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Morphology and Ultrastructure of the Alimentary Canal of the Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (2): The Structure of the Midgut

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Chun-Nu Hung, Tai-Lung Lin and Wen-Yung Lee (2000) Morphology and ultrastructure of the alimentary canal of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (2): The structure of the midgut. *Zoological Studies* **39**(4): 387-394. The midgut of *Bactrocera dorsalis* (Hendel) was studied by using light and transmission electron microscopy. The midgut of this insect, the longest part of the entire alimentary canal, is about 16 mm in length. Its anterior portion is shaped as a tube located in the haemocoel of the thorax, while the most distal part is coiled and embedded in the first three abdominal segments. The midgut epithelium is characterized by a striated border of microvilli, and 2 layers of the peritrophic membrane lie in the gut lumen to protect the midgut cells from possible damage from abrasive food particles. The epithelium is surrounded by 2 layers of muscles and comprised of 4 different types of cells: columnar, goblet, interstitial, and regenerative cells (or nidi). Columnar cells comprise the majority in the epithelium, while a few goblet cells associated with the interstitial cells are found in the middle part of the midgut. Regenerative cells are undeveloped cells lying as a group at the basal portion of the epithelial cells. The structure and function of secretion of digestive enzymes and absorption of nutrients in the midgut epithelium of this fly are discussed.

Key words: Columnar cells, Goblet cells, Interstitial cells, Regenerative cells.

The midgut of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), comprises more than 2/3 of the length of the entire alimentary canal. Although the physiology and the structure of the midgut are well documented for a variety of flies (Miller 1965, De Priester 1971, Ferreira et al. 1981, Gartner 1985, Lehane 1987 1988 1989, Dimitriadis 1991, Wood and Lehane 1991), information on the morphology and ultrastructure of the midgut of the Oriental fruit fly is not available. The purpose of the present study was to provide such data for future research on this insect.

MATERIALS AND METHODS

Mature adults of both males and females of the Oriental fruit fly were used throughout the study. They were reared as described by Lee et al. (1998). For light and electron microscopic examinations, more than 20 individual insects were dissected in Tyrode's solution (Lillie 1965). The entire alimentary canal was photographed under a Wild M8 microscope. The midgut was cut into 10 equal pieces in the fixative with 2.5% glutaraldehyde in 0.1 M cacodylate buffer and fixed with the same fixative at 4-6 °C for 2 h, and then washed with the same buffer twice for 15 min each. Specimens were postfixed in 2% osmium tetraoxide in 0.1 M cacodylate buffer at 4-6 °C for 2 h. After that, specimens were stained with saturated uranyl acetate in 50% ethanol for 3 h, then dehydrated over an ethanol series ranging from 50% to absolute. Two changes of ethanol solution were made for 10-15 min at room temperature for that concentration. Specimens were then infiltrated with mixtures of propylene oxide and Spurr's low-

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viscosity embedding medium at 1:0, 1:1, and 1:2, twice each for 30 to 60 min, before being transferred to pure medium overnight at room temperature. After 2 more changes of the pure medium for 60 min each, the specimens were embedded with fresh medium for 8 h at 70 °C. Sections were made with a Reichard Jung Ultracut-E microtome with a diamond knife at a thickness of 600-1000 Å (golden/gray in color), co-stained with uranyl acetate and lead citrate (Reynolds 1963), and examined in a Hitachi 7000 electron microscope operated at 75 kV.

RESULTS

The midgut (Fig. 1), the longest part of the alimentary canal (about 16 mm) is situated between the foregut and the hindgut. Its anterior region is a straight cylindrical structure located in the thorax, and the posterior region has a coiled shaped which lies in the first three abdominal segments. The straight region has a similar diameter (about 0.45 mm) behind the cardia which follows the foregut. The coiled portion appears to be peristaltic before fixation. Here the diameter is fairly uniform (0.19 to 0.55 mm), but with a constriction in the anterior section. In the beginning, the direction of coiling is downward, then it turns left and upwards, making 2 counter-clockwise turns. Finally, the posterior section runs downward and terminates in the pylorus region that joins the hindgut. The ultrastructure of the midgut (Fig. 2) is composed of a single-layer epithelium resting on the basement membrane. The apical area of the epithelium is characterized by a striated border of microvilli. The epithelium and the basement membrane are surrounded on the haemocoel side by 2 layers of poorly developed muscles. Tracheoles are scattered both inside and outside the cells.

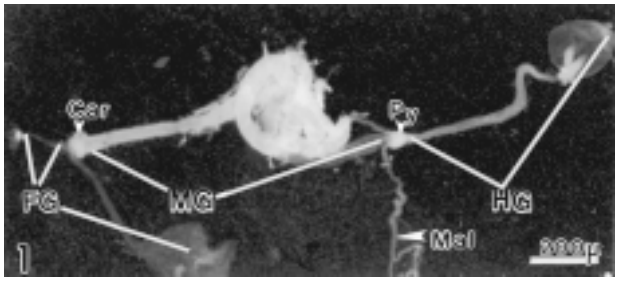

Fig. 1. General view of the alimentary canal of *Bactrocera dorsalis*. Car, cardia; FG, foregut; HG, hindgut; Mal, Malpighian tubules; MG, midgut; Py, pylorus.

The epithelium consists of 4 types of cells: columnar cells, goblet cells, interstitial cells, and regenerative cells.

Columnar cells (Fig. 3) comprise the majority in the epithelium and are trapezoidal with cylindrical or cuboidal shapes from different sections. These cells bear a brushlike border of apical microvilli projecting into the lumen. Their cytoplasmic compartments contain well-developed endoplasmic reticulum, Golgi complexes, mitochondria, secretory granules, and basal infoldings which extend halfway into the cytoplasm. These basal infoldings are highly invaginated by the basal plasma membrane. The mitochondria are mostly scattered both in the apical side and at the base of the cell. Several secretory granules connect with Golgi complexes.

Few goblet cells are found in the midgut of this insect. They are only located in the middle part of the midgut in folds of the central lumen of the midgut and have microvilli with unstable morphology. The tips and bases of the microvilli usually appear with vesicles and many electron-denses. These cells also have endoplasmic reticulum, small mitochondria, and vesicles (Fig. 4). Interstitial cells (Fig. 4) occupy spaces between goblet cells. These cells are large with rich microvilli extending into the gut lumen and tend to cover the apical portion of goblet cells. The cytoplasmic compartments of these cells are similar to those of columnar cells. Their mitochondria are mostly associated with the basal plasma infoldings and with the apical region of the cells. However, most of these structures are not shown in the micrograph.

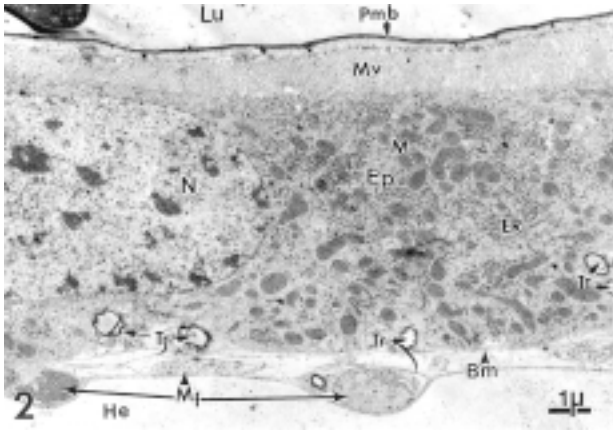

Fig. 2. Ultrastructural micrograph of the epithelium of the midgut in transverse section. Bm, basal plasma membrane; Ep, epithelial cell; ER, endoplasmic reticulum; He, haemocoel; Lu, lumen; M, mitochondria; Mf, muscle fibers; Mv, microvilli; N, nucleus; Pmb, peritrophic membrane; Tr, tracheole.

Regenerative cells are undifferentiated replacement cells (named nidi) located as a group in the basal portion of the epithelium. They do not make contact with the gut lumen (Fig. 5a). These cells have an elongated nucleus, and their cytoplasm is very simple, containing a few endoplasmic reticulum, Golgi complexes and mitochondria as well as short basal infoldings (Fig. 5b). These cells lack microvilli.

The peritrophic membrane of this fly consists of 2 layers. The thick layer is located towards the endoperitrophic space while the thin one is against the ectoperitrophic space. They are loosely connected with each other, but are not parallel to each other (Fig. 6).

Figure 7 shows many small secretory granules which are probably derived from the cisternae of Golgi complexes and the endoplasmic reticulum of the epithelium. Large numbers of secretory granules are distributed in the apical region of cells shown in figure 3. The ultrastructural micrograph in figure 8 shows a cytoplasmic extrusion in the columnar cell. A secretory granule seems to enter the extrusion from the cell body.

Figure 9 shows that small pits at the apical plasma membrane are frequently found between the microvilli. They seem to be pinched off into the cells as pinocytotic-like crypts. A pinocytotic vesicle extends from the apical plasma membrane continuing into the cortex of the cell. A structure of invagination from the plasma membrane and connected to the secretory granule was determined to be exocytosis of the enzyme excreted from the cell to the lumen. Likewise, many openings of the basal plasma membrane show as a connection of the basal infoldings to the extracellular compartment and interrupt the basement membrane (Fig. 10).

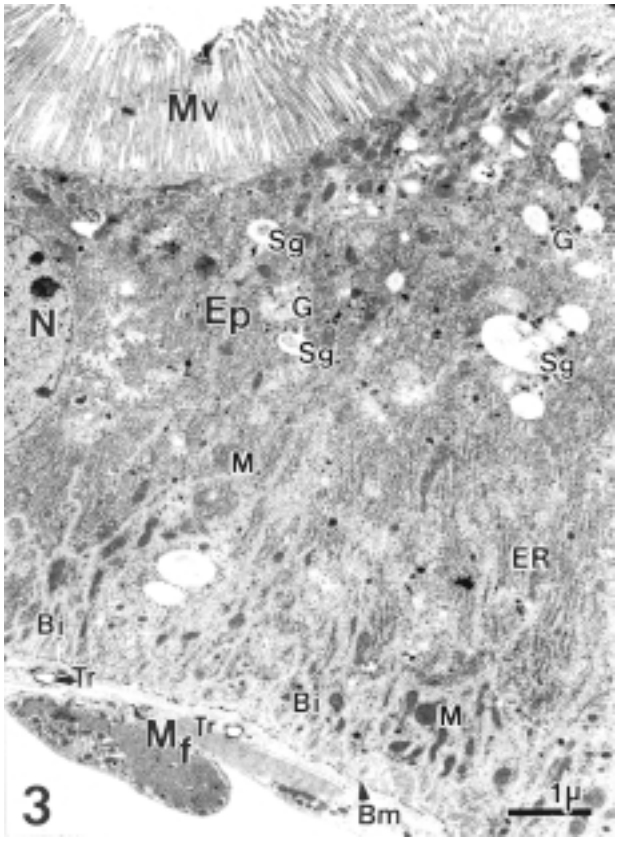

Fig. 3. Transverse section of the ultrastructure of a midgut columnar cell showing the well-developed microvillar border (Mv) on the apical side of the epithelium (Ep), numbers of secretion granules (Sg) together with Golgi complexes (G), most mitochondria (M) distributed in the apical and basal regions of the cell, and the abundant basal plasma infoldings (Bi) in the basal region of the cell. This cell has well-developed rough endoplasmic reticulum (ER). Bm, basal plasma membrane; Mf, muscle fibers; N, nucleus; Tr, tracheole.

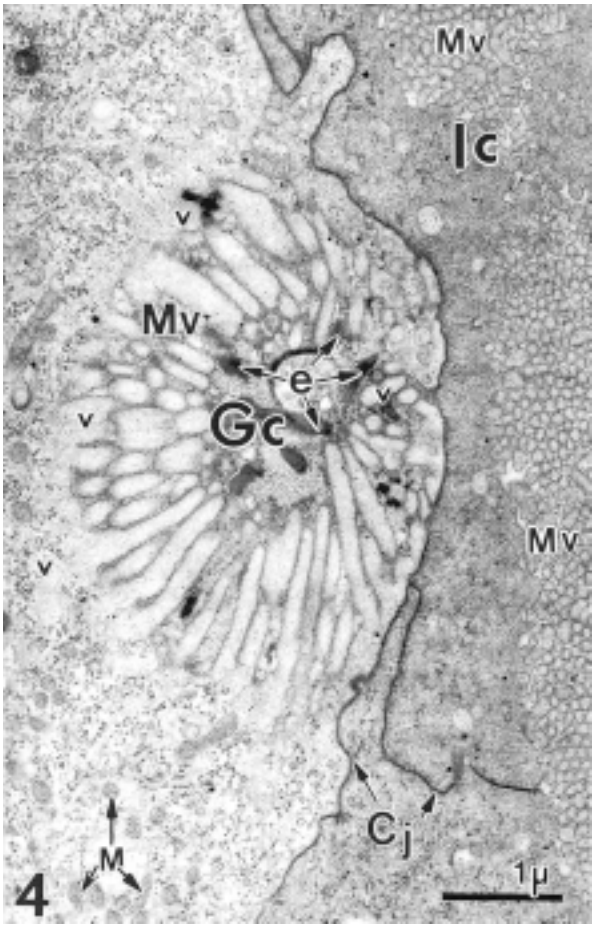

Fig. 4. Transverse section of a goblet cell (Gc) and an interstitial cell (Ic) of the midgut. They are associated with each other. Cj, cellular junction; e, electron-denses; M, mitochondria; Mv, microvilli; v, vesicle.

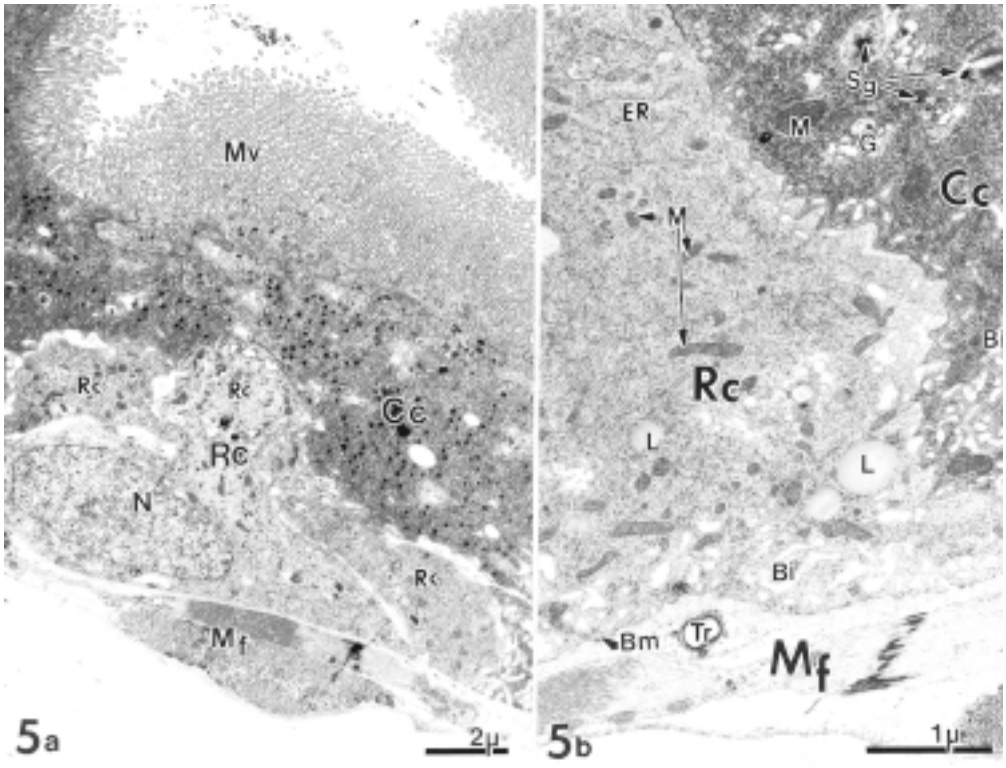

Fig. 5. The regenerative cell. (a) The regenerative cells are together as a group lying at the basal portion of the epithelium and are also called the nidi (Rc). (b) Higher magnification of figure 5a. The organelles in the regenerative cell (Rc) are much less developed than those in the columnar cell (Cc). Bi, basal plasmal infoldings; Bm, basal plasma membrane; G, Golgi complex; L, lipid droplets; M, mitochondria; Mf, muscle fiber; Sg, secretory granules; Tr, tracheole.

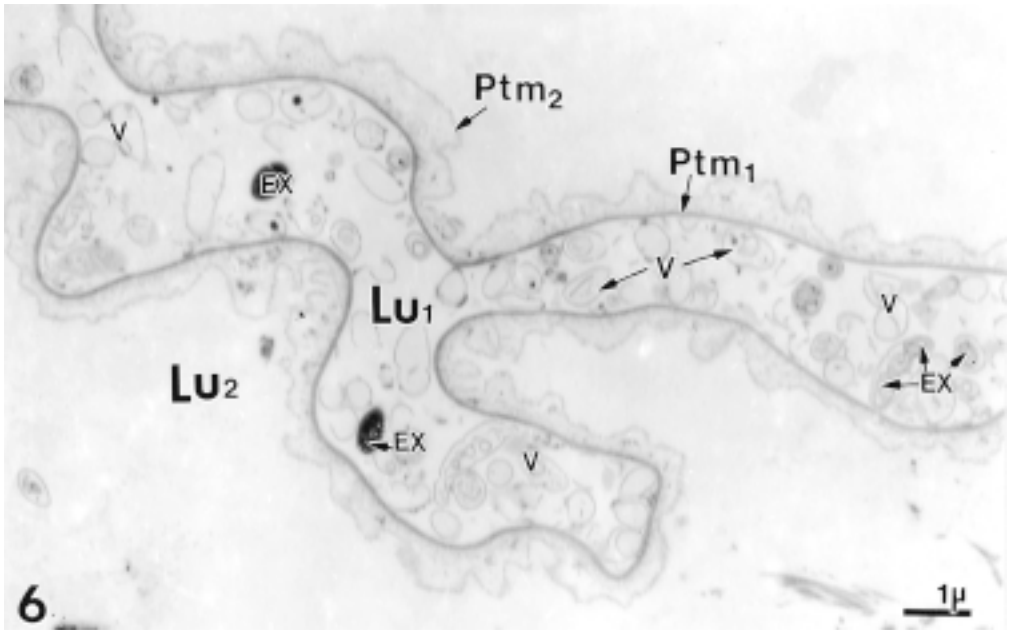

Fig. 6. Micrograph showing 2 layers of the peritrophic membrane in the midgut of the Oriental fruit fly: the thick layer, with greater density, Ptm_1 and the thin layer, Ptm_2. The lumen of the midgut is separated into the endoperitrophic space (Lu_1) and the exoperitrophic space (Lu_2) by these 2 layers of the peritrophic membrane. Ex, probable undigested or excretory materials; V, vesicles.

DISCUSSION

The present report contributes information on the histology and ultrastructure of the midgut of the Oriental fruit fly, *Bactrocera dorsalis*. The architecture of the midgut of this fly is the same as those reported for *Drosophila* (Gartner 1970 1985, Dimitriadis and Kastritsis 1984, Dimitriadis 1991), and similar to those of other dipteran insects: *Musca domestica* (Sohal et al. 1977), *Calliphora erythrocephala* (De Psriester 1971), *Aedes aegypti* (Hecker et al. 1971 1974, Rudin and Hecker 1976, Bauer et al. 1977, Hecker and Rudin 1981), *Anopheles gambiae, An. Stephensi, Culex pipien fatigans* (Hecker 1977), and *Lutzomya longipalpis* (Rudin and Hecker 1982).

The midgut epithelium of insects lacks a permanent uniform intima (cuticle) (Snodgrass 1935). The food in this part of the digestive tract is separated from the peritrophic membrane (Smith 1968). This structure generally plays a role similar to that of mucus in the vertebrate gut, in protecting the midgut cells from mechanical damage caused by abrasive food particles (Richards and Richards 1977). The peritrophic membrane in the Oriental fruit fly midgut contains 2 layers like those in the midgut of *Drosophila* (Dimitriadis 1991), but differs from that in the blowfly which has a 3-layer membrane (Waterhouse and Wright 1960).

The insect midgut is the chief site both for digestion of food and absorption of nutrition, and the epithelium is responsible both for the production of many digestive enzyme and/or the uptake and transfer of nutrients to the haemolymph (Wigglosworth 1965). Aspects of the fine structure of midgut cells have been described in several electron microscopic studies, including the midgut epithelium of Calliphora by Waterhouse and Wright (1960), and of *Aedes* by Bertran and Bird (1961), Roth and Porter (1964), as well as on the ultrastructure and physiology of the midgut epithelium of *Ephestia* by Smith et al. (1969). More recently, Ferreira et al. (1981) examined the midgut intercellular structure of the larvae of *Rhynchgosciara* fly, and Dimitriadis (1991) examined midgut cells of the adult *Drosophila auraria*.

Columnar cells are concerned with enzyme secretion and with the absorption of the products of digestion (Chapman 1985). Smith (1968) stated that the cytoplasm of columnar midgut cells is richly supplied with cisternae of the rough endoplasmic reticulum, and granular cisternae collected into Golgi complexes. Smith et al. (1969) supposed that the dense droplets presenting within the Golgi cisternae

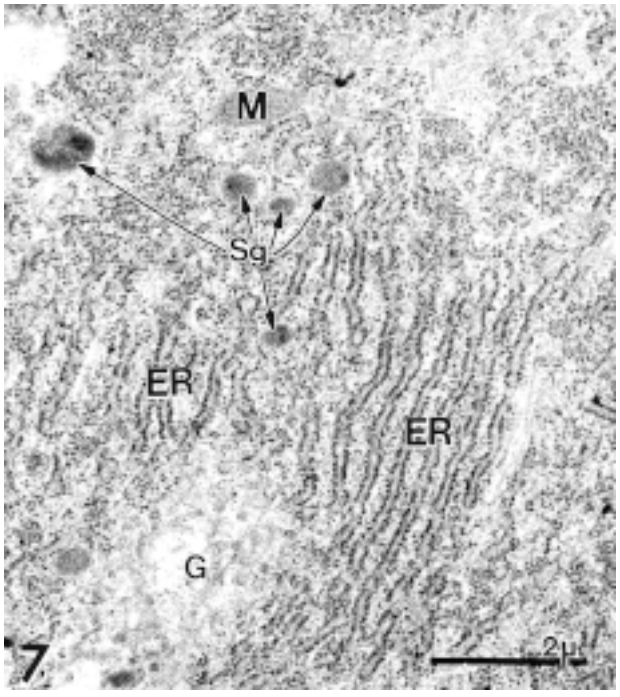

Fig. 7. High-magnification micrograph of part of a columnar cell. Secretory granules (Sg) and endoplasmic reticulum (ER) are indicated. G, Golgi complexes; M, mitochondria.

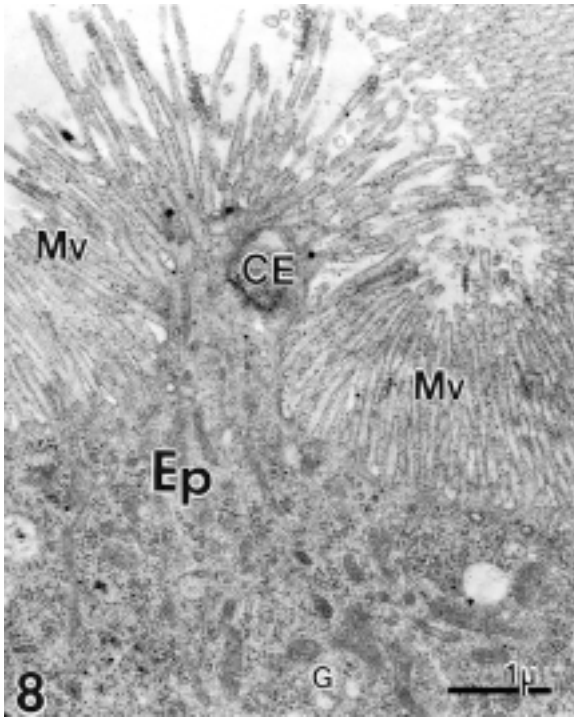

Fig. 8. Micrograph of merocrine secretion from a columnar cell. A secretory granule associated with the cytoplasm (CE) is extruded from the apical side of the cell. Ep, epithelium; G, Golgi complex; Mv, microvilli.

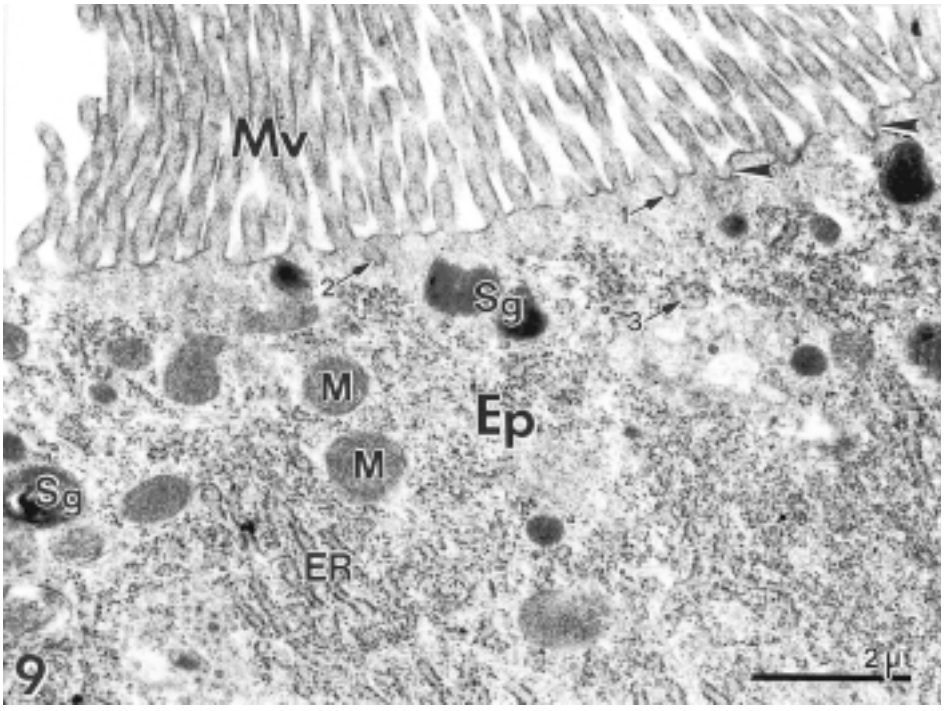

Fig. 9. High-magnification micrograph of the apical region of an epithelial cell (Ep). Note the processes of pinocytosis of the nutrient molecule from the lumen to the cytoplasm of the midgut cell, and exocytosis of the secretion from the cell to the lumen. 1, pinocytotic crypt; 2, pinocytotic crypt separated from the cell membrane and forming a vesicle in the cytoplasm; 3, vesicle, bigger arrow, exocytosis; ER, endoplasmic reticulum; M, mitochondria; Mv, microvilli, Sg, secretory granule.

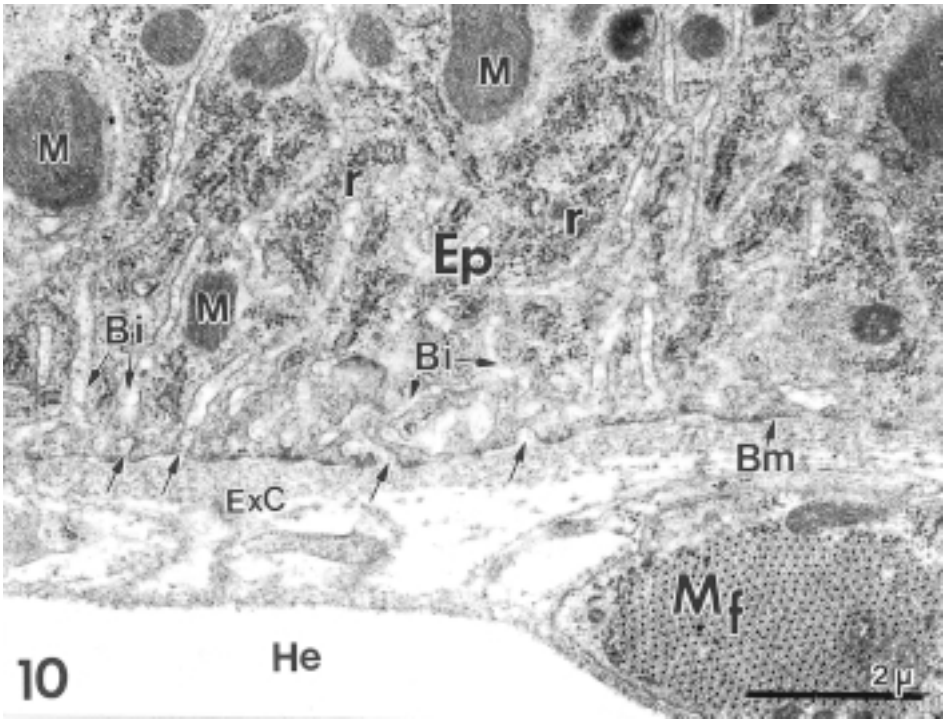

Fig. 10. High-magnification micrograph of the basal region of an epithelial cell (Ep). Note the openings (arrow) of the basal plasma infoldings (Bi) contacting the extracellular space (ExC). He, haemocoel, M, mitochondria; Mf, muscle fiber; r, ribosomes.

in *Ephestia* midgut cells might represent secreted materials. In this study, the midgut columnar cells show many small granules distributed around the endoplasmic reticulum and the Golgi complexes, of which most are probably secretory granules derived from the cisternae of these organelles. Large number of the granules are distributed in the apical part of the cells, and some of them connected to the invagination of the apical plasma membrane might have been released into the gut lumen through the apical plasma membrane by exocytosis. Lehane (1998) stated that the release of the secretory granules from the insect midgut occurs largely by classic exocytosis. Smith (1968) suggested that secretion of digestive enzymes in the midgut is holocrine, or merocrine, involving the splitting off or extrusion of globules of cytoplasm from the columnar cells. Wood and Lehane (1991) studied the relative contributions of apocrine and eccrine secretion to digestive enzyme release from midgut cells of *Stomoxys calcitrans* and found that apocrine secretion occurs immediately following the blood meal , and eccrine is the major means of digestive enzyme secretion. Histological evidence shows that the release of digestive enzymes from midgut cells of the Oriental fruit fly is merocrine secretion where a high-density secretory granule and a considerable amount of associated membrane as well as apical cytoplasm are extruded from the apical region of the cell.

Dimittriadis (1991) stated that the anterior and the posterior midgut regions of the adult *D. auraria* are probably involved in the absorption of nutrients from the gut lumen to the haemolymph. Both of these regions display cells with well-developed microvilli, a large number of mitochondria in their apical portion, and well-developed basal infoldings associated with mitochondria, with relatively few openings to the extracellular compartments. Smith et al. (1969) identified 4 distinct structures from the observation of ferritin uptake in the midgut epithelium of a moth, *Ephestia kuhniella*: 1. Pinocytotic crypts occurred on the apical cell membrane of epithelial cells between the microvilli. 2. These pinocytotic crypts separated from the cell membrane and formed a simple vesicle in the cytoplasm. 3. Multivesicular bodies appeared. 4. Loose whorls of the membranous material were often present in the apical cytoplasm. From ultrastructural studies in the Oriental fruit fly midgut, pinocytotic crypts and vesicles can be observed in the cytoplasm but not in the multivesicular body or the whorled structures. Berridge (1970) reported that the basal plasma membrane resting on the basement membrane is interrupted with an opening into the extracellular space. These fine structures are similar to those in the midgut columnar cells of the Oriental fruit fly. It is suspected that the well-developed microvillar brush border on the apical plasma membrane of the cell and the abundance of the mitochondria have the function of absorbing nutrients from the lumen to the cell. Nutrients might exit from cells to the haemocoel through the infoldings at the distal part of the cell associated with the mitochondria, and distinct openings of the basal plasma membrane to the extracellular compartments.

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果實蠅 *Bactrocera dorsalis* (Hendel) 消化管之形態及超薄顯微鏡結構之研究 (2)：中腸之結構

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本研究係利用光學顯微鏡和穿透性電子顯微鏡研究東方果實蠅 *Bactrocera dorsalis* (Hendel) 中腸之形態和超薄顯微的構造。果實蠅的中腸為全消化管最長部份，全長約 16 mm，前一小部份呈管狀，位於胸部體腔內，後部呈迴轉狀態，位於腹部前三節體腔。在上皮細胞層 (epithelium) 上方著生整齊的絨毛 (microvilli)；腸腔 (lumen) 和絨毛之間，有兩層構造的圍食膜 (peritrophic membrane)，此膜的功能係防止食物碎粒擦破上皮細胞。中腸外表被兩層肌肉包圍著。中腸細胞具四種細胞：柱狀細胞 (columnar cells)、杯狀細胞 (goblet cells)、間介細胞 (interstitial cells), 和再生細胞 (regenerative cell or nidi)；其中以柱狀細胞最多，僅少數杯狀細胞分布於中腸之中段，再生細胞為尚未發育之細胞，分布於上皮細胞基部。此等細胞之微細結構與消化酵素，分泌及營養分子吸收之功能，詳述及討論於本文中。

關鍵詞：柱狀細胞，杯狀細胞，間介細胞，再生細胞。

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