ULTRASTRUCTURAL ASPECTS OF THE ALIMENTARY CANAL IN SOME MAYFLIES

ELDA GAINO[1], MANUELA REBORA[2], ANNA RITA TADDEI[3] & MASSIMO MAZZINI[3]

[1] Istituto di Zoologia, Via Elce di Sotto, 06123 Perugia, Italy
[2] Istituto di Zoologia, Via Balbi 5, 16123 Genova, Italy
[3] Dipartimento di Scienze Ambientali, Università della Tuscia, 01100 Viterbo, Italy

The basic architecture and organization of the alimentary canal in Leptophlebiidae (*Habrophlebia eldae*), Heptageniidae (*Electrogena grandiae, Ecdyonurus venosus, E. helveticus*) and Ephemerellidae (*Ephemerella ignita*) have been investigated. Observations have been carried out by means of both light and electron microscopy. The alimentary canal results from three divisions: foregut, midgut and hindgut, each reflecting particular specializations. In mayflies the foregut is limited to the head. The midgut is the longest tract. The morphology of its cells is highly variable and reflects the multiple performed functions, among which the peritrophic membrane synthesis. The hindgut is thrown up into longitudinal folds delimited by a thick, differently organized cuticle. Some aspects of gut organization and metabolic activity are discussed for the relevance of the trophic route in the bioaccumulation and detoxification mechanisms.

INTRODUCTION

A great number of papers have dealt with the alimentary canal of insects, in terms of both anatomy and fine organization (reviews in MARTOJA & BALLAN-DUFRANÇAIS, 1984; CHAPMAN, 1985; DOW, 1986). A general profile of mayfly digestive tract can be found in old papers (PICKLES, 1931; VON DEHN, 1933). Comparative investigations on the macroscopic differentiation of the alimentary canal have been carried out in a vast number of species (LANDA *et al.*, 1980; LANDA & SOLDÁN, 1985), but no detailed studies on the ultrastructure of the mayfly gut have been recently published, except for some observations on the burrowing *Hexagenia rigida* in relation to contamination mechanisms (SAOUTER *et al.*, 1991a).

Since adult Ephemeroptera are unable to feed, the long-lasting aquatic stages are the only trophic ones. In this phase of the life cycle, the gut-trophic-route represents a contact with the external environment through the ingestion of water, sediment and food. This also arouses an ecotoxicological interest in mayflies (SAOUTER *et al.*, 1991b).

The structural and ultrastructural organization of the gut can provide important information on permanent structural features and yield valuable clues to its function (GREEN, 1979; RATNER & STOFFOLANO, 1984; MARTIN & KIRKHAM, 1989; RYERSE *et al.*, 1992; JARIAL, 1992; CRUZ-LANDIM, 1994; RAES & VERBEKE, 1994; RAES *et al.*, 1994).

The objective of the present study was to illustrate the architecture of the gut in a few mayflies and to characterize its different tracts by comparing cell morphology. This with a view to laying the foundations for further studies on the organizational changes in response to various feeding strategies and physiological demands.

MATERIAL AND METHODS

The gut has been dissected from larvae and nymphs of Leptophlebiidae (*Habrophlebia eldae*), Heptageniidae (*Electrogena grandiae, Ecdyonurus venosus, E. helveticus,*) and Ephemerellidae (*Ephemerella ignita*).

For ultrastructural investigations, selected material was fixed in KARNOVSKY's medium (1965) in cacodylate buffer (pH 7.2) and postfixed in osmium tetroxide (1% in cacodylate buffer).

For scanning electron microscopy (SEM), the specimens were dehydrated with a graded series of ethanol, critical point dried in CO_2 in a Pabisch CPD 750 apparatus, sputter coated with gold with a Balzers Union evaporator, and viewed with a Philips EM 515 electron microscope.

For transmission electron microscopy (TEM), the dehydrated specimens were infiltrated with Epon-Araldyte accelerate mixture. Semithin and ultrathin sections were obtained with a Reichert ultratome using a diamond knife. Ultrathin sections were collected on copper grids, stained with uranyl acetate and lead citrate and examined with a JEOL 1200 EX II electron microscope.

RESULTS

In mayflies, the gut takes up most of the larval hoemocelian cavity and is equipped with a sparse innervation, muscles and extensive tracheolar trunks. As it occurs in any other insect group, the foregut and hindgut are lined

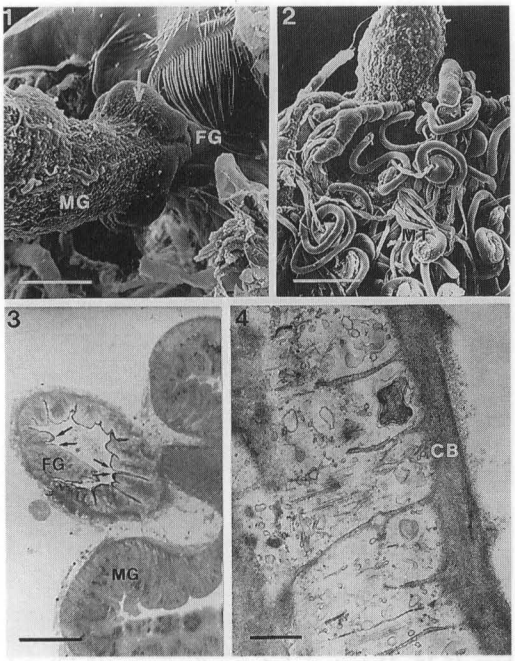
with cuticle. The midgut is devoid of cuticle and the luminal side of its cells protrudes into microvilli which form the typical brush border. The passage from the foregut to the midgut is marked by an enlargement (the cardiac valve) (Fig. 1) and from the midgut to the posterior hindgut by the emptying of the Malpighian tubules (Fig. 2).

Organization of the foregut

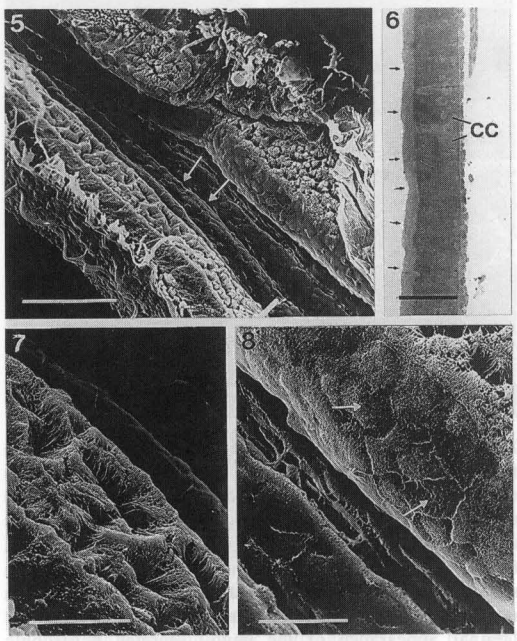
In mayflies the foregut is very short and confined to the head (Fig. 1). In removing the digestive system, it is necessary to enter the head so as not to miss this short region.
The internal epithelium is thrown up into large longitudinal folds (Fig. 3) lined by a well-developed cuticular border (Fig. 4). The foregut projects into the anterior end of the midgut and joins on to this epithelium without a break. Such a passage is marked by the end of the cuticular border (Fig. 3). At this level the midgut bends before straightening. The simple cardiac valve regulates the food passage into the midgut.

Organization of the midgut

The midgut is the longest tract. Sections examined under SEM reveal that the epithelium is thrown up into longitudinal folds (Fig. 5).
In terms of cell differentiation, the line of demarcation between the midgut and the foregut is the cell boundary, where the cuticle ceases and the luminal side of the cells extends into microvilli giving rise to the brush border (Figs 6, 7). Indeed, the epithelium of the midgut is essentially composed of columnar cells locat-



Figs 1-4. SEM view of different regions of the gut of *Electrogena grandiae* (1, 2) and the foregut of *Ecdyonurus helveticus* (3, 4). 1: the short foregut (FG) confined to the head. Note the basal enlargement (arrows) marking the passage into the midgut (MG). Bar = 100 µm; 2: Malpighian tubules (MT) jut out at the midgut/hindgut junction. Bar = 100 µm ; 3: foregut (FG) in a semithin cross section and the anterior portion of the midgut (MG). Note the enfolding of the foregut epithelium (arrows). Bar = 125 µm; 4: TEM view of the outermost epithelial region showing the cuticular border. Bar = 1 µm.

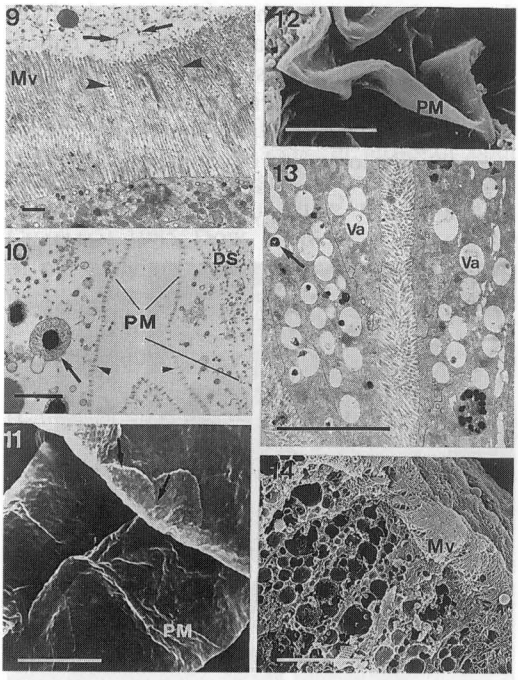


Figs 5-8. SEM view of the midgut of *Ecdyonurus venosus* (5, 7, 8) and semithin section of a midgut region of *Habrophlebia eldae* (6). 5: the enfolding of the midgut walls (arrows). Bar = 100 µm; 6: the columnar cells (CC) with the typical brush border (arrows). Bar = 40 µm; 7: zoomed view of the microvilli forming the brush border; 8: disposition of the columnar cells to give rise to a polygonal pattern (arrows). Bars in 7, 8 = 20 µm.

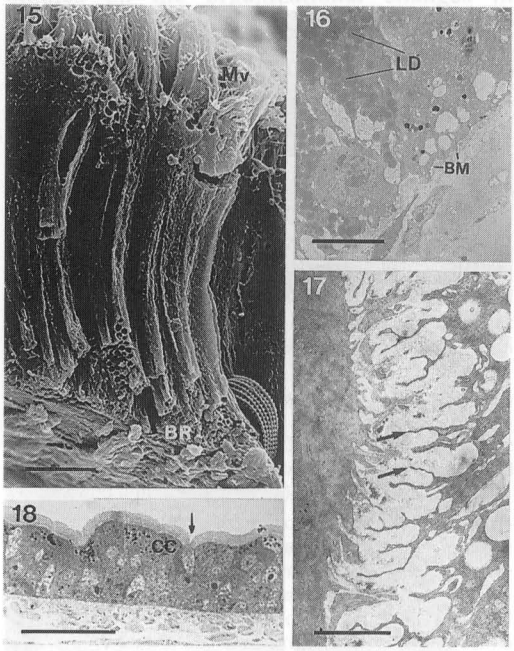
ed side by side to form a polygonal pattern along the cavity lumen (Fig. 8). At TEM level, the microvilli associated to the cell apical surface result from a great number of cylindrical processes (Fig. 9), which project into the gut lumen.

The fine structure of the columnar cells is highly variable and depends upon the multiple functions they perform during the life cycle. Among a wide range of metabolic activities, these cells are competent for the peritrophic membrane synthesis. Indeed, precursor material for the definitive peritrophic membrane appears both in the space between microvilli and accumulates at their apices along the gut lumen (Fig. 9). The peritrophic membrane, secreted along the whole length of the midgut, exhibits a sequence of rod-like elements alternating with coarse strands of dotted appearance (Fig. 10). This membrane is interposed between the cell brush border and the food bolus, and is more easily detectable under SEM (Figs 11, 12). Here it appears as a thin sheet, sometimes including polygonal areas that correspond to the luminal surface of the columnar cells immediately underneath (Fig. 11). In the initial phase of its secretion, the peritrophic membrane is a transparent layer, but it becomes thicker and more folded passing down into the gut (Fig. 12).

The cytoplasm of the columnar cells is filled with lipid droplets and of vacuoles of various



Figs 9-14. TEM (9, 10, 13) and SEM (11, 12, 14) view of the midgut of *Ephemerella ignita*. 9: brush border of the columnar cells with the constitutive microvilli (MV). Note the precursor material for the ensuing peritrophic membrane among microvilli (arrowheads) and along the gut lumen (arrows); 10: peritrophic membrane (PM) resulting from a sequence of rod-like elements (arrowheads) alternated with dotted strands (DS). Note an extruded spherite (arrow); 11: laminar appearance of the newly secreted peritrophic membrane (PM) located above the epithelial cells (arrows). Bar = 10 μm; 12: changes in shape and thickness of the peritrophic membrane (PM) passing down into the gut lumen; 13: vacuoles (Va) sometimes containing electron-dense inclusions (arrow); 14: vacuolated appearance of the cytoplasm of the columnar cells whose microvilli (Mv) face the lumen of the cavity. Bars in 9, 10, 14 = 1 μm; Bars in 12, 13 = 5 μm.



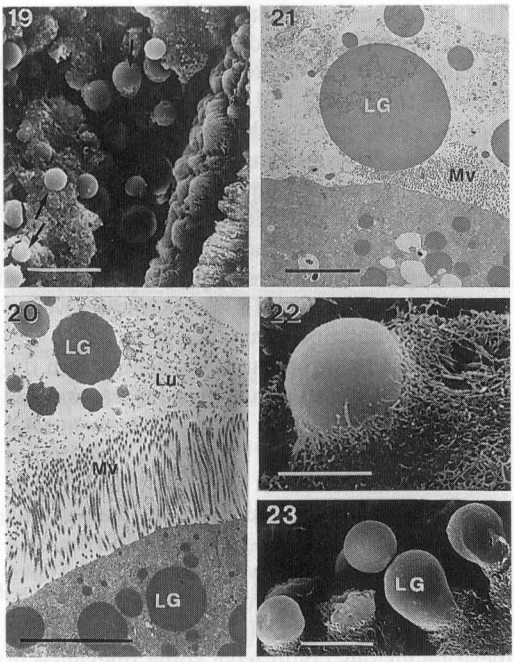
Figs 15-18. Midgut of *Ephemerella ignita* (15-17) and *Ecdyonurus helveticus* (18). 15: SEM view of columnar cells showing both luminal microvillar border (Mv) and basal region (BR); 16: TEM view of the basement membrane (BM) along the basal region of columnar cells. Note the large amount of lipid droplets (LD); 17: TEM view of the labyrinth (arrows) originated by the enfolding of the basal plasma membrane of the columnar cells. Bar = 2 μm; 18: clear cells, interspaced among columnar cells (CC), recalling the morphology of the goblet cells. One of them (arrow) opens into the gut lumen. Bar = 50 μm; Bars in 15, 16 = 5 μm.

sizes sometimes containing electron-dense inclusions (Fig. 13). The morphology of these latter is consistent with that showed by the typical spherites occasionally found into the lumen of the midgut (Fig. 10). The vacuolated appearance of the cytoplasm is particularly evident when sections are examined under SEM (Fig. 14).

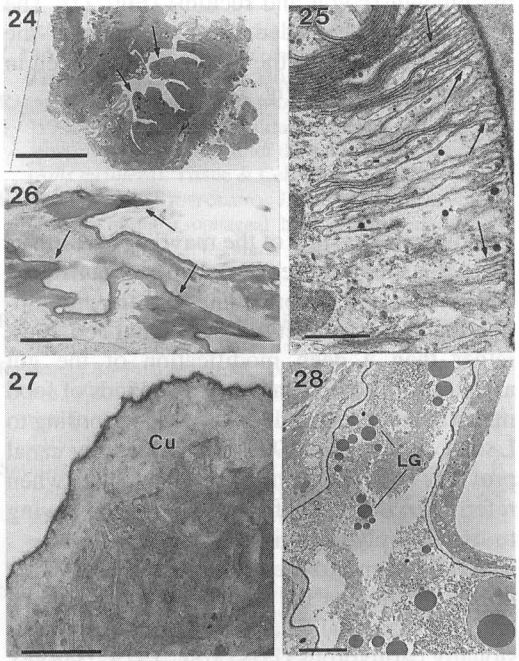
Along the surface opposite to the microvillar border (Fig. 15), the columnar cells lie on a basement membrane (Fig. 16) and the great enfolding of their basal plasma membrane generates a labyrinth (Fig. 17).

Among the columnar cells an other cell type can be occasionally observed. These cells can be distinguished for their clear appearance and nucleus confined to the basal region of the cell (Fig. 18). They presumably represent the so-called goblet cells, whose presence in Ephemeroptera has been repeatedly noticed (MARTOJA & BALLAN-DUFRANÇAIS, 1984; CHAPMAN, 1985). The goblet cells are less frequent. Their presence probably depends upon the region of the midgut under examination, upon the secretory activity of the midgut epithelium or upon the species.

A peculiar metabolic activity has been observed to take place in the columnar cells which give off a great amount of globules (Fig. 19). Before their discharge into the gut lumen, the globules accumulate along the apical region of the cells, immediately below the brush border (Fig. 20). They are smooth and show a uniform electron-dense appearance, a feature consistent with their lipid nature (Figs 20, 21). At the onset of their extrusion they have a rounded shape and



Figs 19-23. SEM (19, 22, 23) and TEM (20, 21) view of lipid globule extrusion by the columnar cells of the midgut of *Ephemerella ignita*. 19: a large amount of globules (arrows) above the luminal border of the cells. Bar = 20 µm; 20: accumulation of the lipid globules (LG) below the microvillar border (Mv) and into the lumen (Lu); 21: section of a big newly discharged lipid globule (LG) partially enveloped by microvilli (Mv); 22: a lipid globule in toto emerging from the microvillar border; 23: the pinched-off appearance of a lipid globule (LG) about to be given off into the lumen. Bar = 10 µm; Bars in 20, 21, 22 = 5 µm.



Figs 24-28. Hindgut of *Ephemerella ignita* (24, 25, 26, 28) and *Ecdyonurus helveticus* (27). 24: semithin section showing the enfolding of the walls (arrows). Bar 100 µm; 25: TEM view of the conspicuous invaginations of the cell apical plasma membrane (arrows); 26: TEM vision of the cuticular denticles (arrows) characteristic of the initial region of the hindgut; 27: TEM view of a portion of the cuticle (Cu) without denticles; 28: lipid globules (LG) extruded from the midgut cells. Bar = 5 µm; Bars in 25, 27 = 1 µm.

are partially enveloped by the microvilli (Figs 21, 22). The pinched-off appearance acquired by the globules in the area close to the brush border documents the gradual release into the gut lumen (Fig. 23).

Organization of the hindgut

The last part of the digestive system begins at the joint with the Malpighian tubules and differs from the midgut epithelium owing to the presence of a cuticular lining.
The hindgut epithelium is thrown on into numerous folds (Fig. 24) and the underlying cells have extensive apical membrane invaginations (Fig. 25). The cuticle shows denticles in the initial tract of the hindgut (Fig. 26). This region, located immediately below the merging of the Malpighian tubules, can be considered the ileum. Denticles are lacking from the rest of the hindgut (colon and rectum) (Fig. 27). The lipid globules extruded from the cells of the midgut descend into the gut and can be seen in the hindgut region (Fig. 28).

DISCUSSION

The digestive system of the mayflies investigated in this study presents a simple organization and this feature is consistent with the assumption that a wide variety of enzymatic activities, rather than a deep modification of the gut architecture, could enable various kinds of food material to be digested. Otherwise, according to LANDA & SOLDÁN (1985) the alimentary canal provides only few comparative characters when referred to other mayfly organ systems, owing to its relatively primitive structure.
Mayflies seem to be generalist feeders that utilize detritus as the main alimentary source but they also qualify as herbivorous and occasionally carnivorous (FONTAINE, 1979; HARVEY *et al.*, 1979; MCCAFFERTY & PROVONSHA, 1986, 1988; MCSHAFFREY & MCCAFFERTY, 1990, 1991). The short foregut confined to the head is a characteristic of continuous feeders (DOW, 1986), which utilize this anterior region of the intestine simply as a passageway for food particles. Mouthparts are efficient in chewing, therefore no particular differentiations are required to enhance food breaking.
The midgut is the longest tract. It is known that cells are primarily concerned with the production and secretion of digestive enzymes and absorption (WIGGLESWORTH, 1974). They undergo continuous changes according to their function and life cycle.
In addition, cells synthesize the peritrophic membrane which is generally assumed to protect the epithelium from the abrasive action of the inorganic particles ingested. Numerous studies have dealt with the origin and structure of this ephemeral structure (SPENCE, 1991; RICHARDS & RICHARDS, 1977; MARTIN & KIRKHAM, 1989; RYERSE *et al.*, 1992). Even though several aspects of the peritrophic membrane synthesis continue to be merely speculative, in mayflies this process takes place along the entire midgut by the activity of the columnar cells, as it has been firstly noticed by VON DEHN (1933) in *Cloeon*.
The brush border of the midgut and the epithelial cells of the hindgut constitutes a wide surface area for absorption and exchange between the animal and its environment. These regions of the gut may have a great function in processes of metal transfer and accumulation, thus stressing that the aquatic stages of mayflies are useful indicators in the ecotoxicological field. As a matter of fact, the great amount of lipid droplets and the release of lipid globules into the gut lumen could have some relevance to bio-accumulation mechanisms.
The process of cytoplasmic extrusion from the midgut cells typically occurs in insects and has been interpreted as the reflex of both normal cell turn-over and enzyme secretion (WIGGLESWORTH, 1974; RYERSE *et al.*, 1992). According to MARTOJA & BELLAN-DUFRANÇAIS (1984), apocrine secretion seems to be the only way to eliminate bulky inclusions. We have observed that lipid globules are apparently extruded from intact cells and we speculate that these inclusions could have some relevance in the ecotoxicological field. Indeed, organic lipophilic xenobiotic compounds (such as, hydrocarbons, pesticides, and toxic aromatic substances) might be stored inside lipid globules; this possibly leads to the elimination of contaminants accumulated via the trophic-route.
In conclusion, ultrastructural details of the cells constituting the alimentary canal other than

giving information on their physiological routine may be viewed as the expression of various sets of metabolic responses in relation to environmental conditions.

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