Bull. Inst. Zool., Academia Sinica **25**(1): 25-38 (1986)

LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS OF THE MIDGUT OF THE LYCHEE STINKBUG, *TESSARATOMA PAPILLOSA* THUN. (HETEROPTERA: TESSARATOMIDAE)

WILKIN WAI-KUEN CHEUNG and LAI-MING LAI

Department of Biology
The Chinese University of Hong Kong
Shatin, N. T., Hong Kong

(Accepted December 26, 1985)

Wilkin Wai-Kuen Cheung and Lai-Ming Lai (1986) Light and electron microscopic observations of the midgut of the lychee stinkbug, *Tessaratoma papillosa* Thun. (Heteroptera: Tessaratomidae). *Bull. Inst. Zool., Academia Sinica* **25**(1): 25-38. The midgut of *Tessaratoma papillosa* can be conveniently divided into 5 regions: stomach, tubular midgut, midgut bulb, posterior bulb, and posterior midgut. Each portion of the midgut has distinct types of epithelial cells and ultrastructural pattern. The stomach cells contain numerous mitochondria, vacuoles, lipoid spheres, endoplasmic reticulum and mineral spherites. The basal plasma membrane is much infolded. The tubular midgut cells have essentially similar fine structure as that of the stomach cells but less lipoid spheres. The midgut bulb is spherical in shape. The cells have scanty rough endoplasmic reticulum. The bulb lumen is filled with a pasty material and some yellow lipoid droplets which are insoluble in water and common organic solvents. The posterior bulb cells have scanty mitochondria and mineral spherites. The bulb lumen has filamentous materials which are broken membranes. The posterior midgut cells have sparse microvilli and the lumen has many rod-like bacteria. The possible functions of each of the above gut regions in relation to their distinct morphology are discussed in this report.

Phytophagous heteropteran insects feed on various parts of plants. In correspondence, they have diverse types of alimentary canal adapted to the specific plant parts which they feed on (Goodchild, 1963, 1966, 1978; Richards and Davies, 1977).

Gastric caecae are usually found in the posterior region of the midgut, and are supposed to have an osmoregulatory function (Goodchild, 1963). Examples of these insects are such as *Nezara* (Malouf, 1933), *Mygdonia* (Goodchild, 1963), *Rhynchocoris* (Cheung, 1977) and *Piezosternum* (Goodchild, 1978). The gastric caecae of these heteropterans also contain a lot of symbiotic bacteria, which can have some nutritional value to their hosts (Goodchild, 1963, 1978).

In other heteropterans, however, gastric caecae are absent. For example, *Oncopeltus* (Hood, 1937), *Dysdercus* (Saxena, 1955), *Leptocoris* (Miyamoto, 1961), and *Tessaratoma* (present authors). It is worthwhile to investigate in detail the gut structure of one of these insects.

Tessaratoma papillosa feeds on lychee and longan trees (Kershaw, 1907). It damages the young shoots and fruits extensively, causing premature fruit drops. A detailed morphological study of the midgut is reported here.

The information obtained is useful to understand the digestive physiology and hydromineral regulation of the pest.

MATERIALS AND METHODS

Female adults of *Tessaratoma papillsa* Thun. were collected from lychee and longan groves in the New Territories of Hong Kong. Dissections were made in 0.2 M phosphate buffer, pH 7.2, and pieces of *Tessaratoma* midgut were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.2, for 1 hr., postfixed in 1.0% osmium tetroxide in phosphate buffer and embedded in araldite, epon or spurr resin after dehydration with acetone series. Sections were cut with a Reichart ultrotome and were stained with uranyl nitrate or acetate and lead citrate. Sections were observed in a Zeiss EM 9S-2 electron microscope. Thick sections for light microscopy were also cut with a Reichart ultrotome and were stained in 1% toluidine blue in 1% borax.

For histological observations material was fixed in Formol saline, embedded in paraplast (56°C) and sectioned at 6μ. Sections were routinely stained with haematoxylin and eosin.

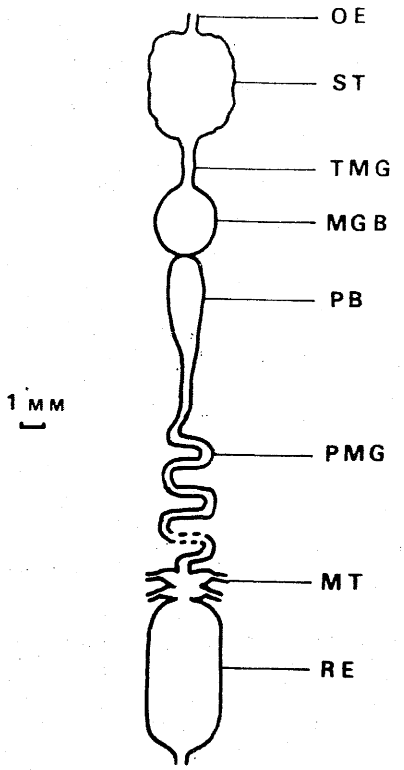


Fig. 1. Diagrammatic representation of the gut of *Tessaratoma papillosa.* MGB, midgut bulb; MT, malpighian tubule; OE, oesophagus; PB, posterior bulb; PMG, posterior midgut, RE, rectum; ST, stomach; TMG, tubular midgut.

OBSERVATIONS

The gross anatomy of the gut of *Tessaratoma* is shown diagrammatically in Fig. 1. In dissection the stomach is brown colour and the posterior bulb is yellow colour.

The midgut can be divided into the following regions: stomach (anterior sac), tubular midgut, midgut bulb (spherical region), posterior bulb, and posterior midgut. Each portion of the midgut has distinct types of epithelial cells (Figs. 2-6).

Stomach

In the light microscope, transverse sections of the stomach (ST) show it consists of one-cell thick epithelium with a prominent brush border (Fig. 2). The epithelium is surrounded by a sheath of circular and longitudinal muscles (Fig. 2). The principal cells contain one or two nuclei, and the ground cytoplasm has numerous granular deposits or mineral spherites (Fig. 2). These mineral spherites stain heavily with haematoxylin or toluidine blue.

Ultrastructure

The principal cells show a brush border consisting of microvilli of 2μ in height (Fig. 7). The microvilli have a surface coat of membranous materials and membrane-bound vesicles (Figs. 7, 8).

The apical cytoplasm contain numerous mitochondria, vacuoles, and rough endoplasmic reticulum (Fig. 7). One or two nuclei are placed centrally in a principal cell (Fig. 7).

The ground cytoplasm has also many mineral spherites (up to 1μ in diameter), lipoid spheres, lysosomes and Golgi bodies (Figs. 7, 9).

The basement membrane is of the amorphous type and is up to 1μ thick (Fig. 10). The basal plasma membrane is much infolded, with numerous mitochondria associated with these infoldings (Fig. 10). A replacement cell with nucleus and mitochondria is also shown in Fig. 10.

Tubular midgut

The tubular midgut (TMG) is a short narrow tube (approximately 0.18 cm long) linking the stomach with the midgut bulb (MGB) (Fig. 1). The principal cells are much elongated and closely packed together (Fig. 3). There are one or two nuclei in each principal cell.

A sheath of circular and longitudinal muscles surrounds the epithelium (Fig. 3). A replacement cell is also shown in Fig. 3.

Ultrastructure

The principal cells of the tubular midgut have essentially similar fine structure as that of the stomach cells. However, fewer lipoid spheres are seen (Fig. 11). The ground cytoplasm has abundant mitochondria, mineral spherites, vacuoles, lysosomes, multivesicular body and Golgi body (Fig. 11).

Midgut bulb

The midgut bulb (MGB) is spherical in shape (Fig. 1). In dissection, the bulb lumen contains a pasty material and some yellow lipoid droplets. This pasty material and the yellow lipoid droplets are insoluble in water and common organic solvents such as ethanol and xylene (Fig. 4).

In the light microscope the principal cells contain one or two basally situated nuclei (Fig. 4). Pasty materials invest the brush border (Fig. 4).

Ultrastructure

The principal cells of the midgut bulb has numerous mitochondria and dense bodies (Fig. 12). The bulb lumen is packed with broken membranous fibrils and 'fuzzy' or pasty material (Fig. 12). There is no sign of secretion of these membranous fibrils from the principal cells.

The basal cytoplasm has scanty rough endoplasmic reticulum (Fig. 13). The basal plasma membrane is much infolded, with occasional mitochondria associated with the ihvaginations (Fig. 13). The amorphous basement membrane is of 1μ thick (Fig. 13).

Posterior bulb

The posterior bulb (PB) is separated from the midgut bulb by a distinct constriction. Thus, it is discontinuous with the midgut bulb (Fig. 1).

In the light microscope, the posterior bulb has filamentous materials investing the brush border (Fig. 5). The principal cells contain 2 nuclei placed in the central position (Fig. 5).

Ultrastructure

The principal cells of the posterior bulb contain short microvilli of 1.2μ in height (Fig. 14). The filamentous materials in the gut lumen are broken membranes (Fig. 14). The apical cytoplasm contain scattered mitochondria, large vacuoles, Golgi bodies, lysosomes and occasionally mineral spherites (Fig. 14).

The basal plasma membrane is deeply invaginated (Fig. 15). There are many elongated mitochondria and small vacuoles associated with the basal infoldings (Fig. 15).

Posteroir midgut

The posterior midgut (PMG) tails off from the posterior bulb. It measures 4.1 cm long and is two-thirds the length of the whole midgut (measuring 6.1 cm in total length in the average adult female, Fig. 1). It is a narrow tube of pale wite colour.

The posterior midgut consists of columnar principal cells with one or two large, oval nuclei (Fig. 6). The brush border is relatively thin (Fig. 6).

Fig. 2. Transverse section of stomach. Light micrograph. Showing principal cells with brush border (bb), mineral spherites (s), nucleus (n), and a sheath (sh) of longitudinal muscles and circular muscles. ×400.

Fig. 3. Transverse section of tubular midgut. Light micrograph. Showing principal cells with brush border (bb), mineral spherites (s), nucleus (n), and a sheath (sh) of longitudinal muscles and circular muscles. A replacement cell (rc) is also shown. ×400.

Fig. 4. Transverse section of midgut bulb. Light micrograph. Showing principal cells with brush border (bb), nucleus (n), and a sheath (sh) of longitudinal muscles and circular muscles. Note pasty materials (p) and lipoid droplets (ld) in the bulb lumen. ×400.

Fig. 5. Transverse section of posterior bulb. Light micrograph. Showing principal cells with brush border (bb), nucleus (n), and a sheath (sh) of longitudinal muscles and circular muscles. Note filamentous materials (f) in the bulb lumen. ×400.

Fig. 6. Transverse section of posterior midgut. Light micrograph. Showing principal cells with brush border (bb), nucleus (n), and a sheath (sh) of longitudinal muscles and circular muscles. A replacement cell (rc) is also shown. ×400.

Fig. 7. Longitudinal section of principal cells of stomach. Showing 'fuzzy' surface coat (sc), microvilli (mv), mitochondria (m), vacuoles (v), mineral spherites (s), Golgi body (G), lipoid spheres (ls), rough endoplasmic reticulum (rer), nucleus (n), and desmosome (d). ×7200.

Fig. 8. High magnification of apical region of principal cell of stomach. Showing mitochondria (m), rough endoplasmic reticulum (rer), microvilli (mv) and a surface coat of vesicles (vs) and broken membranes (arrows). ×23750.

Fig. 9. High magnification of lipoid sphere (ls). Note Golgi body (G), lysosomes (ly), and mitochondria (m). ×13800.

Fig. 10. Longitudinal section of basal region of principal cell of stomach. Showing basement membrane (bm), mitochondria (m), and basal membranous infoldings (arrows). A replacement cell (rc) with nucleus (n) is also shown. ×7200.

Fig. 11. Longitudinal section of apical region of tubular midgut. Showing microvilli (mv) with surface coat (sc), mineral spherites (s), Golgi body (G), lysosomes (ly), multivesicular body (mb), mitochondria (m), vesicles (v), and desmosome (d). ×13800.

Fig. 12. Longitudinal section of apical region of midgut bulb. Showing microvilli (mv), with gut lumen filled with broken membranes (arrows) and paste-like material. Note dense bodies (db), mitochondria (m), and desmosome (d). ×9000.

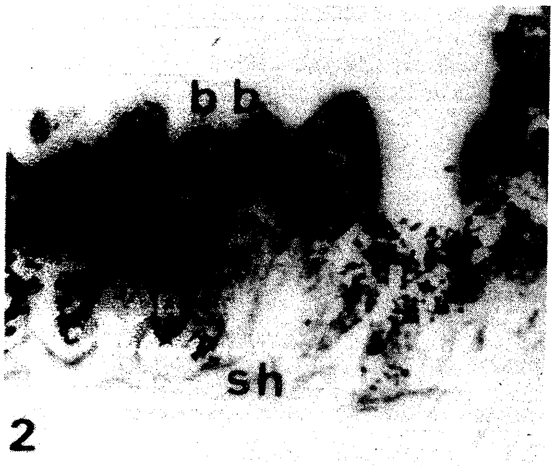
Fig. 13. Longitudinal section of basal region of midgut bulb. Showing numerous membranous invaginations (arrows), mitochondria (m) and basement membrane (bm). ×9000.

Fig. 14. Longitudinal section of apical region of posterior bulb. Showing microvilli (mv) and gut lumen filled with broken membranes (arrows). Note mitochondria (m), Golgi body (G), lysosome (ly), mineral spherites (s), vesicle (v) and desmosome (d). ×13800.

Fig. 15. Longitudinal section of basal region of posterior bulb. Showing numerous membranous invaginations (arrows) with mitochondria (m) associated with them. Note vesicles (v) and basement membrane (bm). ×7200.

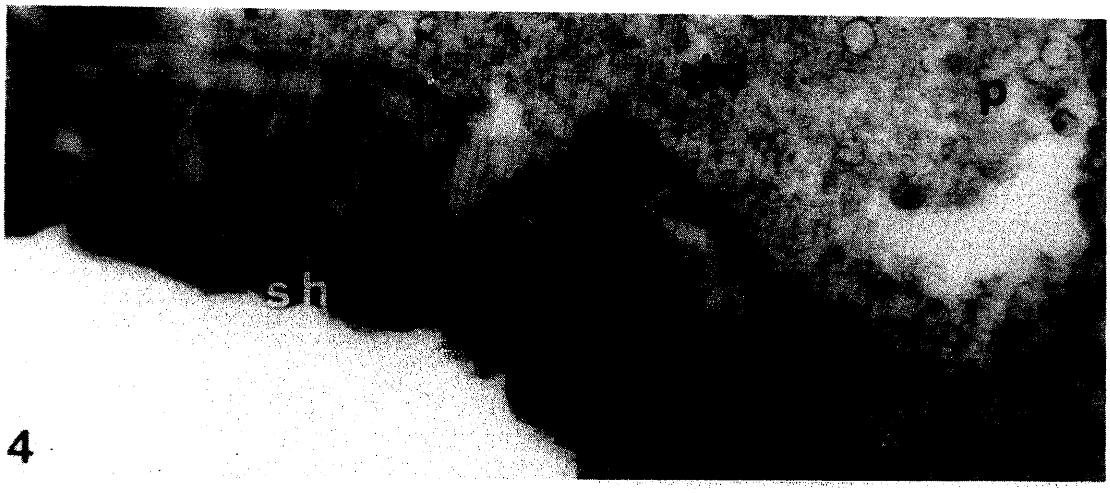
Fig. 16. Longitudinal section of apical region of posterior midgut. Showing bacteria (b) in gut lumen, microvilli (mv), lysosomes (ly), mitochondria (m), Golgi body (G), rough endoplasmic reticulum (rer), and nucleus (n). ×13800.

Fig. 17. Longitudinal section of basal region of posterior midgut. Showing membranous invaginations (arrows) with mitochondria (m), basement membrane (bm), and parts of a nucleus (n) and a lipoid sphere (ls). × 13800.



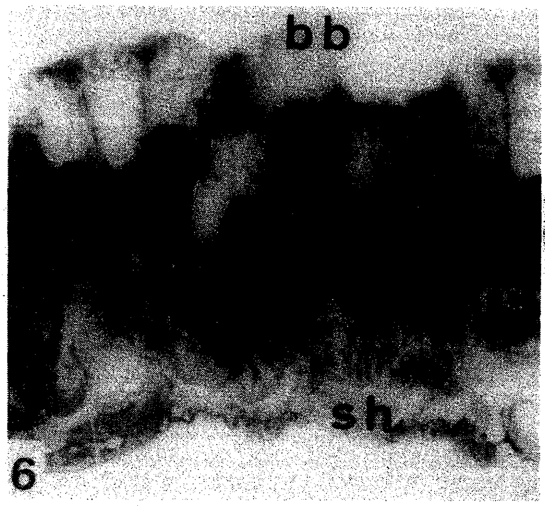
2

3

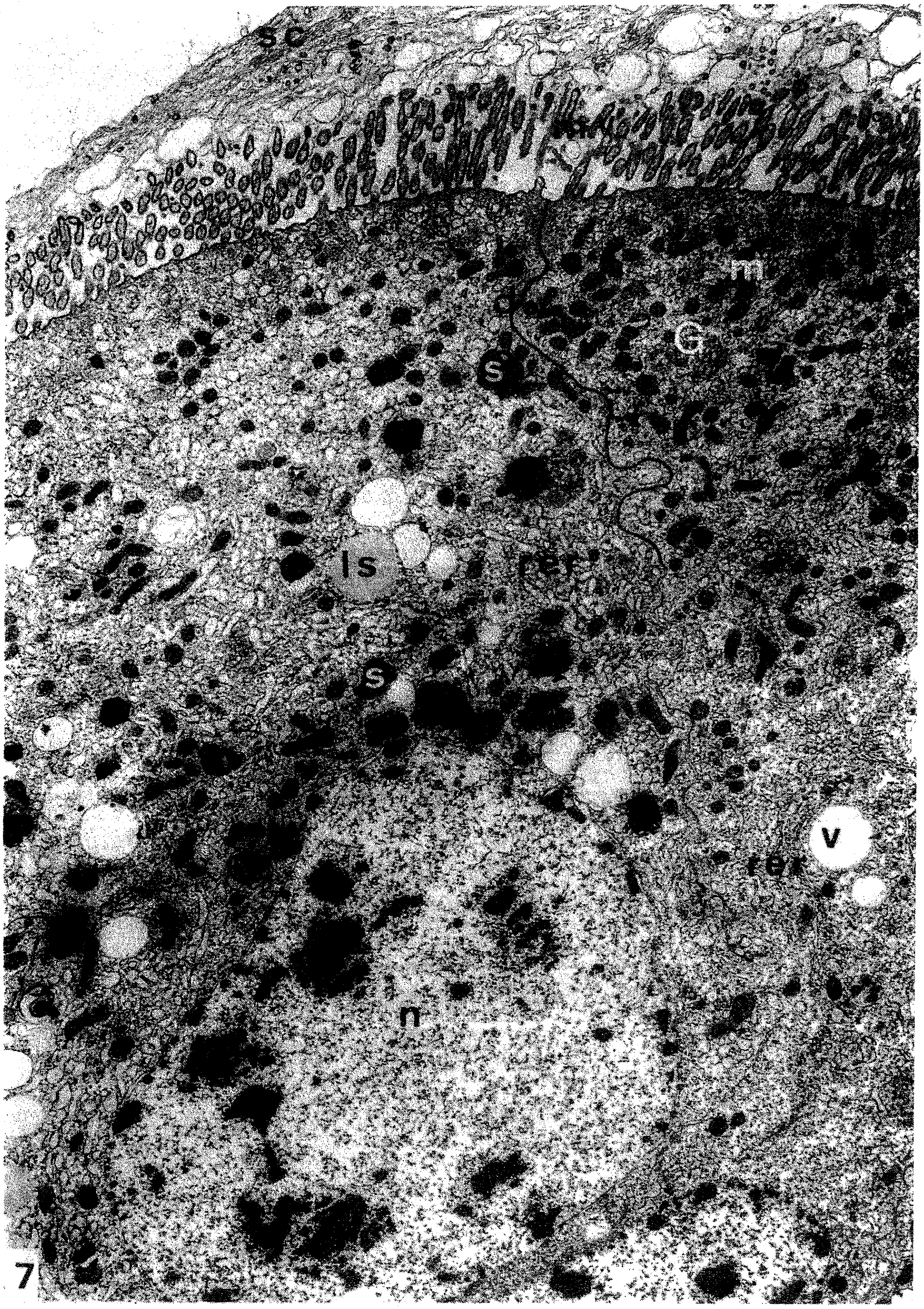


4

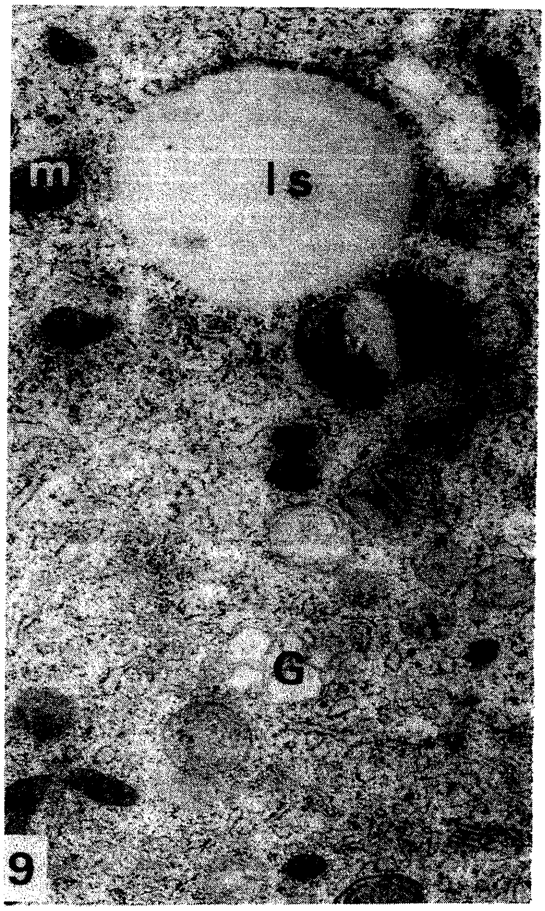
5

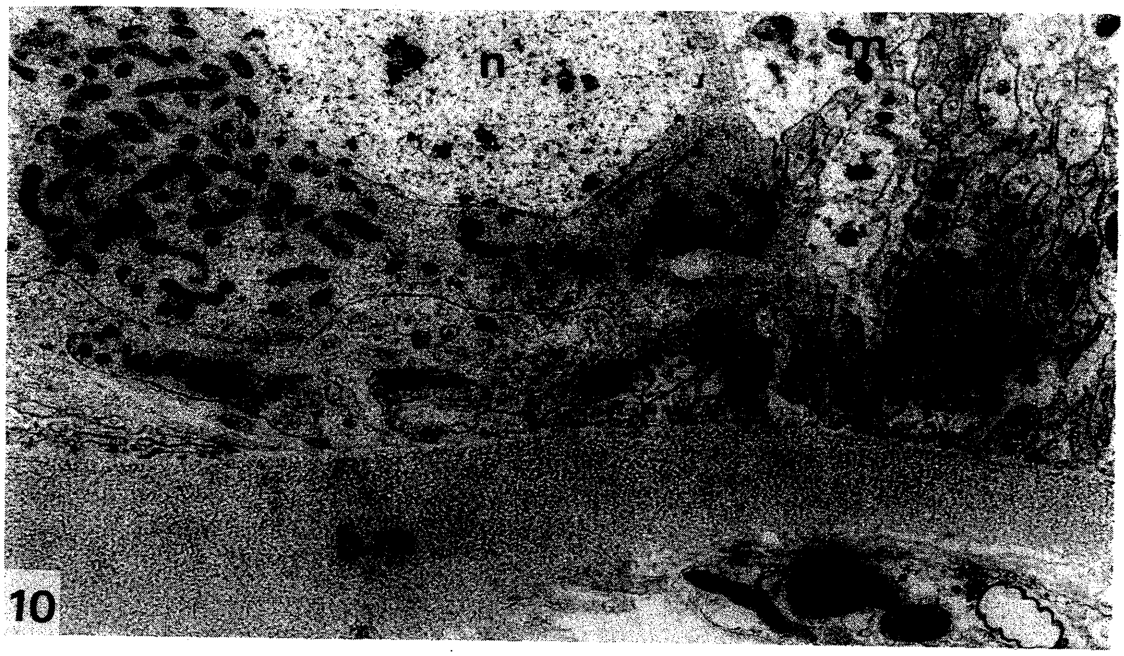


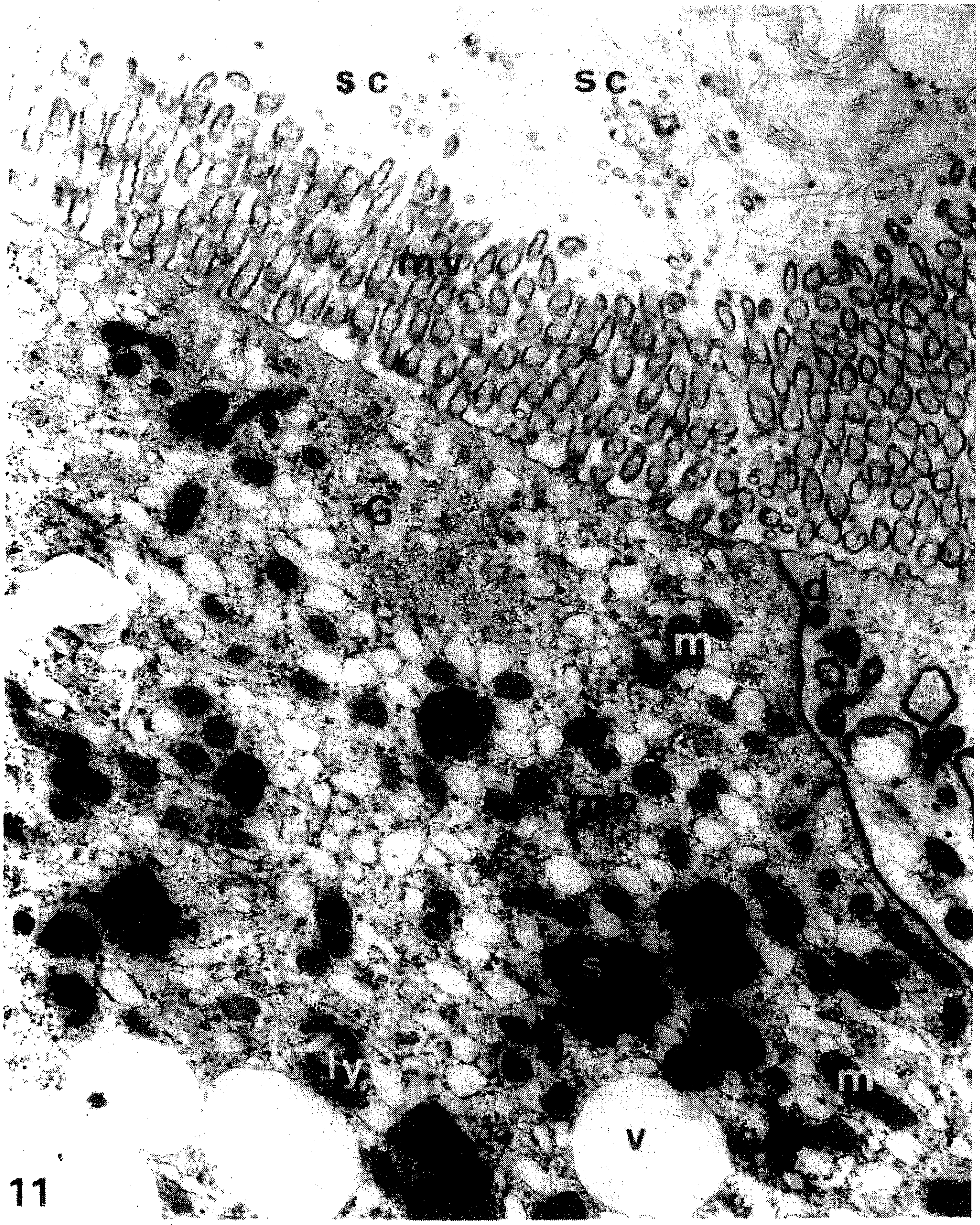
6


sc
m
G
s
ls
rer
s
v
rer
n
7

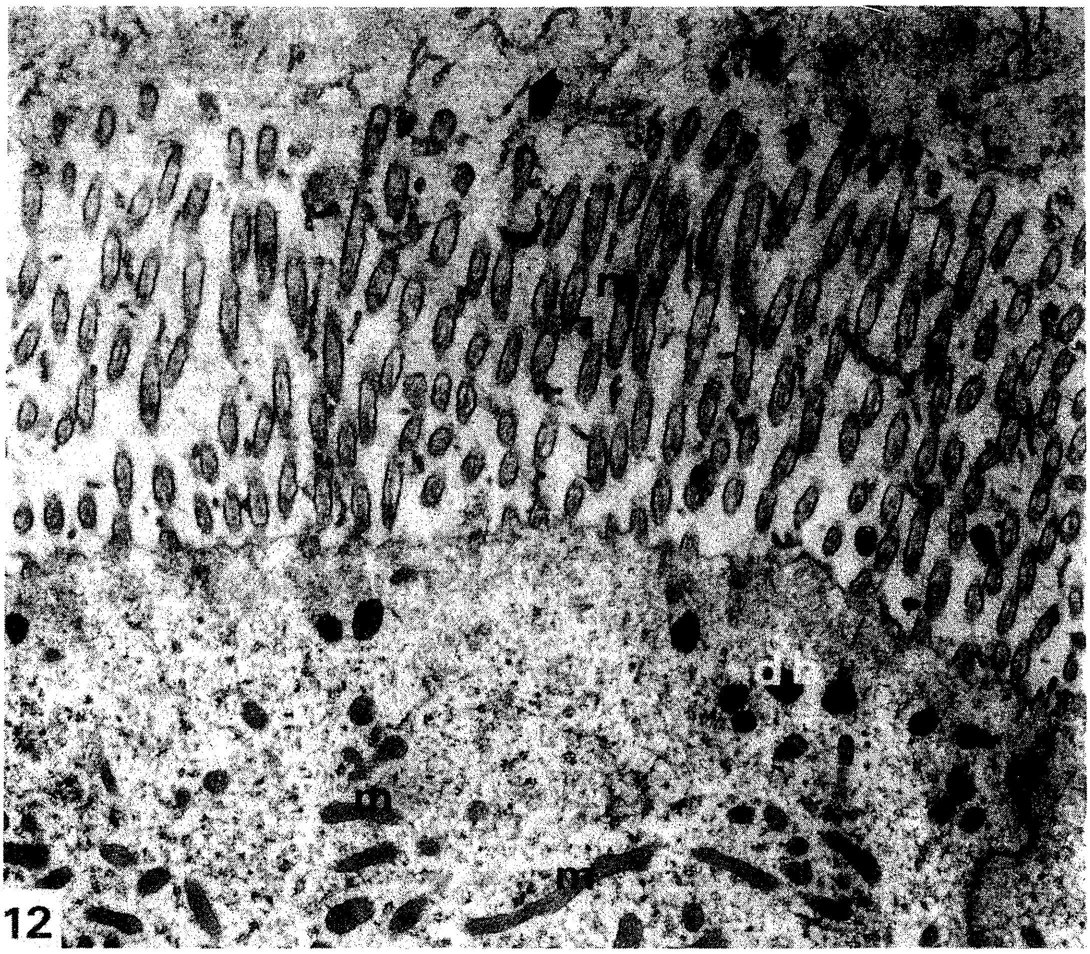

vs
mv
m
re
8


m
ls
G
9

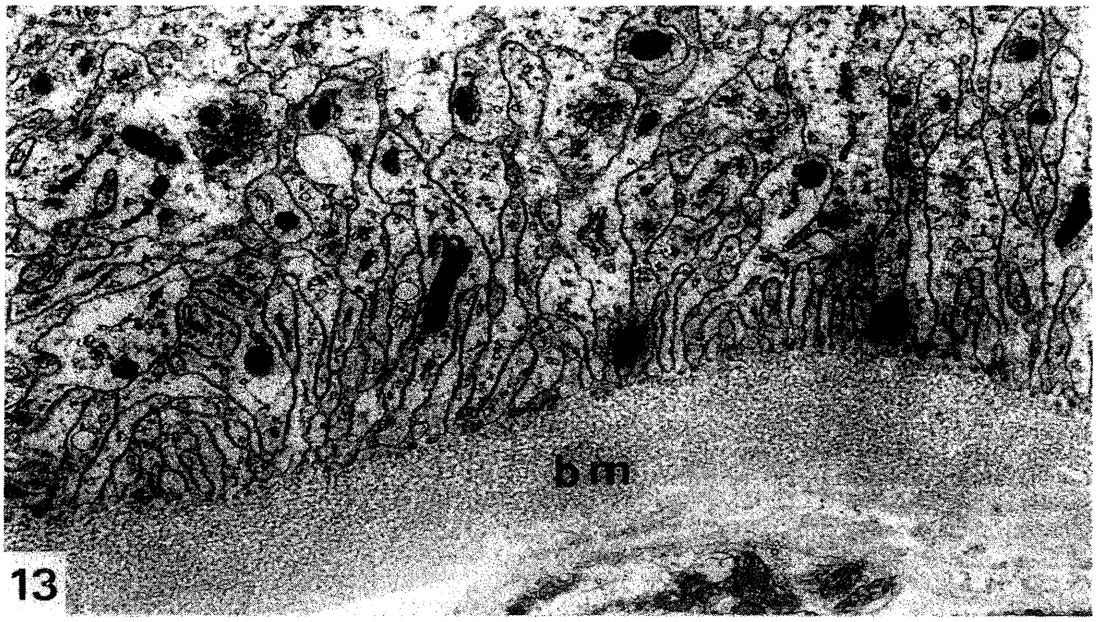

n
m
10



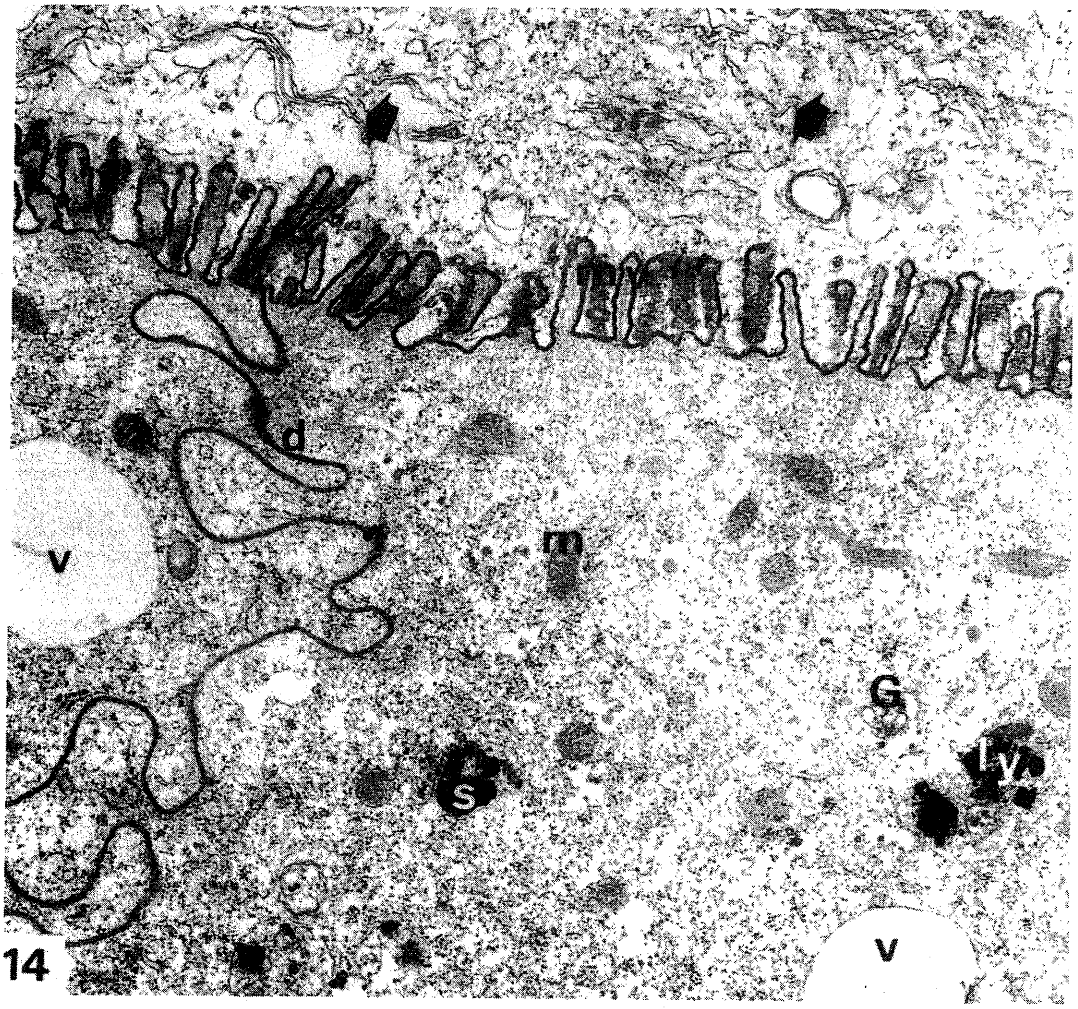
11



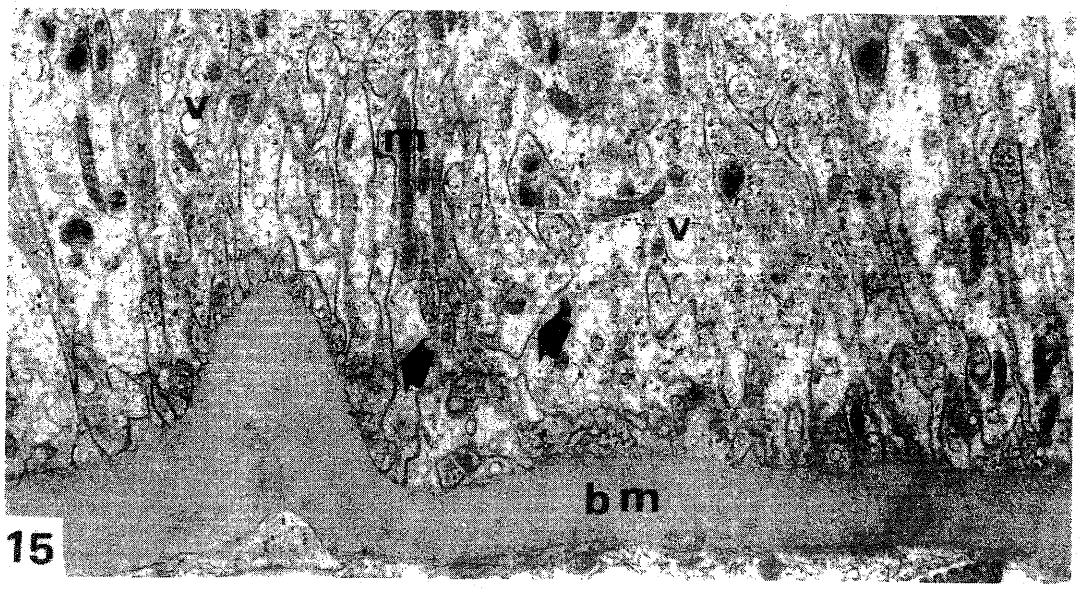
12



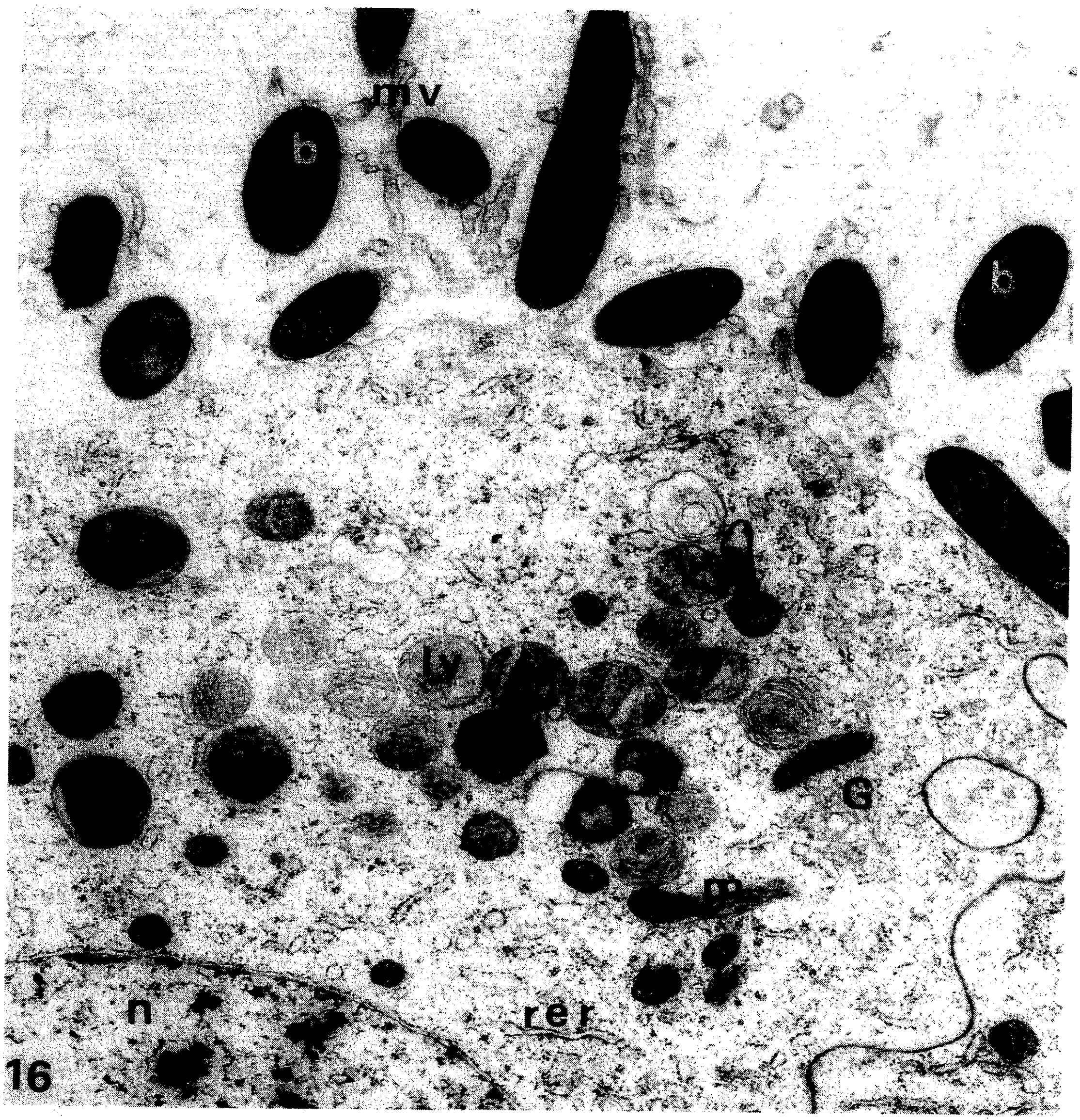
13

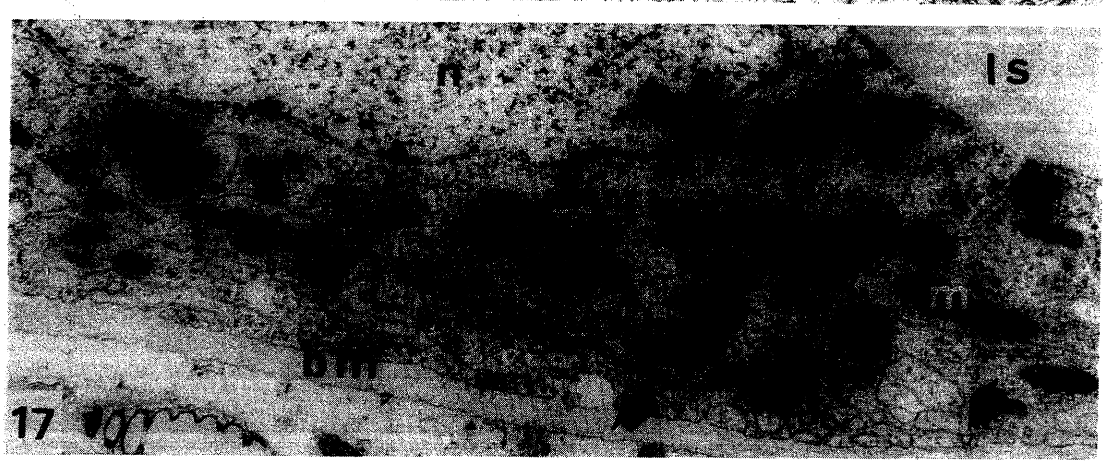


14



15


mv
b
b
ly
g
m
n
rer
16


n
ls
m
bm
17

Ultrastructure

In the electron microscope the lumen of the posterior midgut has many rod-like bacteria, measuring 3μ long and 1μ in diameter (Fig. 16).

The microvilli of the principal cells are sparse, and are of various lengths (Fig. 16). The apical cytoplasm has scattered mitochondria, lysosomes, Golgi bodies and rough endoplasmic reticulum (Fig. 16). Occasionally, lipoid spheres can also be seen (Fig. 17).

The basement membrane is 0.4μ thick. The basal plasma membrane invaginates irregularly to form some intracellullular sinuses (Fig. 17). Many mitochondria are associated with the basal plasma membrane.

DISCUSSION

The insect midgut carries out diverse functions such as enzyme secretion, digestion and absorption of nutrients, intermediary metabolism, and osmoregulation. Reviews of insect midgut functions are given by Smith (1968), Sud (1968), Berridge (1970) and Wigglesworth (1972).

Tessaratoma feeds on the young shoots and fruits of lychee and longan. The midgut is unique in that it is discontinuous between the midgut bulb and the posterior bulb.

The stomach has principal cells similar to columnar cells of midgut of other hemipterans such as cicadas and cercopids (Cheung and Marshall, 1973), the citrus stinkbug *Rhynchocoris* (Cheung, 1977), and the lantern bug *Pyrops* (Cheung and Marshall, 1972). Here both secretion of enzymes and absorption of simple nutrients are carried out. The presence of numerous mitochondria, vacuoles, lysosomes, Golgi bodies and rough endoplasmic reticulum suggest these functions.

The surface coat is less expensive than that of xylem sap feeding insects such as cicadas and cercopids (Cheung and Marshall, 1973) and the cambium sap feeding insect *Pyrops* (Cheung and Marshall, 1982).

Lipoid spheres are found in the stomach cells and other parts of the midgut. These are comparable to those in the citrus stinkbug *Rhynchocoris* (Cheung, 1977) and other hemipterans (Sud, 1968). It is interesting to note that both *Tessaratoma* and *Rhynchocoris* are mainly seed feeders (Cheung, unpublished observation). Thus, they may have excess fatty materials derived from seeds of their hosts. In *Dysdercus*, which is also a seed feeder, Sud (1968) called these lipoid spheres lipochondria. In contrast, xylem sap and cambium sap feeding hemipterans do not have these lipoid spheres (Cheung and Marshall, 1973, 1982).

Mineral spherites are found in the stomach and tubular midgut cells. These spherites are mainly lysosomal in origin, as minerals are seen to accumulate in lysosomes. The minerals sequestered are largely calcium, magnesium and phosphate (Cheung, unpublished observation). These spherites resemble those of the citrus stinkbug *Rhynchocoris* (Cheung, 1977), and other insects such as the housefly *Musca* (Sohal *et al.*, 1977) and the lantern bug *Pyrops* (Cheung and Marshall, 1982).

Basally, the principal cells of the stomach and tubular midgut have numerous membranous infoldings. Mitochondria are found to be associated with them. This ultrastructural feature suggests the function of absorption of nutrients (Berridge, 1970).

The basement membrane is unusually thick, of 1μ in thickness. In other hemipteran insects such as *Rhynchocoris*, it is less than 0.5μ thick (Cheung, 1977). The significance of this for *Tessaratoma* is not known. It may be conjectured that such thick basement membrane may safeguard the haemolymph from intoxicated with alkaloids present in lychee seeds.

The midgut bulb is spherical in shape and is a unique structure. The lumen is filled with a pasty material and some yellow lipoid droplets. The contents are insoluble in water and other organic solvents such as ethanol and xylene. The origin of the lipoid droplets is unknown. They may be derived directly from the insect's food or secondarily as metabolites of midgut cells.

The midgut bulb of *Tessaratoma* somewhat resembles that of *Piezosternum* (Goodchild, 1978). However, it is much larger in *Tessaratoma* and is discontinuous with the posterior bulb. It is tempting to suggest that the pasty materials of the *Tessaratoma* midgut bulb might possibly be derived from the ingested sap of lyche seeds and shoots, and could be some sort of precipitated alkaloids. This is in contrast to the bulb contents of *Piezosternum* (Goodchild, 1978), which are derived from digested caecal flora.

The midgut bulb cells have numerous mitochondria and dense bodies. There could be some absorption in this region. This is further evidenced by the presence of extensive membranous infoldings in the basal portion of midgut bulb cells.

The posterior bulb has extensive broken membranes in the lumen. Since this lumen is only continuous with the posterior midgut, the membranes could be derived from degenerating cells or digested bacterial flora from the posterior midgut. Indeed, Goodchild (1978) has found that digested bacterial flora are swept forward to the anterior part of the midgut of *Piezosternum*.

The posterior midgut in *Tessaratoma* is unusually long as compared to other gut regions. Unlike *Rhynchocoris* (Cheung, 1977) and *Piezosternum* (Goodchild, 1978), *Tessaratoma* has no gastric caecae. Under the electron microscope, a lot of symbiotic bacteria are found in the posterior midgut lumen of *Tessaratoma*.

The apical cytoplasm of the posterior midgut cells has abondant rough endoplasmic reticulum, Golgi bodies, mitochondria and lysosomes. This indicates that some sort of enzyme synthesis and release could occur here.

The enzymes could be released into the posterior midgut lumen so as to digest the symbiotic bacteria there. This process could liberate some vitamins and essential amino acids that are not synthesized by the insect itself, as suggested by Goodchild (1978) for *Piezosternum.*

Summing up, the midgut of *Tessaratoma* is rather unique. Its feeding habit associated strictly with lychee and longan hosts and the corresponding specialisation of the gut probably represent some sort of coevolution with these trees. It is interesting to note that a similar comparison also applies to the citrus stinkbug *Rhynchocoris* with citrus hosts (Cheung, unpublished observation).

REFERENCES

BERRIDGE, M. J. (1970) A structural analysis of intestinal absorption. In *Insect ultrastructure* (A. C. Neville ed.) Blackwell, Oxford.

CHEUNG, W. W. K. (1977) Ultrastructural and func tional differentiation of the midgut of the citrus stinkbug *Rhynchocoris serratus* Don. (Heteroptera: Pentatomidae). *J. Chinese Univ., Hong Kong* **4**: 339–354.

CHEUNG, W. W. K. and A. T. MARSHALL (1973) Studies on water and ion transport in homopteran insects: ultrasturcture and cytochemistry of the cicadoid and cercopoid midgut. *Tissue and Cell* **5**: 651–669.

CHEUNG, W. W. K. and A. T. MARSHALL (1982) Ultrastructural and functional differentiation of the midgut of the lantern bug, *Pyrops candelaria* Linn. (Homoptera: Fulgoridae). *Cytologia* **47**: 325–339.

GOODCHILD, A. J. P. (1963) Some new observations on the internal structures concerned with water disposal in sap-sucking Hemiptera. *Trans. Roy. Entomol. Soc. London* **115**: 217–237.

GOODCHILD, A. J. P. (1966) Evolution of the alimentary canal in the Hemiptera. *Biol. Rev.* **41**: 97–140.

GOODCHILD, A. J. P. (1978) The nature and origin of the midgut contents in a sap-sucking heteropteran, *Piezosternum calidum* Fab. (Tessaratominae) and the role of symbiotic bacteria in its nutrition. *Entomologia, Exp. and App..* **23**: 177–188.

HOOD, C. W. (1937) The anatomy of the digestive system of *Oncopeltus fasciatus* Dall. (Hemiptera: Lygaeidae). Ohio J. Sci. **37**: 151–160.

KERSHAW, J. C. (1907) Life history of *Tessaratoma papillosa* Thun. *Trans. Roy. Entomol. Soc. London* **2**: 253–258.

MALOUF, N.S.R. (1933) Studies on the internal anatomy of the stinkbug, *Nezara viridula*. *Bull. Roy. Entomol. Soc. Egypt* **17**: 96–118.

MIYAMOTO, S. (1961) Comparative morphology of alimentary organs of Heteroptera, with phylogenetic considerations. *Sieboldia* **2**: 197–259.

RICHARDS, O.W. and R.G. DAVIES (1977) *Imms' general textbook of Entomology*. 10th ed. Chapman and Hall, London.

SAXENA, K.N. (1955) Studies in the passage of food, hydrogen ion concentration and enzymes in the gut and salivary glands of *Dysdercus koenigii* Fabr. (Heteroptera: Pyrrhocoridae). *J. Zool. Soc. India* **7**: 145–154.

SMITH, D.S. (1968) *Insect cells, their structure and function*. Oliver and Boyd, Edinburgh.

SOHAL, R.S., P.D. PETERS and T.A. HALL (1977) Origin, structure, composition, and age-dependence of mineralized dense bodies (concretions) in the midgut epithelium of the adult housefly *Musca domestica*. *Tissue and Cell* **9**: 87–102.

SUD, B.N. (1968) The midgut epithelium of insects. In *Cell structure and its interpretation* (S.M. McGee-Russell and K.F.A. Ross eds.) Arnold, London.

WIGGLESWORTH, V.B. (1972) *The principles of insect physiology*. 7th ed. Chapman and Hall, London.

荔枝椿象中腸之顯微光鏡與電鏡觀察

張偉權　黎麗明

荔枝椿象之中腸可劃分爲五部分：胃、管形中腸、中腸球、後球和後中腸。以上所列中腸每一部分都有獨特之上皮細胞及超微結構。胃細胞含有極多粒線體、空泡、脂肪球、內質網，及礦質圓體。基膜呈現極多皺摺。管形中腸細胞基本上與胃細胞有相似之細微結構，但脂肪球比較少。中腸球是圓形狀，其細胞只含有少許顆粒之內質網，球體中腔充滿着黏糊狀物質及若干黃色脂肪小珠，這些物質不能溶於水及普通有機溶劑。後球細胞含有少量粒線體及礦質圓體。後球中腔的線狀物質爲破碎之質膜。後中腸細胞含有稀少之毛狀突物，其中腔有許多桿狀細菌。此文旨在討論中腸各部分之可能功能與其獨特之形態。