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# Light and Electron Microscopic Observations of the Midgut Sheath and Anterior Diverticulum of the Lantern Bug, *Pyrops candelaria* Linn. (Homoptera: Fulgoridae)

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Plant cells have diverse chemical compositions (Steward and Sutcliffe 1959). Plant sap sucking insects feed selectively on different parts of plants. In correspondence, there is modification of the alimentary canal to deal with the specific type of plant sap imbibed (see reviews by Wigglesworth 1972, Richards and Davis 1977).

Homopteran insects that feed on xylem sap have a very specialised filter chamber for dealing with the dilute ingesta that is rich in water and mineral contents (Cheung and Marshall 1973, Marshall and Cheung 1974). On the other hand, phloem feeders such as *Eurymela distincta* have also a distinct filter chamber adapted for short-circuiting excess sugars and water (Lindsay and Marshall 1980). In mesophyll feeders, however, there is generally lack of a filter chamber (Goodchild 1966).

The lantern bug *Pyrops* (*Fulgora*) *candelaria* feeds selectively on cambium sap (Marshall and Cheung 1975, Cheung 1979, 1981). The alimentary canal is modified to have a midgut sheath (intestine sheath) surrounding the long coiled midgut (Cheung 1981, Cheung and Marshall 1982). The ultrastructure of this midgut sheath and also the anterior diverticulum has not been previously reported.

Histological investigations of the midgut sheath and the anterior diverticulum of *Pyrops tenebrosus* were first reported by Goodchild in 1963. He postulated that this sheath acts as a barrier to safeguard the haemolymph from being diluted by the imbibed plant sap.

A detailed study on the fine structure of the midgut sheath and the anterior diverticulum may give useful informations on the functions of these structures.

## 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 sheath and anterior diverticulum 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 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 paraplast (56°C) and sectioned at 6 $\mu$. Sections were routinely stained with haematoxylin and eosin.

For SEM, pieces of midgut sheath and anterior diverticulum were fixed in 2.5%

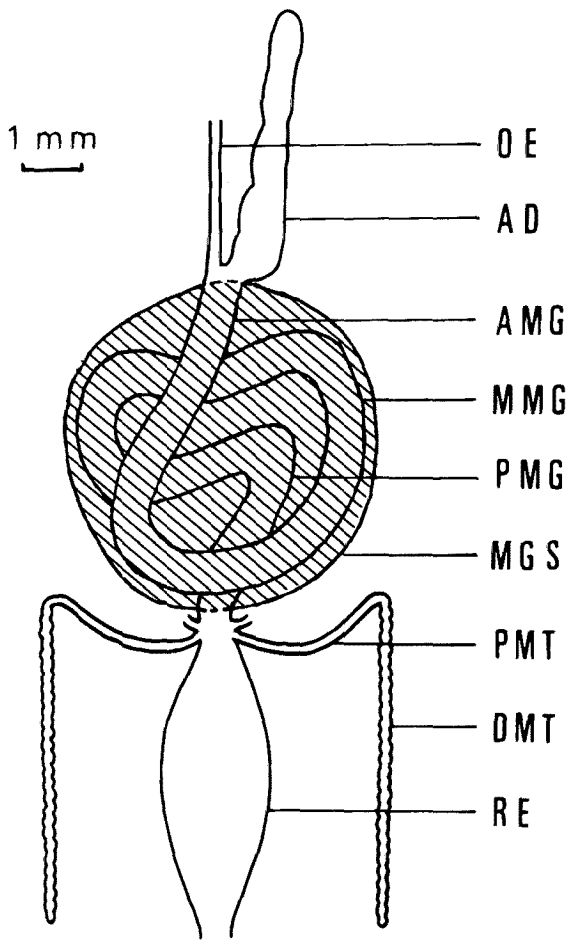
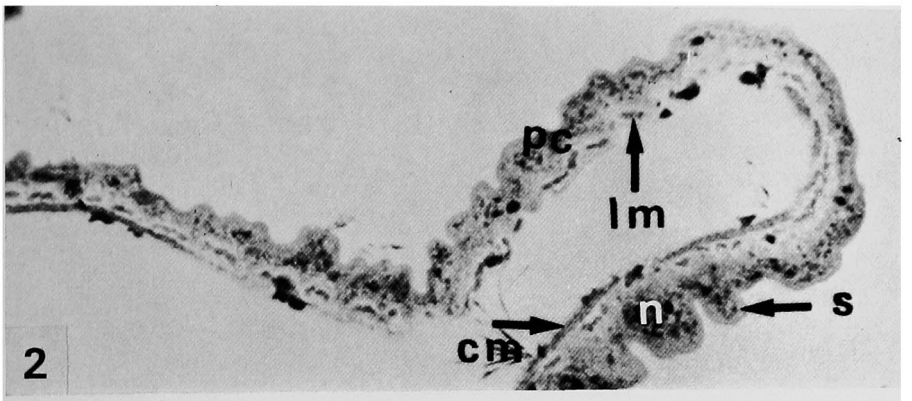



Fig. 1. Diagram of the gut of *Pyrops*. AD, anterior diverticulum; AMG, anterior midgut; DMT, distal malpighian tubule; MGS, midgut sheath; MMG, mid-midgut; OE, oesophagus; PMG, posterior midgut; PMT, proximal malpighian tubule; RE, rectum.

Figs. 2–5. 2, longitudinal section of isolated midgut sheath. Light micrograph of araldite section. Showing cuboidal principal cells (pc) with prominent nuclei (n), osmiophilic granules or spherites (s), circular and longitudinal muscle layers (cm, lm). 800×. 3, longitudinal section of isolated anterior diverticulum. Light micrograph of araldite section. Showing principal epithelial cells (pc) with large oval nuclei (n), numerous osmiophilic granules or spherites (s), and circular and longitudinal muscles (cm, lm). A replacement cell (rc) is also shown. 800×. 4, scanning electron-micrograph of luminal view of principal cells (pc) of the midgut sheath. Microvilli are hardly visible. Note condensed surface coat materials (arrows). 1,000×. 5, scanning electronmicrograph of serosal surface of midgut sheath, showing circular muscle bands and occasional longitudinal muscles (arrow). 1,600×.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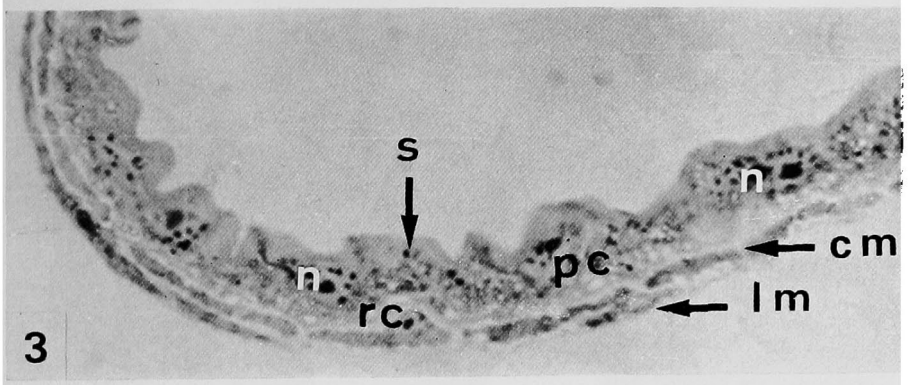
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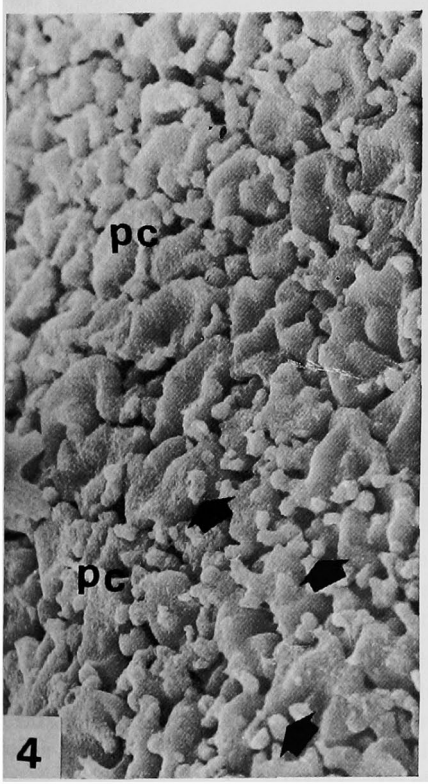
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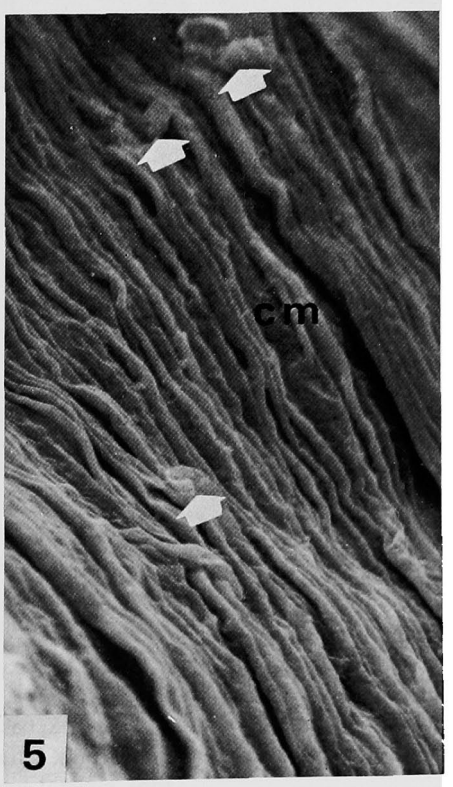
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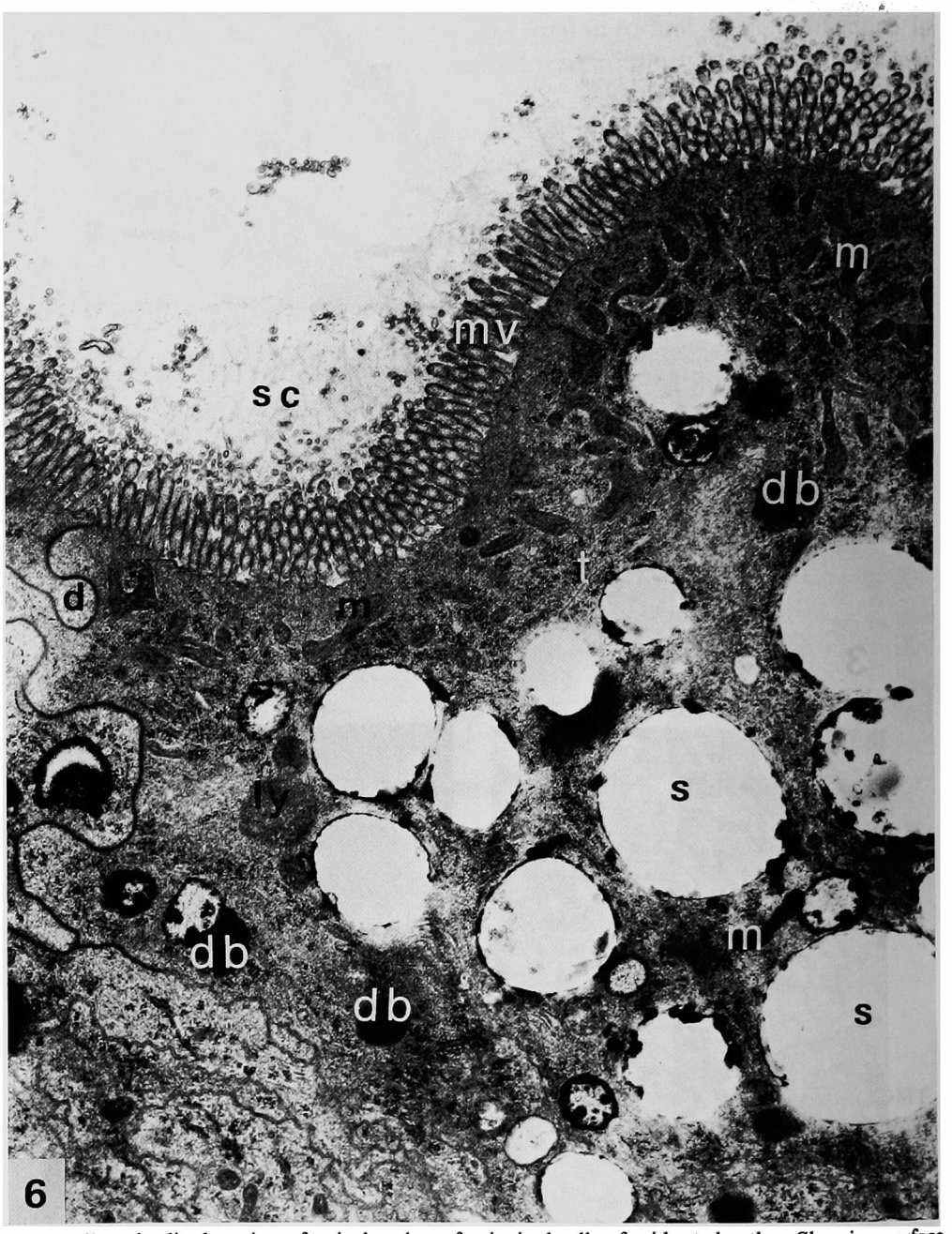
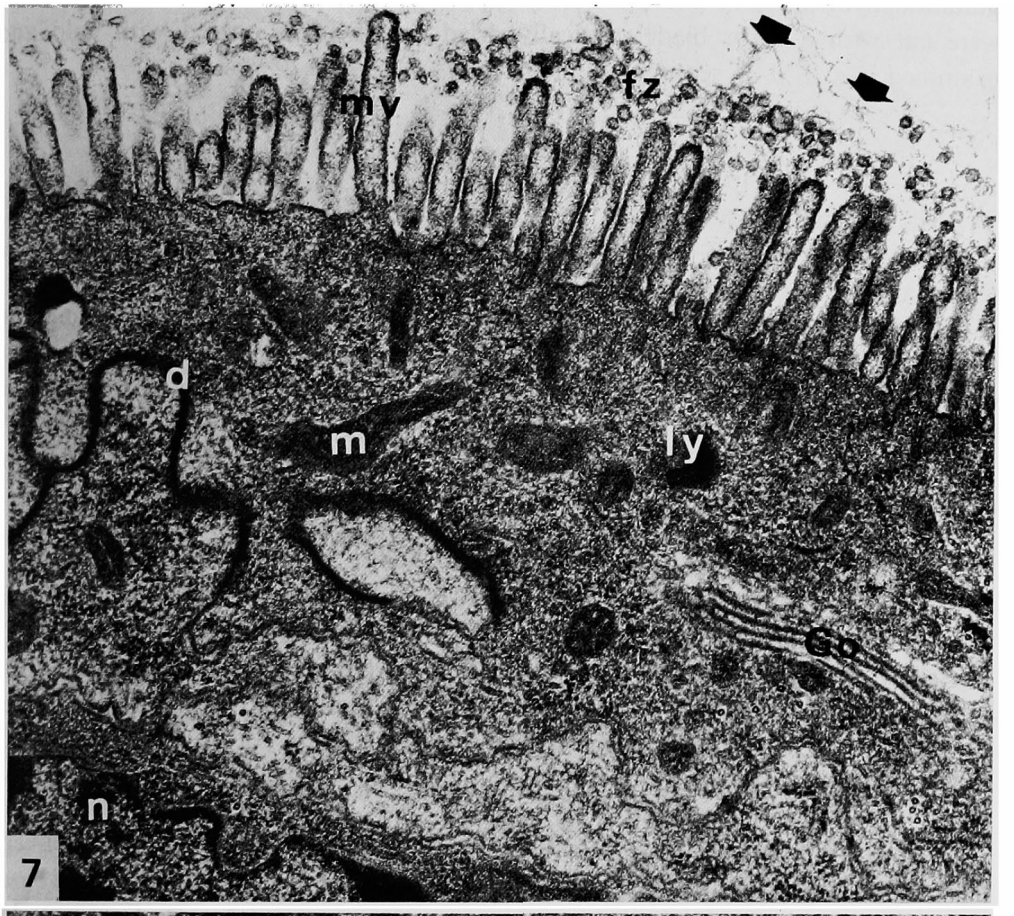



Fig. 6. Longitudinal section of apical region of principal cells of midgut sheath. Showing surface coat (sc), microvilli (mv), mitochondria (m), dense bodies (db), lysosomes (ly), desmosome (d) microtubules (t) and spherites (s) having contents dropped off. 13,800×.

Figs. 7–8. 7, as above. Showing surface coat with fuzz-bearing vesicles (fz) and broken membranes (arrows), microvilli (mv), mitochondria (m), Golgi body (Gb), lysosome (ly), desmosome (d) and part of a nucleus (n). 28,500×. 8, basal region of principal cell of midgut sheath. Showing amorphous basement membrane (bm), intracellular sinuses (is), mitochondria (m), dense body (db), mineral spherite (s). 32,000×.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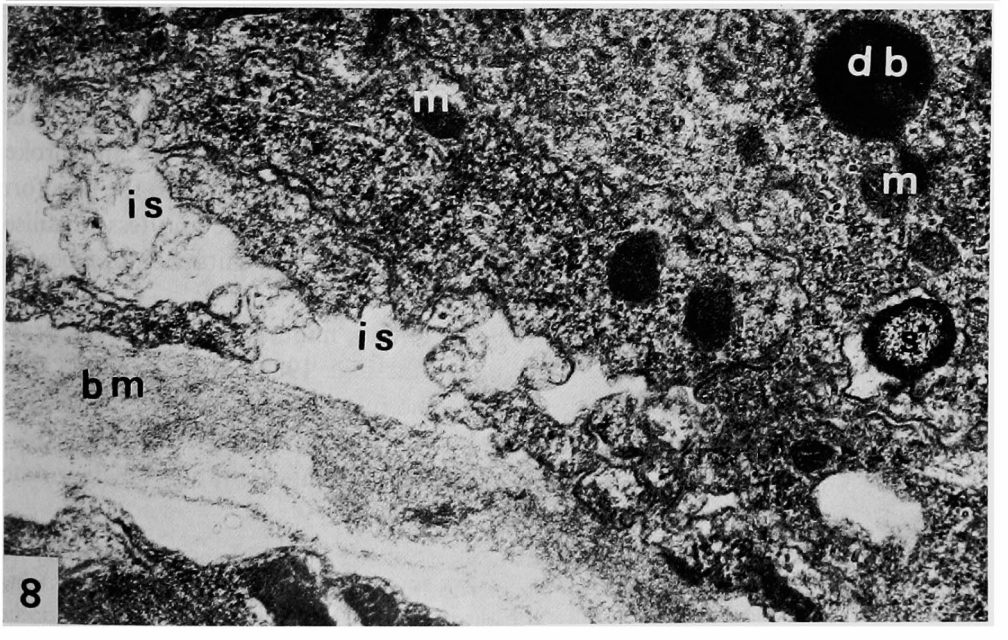
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glutaraldehyde in 0.2 M phosphate buffer, pH 7.2, for 1 hr. Small portions of these were cut with a razor blade, critically dried or air-dried, coated with gold and examined in a JSM-35 scanning electron microscope.

## Observations

The general organisation of *Pyrops* gut is shown diagrammatically in Fig. 1.

The tubular midgut is a coiled mass and is enclosed by a midgut sheath or intestine sheath which is almost transparent in gross dissection.

At the posterior end of the oesophageal valve and the beginning of the midgut, an anterior diverticulum arises as a forward projection into the long snout. On dissection, this anterior diverticulum is air-filled, together with some mucus material inside.

For the sake of convenience, the midgut sheath and the anterior diverticulum are dealt with separately in this study.

### *Histology of the midgut sheath*

The midgut sheath seen under light microscope is a one or two cell thick epithelium of elongated cells or cuboidal principal cells measuring 10 $\mu \times 15$ $\mu$ (Fig. 2). The nucleus is oval shaped. Fine granular materials which are mineral spherites are shown with toluidine blue staining (Fig. 2). Circular and longitudinal muscles are applied to the basal side of the sheath (Fig. 2).

### *Ultrastructure*

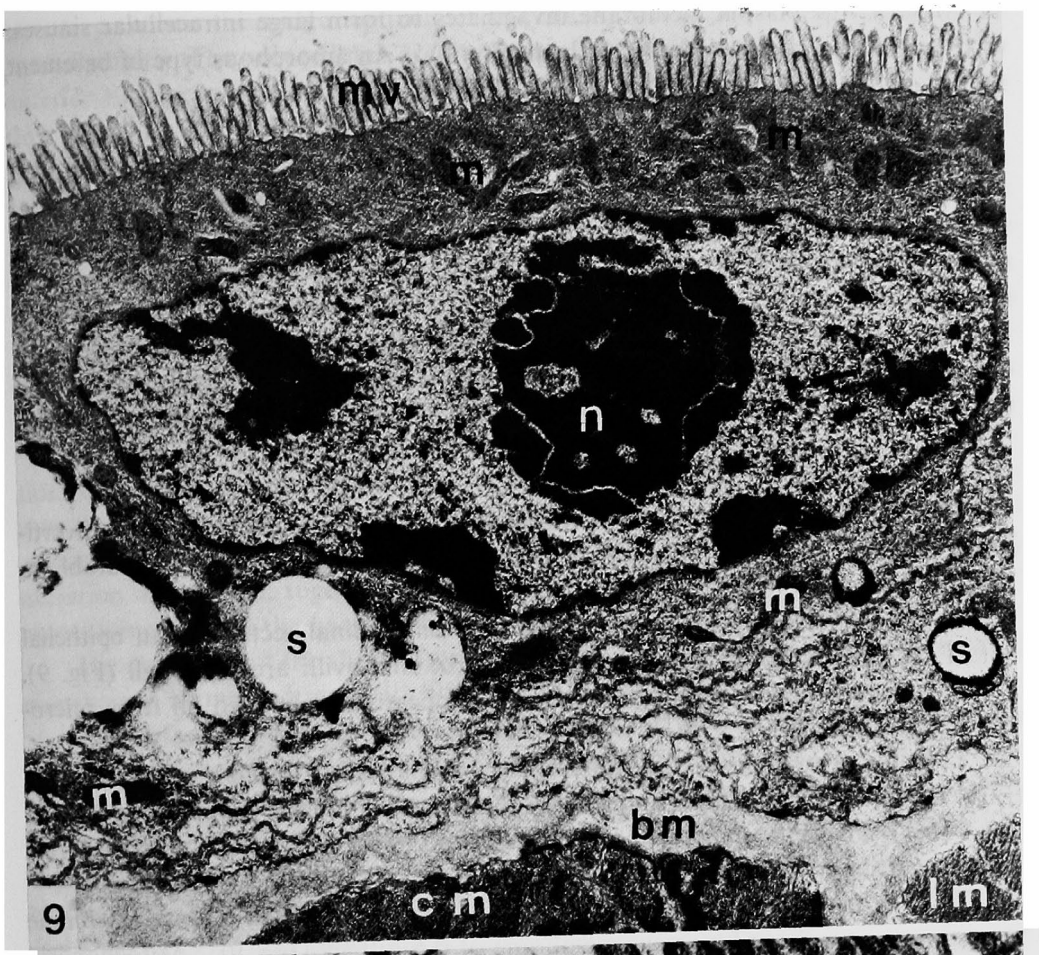
Under SEM observation, the apical surface of the midgut sheath consists of very small microvilli (hardly visible) on broad cuboidal principal cells (Fig. 4) and the basal (serosal) surface has circular muscle bands with occasional longitudinal muscles (Fig. 5). Fibrillar materials of the surface coat condense on the apical surface of cuboidal cells (Fig. 4, arrows).

In the transmission electron microscope the microvilli of the epithelial cells are seen to be 0.6 $\mu$ tall (Fig. 6). A surface coat of fuzz-bearing vesicles and broken membranes are visible (Figs. 6, 7). This surface coat is comparable to the plexiform surface coat of the columnar midgut cells, though less extensive and less organised (Marshall and Cheung 1970). There is a row of polarized mitochondria beneath the microvilli (Fig. 7).

Scattered in the cytoplasm there are dense bodies, mineral spherites (comparable to those in the mid midgut, Cheung and Marshall 1982), microtubules, Golgi bodies, and lysosomes (Figs. 6, 7). Some of the mineral contents of the spherites drop off in sections (Fig. 6).

The nucleus is irregular in shape, with chromatin material randomly distributed (Fig. 7).

Figs. 9–10. 9, longitudinal section of principal cell of anterior diverticulum. Showing microvilli (mv), mitochondria (m), mineral spherites (s), nucleus (n) and basement membrane (bm). Longitudinal and circular muscles (lm, cm) are also shown. 13,800×. 10, apical region of principal cell of anterior diverticulum. Showing surface coat of fuzz-bearing vesicles (fz) and broken membranes (arrows), microvilli (mv), mitochondria (m), lysosome (ly), vesicles (v). 32,000×.

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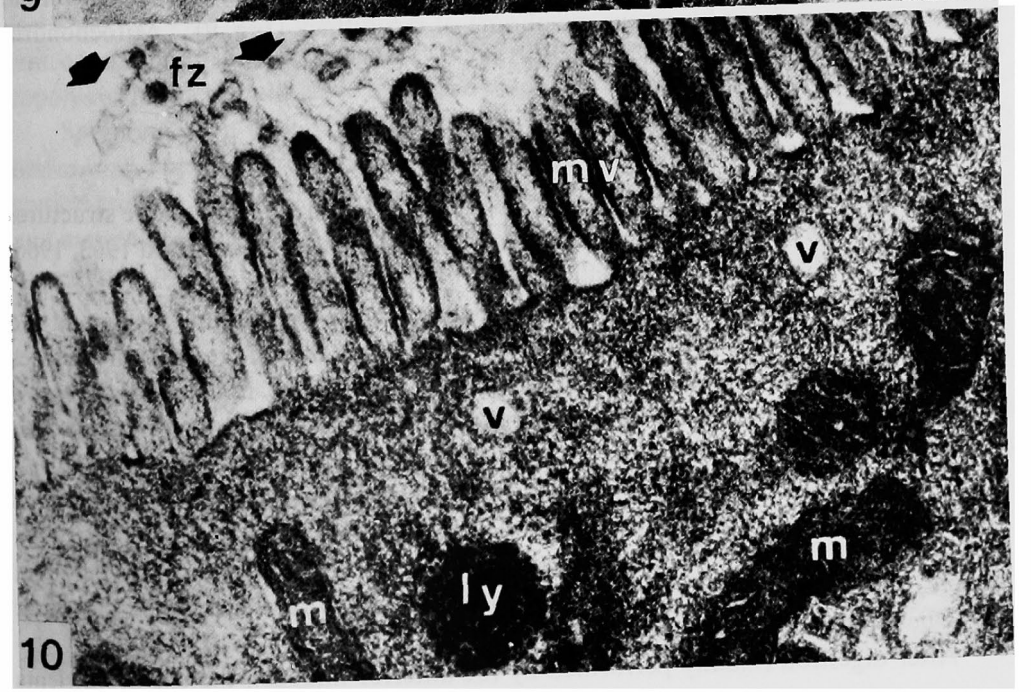


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Basally, the plasma membrane invaginates to form large intracellular sinuses, with mitochondria associated with them (Fig. 8). An amorphous type of basement membrane is also present. This is 0.5 $\mu$ thick (Fig. 8).

*Histology of the anterior diverticulum*

The anterior diverticulum, like the midgut sheath, is endodermal in origin (devoid of a cuticular intima) and consists of very much elongated cells with large, sometimes paired nuclei (Fig. 3). The nuclei have scattered dark-staining chromatin materials (Fig. 3).

The cytoplasm has many small holes which are mineral spherites that drop off during sectioning (Fig. 3). A layer of circular and longitudinal muscles surround the anterior diverticulum, thus confirming that the latter is endodermal (Fig. 3).

*Ultrastructure*

Under SEM observation, the apical and serosal surfaces of the anterior diverticulum have rather similar appearance to those of the midgut sheath (comparable to Figs. 4, 5).

In the transmission electron microscope, longitudinal section of an epithelial cell of the anterior diverticulum shows that the microvilli are 0.6 $\mu$ tall (Fig. 9). Fuzz-bearing vesicles and broken membranes appear to be budded off from microvilli tips (Figs. 9, 10).

The apical cytoplasm has numerous mitochondria, vesicles, and lysosomes (Figs. 9, 10). The nucleus is irregular in outline and is oblong-shaped (Fig. 9).

Some osmiophilic mineral spherites are found in the ground cytoplasm (Fig. 9). Their contents drop off during sectioning.

There are numerous membranous invaginations. The basal plasma membrane rests on an amorphous basement membrane of 0.5 $\mu$ thick (Fig. 9). Mitochondria are associated with these infoldings (Fig. 9).

## Discussion

The midgut sheath and the anterior diverticulum of *Pyrops* are unique structures that have not been reported in insects other than Fulgoroidea (Goodchild 1963, 1966, Cheung 1979, Cheung and Marshall 1982).

Judging from their morphological appearance both the midgut sheath and the anterior diverticulum are endodermal in origin. Since the midgut sheath encloses the long coiled midgut tube, it may be postulated that the midgut sheath has a functional significance in regulating the passage of absorbed nutrients that go into the haemolymph.

The imbibed cambium sap has a high water and mineral content, though less extreme than xylem sap (Steward and Sutcliffe 1959, Marshall and Cheung 1975). The tubular midgut has to perform functions such as digestion, absorption, mineral storage, and water and ion secretion in order to deal with the ingested cambium sap (Cheung and Marshall 1982).

After digestion (mainly performed by the anterior midgut), the useful nutrients

have to go across the midgut epithelium and the midgut sheath in order to reach the haemolymph. The sub-cellular architecture of the midgut sheath (similar to the anterior tubular midgut, Cheung and Marshall 1982) suggests an absorptive function, namely, the presence of microvilli, mitochondria, vacuoles and basal intracellular sinuses (Berridge 1970).

On the other hand, the epithelial cells of the midgut sheath can also carry out the function of storage excretion, as shown by the presence of mineral spherites. These spherites, like those of the mid-midgut, have a high content of calcium, magnesium and phosphate (Cheung, unpublished data). They are comparable to those found in the mid-midgut (Cheung and Marshall 1982), which has a mainly storage excretion function, as contrasted with the digestive function of the anterior midgut and the ion secretion function of the posterior midgut.

The anterior diverticulum also performs the function of mineral storage excretion, since mineral spherites are found there. The minerals for spherite formation may be derived from the haemolymph, comparable to those in the cicadas and cercopids, though much less extensive (Cheung and Marshall 1973). This storage excretion mechanism, together with that of the malpighian tubules, can reduce the excess minerals that the haemolymph does not want (Cheung 1981).

The oenocytes in the sheath space may assist in ion secretion. This is reported elsewhere (Cheung and marshall 1981).

The surface morphology of the midgut sheath and the anterior diverticulum seen under SEM resemble the surface topology of the stomach in man to a certain extent (Pfeiffer 1970), since these tissues have a common endodermal origin. Condensed mucus materials are often shown. The presence of mucus (cytochemically, a mucopolysaccharide, Cheung, unpublished data) in the anterior diverticulum and the lumen of the tubular midgut (associated with the plexiform surface coat, Marshall and Cheung 1970) may contribute to ion binding in relation to the high mineral content of cambium sap, since Bennett (1963) has demonstrated that extracellular mucopolysaccharides could serve as an ion trap, functioning like an ion exchange resin.

Summing up, it may be said that the midgut sheath and the anterior diverticulum perform an important role in hydromineral regulation in addition to the storage excretion and ion secretory activities of the tubular midgut and the malpighian tubules, and may in deed serve as an 'osmotic barrier' to prevent the haemolymph from being overdiluted by the ingested plant sap as originally proposed by Goodchild (1963).

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