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Morphology and Ultrastructure of the Alimentary Canal of Oriental Fruit Fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (I): The Structure of the Foregut and Cardia

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Wen-Yung Lee, Mei-Er Chen and Tai-Lang Lin (1998) Morphology and ultrastructure of the alimentary canal of Oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)(I): The structure of the foregut and cardia. *Zoological Studies* **37**(2): 95-101. The foregut and the cardia of *Bactrocera dorsalis* (Hendel) were investigated by light microscopy and transmission electron microscopy. The foregut of this fly is comprised of a tiny slender tube, the oesophagus, and a lateral diverticulum of the crop. The crop provides a long, smooth duct opening near the end of the oesophagus, and a sac located in the anterior part of the abdomen. The oesophagus and the crop have a thick cuticle (the intima), a thin and simple epithelium, a basement membrane and it is surrounded by a well-developed visceral circular muscle. The cardia is located between the foregut and the midgut, and includes the midgut epithelium in the outer portion of the cardia and the foregut epithelium in the inner portion. The oesophageal epithelium extends into the cardia to form the cardial valve. Both the longitudinal and the circular muscles beneath the oesophageal epithelium form a sphincter to control the entrance of food to the midgut and prevent the regurgitation of the midgut contents. The peritrophic membrane is secreted and forms from the surface of microvilli of the midgut at the anterior part of the cardia.

Key words: Oesophagus, Crop, Cuticle, Epithelium.

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is an important pest of fruit crops. There are no published descriptions of the morphology and ultrastructure of the alimentary canal in this fly, except for the rectal papillae (Lee et al. 1992).

The alimentary canal of insects consists of 3 primary regions, the foregut (stomodaeum), midgut (mesenteron), and hindgut (proctodaeum). The morphology and ultrastructure of the foregut of insects are not well known. A literatural review reveals that a few studies of morphology were conducted in the blow fly, *Calliphora erythrocephala* (Graham and Smith 1934), the grasshopper, *Melanoplus differentialis* (Hodge 1936 1941 1943), *Drosophila melanogaster* (Miller 1965), the mosquito *Aedes aegypti* (Dapples and Lea 1974), and the water bug *Sphaerodema rusticum* (Ameen and Iman 1976). Only 1 paper has dealt with the ultrastructure of the foregut in the blow fly, *C. erythrocephala* (Smith 1968). In view of this lack of detailed information of both the morphology and ultrastructure of the foregut and cardia in insects, the present study was undertaken to provide such data in the oriental fruit fly.

MATERIALS AND METHODS

Oriental fruit flies were reared in the laboratory of the Institute of Zoology, Academia Sinica for many generations. However new gene flow was always introduced into the colony from wild flies which mostly came from damaged guava fruits. Flies were fed with a mixture of sugar: peptone = 3:1, and wet cotton was used as the water supply. A natural light period was contained in the laboratory. The temperature was controlled at 27 ± 2 °C, with RH at about 80%.

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The alimentary canals of 1- to 5-d-old flies were dissected out in Tyrode Ringer solution (Lillie 1965). They were washed thoroughly with the same solution in order to remove the fat body and other adhering particles. The whole alimentary canal was photographed under a WILD M8 microscope.

Foreguts were cut from the mouth to the cardia. All tissue specimens were fixed with 2.5% glutaraldehyde + 0.1 M cacodylate buffer at 4-6 °C for 2 h, then washed with the same buffer twice for 15 min each. Specimens were postfixed in 2% osmium tetraoxide + 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 about 3 h, dehydrated in a series of ethanol concentrations from 30% to 70% over 2 changes with each concentration for 10-15 min at 4 °C, infiltrated in 70% ethanol and LR White medium 1:1 and 1:2 twice each for 30 min at 4 °C, and finally changed to pure LR White medium twice for 30 min each, then overnight at 4 °C. The specimens were embedded in LR White medium for 24 h at 62-65 °C. Sections were made with a Richard Jung Ultracut-E microtome. For the light microscopic studies, sections were cut at 1.5 μm with glass knives and stained with 1% toluidine blue. Preparations were investigated and photographs taken with a Nikon Optiphot Research microscope. For the ultrastructural studies, sections were made with glass knives or a diamond knife at a thickness of 600-1000 Å and co-stained with uranyl acetate and lead citrate, then observed with a Hitachi H-7000 TEM at 75 kV.

RESULTS

The alimentary canal of *Bactrocera dorsalis* is a long tube (Fig. 1). As in other insects, it possesses a foregut, midgut, and hindgut. The present observation only deals with the foregut and cardia. The foregut of *B. dorsalis* includes 2 parts: the oesophagus and the crop.

The oesophagus

The oesophagus is a tiny slender tube about 823 ± 5.3 μm (n = 10) in length and 42 ± 6.5 μm (n = 10) in diameter, located in the cervical region of the anterior part of the thorax. It extends from the cibarial pump to the cardia.

Light micrograph cross sections (Figs. 2, 3) show that the oesophagus is surrounded by a single layer of well-developed circular muscle. Its epithelium is simple with different thicknesses. A cuticle (intima) is present. The structure of the oesophagus of this insect appears to differ from the anterior part to the posterior. In the anterior part (Fig. 2) the thickness of the epithelium is not uniform through the entire cross section, while in the dorsal part it is much thicker. The gut lumen is large. The thickness of the epithelium in the posterior part (Fig. 3) appears almost equal through the entire cross section; the gut lumen is narrower and smaller.

The electron micrograph reveals a very simple epithelium (Fig. 4). The epithelial cells include only a few small mitochondria and some cisternae of endoplasmic reticulum, as well as a small number of unattached ribosomes. A basement membrane lies below the epithelium. The cuticle is differentiated into the electron-dense epicuticle and the microfibrous ultrastructure of the endocuticle. Peripherally the oesophagus is covered with a lattice-work of a well-developed visceral muscle with abundant, rather small mitochondria, and endoplasmic reticulum, and encircled by a sarcolemma.

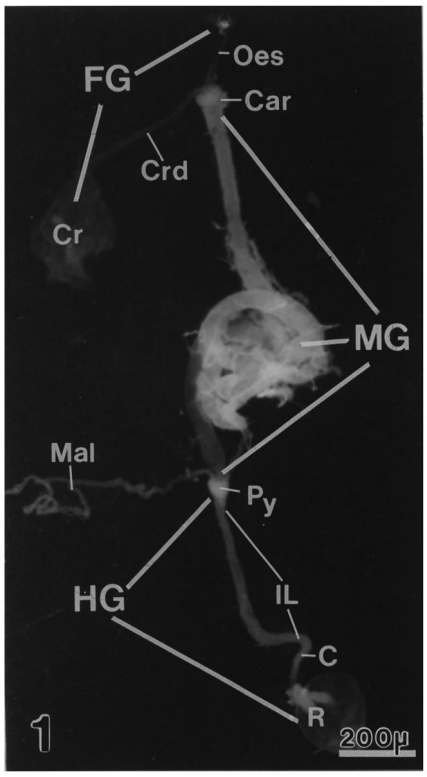

Fig. 1. General view of the alimentary canal of *Bactrocera dorsalis*. C, colon; Car, cardia; Cr, crop; Crd, cardial duct; FG, foregut; HG, hindgut; IL, ileum; Mal, Malpighial tubules; MG, midgut; Oes, oesophagus; Py, pylorus; R, rectum.

The crop

The crop of this fly is a lateral diverticulum of the foregut opening into the oesophagus by a narrow tube known as the crop duct (Fig. 1; Crd). A pedunculate sac, the crop, is connected to the terminus of the duct. This sac lies in the anterior part of the abdomen. The narrow duct is 1.5 ± 0.2 mm (n = 10) in length and 45 ± 5 µm (n = 10) in dia. and opened at the end region of the oesophagus.

The light microscopic structure of the crop appears with numerous folds extending from its

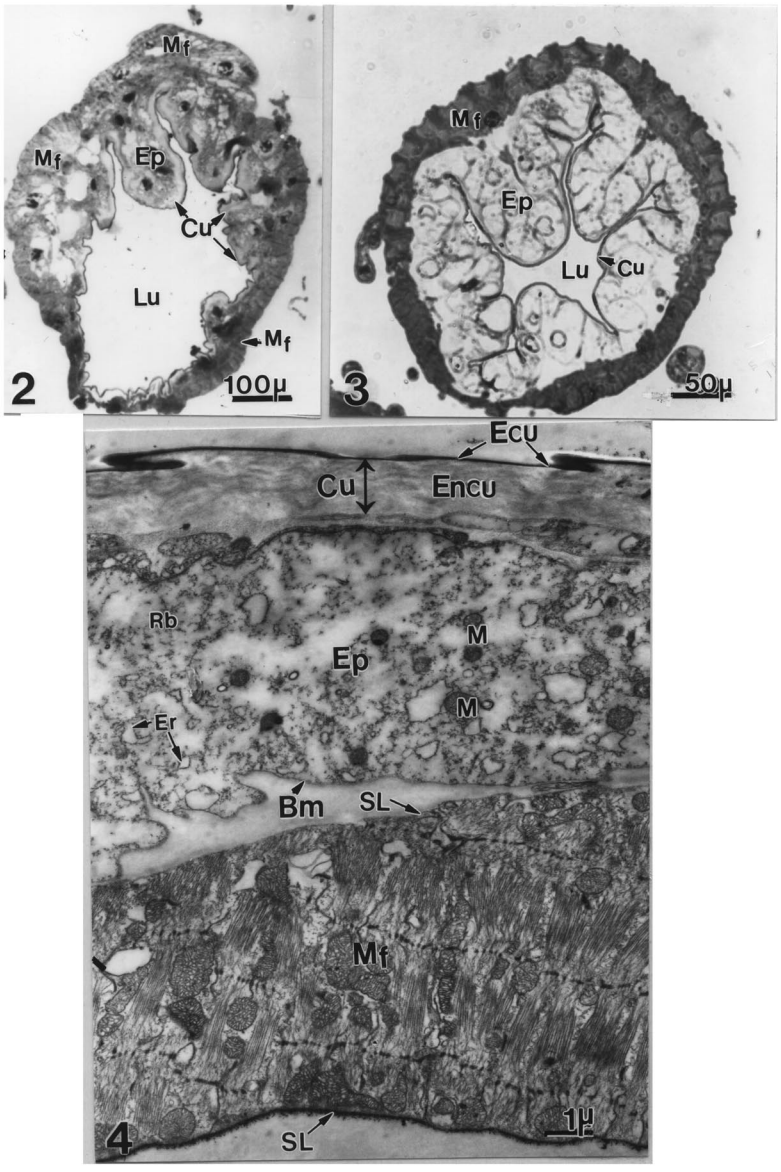

Fig. 2. Cross section of the light micrograph showing the anterior part of the oesophagus surrounded by circular muscle (Mf). The thickness of the gut wall is uneven with 1 side much thicker than the other. Cu, cuticle; Ep, epithelium; Lu, lumen.

Fig. 3. Cross section of the posterior part of the oesophagus showing a single layer of circular muscle (Mf) around the oesophagus. The thickness of the gut wall appears almost uniform. The lumen (Lu) is small. The epithelium (Ep) and cuticle (Cu) protrude into the lumen.

Fig. 4. Ultrastructure of the oesophageal wall. The cuticle (Cu) can be differentiated into an electron-dense epicuticle (Ecu) and a microfibrous endocuticle (Encu). The epithelium (Ep) has a simple structure with few small mitochondria (M), endoplasmic reticulum (Er), and unattached ribosomes (Rb). A basement membrane (Bm) lies underneath the epithelium. The circular muscle (Mf) is well developed.

wall. Fig. 5 shows that the duct is circular and surrounded by a well-developed circular muscle layer. Its epithelium and cuticle are infolded, projecting into the lumen, creating a star-shaped. The wall of the sac (Fig. 6) is also infolded into the lumen. However the lumen in the sac is much wider. The sac is also surrounded by a well-developed layer of circular muscle, and appears wider and more extended when the sac is storing food.

The ultrastructure of the crop (Fig. 7) shows a cuticle with a narrow layer of dense epicuticle and a rather thick layer of microfilament endocuticle. The epithelial layer beneath underneath the cuticle rests on the basement membrane which is close to the sarcolemma of the muscle. The epithelial cells contain few mitochondria, but numerous smooth endoplasmic reticulum. The visceral muscle fibrils are close-packed muscle with distinct I-band, Z-band, and A-band striations.

The cardia

The cardia (Fig. 1; Car) is a swelling ovoid structure between the foregut and midgut. In light microscopic studies of longitudinal sections (Fig. 8), the cardia of *B. dorsalis* shows 2 regions of epithelia. The deep staining outer region connects to the midgut epithelium. The inner region is considered the foregut epithelium which contains 2 parts. The inner epithelium extends from the oesophagus as cuboidal epithelial cells that form the cardial valve (Figs. 8, 9; CarV). The outer part of the epithelium (Figs. 8, 9; FEp) shows many membranous strips. A strong longitudinal muscle (Fig. 9; ML) appears on sides of the cardial valve, and a circular muscle (Fig. 8; MC) on the anterior part of the cardia valve. The electron micrograph (Fig. 10) shows that this is a close-packed muscle with strong myofibrils, endoplasmic reticulum, and abundant mitochondria. In the anterior part of the cardia between the inner and the outer surfaces of the epithelium, ultrastructurally there is a continuous layer with a refringent structure, the formation of the peritrophic membrane (Fig. 11; FPmb) at the outer surface of the microvilli of the midgut epithelium. This is presumably the peritrophic membrane (Fig. 12; Pmb).

DISCUSSION

The alimentary canal of insects comprises structurally and embryologically 3 distinct regions from the mouth to the anus. The 1st region, the foregut, and the 3rd region, the hindgut, are of ectodermal origin, provided with a cuticle, the same as the integument of the body wall. The 2nd region, the midgut, is developed from the endoderm and possesses no cuticular intima (Berridge 1970).

The foregut of *B. dorsalis* begins the alimentary canal, opening from the mouth and extends to the cardia. It is a tiny slender tube, the oesophagus, in the cervical region and its diverticulum, the crop extending to the anterior abdomen. Its morphological structure is similar to that described for *Drosophila melanogaster* (Miller 1965).

The function of the oesophagus of the insect is to pass of food forward to the midgut. The crop serves as a storage depot for ingested food (Wigglesworth 1965). Therefore, the foregut, both the oesophagus and the crop, contains a strong circular muscle layer. There may be little or no absorption of nutrients into the haemolymph across the crop wall (Smith 1968). The muscle layer in *B. dorsalis* is the same as that in *D. melanogaste*r in which there is only 1 layer of circular muscle but lacking any longitudinal muscle. This is different from *Periplaneta* and *Locusta* (Chapman 1984). In which both have circular muscle as well as longitudinal muscle surrounding the foregut.

The crop of *B. dorsalis* has a lateral diverticulum which is similar to that of *D. melanogaster* (Miller 1965). However, the crop of the mosquito protrudes into dorsal diverticula and the ventral diverticula (Dapples and Lea 1974). From light microscopic and electron microscopic observations, the cuticular surface of the foregut appears smooth in both *B. dorsalis* and *D. melanogaster* (Miller 1965), but it has projections of spines or a chitinous lining in the mosquito (Dapples and Lea 1974). Chapman (1984) states that these spines possibly act as a barrier to regurgitation of food from the diverticula back from the midgut. *Bactrocera* and *Drosophila* flies eat food by lapping and sucking the fluid. The action of regurgitation perhaps dose not occur. The pattern of the foregut cuticle of insects is different from that of the cuticle of the body wall. There are 2 major layers in the cuticle of the insect body wall: a very thin outer epicuticle and a thick inner procuticle. The procuticle is differentiated into a hardened exocuticle and a fibrous endocuticle (Romoser and Stoffolano 1994). In the cuticle of the foregut of *B. dorsalis*, only the epicuticle and the endocuticle were visible on ultrastructural micrographs, but it lacked the exocuticle. The structure of the foregut of *P. americana* shows a very thin epicuticle which was not visible in the light microscopic sections, but

the endocuticle was visible throughout the foregut lining (Murthy 1976).

The fine structures of the oesophagus and the crop of *B. dorsalis* are very similar to those of the crop wall in the adult blowfly, *C. erythrocephala*, with a dense epicuticle and a microfibrous endocuticle, with the epithelium underneath. The epithelial cells contain numerous small mitochondria, scattered granular and agranular endoplasmic reticulum, and a large number of unattached ribosomes (Smith 1968).

B. dorsalis does not have a proventriculus. The cardia of *D. melanogaster* was formerly called the "proventriculus", but this designation has been changed because this term is more properly applied in other insects with a chewing mouth part

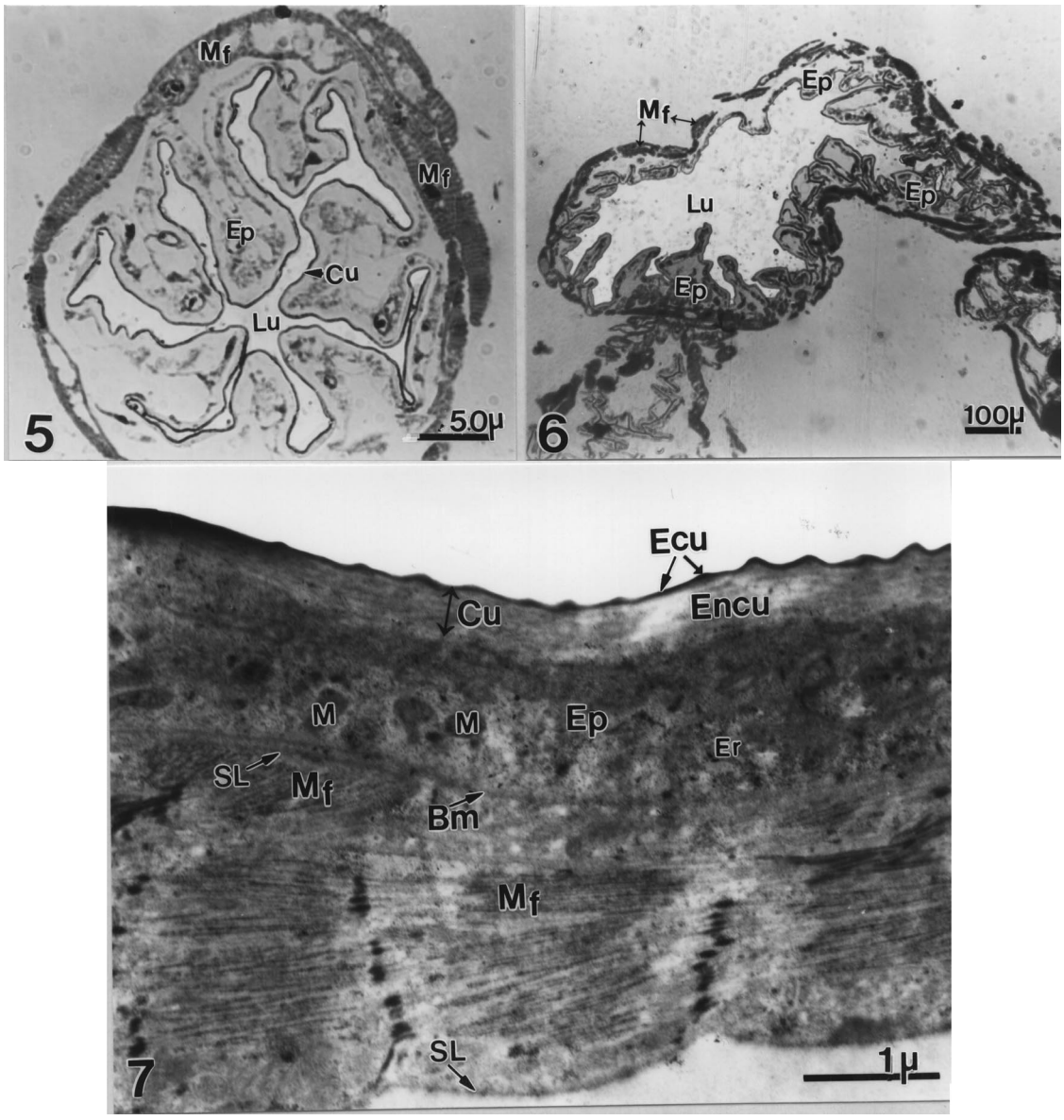

Fig. 5. Light microscopic cross section. The crop duct surrounded by a layer of circular muscle (Mf). The cuticle (Cu) and epithelium (Ep) protrude into the lumen (Lu).

Fig. 6. Light microscopic longitudinal section. The sac of the crop surrounded by muscle (Mf). The epithelium (Ep) and cuticle (Cu) project into the lumen (Lu) of the sac.

Fig. 7. Ultrastructure of the crop, with the cuticle (Cu) differentiated into the epicuticle (Ecu) and endocuticle (Encu). The epithelium (Ep) contains numerous mitochondria (M) and endoplasmic reticulum (Er). The basement membrane (Bm) lies at the bottom of the epithelium. Muscle (Mf) is well developed with strong myofibrils.

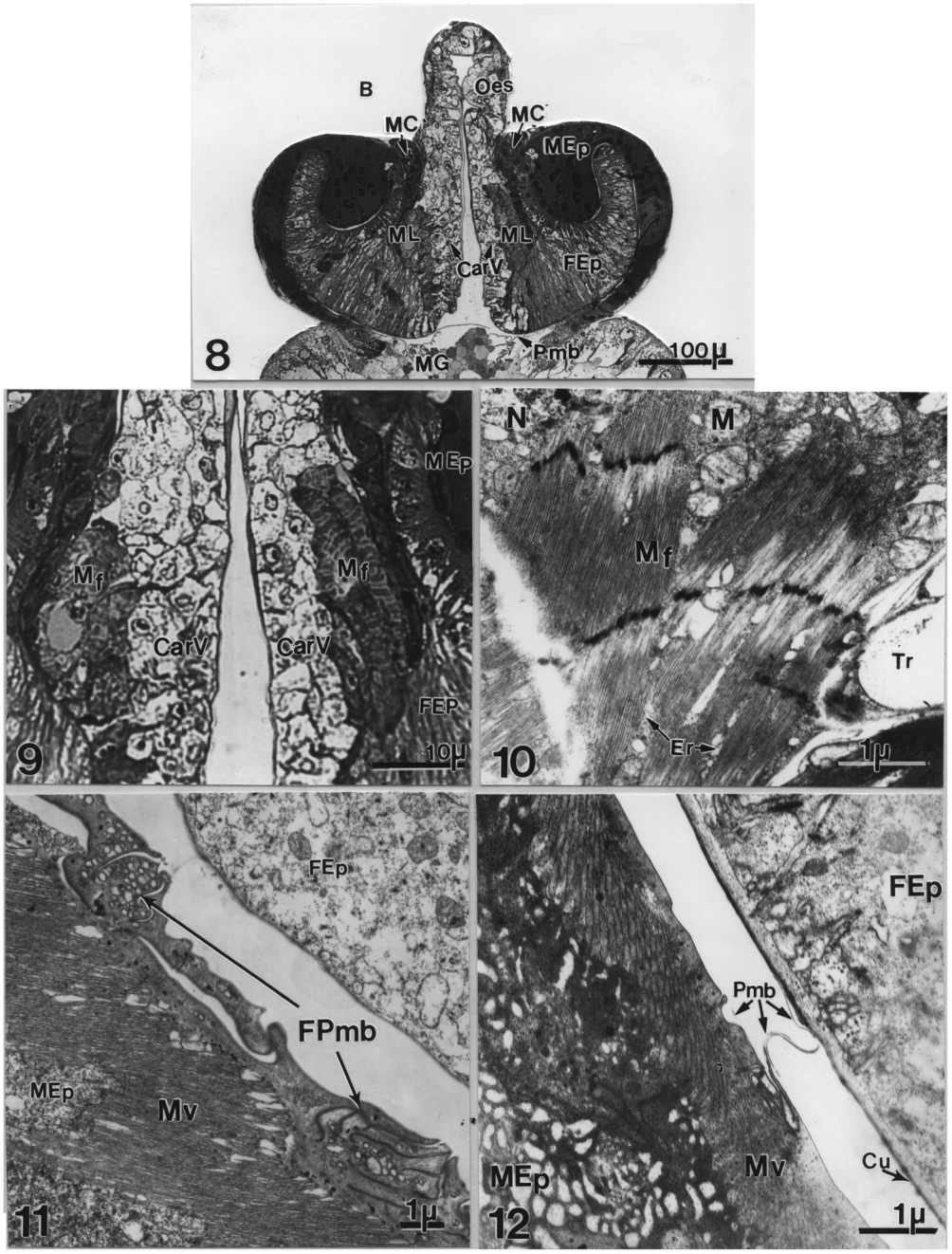

Fig. 8. Longitudinal section, (light microscope) showing the cardia contains 2 regions of epithelium. The outer region is the midgut epithelium (MEp), with deeper staining, and the inner region is the foregut epithelium (FEp). The epithelium of the oesophagus (Oes) with cuboidal cells extends toward the midgut through the cardia forming the cardial valve (CarV). The longitudinal muscle (ML) on the side of the valve and the circular muscle (MC) at the anterior part of the cardia forms a sphincter to prevent the movement of food back to the foregut from the midgut.

Fig. 9. High magnification of the light micrograph of Fig. 8 showing the location of the muscle (Mf) and the cardial valve (CarV). FEp, foregut epithelium; MEp, midgut epithelium.

Fig. 10. Ultrastructural micrograph of the muscle showing the strong myofibrils (Mf) with abundant of mitochondria (M) and endoplasmic reticulum (Er). N, nucleus; Tr, tracheole.

Fig. 11. Ultrastructure of the anterior part of the cardia between the foregut epithelium (FEp) and the midgut epithelium (MEp) showing the refringent structure of the microvilli (Mv) which is considered to be the peritrophic membrane (Fpmb).

Fig. 12. Ultrastructural electron micrograph showing the peritrophic membrane (Pmb) secreted from the microvilli (Mv) of the midgut epithelium (MEp). FEp, foregut epithelium; Cu, cuticle.

(Miller 1965). In fluid feeders, the proventriculus is absent (Chapman 1984). The oesophageal epithelium extends to the beginning of the midgut, forming the cardial valve. Circular muscle forms a sphincter at the entrance to the midgut (Chapman 1984). In *B. dorsalis*, aside from the circular muscle at the anterior part of the cardia, the longitudinal muscle occurs on the sides of the cardial valve in order to control and to prevent food returning to the foregut from the midgut.

There are 2 types of peritrophic membranes in insects, according to their modes of formation. The 1st type occurs in Dipteran insects. This membrane is secreted and formed at the anterior end of the midgut. The 2nd type of membrane is formed by delamination from the entire surface of the midgut and occurs in many orders of insects including Orthoptera and Coleoptera (Chapman 1984). The peritrophic membrane in *B. dorsalis* belongs to the 1st type, which begins to be formed from the anterior part of the cardia, the surface of the microvilli of the midgut epithelium, and extends to the hindgut via the midgut.

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東方果實蠅 *Bactrocera dorsalis* (Hendel)消化管之形態及超薄顯微結構之研究(I)：前腸與盲囊之結構

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本報告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)前腸(foregut)和盲囊(cardia)之形態和超薄顯微的構造。前腸包括一條細管狀的食道(oesophagus)和側旁分出之嗉囊(crop)。嗉囊又分為嗉囊管(crop duct)和嗉囊包(sac)。前者為一細長管，開口於食道末端位於蠅體頭部，後者由嗉囊管延至蠅體腹部的前端。食道和嗉囊之顯微結構均係有一層較厚的表皮(cuticle)和構造簡單的上皮細包層(epithelium)，末端為顯著的基底膜(basement membrane)，整個結構被一層發育良好的內臟橫走肌(visceral circular muscle)包圍。盲囊(cardia)位於前腸與中腸(midgut)間，外部為中腸上皮細胞層，內部屬前腸的細胞層。食道之表皮層由食道延伸至盲囊而形成盲囊瓣(cardial valve)。周圍有縱走肌和橫走肌(longitudinal muscle and circular muscle)，其作用為使食物能從前腸送至中腸，但阻止從中腸回轉到前腸。盲囊前端的中腸部份由該處的微絨毛(microvilli)分泌形成圍食膜(peritrophic membrane)。

關鍵詞：食道，嗉囊，表皮，上皮細胞層。

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