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A HISTOLOGICAL STUDY OF DEVELOPMENT OF THE THORACIC MUSCULATURE FROM LARVAE TO ADULTS IN *TENEBRIO MOLITOR* L. (TENEBRIONIDAE, COLEOPTERA, HEXAPODA).[1]

II. A STUDY OF THE THORACIC MUSCULATURE OF THE LARVAE.

WEN-YUNG LEE[2]

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ABSTRACT

The thoracic musculature of the larvae in *Tenebrio molitor* L. has been investigated by the serial paraffin sections. The nuclei scatter peripherily at the surface of the muscle fibre in the sarcoplasm beneath the sarcolemma and in the fibre among the fibrils. The fibres are well marked with cross striations. The transverse intersegmental pleurospinal tendons appear as fibrinous: no cross striations are shown. The muscle fibres of *T. molitor* larvae differ from those of the larvae of honey-bee and housefly, but similar to the larval muscles of Thymalus beetle. Four types of attachment of the muscles are found from this study. A comparison of the thoracic musculature of the larvae and the adults in *T. molitor* is also made.

INTRODUCTION

The previous work (6) has revealed that the thoracic musculature of the adults of *Tenebrio molitor* L., is consisted of three types of muscles, namely, the general type, the general tubular type and the fibrillar type. The histological structures of these types of muscles are different. The purpose of this study is to disclose the histological structure of the thoracic muscles in *T. molitor* during the larval stage and to compare the thoracic muscles of larvae with those of adults.

MATERIAL AND METHODS

The worms were reared in the laboratory with a medium consisting of the following ingredients: whole wheat flour 300 grams, yeast 15 grams, and corn meal 30 grams.

Mature larvae were fixed alive in Bouin-Duboscq solution (3). This solution penetrates rapidly and preserves the muscles firmly attached to the skeleton. All specimens were fixed for 24 hours and then washed in 70% ethanol for 24 hours to remove excess picric acid (8). After the removal of picric acid, the specimens were dehydrated *via* a butanol series (7), embedded in tissuemat (m. p. 60°C) and cut at 8-10 μ. The sections were stained with Delafield-haematoxylin and 0.5% eosin in 95% ethanol.

Preparations were examined with 10x and 40x Fluoritic, and 90x Fluoritic oil immersion lens of a Leitz Wetzlar Ortholux microscope. Microphotographs

1 This investigation was supported by a research grant from National Science Council.

2 Associate Research Fellow Institute of Zoology, Academia Sinica.

were made with a Leitz Wetzlar Aristophot.

RESULTS

The terms of the muscles adopted for this study are those termed by Lee (5).

The histology of the thoracic musculature of larvae, besides the three of the transverse intersegmental pleurospinal tendon which are located between the prothorax and the mesothorax, the mesothorax and the metathorax, and also the metathorax and the first abdominal segment, all of the larval muscle fibres of *Tenebrio molitor* L. are striated muscles. The longitudinal and the cross sections of them are shown in Figure 1-4. Each fibre is ensheathed in a relatively thick membrane, the sarcolemma. The elongated ovoid shaped nuclei are scattered in the sarcoplasm at the periphery of the fibre beneath the sarcolemma, and are also deposited in the fibre.

The cross striations of the muscle fibre are well marked, the dark disc, the light disc of Wigglosworth (12) are clearly shown (Fig. 2, 4, 5). The dark bands are stained deeply and the light bands lightly. The telophragma is also present when the muscle is expanded (Fig. 4 and 5). However, the median disc and the accessory disc are not distinguished in the light microscope.

Most muscle bundles appear flattened in cross section, but the leg muscles are round in shape. Some fibre are irregular. Therefore the Cohnheim's areas seen in the cross section vary in patterns. The three of the transverse intersegmental pleurospinal tendon are fibrinous and show no cross striations (Fig. 6), all the bands of the fibre dissappeared. They consist of small fibrils and the nuclei are found unevenly distributed among the fibrils. These tendon-like muscles may belong to the tonofibrillae.

There are three types of muscular attachment:

(1) Most inner layer of the ventral longitudinal muscles of the mesothorax and the metathorax are anchored to the transverse intersegmental pleurospinal tendon (Fig. 6).

(2) The first, the second and the third transverse intersegmental pleurospinal tendon and the outer layer of the ventral longitudinal muscles of the mesothorax and the metathorax are attached to the basement membrane of the integument and faintly continued to the epidermis, (Fig. 6).

(3) Some larval thoracic muscles are inserted on or originated from the apodeme; such as the second, the third and the fourth ventral longitudinal muscle of the prothorax and the same muscles of the mesothorax attach to the furcal tendon (Fig. 7), the postoccipital pleural muscle and the pleural muscle of the prothorax attach to the pleural ridge (Fig. 8).

(4) The rest of the larval thoracic muscles are inserted on the integument of the body wall (Fig. 2, 3) with the muscle fibrils seemingly passing through the epidermal cells to attach to the inner surface of the cuticle.

The trachea supplying the larval muscles break up into the fine intracellular tracheoles at the surface of the muscle fibres. From Fig. 1 and 5, these tracheoles penetrate the sarcolemma and remained in the superficial layers of the sarcoplasm.

DISCUSSION

All insect muscles seem to be made up of striated fibres; each fibre always consists of a number of parallel fibrillae (or sarcostyle) which are laid down in a nucleated sarcoplasm. The degree of

differentiation of the fibrillae and their arrangment within the fibre differs in various insects and different kinds of muscles in the same insect. Wigglosworth (12) classified the histological structure of insect muscles into four categories:

(1) The muscles in the larvae of honey-bee and of many Diptera; the fibrils are minute threads with little visible differentiation and are invested by a thick layer of superficial sarcoplasm.

(2) In most insects the fibrillae are larger; they occupy the whole of the cross section of the fibre and are irregularly arranged. Each fibre is ensheathed in a structureless sarcolemma; the nuclei of the sarcoplasm are either scattered throughout the fibre or disposed immediately beneath the sarcolemma.

(3) The tubular muscle which are found in the adult of all the higher Hymenoptera and Diptera, and in the adult *Dytiscus* (Dytiscidae, Coleoptera). The nuclei are arranged in a row through an axial core of the entire length of the fibre; the fibrillae are lamellae radiating from the centre in the transverse section.

(4) The indirect flight muscle of the adult Hymenoptera and many Diptera are called the fibrillar muscle. The fibrils are very large and loosely bound together by the tracheal endings; between the fibrils are rows of nuclei. Sarcolemma is usually wanting.

Histological structure of the larval thoracic muscles of *T. molitor* are most closely related to the second type of the histological muscular categories indicated by Wigglosworth. The muscle fibrillae are comparatively large and the cross striations are distinct; nuclei are scattered throughout the sarcoplasm beneath sarcolemma and embedded deeply in the fibres. This type of muscles differs with the muscles of honey-bee larva. Snodgrass (10) has stated that the fibrils (sarcostyles) of the honey-bee larvae are very small and surrounded by a thick layer of sarcoplasm containing many nuclei on the surface of the fibre. It also differs from the muscles of the house flies which have only few large nuclei lying under the thin sarcolemma. The histological structure of the larval thoracic muscles of *T. molitor* is similar to those of few larval muscles of *Thymalus marginicollis* Chevr studied by Breed (1). Their fibrils are large; nuclei are unevenly distributed in the sarcolemma and frequently embedded deep in the fibres; the cross striations of this muscle are also well marked. However, the muscle fibres of *Thymalus* may show all the usual bands, the light band, the dark band, the telophragma, the accessory disc, the median disc and the mesophragma under a light microscope. In the muscle fibres of *T. molitor*, the mesophragma and the accessory disc are not evident, but the rest of the bands may show up clearly.

The natural types of attachment of muscles to the cuticle of insects have been described by Richards (9) as: (1) sarcolemma is continuous with the basement membrane of the integument, and the fibrils of the muscle penetrate through the epidermis to the cuticle, (2) the attachment of muscles to the cuticle is marked by tonofibrillae. The attachment of most of the muscles of *T. molitor* is similar to the first type illustrated by Richards. However, Richards did not mention type of attachment in which the muscles are attached to the tendon like muscle, such as that occuring in the larvae of *T. molitor*; some ventral longitudinal muscles of the prothorax, the mesothorax and the metathorax are attached to the transverse intersegmental pleurospinal tendon.

When the thoracic musculatures of the larvae and those of the adults in *T.*

molitor (6) are compared; the former have only one type of muscles as described above, and the latter have three types, namely the general type, the general tubular type, and the fibrillar type of muscles. The muscles of the larva are similar to the muscles of the general type in the adult. The fibres are striated with a well-marked sarcolemma and usually with nuclei at the periphery of the fibre or scattered in the fibrils. However, the muscles of the larva are mostly composed of the large fibres, and attached to the epidermis; a few fibres are attached to the tendon like muscle, the transverse intersegmental pleuospinal tendon, or the apodeme of the integument. The general type of muscle in the adult consists of numerous small fibres frequently arranged in a penniform or bipenniform manner and attached to the cuticle through the epidermis, mostly by a common tendon.

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EXPLANATION OF ILLUSTRATION

Fig. 1. Cross section of the tergal promotor of the coxa of the prothorax, 705x.

Fig. 2. Lonitudinal section of the tergal promotor of the coxa of the prothorax. The fibrils (F) of the muscle penetrate through the epidermal cells (E) to the cuticle (C). 705x

Fig. 3. Longitudinal section of the external dorsal longitudinal muscle of the mesothorax. 451.2x

Fig. 4. Longitudinal section of the ternal remotor of the metathorax. The muscle is in extended. 451.2x.

Fig. 5. Longitudinal section of the tergal promotor of the coxa of the metathorax. Tracheoles (tr) penetrate into the muscle. The muscle is in extended. 705x.

Fig. 6. Longitudinal section of the ventral longitudinal muscles of the mesothorax (v^1m_2) and the metathorax (vlm_3), and the second transverse intersegmental pleurospinal tendon (tit_2), the attachment of the ventral longitudinal muscles. The second transverse intersegmental pleurospinal tendon attaches to the basement membrane of the integument. 106x.

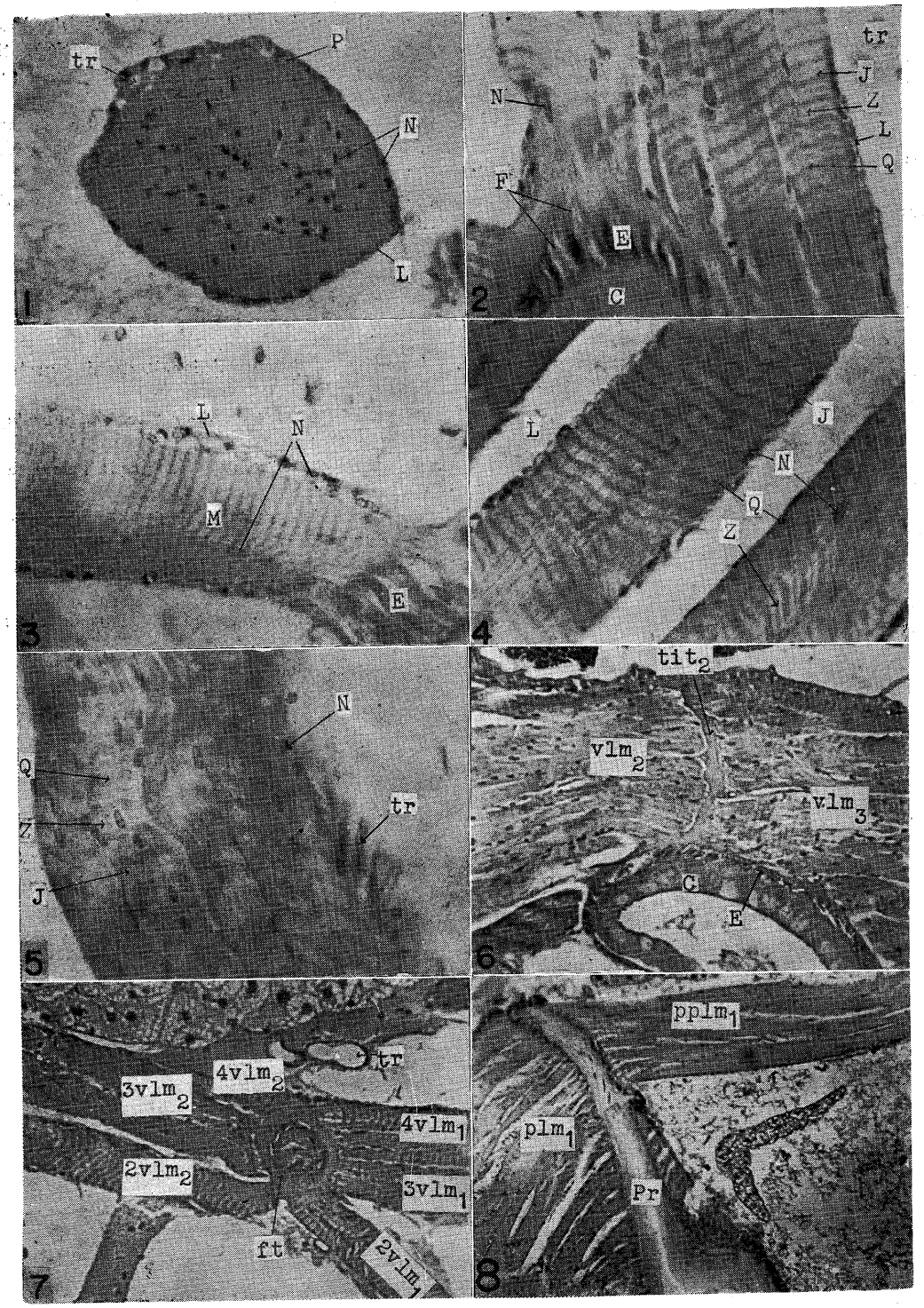
Fig. 7. Longitudinal section of the second, the third and the fourth ventral longitudinal muscle of the prothorax ($2vlm_1$, $3vlm_1$, $4vlm_1$) and the second, third and fourth ventral longitudinal muscle of the mesothorax ($2vlm_2$, $3vlm_2$ and $4vlm_2$) which attach to the furcal tendon (ft). 106x.

Fig. 8. Longitudinal section of the postoccipital pleural muscle and the pleural muscle of the prothorax ($pplm_1$ and plm_1) which attach to the pleural ridge (Pr). 106x.

ABBREVIATION

C—Cuticle
E—Epidermal cells
F—Fibrils
ft—Furcal tendon
J—Light disc
L—Sarcolemma
M—Muscle fiber
N—Nucleus
P—Sarcoplasm
Pr—Pleural ridge
Q—Dark disc
tr—tracheole
Z—Telophragma

plm_1—the pleural muscle of the prothorax
$pplm_1$—the postoccipital pleural muscle of the prothorax
tit_2—the second transverse intersegmental pleurospinal tendon
$2vlm_1$—the second ventral longitudinal muscle of the prothorax
$3vlm_1$—the third ventral longitudinal muscle of the prothorax
$4vlm_1$—the fourth ventral longitudinal muscle of the prothorax
$2vlm_2$—the second ventral longitudinl muscle of the mesothorax
$3vlm_2$—the third ventral longitudinal muscle of the mesothorax
$4vlm_2$—the fourth ventral longitudinal muscle of the mesothorax
vlm_3—the ventral longitudinal muscle of the metathorax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