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Ultrastructural and Functional Differentiation of the Midgut of the Lantern Bug, *Pyrops candelaria* Linn. (Homoptera: Fulgoridae)

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Anatomical studies on hemipteran alimentary canals often reflect the type of diet or plant part (majority being phytophagous) fed on by the insects concerned (Goodchild 1963, 1966). Cicadas and cercopids, which feed on xylem sap, have the most intricate type of gut, i.e. the presence of a filter chamber, for short-circuiting the excess water taken in (Cheung and Marshall 1973a, b, Marshall and Cheung 1974).

In contrast to the cicadas and cercopids, phytophagous hemipterans that feed on seeds allow water to be transferred back to the haemolymph in the rectum for water conservation. These insects usually have a simple, straight gut (Berridge 1965, Goodchild 1966).

Thus, the hemipteran insects mentioned above are on opposite ends of the feeding spectrum, ranging from an extremely high water content diet to a diet of very low water content. Somewhere in the middle of the spectrum, there are insects that feed on plant tissue with a moderate water content. An example of these insects is the lantern bug, *Pyrops candelaria* (formerly known as *Fulgora candelaria*) which feeds on the sap of cambium tissue (Marshall and Cheung 1975).

Preliminary observations on the anatomy and histology of the alimentary canal of fulgorid insects have been carried out by Goodchild (1963, 1966). The plexiform surface coat of the midgut of *Pyrops* have been reported by Marshall and Cheung (1970). The fine structure of the rectum and the malpighian tubules have been described by Cheung (1979, 1981).

The objective of the present study is to find out whether there might be any functional differentiation in the fine structure and cytochemical properties of the midgut. The information obtained may be useful in understanding how the fulgorid gut performs hydromineral regulation in relation to its habit of cambium feeding.

Materials and methods

Adults of *Pyrops* (*Fulgora*) *candelaria* L. were collected from Lychee and Longan groves in the New Territories of Hong Kong Colony. Dissections were made in 0.2 M phosphate buffer, pH 7.2, and pieces of *Pyrops* 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 Porter-Blum ultrotome II or Reichart ultrotome and were stained with uranyl nitrate or acetate and lead citrate. Sections were observed in a JEOL JEM120 of Zeiss EM 9S-2 electron microscope. Thick sections for light microscopy were also cut with a Porter-Blum ultrotome II or Reichart ultrotome and were stained in 1% toluidine blue in 1% borax.

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

For cytochemical studies, tissues were fixed in the appropriate fixatives and subjected to the following tests according to Pearse (1968): Alcian blue (pH 3.0) and toluidine blue for mucoprotein, acid solochrome cyanine for proteins, PAS for glycogen (with diastase digestion as control), Sudan black B for lipids, calcium, magnesium, phosphate and uric acid tests.

Tests for enzymes which are associated with transport and metabolism include α-glucosidase (Rutenburg *et al.* 1960), alkaline phosphatase (Fredricsson 1952), ATPase (Wachstein and Meisel 1957), glucose-6-phosphatase (Wachstein and Meisel 1956), acid phosphatase (Barka and Anderson 1963), leucylaminopeptidase (Nachlas *et al.* 1957), β-glucuronidase (Seligman *et al.* 1954), cytochrome oxidase (Culling 1963) and succinic dehydrogenase (Barka and Anderson 1963). Tests were carried out on freeze-substituted paraffin sections (10 μ on a rotary microtome) or fresh whole gut.

Observations

The midgut of *Pyrops* is a coiled mass. It is enclosed by an intestinal sheath or midgut sheath and has an anterior diverticulum extending forwards (Fig. 1). The whole midgut is tubular in structure, of 120–140 mm length in the female. It shows no variation in width along its length in gross morphology, both in young and old adults (old adults are judged by the maturity state of the ovaries or testes).

For the sake of convenience in this study, the whole midgut is arbitrarily divided into three regions of approximately the same lengths, namely, the anterior midgut, the mid midgut, and the posterior midgut since they differ in histology, ultrastructure and cytochemical properties. The midgut sheath and the anterior diverticulum are dealt with elsewhere (Cheung, unpublished data).

Anterior midgut

The anterior region of the midgut consists of a one-cell thick epithelium of large, binucleate columnar cells. Each columnar cell has a conical apical region having a thick brush border of 2 μ in thickness (Fig. 2). In addition, thread-like structures extend from the brush border, forming an extensive surface coat of another 2–4 μ thick projecting into the gut lumen (Fig. 2).

Replacement cells with prominent nuclei are scattered among the columnar cells or principal cells (Fig. 2). Also, a thin propria tunica of circular and longitudinal muscles surrounds the midgut epithelium (Fig. 2). Frequently oenocytes

with large nuclei, and tracheoles, are found outside the epithelium (Fig. 2).

Ultrastructure

The apical brush border consists of microvilli of 2 μ in height (Figs. 5, 6). The tips of the microvilli are covered with a "fuzz" of filaments and granules (Figs. 5, 6). This is the plexiform surface coat as earlier described by Marshall and Cheung (1970).

The apical portion of anterior midgut cells has well-developed rough endoplasmic reticulum, abundant mitochondria, Golgi bodies, and some lytic bodies or lysosomes (Fig. 5). The nucleus is disc-shaped and irregular in outline. It has dark chromatin materials (Fig. 5).

The basal portion of anterior midgut cells has numerous irregular intracellular sinuses, with mitochondria associated with them (Fig. 7). The basement membrane, which is of the amorphous type, is 0.5 μ thick (Fig. 7).

In Fig. 7 a replacement cell with a prominent nucleus is also shown. There are mitochondria and, occasionally, vacuoles present in the cytoplasm (Fig. 7).

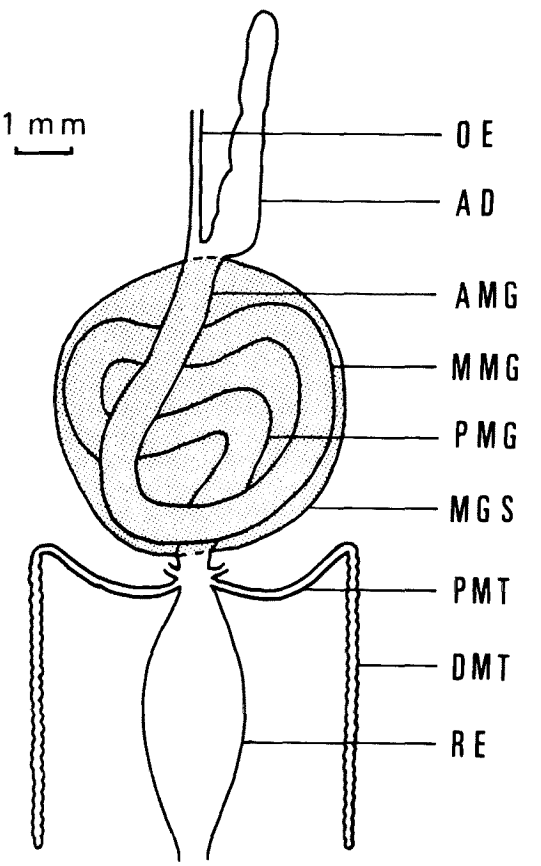
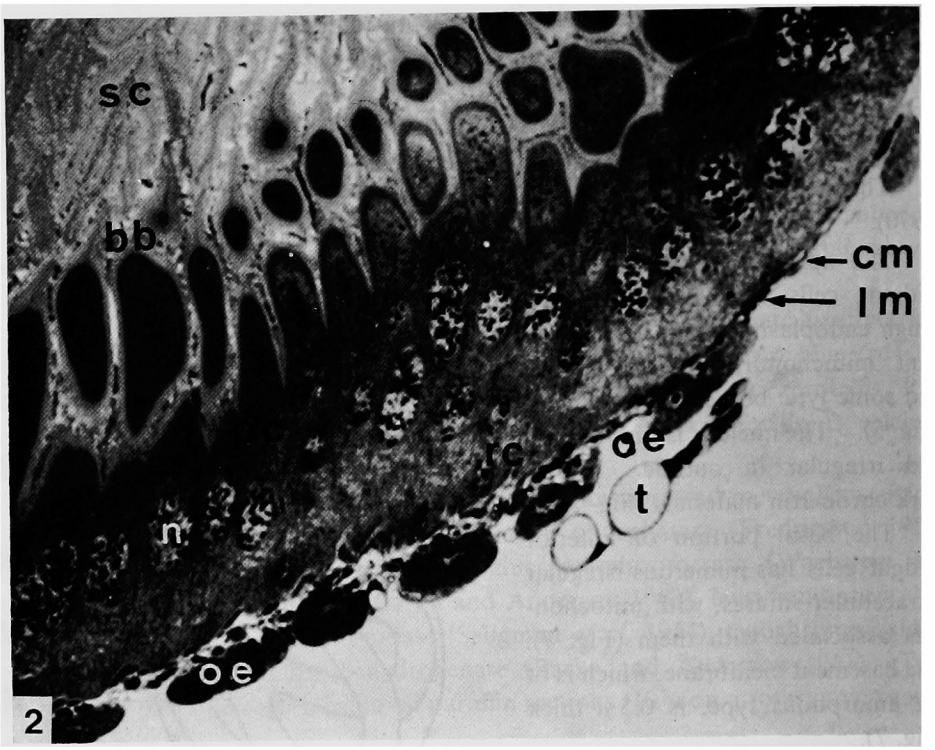


Fig. 1. Diagram of the gut of *Pyrops*. AD, anterior diverticulum; AMG, anterior midgut; DMT, distal malpighian tubule; MGS, midgut sheath; MMG, mid-midgut; OE, oesophagus; PMG, posterior midgut; PMT, proximal malpighian tubule; RE, rectum.

Cytochemistry

The brush border of the anterior midgut stains positively with alcian blue and toluidine blue, with the latter giving intense γ-metachromasia. It also stains positively with PAS reaction, acid solochrome cyanine and Sudan black B tests. Investigations on enzymes such as α-glucosidase, alkaline phosphatase, ATPase leucylaminopeptidase indicate strong positive reactions at the apical cytoplasm. Tests for respiratory enzymes such as glucose-6-phosphatase, cytochrome oxidase and succinic dehydrogenase yield positive results in the ground cytoplasm.

Table 1 summarises results of cytochemical tests on the midgut cells of *Pyrops*.



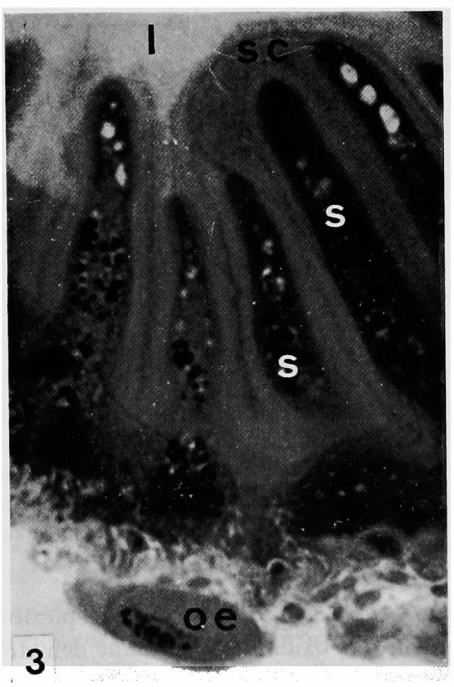


Fig. 2. Transverse section of anterior midgut. Light micrograph of araldite section. Showing principal cells (pc) covered with an extensive surface coat (sc) on the brush border (bb). The nuclei (n) of principal cells (pc) and replacement cells (rc) are visible. Tracheoles (t), oenocytes (oe), circular muscles (cm) and longitudinal muscles (lm) surround the epithelium. ×400.

Fig. 3. Transverse section of mid midgut. Light micrograph of araldite section. Showing principal cells with numerous osmiophilic granules of spherites (s). The surface coat (sc) extends into the lumen (l). A large oenocyte (oe) is visible. ×400.

Fig. 4. Transverse section of posterior midgut. Showing clublike principal cells (pc) with oval nuclei (n). ×400.

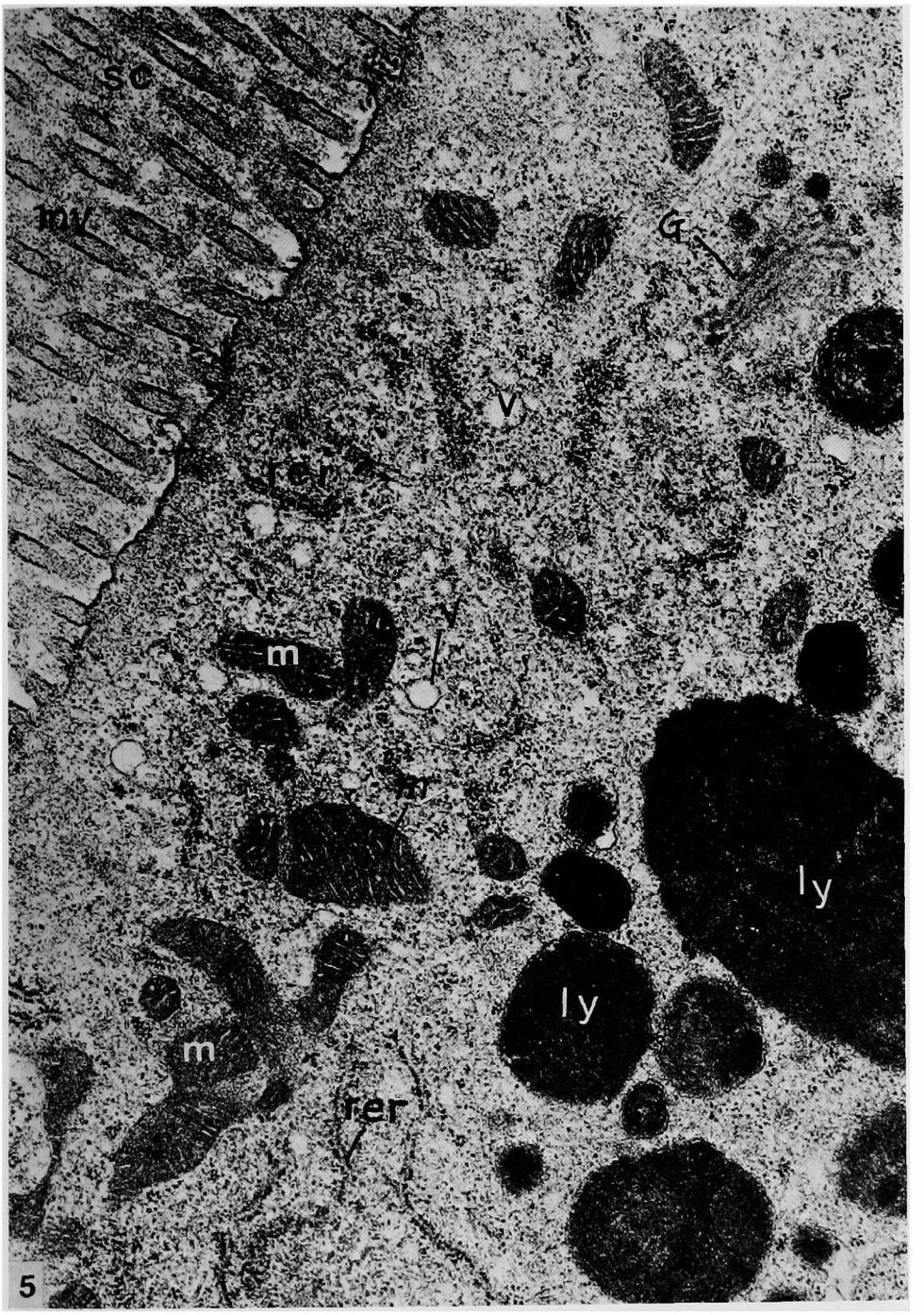
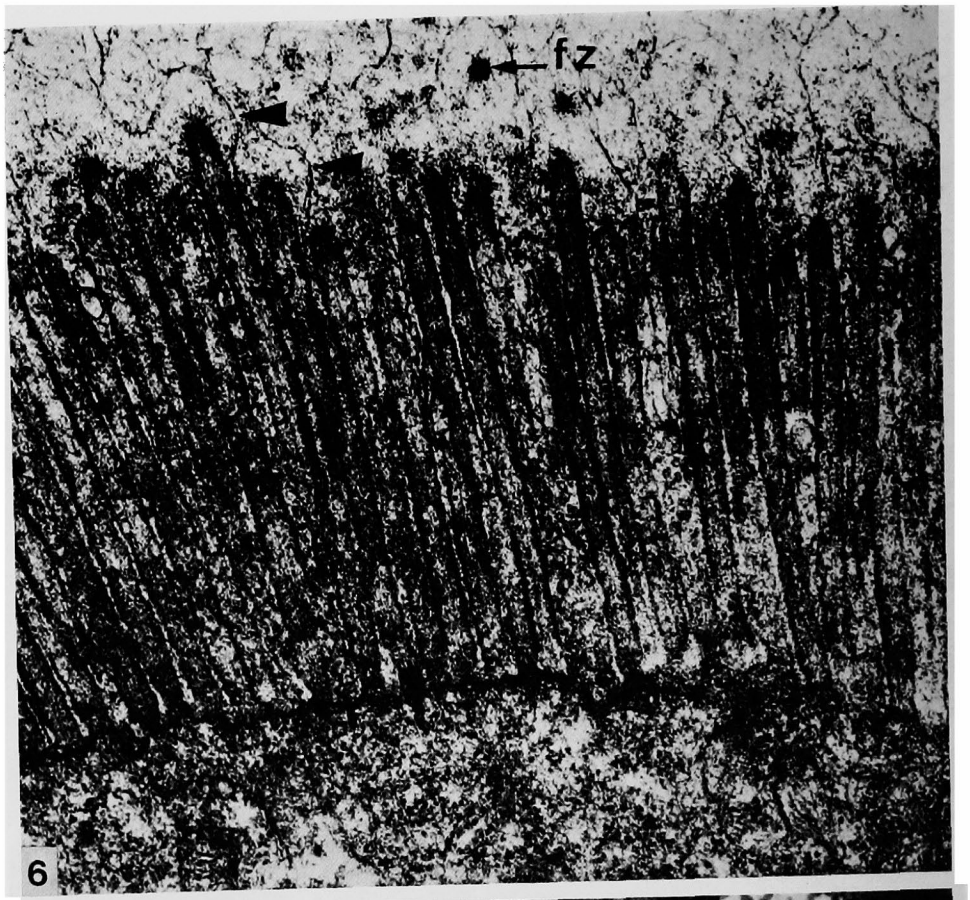


Fig. 5. Longitudinal section of principal cells of anterior midgut. Showing surface coat (sc), microvilli (mv), rough endoplasmic reticulum (rer), lysosomes (ly), mitochondria (m), vesicles (v) and Golgi body (G). ×27,900.



Figs. 6–7. 6, apical region of principal cell of anterior midgut. Showing microvilli (mv) ensheated by membranes (arrow heads) and fuss-bearing vesicles (fz). ×36,750. 7, basal region of principal cell of anterior midgut. Showing basement membrane (bm), mitochondria (m), and intracellular sinuses (is). A replacement cell (rc) with nucleus (n) and mitochondria (m) can also be seen. ×12,500.

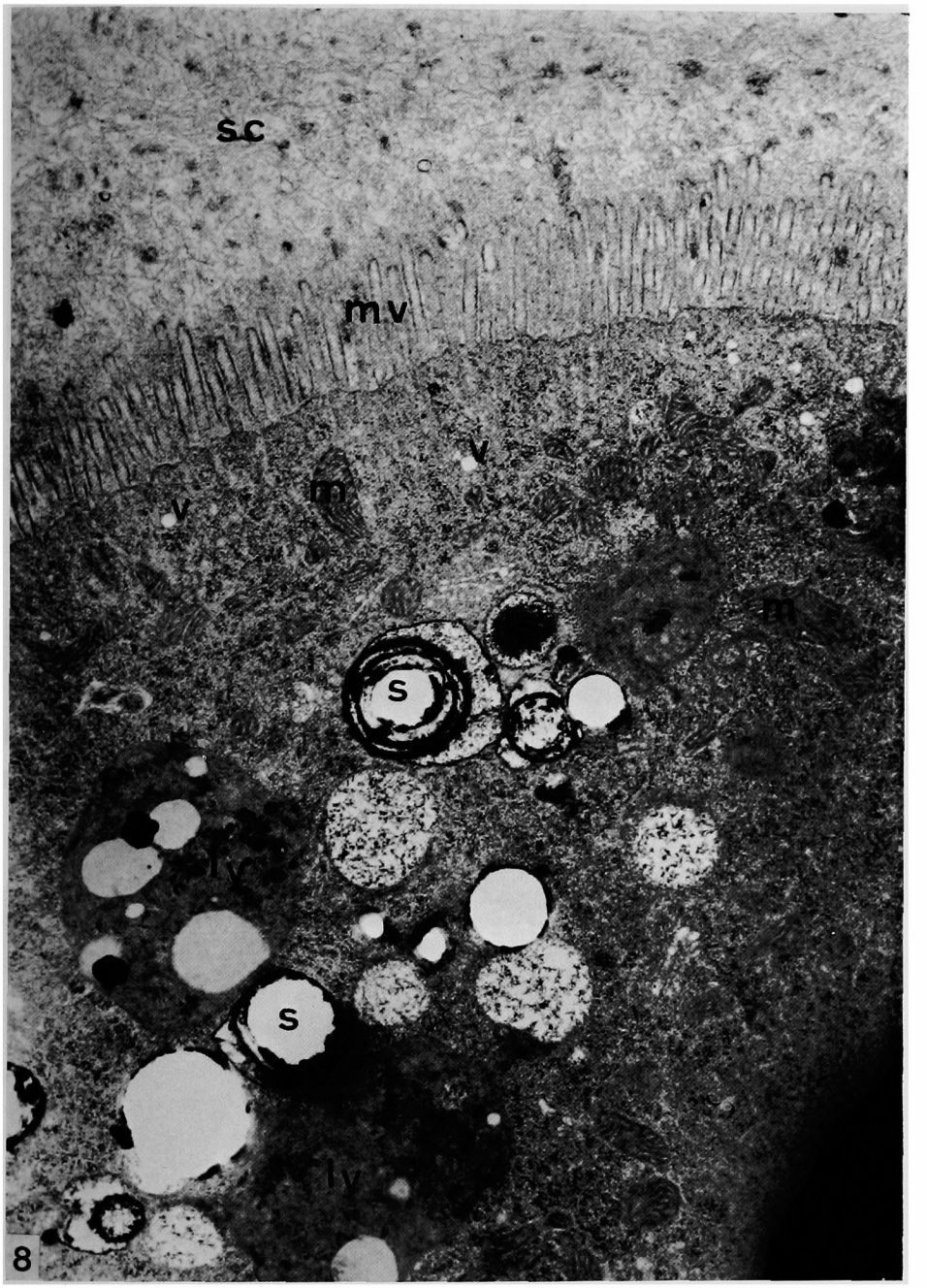
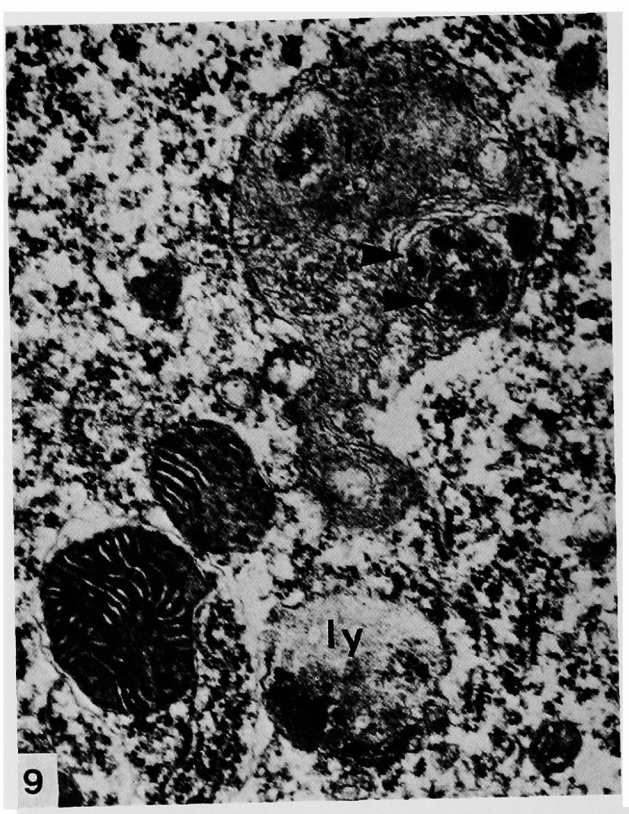
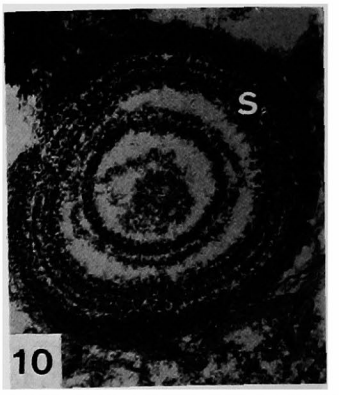
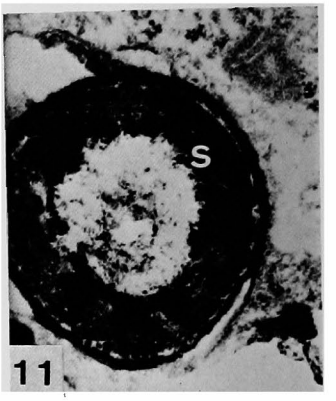


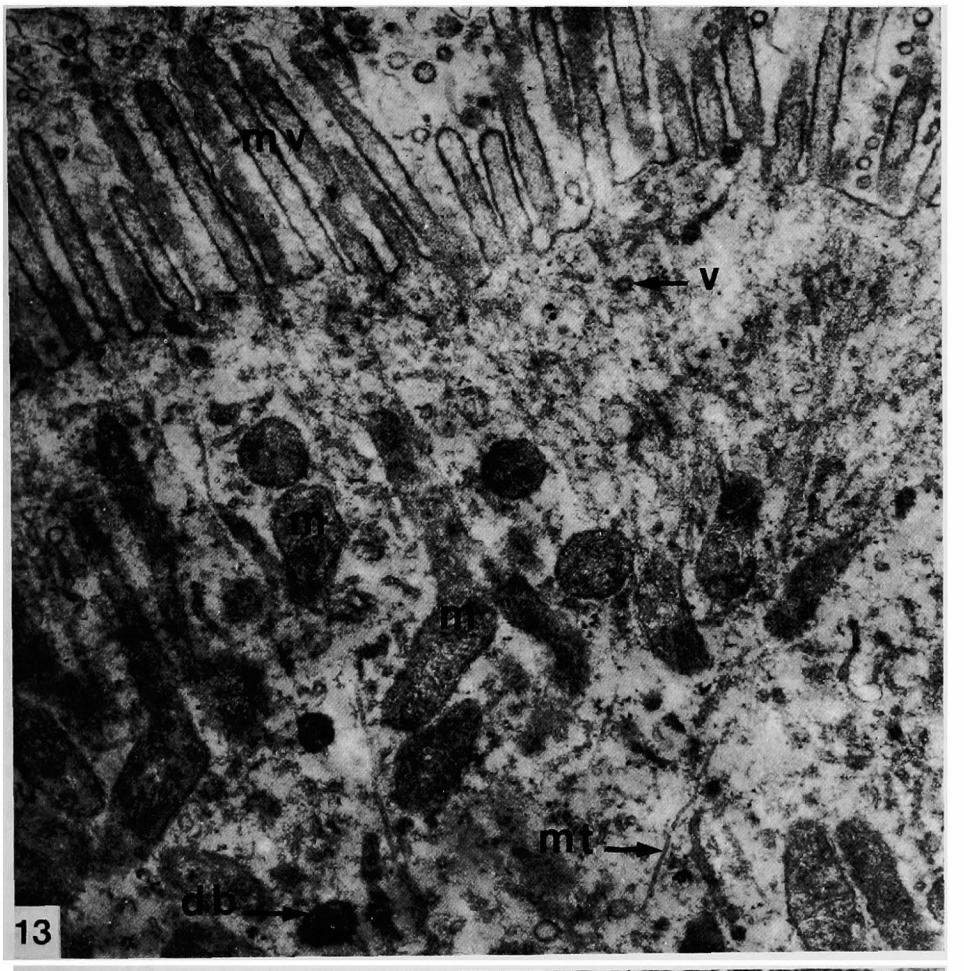
Fig. 8. Apical region of principal cell of mid midgut. Showing microvilli (mv), surface coat (sc), mitochondria (m), vacuoles (v), lysosomes (ly), and osmiophilic granules or spherites (s) which drop off in sections. ×17,250.

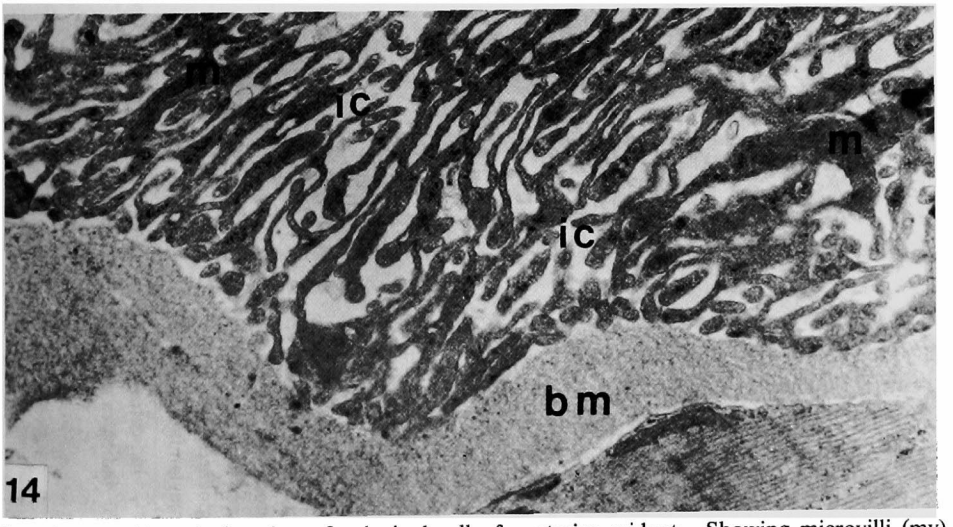






Figs. 9–12. 9, high magnification of a lysosome (ly) undergoing mineralization (arrow heads point to osmiophilic deposits). ×37,500. 10–12, high magnifications of osmiophilic granules or spherites showing various degrees of mineralization. Completely mineralized spherites drop off in sections. ×37,500.





Figs. 13–14. 13, apical region of principal cell of posterior midgut. Showing microvilli (mv), mitochondria (m), dense body (db), vesicles (v) and microtubules (mt). ×33,750. 14, basal region of principal cell of posterior midgut. Showing basement membrane (bm), and intracellular channels (ic) with mitochondria (m) associated with them. ×17,250.

Table 1. Cytochemical tests on the midgut cells of *Pyrops*

	Anterior midgut cells			Mid midgut cells			Posterior midgut cells		
	Microvilli	Apical cytoplasm	Basal cytoplasm	Microvilli	Apical cytoplasm	Basal cytoplasm	Microvilli	Apical cytoplasm	Basal cytoplasm
Toluidine blue	++++	0	0	++++	0	0	+++	0	0
Alcian blue	+++	+	0	+++	0	0	+++	0	0
PAS	++++	0	0	++++	+	0	+++	0	0
Acid solochrome cyanine	++++	+++	+	++++	+	+	+++	+	0
Sudan black B	+++	+	+	+++	+	+	++	+	+
Calcium	0	0	0	0	+	+	0	0	0
Magnesium	0	0	0	0	+	+	0	0	0
Phosphate	0	0	0	0	+	+	0	0	0
Uric acid	0	0	0	0	0	0	0	0	0
α-glucosidase	++	++	0	+	+	0	0	0	0
Alkaline phosphatase	+++	+++	0	+	+	0	0	0	0
ATPase	0	+++	++	0	+	++	0	+	+++
Glucose-6-phosphatase	0	+++	+++	0	++	+++	0	++	+++
Acid phosphatase	0	+	0	0	++	0	0	0	0
Cytochrome oxidase	0	+++	+++	0	++	++	0	++	+++
Succinic dehydrogenase	0	+++	+++	0	++	++	0	++	+++
β-glucuronidase	0	+	0	0	+	0	0	0	0
Leucylaminopeptidase	+++	+++	+	+	0	0	0	0	0

'+' indicates positive reaction, the number of +'s being proportional to the intensity of the reaction.
'0' indicates a negative reaction.

Mid midgut

In the light microscope, the mid midgut consists of tall columnar cells resembling those of the anterior midgut in gross morphology (Fig. 3). In old adults, there are numerous osmiophilic granules or spherites inside the cells (Fig. 3).

Ultrastructure

The mid midgut cells have relatively less endoplasmic reticulum than the anterior midgut cells. There are abundant mitochondria, vesicles, and lysosomes (Fig. 8).

Many lysosomes have granular deposits in them; this is particularly so for old adults (Figs. 8, 9). Old lysosomes are completely 'mineralized' and become spherites which drop off during sectioning (Fig. 8). The sizes of spherites vary from 0.1 μ diameter to as big as 5 μ in diameter (Figs. 3, 8). Figures 9–12 show the possible changes of mineralized lysosomes into spherites with concentric myelin figures, and eventually highly osmiophilic spherites.

Cytochemistry

The brush broder of the mid midgut has the same cytochemical properties as that of the anterior midgut. The ground cytoplasm stains weakly positive with PAS, acid solochrome cyanine and Sudan black B tests. Investigations on the granules or spherites in the mid midgut give positive results for tests of calcium, magnesium and phosphate. Respiratory enzymes such as glucose-6-phosphatase, cytochrome oxidase and succinic dehydrogenase have also been detected in the ground cytoplasm.

Posterior midgut

The posterior midgut cells are low columnar cells, in which the basal zone is poorly developed, with an apical lobe extending into the gut lumen (Fig. 4). The brush border and the filamentous projections (plexiform surface coat) can also be seen in the light microscope (Fig. 4).

Ultrastructure

The microvilli are of 2 μ in height. There are numerous globules and filamentous materials in the surface coat (Fig. 13).

The apical cytoplasm has numerous mitochondria, microtubules and vacuoles (Fig. 13). Dense bodies are occasionally found (Fig. 13). There is little rough endoplasmic reticulum present (Fig. 13).

The columnar cells rest on a basement membrane of 0.5 μ in width. There are abundant intracellular channels invaginated from the basal plasma membrane (Fig. 14). Many mitochondria are seen to be associated with these basal infoldings (Fig. 14).

Cytochemistry

The brush border and the surface coat have the same cytochemical properties as those of the anterior and mid midgut. The apical cytoplasm yields a negative

result with tests for α-glucosidase and leucylaminopeptidase. The ground cytoplasm, however, indicates positive results for tests of ATPase, glucose-6-phosphatase, cytochrome oxidase and succinic dehydrogenase.

Calcium, magnesium, and phosphate have not been detected in the posterior midgut cells.

Discussion

The major functions of the midgut epithelium in insects are the secretion of digestive enzymes, intermediary metabolism, ionic regulation, and the absorption of the products of digestion (Smith 1968, Berridge 1970, Wigglesworth 1972). Ultrastructural and functional differentiation occurs in the midgut in order to deal with the above processes (Filshie *et al.* 1971, Beadle 1972, Cheung and Marshall 1973a, Cheung and Low 1975, Cheung 1977).

The midgut of *Pyrops* is a simple tube, enclosed by a midgut sheath. The midgut presumably has to perform all the essential functions of insect midgut in general as mentioned.

The anterior midgut has columnar cells that are typical of many homopteran insects such as cicadas and cercopids (Cheung and Marshall 1973a). Here both secretion of enzymes and absorption of simple nutrients are carried out.

It is generally believed that enzymes are secreted at the apical region of columnar cells and are bound to the microvilli and the surface coat of columnar cells (Marshall and Cheung 1970, Cheung and Low 1975, Cheung 1977). The extensive surface coat of *Pyrops* is particularly important as a site of enzymatic digestion and may possibly act as a place for cation binding too (Marshall and Cheung 1970).

The presence of well developed rough endoplasmic reticulum, abundant Golgi bodies and mitochondria suggest enzyme synthesis and release (Berridge 1970, Marshall and Cheung 1970). Enzymes associated with digestion, metabolism and transport have been detected, notably α-glucosidase, leucylaminopeptidase, alkaline phosphatase, glucose-6-phosphatase, cytochrome oxidase and succinic dehydrogenase.

Basally, there are numerous infoldings forming intracellular sinuses. Mitochondria are found to be associated with them. This ultrastructural feature suggests the function of absorption of nutrients, and is comparable to that of the anterior midgut cells of many insects such as *Periplaneta* (Berridge 1970). *Gaeana* (Cheung and Marshall 1973a), *Protaetia* (Cheung and Low 1975) and *Rhynchocoris* (Cheung 1977).

The mid midgut cells have less endoplasmic reticulum than the anterior midgut cells. However, abundant lysosomes are found. In old adults many lysosomes are in the process of 'mineralization' to form spherites. Spherites have not been observed in young adults (Marshall and Cheung 1975). As the insect grows mature there is gradual accumulation of spherites in the midgut. This could be a form of storage excretion (Gouranton 1968, Cheung and Marshall 1973a, Sohal *et al.* 1977).

Spherites of similar structure have been reported in the midgut of many other insects, such as cicadas and cercopids (Cheung and Marshall 1973a), silkworm

(Waku and Sumimoto 1974, Turbeck 1974) and housefly (Sohal *et al.* 1977). In cicadas and cercopids, spherites persist throughout the lifespan of the insects (Cheung and Marshall 1973a), but in housefly spherites are only found with increasing age of the adult (Sohal *et al.* 1977).

In most insects, spherites are mainly found in the malpighian tubules, for example, in *Gryllus* (Berkaloff 1960), *Rhodnius* (Wigglesworth and Salpeter 1962), *Cenocorixa* (Jarial and Scudder 1970), *Musca* (Sohal 1974), *Periplaneta* (Wall *et al.* 1975) and *Arachnocampa* (Green 1979). This is also the case in *Pyrops* (1981), since malpighian tubules are the chief organs for the elimination of nitrogenous wastes and excess minerals (Wigglesworth 1972).

Sohal *et al.* (1977) have pointed out that spherites of the *Musca* midgut appear to originate mainly from dense bodies and lysosomes rather than from 'mineralization' of mitochondria, which is the case in *Rhodnius* malpighian tubles (Wigglesworth and Salpeter 1962) and *Pyrops* malpighian tubules (Cheung 1981). The present data support the view of Sohal *et al.* (1977), though these two processes and, possibly other ways of mineral sequestration, could occur together.

In general, the spherites of insect midgut and those of the malpighian tubules have similar major minerals present i.e. calcium, magnesium and phosphate, as studied cytochemically and by X-ray microanalyses in several insects (Cheung and Marshall 1973a, Waku and Sumimoto 1974, Turbeck 1974, Sohal *et al.* 1976, 1977, Green 1979).

Sohal *et al.* (1977) have found that the spherites of *Musca* midgut sequester more copper than those of the malpighian tubules. Also, there is absence of uric acid in the spherites of insect midgut (Gouranton 1968, Cheung and Marshall 1973a, Sohal *et al.* 1977), but uric acid is generally present in most insect malpighian tubules (Berkaloff 1958, Wessing and Eichelberg 1975, Wall *et al.* 1975, Green 1979). These minor differences in chemical composition indicate that the spherites of insect midgut and those of the malpighian tubules perform slightly different types of minerals and solutes transport, and they could have different modes of origins.

The posterior midgut cells have a plexiform surface coat similar to that of the anterior and mid midgut cells, but it is less extensive. The apical cytoplasm has a row of polarized mitochondria, numerous microtubules and some scattered rough endoplasmic reticulum. Basally, the cells have abundant invaginations forming long, narrow intracellular channels extending deep into the luminal side.

This ultrastructural pattern shows that the posterior midgut cells resemble those of cicadas and cercopids which are specialised for the secretion of ions from the haemolymph to the gut lumen (Cheung and Marshall 1973a).

Cytochemically, the posterior midgut cells stain positively with enzymes that are associated with transport and metabolism, namely ATPase, glucose-6-phosphatase, cytochrome oxidase and succinic dehydrogenase.

Thus, it many be postulated that the secretory functions of the posterior midgut cells may play an important role in ionic regulation. The posterior midgut, together with the midgut sheath, the malpighian tubles and the rectum maintain the *milieu interieur* of the body fluid (Marshall and Cheung 1975, Cheung 1979, 1981).

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