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The histology of the third instar larva of the apple maggot, Rhagoletis pomonella (Walsh).

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THE HISTOLOGY OF THE THIRD INSTAR LARVA OF THE APPLE MAGGOT, RHAGOLETIS POMONELLA (WALSH)

A Thesis Presented

By

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I. INTRODUCTION

Since its original description under the name Trypeta pomonella by Walsh (1867), the apple maggot, Rhagoletis pomonella has remained an orchard pest of some importance in much of the U. S. and southeastern Canada. In temperate climates the apple maggot is univoltine. Adults emerge from late June through early August (Rivard, 1968) and apparently feed on aphid honeydew adhering to the surfaces of apple leaves and fruit (Neilson and Wood, 1966). After a pre-oviposition period of about 12 days, the females mate and begin to lay eggs singly, just beneath the skin of the fruit with the aid of a sharp ovipositor. The larvae tunnel through the fruit spreading the bacterium Pseudomonas melophthora which causes a brown rot to develop in the apple flesh. Growth of both larvae and bacteria is slow until the fruit ripens and drops after which the apple maggot develops rapidly, leaves the rapidly decomposing apple and pupates in the soil. Winter is passed in the pupal stage, a number of pupae diapausing for two years.

Due to the detrimental environmental effects of chemical insecticides, it would seem that future emphasis must be placed on alternative control measures. Many of these will depend on exploitation of certain aspects of the biology of target species, thus making basic research in this area desirable. The higher flies (cyclorrhapha) include a number of important pest species and often (for instance, the apple maggot) the larva is the important stage. However, little basic work has been done with maggots other than Drosophila, Musca and large Calliphorids, especially with regard to histology. In fact, so far only the work of Pérez

(1910) on Calliphora has dealt with the normal histology of the organ systems of the entire animal. The present study will attempt to rectify this by presenting a description of the histology of a typical cyclorrhaphan larva, the apple maggot.

Since maggots are very similar morphologically, this work will mitigate interpretation of histological sections of the larvae of most cyclorrhapha. A knowledge of the appearance of the normal tissues would thus allow recognition of pathological conditions brought about through a biological control such as a pathogen or parasite.

The major damage to fruit inflicted with apple maggot is actually caused by its associated symbiote, P. melophthora. This bacterium is introduced through oviposition and produces the brown rot always associated with apple maggot tunneling which eventually destroys the fruit. Therefore, a second objective of this study is to locate P. melophthora in the body of the animal and to determine if the larva contains any special structures for carrying its symbiotes into the pupal stage. In order to avoid the difficulty involved in working with the very small first and second instars, this study will concern only mature (third instar) larvae.

II. LITERATURE REVIEW

A. Integument

The general organization of larval cyclorrhaphan cuticle into endocuticle and epicuticle has been known since the work of Lowne (1890-92) on Calliphora. Dennell (1946) performed a variety of histochemical tests on the developing larval cuticle of Sarcophaga falculata and found that each of the two original layers could be further subdivided into two layers, the inner and outer endocuticle and the inner and outer epicuticle. Locke (1957) studied the epicuticle with the electron microscope and determined that the outer layer, which he termed the cuticulin, was present over most of the body surface of insects being absent only in some sense organs (Slifer, 1961) and the gut (Bertram and Bird, 1961). The cuticulin is extremely important as it essentially determines the surface characteristics of the cuticle as well as protecting the developing deeper layers from the potent molting fluid enzymes (Locke, 1966).

The chemical composition of maggot cuticles was investigated by Pryor (1940) who was mainly interested in the localization of phenols during cuticle tanning. His histochemical tests on Calliphora showed phenol restricted to the epicuticle in the feeding larva. Fraenkel and Rudall (1940, 1947) studied the composition of the larval cuticles of Calliphora erythrocephala and Sarcophaga falculata using a variety of chemical tests as well as X-ray diffraction and concluded that the larval cuticle is a polysaccharide-protein complex, 60% of which is chitin, and that in the larva these chitin filaments are oriented ran-

domly allowing flexibility. Richards (1956) performed microincineration and elemental analysis studies on dried cuticles of Sarcophaga bullata and found traces of 25 elements left in the ash with magnesium being the predominant cation. The cuticulin layer left a relatively large amount of ash in which most of the iron content of the cuticle was present.

A number of authors (Dennell, 1946; Wolfe, 1954; Kennaugh, 1965; Filshie, 1970) have described pore canals from the larvae of cyclorrhaphous diptera. Locke (1961) reported that no pore canals are present in the larva of Calpodes ethlius (Lepidoptera:Hesperiidae).

B. Anal organ

This plate of thin endocuticle has been used by taxonomists for some time as a diagnostic character (Zimin, 1951) but its function is still in doubt. El Shatoury (1955) claimed that in larval Drosophila the thin cuticle contracts rhythmically thus acting as an accessory pulsating organ. Gloor (1949) found that the anal organ of Drosophila darkened after immersion in a dilute solution of silver nitrate indicating that this area is more permeable to ions than the surrounding integument. On the basis of Gloor's work, Quintart (1961) proposed an osmoregulatory function for the anal organ, a view also held by Stoffolano (1970).

C. Tracheal system

The most thorough study of the morphology of the tracheal system in larval Diptera is that of Whitten (1955, 1957). Whitten's detailed (1955) paper deals comparatively with this system and reveals a common pattern occurring through the order which may still be recognized in the highly modified cyclorrhapha. Her later (1957) paper follows the development of the tracheal system of Drosophila through larva, pupa and adult. Recently, Whitten (1972) reviewed the morphology of the insect tracheal system. Keilin (1944) also studied the comparative morphology of this system in larval Diptera but was more concerned with the structure, location, number and development of the spiracles. His lengthy paper includes a section on the paraspiracular (peristigmatic) glands in dipterous larvae. These glands were first reported by Leydig (1859). Gazagnáire (1886) noted the long, coiled duct in the gland of Eristalis tenax and rightly theorized its function to be to conduct a hydrophobic oil to the cuticle surrounding the spiracular openings. Keilin et al. (1935) found these glands in mosquito larvae and were able to discern oil droplets in the ducts leading to the spiracles.

Bates (1934) described these glands in the anterior and posterior spiracles of the apple maggot while Phillips (1939) found them in the anterior spiracles of the walnut husk fly, Rhagoletis suavis. Butt (1937) also shows these glands in his paper on the posterior felt chambers of the apple maggot.

D. Alimentary tract

Snodgrass (1924) provides a detailed account of the morphology of the cephalopharyngeal skeleton of the apple maggot but merely mentions the pharyngeal lamellae as being visible through the pharyngeal floor. Previously Keilin (1912) had examined a variety of maggots representing many different families of higher Diptera and had determined that pharyngeal lamellae are present only in larvae living on dead plant or animal tissue. Tephritid larvae develop in living fruit but Keilin (1913) theorized that they actually feed on tissue killed by either oviposition or an introduced bacterium and are thus, saprophages.

Becker (1910), working with house fly larvae, felt that food particles were wedged between the lamellae then digested by salivary enzymes. However, Dowding (1967) performed extensive feeding experiments on Calliphora larvae and concluded that food particles were strained out above the lamellae and only fluid coursed through the lower channels. In a later (1968) paper she studied the development of these ridges through light and electron microscopy, discovering that they are formed via cytoplasmic extensions of the pharyngeal epidermis. She also reported the presence of a thick epicuticle which may serve to protect the deeper layers from potent salivary enzymes (Dennell, 1950).

Dean (1932) described the morphology and histology of the alimentary canal of R. pomonella posterior to the pharynx and found it similar to those of other maggots (Lowne, 1890-92; Hewitt, 1914). Hobson (1931) reported three histolcgically distinct zones in the larval midgut of Lucilia sericata and made a careful study of the pH

through this tube. He also determined the location and action of a number of enzymes found in the larval blow fly alimentary tract. Waterhouse and Stay (1955) found further differentiation in the midgut of Lucilia cuprina by using a variety of histochemical tests. The tests revealed that lipids, glycogen, various metals, ascorbic acid, phosphatases, dehydrogenase and cytochrome oxidase are present in well defined bands along the midgut. They also demonstrated the existence of an area christened the "mosaic midgut" in which two histologically and histochemically distinct cell types are present. Waterhouse and Wright (1960) studied this area through the electron microscope and classified the two cell types as either lipophilic or cuprophilic. The former contains much lipid, glycogen and acid phosphatase and has a well developed brush border composed of parallel lamellae. The cuprohilic cell is rich in iron, copper, dehydrogenase, cytochrome oxidase and esterase but has a very poorly developed brush border.

E. Fat body

Wigglesworth (1965, pg. 401) states that the "most obvious" function of the insect fat body is storage of fats, proteins and glycogen. This organ is also an important center of intermediary metabolism possessing for example, the complete citric acid cycle (Wigglesworth, 1965, pg. 404). Snodgrass (1924) determined that the vacuoles in the fat body cells of R. pomonella contain lipid.

When present, oenocytes are generally found associated with the fat body (Hewitt, 1914; Bodenstein, 1950; Wolfe, 1954). Wolfe (1954) in his study of molting in Calliphora erythrocephala reports that at this time the oenocytes migrate to just below the epidermis from where they seem to secrete at least part of the epicuticle.

Crystalline material is often found within the fat body cells. Pérez (1910) considered the crystals found in Calliphora erythrocephala to be evidence of excretory activity. Calcium carbonate crystals (calcospherites) have been reported from the fat body of Syrphidae (Bhatia, 1939) and other dipterous larvae (Keilin, 1921).

F. Nervous system

1. Central nervous system

The central nervous system of larval cyclorrhapha exhibits an extreme degree of fusion, all of the thoracic and abdominal ganglia being represented by the large ventral ganglion (Lowne, 1890-92; Hewitt, 1914; Keilin, 1917; Hertweck, 1931). The ancestral segmented nature of the ganglion is revealed by the eleven pairs of segmented nerves which originate from its sides. One pair of lateral nerves runs to each segment where they supply the integumental musculature (Hewitt, 1914; Hertweck, 1931; Osborne, 1963), cuticular sense organs (Osborne, 1963) and chordotonal organs (Whitten, 1963a). In larvae of higher Diptera there are three pairs of cephalic nerves (Ludwig, 1949). The antenno-labral nerves which arise from the brain, innervate two pairs of sense organs and also enter the stomatogastric system at the frontal ganglion. A second pair, the mandibulo-maxillary-labial nerves originate from the ventral ganglion and innervate three pairs of sense organs and the mouth-hook musculature. The third pair is the prothoracic accessory nerve which arises near the preceding and which ramifies to supply the pharyngeal extensor muscles (Ludwig, 1949).

The ventral ganglion also gives rise to from four (Hewitt, 1914) to seven (Osborne, 1964) median unpaired nerves. Whitten (1963b) considered them to be giant neurosecretory cells located outside the ventral ganglion, however, Osborne's (1964) electron microscopic work showed them to be typical nerves similar in fine structure to the peripheral nerves described by Edwards *et al*. (1958) from the wasp leg.

The brain and ganglion show histological features common to insects. The outer neural lamella which is freely permeable to ions (Chapman, 1969, pg. 525) is contiguous with the basement membrane covering the nerves (Whitten, 1964a). Histochemical and ultrastructural studies have shown that this sheath is composed of a mucopolysaccharide in which collagen fibrils are embedded (Ashurst, 1961a, b). Directly beneath this sheath is a single layer of cells, the perineurium which is responsible for regulating the flow of ions into the underlying nerve cells (Hoyle, 1952). Histochemical and ultrastructural studies were performed on this layer by Ashurst (1961b, c) in Periplaneta americana and Locusta migratoria, respectively. Lowne (1890-92) and Hertweck (1931) have briefly described the histology of the cortical region of fly larvae. Ashurst (1961a) found that the neurons of Periplaneta have many lipid containing bodies and highly developed Golgi apparatus which Wigglesworth (1960) considered to be the origin of microtubules which extend through the axons of these cells.

A number of authors (Thomsen, 1951; Possompes, 1953; Fraser, 1959a) have found neurosecretory cells in the cortex of the brain of blow fly larvae and Fraser (1959b) also reported them from the ventral ganglion. Smith's (1968) electron microscopy of insect neurons shows large nuclei enveloped by a thin layer of cytoplasm. The neurons are enmeshed in a tangle of axons and glial cell processes.

The neuropile region of the insect ganglia is composed mainly of axons and glial elements. Osborne's (1966) electron microscopy of

larval Phormia terrae-novae has shown that it is here that interneuronal contacts are made.

2. Stomatogastric system

Ludwig (1949) has made a careful study of the cephalic nerves of larval Calliphora erythrocephala including the stomatogastric system. Fraser (1957, 1959c) described the endocrine organs and stomatic system (retrocerebral organs) of Protophormia, Lucilia and several Muscids.

3. Sense organs

The cuticular sense organs of larval cyclorrhapha may be conveniently divided into two groups, the five pairs of large cephalic organs, and the many smaller organs scattered over the body wall. The most conspicuous of the cephalic organs are the two pairs located at the anterior tip of the body. The dorsal pair had long been considered to be photoreceptors (Lowne, 1890-92; Hewitt, 1914; Ellsworth, 1933) until Bolwig's (1946) behavioral and histological study proved them to be olfactory receptors in larval house flies. The actual photoreceptors were found to be a small cluster of cells lying in a pocket in the cephalopharyngeal skeleton.

Bolwig's (1946) light microscopic study has been extended through the scanning and transmission electron microscopy of Chou and Axtell (1971). These workers have shown the dorsal sense organ to be composed of a proximal cluster of neurons which send axons around the periphery of a large central vacuole into the apical dome. Here the axons branch profusely. The apical dome is perforated by many small pores but the axons do not seem to be connected to them. The basal cells are covered by a layer of glial cells which send processes around the neurons to form the "tunicated sheath" typical of the insect nervous system (Smith, 1967).

The ventral terminal organs of house fly are considered to be gustatory in function (Bolwig, 1946). They are histologically simpler than the dorsal organs, being composed of an oblong cluster of fusiform neurons which send axons into a somewhat smaller papilla surrounded by

several concentric rings of oblong plates (Bolwig, 1946; Chou and Axtell, 1971). In Rhagoletis the apical portion is composed of several small papillae (Snodgrass, 1924).

Snodgrass (1924) and Bolwig (1946) report the presence of sense organs at the corners of the mouths of Rhagoletis and Musca, respectively, which seem to be similar to although smaller than the ventral terminal organ.

Two more pairs of cephalic organs are located in the pharynx of fly larvae, one in its floor and the other in the pharyngeal roof. These have been described by Pantel (1898), Keilin (1915), Hertweck (1931) and Bolwig (1946) in a variety of maggots. Ludwig (1949) has described the innervation of the cephalic organs in the larva of Calliphora erythrocephala.

The small cuticular sense organs have been studied most thoroughly by Hertweck (1931) for Drosophila melanogaster. Osborne (1963) was able to trace the innervation of the cuticular organs of Phormia through a sub-cuticular nerve plexus to the lateral segmental nerves.

Maggots are also equipped with various types of chordotonal organs. Keilin (1927) found these receptors in association with the terminal sense organs in Drosophila amphilophila and Platychirus scutatus Meig. (Diptera:Syrphidae), while Hertweck (1931) described a series of segmentally arranged stretch receptors in the thorax and abdomen of D. melanogaster. More recently Osborne (1963) and Whitten (1963a) found stretch receptors attached to integumental muscles and tracheae innervated through the lateral segmental nerves. Also, Osborne (1964)

found muscular receptors innervated through the dorsal unpaired nerves of blow fly larvae.

G. Imaginal discs

The imaginal head of higher Diptera is represented in the larva by a pair of diverticula from the roof of the pharynx. These are known as the frontal sacs and contain rudiments of the compound eyes, antennae and head capsule (Snodgrass, 1924). During metamorphosis these sacs evert through the mouth to form the imaginal head.

A number of light microscope studies have been made of the developing eye of D. melanogaster. Krafka (1924) compared the development of the normal eye with that of the mutant bar eye. These studies were expanded through the work of Steinberg (1941, 1943), while Enzmann and Haskins (1938) concerned themselves with the histology of the normal eye. The work of these authors was summarized by Bodenstein (1950) who noted that the presence of recognizable ommatidial rudiments in third instar eye discs was somewhat in doubt. Their presence was later confirmed by the ultrastructural studies of Waddington and Perry (1960).

Little attention has been paid to the antennal disc but Lowne (1890-92) determined that the central papilla seen in mature Calliphora larvae forms the flagellum of the imago while the encircling ring forms the scape and pedicel.

Lowne (1890-92) and Bodenstein (1950) report similar structures for the developing leg discs of Calliphora and Drosophila, respectively. Here there are more segments visible in mature larvae but the mode of development is similar to the antennal disc. Chiarodo and Denys (1968) studied the ultrastructure of the metathoracic leg disc of Sarcophaga bullata and found a dramatic increase in cytoplasmic volume and com-

plexity during the last larval instar.

Auerbach (1936) has studied the development of both wings and halteres in Drosophila. In the third instar these structures are simple sacs, the mesial wall of which is thickened and occasionally somewhat folded. The wing and haltere later develop from this thickened portion.

H. Reproductive system

In Drosophila larvae the reproductive system is represented by an unpaired genital disc located in the last abdominal segment, and a pair of gonads located in the fifth abdominal segment (Bodenstein, 1950). In Dacus tryoni (Anderson, 1963b), Musca domestica and Phormia regina (Dubendorfer, 1970) the unpaired median disc is flanked by a pair of lateral genital discs. Genitalia and gonads do not join until after pupation (Dobzhansky, 1930). Newby (1942) and Bodenstein (1950) claim that the genital disc in Drosophila does not differentiate from the larval epidermis until the second instar. However, Anderson (1963a) reports differentiation of the disc of Dacus tryoni in the late embryo. Newby (1942) was able to sex mature Drosophila larvae by the histology of the genital disc.

The gonads develop from the pole cells of the embryo (Anderson, 1963a). In Drosophila larvae the testes are significantly larger than the ovaries throughout larval life. During the third instar they are also distinguishable histologically (Kerkis, 1933).

Bush (1966) using adult gonad as well as larval brain tissue was able to determine that the apple maggot has six pairs of chromosomes.

I. Circulatory system

The heart and aorta of a pair of cyclorrhaphan larvae, Musca domestica and Calliphora erythrocephala have been described by Hewitt (1914) and Lowne (1890-92), respectively. Essentially the heart is a muscular tube lying in the last three abdominal segments with one pair of ostia in each segment. The structure of the ostia has largely been overlooked but Tzonis (1936) reports them to be simple verticle slits in the larva of Corethra, each lip being equipped with a median unicellular thickening connected to a system of fibrils. These thickenings and the associated fibrils play a role in the action of the ostia. The heart is dilated by the contraction of three pairs of alary muscles (Lowne, 1890-92; Hewitt, 1914) which are attached to the heart through a series of fibers. In Sarcophaga bullata similar fibers connect the double row of pericardial cells with the heart (Whitten, 1964a) and contain secretion filled channels. In Calliphora the pericardial cells are connected to the corpus cardiacum by neurosecretory axons suggesting transfer of neurosecretory hormones to these cells (Chapman, 1969, pg. 697).

Lowne (1890-92) has termed the space dorsal to the pericardial cells and between the dorsal oblique muscles (Fig. 58 PS) the pericardial sinus although no dorsal diaphragm is present. The ventral diaphragm is also lacking among the larvae of higher Diptera (Richards, 1963).

The small cells flanking the aorta have been interpreted in several ways. Anderson (1963a) refers to them as the "lymph gland cells," and Stark and Marshall (1930) consider them to be blood forming organs

while Bhatia (1939) and Keilin (1924) classify them as nephrocytes. Keilin (1924) reported phagocytosis occurring in these cells in the larva of Lonchaea chorea (Diptera:Lonchaeïdae).

A row of nephrocytes may also be found between the salivary glands in larval diptera constituting the "garland like cell chain" of Weissmann (Keilin, 1917). These structures have been described in Phaonia cincta (Diptera:Lonchaeidae) by Keilin (1917, 1924) and in Calliphora (Chapman, 1969, pg. 495).

According to Arvy (1953), three basic types of hemocytes are present in house fly larvae. The small basophilic cells containing little cytoplasm are prohemocytes, those with relatively greater amounts of cytoplasm are termed plasmatocytes while the large, eosinophilic forms are oenocytoids. Several other types of blood cells have been described from dipterous larvae by investigators using phase contrast microscopy (Rizki, 1953; Jones, 1956; Whitten, 1964b, 1969; Nappi, 1970).

Few blood cells circulate freely before pupation in the larvae of higher Diptera. Instead, most of the cells may be found in aggregations in the last abdominal segments. Arvy (1954) considers these aggregations to be hemopoietic organs while Anderson (1963a) feels that they are artifacts of fixation. However, Nappi and Stoffolano (1972) using liquid nitrogen to rapidly kill and fix larvae of M. domestica and M. autumnalis, found blood cells in the same area. Thus it would seem unlikely that these aggregations are artifacts.

J. Endocrine system

Burtt (1938) found that when a portion of the ring gland of third instar Calliphora larvae is destroyed, the animals are unable to pupate. Thus, Burtt determined that this ring of tissue encircling the aorta is an important endocrine organ in higher Diptera. Comparative studies (Burtt, 1937; Thomsen, 1951) show that the ring gland represents the fused corpora allata, corpora cardiaca, and prothoracic gland of more generalized insects. The three glands are still recognizable as they differ in location and (usually) histology. The lateral portions of the ring contain large prothoracic gland cells while the distinctly smaller cells of the unpaired corpus allatum are situated dorsally (Scharrer and Hadorn, 1938; Day, 1943; Thomsen, 1951; Fraser, 1959c). The position of the corpus cardiacum varies. In Lucilia, Drosophila and Eristalis tenax it is fused to the ventral ends of the prothoracic glands ventral to the aorta (Day, 1943; Bodenstein, 1950; Cazal, 1948). In Musca and some Calliphorids, however, the corpus cardiacum forms a patch in the aortal floor and is not connected to the prothoracic glands (Fraser, 1959c).

In Eristalis (Cazal, 1948) and Lucilia (Day, 1943) the corpora cardiacum and allatum are connected through nerves. Whitten (1964a) reported the presence of secretion filled channels in the basement membrane of the ring gland of Calliphora larvae.

Herman (1967) has reviewed the literature pertaining to the ecdysial (prothoracic) glands of all Arthropods.

K. Excretory system

The most important excretory organs of insects are the Malpighian tubules. These structures are unusually sensitive to fluids other than hemolymph, thus many artifacts are formed during fixation (Wigglesworth, 1965, pg. 511). These include intense vacuolation (von Gorka, 1914) and swelling of the cells resulting in an occluded lumen and eradication of microvilli (Metalnikov, 1908; Wigglesworth and Saltpeter, 1962).

Dean (1932) described and figured the Malpighian tubules in his histological investigation of the alimentary tract of Rhagoletis pomonella. He mentions that two cells border the lumen of the tubes but often in sections it appears as if only one is present due to indistinct cell membranes and staggered nuclei. Snodgrass (1924) found white crystals occupying the lumen of these organs in the apple maggot which may be uric acid (Chapman, 1969, pg. 498).

The cells occupying the ampullar region of the Malpighian tubules of Drosophila and Rhodnius have their apical borders produced into long filaments which extend into the hindgut (Eastham, 1925; Wigglesworth, 1931). Wigglesworth (1931) presented experimental evidence indicating that these filaments function in reabsorption of water from the rectum in Rhodnius.

The proximal region of the Malpighian tubules of Drosophila and Calliphora is equipped with a layer of circular muscles the contractions of which make peristaltic movements possible. Presumably excretory products move via peristalsis into the hindgut. Little is known about the excretory products of fly larvae. Stobbart and Shaw (1964) reported

that Lucilia larvae excrete nitrogen mainly in the form of ammonia and to a lesser extent, allantoin. Chapman (1969, p. 498) has reported the presence of uric acid in the tubules of unspecified larval Diptera.

The nephrocytes or pericardial cells are also thought to have an excretory function. Hollande (1921) theorized that they absorbed complex materials from the blood and broke them down enough to be handled by the Malpighian tubules. Lesperon (1937) claimed that they were able to phagocytose colloids. Mills and King (1965) and Aggarwal and King (1967) described the fine structure of these cells in adult and larval Drosophila. Both papers show many lysosome like bodies in the pericardial cells and other large vesicles containing electron dense material in various states of decay. Aggarwal and King (1967) were able to locate macromolecules adhering to the cell membranes which were actively engaged in pinocytosis and cited these observations as being evidence of an excretory function.

L. Bacterial symbiotes

Petrie (1909, 1910) first reported the presence of symbiotic bacteria in the digestive tract of the Tephritid Dacus oleae. The two species of bacteria involved, Pseudomonas savastanoi and Ascobacterium luteum, are located in the gastric caeca of the larva and after emergence the flora is restablished via a special oesophageal diverticulum located immediately anterior to the brain of the adult.

The association of the plant pathogen Pseudomonas melophthora with the apple maggot was first reported by Allen and Riker (1932). Allen et al. (1934) using culturing techniques, were able to demonstrate the presence of P. melophthora in all life stages of Rhagoletis. Although the alimentary tract of Rhagoletis is similar to that of D. oleae, having caeca in the larva (Dean, 1932), and an "oesophageal bulb" in the adult (Dean, 1933), the exact location of the symbiotes is not known. Baerwald and Boush (1968) used the fluorescent antibody technique in an attempt to locate P. melophthora in sections and smears of Rhagoletis eggs, larvae, pupae, and adults but obtained very inconsistent results due to the small numbers of bacteria present.

III. MATERIALS AND METHODS

A. Apple maggot colony

The apple maggot colony was started with several hundred pupae from the New York State Agricultural Research Station in Geneva, New York. The flies were cultured according to methods described by Neilson (1965) and Prokopy (1968). Adults were kept in a constant temperature chamber (Sub Zero) in screen covered wooden cages (12"x12"x12") at 27°C and approximately 50% R.H. The flies were exposed to a 16 hour photophase. Food consisted of sucrose, vitamins (Vitamin Diet Fortification Mixture)^{R} and an amino-acid mixture provided in separate wax paper dishes. The amino-acid mixture consisted of vitamin and salt free casein hydrolysate-93 g.; tryptophane-2g.; arginine-2g.; histidine-2g.; and cystine-1g.; mixed with minerals (Salt mixture number 2 U.S.P. xiii) in a 4:1 ratio. Water was provided in plastic containers equipped with dental cotton wicks.

As soon as mating was observed (about eight to ten days after emergence) oviposition material was provided. Depending of the size of the population, 2 to 5 Red Delicious apples were placed in the cage daily. Infested apples were dated and placed in 18"x9"x5" white enameled metal pans containing a layer of damp sand. These were maintained at the same parameters of light, heat and humidity as the adults. When larval development was completed (20-25 days), the maggots left the apples to pupate in the sand and could be collected by flooding the pans with water causing the pupae to float to the surface. The water

was strained and the pupae were dried on paper towels to prevent growth of fungus. They were then placed in petri dishes lined with paper tissue (Kimwipes)^{R} and were placed in a humidity chamber to avoid desiccation. The chamber used consisted of a one liter desiccator jar containing a saturated solution of KCl which results in a R.H. of approximately 95% at 27°C, the optimum conditions for pupal development (Neilson, 1962). The desiccator was kept in the growth chamber with the adults and larvae and was opened periodically to allow exposure to fresh air. Adults started to emerge about 30 days later and were immediately transferred to the screened cages.

During August and September, the colony was supplemented with infested field collected apples. These were then placed in either the white pans mentioned above or in plastic 12 quart pails containing moist sand. The pupae were collected as described and placed in the growth chamber.

B. Histological techniques

1. Killing, relaxation and fixation

Apple maggot larvae molted to the third instar after 13 days under the conditions provided. Since apples taken from the cages were dated, the age of each maggot was known to within 12 hours. The insects were killed and relaxed by plunging them into water at 60-65°C. The temperature of the water was important as hotter water caused the larvae to burst while lower temperatures did not produce proper relaxation. The larvae were then placed in cold fixative for approximately 30 minutes before being cut in half to insure penetration. A variety of fixatives including Kahle's, Zenker's, Steive's, Carnoy's, Susa, Petrunkevitch's and Bouin's were used. However, adequate sectioning was possible only after fixation in Bouin's as all others caused crumbling of the tissue. The tissue was fixed in Bouin's for approximately 24 hours.

2. Dehydrating, clearing, infiltrating and embedding

Two separate procedures were employed for dehydrating, clearing and infiltrating. In the first, the maggots were dehydrated in three 30 minute washes of 100% isopropyl alcohol. Clearing was accomplished by three washes in beechwood creosote over a 24 hour period after which the specimens were infiltrated with paraffin (Tissuemat, M. P. 56°C) in a vacuum oven for 24 hours during which time the paraffin was changed twice. The larvae were then embedded in paraffin in disposable molds. After orientation of the tissue under a binocular microscope, the molds were placed in cold water to harden.

The second method was suggested by Dr. H. Potswald and consisted of passing the tissue through the following solutions after fixation:

1. 50% solution (40 parts 95% EtOH; 10 parts tertiary butyl alcohol (TB); 50 parts distilled water), 2 hours.
2. 70% solution (50 parts 95% EtOH; 20 parts TB; 30 parts DHOH), overnight.
3. 85 % solution (50 parts 95% EtOH; 35 parts TB; 15 parts DHOH), one hour.
4. 95% solution (45 parts 95% EtOH; 55 parts TB), one hour.
5. 100% solution (25 parts 100% EtOH; 75 parts TB), one hour.
6. three changes in 100% TB, leave overnight.

The tissue was infiltrated with a mixture consisting of 300 grams of paraffin (M. P. 60-62°C) and 45 grams of dry, powdered piccolyte resin (Cloney, 1961). Maggots were transferred from the 100% TB to a 50-50 mixture of TB and the infiltration medium and left in a 60-62°C vacuum oven for one hour, after which they were placed in pure infiltration medium and returned to the oven. This was changed twice over the course of the next six hours at which time the larvae were removed from the oven to be oriented in blocks under the binocular and trimmed. Since the piccolyte makes an extremely hard block and ribbons do not form well, the trimmed blocks were coated with paraffin (M. P. 56°C) and retrimmed. This procedure allowed ribbons to be formed with a minimum of static electricity.

3. Sectioning and staining

Sections were cut at 5-8 microns on an American Optical rotating microtome and were affixed to slides with either albumin-glycerine or Haupt's adhesive. Ribbons were floated on distilled water when the former adhesive was used and 2% formalin with the latter and were flattened by placing the slides on a 40°C warming table for 48 hours.

The tissue was stained in either Delafield's hematoxylin and eosin Y, Masson's trichrome as modified by Rae (1955), Mallory's triple, or the trichrome stain devised by Lower (1955).

4. Gross dissections

Larvae were killed and relaxed by plunging them into hot (60°C) water for a few seconds. They were then pinned through the last abdominal segment to a wax filled petri dish and covered by a drop of water or insect saline. Under a dissecting microscope the cuticle was ripped open along the longitudinal axis of the body with two pairs of fine forceps and secured with insect pins. Internal organs were then manipulated with insect pins or minuten pins taped to thin dowels.

Staining, when necessary, was accomplished by flooding the dissection with a 1:1000 solution of methylene blue (Ludwig, 1949) or Delafield's hematoxylin for a few minutes. Excess stain was washed away with water.

5. Microscopy and photomicroscopy

Sectioned material was examined under a Wild M-20 microscope equipped with both flat field and phase contrast objectives at 125, 500, and 1250X. Fresh tissue was examined under phase contrast or through a Wild M-5 dissecting microscope. Micrographs were taken through either of the Wild scopes with an Asahi Pentax double reflex camera on either Tri-X or Ektachrome-B film.

IV. RESULTS AND DISCUSSION

A. Integument

The integument includes a tough, flexible cuticle and an underlying layer of cells, the epidermis or hypodermis. The cuticle is readily distinguishable into an outer epicuticle (Fig. 1, Ep) and a much thicker endocuticle (Fig. 1, En). The epicuticle, which remains about 2 thick during the third instar, is composed of two layers, a very thin (less than 0.5μ) black staining outer layer (Fig. 2, Ep_1) and a thicker inner layer which stains yellow or red in Mallory's stain (Fig. 2, Ep_2). The endocuticle is also a bilaminar structure but is increases in thickness during the third instar from 4μ in the 13 day old larva to 30-40μ in the mature larva. It stains blue in Mallory's stain, green with Masson's and Lower's stain and light grey with hematoxylin and eosin Y. The inner portion is the thicker of the two, increasing from 6-7 to 20-30μ during the third instar (Fig. 2, En_2) It shows distinct layers near the epidermis which become less well organized distally and are convoluted toward the epidermis into light staining bands (Fig. 2, LB). The outer endocuticle is composed of a featureless material in which no layers can be seen and it increases in thickness from 1μ to 2-4μ from the 13 day old larva to the 20 day old larva, respectively (Fig. 2, En_1). The endocuticle invaginates along the intersegmental lines to form apodemes to which are attached the muscles of the body wall (Fig. 3, A). The small (4μ high) hooks (Fig. 2, h) on the circular swellings of the larvae generally stain like the epicuticle as they are usually not sectioned, but when seen in cross

section, the endocuticular core is readily discernable.

The epidermis is composed of flattened cells with large round nuclei and bulge conspicuously into the hemocoel (Figs. 1, 2, EC). In most sections the cell boundaries are indistinct, however, tangential sections show them clearly. In early third instars the cells measure 25μ in diameter with a 10μ nucleus. At the end of the third instar these dimensions have increased to 50-60μ and 15-20μ. Both the nucleus and the cytoplasm are basophilic, the cytoplasm appearing finely granular and the nucleus showing coarsely precipitated chromatin material. Often a space (probably an artefact) is present between the nucleus and the cytoplasm. Under the oil immersion, phase contrast lens a thin (less than 0.5μ), blue staining basement membrane may be seen in Mallory treated sections.

The epicuticle of Rhagoletis is similar to those described by Dennell (1946) and Wolfe (1954) for Sarcophaga and Calliphora, respectively. Dennell (1946) demonstrated through the use of histochemistry that the outer layer is composed mainly of lipids and sterols while the inner layer is exclusively proteinaceous. No chitin is present in the epicuticle. In a later paper (Dennell, 1950) showed that the epicuticle protects the larva of Calliphora from enzymes secreted by the salivary glands.

Locke (1957) has termed the dark staining outer layer the cuticulin layer. It is present over the entire surface of all insects including tracheoles, gland ducts, hairs and scales and is absent only in some sense organs (Slifer, 1961) and the gut (Bertram and Bird, 1961). According to Locke (1966) the cuticulin is one of the most important

layers of the insect cuticle as it essentially determines the surface characteristics of the cuticle. Also, it is the first layer produced by the epidermis and serves to protect the developing endocuticle from the powerful molting fluid enzymes. In electron micrographs several layers superficial to the cuticulin may be detected (Locke, 1966; Filshie, 1970). However, these are not preserved in light microscope preparations and thus do not appear in the apple maggot sections.

Distinct pore canals containing cytoplasmic filaments have been described by several authors (Dennell, 1946; Wolfe, 1954; Kennaugh, 1965) in larval cyclorrhapha. Since these canals range in size from 0.15μ in Periplaneta to 1.0μ in Sarcophaga, the thickness of the sections used in this study (5-8μ), may have rendered them inconspicuous. The light staining bands described above may represent chitin filled pore canals, however, Dennell (1946) describes them only in the outer endocuticle of Sarcophaga and states that they do not reach the epidermis in the mature larva. Since these bands do reach the epidermis in the apple maggot, it is possible that they merely represent stressed areas. Supporting this idea is the fact that each band extends into the center of the folds of the inner surface of the endocuticle (Figs. 1, 2, LB). Locke (1961) has reported that no pore canals are present in the larva of Calpodes ethlius (Lepidoptera:Hesperiidae), thus a similar condition may exist in Rhagoletis.

A number of authors (Pryor, 1940; Fraenkel and Rudall, 1940, 1947; Dennell, 1946; Richards, 1956) have studied the chemical composition of the endocuticle of cyclorrhaphous larvae; the general consensus being

that it is composed of chitin, proteins, minerals, and water in varying proportions.

B. Anal organ

The anal organ of Rhagoletis is composed of two hemispheres of modified integument flanking the anus in the last abdominal segment (Fig. 4, ao). Sections through these projections show the endocuticle of the anal organ (Fig. 5, ao En) to be reduced in thickness to approximately one half to one third that of the surrounding endocuticle (Fig. 5, En), and that the organ is delineated by an invagination of the epicuticle (Figs. 5, 6, i). The epidermis in this area stains like the surrounding integument, but the individual cells are much enlarged, measuring 25-30μ thick through the nucleus in the 20 day old larva compared to 12-15μ for the surrounding epidermal cells (Figs. 5, 6, EC). Each cell is equipped with a large round nucleus 16-20μ in diameter which bulges into the hemocoel and which contains coarsely precipitated chromatin material (Fig. 6, n). Each protuberence is provided with a pair of muscles, both inserting near its center. The smaller of the two inserts mesially and extends dorsally joining a larger group of muscles associated with the rectum (Fig. 6, m), all of which originate on the body wall directly above the large tracheal commissure of the last abdominal segment. The larger muscle extends anteriorly to its origin on the ventral apodeme between the seventh and eighth abdominal segments (Fig. 6, M).

The exact function of this structure has not yet been adequately demonstrated. Quintart (1961) considers it to be an osmoregulatory organ, a function strongly suggested by the histology of the area. The thin endocuticle would tend to be more permeable than the surrounding integument while the epicuticular invagination might prevent passage of

salts into the surrounding endocuticle and allow only the large epidermal cells to control this flow. El Shatoury (1955) claims that the anal organ of Drosophila contracts rhythmically, acting as an accessory pulsating organ. Although equipped with muscles, the anal organ of Rhagoletis does not exhibit such contractions. During locomotion however, muscle contraction causes this area to be vigorously invaginated. These movements occur simultaneously with the general contractions of the abdomen and must contribute to the increase in hemolymph pressure which extends the anterior portion of the body.

Although histologically similar to the Muscids described by Stoffolano (1970) and to Drosophila (Gloor, 1949), the anal organ of Rhagoletis differs morphologically as it conspicuously bulges from the integument as does that of Stomoxys calcitrans (Zimin, 1951). The organs of Musca, Orthellia, and Drosophila are contiguous with the surrounding integument and are difficult to detect in the living animal.

C. Tracheal system

Like all larval cyclorrhapha, the apple maggot is amphineustic in the third instar, that is, there are two pairs of spiracles, one located in the prothorax (Fig. 7, ASp) and the other in the last abdominal segment (Fig. 7, PSp). Basically the tracheal system consists of a pair of large dorsal longitudinal trunks (Fig 7, Tra) into which the larval spiracles open, and a smaller pair of lateral longitudinal trunks which join the dorsal pair in the metathorax and last abdominal segments (Fig. 7, t). The dorsal longitudinal trunks are connected by a series of ten segmentally arranged dorsal anastomoses (Fig. 7, r), the last of which occupies the eighth abdominal segment (Fig. 7, s). The most anterior and posterior anastomoses are much larger in diameter than the rest (Figs. 7, 9, s). The longitudinal trunks communicate through eight segmentally arranged pairs of transverse connectives, the most anterior of which is located in the metathorax (Fig. 7, Tc).

The tracheae, regardless of size, show a similar structure in sections. There is a thin, chitinous intima which stains similar to the endocuticle and which is reinforced by a spiral thickening of the walls, the taenidia (Fig. 8, ta). This intima is covered by a thin layer of cytoplasm which contains an occasional nucleus (Fig. 8, n). These appear oval in sections tangential to the trachea while cross sections show them to be flat discs contoured to fit around the associated trachea.

The larval spiracles are highly developed in Rhagoletis. The anterior pair located in the prothorax are flap like, yellow colored structures from which extend approximately twenty small papillae

(Fig. 9, ASp). Each papilla bears a slit like spiracular opening at its tip leading into an air passage which runs the length of the papilla and enters a cylindrical stigmatic chamber located in the basal flap (Fig. 10, sc). The stigmatic chanber is in turn confluent with the dorsal longitudinal trunk. Sections show that both papillae and stigmatic chamber are composed of endocuticle lined with a 5-8μ thick layer which appears yellow regardless of the stain employed and which is responsible for the color of these structures in the living animal. This inner layer shows definite striations oriented perpendicular to the spiracular wall.

Ridges of endocuticle extending into the stigmatic chamber form a series of baffles which serve as a filtering device (Fig. 10, ER). The portion of the stigmatic chamber that projects into the body cavity is covered by a single layer of cuboidal cells which increase from 7-8μ thick in the young third instar to 20μ in the mature larva (Fig. 10, EC). These cells stain similar to the hypodermis but are rather darker and have small round nuclei embedded in a homogeneous cytoplasm. Along the anterior surface of the stigmatic chambers is a row of twenty to twenty-five paraspiracular (peristigmatic) cells (Fig. 10, PC). These are somewhat larger than the cuboidal cells although they stain similarly. Under oil immersion a single fine channel may be detected in each para-spiracular cell which penetrates the cuticle of the stigmatic chamber (Fig. 11, ch).

Each posterior spiracle consists of an oval plate of yellow colored endocuticle, the stigmatic plate (Fig. 12, ST, PL) pierced by three elongated openings (Figs. 12, 13, SL). The stigmatic plate is also

equipped with four tufts of hairs located between the lateral ends of the spiracles (Fig. 12, CP), and a clear disc shaped area, the "stigmatic scar" located at the mesial edge (Fig, SC). The edges of the spiracular slits are provided with a series of interdigitating bars which form a filtering device (Fig. 13, IB). The spiracles lead into a short cylindrical stigmatic chamber which communicates with the longitudinal trunks.

The posterior stigmatic chambers are also composed of endocuticle coated with a striated, yellow substance (Fig. 13, sy). Sections show many endocuticular ridges extending to the interior of the stigmatic chamber where they branch profusely forming an effective filtering device termed the "felt chamber" by Keilin (1944) (Fig. 13, ER). Ventral to the felt chamber are three large, rounded paraspiracular cells or "glands" which increase in diameter from 40 to 50 during the third instar (Figs. 13, 14, PC). In Masson's preparations a well defined green staining channel can be seen giving off branches through the brown cytoplasm (Fig. 14, ch). The paraspiracular cell also contains a large round nucleus located somewhat ventrally (Fig. 14, n). The nucleus contains many large aggregations of red-brown staining chromatin material as well as a distinct nucleolus (Fig. 14, nu). Under high-dry and oil immersion lenses a thin, green staining basement membrane may be seen (Fig. 14, bm). The epithelium of the posterior stigmatic chambers is essentially the same as that of the anterior pair.

The basic morphology of the tracheal system of the apple maggot has been described by Snodgrass (1924). A much more detailed study has been made of this system using Calliphora erythrocephala (Whitten, 1955) and

Drosophila melanogaster (Whitten, 1957). Whitten (1972) has recently reviewed the comparative morphology of the insect tracheal system. The findings of this study are in complete agreement with Snodgrass (1924) and differ very little from the basic cyclorrhaphan plan presented by Whitten (1955).

The present study of the spiracular apparatus has merely confirmed the descriptions of Snodgrass (1924) and Butt (1937) for Rhagoletis pomonella. The peristigmatic cells have been found to be very similar to the descriptions of Bates (1934) for the apple maggot and Phillips (1939) for the anterior peristigmatic glands of Rhagoletis suavis. These glands seem to be present in all immature cyclorrhapha (Keilin, 1944), although Bates (1934) reported the anterior ones absent in "hibernating" larvae of Eurosta solidaginis (Diptera:Tephritidae). They have been known since 1859 from the work of Leydig, and although their exact function has not been demonstrated, Gazagnáire (1886) offered the most widely accepted theory, considering them to be the origin of a hydrophobic substance used to keep water out of the spiracles in the aquatic larvae of Eristalis tenax. The cells of this species also contain convoluted ducts through which the hydrophobic material reaches the spiracular openings (Keilin et al., 1935).

D. Alimentary tract

The anterior portion of the digestive system contains the only sclerotized chitin found in cyclorrhaphous larvae. The mouth, located ventrally at the extreme anterior end of the animal, is flanked by the curved, sclerotized mouth hooks or mandibles (Figs. 15, Hk; 71, Hks). Like all sclerotized chitin, these hooks do not stain but appear black in sectioned material. They are apparently very hard, for they generally crack when sectioned. The hooks are hollow, possessing a cytoplasm filled channel towards their mesial border and at their base they widen and form an articulation with a pair of triangular plates embedded in the lateral pharyngeal wall, the hypostomal sclerites (Figs. 15, 71, A). The mandibular extensor and flexor muscles insert on the base of the hooks above and below this articulation and originate on a phragma farther back on the lateral pharynx (Figs 15, 71, b). The two hypostomal sclerites are connected by a bar passing ventral to the pharyngeal floor. Saggital sections indicate this bar may be poorly sclerotized as although it does not stain it always appears yellow (Fig. 15, e). Posteriorly the hypostomal sclerites are narrowly joined to a much larger pair of plates, the pharyngeal skeleton proper (Figs. 15, 71, C, B). A pair of slender arms extend anteriorly from this junction to support the pharyngeal roof (Fig 15, a). At their ends, oriented transversely is another thin sclerotized bar, the epipharyngeal sclerite (Fig. 15, D).

The cephalopharyngeal skeleton is essentially a sclerotization of the lateral walls and roof of the cylindrical pharynx. There are three

deep, posterior, unsclerotized clefts, two lateral and one dorsal, which divide the posterior portion of the skeleton into four plates (Figs. 15, 71, C, B). The lateral surface of the ventral plates bear a non-sclerotized phragma (Figs. 15, 71, b) from which originates the mandibular musculature (Fig. 71, FMcl). Each dorsal wing also bears a phragma (Figs. 17, 71, c), the origins of the dorsal and ventral pharyngeal extensors (Fig. 71, DPMcl, LPMcl). Sections through this area show that only the inner layer of the pharyngeal skeleton is sclerotized.

Overlying the hard parts is a layer of unsclerotized chitin which is in turn covered by a layer of cuboidal epithelium. In the posterior region of the pharyngeal clefts, only these cells may be present, supported by a thin basement membrane.

Internally the pharynx is divided into upper and lower chambers by a flexible chitinous membrane (Fig. 16, m). The lower chamber like the preceding buccal cavity is composed of chitin equipped with a layer which stains similar to epicuticle. The floor of the pharynx is marked by the presence of seven longitudinally oriented chitinous bars, the pharyngeal lamellae (Figs 16, 17, l). Anteriorly these lamellae jut into the pharyngeal lumen but become gradually reduced in height until they fade out before reaching the oesophagus. The lamellae are capped by a series of thin, transversely oriented plates which, when seen in cross sections, stain like epicuticle (Fig. 17, p). The lateral tips of these plates appear to touch those of adjacent lamellae thus forming an efficient filtering device. Cross sections through the lamellae show differential staining when Masson's trichrome is used. The layer immediately above the epithelium stains yellow-orange, indicating a high protein con-

tent. Next comes a layer of green staining chitin which becomes thicker directly beneath each lamella. The major portion of the longitudinal plates is composed of a yellow staining fibrous material which extends into the pharyngeal floor (Fig. 17, 1). The dorsal regions of the lamellae stain green indicating a high chitin content, while the transverse plates stain yellow like epicuticle. Occasionally food material wedges between the lamellae and causes them to bend. This bending always occurs in the green staining region, indicating that the yellow fibers have a strengthening function (Fig. 17, F).

The upper chamber, the epipharyngeal sinus, contains the cibarial muscles which cause flexing of the dividing membranes and constriction of the oesophageal opening. There are 20-25 pairs of the former (the cibarial muscles), all of which originate from the sclerotized dorsal wings of the pharyngeal skeleton and insert on the dividing membrane (Fig. 16, cm). The constrictors are present in two groups, an anterior pair and a larger posterior group of five (Fig. 16, con). The muscles in the epipharyngeal sinus are innervated by the large procurrent nerve running between the two rows of cibarial muscles. Figure 18 (nj) shows a neuromuscular junction between a branch of the procurrent nerve and one of the anterior oesophageal constrictor muscles (con). Oxygen is provided by a pair of tracheae which penetrate the posterior pharynx and run between the cibarial muscles and the pharyngeal wall.

The oesophagus (Figs. 19, O; 71, OE) extends from the posterior pharynx between the cerebral hemispheres (Figs. 19, CH; 71, Br) to the bulb like proventriculus located in the first abdominal segment (Figs. 20, P; 71, Pvent). Cross sections reveal several concentric layers.

Outermost is a thick layer of poorly differentiated muscle fibers (Fig. 19, m). These support a single layer of poorly differentiated epithelial cells which send spike like processes into the lumen (Fig 19, e). Lining the lumen is a chitinous intima (Fig 19, i).

As the oesophagus enters the proventriculus, its muscular layer becomes reduced in thickness and disappears. The epithelium eventually doubles back on itself accompanied by the chitinous intima (Fig. 20, e) and extends anteriorly just past the base of the gastric caeca where it merges into a ring of large columnar cells which have very light staining cytoplasm (Fig. 20, en). Immediately anterior is the ring of imaginal proventricular cells which will be described in conjunction with the imaginal discs. The proventricular tissue next takes the form of a single layer of flattened, dark staining cells which extend posteriorly to the base of the gastric caecae (Fig. 20, fc). Sections show the cells to be highly irregular in shape with round nuclei located centrally in the finely granular cytoplasm. These cells stain similar to muscle tissue.

The four gastric caeca are short tubes ending blindly in the hemocoel (Figs. 20, gc; 71, GC). They are composed of irregularly shaped cells which bulge into the small lumen and are anchored to a basement membrane confluent with that covering the proventriculus. The cells are histologically similar to those of the anterior midgut, termed the chyle stomach by Dean (1932).

The remainder of the midgut goes through a complex series of convolutions before its junction with the hindgut. Dean (1932) describes its course in Fig. 21 as follows: "The chyle stomach extends caudad

close to the ventral body wall as far as the eighth segment and then bends abruptly back and to the left for a short distance. It then forms a wide loop to the right and passes above the first straight portion posterior to the proventriculus, becoming somewhat narrower. Then, extending caudad and to the right of the median plane as far as the first bend, over which it forms a short loop to the left, it bends toward the ventral body wall. At this point there is a more or less distinct contraction, the tube enlarging again immediately. The tube then proceeds cephalad, shortly looping to the left and contracting in diameter for a short distance. After forming a loop to the right and upward, the tube extends caudad, loops to the right again, proceeding cephalad a short distance before bending abruptly back on itself and to the left. It next turns toward the ventral surface within the next to the last loop and proceeds cephalad to the middle of the body where it joins the hind-intestine."

Sections through the midgut show thin muscle fibers oriented both longitudinally and transversely, forming a lattice like network outside the basement membrane (Fig. 22, m). The epithelial cells vary histologically along the length of the midgut. Anteriorly the cells are very large and appear almost columnar as they bulge into the lumen (Fig. 20, ec). This condition is most pronounced in the enlarged portion of the midgut immediately posterior to the proventriculus which Dean (1932) has termed the chyle stomach. The cells here have granular cytoplasm which stains similar to muscle tissue. The nuclei are round and contain coarsely precipitated chromatin material as well as a conspicuous nucleolus. The apical cytoplasm is filled with light staining

vacuoles and each cell is bordered by a very thin layer of microvilli (brush border).

Posterior to the chyle stomach the epithelium becomes somewhat flatter, the cells showing a thick distinctly striated brush border and no vacuoles. In the middle portion of the midgut the epithelium is composed of highly flattened cells having finely granular cytoplasm in which an occasional vacuole may be found. Nuclei are scarce in this region and microvilli are lacking (Fig. 23, ec). In the posterior portion of the midgut (most of which lies coiled in the last three abdominal segments), the cells again become rounded. A heavy, striated brush border is present and the cells are often highly vacuolated (Fig. 24, ec).

A peritrophic membrane occupies the lumen of the midgut (Figs. 23, 24, PM). It stains similar to chitin and appears in sections as a very thin film, often containing particles of food matter.

The posterior midgut abruptly merges into a band of very light staining cells (Fig. 25, lc) which border the junction of the Malpighian tubules and midgut. The hindgut is distinguishable in sections by its layer of columnar epithelium which is quite distinct from the light staining band (Fig. 25, ce). The hindgut itself actually runs anteriorly for a short distance before doubling back to the anus. Its anterior portion is of similar diameter to the midgut and has a layer of columnar epithelium surrounded by a lattice of thin muscle fibers similar to that of the midgut. The epithelium of this swollen area (termed the sinus by Dean, 1932) shows homogeneously granular, non-vacuolated cytoplasm and no brush border (Fig. 25, ce). The sinus abruptly merges

into the rectum which is similar in histology to the oesophagus (Fig. 5, R). There is a thick, circular layer of muscle, a basement membrane, and a reduced epithelium which supports a thick chitinous intima. Near the anus this intima is thrown into deep folds (Fig. 5, i).

The pharyngeal lamellae of Rhagoletis were alluded to by Snodgrass (1924) in his morphological study as a series of parallel striae visible through the floor of the pharynx. Keilin (1912) observed that these structures are found only in maggots which live on dead plant or animal material. They are absent in predacious and parasitic larvae. The apple maggot fits this pattern for although the larvae develop in living fruit, they actually feed on tissue killed and decomposed by Pseudomonas melophthora. This condition is shared by the olive maggot, Dacus oleae (Hagen, 1966) and probably most other fruit inhabiting Tephritidae (Dowding, 1967).

The most complete study of the structure and function of the pharyngeal lamellae has been conducted by Dowding (1967-1968) on Calliphora erythrocephala. Her drawings of this filter apparatus strongly resemble that of the apple maggot except for the anterior portion. In Calliphora the tops of the anterior ends of the lamellae fuse, forming a solid roof while in Rhagoletis the transverse plates extend to the ends of the lamellae.

Dowding (1968) used both Mallory's triple and Masson's trichrome stains on the blow fly pharynx and reported staining identical to that of Rhagoletis. She also reports the presence of fibrous material originating in the pharyngeal floor and extending well into the lamellae. As can be seen from Fig. 16 (l), the apple maggot is also equipped with

these fibers. Figure 17, which shows the non-fibrous portion of the lamellae bending, supports Dowding's (1968) contention that they have a strengthening function. These fibers are embedded in a chitinous matrix. The apical transverse plates have been determined by the electron microscopic work of Dowding (1968) to be composed entirely of epicuticle, hence the red color in Mallory's and Masson's preparations. The highly proteinaceous secondary floor mentioned by Dowding can easily be seen in Masson's trichrome stained sections of Rhagoletis as an orange staining band lying over the epithelial cells. According to Dowding (1968), only this layer is formed by the same process as integumental endocuticle. The major part of the filtering apparatus is formed through the action of cytoplasmic extensions from the epithelium which recede as the lamellae are formed. The entire pharyngeal and buccal cavities are lined with a thick epicuticle which may serve to protect the endocuticle from potent salivary enzymes (Dennell, 1950).

It is not exactly known how these filters work. Becker (1910) concluded that in larval Musca and Anthomyia solid material mixed with saliva are directed into the lower channels only. Liquified material then would pass through the transverse plates to be swallowed. Dowding (1967) fed pollen grains and spores of varying diameters to Calliphora larvae. The maggots were killed while feeding and when sectioned, it was found that particles too large to pass between the transverse plates were most often found in the upper chamber. Dowding concluded that the suspended food particles are directed into the upper chamber when the pharyngeal muscles contract. Relaxation of these muscles allows the pharyngeal roof to move down toward the lamellae forcing liquid through

the filter while leaving particulate matter concentrated on top of the lateral plates. Presumably the next inflow of food carries this material into the oesophagus.

The filter of Rhagoletis, however, does not seem to possess any mechanism for directing food exclusively to either chamber. As can be readily seen from Fig. 16, the anterior ends of the channels, as well as the upper lumen, open directly into the buccal chamber. Therefore, it seems likely that food enters both chambers simultaneously. Relaxation of the pharyngeal muscles would then cause excess liquid to be forced across the sieve through the channels and back into the buccal cavity. Food lying on top of the filter is most likely pushed into the oesophagus by the next influx of liquid. Unusually large particles might occasionally be jammed through the filter by the descending pharyngeal roof and remain lodged between the lamellae as seen in Fig. 17. In this event, salivary enzymes could be expected to eventually break up the obstruction. More serious blockage would be rectified when the entire pharyngeal lining is shed during ecdysis, and in any event, Dowding (1968) reported that completely plugging these channels causes no apparent harm to the third stage larva of Calliphora.

The histology of the remainder of the larval alimentary canal of Rhagoletis has been described by Dean (1932). Dean's paper does not mention the small imaginal cells in the midgut but otherwise the present study agrees with his observations.

The histology of the midgut strongly suggests differential cell functions. The epithelium of the chyle stomach contains many vacuoles while the brush borders of these cells are poorly developed (Fig. 20)

indicating that secretion is the main function of this area. The function of the thin mid, midgut cells is not so obvious but those immediately posterior to the chyle stomach and the hindgut carry a thick, striated brush border, indicating an absorptive function. In addition, the posterior midgut cells are highly vacuolated, a condition usually associated with fat storage (Fig. 24, ec).

These suggestions are supported by the work of Hobson (1931) and Waterhouse and Stay (1955) of the larvae of Lucilia sericata and L. cuprina, respectively. Hobson (1931) reported that normally food passes very quickly through the anterior, midgut (chyle stomach) where it is supplied with various enzymes. In the mid, midgut much of the fluid is absorbed and the pH drops from near neutrality to acidity (3.0-3.5). The hind, midgut of Lucilia is the most active site of digestion and absorption. Here are found the widest variety of enzymes and a reversal of pH to near neutrality, a condition more conducive to the activity of most digestive enzymes (Hobson, 1931).

The histochemical work of Waterhouse and Stay (1955) shows the mid, midgut of Lucilia to be concerned with uptake of copper and iron as well as glycogen storage. Poulson (1950) demonstrated a copper absorbing region in the mid, midgut of Drosophila, thus a similar situation may exist in Rhagoletis.

Waterhouse and Stay (1955) have also reported the presence of a curious region in which two histologically distinct cell types are present. Waterhouse and Wright (1960) explored the fine structure of this "mosaic midgut" in a later paper. This condition appears to be restricted to Lucilia as it is not found in many other larvae including

Drosophila and Rhagoletis.

The imaginal midgut cells were mentioned by Bodenstein (1950) as clusters of small, dark staining cells interspersed between the larger midgut epithelial cells in Drosophila. In the pupa the large cells slough off while the imaginal cells proliferate reforming the midgut epithelium. Anderson (1964) reports that both imaginal and larval gut epithelium are derived from the original strands of endoderm found in the embryo of Dacus tryoni.

E. Fat body

The larval fat body is essentially located in the abdomen between the body wall and the viscera. In the third instar it makes up much of the bulk of the abdomen and also is responsible for the white color of the maggot. The cells are very large increasing in diameter from 50-60μ to 90-110μ during the third instar. They have a very characteristic honeycombed appearance in sections due to extraction of the fat droplets during fixation (Fig. 24, FB). The little cytoplasm left generally stains like muscle tissue and the centrally placed nucleus a darker shade of the same. The nucleus is round and contains finely precipitated chromatin and a nucleolus.

Chapman (1969, pg. 83) assigns several important functions to the fat body of insects. These include storage of both food and excretory products as well as being the site of many important metabolic processes. The cells concerned with storage are termed trophocytes (Chapman, 1969) and the fat body of Rhagoletis seems to be composed exclusively of them. Snodgrass (1924) subjected these cells to Soudan III and osmic acid and found that the large vacuoles seen in Fig. 24 contain lipids in the living animal. These lipids are extracted during classical histological processing; thus, the characteristic honeycombed appearance in sectioned material.

A number of authors (Hewitt, 1914; Bodenstein, 1950; Wolfe, 1954) have found oenocytes associated with the fat body of cyclorrhaphous larvae. Wolfe (1954) reports that during molting these cells concentrate just below the epidermis and appear to be involved in formation

of the epicuticle. However, such cells could not be located by this author in sections nor were they seen by Snodgrass (1924) in dissected larvae. Possibly oenocytes would be easier to detect in Rhagoletis if molting larvae were studied.

Perez (1910) found crystals in the pupal fat cells of Calliphora erythrocephala during formation of the imaginal Malpighian tubules and concluded that they had temporarily usurped the function of excretion. Snodgrass (1924) did not find such crystals in the developing pupa of Rhagoletis.

Bhatia (1939) and Keilin (1921) report the presence of calcospherites in the fat body of fly larvae but these structures do not appear in sectioned apple maggots, having been dissolved during fixation. Snodgrass (1924) found them only in the Malpighian tubules during his morphological study of this insect.

F. Nervous system

1. Central nervous system

Like all insects, the apple maggot is equipped with both a central and a stomodeal or stomatogastric nervous system. The central nervous system includes the ventral ganglion, cerebral hemispheres or brain, and associated nerves. The former two structures are located in the meta-thorax and first abdominal segments, the hemispheres (Figs. 19, CH; 71, Br) flanking the oesophagus and proventriculus (Figs. 26, 27, VG; 71, Gng). The ventral ganglion represents the fused thoracic and abdominal ganglia. It is an oblong structure 70μ thick and 450μ long tapering from 85μ wide anteriorly to 55μ posteriorly in the early third instar. Just before pupation these dimensions increase to 100μ thick, 550μ long, 120μ wide anteriorly, and 90μ wide posteriorly.

The two rounded cerebral hemispheres are broadly joined to the anterior-dorsal portion of the ganglion (Fig. 27, CH) and increase in diameter during the third instar. The pars intercerebralis connects these lobes mesially, forming an aperture through which runs the oesophagus (Figs. 19, 26, 31, PI).

The ventral ganglion and cerebral hemispheres are histologically similar. Each is covered by a non-cellular neural lamella which stains green in Masson's and blue in Mallory's triple and which is easily dislodged during dissection (Fig. 28, NL). Underlying the neural lamella is the perineurium, a single layer of cells having very light staining cytoplasm (Fig. 28, Pe). The perineurium surrounds a layer of dark staining perikaryons and glial cells which appear to have very little

cytoplasm. This layer stains brown in Masson's, red or light blue in Mallory's triple, pink in hematoxylin and eosin Y, and greenish-grey in Lower's. The cells are so tightly packed the boundaries are difficult to determine and the layer is concentrated in the ventral and lateral portions of the ventral ganglion (Figs. 27, 28, Pk). In the cerebral hemispheres this layer is of uniform thickness and unlike that of the ventral ganglion it shows some organization. This takes the form of several aggregations of cells in the posterior portion of the brain near the optic stalk (Fig. 28, OR).

The innermost tissue is the featureless neuropile which is composed of axons from the surrounding neurons and which stains much lighter than the perikaryons (Figs. 27, 28 Np). Periodically strands of the neuropile traverse the outer tissues and give rise to the nerves of the central nervous system.

Eleven pairs of nerves issue from the lateral edges of the ventral ganglion and innervate the body wall musculature of the eight abdominal and three thoracic segments (Fig. 29, th, a). All but those of the last abdominal segment are accompanied by a trachea which enters the ganglion just posterior to the corresponding nerve. The two anterior pairs of segmental nerves emerge near the stalks of the pro and mesothoracic leg discs. An additional two pairs of nerves leave the anterior end of the ganglion and supply the head (Fig. 29, MN, PN).

In addition to these lateral nerves the ventral ganglion gives rise to ten small nerves which originate from its dorsal midline, the dorsal unpaired nerves. All of these nerves eventually fork but the posterior-most extends well into the abdomen before this happens. The next two

extend to the third abdominal segment before forking while the rest split soon after leaving the ganglion. Each of these fibers follows one of the larger lateral nerves and both enter the body wall and branch at about the same place. They are inconspicuous in sectioned material and do not arise from a strand of neuropile as do the other nerves.

Three pairs of nerves originate from the cerebral hemispheres. The thick optic nerves arise from the posterio - ventral area of each hemisphere and extend anteriorly to supply the imaginal eye disc (Fig. 29, AS). The second pair originate from the anterior surface of each lobe (Fig. 29, AN). They are fused to the ventro-mesial surfaces of the eye-antenna histoblast (Fig. 42, N) until they enter the posterior portion of the pharynx. The third pair are the nervi corpora cardiaci which enter the brain immediately posterior to the pars intercerebralis. They will be discussed in connection with the stomatogastric nervous system.

In sections the lateral segmental and cephalic nerves are rather featureless strands of tissue. The non-granular cytoplasm stains similar to muscle and a few regularly spaced nuclei may be seen just beneath the thin basement membrane (Fig. 42, n). The median unpaired nerves are similar except for a smaller diameter and an apparent lack of nuclei. Under phase contrast these two types of nerves are readily distinguishable. The lateral nerves show a grey, highly granular cytoplasm in which many dark streaks may be seen (Fig. 30, LN). The nuclei are oval in shape and contain a distinct nucleolus. The median unpaired nerves appear uniformly black under low power phase contrast (Fig. 30, UN). The only nuclei present are located at or near the points of bifurcation and resemble those in the lateral nerves.

A number of authors (Lowne, 1890-92; Hewitt, 1914; Keilin, 1917; Hertweck, 1931) have described the basic morphology of the larval cyclorrhaphous central nervous system. The larva of Rhagoletis has proven similar to these accounts. So far, only Ludwig (1949) has studied the cephalic nerves of cyclorrhaphous larvae in detail and the present investigation has shown that Rhagoletis differs only in small details. These mainly concern the stomatogastric nervous system and will be discussed later. As regards the innervation of the maggot head, it is interesting from a comparative viewpoint that Bhatia (1939) found a much larger number of nerves originating from the brain and ganglion in several species of Syrphidae, indicating that a large amount of fusion has occurred in the more advanced Calliphora and Rhagoletis.

Lowne (1890-92) and Hertweck (1931) have described the histology of the brain and ventral ganglion in Calliphora and Drosophila, respectively. Lowne (1890-92) describes the neural lamella as "varying in thickness but (differing) in no way from the . . . tissue covering the other internal organs." Whitten (1962, 1964a) has shown that this sheath is indeed continuous with that covering the internal organs. In an earlier series of papers, Ashurst (1961b, c) demonstrated through the use of histochemistry and ultrastructural studies that this sheath contains collagen fibers and mucopolysaccharides. This would help account for its color in Masson's preparations as this causes connective tissue to stain green.

According to Chapman (1969, pg. 525), the neural lamella has a supportive function and offers no resistance to diffusion of ions. Osmoregulation in the ganglia is instead controlled by the underlying

perineurium (Hoyle, 1952). The histochemistry and ultrastructure of this single layer of cells was studied by Ashurst (1961b, c) in Periplaneta and Locusta, respectively. As in the present study, Ashurst reported having difficulty seeing cell membranes through the light microscope. The cells of Periplaneta contained large amounts of glycogen and lipids (lipochondria) and stained red in Masson's and Mallory's stains as do those of Rhagoletis.

The layer of perikaryons in the brain and ventral ganglion of Rhagoletis is very similar to the description of Lowne (1890-92) and Hertweck (1931). Lowne (1890-92) was able to identify many of the cell clusters in the brain of larval Calliphora. In the apple maggot only the optic rudiments are obvious (Fig. 28, OR). The large peripheral cells in this region were considered by Hertweck to be the larval neurons and the rest imaginal cells. Lowne was also able to determine that the cells in the ventral ganglion were stellate shaped, however, the shape of corresponding cells in Rhagoletis could not be determined from sectioned material.

Ashurst (1961a) has made a histochemical study of the neurons of the cockroach Periplaneta americana and found the cytoplasm to be filled mainly with lipid containing bodies which she called lipochondria and a large quantity of Golgi bodies. Wigglesworth (1960) in a study of the Golgi apparatus of Periplaneta expressed the idea that these organelles are responsible for formation of the microtubules found extending through the axons.

Ashurst (1961a) also described two distinct sizes of neuron in the roach ganglia, a situation similar to the brain of Rhagoletis.

Electron microscope studies of the cortical region of insect ganglia have been made by a number of authors. Recently, micrographs prepared by Smith (1968) show nuclei of both neurons and glial cells covered by a very thin layer of cytoplasm. The area between these cells is composed of axons and glial cell processes. In the Rhagoletis sections these nuclei stain darkly and are covered by a light staining ring of cytoplasm. Neurons and glial nuclei are indistinguishable histologically, the cells themselves being embedded in a matrix of light staining glial processes and axons.

The neuropile region of the larva of Phormia terrae-novae has been investigated by Osborne (1966) with the electron microscope. This region is composed mainly of axons and glial cell processes and it is here that interneuronal contacts are made.

Thomsen (1951) and Possompes (1953) found neurosecretory cells in the pars intercerebralis of the larva of Calliphora erythrocephala. Fraser (1959a, b) demonstrated histochemically the presence of several groups of these cells in both brain and ganglion of Lucilia caesar. However, no cells of this type could be differentiated in sectioned Rhagoletis as no histochemical procedures were performed.

The electron microscopic work of Edwards (1958) on the peripheral nerves of the hornet has shown that the nuclei seen by light microscopists are those of enveloping glial cells. Nerve cell nuclei are entirely contained within the brain or ganglion and only the fibers extend into the lateral nerves. The glial cells enveloping these fibers are themselves covered by the ubiquitous basement membrane.

Since the lateral nerves can be seen innervating the segmental muscles it is reasonable to assume they contain motor fibers. Osborne (1963) discovered a subcuticular nerve plexus innervating chordotonal and cuticular sense organs in larval blowflies supplied by the lateral segmental nerves. Whitten (1963a) found stretch receptors associated with both muscles and tracheae sending fibers through the lateral nerves. Thus, these nerves contain both motor and sensory fibers. It has not been possible to accurately trace these nerves in Rhagoletis due to its much smaller size. Nevertheless, these animals are equipped with cuticular sense organs; thus a common function seems likely.

The dorsal unpaired nerves were considered to have a neurosecretory function by Whitten (1963b) who observed formation of granules in them during pupation in Calliphora. The work of Osborne (1964) has shown, however, that they are structurally similar to the lateral nerves. Osborne (1964) also determined that these nerves supply a muscle and a sense organ in the blowfly larva; thus they have both a motor and a sensory function.

2. Stomatogastric system

The stomatogastric nervous ststem includes a series of nerves and ganglia associated with the anterior portion of the digestive tract. Its most conspicuous feature is an elongated, highly nucleated ganglion (Figs. 26, 31, G) the most anterior portion of which is fused to the ventral surface of the ring gland (Figs. 26, 31, RG). At the level of its union with the ring gland, three nerves emerge from this ganglion. The anterior pair are very short and they plunge ventrally into the mesial surfaces of the cerebral hemispheres (Fig. 31, ncc). The posterior nerve is the unpaired recurrent nerve which slants forward to pass between the oesophagus and the pars intercerebralis connecting the cerebral hemispheres (Fig. 31, RN). It then passes through a small ganglion attached to the dorsal wall of the oesophagus and eventually enters the caudal end of the pharynx. Here it joins the large "procurrent nerve" of Ludwig (1949) which passes through the epipharyngeal sinus to innervate the cibarial muscles.

Posteriorly, the ganglion gives off two small nerves. The ventral nerve is very short, ending at the junction of oesophagus and proventriculus (Fig. 26, vn). The dorsal nerve courses over the proventriculus and eventually attaches to the dorsal surface of the chyle stomach at the level of the gastric caeca (Fig. 26, dn). From here it sends several posterior branches along the surface of the chyle stomach.

The stomatogastric nervous system of Rhagoletis shows some variation from the descriptions of Ludwig (1949) for Calliphora and Fraser (1957, 1959c) for Protophormia, Lucilia and several Muscids. In these

forms the recurrent nerve passes through a small hypocerebral ganglion which is distinctly separate from the ring gland, the two structures communicating through a single nerve. The recurrent nerve then extends posteriorly to terminate in a proventricular ganglion located just anterior to the proventriculus.

In Rhagoletis both of these ganglia have fused into a relatively large, elongated structure attached to the ventral surface of the ring gland (Figs. 26, 31, RG). Also lacking in the apple maggot is the distinct frontal ganglion shown by Ludwig (1949) at the junction of the recurrent and "procurrent" nerves.

3. Sense organs

The sense organs of the apple maggot include five pairs of large cephalic organs and many smaller ones scattered over the integument. Most prominent and largest of the cephalic organs, all of which stain similar to nervous tissue, are the two pairs located at the extreme anterior end of the maggot (Figs. 32, 33, DO, VO; 71, g, h). The dorsal pair (Figs. 32, 33, DO; 71, g) are composed of a basal spherical region measuring about 25μ in diameter through which the organ is innervated (Figs. 32, bs), joined to an oblong central structure (Fig. 32, mp) which sends cytoplasmic (Fig. 32, cf) filaments into the yellow staining thin walled external dome (Fig. 32, ed). No trace of cell membranes were seen in the basal sphere thus the shape of the cells composing it could not be determined. The 5μ diameter nuclei are scattered evenly through the granular cytoplasm (Fig. 32, n). The 50μ long median portion (Fig. 32, mp) is composed of a central core of about six fusiform cells covered by a single layer of flattened cells. The nuclei of the fusiform cells are located basally, the terminal ends of the cell tapering into the thin filaments which extend to the rim of the apical dome (Fig. 32, cf). Cross sections through this region show a number of small vacuoles forming between the cells which eventually coalesce into one large central vacuole located immediately beneath the apical dome (Fig. 32, cv). The large vacuole is confluent with the interior of this dome which is the only external part of the sense organ.

The walls of the external dome are extremely thin, stain like epicuticle, and rest on a ring of similar material embedded in the

cuticle. The dome rises 5μ from the surface of the integument and has a diameter of 8μ. The cytoplasmic filaments from the median fusiform cells (Fig. 32, cf) seem to be attached to the basal rim of this structure while its interior seems to be empty. The ventral organ is somewhat simpler in structure, its internal portion being a cylinder of cells 70-80μ long and 25μ in diameter (Figs. 33, VO; 71, h). Nuclei 5μ in diameter are scattered evenly through the granular cytoplasm, but the cell membranes are indistinct making it impossible to determine the cells' true shape. Distally several cytoplasmic filaments run through a yellow staining ring in the cuticle and terminate at the tip of the external portion of the sense organ (Fig. 33, cf). This structure consists of a low, yellow staining cylinder 20μ in diameter projecting 6-8μ from the cuticle (Fig. 33, ed). The terminal end is covered with a thin membrane in which the filaments described above terminate.

The third pair of cephalic organs may be found at the edge of the buccal cavity immediately lateral to several small tooth like structures (Fig. 34, t). Structurally they are similar to the ventral terminal organ although smaller, being composed of an internal cylinder of cells of undeterminable shape (Fig. 34, ic) which send cytoplasmic filaments through the cuticle (Fig. 34, cf) to the external papilla. This papilla is almost identical to that of the ventral organ except for its slightly smaller size.

The fourth pair of organs are attached to the floor of the pharynx immediately anterior to the chitinous bar connecting the hypopharyngeal sclerites (Fig. 35, B). These have a comparatively simple structure being composed of an oblong mass of cells similar to those of the ven-

tral organ and their communication with the pharyngeal lumen is inconspicuous in sectioned material.

The final pair of large sense organs is located in the extreme anterior end of the suprapharyngeal sinus. Internally they are similar to the last pair of organs except that two distinct cytoplasmic filaments are given off, one of which (Fig. 35, f_1) enters a sclerotized area in the dorsal pharyngeal wall (Fig. 35, es), the other traversing this wall to terminate in a small papilla just behind the first (Fig. 35, f_2).

In addition to the large, complex cephalic sense organs the apple maggot is equipped with a large number of smaller, much simpler organs scattered over the integument. Due to their small size it is difficult to determine distribution with any accuracy. However, there are four pairs located on the protuberences of the last abdominal segment, and at least one longitudinal row (one pair per segment) in the lateral walls of the anteriormost segments. Generally these structures are represented by a thin filament of cytoplasm traversing the cuticle (Fig. 36, cf). Distally the filament enlarges and enters the external portion which has a variety of shapes including pegs, cones, and thin spikes.

The dorsal cephalic sense organs of larval cyclorrhapha, considered by many early workers (Lowne, 1890-92; Hewitt, 1914; Ellsworth, 1933) to be photoreceptors, have been shown by Bolwig (1946) to be olfactory in function. Bolwig's detailed histological studies have since been supplemented by the scanning and transmission electron microscopy of Chou and Axtell (1971) on the dorsal organ of house fly larvae. The sectioned olfactory organs of Rhagoletis seen in Fig. 32, except for a

few details, are remarkably similar to the micrographs of Bolwig (1946) and the diagram presented by Chou and Axtell (1971).

Since no cell membranes were preserved in the apple maggot slides, the shape of the cells in the basal sphere and the presence of any nervous connection with them could not be determined. In house fly (Bolwig, 1946; Chou and Axtell, 1971) and Calliphora (Ellsworth, 1933) these cells are fusiform in shape and Chou and Axtell (1971) have shown that they are the origin of dendrites which branch profusely in the apical dome (Fig. 32, bs).

The fusiform cells in the median portion of the organ which surround the central vacuole have been noted by Ellsworth (1933) and also by Bolwig (1946) who termed them "enveloping cells." Chou and Axtell (1971) later termed them the "formative cells" and found them to be equipped with a mesial layer of microvilli. They contend that these cells secrete a fluid into the central "fluid filled" vacuole but do not elaborate on the function of this liquid (Fig. 32, cv).

The presence of the glial cells covering the dorsal organ of Rhagoletis is revealed only by the position of nuclei in cross sections, but Chou and Axtell (1971) were able to differentiate between them and the underlying neurons. Furthermore, they report that processes from the glial cells envelope the underlying neurons forming the "tunicated" type of sheath typical of insects (Smith, 1967). Chou and Axtell (1971) found many pores penetrating the apical dome of the house fly but none could be seen in the apple maggot sections. Also, the chordotonal organs mentioned by Keilin (1927) and Bolwig (1946) as being associated with the cephalic sense organs were not seen.

The ventral organ of Rhagoletis seems to agree with Bolwig's (1946) description for the house fly "terminal organ." He describes a distinct neurilemma covering the central core of sensory cells but again the absence of cell membranes in sectioned material made differentiation impossible for the apple maggot. Bolwig also describes a complex series of structures embedded in the integument through which the "sense rods" pass, but in Rhagoletis only a ring of epicuticle is present. Drawings of this organ show a number of small papillae projecting from its surface, the presence of which could not be positively confirmed (Snodgrass, 1924).

The organs at the corners of the mouth of Rhagoletis correspond to what Bolwig (1946) calls the "ventral organ" of Musca. Except for the usual lack of distinct cell membranes the descriptions of these structures are similar. Drawings of the external papilla of this organ (Snodgrass, 1924) show it to be very similar to the larger ventral terminal organ, but as before, this could not be positively confirmed from sectioned material.

The pharyngeal sense organ seen in Fig. 35 probably corresponds to the epipharyngeal organs of Pantel (1898) and the epiphyral organs of Keilin (1915), and Hertweck (1931), as one cytoplasmic filament enters the epipharyngeal sclerite (Fig. 35, es). Bolwig (1946) calls the organs in the floor of the pharynx the hypostomal organs (the hypostomal organs of Pantel, 1898; and hypophyreal organs of Keilin, 1915, and Hertweck, 1931). The structures of both of these organs are similar to the description of Bolwig (1946) and although there is no experimental evidence, their position indicates a gustatory function.

Bolwig (1946) mentions several small sense organs located in the rear of the pharynx of Musca which could not be found in Rhagoletis.

The many small sense organs scattered over the body wall resemble those in Hertweck's (1931) drawings for Drosophila and Osborne's (1963) description for Phormia terrae-novae. Osborne (1963) has shown that these organs are innervated through the lateral segmental nerves but the small size of the apple maggot has made similar observations impossible. Osborne (1963, 1964) and Whitten (1963a) found stretch receptors associated with segmental muscles and tracheae in a number of cyclorrhaphous larvae innervated by both lateral and median dorsal nerves. In dissections of the apple maggot, branches from lateral nerves were occasionally seen to fuse with the median portion of segmental muscles, a situation reminiscent of the structures seen by Osborne (1963, 1964) and Whitten (1963a). Unfortunately, it was not possible to find any evidence of chordotonal organs in sectioned material.

No trace of the photoreceptors found by Bolwig (1946) in the pharyngeal skeleton of Musca could be detected in sectioned apple maggots.

G. Imaginal discs

The imaginal discs of Rhagoletis may be conveniently divided into the principle discs, which include the frontal sacs, leg, wing, haltere, labral, tracheal and genital discs, and the imaginal cell patches which include rudiments of the adult proventriculus, salivary glands, and Malpighian tubules.

The principle discs of the 13 day old larva are histologically similar, each being a sac like cluster of cells containing a slit like lumen connected to the hypodermis by a thin stalk (Figs. 37, i; 71, L_1, L_2, L_3). The cells are small, dark staining, and closely packed having a thin coat of non-granular cytoplasm and indistinguishable cell membranes. They stain brown in Masson's, red or blue in Mallory's, green in Lower's and dark blue in Hematoxylin and eosin Y. The nucleus stains darker than the cytoplasm. By day 20, the discs have increased in size and in some, the original cuboidal cells have started to form columnar epithelium. The frontal sacs and leg discs now show organ rudiments and it can be seen that the principal discs are covered by a thin (less than 0.5) membrane which stains green in Masson's trichrome stain.

The largest of the principle discs are the two frontal sacs which contain rudiments of the eyes, antennae and head capsule. These structures are roughly oblong masses of tissue located centrally in the meso- and metathorax, and laterally along the oesophagus (Figs. 40, fs; 71, FS). They are connected anteriorly with the posterio-dorsal epithelium of the pharynx and posteriorly with the cerebral hemispheres by a thick nerve which connects the posterior portion of the sac with the

posterio-dorsal region of the brain (Figs. 38, 39, ON). Cross sections through the thorax show the anterior end of the aorta (Fig. 40, Ao) fusing with the mesial wall of the frontal sacs (Fig. 40, fs) and their relation with the oesophagus (Fig. 40, o). In the 13 day old larva these discs are relatively featureless and measure 250μ long and 30-60μ thick. By day 20 these dimensions have increased to 400μ long and 90 to 110μ thick. The rudiments of the compound eyes and antennae may be recognized. The eyes appear as cup-shaped layers of columnar epithelium located in the extreme posterior end of each frontal sac (Figs. 38, 39, E; 71, ob). Cross and frontal sections show this epithelium to be located on the mesial surfaces of the frontal sac (Fig. 41, E).

The antennal rudiments develop anterior to the eye (Figs 38, A; 71, ab). In the 20 day old animal these structures appear as a peg of columnar epithelium (Fig. 42, CP) growing from the mesial wall of the frontal sac 45μ high and 90μ wide which may be surrounded by one or two concentric rings of tissue (Fig. 42, CR).

The remainder of the adult head is formed from the paired labral discs. These are sac shaped structures attached to the latero-ventral surface of the pharynx immediately anterior to the junction of the salivary duct and the pharyngeal floor (Figs. 43, ld; 71, LbB). In the 13 day old larva these histoblasts measure 40μ in diameter and increase to 60μ in the 20 day old animal. In both cases, the walls of the disc are composed of cuboidal or columnar cells arranged around an irregularly shaped lumen. No recognizable organ rudiments are present before pupation.

The pro- and mesothoracic leg discs are located in the metathoracic

segment between the salivary glands and ventral to the large ventral ganglion (Figs. 44, LD; 71, L_1, L_2). Each disc is a spindle shaped structure attached ventrally to the hypodermis by a long slender stalk, and dorsally to the ventral ganglion by a much shorter, thicker stalk (Fig. 71). The two prothoracic discs are fused mesially while the meso- and metathoracic discs are free. The metathoracic discs have no connection with the ventral ganglion and are located dorsolaterally in the first abdominal segment where they are closely associated with the wing and haltere discs, usually lying between them (Figs. 41, LD_3; 71, L_3).

In the 13 day old larva these structures are simple clumps of cuboidal cells 40-50μ thick in which a small cup shaped slit-like lumen can be seen in frontal and cross sections (Figs. 37, 45, lu). No recognizable organ rudiments have developed at this time. By the twentieth day each leg disc has increased in thickness to 100μ and is equipped with a central papilla (Fig. 46, ts) surrounded by a series of concentric rings all of which grow into the lumen from the lateral wall (Fig. 46, cr). These are the rudiments of the leg segments, and both rings and papilla are composed of a central core of light staining irregularly shaped cells supporting a much darker staining layer of columnar epithelium. The epitheliar cells measure 25μ high and 4μ wide, the elongate oval nucleus measuring 6μ long and 2-3μ wide. The columnar epithelium extends part way along the mesial wall of the disc (Fig. 46, ce) but the remainder of the mesial wall has not differentiated at this time.

The wing and haltere discs are located dorsolaterally in the meta-

thorax and first abdominal segment, respectively. They are bounded mesially by the fat body, laterally by the muscles of the body wall and dorsally by the dorsal tracheal trunk, the metathoracic leg disc being located between them (Fig. 41, W, H). Each disc is attached to the hypodermis of the thorax by a slender stalk (Fig. 47, s); however, both are also anchored by the lateral tracheal branch (transverse connective) of the segment in which they are located (Figs. 47, 48, tc). In addition, the wing disc is partially fused to the metathoracic leg disc (Figs. 41, 48, W, LD_3).

Both of these structures are histologically similar and except for a two fold increase in size from 30-40x100-110μ to 60-70x180-200μ, they change little during the third instar. Cross and frontal sections show the mesial wall of the disc to be much thicker than the lateral surface and reveal a curved, slit-like lumen (Fig. 49, lu). Some frontal sections show the mesial wall of the wing disc thrown into a series of folds (Fig 50). The thickened mesial wall of these discs is composed of dark staining columnar cells which gradually decrease in size toward the edges of the disc, eventually forming the cuboidal cells of the lateral walls (Figs. 49, 50, ce). In the 20 day old larva the columnar epithelium rests on a core of light staining cells similar to those of the leg discs (Fig. 47, co).

There are two pairs of histoblasts associated with the tracheal system in this stage of Rhagoletis. They are sac-like structures having a small slit shaped central lumen; the walls being composed of several layers of cuboidal cells. Each anterior stigmatic chamber is endowed with a dorsal and a ventral disc both of which are anchored by a short

thick stalk to the chamber near the large peristigmatic cells. The tracheal discs do not change in structure during the third instar but do change in size increasing from 40μ to 60μ in diameter (Fig. 51, TD).

Imaginal cell patches are present in the salivary glands, proventriculus, and at the junction of the midgut and the Malpighian tubules. In the salivary gland they form the junction between the large secretory cells of the gland and the salivary duct (Fig. 53, ic). The cells are easily recognized as they are much smaller than the adjoining cells and stain slightly darker. There is no substantial increase in size from approximately 20-25μ in diameter during the third instar.

The imaginal cells of the proventriculus form a ring in the anterior wall of that organ (Fig. 52, ic). They appear to be elongated cells with oval nuclei oriented in a radial fashion along the inner wall of the spherical proventriculus. The cytoplasm stains similar to the rest of the proventriculus but more faintly. The imaginal proventricular cells measure 20-25μ long in the 13 day old larva and 50μ long after 20 days. There is no change in structure during this time.

The imaginal cells of the ampulla form a ring around the mesenteron at its anterior junction with the ampulla of the Malpighian tubules. They are atypically light staining and thus are readily discernible in sections (Fig. 25, 1c).

Although appearing to originate from the pharynx, the lumens of the frontal sacs are actually confluent with the mouth, a condition which allows eversion of these structures during metamorphosis (Snodgrass, 1924). A green staining line running along the dorsal surface of the pharynx in Masson's preparations represents this obscured passageway.

A number of authors (Krafka, 1924; Enzmann and Haskins, 1938; Steinberg, 1941, 1943; Bodenstein, 1950) have studied the development of the compound eyes in Drosophila using light microscopy. All agree that the ommatidia have started to differentiate in the third instar. These structures are generally described as being clusters of four fusiform cells regularly arranged over the mesial surface of the disc (Bodenstein, 1950). In Rhagoletis no cell clusters can be seen in sections through the columnar epithelium of the optic disc (Fig. 39) of the mature larva. It is possible that differentiation begins after pupation in this insect, but Waddington and Perry (1960) who noted these clusters in Drosophila with the electron microscope stated that cell membranes in the optic disc fix poorly even with osmium. Thus, the absence of such clusters in the apple maggot may be an artifact.

The development of the antennal discs of Rhagoletis is very similar to Lowne's (1890-92) description of this organ in Calliphora. Thus, the central papilla present in the 20 day old larva represents the third antennal segment of the adult while the encircling rings eventually form the two basal segments.

The leg discs conform to the descriptions of Lowne (1890-92) for Calliphora and Bodenstein (1950) for Drosophila melanogaster and D. virilis. The central papilla present in both young and old third instars is the rudiment of the tarsal segments, while the adjacent ring of tissue forms the tibia. The second ring forms the imaginal femora and trochanter while the third ring gives rise to the coxa. During metamorphosis the stalks of the leg discs shorten and spread over the ventral region of the thorax, the cells replacing the histolysed larval

epidermis and forming the imaginal tissue.

The ultrastructure of the metathoracic leg disc of Sarcophaga bullata has been studied by Chiarodo and Denys (1968). In this species the discs of the early third instar are composed of very small, irregularly shaped cells having relatively large nuclei covered by a thin layer of cytoplasm. Toward the end of the third instar when the discs have begun to differentiate, the cells become columnar in shape and are endowed with a larger amount of cytoplasm. As can be seen from Figs. 45 and 46, a similar sequence of events takes place in the developing leg discs of Rhagoletis.

Auerbach (1936) has studied the development of the wings and halteres in Drosophila. Her drawings of these discs in third instar larvae are almost identical to Figs. 49 and 50, thus the mesial thickened areas form the actual wing and haltere while the thin lateral wall is the peripodial sac which degenerates in the pupa.

H. Reproductive system

In the third instar larva the reproductive system consists of the genital disc and the gonads. The genital disc is an unpaired, dumbbell shaped structure oriented transversely near the ventral epidermis in the seventh abdominal segment (Fig. 5, gd). It is anchored by a pair of filaments which join the epidermis immediately before the two projections of the anal organ. During the third instar, this disc increases in size from 130μ long and 30-35μ thick to 250μ by 50-55μ. The internal organization which consists of a narrow, elongate lumen lined by several layers of cuboidal cells, does not change during the third instar (Fig. 54, gd).

The gonads are not as yet closely associated with the genital disc. They appear in sections as round bodies which stain similar to the imaginal discs and are composed of a central region occupied by densely packed cells with light staining, non-granular cytoplasm and a peripheral layer of much darker staining cells (Fig. 55, go). These structures are located in the fifth abdominal segment, partially embedded in the mesial surface of the dorso-lateral fat body, just ventral to the junction of the lateral tracheal branch and the great dorsal tracheal trunk (Figs. 56, go). Apparently, the gonads develop very slowly or not at all in the third instar as they have a diameter of 40-50μ in both 13 and 20 day old larvae.

Dobzhansky (1930) demonstrated the development of the genital ducts, accessory glands and external genitalia from the unpaired median genital disc of both sexes of Drosophila melanogaster. Such development takes

place in the pupal stage, however, and through most of the third instar this disc is as seen in Rhagoletis (Fig 54, gd). Newby (1942) found that the genital disc of Drosophila virilis begins to develop just before the third day of larval life (second instar) while Bodenstein (1950) states that in D. melanogaster this disc is present in early third instars and probably develops at the same stage as D. virilis. However, Anderson 1963) reported that differentiation of the median genital disc in the embryo of Dacus tryoni (Diptera:Tephritidae) is concurrent with dorsal closure, that is, approximately two thirds of the way through embryogenesis. He further describes a pair of "lateral genital discs" which do not differentiate until the second instar. These are not present in Rhagoletis but Dubendorfer (1970) has described them from Musca domestica and Phormia regina.

Newby's (1942) account mentions that by day four the cells at the surface of the genital disc of D. virilis have formed an epithelial plate which separates from the underlying tissue thus forming a lumen as seen in Fig. 54. This later becomes the lumen of the reproductive tract. Newby (1942) describes the discs of third instars of both sexes as being histologically indistinguishable until before pupation when the primordia of the vasa efferentia and accessory glands become apparent in the males. In the apple maggot these discs are similar in both sexes until after pupation.

The gonads differentiate very early in embryonic life in the form of the pole cells (Anderson, 1963). Throughout larval life they are widely separated from the genital disc and only after pupation do the two structures make contact. In Drosophila the testes are significantly

larger in all three instars although the disparity in size is greatest immediately following eclosure (Kerkis, 1931), and are also distinguishable histologically from the ovaries by the third instar (Kerkis, 1933). In Rhagoletis it is not possible to distinguish ovary and testes through size or histology before pupation.

Bush (1966) used larval gonad as well as brain tissue to study the chromosomes in the genus Rhagoletis. He reports that in R. pomonella there are twelve chromosomes including three pairs of metakinetic, two of rod-shaped acronetic and a single pair of acronetic dot chromosomes. No morphologically distinct heterochromosomes (XY) were seen.

I. Circulatory system

The circulatory system consists of the heart, aorta, pericardial cells or nephrocytes, hemolymph and the hemocytes. The heart is a tubular structure lying just below the dorsal integument in the last three abdominal segments. Cross sections through this organ reveal 2-3μ thick walls composed of a featureless tissue which stains red in Mallory's, brown in Masson's, pink in hematoxylin and eosin Y, and orange in Lower's. The lumen is irregular and increases in diameter from 75-100μ in the 13 day old larva to 100-125μ in the 20 day old larva. Nuclei may be found in the lateral walls of the heart measuring 10-12μ thick in the 13 day old animal and 12-15μ thick in the mature form. Tangential sections through the heart show transversely oriented muscle fibers (Fig. 57, m) and show that the nuclei are oval organelles measuring 18x25-30μ (Fig. 58, n). They are regularly spaced about 50μ apart in the lateral walls of the heart. Figure 59 shows that the nuclei (n) on each side of the heart are located directly opposite each other.

There are three pairs of ostia in the heart, one in each of the last three abdominal segments. Each is a slit in the lateral wall of the heart, the edges of which are equipped with flaps of tissue acting as valves (Fig. 60, v). Each ostial flap has a centrally placed, unicellular thickening (Fig. 60, t), but above and below this the valves are composed of unmodified cardiac tissue.

Closely associated with the ventral surface of the heart is a double row of pericardial cells (Fig. 61, PC). These are rounded cells measuring 21-30μ in young larvae and eventually increasing to 35-40μ in late

third instars while the oval nucleus increases in length from about 10 to 18-22μ. The cytoplasm is distinctly granular and stains blue in Mallory's, brown in Masson's, pink in hematoxylin and eosin Y, and green in Lower's trichrome. The nucleus stains dark blue in hematoxylin and eosin Y and red or orange with the others. These cells are attached to the ventral surface of the heart by fine strands of connective tissue which stains blue in Mallory's triple.

The aorta extends from its union with the heart in the sixth abdominal segment to the metathorax. Here it enters Weissmann's ring (ring gland) where its ventral surface presumably fuses with the corpora cardiaca. The dorsal and lateral walls pass through the ring (Fig. 19, Ao), the dorsal wall (Fig 62, Ao) eventually fusing with the posterior portion of the pharynx (Fig. 62, Ph) and the lateral portions with the mesial surfaces of the frontal sac (Fig. 40, Ao). Thus, the blood is directed ventrally and anteriorly over the oesophagus and flows into the portion of the hemocoel surrounding the pharynx which Lowne (1890-92) has termed the cephalopharyngeal sinus. Blood is also directed into the suprapharyngeal sinus (Fig. 62, SS).

Except for its smaller diameter the aorta is histologically similar to the heart showing transverse muscle fibers and oval nuclei (Fig. 66, Ao). The aorta is flanked laterally by a double row of cuboidal or rounded nephrocytes extending from the heart to the second abdominal segment (Fig. 63, ne). These cells stain similar to those near the heart but are much smaller measuring only 20μ in diameter in late third instars, have non-granular cytoplasm, and are occasionally binucleate.

A few nephrocytes histologically similar to the cells flanking the

aorta may be found between the salivary glands in Rhagoletis larvae. They form a rough crescent with its open end facing anteriorly and are often binucleate (Fig. 64, ne).

The hemolymph of the apple maggot is a clear fluid in the living animal which forms a light staining, granular material in sections, and in which very few hemocytes circulate freely before pupation. In early third instars blood cells seem to be present only in aggregations in the last abdominal segment. Just prior to pupation, however, they may be found adhering to the imaginal discs (Figs. 41, 42, bc) and muscles and in the suprapharyngeal sinus. They also appear near, but never in the heart.

In Masson's trichrome stained sections they are readily distinguishable into two types, small basophilic 5-7μ diameter prohemocytes having dark staining nuclei measuring 3-4μ (Fig. 65, Pr) and larger 12-20μ acidophilic oenocytids with 5-7μ nuclei (Fig. 65, oe).

The heart of Rhagoletis is similar to that of Musca domestica (Hewitt, 1914) and Calliphora (Lowne, 1890-92). Lowne described many ostia in the heart of C. erythrocephala larvae, however, only three pairs are present in the apple maggot and Musca (Fig. 60, os). The unicellular thickenings seen in the ostia (Fig. 60, t) have been described by Tzonis (1936) in the larva of Corethra (Chaoborus) (Diptera:Chaoboridae). Wigglesworth (1965, pg. 371) describes their role in the action of the heart as follows: ". . . each ostial lip has a unicellular thickening which runs into a thread attached to the inner wall of the heart. When the heart dilates the valves are widely separated and the blood enters; when dilation is complete (diastole) the valves are closed

and stand out at right angles to the wall; during contraction or systole they become evaginated as far as their attached threads will permit, and are forced together so that no blood can escape." So far these thickenings have not been reported from other cyclorrhapha.

The regularly spaced nuclei located inside the heart muscle fibers of Rhagoletis were also noted by Lowne (1890-92) in larval Calliphora. No trace of alary muscles could be found in the apple maggot sections although they are easily seen in gross dissections. The fibers connecting them with the heart were often present in frontal sections through the abdomen (Fig. 58, f). Similar fibers connect the pericardial cells with the heart.

Whitten (1964a) has demonstrated the presence of analogous fibers in larval Sarcophaga bullata. Furthermore, she discovered under phase contrast the presence of fine, secretion filled channels in the fibers connecting pericardial cells and heart. Thus, it would appear that in Sarcophaga these cells may chemically influence the heart in addition to their more traditional functions of phagocytosis and excretion. Chapman (1969, pg. 697) reports that in Calliphora the pericardial cells are connected to the corpora cardiaca by neurosecretory axons, a condition which would suggest transfer of neurohormones to these cells. However, no channels or neurosecretory axons of this type could be seen in sectioned or dissected apple maggot larvae.

Lowne (1890-92) has termed the space dorsal to the pericardial cells and between the dorsal oblique muscles (Fig. 61, PS) the pericardial sinus although no dorsal diaphragm is present. The ventral diaphragm is also lacking, a condition common among larvae of higher

Diptera (Richards, 1963).

The small cells flanking the aorta have been interpreted in several ways. Anderson (1963) refers to them as the "lymph gland cells," and Stark and Marshall (1930) consider them to be a blood forming organ while Bhatia (1939) and Keilin (1924) classify them as nephrocytes. Keilin (1924) reported phagocytosis occurring in these cells in the larva of Lonchaea chorea (Diptera:Lonchaeidae).

A row of nephrocytes may also be found between the salivary glands in larval Diptera constituting the "garland-like cell chain" of Weissmann (Imms, 1957). These structures have been described in Phaonia cincta (Diptera:Lonchaeidae) by Keilin (1917, 1924) and in Calliphora (Chapman, 1969, pg. 495).

The appearance of the hemocytes of Rhagoletis in sections is very similar to those described by Anderson (1963) in Dacus tryoni (Diptera: Tephritidae). Using the nomenclature of Arvy (1953) the small, basophilic cells correspond to prohemocytes while the larger, eosinophilic forms are oenocytoids. Several other types of blood cells have been described from dipterous larvae by investigators using phase contrast microscopy (Rizki, 1953; Akesson, 1953; Jones, 1956; Whitten, 1964b, 1969; Nappi, 1970).

The aggregations of blood cells in the last abdominal segment of dipterous larvae have previously been noted by Anderson (1963a) in Dacus and by Arvy (1954) for Musca domestica. Arvy considers them to be hemopoietic organs while Anderson (1963) feels that they are artifacts of fixation. Nappi and Stoffolano (1972), however, using liquid nitrogen to rapidly kill and fix larvae of M. domestica and M. autumnalis

found the blood cells in the same area. This precludes that they are an artifact but does not exclude the fact that they may be concentrated there because of hemodynamics.

J. Endocrine system

The endocrine organs of cyclorrhaphan larvae are located in a structure known as the ring gland or Weissmann's ring. As its name suggests, this structure is composed of a ring of tissue encircling the aorta in the last two thoracic segments. The aorta (Fig. 66, Ao) is fused dorsally and ventrally to the basement membrane of the gland (Fig. 66, RG) but the lateral walls are free. The cephalic end of the gland is somewhat thickened and is pierced by the commissure of the pair of tracheae supplying the pars intercerebralis. Two posterior arms, two or three cells thick (Fig. 67, pg), reach around the aorta and meet in another swollen area beneath the aorta. This ventral thickening is fused dorsally to the aorta and ventrally to an elongate, histologically distinct structure which receives the recurrent nerve and eventually sends two nerve branches to the proventriculus. This structure is probably the fused hypocerebral and proventricular ganglia and is described more fully in the section dealing with the stomatogastric nervous system.

The one pair of nervi corpora cardiaci connect the pars intercerebralis with the lateral edges of the ventral portion of the ring gland (Fig. 31, ncc). Two distinct cell types are present in the ring gland. The bulk of the organ is composed of relatively large cells having granular cytoplasm which stains like muscle tissue and indistinct cell membranes. The nuclei are round and show coarsely precipitated chromatin and a distinct nucleolus as they increase from 5μ to 12-17μ in diameter during the third instar (Fig. 67, pg). The median area of

the anterior thickening is composed of smaller cells having rather homogeneously dark staining nuclei (Fig. 67, ca). The cells themselves cannot be measured due to the indistinct cell membranes, but the nuclei increase in diameter from 3μ to 5μ in the 20 day animal. The trachea passing through this area is covered with a layer of tracheal cells which stain similar to the small gland cells but are easily differentiated due to their location (Fig. 67, te).

It has been known since the work of Burtt (1938) that the ring gland (Weissmann's ring) is an important endocrine organ in the larvae of higher Diptera. Comparative studies (Burtt, 1937; Thomsen, 1951) indicate that this structure represents the fused corpora allata, corpora cardiaca, and prothoracic glands of more generalized insects. Although fused, the three glands have been shown by many authors to be histologically distinct. Thus, the large cells of the lateral arms and dorsal thickened area represent the prothoracic glands (Fig. 67, pg). The small dorsal cells clustered near the penetrating trachea represent the fused corpora allata (Fig. 67, ca), while the cells fused to the elongate hypocerebral ganglion and aortal floor may be the corpora cardiaca. The corpus cardiacum receives a pair of nerves, the nervi corpora cardiaci (Fig. 31, ncc) from the pars intercerebralis which convey neurosecretions to this storage organ.

Day (1943) reported five distinct cell types from larval Lucilia sericata (including nerve and tracheal cells) while Scharrer and Hadorn (1938) were able to find only two in Drosophila. In this regard, Rhagoletis is similar to Drosophila as only two cell types may be distinguished histologically. Thus, the small pocket of cells in the

dorsal ring (Fig. 67, ca) correspond to published descriptions of the corpora allata and the large peripheral cells (Fig. 67, pg) to the prothoracic gland (Thomsen, 1951; Fraser, 1959c). Most workers state that the corpus cardiacum cells are also distinguishable due to their small size and ventral location in the ring. In the apple maggot the ventral cells are similar in size and histology to those of the lateral arms rendering the corpus cardiacum of this species indistinguishable histologically from the prothoracic gland.

Fraser (1959c) has investigated the endocrine organs of seven species of Muscidae and Calliphoridae and found that in all cases the prothoracic gland cells are confluent beneath the aorta. The corpus cardiacum cells form a patch of the aortal floor immediately anterior to the ring cells and are innervated by the nervi corpora cardiaci and a branch from the hypocerebral ganglion. Thus, in these species the corpus cardiacum is not an integral part of the ring as is the case in Drosophila virilis (Bodenstein, 1950), Eristalis tenax (Cazal, 1948) and the apple maggot.

Cazal (1948) shows a pair of nerves connecting corpora allata and cardiaca through the lateral walls of the ring in Eristalis and Day (1943) reported the presence of nerve cells in the prothoracic gland region of Lucilia sericata. Only gland cells compose the prothoracic gland of Rhagoletis. Whitten (1964a) has demonstrated the presence of many secretion filled channels in the basement membrane covering the ring gland of Calliphora larvae. Possibly the two glands communicate through these channels in the absence of nerves.

K. Excretory system

The excretory system consists of two pairs of Malpighian tubules and the nephrocytes or pericardial cells. The Malpighian tubules can easily be seen in living larvae as they have a distinctive white color and moniliform shape. Each pair originates from a common duct which empties at the junction of mid- and hind-intestine in the fifth abdominal segment. At the base of each common duct is a swelling termed the ampulla by Snodgrass (1924) (Fig. 25, amp). Of the two tubules which branch from each common duct, one extends anteriorly staying in close proximity to the midgut, to the second abdominal segment where it ends blindly near the posterior end of the salivary gland. The other tubule courses posteriorly to the eighth abdominal segment, doubles back, and ends near the segment of its origin.

These tubules are easily recognized in sectioned material from their distinct beaded shape and the yellow-colored, occluded, intracellular lumen (Fig. 68, lu). Cross sections show a single cell having highly vacuolated granular cytoplasm which stains similar to muscle tissue. The cells increase in diameter from 50μ to 70μ during the third instar. The round nucleus is located somewhat peripherally due to the presence of the central lumen, and shows a rather indistinct aggregation of chromatin as it increases in diameter from 12-15μ to 20μ. The lumen as previously stated is readily distinguished due to its yellow color. It is slit-like in shape and gives off several short protrusions into the cytoplasm (Fig. 68, lu). The cells are invested in the usual thin basement membrane and the tubules themselves are devoid of musculature.

The common ducts of the Malpighian tubules are quite different histologically from the tubules. Here the lumen is obviously extracellular, being surrounded by small rounded cells with very highly developed brush borders (Fig. 69, bb). The nuclei are relatively large and comprise much of the bulk of the cell. The cells rest on a basement membrane and a layer of circular muscle is present.

The cells of the ampullar region are also rounded but are smaller than those of the common ducts and have no brush borders. In sections, long tapering filaments are seen to originate from the apical region of these cells and extend into the hindgut (Fig. 70, f).

The nephrocytes have been described in conjunction with the circulatory system.

According to Wigglesworth (1965, pg. 511) ". . . no artificial fluid is inert towards the Malpighian tubes which swell up and discharge droplets when so treated." Thus, fixation of these delicate tissues may be expected to result in the formation of many artifacts, and in fact, published accounts reveal changes in these organs during routine histological processing which are similar to the results seen in the present study. Intense vacuolation of these cells as seen in Fig. 68 (v) has been noted by von Gorka (1914) in Coleoptera. The microvilli seen lining the lumen of the tubules in the electron micrographs of many authors (Wigglesworth and Saltpeter, 1962) do not appear in the terminal tubules of Rhagoletis, an artifact also noted by Metalnikov (1908) in larval Galleria. Finally, the occluded lumen seen in Fig. 68 is most likely the result of severe swelling of the cells during immersion in the various fluids involved in histological process-

ing.

Although cell preservation is undoubtedly poor in the present study, the material used by previous authors was subjected to similar insults, thus, the Malpighian tubules of Rhagoletis are similar histologically (and morphologically) to other cyclorrhaphan larvae. Dean's (1932) treatment of these organs is somewhat superficial but he does mention that two cells are present bordering the lumen but generally appear as one doe to the staggered position of the nuclei and indistinct cell membranes. The present investigation seems to indicate, however, that the tubules are composed of rows of single cells having intracellular lumens, a situation similar to that shown by Wigglesworth (1965, pg. 513) for Rhodnius.

The white crystals occupying the lumen of the Malpighian tubules described by Snodgrass (1924) do not appear in sections. They are routinely seen during gross dissections, however, and probably dissolve during fixation. Little is known of the excretory products of fly larvae. It has been determined that larval Lucilia excrete nitrogen mainly in the form of ammonia and to a much lesser extent, allantoin (Stobbart and Shaw, 1964). Chapman (1968, pg. 498) reports that in other dipterous larvae uric acid crystals occur throughout the Malpighian tubules. Thus, it seems likely that the crystals seen by Snodgrass were uric acid.

The long filaments originating from the ampullar cells have been previously described from Drosophila (Eastham, 1925) and Rhodnius prolixus (Wigglesworth, 1931). Wigglesworth (1931) has shown that in Rhodnius these filaments are capable of picking up the dye neutral red

from the rectum and cites this as evidence for reabsorption of water by the ampullar cells. Since the ampullar processes of Rhagoletis also trail near the hindgut and are very similar to those of Rhodnius, it seems likely that they have a common function.

Eastham (1925) has reported seeing peristaltic movements in the common ducts of Drosophila and Calliphora larvae. The musculature of the common ducts of the apple maggot is so similar to that figured by Eastham that a similar mechanism seems certain.

The excretory function of the nephrocytes was suspected before the turn of the century. Hollande (1921) working with various Lepidoptera noticed that the pericardial cells displayed regular cycles of vacuole formation, coalescence, growth, and finally resorption. He concluded that complex materials circulating in the blood were taken up by these cells and degraded enough to pass through the Malpighian tubes. Lesperon (1937) showed that pericardial cells were active in phagocytosis of colloids, the efficiency of the process depending on sizes and the charge of the particle.

Mills and King (1965) and Aggarwal and King (1967) have described the ultrastructure of these cells in adult and larval Drosophila. Both papers show the nephrocytes to contain many lysosome-like bodies as well as large vacuoles containing electron dense material in various states of decay. Aggarwal and King were able to locate macromolecules adhering to the cell membranes which were actively engaged in pinocytosis. As a result of this discovery, they suggested the name pinocytes be used in lieu of earlier synonyms.

The pericardial cells of Rhagoletis resemble those of Drosophila

under the light microscope. In both species, the cells near the heart are histologically distinct from those flanking the aorta and lying between the salivary glands. Ultrastructural studies show, however, that in fact the only major difference is one of size in Drosophila (Mills and King, 1965; Aggarwal and King, 1967).

L. Bacterial symbiotes

The association between the apple maggot and its bacterial symbiote, Pseudomonas melophthora, has been known since the work of Allen and Riker (1932). However, virtually nothing is known of the location of this bacterium inside the various stages in the life history of Rhagoletis and if the insect possesses any specialized structures for storage and dissemination of the symbiote. The olive maggot, Dacus oleae (Diptera:Tephritidae), maintains a colony of its bacterial symbiotes, Pseudomonas savastanoi and, Ascobacterium luteum, in the digestive tract in larvae and adults. In the larva the microbes are concentrated in the gastric cecae while after emergence the flora is reestablished via a special oesophageal diverticulum located just before the brain (Petri, 1909, 1910).

The alimentary tract of Rhagoletis is similar to that of Dacus having both cecae in the larva (Dean, 1932) and the "oesophageal bulb" in the imago (Dean, 1933). Since Allen et al. (1934), using culturing techniques, were able to demonstrate the presence of P. melophthora in all life stages of the insect, it would seem that the apple maggot is similar to D. oleae. However, Baerwald and Boush (1968) using the fluorescent antibody technique were able to locate the symbiote only in the thoracic and abdominal hemocoel of two of the eight female flies tested. P. melophthora was found occasionally in larval and pupal smears but not at all in whole eggs and infected apple tissue. The authors attribute these rather curious results to the small numbers of bacteria present in the insect as dissected larvae and infected apple

tissue yielded positive fluorescent antibody tests after incubation on nutrient agar for 48 hours.

In the present study no material of any kind was found to be present in the gastric cecae of third instar larvae. The lumen of the midgut often contained much particulate matter adhering to the peritrophic membrane (Figs. 23, 24, PM) so P. melophthora may be harbored in this area. However, the stains used are not specific for bacteria so P. melophthora could not be positively identified in midgut sections.

V. SUMMARY

The histology of the organ systems of third instar Rhagoletis pomonella have been described from paraffin sections. A culture of R. pomonella was maintained in the lab on artificial diet. Early and late third instar larvae were removed from infested apples, fixed, embedded in paraffin and sectioned. Sections were stained with hematoxylin and eosin, Mallory's triple, Masson's trichrome or Lower's stain and examined under both flat field microscopy and phase contrast microscopy. Histological features were noted for tissues of the integument, tracheal system, alimentary tract, fat body, nervous system, imaginal discs, reproductive system, circulatory system, endocrine glands and excretory system.

An unsuccessful attempt was made to locate the bacterial symbiote P. melophthora in the body of the third instar apple maggot.

VI. CONCLUSIONS

Both the histology and basic morphology of the third instar larva of R. pomonella have been found to be similar to published descriptions of other larval cyclorrhapha. The only differences noticed were an apparent fusion of the hypocerebral and proventricular ganglia of the stomatogastric nervous system and the rather bulbous anal organ.

No special structures for harboring the bacterial symbiote Pseudomonas melophthora could be found in R. pomonella larvae. The gastric cecae, the site of symbiote storage in Dacus oleae, were always empty even in late third instar apple maggots.

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Fig. 1. Longitudinal section through integument (1250x phase contrast). EC, epidermal cell; En, endocuticle; Ep, epicuticle; LB, light staining band.

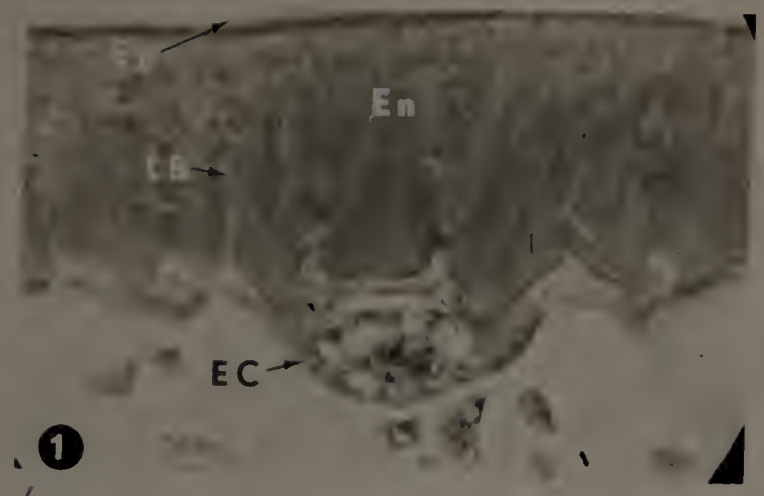
Fig. 2. Longitudinal section through integument (1250x phase contrast). EC, epidermal cell; En_1, outer endocuticle; En_2, inner endocuticle; Ep_1, outer epicuticle; Ep_2, inner epicuticle; h, hook on surface of integument; LB, light staining band.

Fig. 3. Longitudinal section through integumental apodeme (500x). A, apodeme; IM, integumental muscle.

Fig. 4. Posterior four segments of apple maggot (25x). ao, anal organ.

Fig. 5. Saggital section through last two segments showing anal organ (125x). ao En, anal organ endocuticle; EC, epidermal cells; En, endocuticle; gd, genital imaginal disc; in, epicutivular invagination; i, intima of rectum; R, rectum.

Fig. 6. Saggital section through anal organ (500x). EC, epidermal cells of anal organ; in, epicuticular invagination; M, dorsal muscle of anal organ; m, ventral muscle of anal organ; n, nucleus of epidermal cell.



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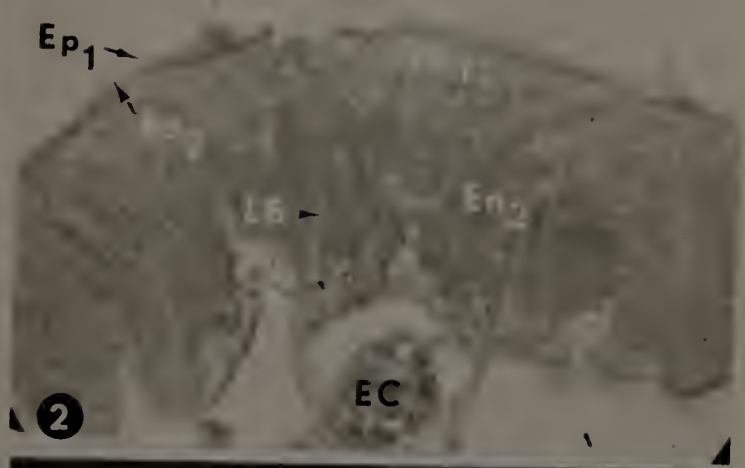

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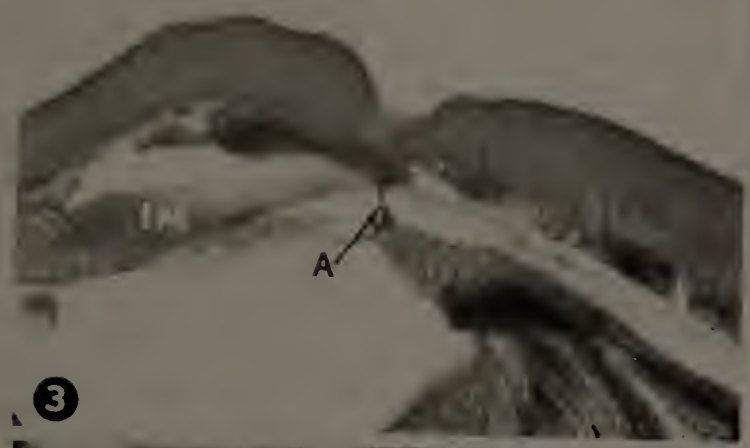

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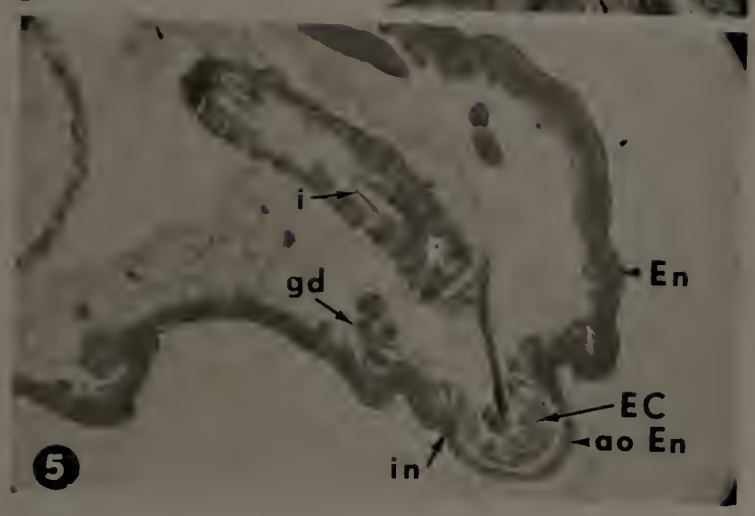

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Fig. 7. Drawing of main tracheal elements of apple maggot (modified from Snodgrass, 1924). ASp, anterior spiracle; r, dorsal anastomosis; q, anteriormost dorsal anastomosis; s, posteriormost dorsal anastomosis; PSp, posterior spiracle; t, lateral tracheal trunk; Tc, transverse connective; Tra, longitudinal tracheal trunk.

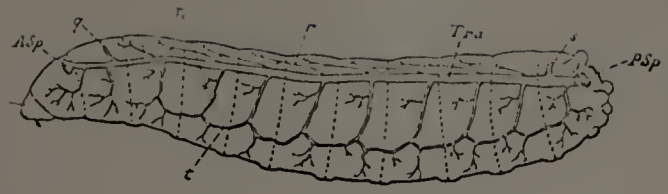
Fig. 8. Tangential section through longitudinal tracheal trunk (500x). n, nucleus of tracheal epithelial cell; ta, taenidia.

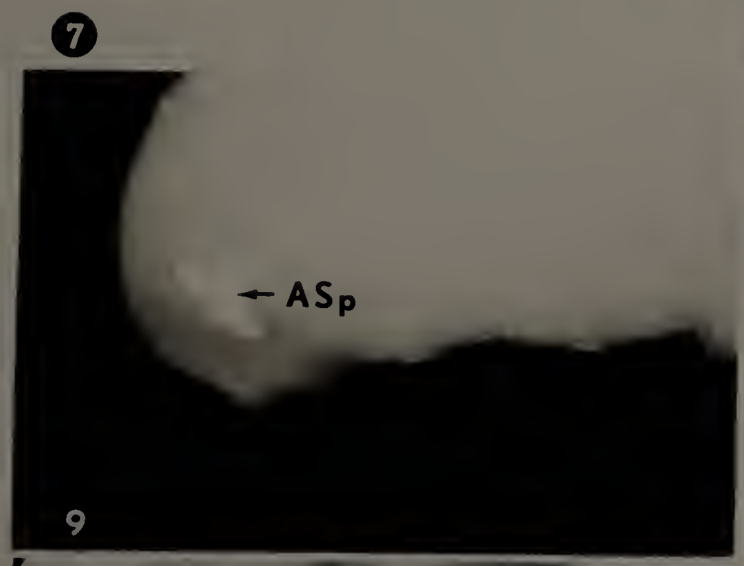
Fig. 9. Lateral view of anterior segments of apple maggot (25x). ASp, anterior spiracle.

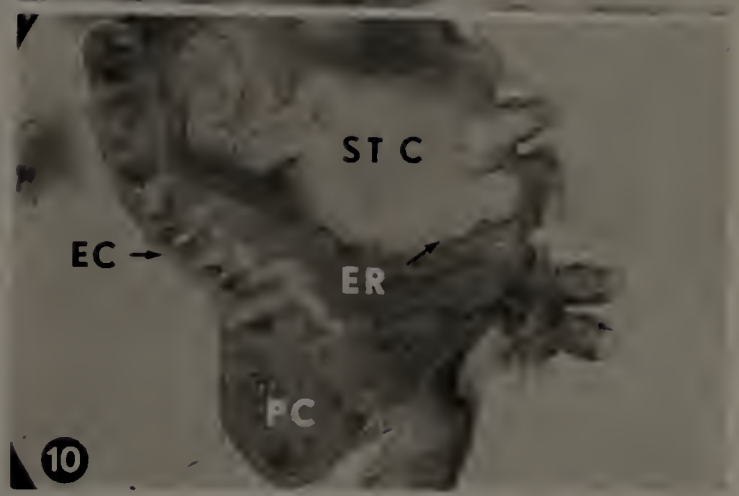
Fig. 10. Cross section through anterior spiracle (500x). EC, epidermal cells; ER, endocuticular ridge; PC, perispiracular cell; STC, stigmatic chamber.

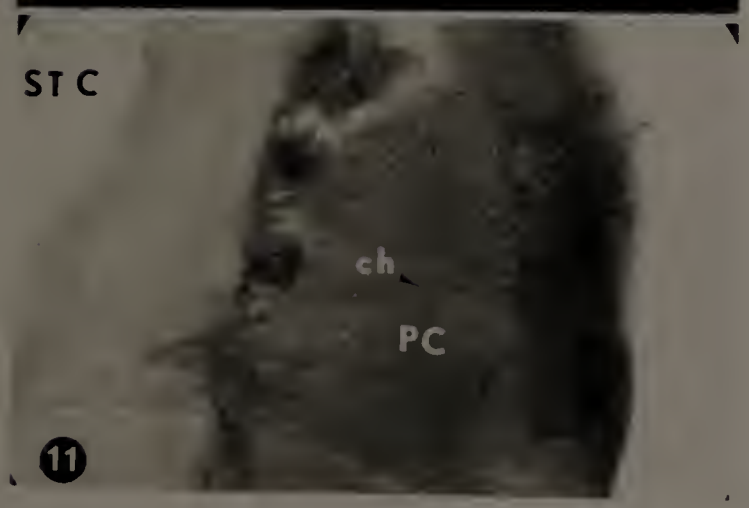
Fig. 11. Cross section through anterior perispiracular cell (125x). ch, channel running through cell; PC, pericardial cell; STC, stigmatic chamber.

Fig. 12. Drawing of posterior spiracle of apple maggot (from Butt, 1937). CP, tuft of hairs on stigmatic plate; SC, stigmatic scar; SL, spiracular opening, ST PL, stigmatic plate.









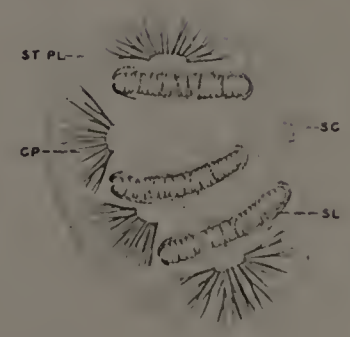


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Fig. 13. Saggital section through posterior spiracle (500x). ER, endocuticular ridges; IB, interdigitating bars forming filter at spiracular opening; PC, perispiracular cell; SL, spiracular openings; ST C, stigmatic chamber; SY, striated yellow coat of stigmatic chamber.

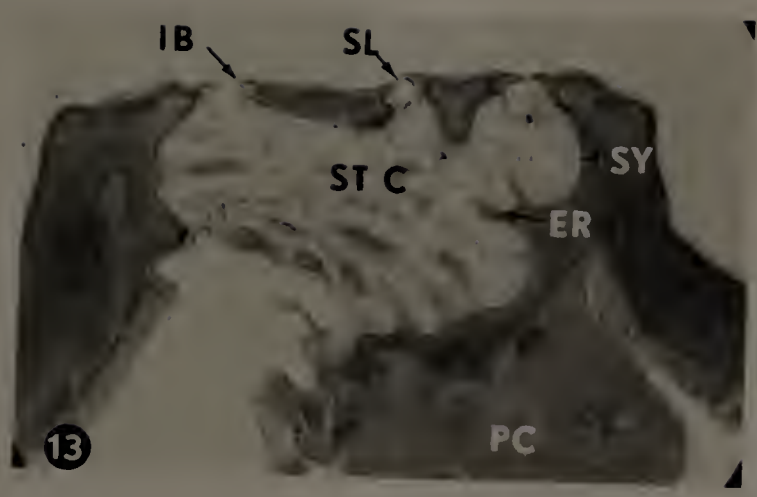
Fig. 14. Cross section through posterior perispiracular cell (500x). bm, basement membrane; ch, intracellular channel; PC, perispiracular cell; n, nucleus; nu, nucleolus.

Fig. 15. Drawing of cephalopharyngeal skeleton of apple maggot (from Snodgrass, 1924). A, hypostomal sclerites; a, arms of plate A supporting roof of pharynx; B. ventral plate of pharyngeal skeleton; b, phragma from which mouth hook muscles originate; C. dorsal plate of pharyngeal skeleton; c, phragma on which insert pharyngeal extensor muscles; D, epipharyngeal sclerite; Hk, mouth hook; OE, oesophagus.

Fig. 16. Saggital section through pharynx (125x). con, constrictor muscles; cm, cibarial muscles; l, pharyngeal lamellae; m, membrane dividing pharynx into upper and lower chambers.

Fig. 17. Cross section through pharynx (500x). l, pharyngeal lamella; m, membrane dividing pharynx; p, transversely oriented plate; F, food material wedged between lamellae.

Fig. 18. Saggital section through neuromuscular junction of pharyngeal constrictor muscle (1250x). con, constrictor muscle; N, nerve; nj, neuromuscular junction.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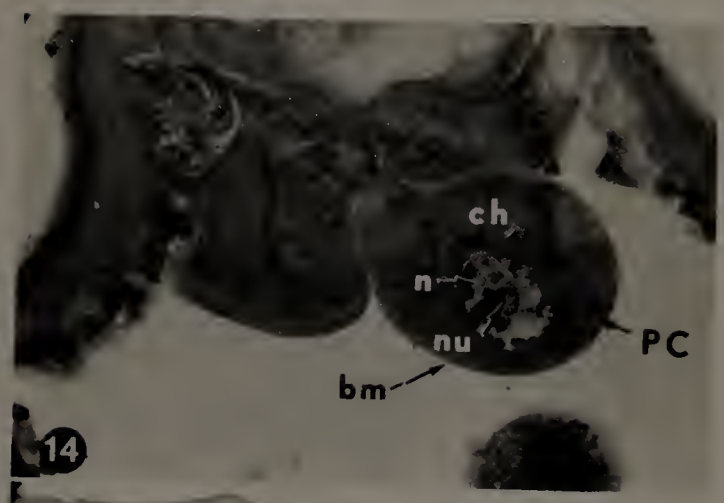
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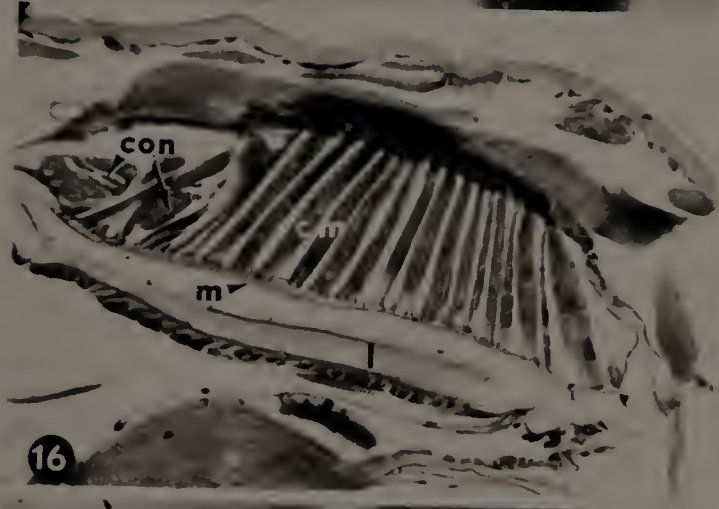
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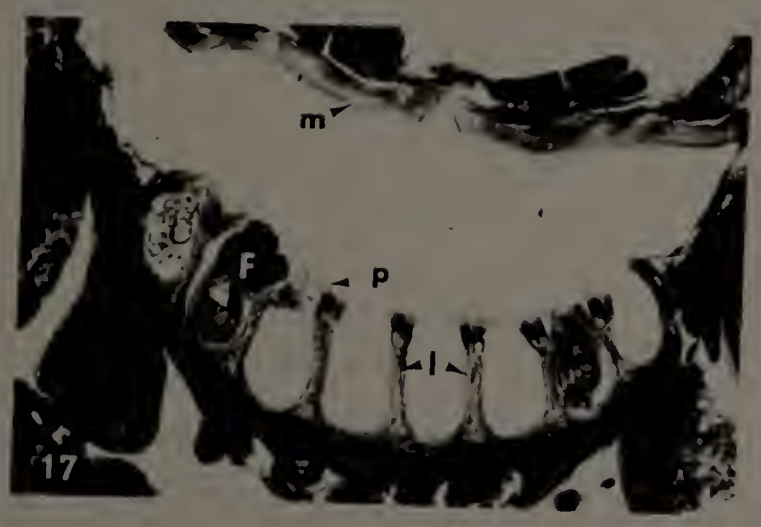
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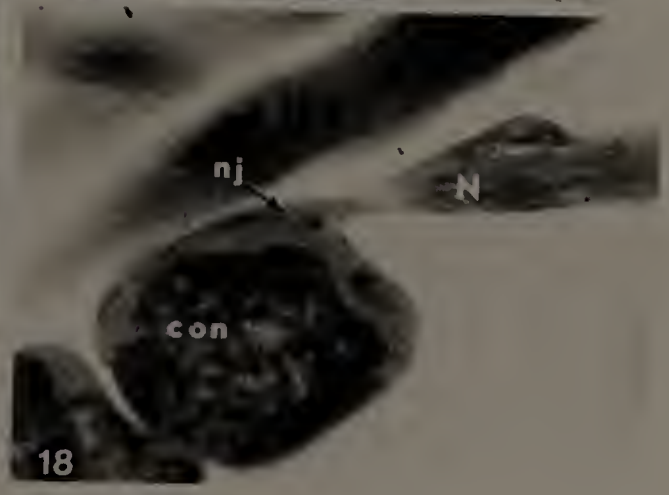
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Fig. 19. Cross section through oesophagus at level of brain (500x). Ao, aorta, CH, cerebral hemisphere; e, oesophageal epitheliar cell; i, chitinous intima; m, layer of circular muscle; O, oesophagus; PI, pars intercerebralis; RG, ring gland.

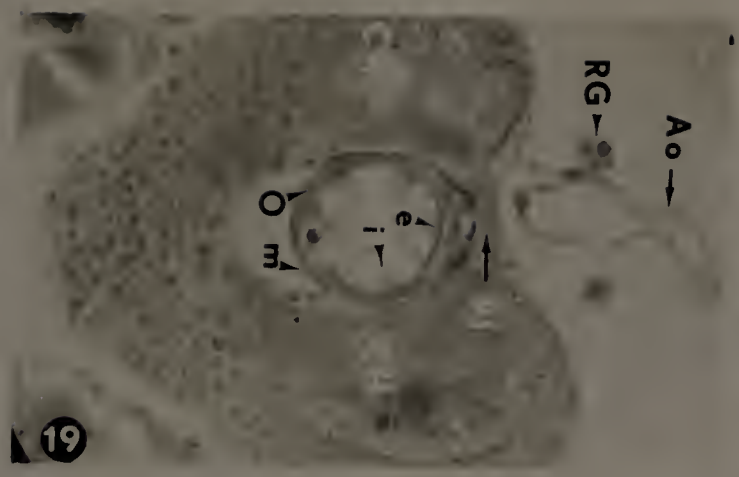
Fig. 20. Frontal section through proventriculus and chyle stomach (125x). e, oesophageal epithelium; en, large columnar cells of proventriculus; ec, epithelium of chyle stomach; fc, flattened cells covering proventriculus; gc, gastric caecum; P, proventriculus.

Fig. 21. Diagram of alimentary canal of apple maggot (from Dean, 1932). G.COE, gastric cecum; H.INT, hind-intestine; M.INT, mid-intestine; M.TUB, Malpighian tubule; OES, oesophagus; PH., pharynx; P.VENT, proventriculus; REC., rectum; S.GL., salivary gland.

Fig. 22. Tangential section through midgut showing lattice of small muscle fibers (500x). m, muscle fiber.

Fig. 23. Frontal section through mid, midgut (125x). ec, epithelial cells; PM, perithrophic membrane.

Fig. 24. Saggital section through hind, midgut (500x). ec, epithelial cells; PM, peritrophic membrane; FB, fat body.



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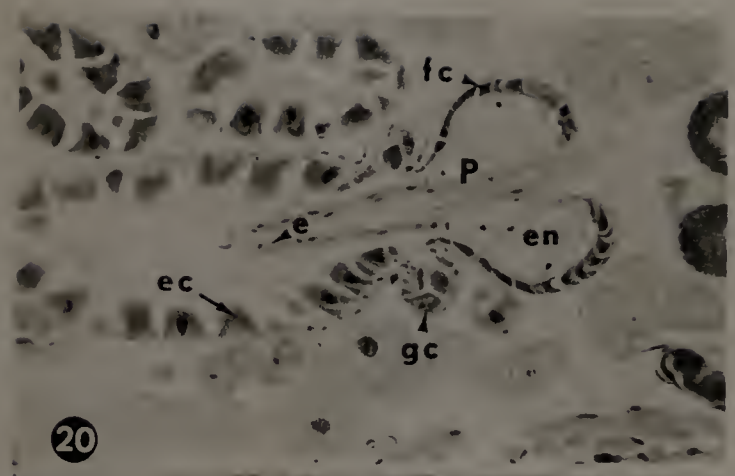

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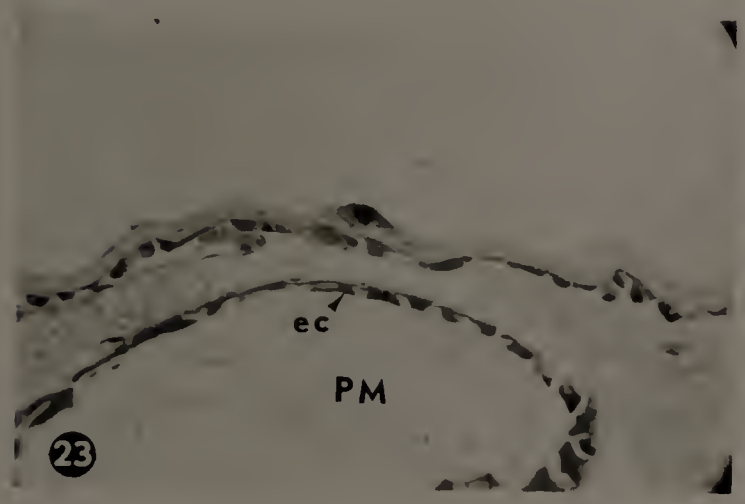

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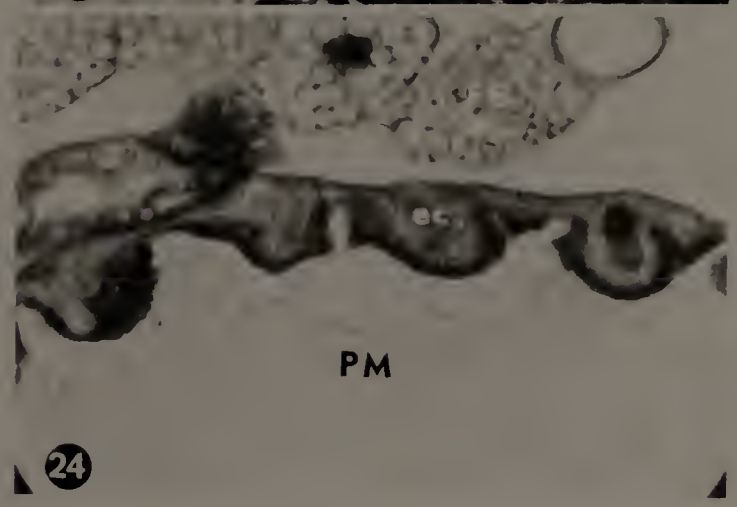

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Fig. 25. Saggital section through junction of midgut and hindgut (125x). amp, ampulla; ce, columnar epithelium of hindgut; lc, light staining cells at junction of midgut and hindgut.

Fig. 26. Saggital section through ventral ganglion (125x). dn, dorsal nerve; G, fused hypocerebral and oesophageal ganglia; O, oesophagus; PI, pars intercerebralis; Pv, proventriculus; RG, ring gland; VG, ventral ganglion; vn, ventral nerve.

Fig. 27. Saggital section through brain (125x). CH, cerebral hemisphere; Np, neuropile; Pk, perikaryal layer; VG, ventral ganglion.

Fig. 28. Frontal section through cerebral hemisphere (500x). NL, neurilemma; Np, neuropile; Pe, perineurium; Pk, perikaryal layer; OR, optic rudiment.

Fig. 29. Drawing of brain and ganglion of typical cyclorrhaphan larva showing origins of nerves (modified from Hertweck, 1931). AS, optic stalk; AN, MN, PN, cephalic nerves; th, thoracic nerves; a, abdominal nerves.

Fig. 30. Nerves of apple maggot (500x phase contrast). LN, lateral nerve; UN, median unpaired nerve.


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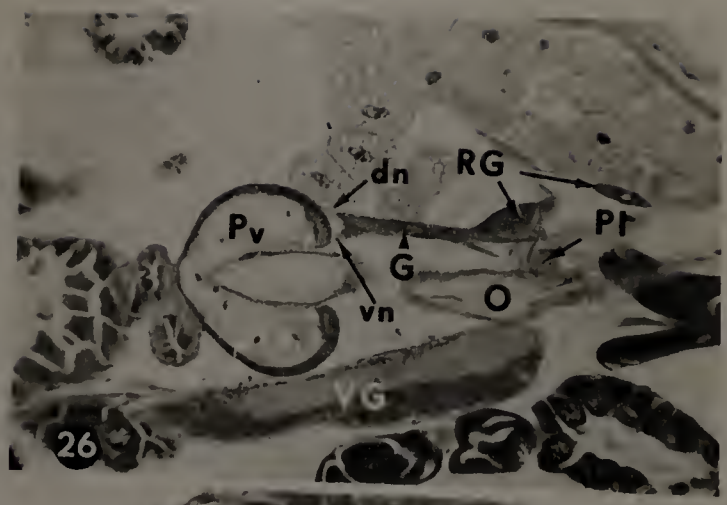
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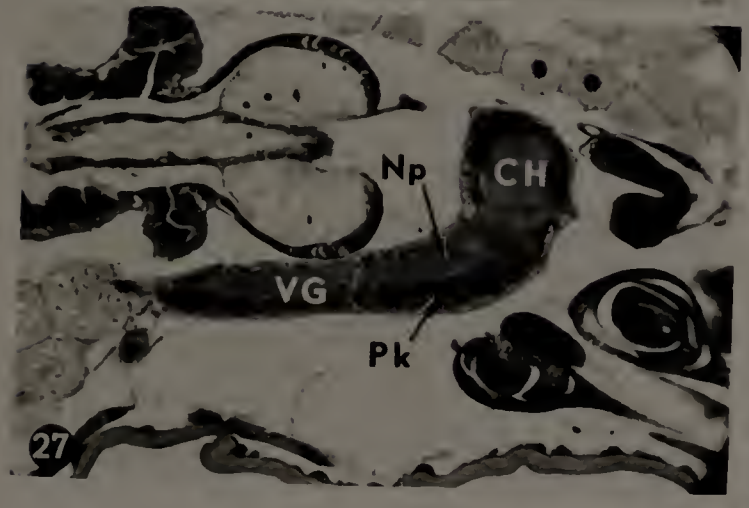
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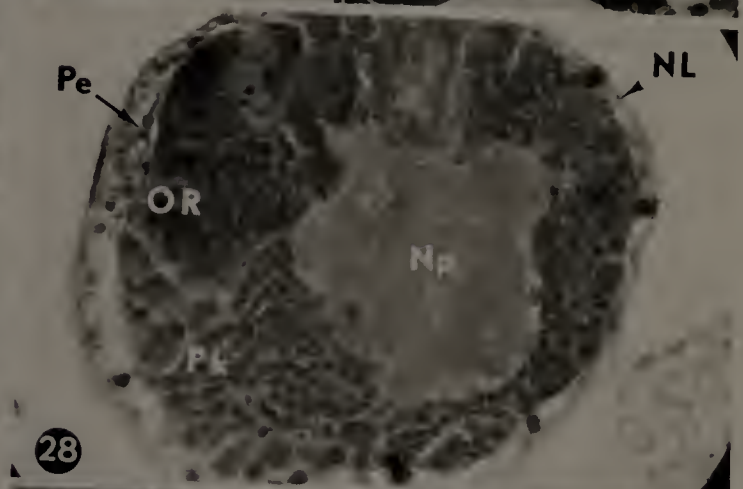
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Fig. 31. Saggital section through fused hypocerebral and proventricular ganglia (500x). G, ganglia; ncc, nervi corpora cardiacum; PI, pars intercerebralis; RG, ring gland; RN, recurrent nerve.

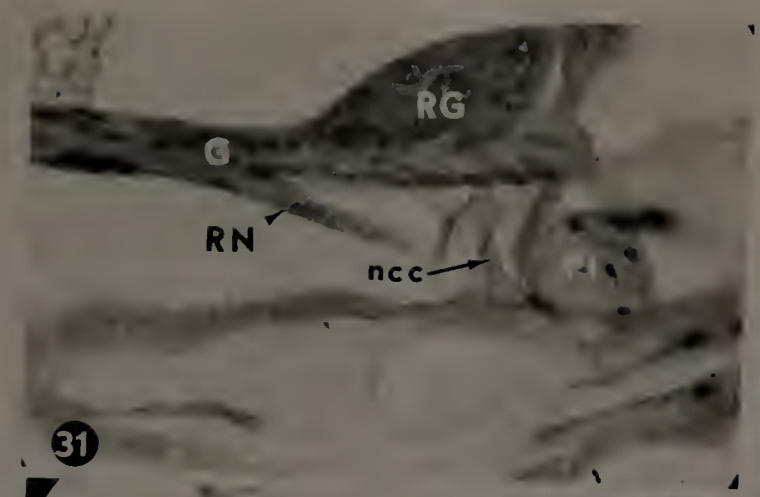
Fig. 32. Saggital section through anterior sense organs (500x). bs, basal sphere; cf, cytoplasmic filaments; cv, central vacuole; DO, dorsal organ; ed, external dome; mp, median portion of organ; n, nucleus of basal sphere cell; VO, ventral organ.

Fig. 33. Saggital section through ventral sense organ (500x). cf, cytoplasmic filament; DO, dorsal organ; ed, external dome; VO, ventral organ.

Fig. 34. Saggital section through sense organ at edge of buccal cavity (500x). cf, cytoplasmic filament; ic, internal cylinder of cells; t, small tooth at edge of buccal cavity.

Fig. 35. Saggital section through pharyngeal sense organ (500x). f_1, anterior cytoplasmic filament; f_2, posterior cytoplasmic filament; es, epipharyngeal sclerite; PSO, pharyngeal sense organ; B, bar connecting hypopharyngeal sclerites.

Fig. 36. Frontal section through cuticular sense organ (125x). cf, cytoplasmic filament.



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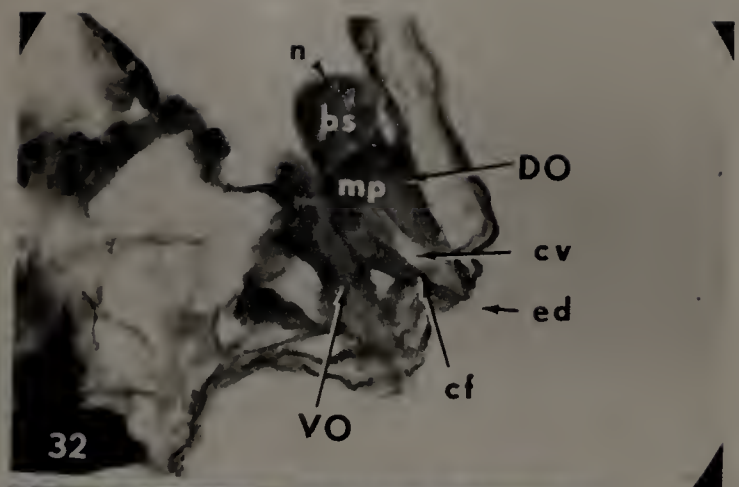

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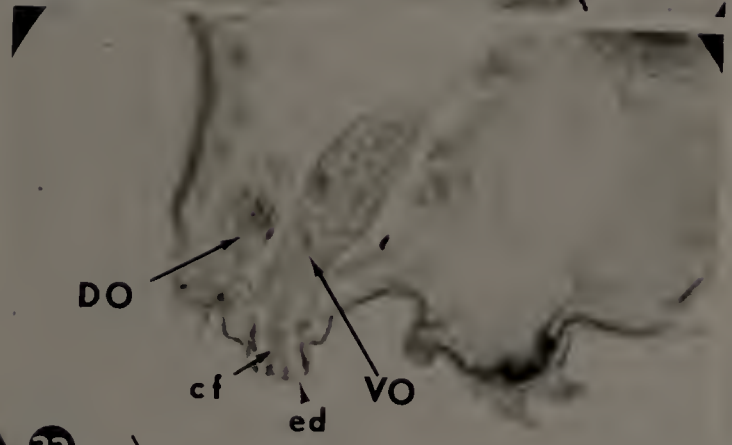

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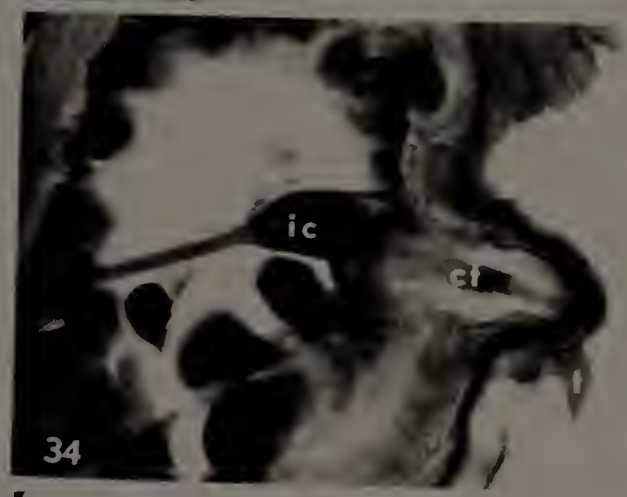

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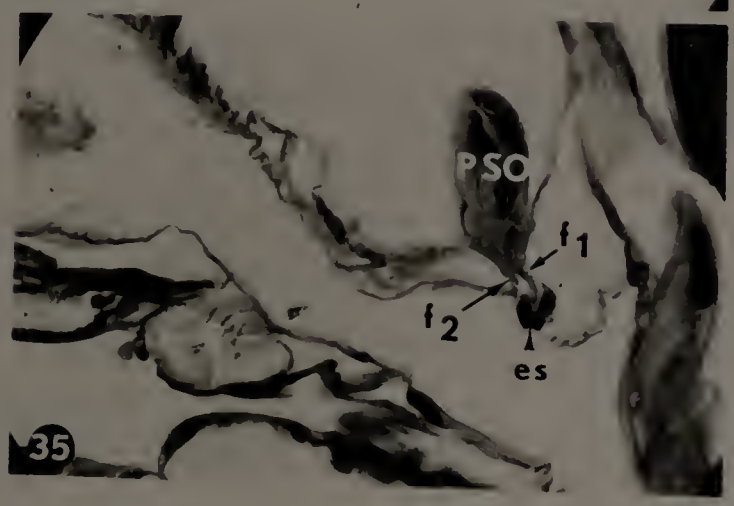

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Fig. 37. Cross section through imaginal disc of prothoracic leg of 13 day old larva (125x). i, imaginal leg disc; VG, ventral ganglion; lu, lumen of disc.

Fig. 38. Saggital section through frontal sac of 20 day old larva (125x). A, antennal disc; E, eye disc; fs, frontal sac; ON, optic nerve; CH, cerebral hemisphere; SG, salivary gland.

Fig. 39. Saggital section through imaginal eye disc (500x). E, eye disc; ON, optic nerve.

Fig. 40. Cross section through frontal sac of 13 day old larva at level of antennal disc (125x). Ao, aorta; fs, frontal sac; O, oesophagus.

Fig. 41. Frontal section through thoracic segments of 20 day old larva at level of frontal sac (125x). A, antennal disc; bc, blood cells; E, eye disc; H, haltere disc; LD_3, meta-thoracic leg disc; O, oesophagus; W, wing disc.

Fig. 42. Frontal section through antennal disc of 20 day old larva (500x). bc, blood cells; CP, central papilla; CR, concentric rings of histoblast tissue; N, nerve; n, nucleus of nerve.

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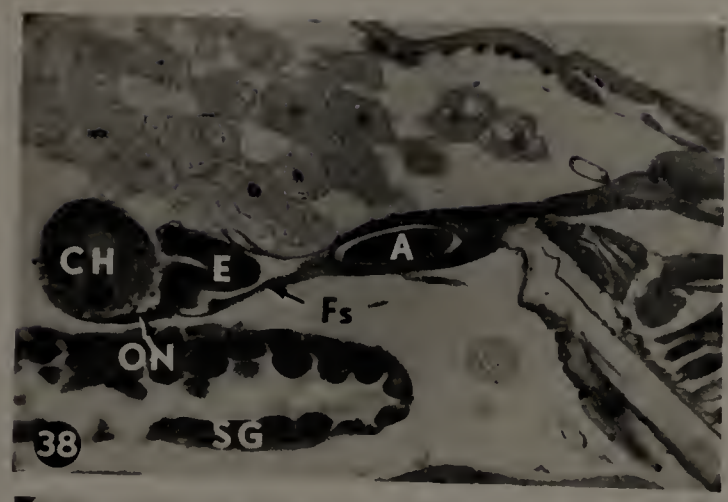

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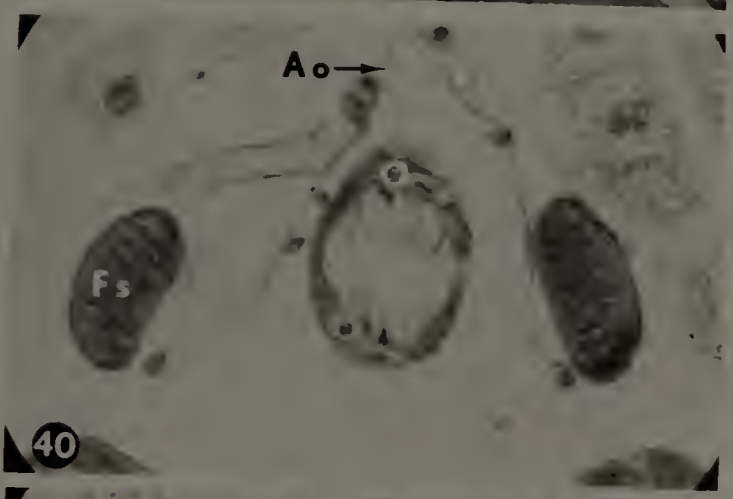

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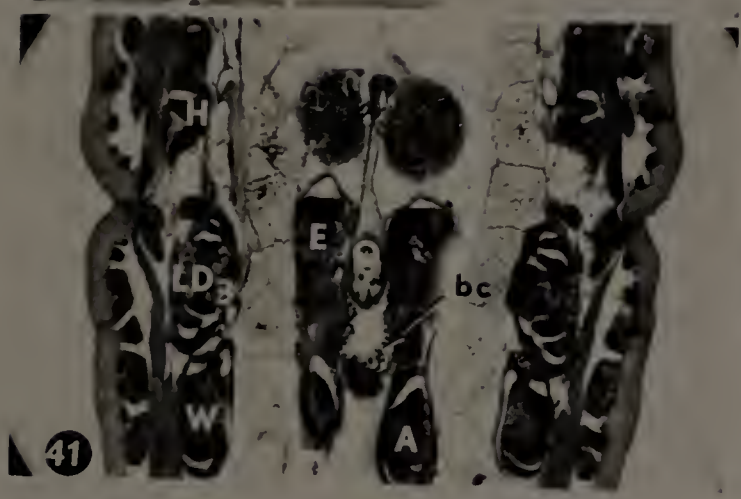

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Fig. 43. Frontal section through ventral portion of 20 day old larva showing labial discs (125x). ld, labial disc.

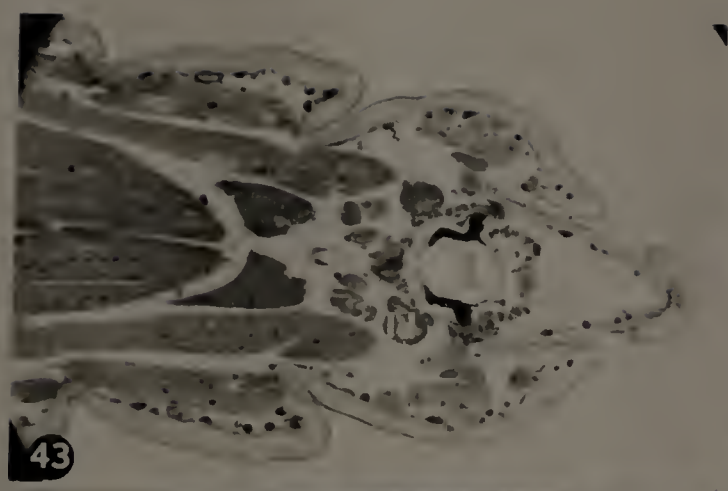
Fig. 44. Saggital section through pro- and mesothoracic leg discs of 20 day old larva (125x). LD, leg discs.

Fig. 45. Saggital section through prothoracic leg disc of 13 day old larva (500x). lu, lumen of disc.

Fig. 46. Frontal section through prothoracic disc of 20 day old larva (500x). ce, columnar epithelium of peripodal sac; CR, concentric ring of histoblast tissue; PS, peripodal sac; ts, tarsal segment.

Fig. 47. Frontal section through haltere disc of 13 day old larva (500x). H, haltere disc; s, stalk attaching disc to integument; tc, transverse connective.

Fig. 48. Frontal section through thoracic segments of 13 day old larva (125x). H, haltere disc; LD_3, metathoracic leg disc; tc, transverse connective; W, wing disc.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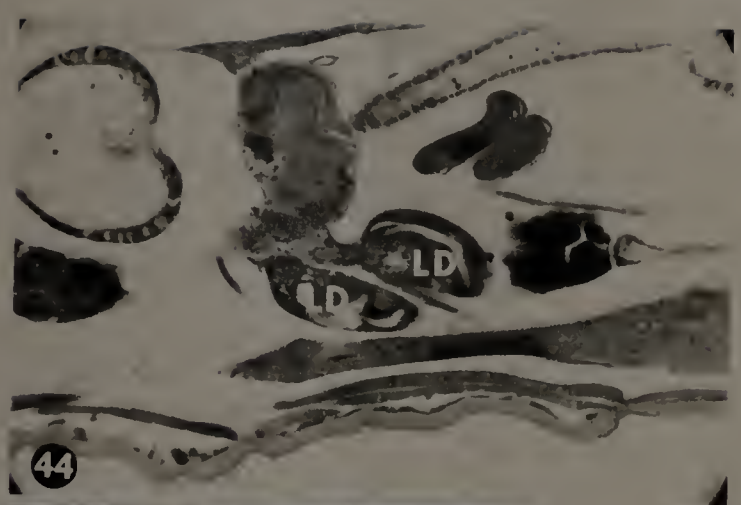
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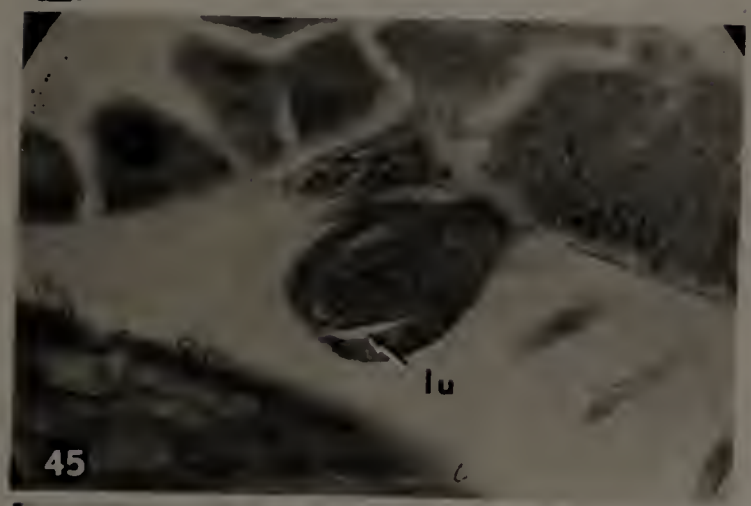
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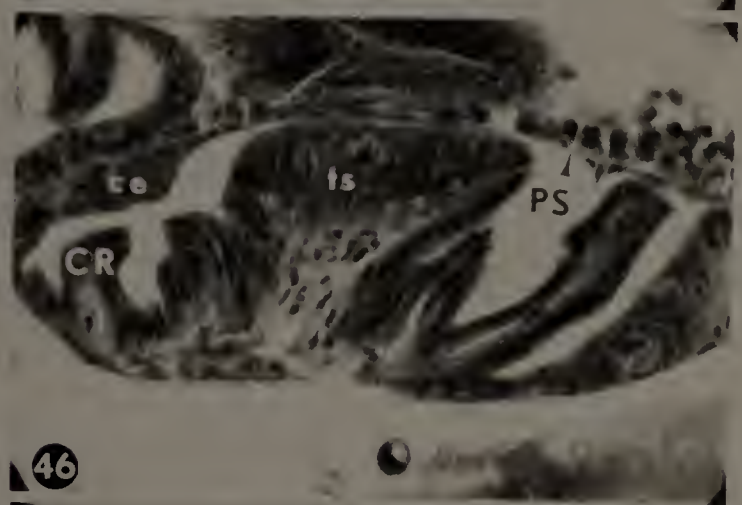
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Fig. 49. Frontal section through haltere disc of 20 day old larva (500x). ce, columnar epithelium; co, core of light staining cells; lu, lumen.

Fig. 50. Frontal section through wing disc of 20 day old larva (500x). ce, cuboidal epithelium of peripodial sac.

Fig. 51. Frontal section through anterior spiracles (125x). TD, tracheal disc.

Fig. 52. Saggital section through proventriculus of 20 day old larva (500x). ic, imaginal cells.

Fig. 53. Frontal section through anterior portion of salivary gland of 20 day old larva (500x). ic, imaginal cells.

Fig. 54. Cross section through genital disc of 20 day old larva (500x). gd, genital disc.

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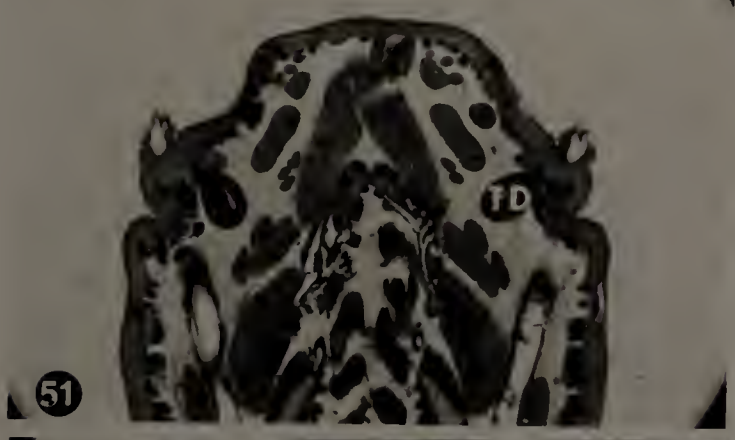

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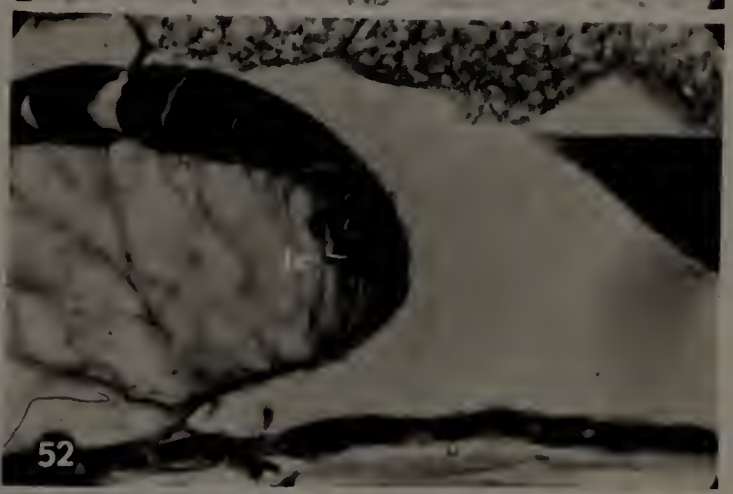
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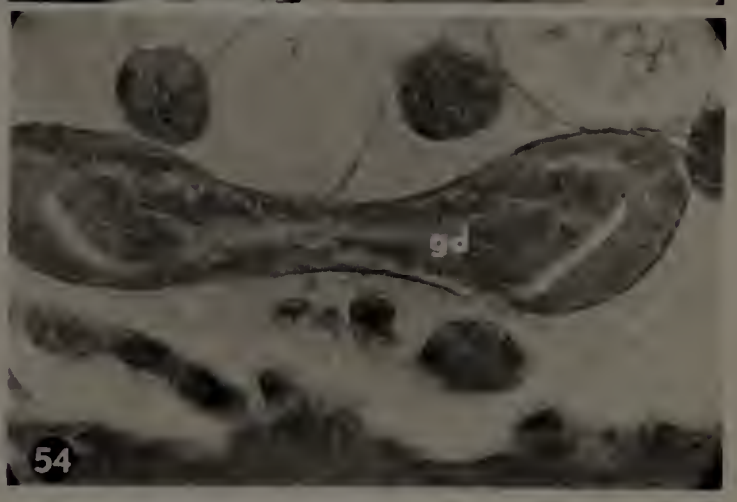

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Fig. 55. Frontal section through gonad of 20 day old larva (500x). FB, fat body; go, gonad; tc, transverse connective.

Fig. 56. Cross section through gonad of 20 day old larva (500x). FB, fat body; go, gonad; Dt, dorsal tracheal trunk.

Fig. 57. Frontal section tangential to heart showing transversely oriented muscle fibers (500x). m, muscle fibers.

Fig. 58. Frontal section tangential to heart (1250x). f, fibers connecting heart with alary muscles; n, nucleus of heart muscle.

Fig. 59. Cross section through heart (500x). n, nucleus.

Fig. 60. Frontal section through heart showing ostia (500x). os, ostia; t, unicellular thickening at end of valve; v, ostial valve.


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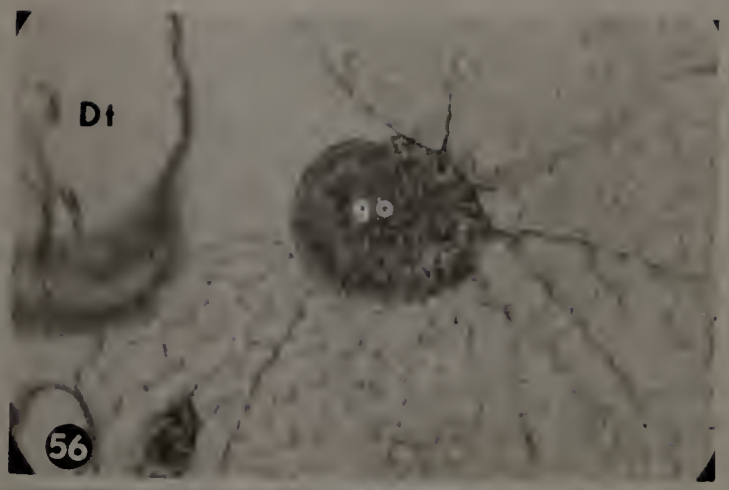
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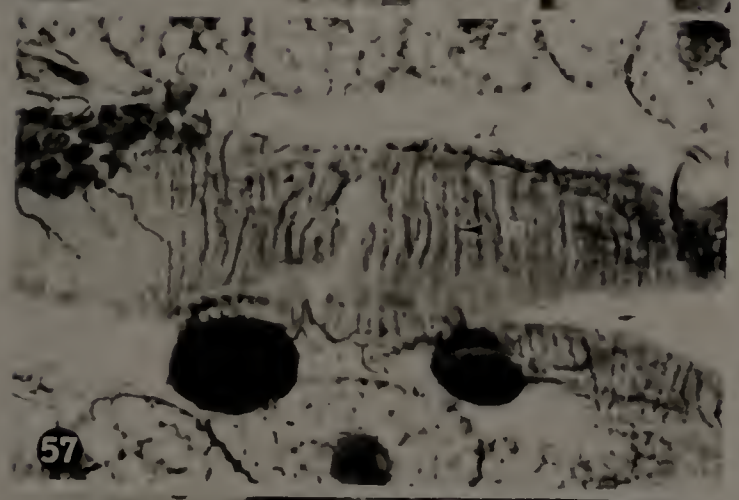
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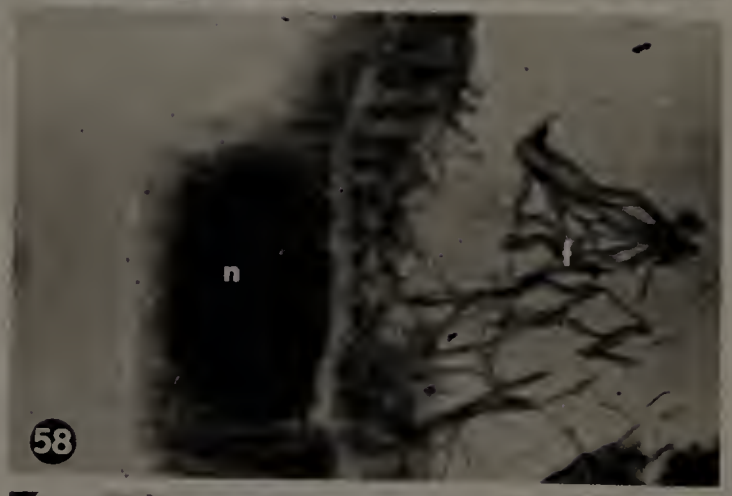
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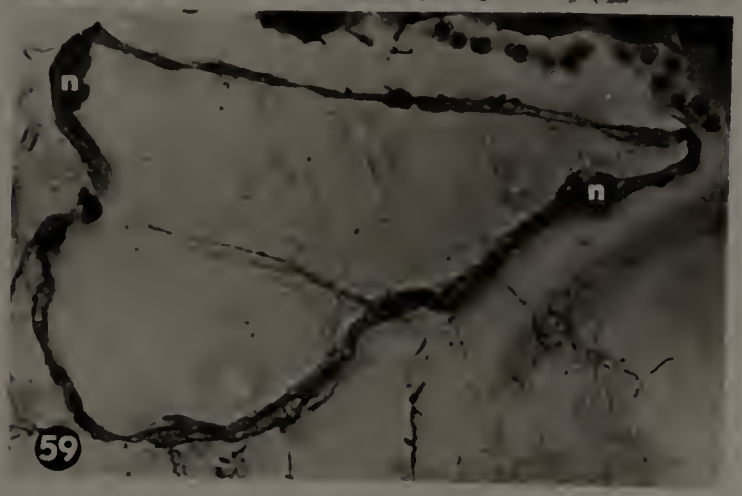
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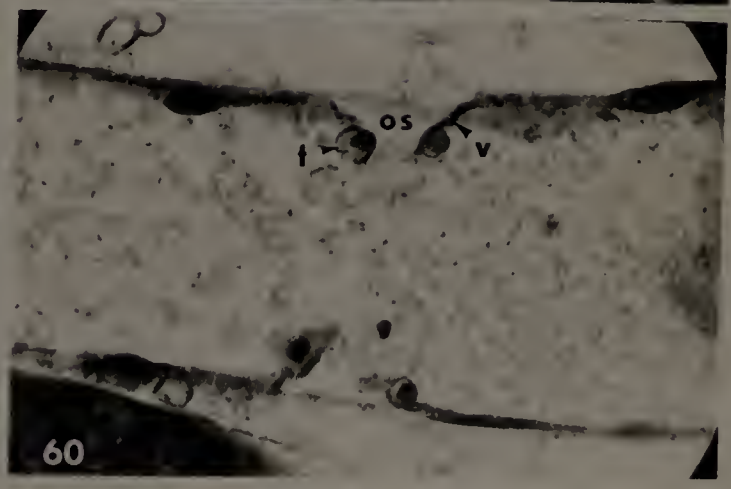
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Fig. 61. Cross section through heart showing pericardial cells (500x). PC, pericardial cells; PS, pericardial sinus.

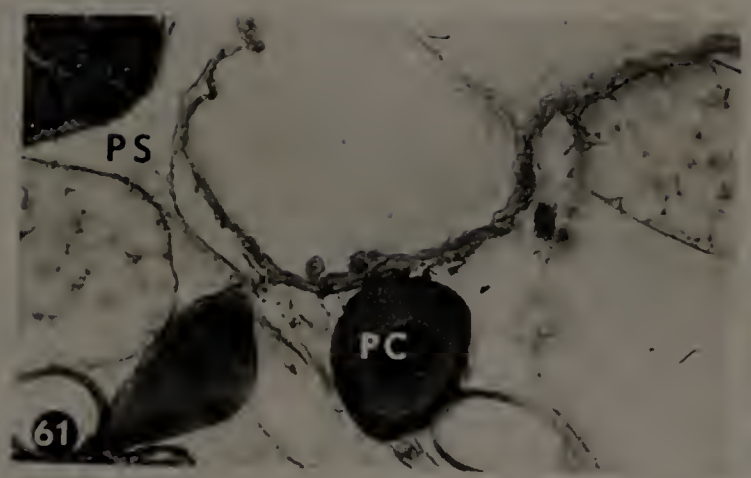
Fig. 62. Saggital section through anterior end of aorta (125x). Ao, aorta; O, oesophagus; Ph pharynx; RG, ring gland; SS, suprapharyngeal sinus.

Fig. 63. Frontal section through nephrocytes flanking aorta (500x). Ao, aorta; ne, nephrocytes.

Fig. 64. Frontal section through salivary gland showing nephrocytes (500x). ne, nephrocytes; SG, salivary gland.

Fig. 65. Cross section through blood cell aggregation in last abdominal segment (1250x). oe, oenocytoid; pr, prohemocyte.

Fig. 66. Saggital section through ring gland (125x). Ao, aorta; O, oesophagus; RG, ring gland.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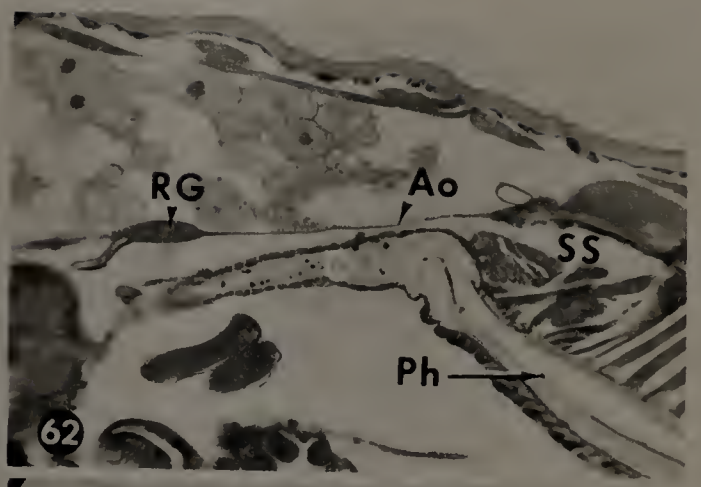
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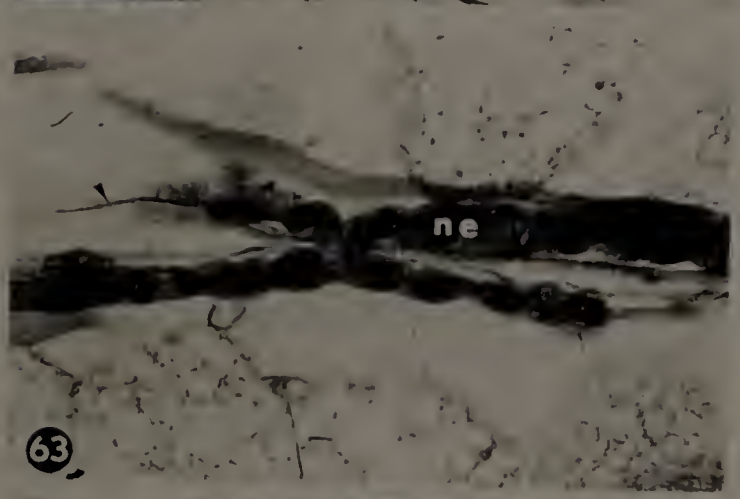
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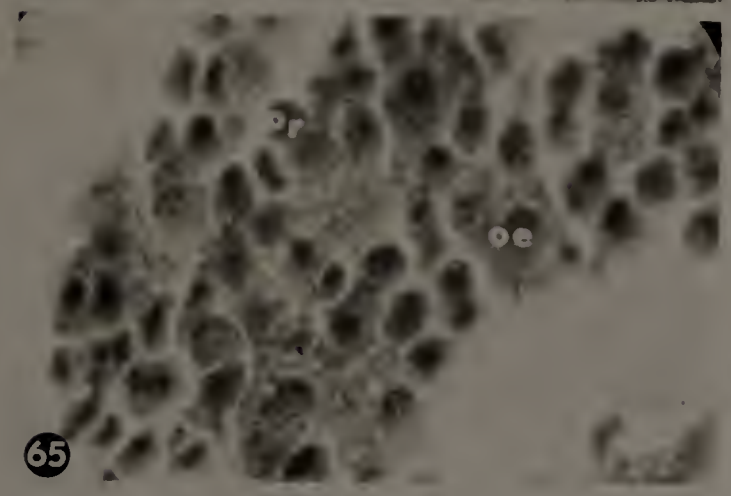
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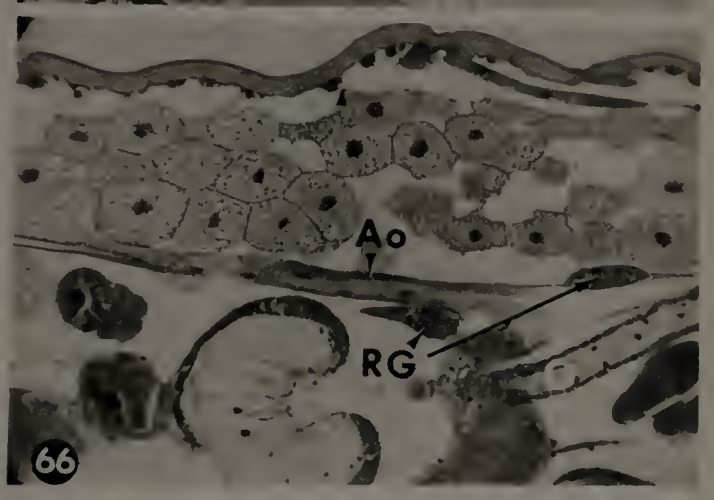
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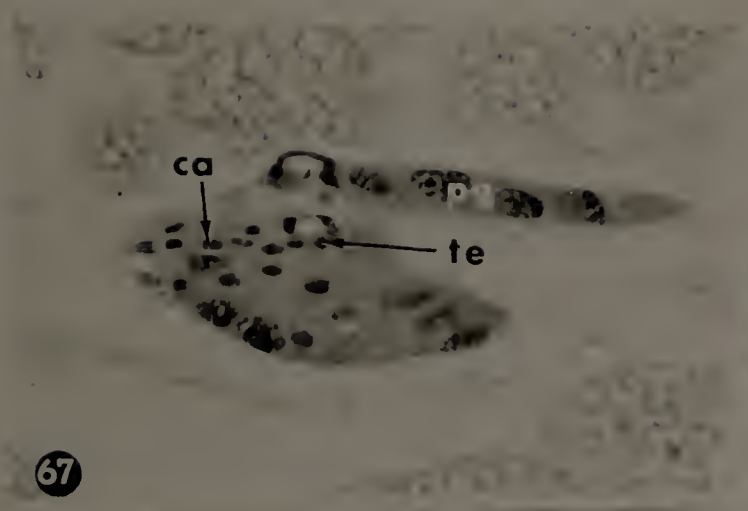
Fig. 67. Frontal section through dorsal portion of ring gland (500x). ca, corpus allatum; pg, prothoracic gland; te, tracheal epitheliar cell.

Fig. 68. Section through Malpighian tubule (1250x). lu, lumen; n, nucleus; v, vacuolated area.

Fig. 69. Section through common duct of Malpighian tubule (500x). bb, brush border of epithelial cell; ec, epithelial cell of common duct; n, nucleus.

Fig. 70. Section through ampulla (500x). ce, columnar epithelium of hindgut; f, cytoplasmic filaments of ampullar cells.



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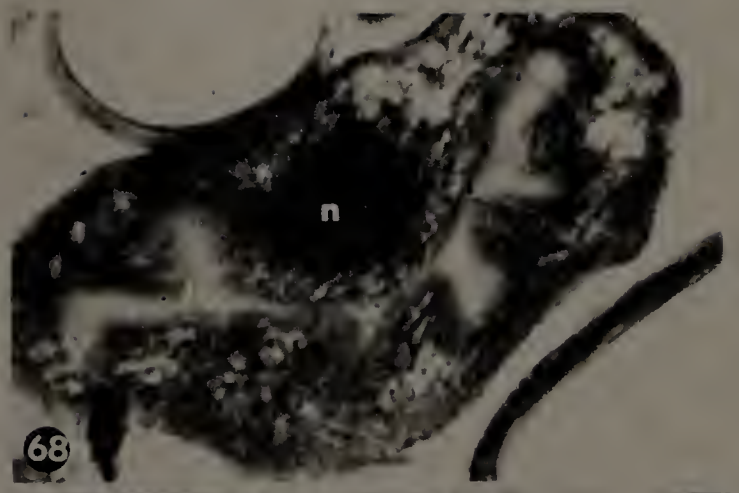

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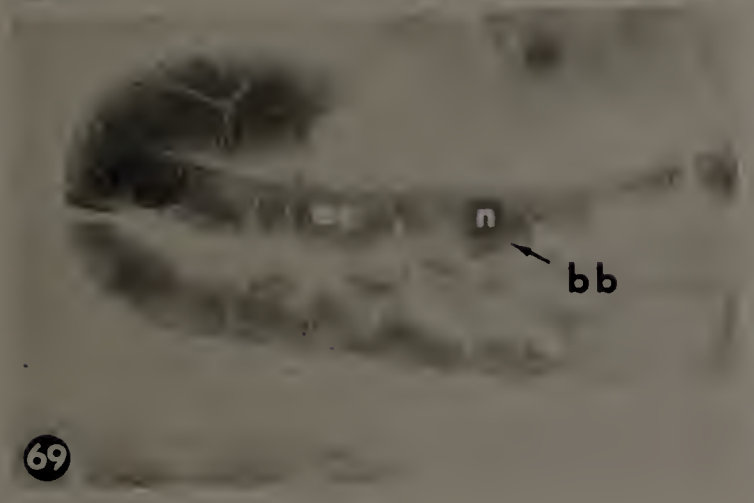

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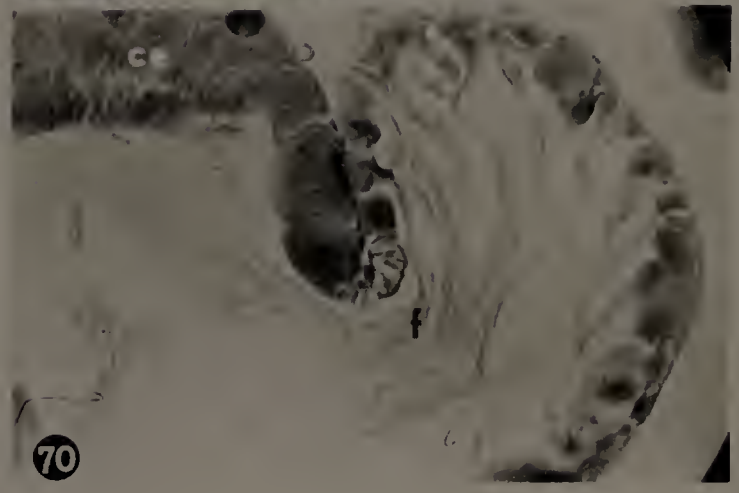

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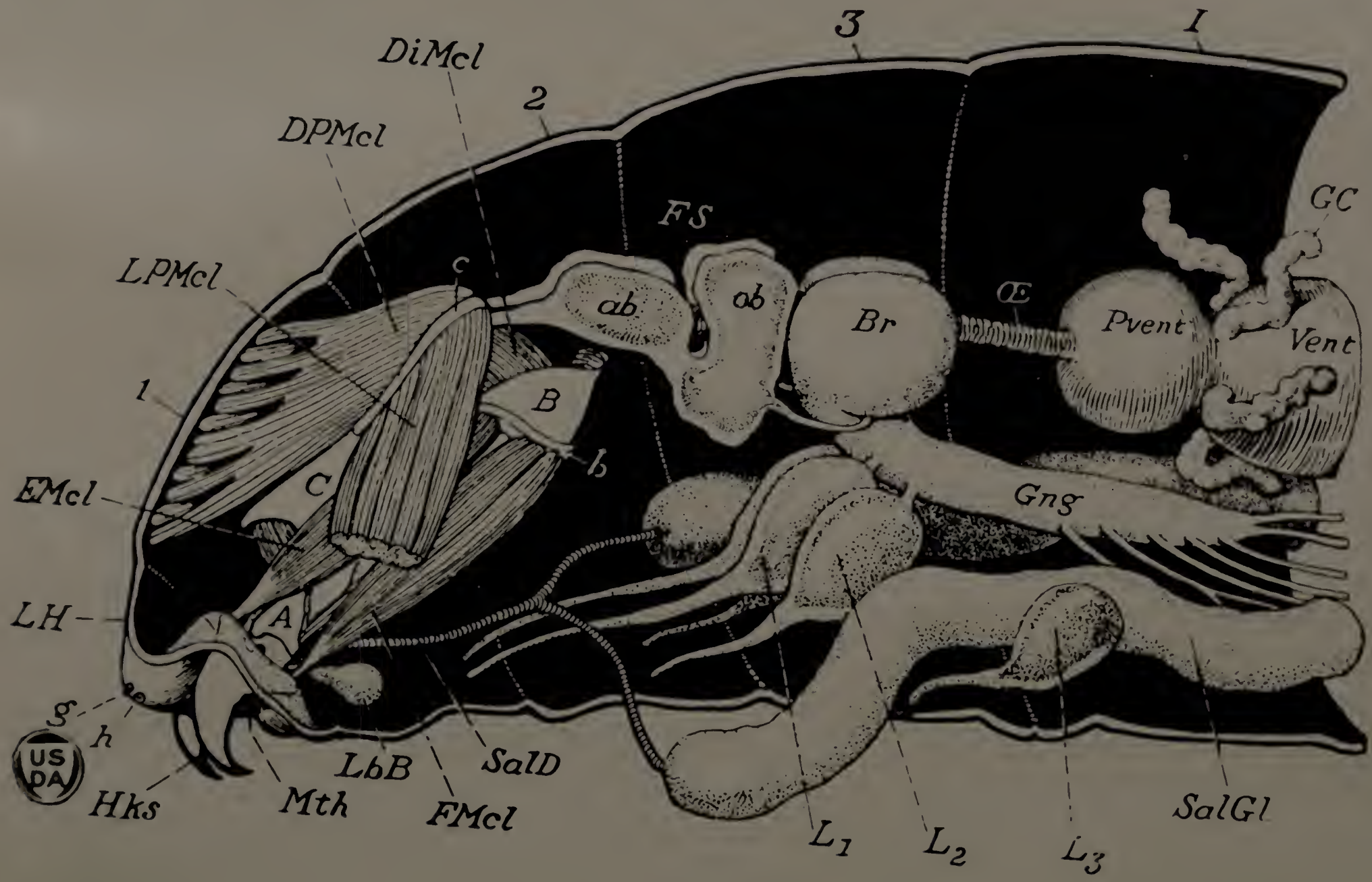
Fig. 71. Diagram lateral view of dissection of head and first four segments of mature apple maggot (from Snodgrass, 1924). 1, prothorax; 2, mesothorax; 3, metathorax; I, first abdominal segment; A. hypopharyngeal sclerite; ab, antennal imaginal disc; B, ventral plate of cephalopharyngeal skeleton; b, phragma on plate B from which originate muscles of mouth hooks; Br. brain; C, dorsal plate of cephalo-pharyngeal skeleton; c, phragma on plate C on which insert the pharyngeal extensor muscles; DiMcl, cibarial dilator muscles; DPMcl, dorsal extensor muscle of pharynx; EMcl, extensor muscle of mouth hook, FMcl, flexor muscle of mouth hook; FS, frontal sacs; GC, gastric caecum; Gng, ventral ganglion; g, dorsal sense organ; h, ventral sense organ; Hks, mouth hooks; L_1, L_2, L_3, imaginal discs of prothoracic, mesothoracic, and metathoracic legs, respectively; LbB, labial imaginal disc; LH, larval head; LPMcl, lateral extensor muscles of pharynx; Mth, larval mouth; ob, imaginal eye disc; OE, oesophagus; Pvent, proventriculus; SalD, salivary duct; SalGl, salivary gland; vent, ventriculus (midgut).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