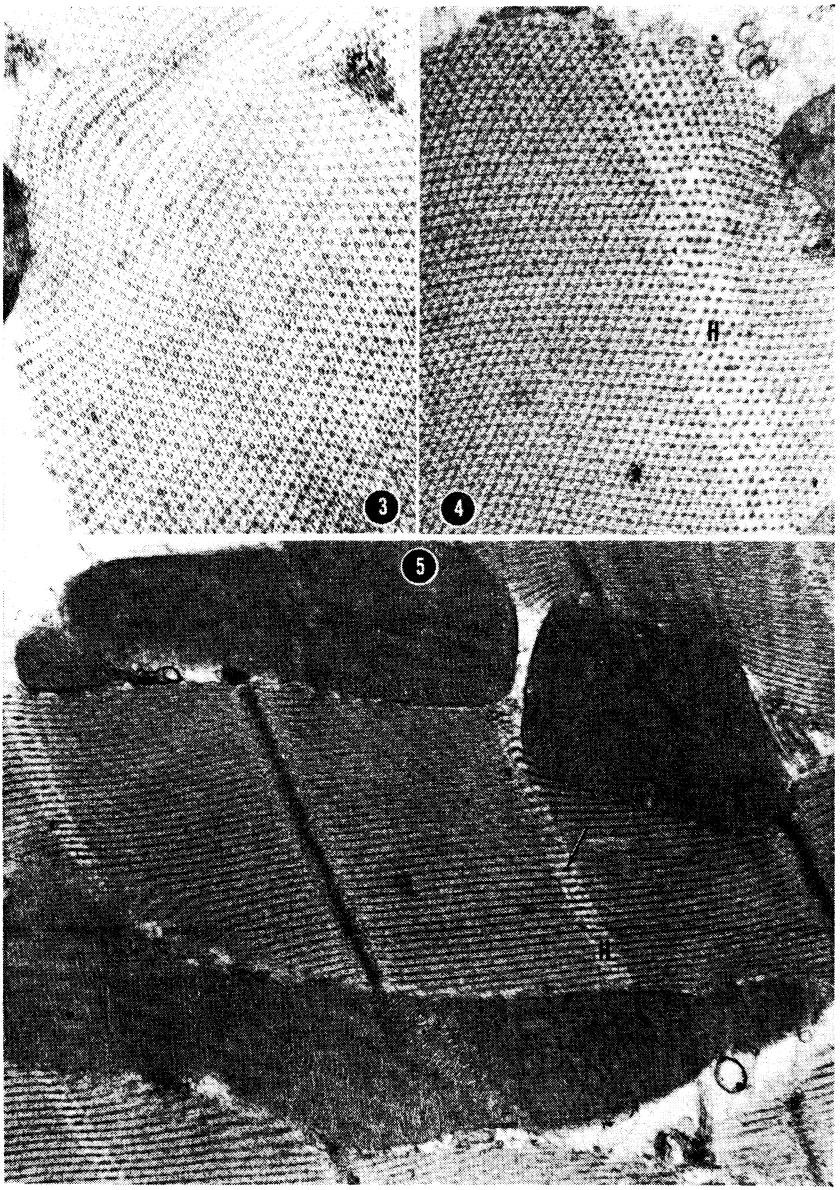
FIG. 3. Transverse section through the A-band region of a myofibril from transverse thoracic muscle. Thick filaments are hollow and each thick filament is surrounded by six thin filaments × 75,600.

FIG. 4. A transverse section through the saliva incubated transverse thoracic muscle. The thick filaments in H— band (H) region are uniformly dense and the surrounding thin filaments are absent × 70,000.

FIG. 5. A longitudinal section through the saliva incubated transverse thoracic muscle showing a complete digestion of glycogen particles. The H— band (H) region is in the middle of the A— band. M— line (⟶) seems to arise from local thickenings of thick filaments in the H— band × 51,000.


3
4
A
5
H

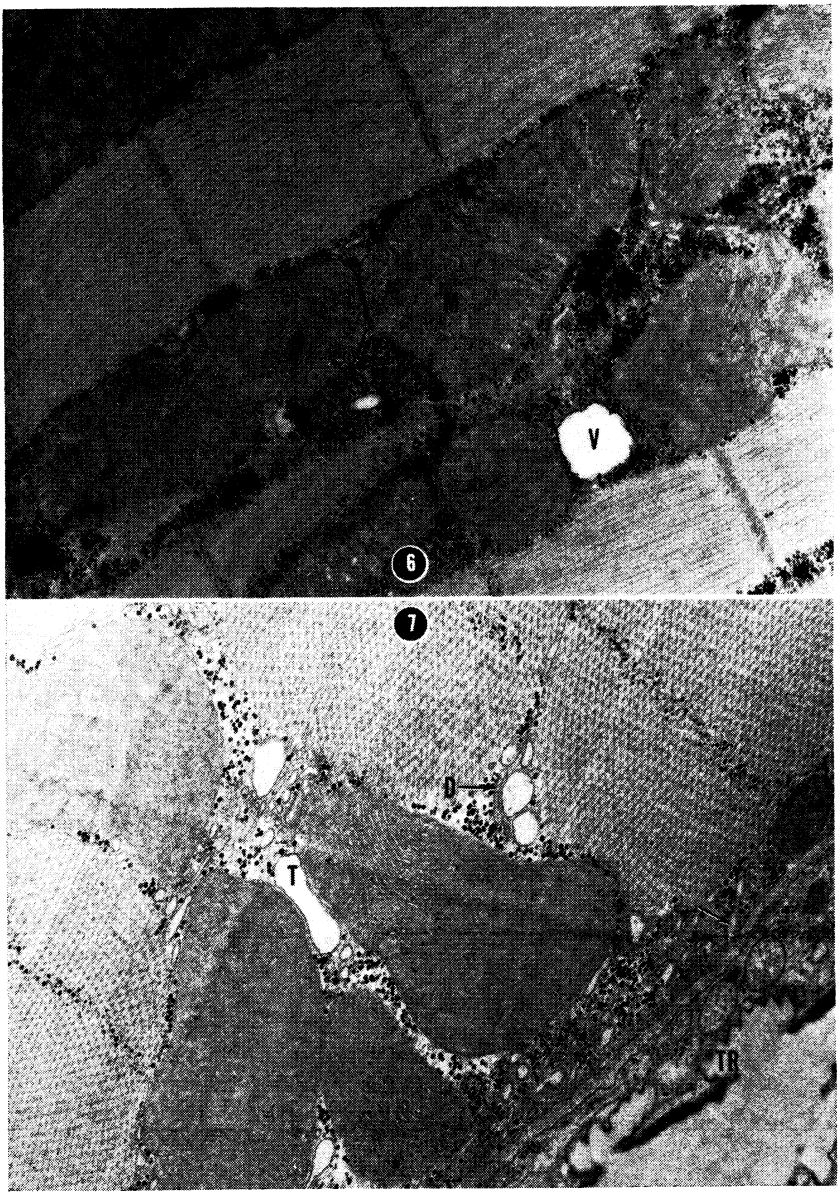
by the incubation of glutaraldhyde fixed tissues in saliva. In saliva-treated tissues these particles were absent (Fig. 5). Glycogen particles were seen in the subsarcolemmal and interfibrillar spaces where they surround the mitochondria. Glycogen particles are regularly present along the H- band region of the myofibrils (Fig. 2). The myofibres are multinucleated and the nuclei are seen both at the periphery as well as in the interior regions of the myofibre. The nuclei are elongated along the longitudinal axis of the myofibre. The chromatin is present in the form of dense heterochromatin patches along the nuclear periphery and the finely granular and fibrillar euchromatin.

DISCUSSION

The fine structure of the fibrillar muscles in different insectan orders has been previously described by other authors (Huxley and Hanson, 1957; Shafiq, 1963, 1964; Smith, 1961, 1962, 1965; Ashhurst, 1967). The transverse tubular system has been reported to be well developed in the fibrillar muscles of Coleoptera (Smith, 1961), Hymenoptera (Smith, 1962), Hemiptera - Homoptera (Smith, 1965) and Diptera (Shafiq, 1964). In these muscles the T- tubular system is formed by the invaginations of the circumtracheolar sheath of the surface muscle cell membrane into the sarcoplasm. The diameter of these extra cellular tubular extensions is highly varied (Smith, 1965). Whether such extracellar channels are comparable to the T- tubular system is debatable. In *M. domestica* similar extracellular channels were seen as vesicular structures of various dimensions. Unlike the smooth appearance of the plasma membrane, the limiting membranes of many of these structures were rather thick and irregular. Based on the present studies, it is difficult to say if the limiting membranes of these vesicular structures are membranes of T- tubules or are related to the trachaeolar system. In this study only those tubular elements which directly extend from extracellular channels (Fig. 7) or form dyadic configurations with the cisternae of the sarcoplasmic reticulum were identified

FIG. 6. A longitudinal section through the dorsal thoracic muscle showing vesicular structures (V) bounded by dense irregular membranes. The exact nature of these structures is unknown. Large mitochondria (M) are seen in the interfibrillar spaces × 36,000.

FIG. 7. An oblique section through the dorsal thoracic muscle showing the transverse tubular invagination (⟶) of the sarcolemma. T— tubules (T) appear as vesicular structures of variable diameter. A dyad (D) and a tracheole (TR) are identified × 45,750.


V
6
7
D
T
TR

as T- tubules. Most of the studies on the fibrillar muscle indicate a poor development of sarcoplasmic reticulum (Smith, 1965, 1966).

It is now relatively well established at least in the vertebrate skeletal muscle that the T- tubules act as a pathway for the inward spread of the electrical impulse from the surface sarcolemma to the interior of the sarcoplasm. The cisternae of sarcoplasmic reticulum are involved with the contraction of the myofibres in, as yet, an unknown fashion, but probably by releasing and capturing calcium ions (Hasselbach, 1964; Freygang, 1965; Peachey, 1966). Fast-beating muscles like the cricothyroid muscle of the bat (Revel, 1962) and the muscle in the toad-fish swim bladder (Fawcett and Revel, 1961) have a highly developed sarcoplasmic reticulum. It is thought that the fast contraction rate in these muscles is structurally reflected in the SR hypertrophy.

Fibrillar muscles in insects have a very fast rate of contraction and relaxation. The rate of contraction in these muscles greatly exceeds the rate of neurogenic depolarization (Roeder, 1951; Boettiger, 1957; Pringle, 1965). The nerve impulse creates an active state in the muscle, which undergoes many contraction cycles in an oscillatory fashion. In spite of the rapid contraction rate of the fibrillar muscles, the excitation-contraction coupling appears to be rather slow and is reflected in the poor development of the sarcoplasmic reticulum (Pringle, 1965). Sarcoplasmic reticulum may be involved in the initial activation of the muscle by neurogenic stimuli but is probably not concerned with the oscillatory contraction cycles (Jewell and Ruegg, 1966). Our results are in agreement with others, in that the sarcoplasmic reticulum in *M. domestica* is very poorly developed. However, the mechanism of the excitation-contraction coupling in the fibrillar muscle is poorly understood at this time so as to warrant any conclusions regarding the specific role of the T- tubular system and the sarcoplasmic reticulum.

The myofilament arrangement of the insect fibrillar muscle was first described by Huxley and Hanson in *Calliphora* (1957). The basic myofilament pattern was similar to the vertebrate striated muscle, however, in *Calliphora* the thin filaments were more numerous and each thin filament was shared by two thick filaments. In the A- band of *M. domestica* each thick filament is surrounded by six thin filaments in a hexagonal pattern and each thin filament is shared by 2 thick filaments. The M- lines could not be identified by Shafiq (1963) in *Drosophila melanogaster,* however, in *M. domestica* the M- line appears as local thickenings of the thick filaments in the center of the H- band.

SUMMARY

The organization of the fibrillar flight muscle of *Musca domestica* was studied by electron microscopy. The transverse tubular system and the sarcoplasmic reticulum are poorly developed. Dyads did not show any preferential orientation in regard to the myofibrillar striation bands. I- band is quite narrow. In transverse sections the thick filaments have a hollow interior and each thick filament is surrounded by six thin filaments in a hexagonal pattern. H- band is seen in the middle of the A- band and the M- line appears as local thickenings of the thick filaments in the center of the H- band. Large elongated mitochondria occur in the interfibrillar regions of the myofibre. The sarcoplasm is heavily populated with glycogen particles. Elongated nuclei are situated in the subsarcolemmal and the central region of the myofibre.

ACKNOWLEDGMENTS

Technical assistance of Mr. James Mitchell is gratefully acknowledged.

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