*Bull. Inst. Zool., Academia Sinica* 12(1): 27-37 (1973)

# ULTRASTRUCTURE OF THE LARVAL AND THE ADULT ABDOMINAL MUSCLE OF THE YELLOW MEAL WORM *TENEBRIO MOLITOR* L.*

WEN-YUNG LEE AND MEI-YU YOUNG

Research Fellow Institute of Zoology, Academia Sinica.
Assistant. Institute of Zoology, Academia Sinica.

*Received for Publication, May 9, 1973*

## SUMMARY

**W. Y. Lee and M. Y. Young** (1972) *Ultrastructure of the Larval and the Adult Abdominal Muscle of the Yellow Meal Worm* Tenebrio molitor L. Bull. Inst. Zool., Academia Sinica 11(2): (27-37). The Ultrastructures of *Tenebrio molitor* larval and adult abdominal muscles are similar. Bands zigzag irregularly across the myofibrils. Sarcoplasmic reticulum formed cisternae around, and in places penetrates into the myofibrils. Dyads are mostly located at the A-I band junctions. More dyads appear in the larval muscle than in the adult. Sarcolemma of amorphous materials and a basement membrane, is thicker in larval muscle than in the adult. The ratio of thin and thick myofilaments is 6:1. Sarcosomes are located at both sides of Z-bands. Fine structures of the larval and adult abdominal muscles are compared with the muscles of other insects.

The ultrastructure of the metathoracic muscles in the larvae and adults of *Tenebrio molitor* L. has been studied[11]. The transformation of the flight muscles from larval muscles has also been investigated[12]. The present report is the continuous research concerning the fine structure of abdominal muscles in larvae and adults of *T. molitor* L.

## MATERIALS AND METHODS

*Tenebrio molitor* L., have been reared in our laboratory for more than seven years. Fully grown larvae and adults with brownish integument were killed by injecting each individual 0.3-0.5 ml. 6% glutaraldehyde in 0.1 M cacodylate buffer, at pH 7.2. The pretreatment prevents muscle contraction, thus minimizes possible artifacts. The dorsal longitudinal muscles of the second and third abdominal segments were obtained by severing the injected insects in the same fixative.

The dissected muscles were further fixed in the same glutaraldehyde fixative for 3 hours. Subsequent treatments of the specimens for Electron Microscopy were conducted as before[11]. Sections were examined under a Hitachi Electron Microscope type 11A operated at 50 kv or 75 kv.

## RESULTS

The structural components of both larval and adult muscles of *Tenebrio molitor* L. are

* This investigation was supported by a research grant from Biological Research center, Nationl Seience Councel, R. O. C.

summarized in Table I.

TABIE I

The structural components of *Tenebrio molitor* larval and the adult abdominal muscles.

| Muscle | Average Sarcomere length (μ) | Ratio of Thick and Thin filament | *Dyads* sarcomere bet. adjacent myofibrils | Thickness of sarcolemma (μ) | Average length of sarcosomes (μ) | | shapes of sarcosome |
|---|---|---|---|---|---|---|---|
| | | | | | L-plane | T-plane | |
| larval | 6.08 | 6:1 | 1-2 | 0.174 | 1.21×0.36* | 0.99×0.22* | rod |
| adult | 4.88 | 6:1 | 0-2 | 0.069 | 1.02×0.28* | 0.51×0.29* | various |

* lengrh by width

**Sarcomeres:**

The striation pattern of the sarcomere in the larval abdominal muscle appears similar to that of adult. Bands zigzag irregularly across myofibrils (Figs. 1 an 2). The alignments of Z bands (Figs 1 and 2) are continuous and clear in the larval as well as adult muscles. However Z bands also appear zigzag. Both A and I bands (Figs 1 and 2) are similar in length, but in some ultramicrographs. I bands appear shorter, possibly due to the contraction during fixation. H bands can not be defined in the longitudinal sections. They can be identified, however, in the transverse sections by the absence of thin filaments (Figs 5 and 8). No. M band is present in muscles of the larvae and adults.

The sarcomere of the larval abdominal muscle is ca. 6.08 μ long whereas that of adult ca. 4.88 μ (Table I).

**Sarcoplasmic Reticulum:**

Transverse sections of both larval and adult abdominal muscles (Figs 11 and 13) revealed that some myofibrils are incompletely separated by the cisternae of sarcoplasmic reticulum while others are completely divided into an irregular shape. Dyads are more frequently found at the A-I band junctions of larval muscles than in those of adults (Figs 1 and 13). In all cases, sarcolemma appears to consist of two layers; amorphous material and a thin layer of basement membrane (Figs 11 and 12). The amorphous material of larval muscle, however, much thicker than of adult (Table 1). T tubules arising from invaginations of the basement membrane are evident in all of the sarcolemma observed (Figs 11 and 12).

Microtubules mostly scatter in the I-band and associated with elements of sarcoplasmic reticulum (Figs 9, 10, 11, 12 and 13).

**Myofibrils:**

In the transverse sections (Figs 3-6), no difference in the arrangment of myofilaments can be detected for larval and adult abdominal muscles. A thick filament is surrounded by 11 to 13 (usually 12) thin ones. Thus the overall ratio between them is 6:1.

**Sarcosomes:**

The distribution of sarcosomes in both larval and adult abdominal muscles is similar. The organelle is generally confined to the I-band level. The measurement of the organelle in longitudinal sections (Figs 1 and 2) and transverse sections (Figs 3, 6, 9 and 10) are summerized in table 1. Sarcosomes appear frequently elongate in the longitudinal and transverse sections of both larval and adult muscles. However, y-shape, u-shape and irregular sarcosomes are also observed in the transverse sections of the adult muscle (Figs 6, 10 and 11). The cristae of sarcosomes in larval and adult muscles are remarkably similar. There are generally 11-13 of cristae per 0.36 μ length of sarcosomes and the cristae are parallelly arranged.

## DISCUSSION

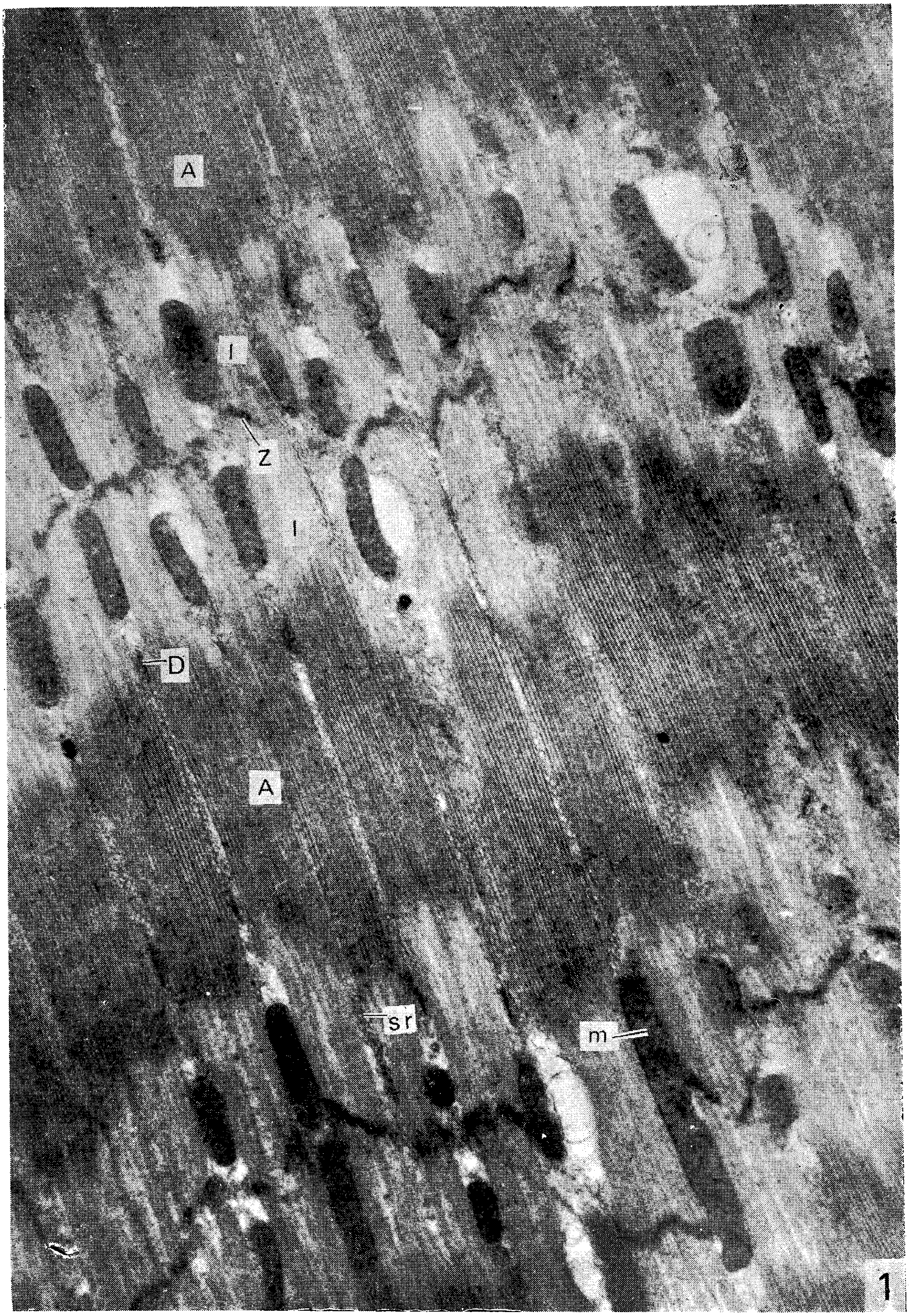
The fine structures of metathoracic dorsal longitudinal muscles of larval *Tenebrio molitor* .L can be easily distinguished from those of the adult[11]. However, the abdominal muscles of the larvae are not only similar to those of adults according to the Electron microscope studies, but also appear resembling to the larvae metathoracic muscles previously reported[11]. Breed[2], studied the beetle, *Thymalus marginicollis* chevr., with light microscope and found that abdominal muscles of the thymalus beetle remain unaltered from larvae to imago. Crossley[5] reported that abdominal intersegmental muscles of *Calliphora* larvae and adults are extremely similar in fine structure. The tenebrio abdominal muscles are ultrastructurally similar to the abdominal intersegmental muscles of larval *Calliphora*[4], to the abdominal intersegmental muscles of adult male *Periplanata americana*[16] and the visceral muscles of insects[17]. In all of the aforementioned muscles, bands of sarcomere zigzag irregularly across the muscle cells, and sarcosomes scatter at I-bands, both sides of Z-bands.

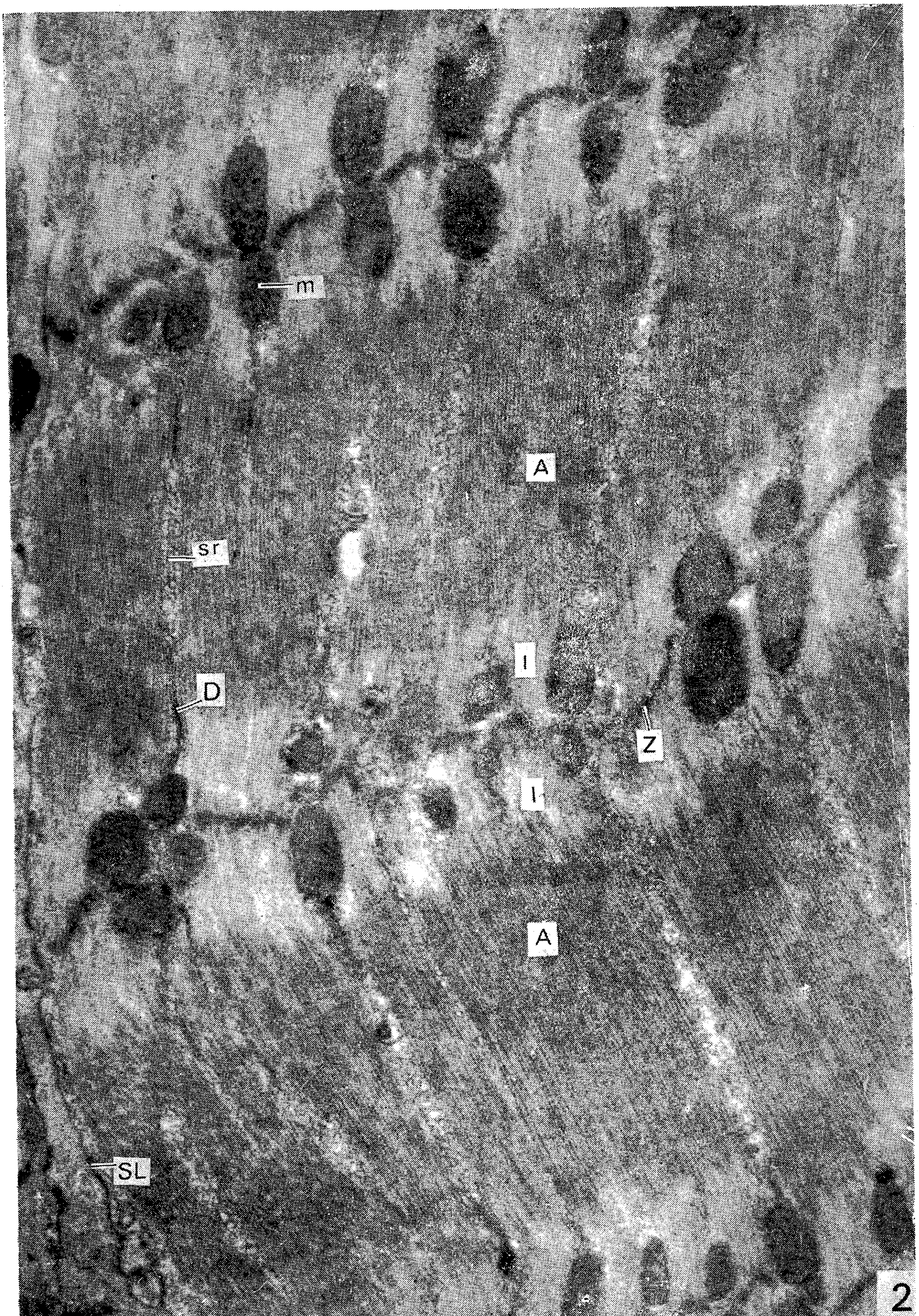
Huxley and Niederberke[9] proposed that the relationship among sarcomere length, contraction speed, and total tension is in qualitative agreement with each other. The hypothesis was substantiated by the work with *Periplanata americana* in which the long-sarcomere fibers in coxal muscle 135b and the proximal part of the tibial extensor muscle were found to receive innervation from "slow" motor axons whereas the other muscles of coxa which have shorter sarcomere fibers were not[10]. For the tenebrio, the average sarcomeres of larval abdominal muscle are slightly longer than those of the adult. However, whether the structural difference has any locomotive significance is not known.

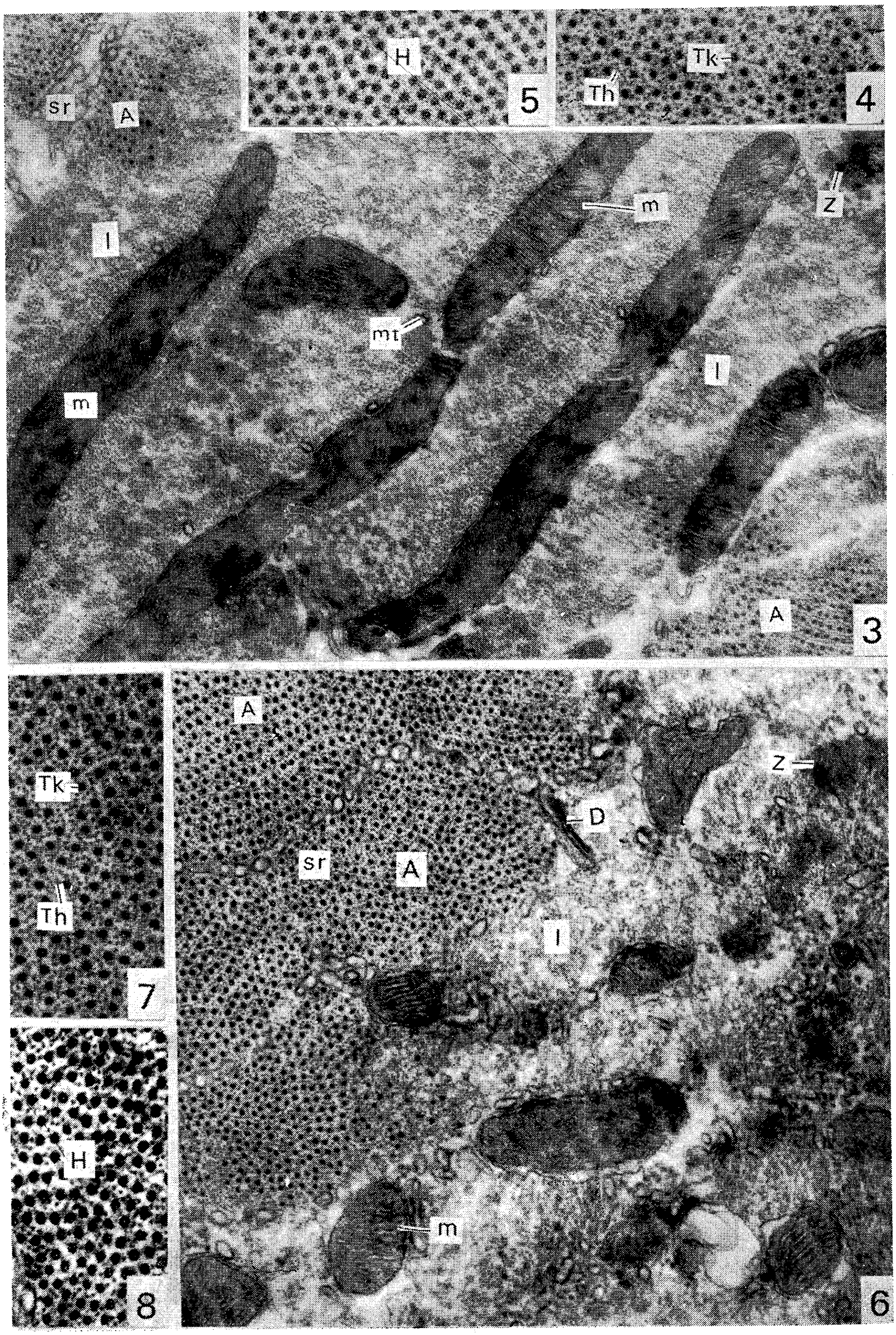
It has been well established that the thin filaments (action filaments) are arranged in hexagonal array in flight muscles whereas in less active muscles such as the abdominal, intersegmental muscles and the visceral muscles of insects, the number of thin filaments relative to thick filaments (myosin filaments) are usually more than 5:1[4,16,17]. We have previously demonstrated that the flight muscles of *T. molitor* have hexagonally arranged thin filament[11]. Since our results here showed that the ratios of thin to thick filaments for the abdominal as well as metathoracic muscles[11] are 6:1, it is concluded that the aforementioned general rule also holds true for *T. molitor*.

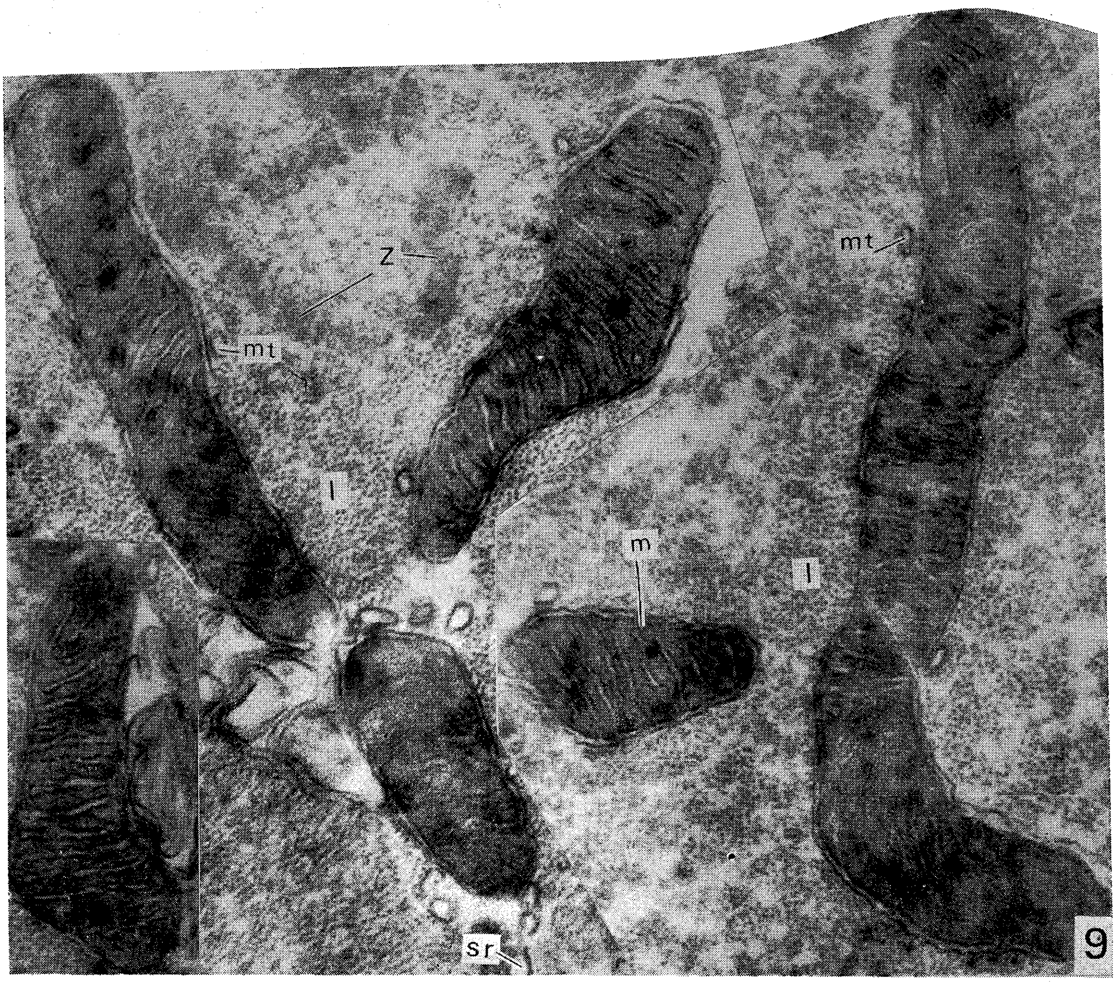
Sarcoplasmic reticulum found in the abdominal muscles of *T. molitor* are much more abundent than those in the flight muscles previously reported[11]. In the synchronized muscles such as the cockroach femoral muscles[7], intersegmental muscle of the american cockroach[16] as well as flight muscles of odonata[15] and Lepidopterans[13], abundant sarcoplasmic reticulum are found along the myofibrils. In the asynchronized flight muscles of the giant water bug[1] and Drosophila flies[14], on the other hand, the organelle are markedly reduced, and the sheath of cisternae around the fibrils is absent. Our results, therefore, suggest that the abdominal muscles of the tenebrio beetle are most likely synchronized whereas the flight muscles asynchronized. Smith[18] stated that calcium movement between the sarcoplasmic reticulum and the fibres synchronized with motor impulse may control contraction and relaxation in most muscles. The frequency of oscillatory length changes in asynchronous fibres is determined by the mechamical properties of the wings and thorax, and by an intrinsic oscillatory mechanism residing in myofibrils.

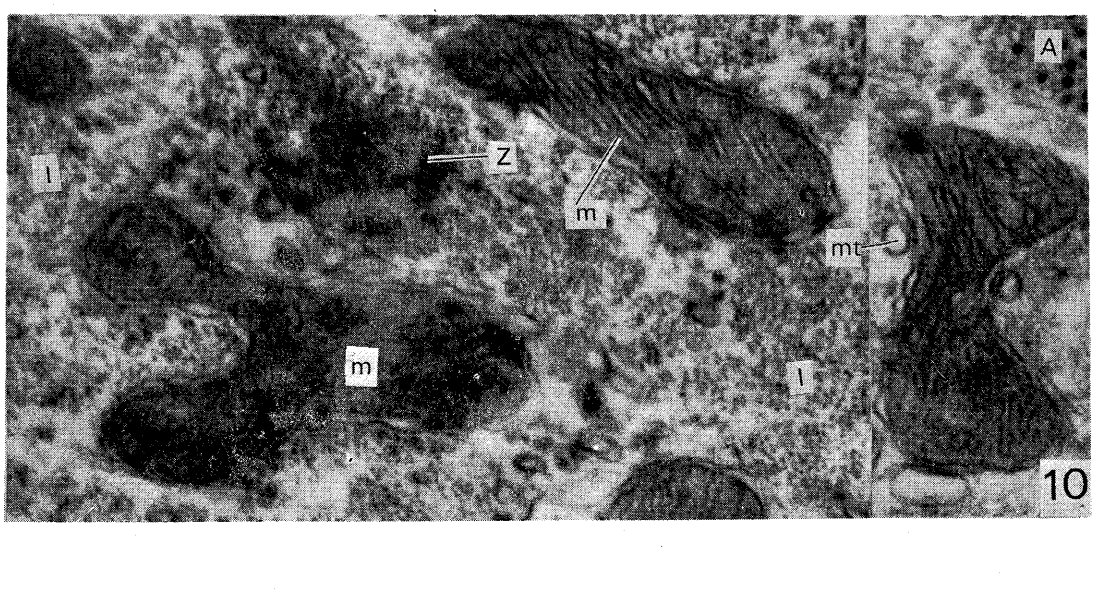
The sarcolemma of most insects has a single layer of basement membrane and amorphous materials. T-tubules clefts extend into myofibrils from the basement membrane. In our observation, amorphous materials of sarcolemma in the larval abdominal muscle are thicker than that of the adult. The sarcolemma of larval intersegmental muscle in *Calliphora* is also thicker than that of adults intersegmental muscle[4,6]. The latter bears a thin basement membrane and occasional hemidesmosomes, whereas the former does not. Sarcolemma of flight muscles of the giant water bugs[1] consists of cell membrane and layer of amorphous materials about 500 Å thick where a tracheol is frequently present. The


A
I
Z
I
D
A
sr
m
1


m
A
sr
I
D
Z
I
A
SL
2


sr
A
H
5
Tk
Th
4
I
m
Z
mt
I
m
A
3
A
Tk
Th
7
H
8
A
Z
D
sr
A
I
m
6


Z
mt
mt
I
m
I
sr
9


A
I
Z
m
mt
m
I
10

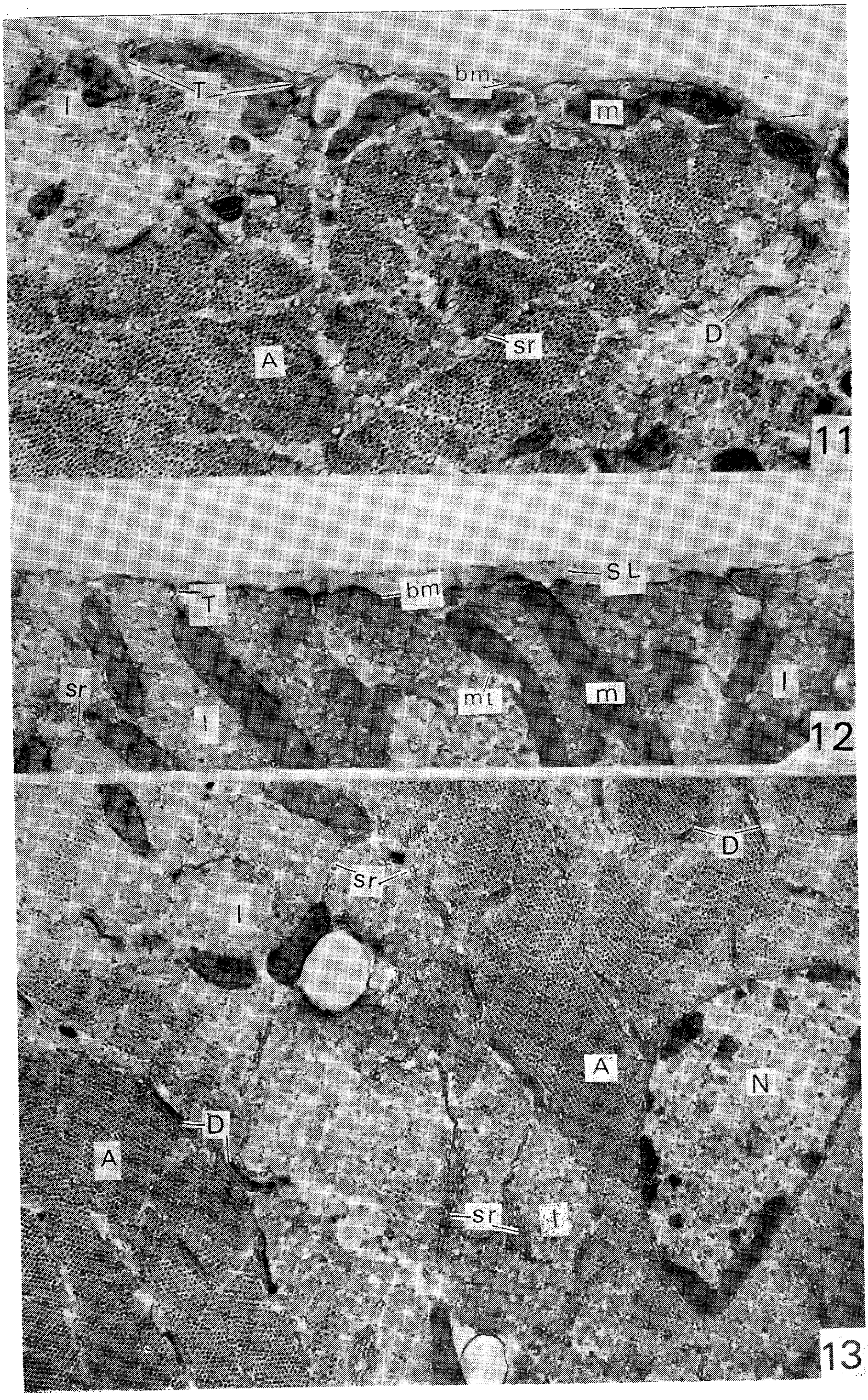

I
T
bm
m
D
sr
A
11
SL
T
bm
sr
I
mt
m
I
12
D
sr
I
A
N
D
A
sr
I
13

Fig. 1. Electron micrograph of the Longitudinal section of the Larval abdominal muscle. The Z-band (Z) zigzag across the myofibrils. The sarcosomes (m) locate in the I band (I), both sides of Z-band. The sarcoplasmic reticulum (Sr) surrounds the myofibrils as the cisternae. Dyads (D) locate at the junction of the A-band (A) and the I-bands. 20000×.

Fig. 2. Electron micrograph of the Longitudinal section of the adult abdominal muscle. Same as the figure 1. A. A-band; I. I-band; D. Dyads; E. Electron dense; Sr. Sarcoplasmic reticulum. Z. Z-band. 20000×.

Fig. 3. Electron micrograph of the transverse plane of section of the larval abdominal muscle. The sarcosomes (M) deposite in the I-band (I). Few numbers of the microtubulae (mt) Scatter around the sarcosome. A. A-band; Sr. Sarcoplasmic reticulum; Z. Z-band. 61,500×.

Fig. 4. Electron micrograph A-band of the transverse plane of section of the larval abdominal muscle shows the relationship of the thick myofilaments (Tk) and the thin myofilaments (Th). 102,500×.

Fig. 5. Electron micrograph of the transverse section shows the H-band (H) of the sarcomere of the larval abdominal muscle. 102,500×.

Fig. 6. Electron micrograph of the transverse section of the adult abdominal muscle. The sarcosome (M) scatter in the I-band (I) near the Z-band (Z). The myofibrils are incompletely divided by the sarcoplasmic reticulum (Sr). The microtubules mostly locate in the I-band and associate with the sarcoplasmic reticulum. A. A-band; D. Dyads. 61,500×.

Fig. 7. Electron micrograph of the transverse section indicate the relationship of the Thick (Tk) myofilaments and the thin myofilaments in the A-band of the adult abdominal muscle. This relationship is same as that of the larval abdominal muscle. 102,500×.

Fig. 8. Electron micrograph of the transverse section shows the H band (H). 102,500×.

Fig. 9. The photomicrograph of the transverse section shows that the sarcosomes (m) of the larval abdominal muscle are elongated in shape with few number of cristae in the parallel arrangement. I. I-band; mt. microtubules; Sr. sarcoplasmic reticulums; Z. Z-band. 102,500×.

Fig. 10. The photomicrograph of the transverse section shows the sarcosomes (m) of the adult abdominal muscle with the elongated shaped, the u-shaped and the y-shaped. A. A-band; I, I-band; mt. microtubules; Z. Z-band. 102,500×.

Fig. 11. Electron micrograph of the transverse section of the adult abdominal muscle. The sarcolemma consists of a thin layer of the amorphous material (SL) and the basement membrane (bm). The T-tubules (T) are formed from the invagination of the basement membrane. The myofibrils are imcompletely or completely divided by the cisternae of the sarcoplasmic reticulum (sr). Dyads (D) appear mostly in the junction of the A-bands (A) and the I-bands (I). m. sarcosomes. 28,750×.

Fig. 12. The photomicrograph of the transverse section of the larval abdominal muscle shows the sarcolemma consisting of a thick amorphous materials (SL) and the basement membrane (bm), the invaginations of the T-Tubules (T) from the basement membrane. I. I-band; m. sarcosomes; mt. microtubules; sr. sarcoplasmic reticulum. 28,750×.

Fig. 13. Electron micrograph of the transverse section of the larval abdominal muscle. The myofibrils are divided by a abundand sarcoplasmic reticulum (sr) incompletely or completely. The sarcoplasmic reticulum (sr) forms a fenestrated and clain structure. Dyads (D) in this micrograph show more in number than those in the figure 11. A. A-band; I. I-band; N. nucleus. 12,500×.

significance for the differences of the sarcolemmal structure is not yet clear.

Most sarcosomes in insects and other animals are elongate. However, in the abdominal muscles studied here, oval and irregular sarcosomes are found in addition to the elongate ones which are of predominent type. The sarcosomes in the femoral muscles of cockroach, *Leucophaea maderae* Fab. are also of many types; elongate, oval and Y-shaped[8]. Measurements are compared with those obtained from *T. molitor* in Table 2.

TABLE 2.

The comparison of sizes in the sarcosomes of *T. molitor* and *Leucophaea maderae.*

| | longest size of elongate sarcosomes | longest size of y-shape sarcosomes |
|---|---|---|
| *T. molitor* | 4.24×0.04 μ | 0.74×0.047 μ |
| *L. maderae* | 6 ×0.5 μ | 2.7 ×0.3 μ |

Vogel[19] suggusted that quantitative variative in sarcosomal enzymes can be associated mainly with the differenes of both sarcosomal size and ratio of their total cristal surface to their matrix volume. According to this suggestion, quantity of sarcosomal enzyme of the abdominal muscles is apparently smaller than that of the femoral muscles. The sarcosomal enzyme of the elongate sarcosomes in the adult abdominal muscle is also quantitatively smaller than that of the larva. However, the y-shape sarcosome of adult abdominal muscles provides a large contact area between sarcosomes and the region where the myofibrils undergo profound alternation during muscular contraction. Therefore, the quantitative sarcosomal enzyme for supporting the muscular contraction may be considered similar in the larval and adult abdominal muscle of *T. molitor.*

## ACKNOWLEDGEMENT

The authurs wish to express their heartly thankful to Drs. Woo, S. C. and Huang, C. S. for their kind help and critical reading the preparation of this manuscript.

## REFERENCES


1. Ashhurst, D. E. (1967). The flight muscle of gaint water bugs: an electron microscope study. *J. Cell. Sci.* **2**: 435-444.
2. Breed, R. S. (1903). The changes which occur in the muscles of a beetle, *Thymalus marginicollis* chevr, during metamorphosis. *Bull. Mus. Comp. Zool.* XL (7): 1-382.
3. Cochrane, D. G., H. Y. Elder and P. N. R. Usherwood (1972). Physiology and ultrastructure of phasic and tonic skeletal muscle fibres the locust, *Schistocerca gregaria. J. Cell Sci.* **10**(2): 419-441.
4. Crossley, A. E. (1968). The fine structure and mechanism of breakdown of larval intersegmental muscles in the blowfly, *Calliphora erythrocephala. J. Insect Physiol.* **14**(10): 1389-1407.
5. Crossley, A. C. (1972). Ultrastructural changes during transition of larval to adult intersegmental muscle at metamorphosis in the blowfly *Calliphora erythrocephala*, I. Dedifferentiation and myoblast fusion. *J. Embry. Exptl. Morph.* **27**(1): 43-74.
6. Crossley, A. C. (1972). Ultrastructural changes during transition of larval to adult intersegmental muscle at metamorphosis in the blowfly *Calliphora erythrocephala*, II. the formation of adult muscle. *J. Embry. Exptl. Morph.* **27**(1): 75-101.
7. Hagopian, M. (1966). The myofilament in the femoral muscle of cockroach, *Leucophaea meaderae* Feb. *J. Cell Biol.* **28**: 545-562.
8. Hagopian, M. (1967). Three shapes of mitochondria in femoral muscle of the cockroach, *Leucophaea meaderae* Feb. *J. Morph.* **122**: 147-168.
9. Huxley, A. F. and Niederberke, R. C. (1954). Interference microscopy of living muscle fibres. *Nature, Lond* **173**: 971-973.
10. Jahromi, S. S. and H. L. Atwood (1969). Structural features of muscle fibres in the cockroach leg. *J. Insect Physiol.* **15**(12): 2255-2262.
11. Lee, Wen-yung, Yi-chang Lin and Fei-ying Shen (1970). Comparative studies on ultrastructures of the thoracic muscles of the larval and the adult stages in *Tenebrio molitor* L. *Bull. Inst. Zool.*, Academia Sinica, **9**(1): 15-21.
12. Lee, Wen-yung, Yi-chang Lin and Jia-shyang Su (1970). A study on the ultrastructure of flight muscle during the development in the pupal stage of *Tenebrio molitor* L. *Bull. Inst. Zool.* Academia Sinica **9**(2): 83-106.
13. Reger, J. F. and D. P. Cooper (1967). A comparative study on the fine structure of the basalar muscle of the wing and the tibial extensor muscle of the leg of the lepidopteran, *Achalarus lyciades. J. Cell. Biol.* **33**: 531-542.
14. Shafio, S. A. (1964). An electron microscopical

study of the innervation and sarcoplasmic reticulum of the fibrillar flight muscle of Drosophila melanogaster. *Q. J. Microsc. Sci.* **105**: 1–6.
15. Smith, D. S. (1966a). The organization of flight muscle fibers in the odenata. *J. Cell. Biol.* **28**: 109–126.
16. Smith, D. S. (1966b). The structure of intersegmental muscle fibers in an insect, *Periplanata americana* L. *J. Cell Biol.* **29**(3): 449–459.
17. Smith, D. S. (1966c). The organization and myofilament array of insect visceral muscles. *J. Cell. Sci.* **1**: 49–57.
18. Smith, D. S. (1968). Insect cells, their structure and function, p. 31–58, Oliver and Boyd, Edinburgh.
19. Vogel, W. (1963). Struktur und funktionelle Biochemie der Mitochondrion. I. Die Morphologie der Mitochondrien, in Funktionelle und Morphologische Organisation der Zelle. Springer-Verlag, Berlin 56–92.

# 麵粉甲蟲 (*Tenebrio molitor* L.) 幼蟲和成蟲腹部肌肉之超顯微構造

李 文 蓉　　楊 美 玉

本研究為利用電子顯微鏡觀察麵粉甲蟲 (*Tenebrio molitor* L.) 幼蟲和成蟲的腹部肌肉，比較其異同，電子顯微鏡下的結果，兩蟲期的腹部肌肉大致相同，肌帶 (Bands) 顯示成為波浪狀明帶 (I-Band) 和暗帶 (A-Band) 明晰，兩帶的長度相同，亨氏線 (H-Band) 在橫切面才明顯，肌質網絲 (Sarcoplasmic reticulum) 豐富成網狀圍繞着顯微肌絲 (myofibril) 更挿入肌絲內，運輸系統 (Dyads and T-system) 多顯現於明帶和暗帶交接間，這種構造在幼蟲腹部肌內較成蟲者多，肌膜 (Sarcolemma) 具兩層構造，無定形構造層 (amorphous material) 和基膜層 (basement membrane)，幼蟲腹肌的肌膜較成蟲者厚，細肌原纖維和粗肌原纖維 (Thin and thick myofilament) 的比例為 6:1，肌肉粒的構造在幼蟲和成蟲腹肌非常相似多數呈長筒形，但成蟲腹肌之肌肉粒除長筒形，圓形外，仍有Y形和U形，麵粉甲蟲幼蟲在成蟲腹部肌肉與其他昆蟲肌肉比較於原文。