

A STUDY ON THE ULTRASTRUCTURE OF FLIGHT MUSCLE DURING THE DEVELOPMENT IN THE ADULT STAGE OF *TENEBRIO MOLITOR* L.

WEN-YUNG LEE, YI-CHANG LIN AND JIA-SHYANG SU

Biological Research Center, Academia Sinica, Taipei, Taiwan, Republic of China

Received for publication August, 1970

ABSTRACT

W. Y. Lee, Y. C. Lin and J. S. Su (1970) *The Study of Ultrastructure of Flight Muscle on the Development in the Adult Stage of Tenebrio molitor L.* Bull. Inst. Zool. A. S. 9(2): 107-117 The developmental and ultrastructural changes of the indirect flight muscles of *Tenebrio molitor* L. within eight days after adult emergence were studied. The diameter of the myofibril increases during the process of adult maturation, the sarcosomes increase in size and the cristae in number. The methods of the multiplication and the growth of the sarcosomes are observed.

The ultrastructural changes of sarcosomes of the flight muscle in honey bee during the process of adult maturation have been observed by Herold (4). Herold and Borei (3) showed that major qualitative and quantitative changes occurred in cytochrome components of the indirect flight muscles of the honey bee during development. Watanabe and Williams (14) observed changes in the cytochrome pattern and the ultrastructural changes of the indirect flight muscle. In developing sarcosomes of the house fly Rockstein and Bhatnagar (12) have reported that the size and the number of the giant mitochondria in the flight muscles of the common housefly, *Musca domestica* L. are changing with the ages.

The development of the indirect flight muscles of *Tenebrio molitor* L. has been studied in anatomical and histological aspects under the light microscope in the previous papers (6,7). In this report, the

developmental changes in the myofibrils and the sarcosomes during the process of the adult maturation have been examined by means of electron microscope.

MATERIALS AND METHODS

Tenebrio molitor L. used in this study was reared in this laboratory. The adults of known ages were obtained. The indirect flight muscles were removed from metathorax in the fixative solution of 6% glutaraldehyde with 0.1M cacodylate and were torn into small pieces. The preparation for electron microscope was accorded with the methods described in the previous paper (8). The gold and the silver thin sections were cut on a Sorvall Porter-Blum Microtome. All electron micrographs were taken from Hitachi Electron Microscope Type 11-A.

RESULTS

In the newly emerged adults *Tenebrio molitor* L., the myofibrils (Mfb, Fig. 1, 10) are well developed and their diameter increased gradually with the increase of age. The increase of the diameter is due to the gradual addition of new myofilaments to the growing myofibrils daily (up to the eighth day adult) (Mfb, Fig. 10-13). In the electron micrograph of a seventh day adult, H-bands (H,) are beginning to appear faintly.

The sarcosomes are disorderly scattered in the intermyofibrillar space, and they varied in shape and in size. The number and orientation of the cristae are varied from newly emerged adult to the fifth day adult (M, Fig. 1, 6, 7, 10, 11).

Besides the sarcosome fission which frequently occurs in the adult stage, there are several ways of their propagation and growth as showed in the electron micrographs. In Figure 2, the sarcosome is divided into two partitions by the growth of a transverse crista, then more cristae in the part of vacuolate will produce from the inner membrane of the sarcosome to fill up the vacuole. In Figure 3, a new partition of the sarcosome formed from the extension of the outer membrane of the sarcosome can be observed and the inner membrane of the new partition infolds to form the cristae afterward. Figure 4 shows that the inner and the outer membrane are extended through swelling, and cristae grow to fill the swelling partition. Figure 5 also shows the swelling sarcosome. Although, it seems that the newly formed sarcosomes are mostly from the swelling of outer membrane and newly formed cristae stem from the inner membrane, yet in some electron micrographs, there are budding, which leads to the formation of sarcosome can also be observed.

That swelling of sarcosomes is filled

up by the cristae can be found in the fifth and the sixth day adult. The cristae of the sarcosome in the seventh day adult become much more increasing in their number and most of them are in compact parallel arrangement (M, Fig. 8, 12).

In the electron micrographs of the eighth day adult (Fig. 9, 13) the structure of muscle fibrils can be seen and it is almost the same as that of the fully grown adult. The sarcosomes are arranged in one or more rows between the myofibrils, and they contain a large number of cristae in compact arrangement. H-band, I-band, and Z-band are distinctly seen in each sarcomere.

DISCUSSION

In anatomical and histological observations, the indirect flight muscles of *Tenebrio molitor* L. have completely grown before emergence (6,7). However, in the present investigation, the electron micrographs show that the indirect flight muscles are still in the process of development as of newly emerged honey bee workers, and they are different from that of the mature adults (4). They appear as progressive growth.

The length of the sarcomere does not show any increment between newly emerged adult and those of the mature adult. However, the diameter of the myofibrils obviously keeps increasing up to the sixth and the seventh day adult. The increase of the size of myofibrils is a result of the new formation of myofibrilament in the periphery of the myofibril. Breuer *et al* (1) isolated large polyribosomes containing 60 to 80 ribosomes from the postnatal skeletal muscle of rat. The large aggregates of particles of ribosomes have also been seen in sections of chick embryo (2) as those in the developing myofibrils of *Tenebrio* adult. Although it has been proposed that such

large polyribosomes may mediate myosin synthesis (1), yet no supporting evidence exists.

The development of the indirect flight muscle sarcosome of *Tenebrio molitor* L. as described in this report is in process with adult maturation up to eight-day-old adults. But in the house flies, the flight muscles as reported by Rochstein (12) begin in the process of maturation soon after emergence. The smaller sarcosomes diminish in number and are replaced by the increased population of the larger sarcosomes during the first week of adult. Herold (4) found that the sarcosomes of honey bee continue to increase in size and in number of interior tubules (cristae) during the process of adult maturation.

The methods of the propagation and the growth of sarcosome are also observed in this paper. Leduc *et al* (5) observed the cyclic changes in mitochondria of the hamster tumor cell in culture. They found that there are no atypical mitochondria (swelling) just after inoculation, relatively fewer in number for the first day but more and larger for the second and third day after inoculation. The mitochondria become typical with moderately dense matrix in the older culture, the atypical mitochondria are of growing form. Luck (10) has clearly demonstrated that the mitochondria are self-replicating bodies in the lower organism, *Neurospora*. Same evidence has been described to mitochondria of higher organisms (11). Tandler *et al* (13) indicated that mammalian mitochondria make division or fusion and the membraneous partitions within the mitochondria play a cardinal role in this process. However, as to the authors' knowledge there is no reference about the growth of mitochondria by the extension of only outer membrane and the cristae formed by inner membrane.

This investigation was supported by the research grant from the Biological Research Center, Republic of China.

REFERENCES

1. Breuer, C. B., M. C. Davies and J. R. Flerin: (1964) Amino and Incorporation Cell-free Preparations from Skeletal Muscle. II. Preparations and Properties of Muscle Ribosomes and Polysomes. *Biochemistry* 3:1713-1720.
2. Fischman, D. A.: (1965) The Fine Structure of Embryonic Chick Skeletal Muscle. *Anat. Record*. 151:350-372.
3. Herold, R. C. and H. Borei: (1963) Cytochrome Changes during Honey Bee Flight Muscle Development. *Devel. Biol.* 8:67-79.
4. Herold, R. C.: (1965) Development and Ultrastructural Changes of Sarcosomes during Honey Bee Flight Muscle Development. *Devel. Biol.* 12:269-286.
5. Leduc, Elizabeth H., W. Bernhard and P. Tournier: (1955) Cyclic Appearance of a Typical Mitochondria Containing DNA Fibers in Cultures of an Adenovirus 12-induced Hamster Tumor. *Exptl. Cell Research* 42:597-616.
6. Lee, Wen-Yung: (1964) A Study of Development of the Thoracic Musculature from Larvae to Adult in *Tenebrio molitor* L. *Ph. D. Thesis in the University of Minnesota, U. S. A.*
7. Lee, W. Y. and K. K. Chang: (1958) A Histological Study of Development of the Thoracic Musculature from Larvae to Adult in *Tenebrio molitor* L. (Tenebrionidae, Coleoptera, Hexapoda) III. A Study on the Development of Thoracic Musculature at the Pupal Stage. *Bull. Inst. Zool. Academia Sinica, Republic of China*. 7: 71-81.
8. Lee, W. Y., Y. C. Lin and F. Y. Shen: (1970) Comparative Studies on Ultrastructures of the Thoracic Muscles of the Larval and Adult Stages in *Tenebrio molitor* L. *Bull. Inst. Zool. Academia Sinica, Republic of China*. 9(1): 15-21.
9. Lee, Wen-Yung and Yi-Chang Lin: (1970) The Study of Ultrastructure on the Histolysis of the Larval Muscle in *Tenebrio molitor* L. *Bull. Inst. Zool. Academia Sinica, Republic of China*. 9(1): 23-29.

10. Luck, D.J.L.:(1964) Formation of Mitochondria in *Neurospora erassa*. A Study Based on Mitochondrial Density Changes. *J. Cell Biol.* 24:461-475.
11. Novikoff, A.B.:(1961) Mitochondria (Chondriosomes) In *Cell*. J. Brachet and A.E. Mirsky, Editors. Academic Press Inc. New York 2:299.
12. Rockstein, M. and Bhatnagar, Prem Lata: (1965) Age Changes in Size and Number of the Giant Mitochondria in the Flight Muscle of the Common Housefly (*M. domestica* L.). *J. Insect Physiol.* 11:481.
13. Tandler, B., R. A. Erlandson, A. L. Smith and E. L. Wynder: (1939) Riboflavin and Mouse Hepatic Cell Structure and Function. II. Division of Mitochondria during Recovery from Simple Deficiency. *J. Cell Biol.* 41:471-493.
14. Watanabe, M. J. and C. M. Williams: (1951) Mitochondria in Flight Muscles of Insect. I. Chemical Composition and Enzymatic Content. *J. Gen. Physiol.* 34:657-688.

Fig. 1. Electron micrograph of a longitudinal section of the newly emerged adult. The structure of myofibrils (Mfb) is well developed. The sarcosomes (M) are varied in sizes and shapes. Some small sarcosomes fuse to become larger ones. A sarcosome in the photograph swells in one position in order to increase the size. 25,000. x.

Fig. 2. The photograph shows one sarcosome (f) being on the process of division. Another is going to be divided into two partitions by the growing cristae (d). 50,000. x.

Fig. 3. A new partition of the sarcosome is formed from the extension of the outer membrane of the sarcosome (Ou). In, Inner membrane. 50,000. x.

Fig. 4. The photograph shows that the inner membrane (In) and the outer membrane (Ou) of the sarcosome are extended by swelling. 50,000. x.

Fig. 5. The photograph shows the outer membrane (Ou) of the sarcosome extended to form a vesicular portion. 50,000. x.

Fig. 6. Electron micrograph of a fourth day adult. Various sizes of sarcosomes (M) are disorderly oriented in the intermyofibrillar space. Mfb, Myofibril. 25,000. x.

Fig. 7. Electron micrograph of a fifth day adult. The sarcosome (M) are grown by swelling. The number of cristae are increased and oriented more in order. Which divided the sarcosome into two partition. d, Transverse crista. Mfb, Myofibril. Tr, Tracheole. 25,000. x.

Fig. 8. The photograph shows the size of sarcosomes (M) on the seventh day pupa much increasing, even these are still some small sarcosomes. The cristae almost grow as those of the adult. Mfb, Myofibril. 25,000. x.

Fig. 9. Electron micrograph of the eighth day adult. The structure (M) of the muscle fibril is completely grown as the mature adult. The sarcosomes are arranged as rows parallel between the myofibrils (Mfb). Sr, Sarcoplasmic reticulum. 25,000. x.

Fig. 10. Electron micrograph of the Transverse section of the newly emerged adult. The myofibrils (Mfb) and the sarcosomes (M) are growing large amount of ribosomes surround the myofibrils and scatter in the sarcoplasm. Some sarcosome are divided at the base of the end (b). A, A-band, I, I-band. Z, Z-band. D, Dyad. Pm, Plasma membrane. Tr, Tracheol. 25,000. x.

Fig. 11. The transverse section of the fourth day adult. The diameter of myofibril (Mfb) is much increased. Various size of sarcosomes (M) are distributed in the intermyofibrillar space. A, A-band. Sr, Sarcoplasm reticulum. Tr, Tracheole. 25,000. x.

Fig. 12. The Transverse section of the seventh day adult. The sarcosomes (M) are almost growth as those of the mature muscle. The large number of cristae arrange in compact parallel. The sarcoplasmic reticulum (Sr) surrounds the myofibril instead of the ribosomes. A, A-band. I, I-band. Pm, Plasma membrane. Sr, Sarcoplasmic reticulum. Tr, Tracheole. 25,000. x.

Fig. 13. Electron micrograph of the eighth day adult. The structure of muscle fibril is almost completely grown as that of the mature adult. A, A-band. Z, Z-band. M, Sarcosome. Mfb, Myofibrils. Sr, Sarcoplasmic reticulum. Tr, Tracheole. 25,000. x.









