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THE SUSPENSORY MUSCLES IN A TIPULID LARVA,  
*TIPULA ABDOMINALIS*<sup>1</sup>  
(DIPTERA: TIPULIDAE)

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ABSTRACT

There are three pairs of suspensory muscles in the larva of *Tipula abdominalis* which originate dorso-laterally between body segments one through four and insert laterally along the mid-gut and on the mid-portion of the Malpighian tubules. Cytologically these muscles resemble body wall muscles more than gut muscles.

In the living animal, the muscles probably take up slack and keep the gut and Malpighian tubules from being displaced unduly.

Although the existence of suspensory muscles in some insect larvae has been recognized for some time, few descriptions of these muscles have actually been published. Lyonet (1762) was the first to describe such muscles, in the carpenter moth, *Cossus cossus*. He observed seven pairs of these muscles which extended from intersegmental portions of the body wall to various places on the mid-gut, hind-gut, Malpighian tubules, and silk glands. He called these "trunk" muscles.

Much later, a second observation of such muscles was made in the silkworm, *Bombyx mori*. Tanaka (1911) described nine pairs of muscles which he called "dermo-visceral" muscles. They originated on the body wall between segments

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one and two through segments nine and ten. The second pair was attached dorsally to the gut. The fourth and eighth pairs sent branches to the silk gland.

Further work was done on the "dermo-visceral" muscles in *Bombyx mori* in order to discover their action. Mori (1928) noted that contractions and relaxations of these muscles produced vermicular movements and a pulling forward of the gut. He described also a dimpling of the body wall at the dorsal attachment of the large second pair of muscles. This dimpling alternated from one side to the other.

About the same time that the original work was done on *Bombyx mori*, Peterson (1912) described one pair of such muscles (which he called suspensory muscles) in the tomato hornworm, *Protoparce quinquemaculata*. They extended from the ventral side of the cephalic margin of the caudal enlargement of the large intestine to the latero-ventral portion of the transverse conjunctiva, between the sixth and seventh abdominal segments.

Later, Snodgrass (1928) recognized one similar pair of muscles in the eastern tent caterpillar, *Malacosoma americana*, and called it the first suspensory muscle of the ventriculus. He also illustrated, but did not name, a suspensory muscle in the grasshopper, *Dissoteira*.

In a later paper, Snodgrass (1931) showed a suspensory muscle in the fourth abdominal segment of *Eshigmene acraeae*, the salt-marsh caterpillar, but did not label it.

The most recent mention of suspensory muscles was made by Graham-Smith (1934) who described a pair of long, thin muscles in the blowfly, *Calliphora erythrocephala*. These muscles extend from each side of the anterior portion on the thoracic wall and are inserted near the side of the "neck." He called these the "adductor muscles of the ventriculus."

Although there are few published accounts of the suspensory muscles, they appear to be wide-spread among the insects. They are present in other Orthoptera and in the larvae of many Lepidoptera, Trichoptera, Neuroptera, and other related Diptera (Weaver, 1963).

In the present paper, the cytology of three pairs of suspensory muscles in a crane-fly larva, *Tipula abdominalis*, (Diptera: Tipulidae) has been studied in order to determine if the muscles are derived from body wall or gut muscles. It was also hoped that through this study a possible related function for the suspensory muscles could be suggested.

#### EXPLANATION OF FIGURE 1

FIGURE 1. Dorsal view of dissected larva of *Tipula abdominalis* showing the three pairs of suspensory muscles.

#### LEGEND

- car—cardia
- cmcl—circular muscles of mid-gut
- cr—crop
- di—dorsal internal muscles of body wall
- ep—epithelium of body wall
- eso—esophagus
- gca—gastric ceca
- il—internal lateral muscles of body wall
- lmcl—longitudinal muscles of mid-gut
- Mal—Malpighian tubule
- 1S—first pair of suspensory muscles
- 2S—second pair of suspensory muscles
- 3S—third pair of suspensory muscles
- tr—trachea
- vent—ventriculus
- vi—ventral internal muscles of body wall

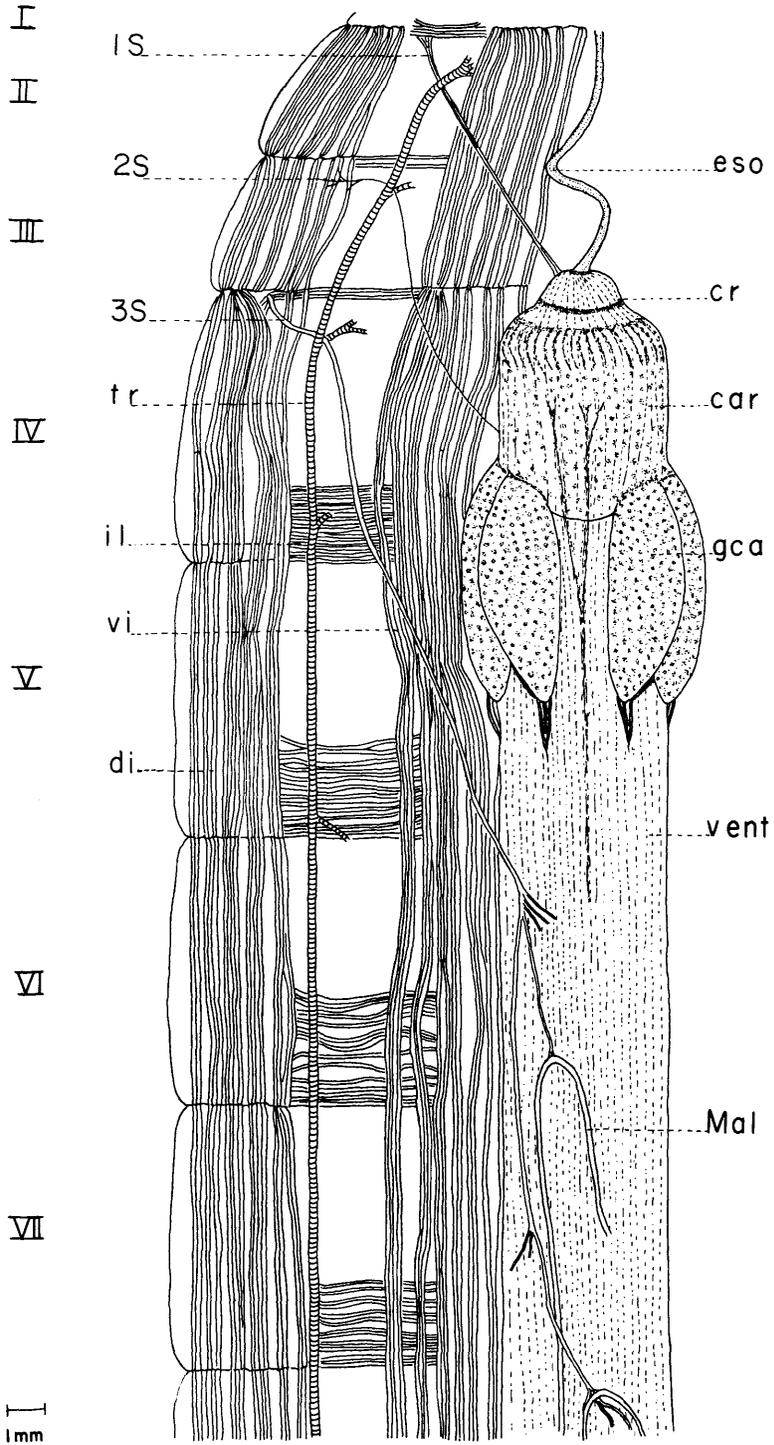


Fig.1

## MORPHOLOGY AND HISTOLOGY OF SUSPENSORY MUSCLES

*Materials and Methods*

The animal used in this study was a common, large crane-fly larva, *Tipula abdominalis*, chosen because previous work by Weaver (1963) indicated the presence in it of suspensory muscles. The larvae were collected in late April in masses of partially submerged, dead leaves in a spring-fed stream which flows into Little Applecreek stream in Highland Park, Wooster, Ohio. They were found more abundantly in the rapidly moving parts of the stream.

Several other collections were made in September, November, and February, and the larvae obtained were put into an aerated aquarium. There was about 50 per cent mortality, but some larvae lived throughout the winter, feeding on dead leaves taken from the collection site. The larvae collected in September were small (1 inch long), but those that survived molted and grew, attaining a size of about 2 inches by January. After the February collection, about 100 larvae were added to the aquarium. This addition evidently destroyed the balance in the aquarium because all the larvae died shortly afterwards.

*Fixation.*—The larvae were injected by a hypodermic needle with various fixatives and then dropped into the fixative. After 18 hrs, the animals were slit half-way longitudinally to aid in removal of the fixative and then stored in 70 per cent ethyl alcohol. Two fixatives, Bouin's Fluid and Heidenhain's Fluid, gave the best results with both sections and whole mounts.

*Dissection.*—Whole-mount preparations consisted of individual suspensory muscles attached to a small piece of gut or some body wall muscle. A small longitudinal portion of the entire mid-gut with suspensory muscle attached or a small square of body wall with suspensory muscle were embedded in paraffin.

Finding the thin suspensory muscles in sections was facilitated by removing as small a portion of tissue surrounding the suspensory muscle as possible. For body-wall preparations, all body wall muscles, except the ones to which the suspensory muscles were attached, were dissected out of a small square of body wall tissue. For gut preparations, a longitudinal portion of the mid-gut just wide enough to include the suspensory muscle attachment was used.

*Histological Technique.*—The basic histological technique used was obtained from Weaver (1961). *Paraplast*, a new plastic-paraffin, previously found beneficial when muscle tissue was hard to section, was used for embedding.

*Results*

In the larva, three pairs of thin muscles (fig. 1, 1S, 2S, 3S) extend anteriorly from their various lateral connections on the mid-gut and attach dorso-laterally to different body wall muscles and, in the case of the third pair, also directly to the body wall. The paired muscles are of varying thicknesses. The thick third pair is almost as wide as a group of dorsal internal body wall muscles. The thin second pair is only a few cells wide. The first and second pairs of muscle each attach to the mid-gut at only one place, whereas the third pair has several attachments to the mid-gut and sends branches to the mid-portion of each Malpighian tubule (fig. 1).

The fibers of the first pair of muscles fan out and are attached to internal lateral muscles on the body wall between the first and second segments (fig. 2). Although the whole-mount preparations do not show clearly how all the fibers of the first pair attach to the internal lateral fibers, some of the fibers appear to become continuous with the body wall fibers (fig. 3).

The first pair of suspensory muscles attaches to the gut just posterior to the junction of the esophagus and the crop (fig. 4). The muscle fibers taper, branch, and spread over the gut, but do not become continuous with the longitudinal, or circular muscle fibers of the gut (fig. 5).

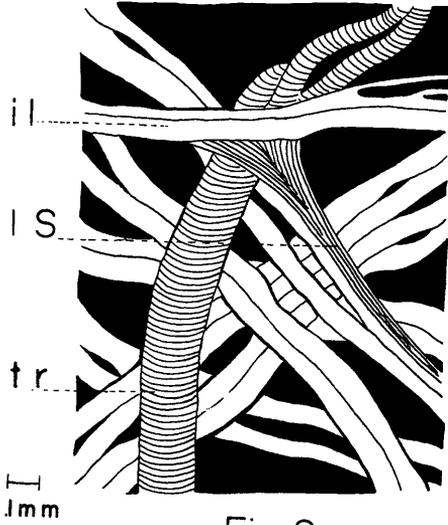


Fig.2

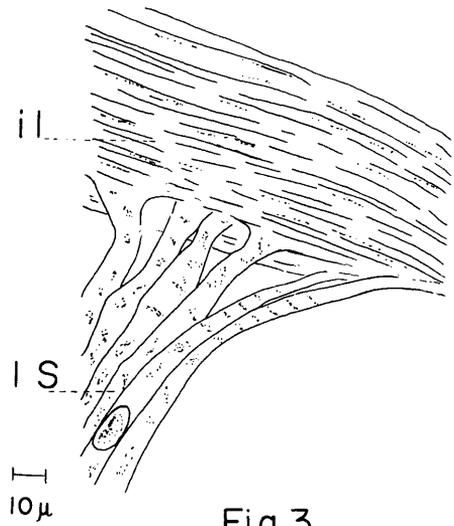


Fig.3

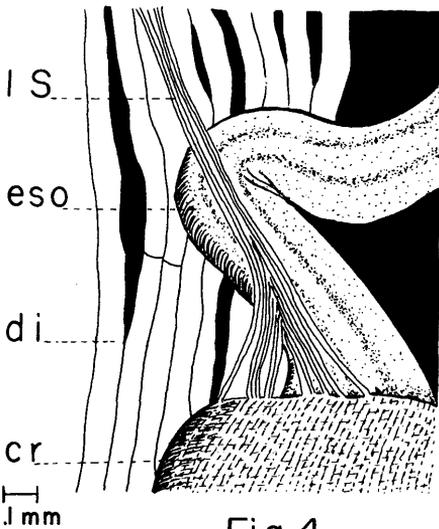


Fig.4

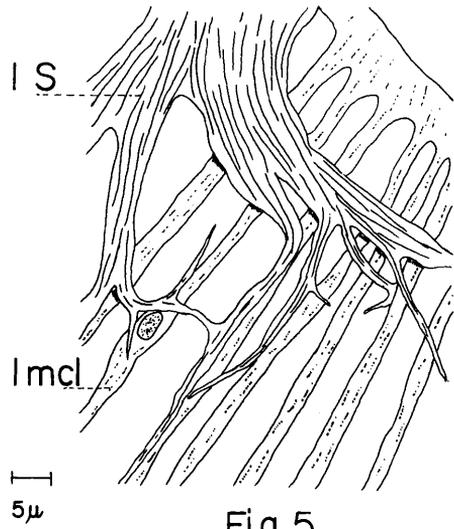


Fig.5

FIGURE 2. Attachment of the first suspensory muscle to the internal lateral muscles of the body wall.

FIGURE 3. Whole mount preparation of the attachment of the first suspensory muscle to the internal lateral muscles.

FIGURE 4. Attachment of the first suspensory muscle to the crop.

FIGURE 5. Whole mount preparation of the attachment of the first suspensory muscle to the crop.

The fibers of the second pair of muscles are attached to several dorsal internal muscles on the body wall between the second and third segments (fig. 6). As seen in a whole-mount preparation, the fibers of the second pair appear to become continuous with the fibers of the dorsal internal muscles (fig. 7).

The second pair of suspensory muscles attaches to the gut just anterior to the gastric ceca (fig. 8). As with the first pair (fig. 5), the muscle fibers taper extensively, branch, and spread over the gut, but do not become continuous with the longitudinal muscle fibers of the gut.

The fibers of the third pair of muscles attach directly to the body wall, as well as to some of the body wall muscles (fig. 9). Whole-mount preparations of the third muscle show that some of the fibers become continuous with internal lateral muscles (fig. 10).

The third pair is attached to the mid-gut in three places. The first attachment is about half-way along the mid-gut, the second attachment is more posterior, and the third is close to the junction of the mid-gut and hind-gut (fig. 1). Each of these attachments is similar with many fibers extending from each branch of the thick third muscle to the gut (fig. 11). A branch of the third muscle attaches to the mid-portion of each Malpighian tubule (fig. 1). As with the first pair of muscles (fig. 5), the fibers fan out over the gut and do not become continuous with the longitudinal or circular gut muscle fibers, but attach directly to the gut.

The cytology of the suspensory muscles, body wall muscles, and gut muscles was studied, and the attachments of the suspensory muscles to the body wall and gut were examined. Whole-mount preparations show that the body wall muscles consist of multinucleate fibers with nuclei of varying sizes and shapes which range from ovoid to spherical (fig. 12). The nuclei appear roughly spherical in all sections, indicating that they are not disc-shaped.

The longitudinal gut muscles are multinucleate fibers which thicken and bulge slightly where the large ovoid nuclei are located (fig. 13). Each longitudinal fiber extends the entire length of the mid-gut and contains four large nuclei arranged in two pairs, one pair at the anterior end of the mid-gut and the other at the posterior end. In a whole-mount preparation of the entire mid-gut, these individual pairs of nuclei seem to form four rows of nuclei around the mid-gut.

The circular muscles of the gut are multinucleate and consist of several fibers, which unite forming a single fiber where the large, circular nuclei are located (fig. 13). Each muscle fiber, which appears to completely encircle the gut, contains four nuclei arranged in two pairs along the fiber. These individual pairs of nuclei of all the circular fibers form four longitudinal rows of nuclei along the mid-gut. The nuclei of both the circular and longitudinal fibers are of constant size and are disc-shaped, since in sections of the fibers the nuclei are clearly flattened.

The suspensory muscles are made up of multinucleate fibers with nuclei of varying sizes and shapes which range from ovoid to spherical (fig. 14). In longitudinal sections, the nuclei appear approximately spherical, indicating that they are not disc-shaped.

In most preparations of the muscles, only the suspensory muscles show striations clearly. Examination of unstained muscle tissue under a phase contrast microscope, however, shows that all three kinds of muscle, of body wall and gut, as well as suspensory muscle, are striated.

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#### EXPLANATION OF FIGURES

- FIGURE 6. Attachment of the second suspensory muscle to the dorsal internal muscles of the body wall.
- FIGURE 7. Whole mount preparation of the attachment of the second suspensory muscle to the dorsal internal muscles.
- FIGURE 8. Attachment of the second suspensory muscle to the mid-gut.
- FIGURE 9. Attachment of the third suspensory muscle to the internal lateral muscles and the dorsal internal muscles of the body wall and the body wall.
- FIGURE 10. Whole mount preparation of the attachment of the third suspensory muscle to the dorsal internal muscles and the body wall.
- FIGURE 11. One attachment of the third suspensory muscle to the mid-gut.

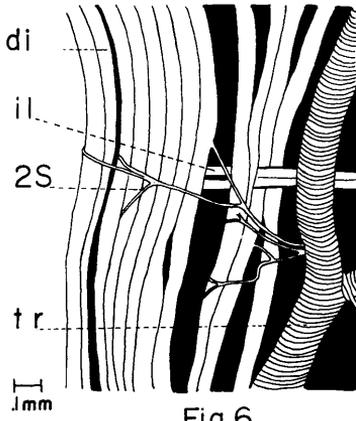


Fig 6

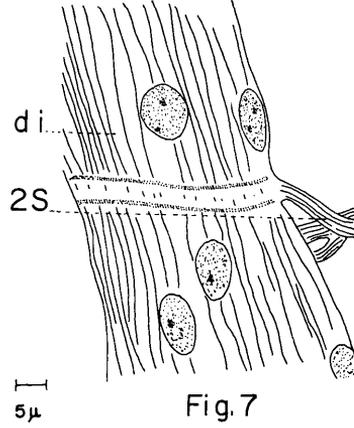


Fig. 7

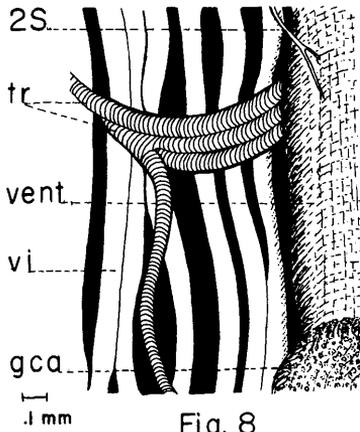


Fig. 8

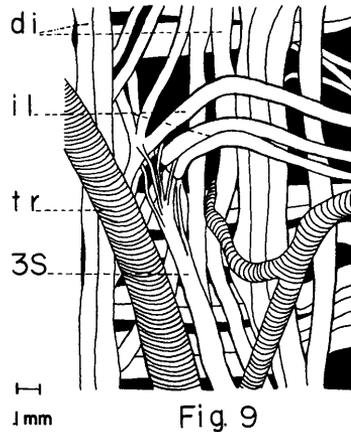


Fig. 9

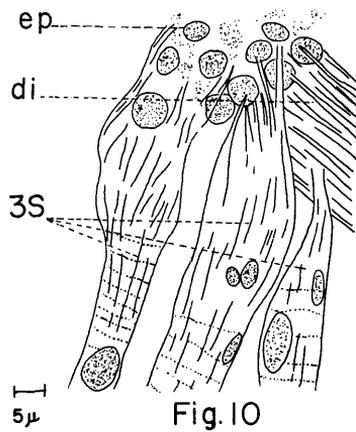


Fig. 10

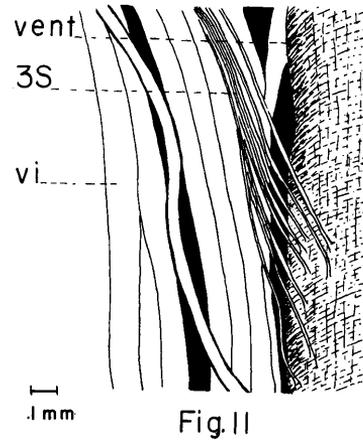


Fig. 11

### Discussion

Cytological examination of the attachments of the suspensory muscles to the gut and body wall, and comparison of the size and shape of the nuclei of body wall muscles, gut muscles, and suspensory muscles indicates that the three pairs of suspensory muscles originate from body wall muscles and body wall, and insert on the mid-gut. This does not agree with Graham-Smith (1934), who describes the adductor muscle of the ventriculus as being derived from several of the longitudinal and some of the transverse bands of the ventriculus. Although he shows the fibers of the adductor muscles becoming continuous with the longitudinal gut fibers, he makes no histological comparison of these two kinds of muscles.

In *Tipula abdominalis*, however, the fibers of all three pairs of suspensory muscles are continuous with the body wall fibers, so one can assume this is the origin. The suspensory muscles branch only slightly where they become continuous with the body wall muscles, with little tapering of fibers (figs. 3, 7, 11). Where all three pairs of muscles join the gut, however, the fibers branch extensively, taper, and end directly on the ventricular epithelium of the gut, and do not join the muscle fibers of the gut (figs. 5, 9, 13). This can be considered the insertion of the suspensory muscles.

Size and shape of the nuclei give further support to the idea that the suspensory muscles are body wall muscles. Both the nuclei of body wall muscle and those of the suspensory muscle range from ovoid to spherical and are of varying sizes (fig. 12, 14). The nuclei of the longitudinal and circular muscles of the gut are ovoid and circular, respectively, and of constant size (fig. 13).

In comparing the findings of the present study with previous work, one finds there are few generalizations which can be made. Although the suspensory muscles in *Tipula abdominalis* attach dorso-laterally to the body wall, there is some variation in places of attachment among the muscles described in other insects. Most of the muscles described have lateral attachments, but some are ventro-lateral (Lyonet, 1762; Tanaka, 1911; Peterson, 1912). All these studies include a description of the body wall attachments and indicate that the muscles attach between body segments. The body wall attachments in *T. abdominalis* are also intersegmental.

There is some variation in attachment of the suspensory muscles to the gut. In *Tipula abdominalis*, the muscles attach only to the mid-gut, with two branches of the third pair extending to two Malpighian tubules (fig. 1). Lyonet (1762), however, described suspensory muscle attachments to the mid-gut, hind-gut, Malpighian tubules, and silk glands in *Cossus cossus*. Tanaka (1911) also described mid-gut, hind-gut, and silk gland attachments of these muscles in *Bombyx mori*.

A cytological study of the suspensory muscles permits a greater understanding of their structural detail. It brings no insight as to the action of the muscles, however. The second part of the study, a physiological approach, is an attempt to find out more about the function of the suspensory muscles.

#### ACTION OF SUSPENSORY MUSCLES OF TIPULA ABDOMINALIS

##### *Materials and Methods*

The following procedure was used to prepare small platinum electrodes (Wise, 1964): A small piece of platinum wire was bent to form a right angle, and the vertex of the angle was fused to the tip of a glass rod. A copper wire connected

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#### EXPLANATION OF FIGURES

- FIGURE 12. Dorsal internal muscles of the body wall.  
 FIGURE 13. Longitudinal and circular muscles of the mid-gut.  
 FIGURE 14. The third suspensory muscle.

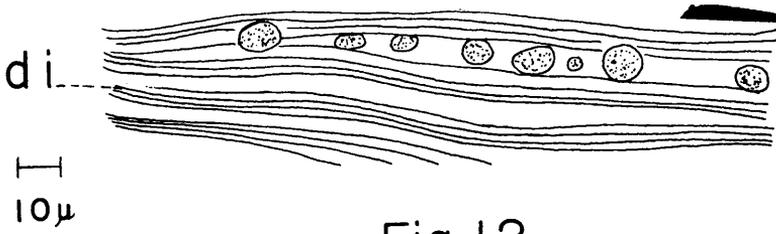


Fig.12

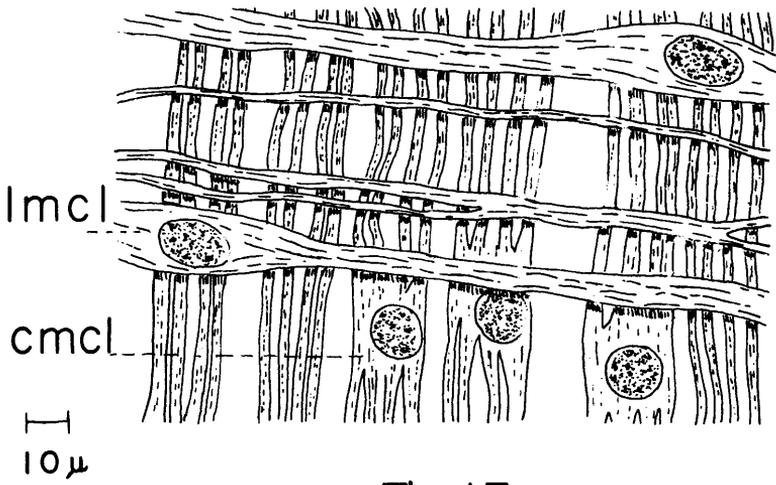


Fig.13

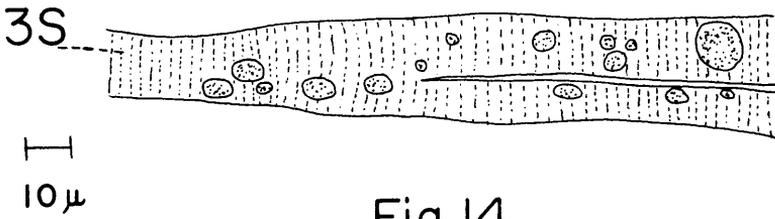


Fig.14

to an SD-5 stimulator (Grass Instrument Co.) was then attached to the end of the platinum wire perpendicular to the glass rod. The other end of the platinum wire was used to touch the muscle being stimulated. Two electrodes were prepared in this manner.

The animal was kept alive for 4 hrs (could have been longer) in an ice-cold dilute insect saline consisting of 7.5 g NaCl, 0.35 g KCl, and 0.21 g CaCl<sub>2</sub> to 1 liter of water (Williams, 1946). A mid-dorsal incision was made and the body wall was teased away from the gut and pinned down. The third suspensory muscle was easily exposed after partial removal of the fat body. The stimulator was set on "repeat" so that there would be automatic stimulation of the muscle, and the two electrodes were held on different portions of the third muscle.

About 72 volts of electricity were sufficient to stimulate the third suspensory muscle. The frequency was one pulse per 1.7 sec, the duration was 10 msec, and there was no delay. The output was monophasic and the polarity was normal.

The third pair of muscles was the only one wide enough and long enough to be stimulated with the electrodes prepared.

### Results

Stimulation of the third suspensory muscle with the platinum electrodes led to contraction of the muscle and a subsequent forward movement of the gut. When the suspensory muscles relaxed, the gut returned to its original position. When the gut muscles themselves were stimulated, there was only a rippling of the gut and no displacement of it.

When the dissected animal was observed in saline, but with no electrical stimulation, there was an irregular contraction of a branch of the third suspensory muscle which attached to the Malpighian tubule, and this resulted in an elongation of the Malpighian tubule. When the muscle relaxed, the tubule shortened again.

### Discussion

Although the gut moved forward after stimulation of the third suspensory muscle, this is not conclusive evidence that it does this under normal conditions, since the animal was in an unnatural (pinned out) condition. In the living animal, the muscles probably just take up slack and keep the gut from being displaced unduly, as suggested by Graham-Smith (1934). Since the gut is quite thin and delicate, these muscles might keep it from twisting completely around during the swimming and crawling activity of the larva (Weaver, 1964). The attachments of the muscles to the Malpighian tubules probably keep them from becoming excessively twisted or tangled also.

### ACKNOWLEDGMENTS

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