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A TRANSMISSION ELECTRON MICROSCOPIC STUDY OF THE PHARYNGEAL ARMATURE IN THE LARVAL *DROSOPHILA MELANOGASTER*

Mohinder S. Jarial: Muncie Center for Medical Education, Ball State University, Muncie, Indiana 47306

ABSTRACT. The ultrastructure of pharyngeal armature was investigated in the larval *Drosophila melanogaster,* which principally feeds on yeasts and bacteria. The pharyngeal epithelial cells have large nuclei with prominent nucleoli. Their cytoplasm is rich in ribosomes, vesicles, vacuoles containing electron-dense material and large mitochondria; but the rough endoplasmic reticulum and Golgi complexes are sparse. These cells apparently secrete the pharyngeal cuticular intima, consisting of three layers: the lamellate endocuticle, the exocuticle with trabeculae-bound spaces and the thin epicuticle lining the luminal surface. The dorsal cuticular intima of the pharynx displays blunt teeth. The ventral cuticular intima is folded to form nine vertically-oriented pharyngeal ribs with bifurcated tips. Each bifurcation is further divided into four to five sharp, intertwining, finger-like processes with food particles trapped between them. It is suggested that the blunt cuticular teeth and sharp finger-like processes of the ribs of pharyngeal armature of *Drosophila* larvae are most suited for breaking down the rigid cell walls of food particles such as yeasts and bacteria, thus releasing their cellular contents for digestion and absorption in the midgut.

Keywords: Epithelial cells, mitochondria, ribosomes, cuticular intima, teeth, ribs, food particles

In the majority of insects, the alimentary canal comprises three regions: the foregut, midgut and hindgut. The foregut and hindgut are ectodermal in origin, while the midgut originates from endoderm. The foregut is lined by the cuticular intima and is mainly concerned with the passage, storage and fragmentation of food material before it reaches the midgut (Snodgrass 1935; Chapman 1985; Lehane 1998).

In the larvae of *Drosophila melanogaster*, the foregut consists of the buccal cavity (atrium), pharynx, esophagus and proventriculus. The pharynx is the dilated portion of the foregut situated between the oral cavity and esophagus. In addition to the typical muscular coat of the foregut, the pharynx has dilator muscles that aid in conveying food from the buccal cavity to the esophagus (Chapman 1985; Skaer 1993).

The cuticular intima of the foregut in insects displays teeth, spines, piercing stylets and complex triturating devices which apparently breakdown and filter food material (Snodgrass 1935; Parsons 1972; McGreevy et al. 1978; Coluzzi et al. 1982; Kapoor 1997; Lehane 1998). In *Drosophila* larvae the cephalopharyngeal armature is a complex structure consisting of a pair of hooks which posteriorly articulate with the so-called H-piece (Jurgens et al. 1986). Located behind the H-piece is a pair of large vertically-placed cephalopharyngeal plates with dorsal and ventral arms which support the buccal cavity and pharynx. In addition, the floor of the pharynx displays a series of cuticular bars or ribs (Strasburger 1932; Bodenstein 1965; Jurgens et al. 1986).

The studies of Wheeler (1947; 1950) demonstrated that radioactive iodine (I^{131}) was concentrated in the buccopharyngeal cells and armature of *Drosophila gibberosa* larva but no concentration of I^{131} appeared in its endocrine structures. These findings clearly indicated that the *Drosophila* larva was able to take up iodine in the food and metabolically incorporate it in the protein component of the cuticular intima of the pharynx. It seems probable that members of large phyla of invertebrates, with the exception of protozoans and echinoderms, synthesize iodoproteins to some extent, supporting the theory that perhaps the pharynx in an invertebrate might be specialized and become associated with thyroid-like iodine transporting structure found in the vertebrates (Gorbman et al. 1983).

The aim of the present study was to examine the fine structure of the posterior pharyngeal armature (skeleton) in full-grown larva of *D. melanogaster* to elucidate its role in the breakdown of food material.

METHODS

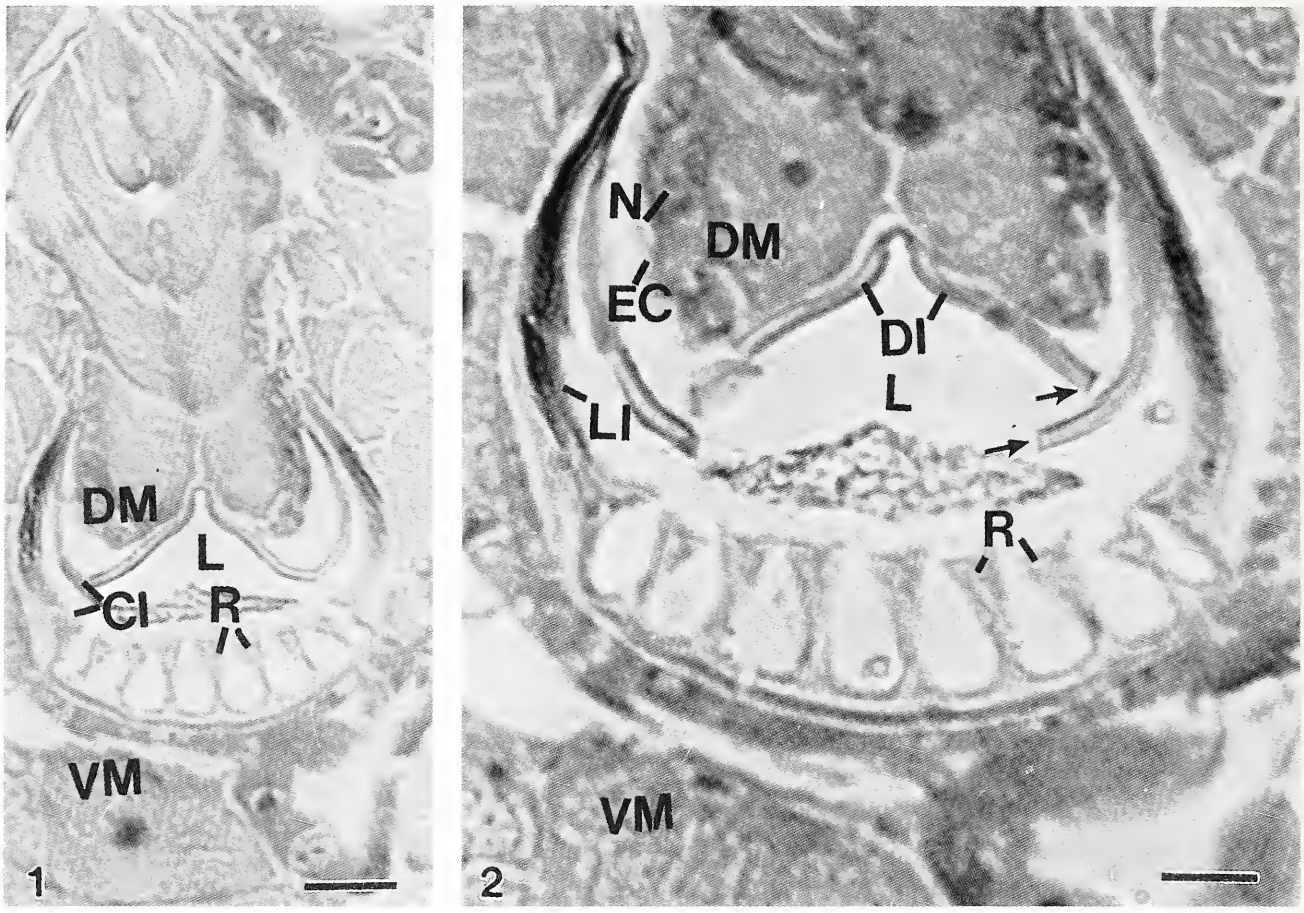
Cultures of wild-type *D. melanogaster* (Oregon R-P2) were maintained on instant *Drosophila* medium (formula 4-24, Carolina Biological). Large "wandering" third-instar larvae were isolated from these cultures and used in this study. The larvae were dissected into insect Ringer's (Hoyle 1953), and the posterior pharynx was excised at the level of the anterior spiracles. The pharynges were immediately fixed by immersion at room temperature in a cocktail of 2.5% glutaraldehyde and 2% paraformaldehyde (1:1) in O.1 M cacodylate buffer at pH 7.4 (Millonig 1976) and subsequently post-fixed in 1% osmium tetroxide in the same buffer. The pharynges were dehydrated in an ethanol series to propylene oxide and embedded in LX112 (Ladd Industries) (Luft 1961). Polymerization was carried out overnight at 60° C. Ultrathin sections were cut on a Porter-Blum MT2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Hitachi H-600 transmission electron microscope. Some pharynges of larvae were fixed in Bouin's fluid and embedded in paraplast. Transverse (6 μm) sections were cut and stained with Harris hematoxylin/eosin and examined with an American Optical light microscope.

RESULTS

General morphology and histological observations.—The pharynx in a *D. melanogaster* larva is situated between the buccal cavity and the esophagus. In the absence of a crop, the esophagus communicates directly with the spherical proventriculus, which in turn opens into the midgut. In transverse sections cut at the level of the anterior spiracles, the epithelium of the pharynx consists of a single layer of flat cells with prominent nuclei. Their cell membranes are indistinguishable, hence the epithelium appears syncytial. The epithelium is invested by the visceral and dilator muscles. It is lined with a 2.5 μm thick dorsal cuticular intima, the middle portion of which is curved upwards with right and left halves connected by an elevated bridge. The dorsal cuticular intima becomes continuous with a much thicker (5 μm) lateral intima. In the pharyngeal floor, the cuticular intima is folded to form nine stout bars or ribs with bifurcated tips which extend dorsally into the pharyngeal lumen (Figs. 1, 2).

Transmission electron microscopic observations.—The flat epithelial cells measure about 1.5 μm in height and have large nuclei with prominent nucleoli (Figs. 3, 4). The cytoplasm contains numerous free ribosomes, large mitochondria, many vesicles and vacuoles containing electron-dense material; but the rough endoplasmic reticulum and Golgi complexes are sparse (Figs. 3–5, 7–9). The delicate lateral membranes of the epithelial cells are folded (Fig. 9). The apical plasma membrane facing the cuticular intima is thrown into narrow infoldings, thereby increasing the surface area (Fig. 10).

The dark cuticular intima of the dorsal pharyngeal wall, which at some locations is separated from the adjoining epithelium, measures about 2 μm in thickness and displays short, stout teeth especially in the middle region (Fig. 3). It consists of the lamellate endocuticle and epicuticle layers, which become more discernible in the lateral regions (Fig. 6). The cuticular intima of the lateral wall measures about 5 μm in thickness and displays at regular intervals small, circular empty spaces (Fig. 5). The ventral cuticular intima is composed of three layers. The lamellate endocuticle is the outermost layer and is directly apposed to the epithelium. The exocuticle, the middle layer, consists of trabeculae and irregular spaces. The thin epicuticle lines the lumen of the pharynx (Figs. 8, 9). The ventral intima is folded to form vertically-oriented pharyngeal ribs that are about 30 μm long and project into the lumen (Figs. 8, 11). The ribs are bifurcated at the tips and each bifurcation is further divided into 4–5 sharp intertwining finger-like processes that strikingly resemble the design of a claw hand (Figs. 11, 12, 14). Bacteria-like particles appear to be trapped between the sharp finger-like processes of the ribs (Fig. 11). Whole or fragmented bacteria-like particles, presumably food, are observed in the lumen of the pharynx in close proximity to the tips of the rib processes. Intact food particles are not seen in the lumen below the tips of the ribs (Figs. 11–14).



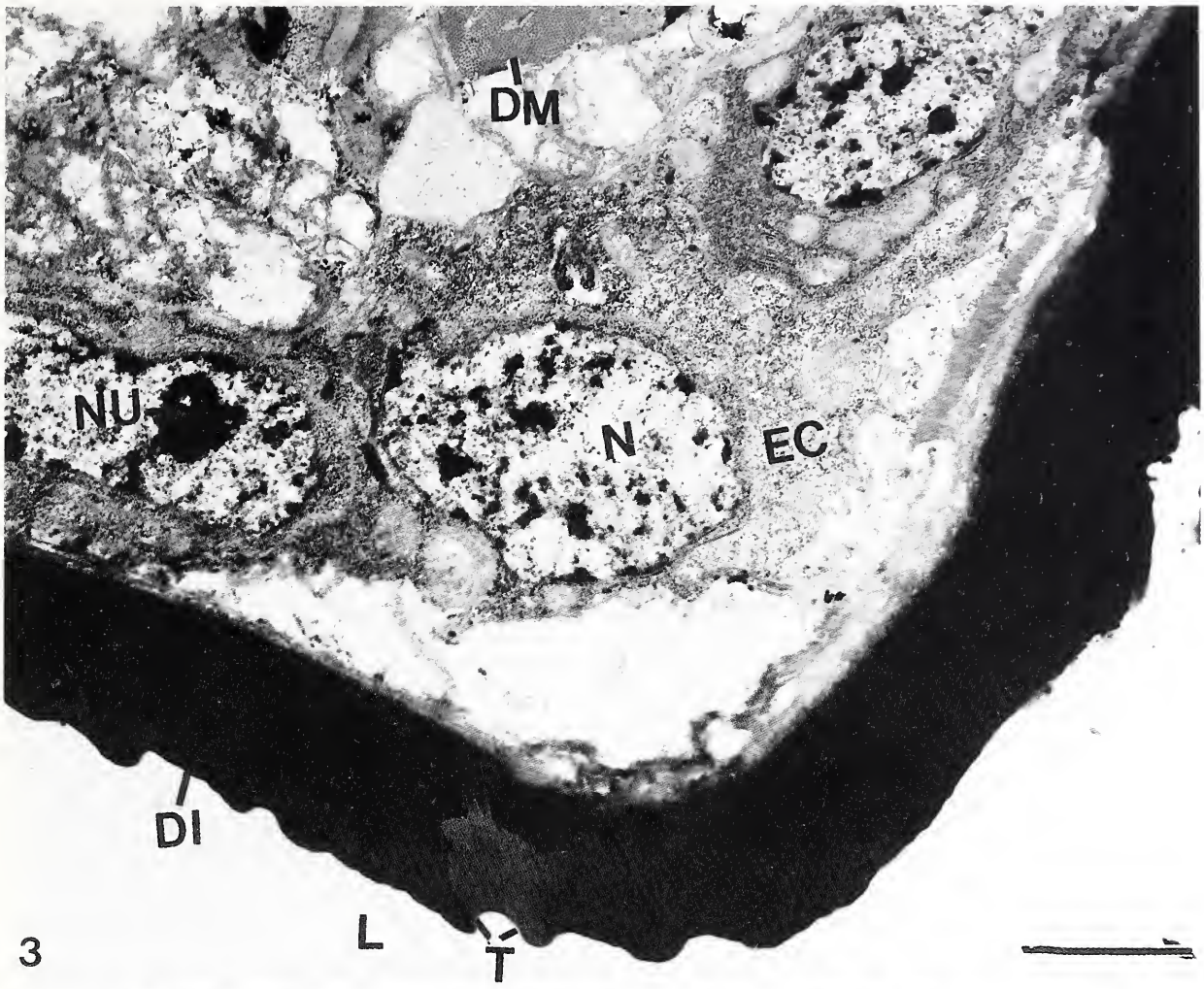
Figures 1, 2.—Light micrographs of the pharynx. 1. Transverse paraplast section (6 μm) at the anterior spiracles level, stained with hematoxylin/eosin; Scale bar = 25 μm; 2. Magnified image of a transverse section of pharynx showing torn dorsal cuticular intima (DI) (arrows). *Abbreviations:* CI, cuticular intima; DM, dilator muscle; EC, epithelial cells; L, lumen; LI, lateral cuticular intima; N, nucleus; R, ribs; VM, visceral muscle; Scale bar = 10 μm.

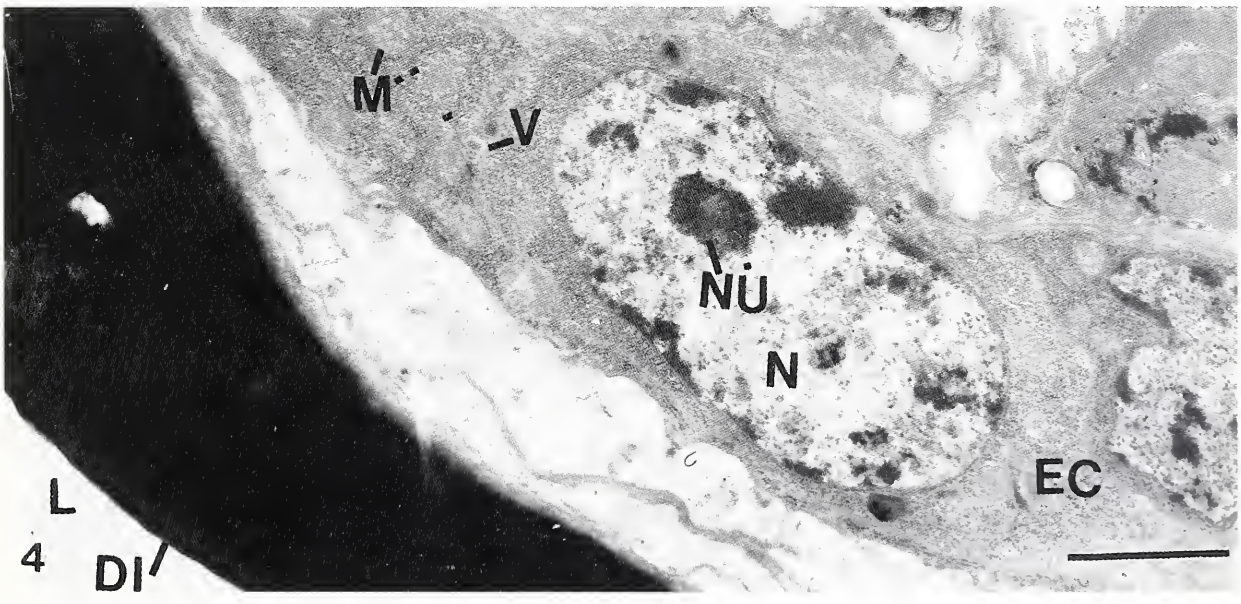
DISCUSSION

The epithelial cells of the *D. melanogaster* larval pharynx possess ultrastructural features that are characteristic of epithelia which specialize in protein synthesis (Palade 1975). The presence of abundant free ribosomes in the cytoplasm suggests their role in the synthesis of proteins that are segregated in the rough endoplasmic reticulum and packaged in vesicles of the Golgi complexes for export from the cells. The presence of apical membrane infoldings provides increased surface area for export of proteins to form the cuticular intima of the pharynx.

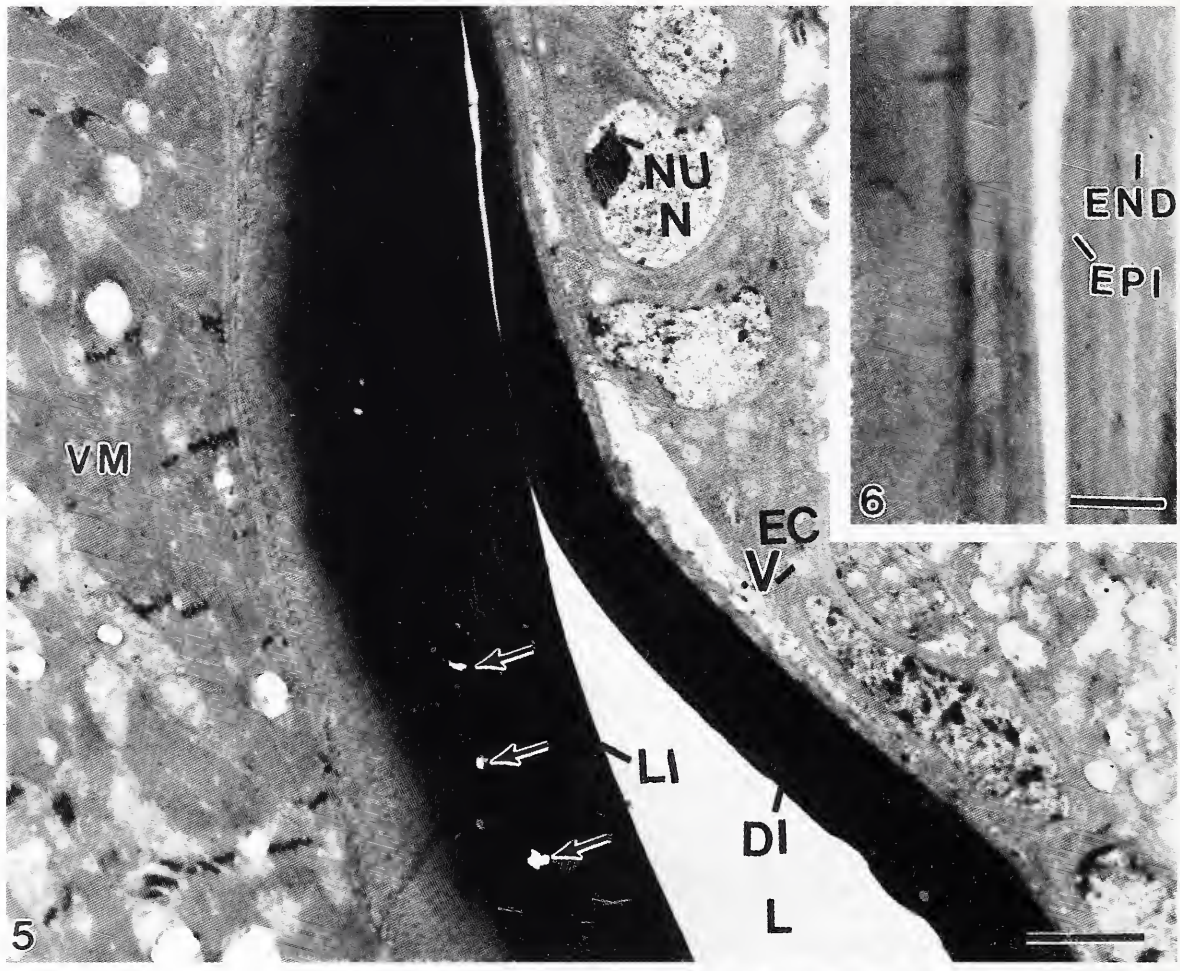
The results of the present study clearly support the hypothesis that the elaborate cuticular pharyngeal armature of the *D. melanogaster* larva, secreted by the epithelial cells, mechanically acts on food material to break it down for digestion and absorption in the midgut. Baumberger (1917) has shown that the larvae of *D. melanogaster* principally feed on yeast cells. There is also evidence that other microorganisms such as bacteria provide nutrition for these larvae (Baumberger 1917; Sturtevant 1921). The spherical yeasts are about 5 μm in diameter and bacteria are about 1–2 μm in diameter. The rigid cell wall of yeasts is composed of cross-linked polysaccharides (cellulose and chitosan), proteins and glycoproteins (Walker 1998). The structural features of the cuticular parts of the pharynx of *D. melanogaster* larvae revealed by this study suggest that the food particles like yeasts and bacteria become entrapped between the stout teeth of the dorsal cuticular intima and the sharp, intertwined finger-like processes at the tips of the vertically oriented pharyngeal ribs. The rigid cell walls of yeasts and bacteria are then torn apart by the sharp processes of the ribs, thereby releasing their proteinaceous cellular contents into the lumen of the pharynx.

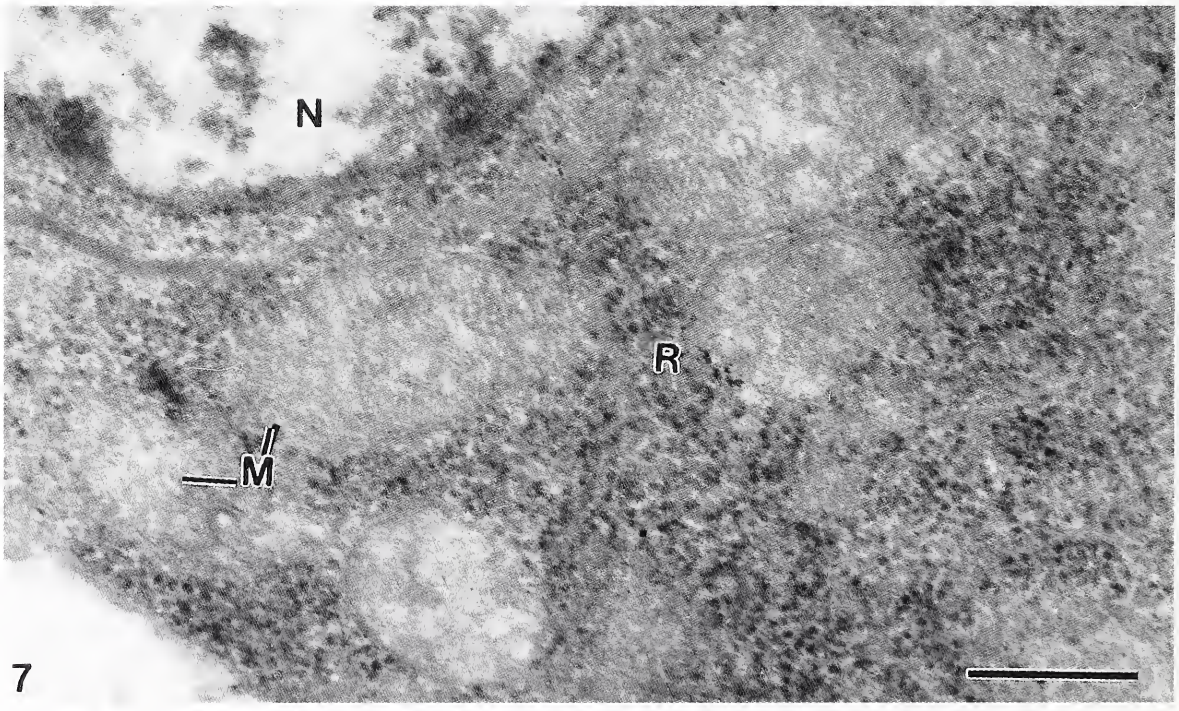
The cuticular armature of the foregut and its role in breakdown of food material and de-



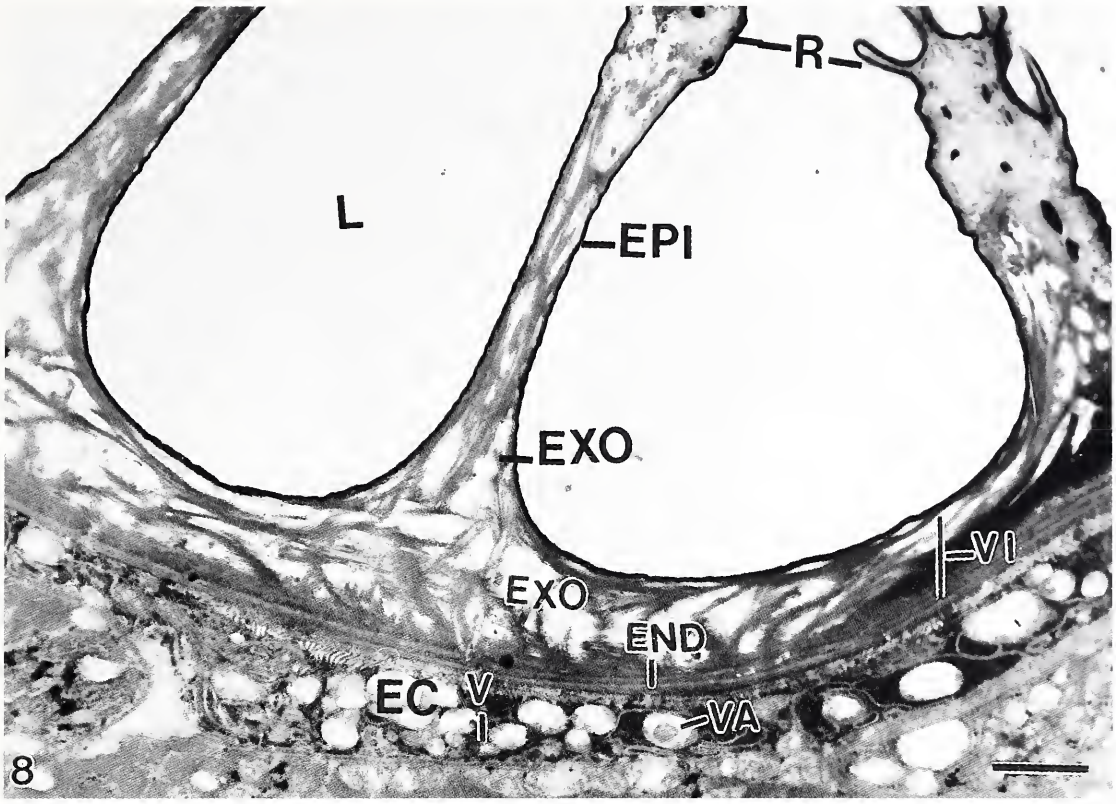


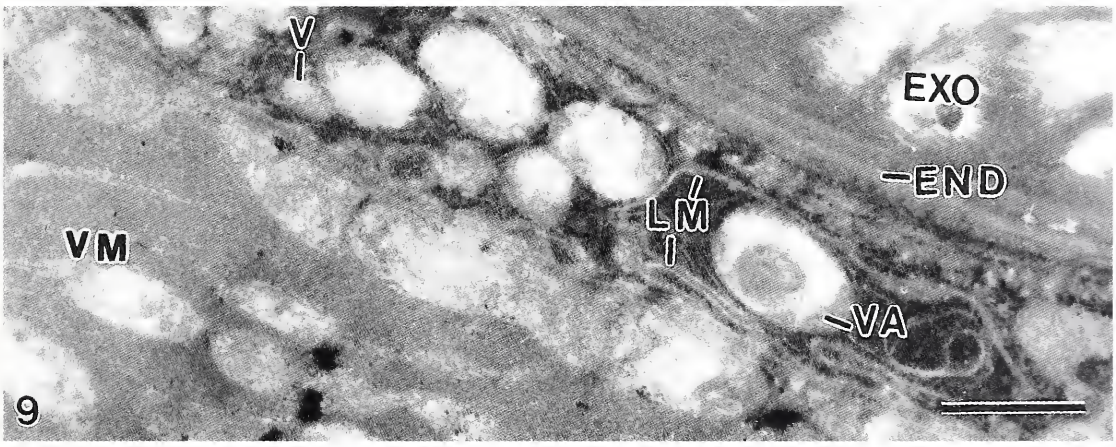
Figures 3, 4.—Transmission electron micrographs of the pharynx. 3. Transverse section of the dorsal wall showing epithelial cells (EC) with large nuclei (N) lined by thick dorsal cuticular intima (DI) displaying teeth (T), DM, dilator muscle; L, lumen; NU, nucleolus; Scale bar = 2 μm; 4. Portion of the pharyngeal wall lateral to that seen in Figure 3. Epithelial cells (EC) appear separated from the smooth dorsal cuticular intima (DI). *Abbreviations:* L, lumen; M, mitochondrion, N, Nucleus; NU, nucleolus, V, vesicle; Scale bar = 1 μm.

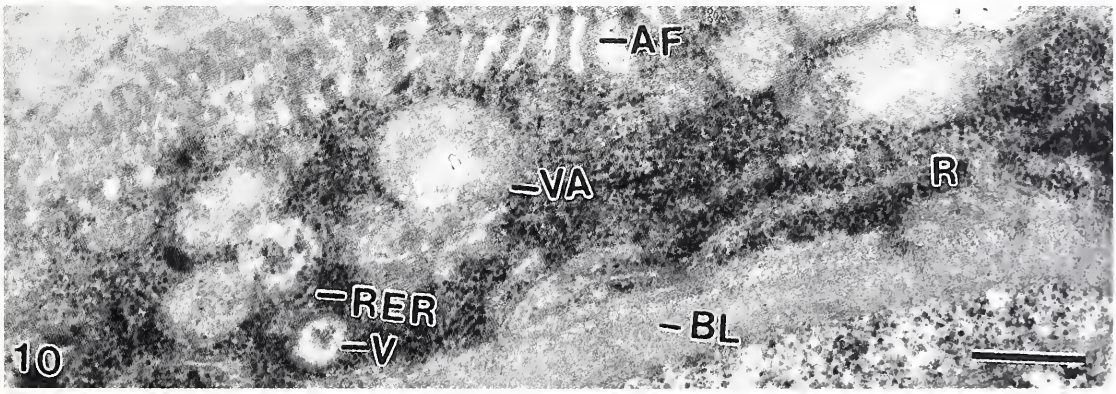




Figures 5–7.—5. Junction of dorsal and lateral walls. Note the difference in thickness of dorsal cuticular intima (DI) and lateral intima (LI) that has small empty spaces (arrows). EC, epithelial cells; L, lumen; N, nucleus; NU, nucleolus; V, vesicle; VM, visceral muscle; Scale bar = 0.25 μm; 6. Dorsal cuticular intima showing endocuticle (END) and epicuticle (EPI) layers; Scale bar = 0.5 μm; 7. Nuclear region of an epithelial cell showing a portion of the nucleus (N), free ribosomes (R) and large mitochondria (M); Scale bar = 0.25 μm.







Figures 8–10.—Electron micrographs of the ventral wall of the pharynx. 8. Transverse section showing ventral cuticular intima (VI) displaying endocuticle (END), exocuticle (EXO) and epicuticle (EPI), ribs (R) and epithelial cells (EC). L, lumen; V, vesicles; VA, vacuoles; Scale bar = 2 μm; 9. Magnified view of an epithelial cell displaying delicate lateral membranes (LM), vesicles (V) and vacuoles (VA) containing electron dense material. END, endocuticle; EXO, exocuticle; VM, visceral muscle; Scale bar = 0.25 μm; 10. A portion of epithelial cell showing apical membrane infoldings (AF), ribosomes (R), rough endoplasmic reticulum (RER), vesicle (V) and vacuole (VA) containing electron-dense material. BL, basal lamina; Scale bar = 0.5 μm.

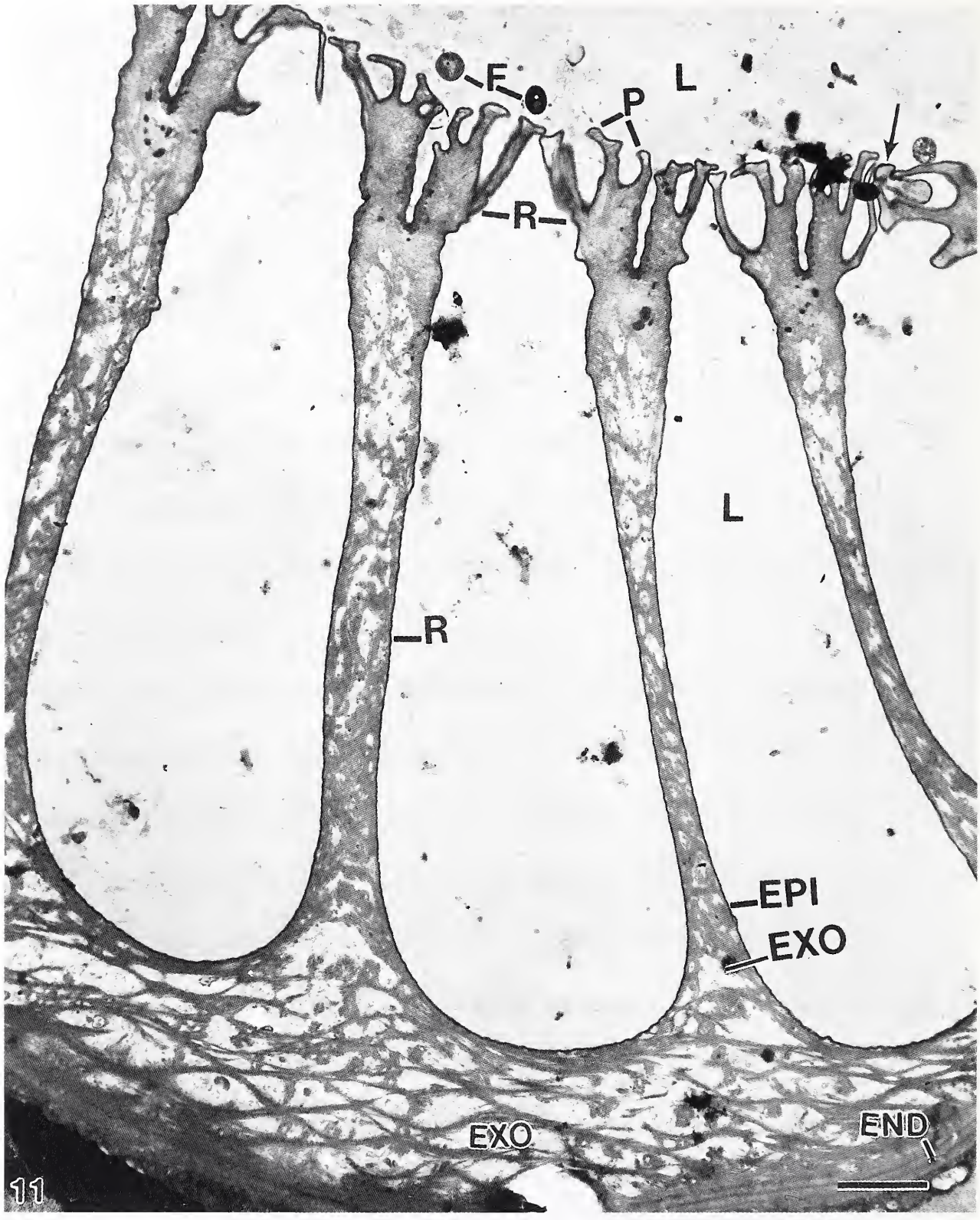
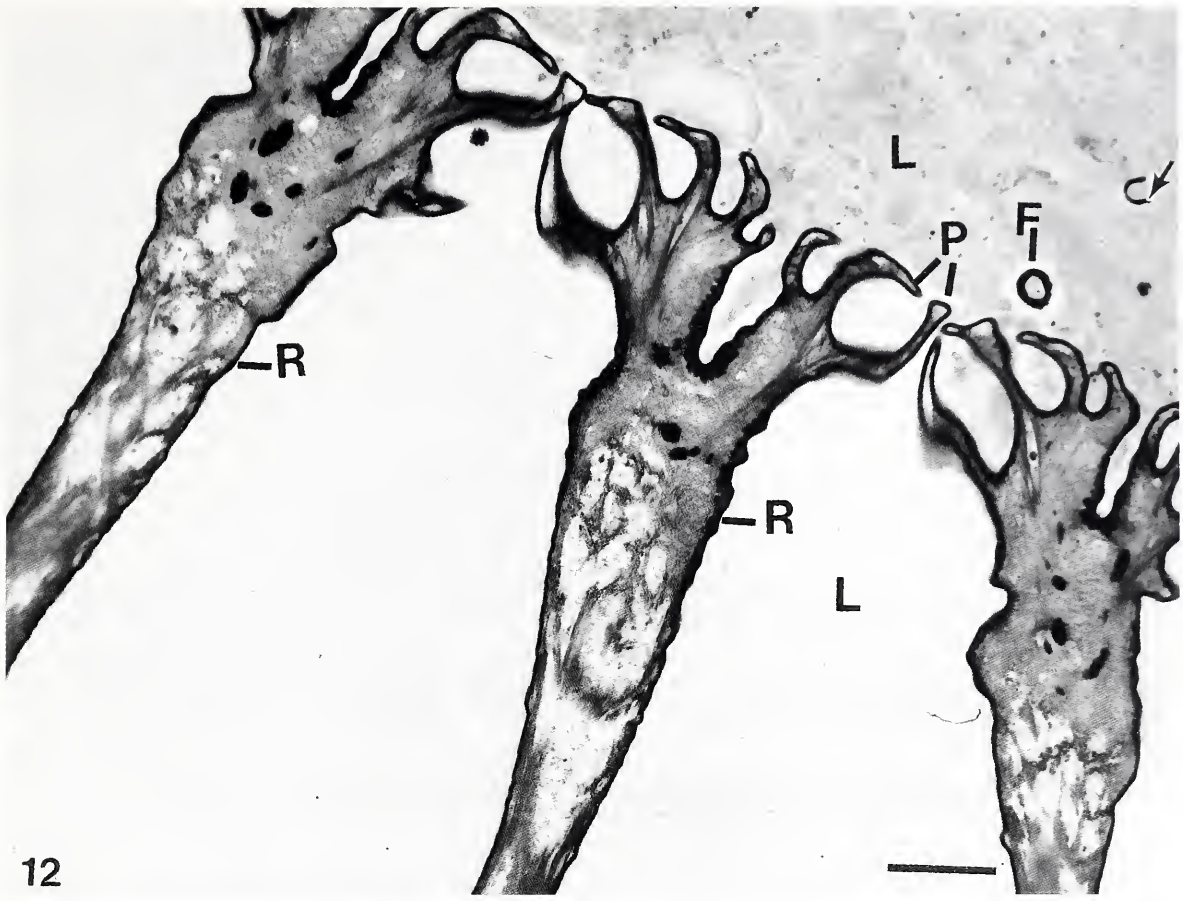
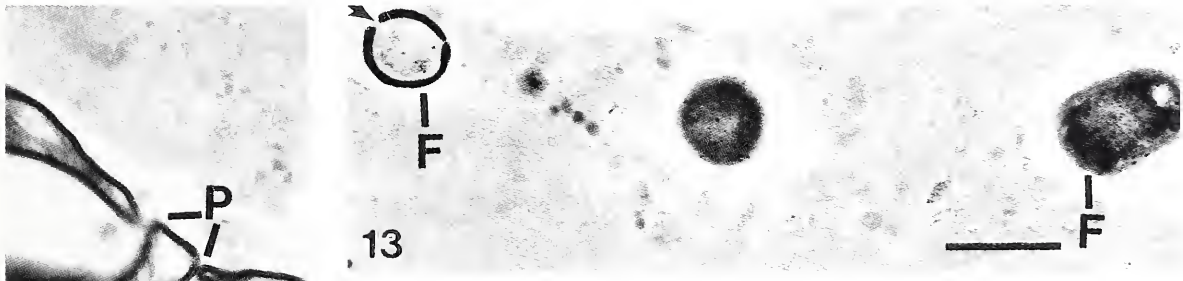
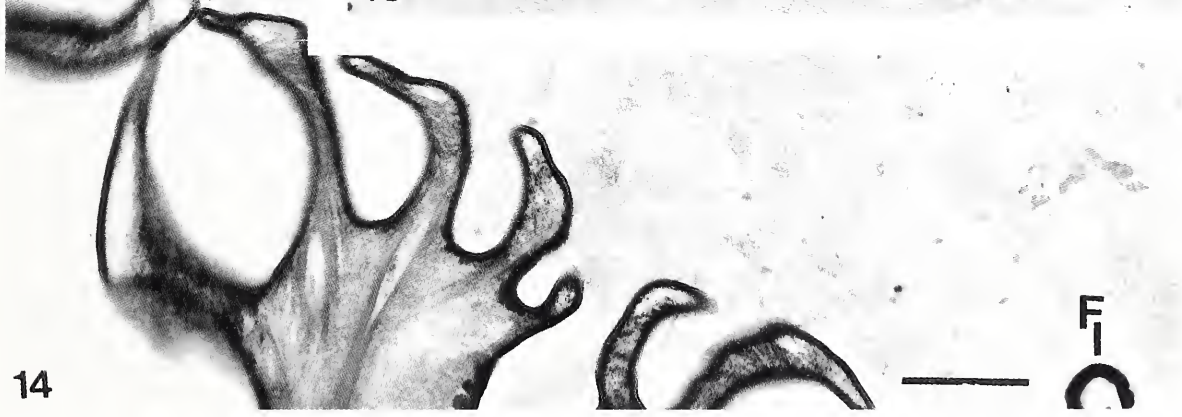


Figure 11.—Transverse section of the ventral cuticular intima showing ribs (R) with bifurcated tips ending in sharp finger-like process (P). Note a bacterium-like food particle trapped by the processes (arrow). *Abbreviations:* F, food particles; END, endocuticle; EPI, epicuticle; EXO, exocuticle; L, lumen. Scale bar = 3 μm.

struction of parasites has been described in different insects. Parsons (1972) demonstrated the presence of nodes and bifurcating longitudinal ridges in the cibarial food pump of *Notonecta* (Heteroptera) which act as triturating devices grinding and filtering particulate matter in the food. The cibarial and pharyngeal armatures have been reported in blood-sucking mosquitoes. Caluzzi et al. (1982) have shown that in seven species of the *Anopheles* mosquito the spines present in the posterior end of the cibarial and pharyngeal armature







Figures 12–14.—Electron micrographs of the pharyngeal ribs and food particles. 12. Detail of the bifurcated tips of the ribs (R) ending in sharp finger like processes (P). Note a fragment and empty-looking food particle (arrow) near the ends of the processes. F, food particle; L, lumen; Scale bar = 2 μm; 13. Bacteria-like food particles (F), one empty-looking with a break in its cell wall (arrow); Scale bar = 1 μm; 14. Magnified view of the processes (P) of the tips of ribs almost touching each other. F, food particle; Scale bar = 1 μm.

are mechanically capable of the hemolysis of ingested red blood cells. In addition, microfilaria parasites have been reported to be killed by the mechanical action of the cibarial and pharyngeal armature and other spines, teeth and papillae in the foregut of *Culex*, *Anopheles* and *Aedes* mosquitoes (McGreevy 1978; Lehane 1998).

The proventriculus, which is the terminal part of the foregut, is variously modified in different insects. In insects which feed on solid food, the inner wall of the proventriculus becomes armed with strong cuticular plates or teeth which grind the food (Snodgrass 1935). Since the proventriculus in the larvae of mosquitoes and *Drosophila* secretes the peritrophic membrane (Wigglesworth 1931; Rizki 1956), it is suggested that in these insects its usual role in breakdown of food material has been relegated to the elaborate pharyngeal armature.

The arrangement of cuticular layers in the pharyngeal ribs with lamellate endocuticle forming the base and exocuticle with trabeculae and spaces forming the core and covered by the thin epicuticle lining the luminal surface, appear to resemble the design of bone structure of vertebrates and may provide mechanical support to them. The lateral cuticular intima, which is much thicker than the dorsal intima and devoid of teeth, apparently provides support for the lateral walls of the pharynx.

The pharyngeal cuticular intima is devoid of depressions, pores or canals suggesting that absorption of food does not take place in the pharynx and that the released cellular contents of yeasts and bacteria are transported to the midgut for digestion and absorption. This view is supported by the finding that the cuticular intima of the foregut in locust and cockroaches has very low permeability to substances like amino acids, atropine, glucose and sucrose (Maddrell & Gardiner 1980).

In conclusion, the ultrastructural features of the pharyngeal armature of the larval *D. melanogaster* revealed by this study suggest that it is engaged in the breakdown of rigid cell walls of yeasts and bacteria in the food.

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