The larval alimentary canal of the Antarctic insect, *Belgica antarctica*

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**A R T I C L E   I N F O**

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**A B S T R A C T**

On the Antarctica continent the wingless midge, *Belgica antarctica* (Diptera, Chironomidae) occurs further south than any other insect. The digestive tract of the larval stage of *Belgica* that inhabits this extreme environment and feeds in detritus of penguin rookeries has been described for the first time. Ingested food passes through a foregut lumen and into a stomodeal valve representing an intussusception of the foregut into the midgut. A sharp discontinuity in microvillar length occurs at an interface separating relatively long microvilli of the stomodeal midgut region, the site where peritrophic membrane originates, from the midgut epithelium lying posterior to this stomodeal region. Although shapes of cells along the length of this non-stomodeal midgut epithelium are similar, the lengths of their microvilli increase over two orders of magnitude from anterior midgut to posterior midgut. Infoldings of the basal membranes also account for a greatly expanded interface between midgut cells and the hemocoel. The epithelial cells of the hindgut seem to be specialized for exchange of water with their environment, with the anterior two-thirds of the hindgut showing highly convoluted luminal membranes and the posterior third having a highly convoluted basal surface. The lumen of the middle third of the hindgut has a dense population of resident bacteria. Regenerative cells are scattered throughout the larval midgut epithelium. These presumably represent stem cells for the adult midgut, while a ring of cells, marked by a discontinuity in nuclear size at the midgut-hindgut interface, presumably represents stem cells for the adult hindgut.

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1. Introduction

Despite the dominance of insects and other terrestrial arthropods throughout the world, only a few species are found in Antarctica. Most are collembolans and mites, with the Class Insecta represented by only two endemic species of midges (Diptera: Chironomidae) (*Convey and Block, 1996*). Of these, the range of the more abundant midge *Belgica antarctica* extends further south on the Antarctic Peninsula than that of any other insect.

*B. antarctica* has a patchy distribution on the Peninsula, but it is particularly abundant near penguin rookeries on the small, offshore islands near Palmer Station. In this habitat, midge larvae are subjected to a range of environmental stresses including desiccation, freezing, anoxia, pH fluctuation from the nitrogenous run-off, and inundation from seawater as well as fresh water from precipitation and ice melt. Numerous physiological adaptations are apparent in the larvae: heat shock proteins are continuously up-regulated (*Rinehart et al., 2006*), agents to counter oxidative stress are present in abundance (*Lopez-Martinez et al., 2008*), loss of a high percentage of body water is tolerated and in fact contributes to enhanced freeze tolerance (*Hayward et al., 2007; Elnitsky et al., 2008*), dramatic changes in metabolites (*Michaud et al., 2008*) and gene expression (*Lopez-Martinez et al., 2009*) accompany these physiological responses to environmental stress.

In addition to these distinctive biochemical features of cells of *B. antarctica*, it is quite possible that structural features of this insect may also deviate somewhat from the patterns observed in insects at lower latitudes. The harsh environment of Antarctica obviously places great demands on the resiliency of cells that are exposed to periodic desiccation and freezing. To promote formation of ice crystals within extracellular spaces rather than within cells, insect cells must rapidly exchange water with their extracellular environments. Expansion of luminal and/or basal surface areas of epithelial cells would facilitate this rapid exchange.
Special emphasis in this manuscript has been placed on the cellular architecture of the three well-delineated epithelial regions of the insect alimentary canal – foregut, midgut, and hindgut. Secretion from the midge's large salivary gland cells aggregates detritus particles and facilitates uptake of detritus into the gut (Oliver, 1971). After passage of ingested food through the midge larva’s short and narrow foregut, digestion and absorption of ingested material occurs across the peritrophic membrane and microvilli that line the lumen of the midgut. Subsequently digested food passes into the hindgut where ions, small molecules and water are absorbed across a cuticular lining.

Comparisons of internal gut morphology of arthropods complement existing studies of external morphological features and molecular characters on which traditional phylogenetic relationships, representing genetic differences among organisms, are based. Although the alimentary canals of other species in the family Chironomidae have not been examined at the cellular level of organization examined in this manuscript, a comprehensive comparison of internal morphological characters among related species would reveal differences that presumably are based on environmental adaptations. In this study we explore the possibility that the extreme environment in which these midge larvae live shapes the architecture of their gut epithelial cells.

2. Materials and methods

Larvae of *B. antarctica* were collected from sites near penguin rookeries on Cormorant and Humble Islands, near Palmer Station, Antarctica (64°-46'S, 64°-04'W) in January and February 2007. The high nitrogen run-off from penguin rookeries provides the nutrient base for the microbes, algae (*Prasiola crispa*), lichens and moss with which midge larvae are usually associated. Larval development is restricted to the brief austral summer, from late December until mid-February; and larvae remain immobilized in the frozen substrate for the remainder of the year. Two years are required for the larva to complete its development (Usher and Edwards, 1984), and adult life is compressed into a 1–2 week period in late December/early January (Sugg et al., 1983). Larvae collected in Antarctica were shipped to the laboratory at Ohio State University where they were maintained with their substrate at 4°C.

Digestive tracts and salivary glands from 32 individuals were dissected with fine watchmaker’s forceps in Grace’s insect culture medium. Tissues were immediately fixed at 4°C in a primary fixative of 2.5% glutaraldehyde (v/v) and 0.5% paraformaldehyde (w/v) dissolved in a rinse buffer of 0.1 M cacodylate (pH 7.4) containing 0.18 mM CaCl₂ and 0.58 mM sucrose. After 3 h in this fixative, tissues were washed three times with rinse buffer before being transferred to secondary fixative (2% osmium tetroxide dissolved in rinse buffer, w/v) for 4 h. After thoroughly washing with rinse buffer, tissues were gradually dehydrated in a graded ethanol series (10–100%, v/v). From absolute ethanol, tissues were transferred to propylene oxide and infiltrated with mixtures of propylene oxide and resin before being embedded in pure LX112 resin.

Semithin sections for light microscopy were mounted on glass slides and stained with 0.5% toluidine blue in 1% borax (w/v). Ultrathin sections were mounted on grids and stained briefly with saturated aqueous uranyl acetate and Luft’s lead citrate to enhance contrast. Images were taken with a Hitachi 600 transmission electron microscope operating at 75 kV.

Whole mounts were prepared by fixing tissues at room temperature for 30 min in a 4% solution of paraformaldehyde (w/v) dissolved in phosphate-buffered saline (PBS). After...
several rinses in PBS, nuclei of cells were labeled with 1:1000 dilution of 4',6-diamidino-2-phenylindole (DAPI, 1 mg/ml water) after first permeabilizing cells for 30 min in a solution of 0.1% Triton X-100 in PBS (v/v). Tissues were mounted under cover glasses in a solution of 70% glycerin in 0.1 M Tris at pH 9.0 (v/v).

3. Results

3.1. Global organization of the Belgica larval gut

The larval gut is a straight alimentary canal that is associated with a pair of salivary glands at its anterior end and four Malpighian tubules that converge with the alimentary canal at the junction of midgut and hindgut (Fig. 1, mh). A prominent stomodeal valve occupies the interface between foregut and midgut (Fig. 1, fm). The foregut occupies only about 5% of the total length of the alimentary canal, while the endodermal midgut that occupies the region between fm and mh in Fig. 1 clearly represents more than half the length of the alimentary canal.

3.2. Salivary glands and foregut

Conspicuous salivary glands occupy the anterior end of the larval midge. These glands are both polyplloid and polytene (Fig. 2).

Fig. 2. Whole mount of Belgica salivary gland as viewed with Nomarski optics (a) and after labeling DNA with DAPI (b). The salivary duct is located at the arrow. Scale bar – 50 μm.

Fig. 3. Cuticles of foregut and epidermal epithelia have different stratification. At higher magnification, the internal folded foregut cuticle (a) is compared with the cuticle of the external epidermis (b). A bracket extends across the foregut cuticle. The gut lumen (*) is surrounded by relatively thin cuticle compared to the thicker epidermal cuticle in (b) epithelial cells (e), pigmented fat body (f), In (c) the convoluted integument of the foregut (arrowhead) is surrounded by muscles (arrows), tracheoles (t), neural tissue (n) and salivary gland (g). Scale bars: (a) 1.0 μm, (b) 5 μm, and (c) 20 μm.
Like glands found in other larval members of the Chironomidae, these salivary glands secrete silken threads that entrap food particles.

On the foregut’s apical surface, the cuticle lining the narrow foregut lumen is about 0.3–0.5 μm thick (Fig. 3a). Unlike the thicker, contiguous cuticle of the larva’s exoskeleton with its inner electron-dense layer (Fig. 3b), this foregut cuticle is highly convoluted and its outermost layer is electron-dense. Conspicuous muscle layers surround the foregut. The large salivary glands and larval brain in turn surround these muscles on the basal surface of the larval foregut (Fig. 3c).

3.3. Stomodeal valve at foregut–midgut interface

After being channeled through a foregut lumen lined by a convoluted cuticle, the contents of the gut pass through a conspicuous stomodeal valve into the endoperitrophic space of the midgut epithelium. The stomodeal valve represents an intussusception of the foregut epithelium into the midgut (Figs. 4a–d and 5a–c). This folding of the foregut epithelium and its cuticle creates a caecum that is lined centrally by foregut cuticle and peripherally by midgut microvilli. The interface of foregut and midgut lies at the anterior end of the caecum. At this junction of foregut and midgut, a peritrophic membrane originates and lines the lumen of the more posterior midgut epithelia.

3.4. Spatial differentiation of the midgut epithelium

An abrupt epithelial discontinuity marked by disparity in midgut microvillar length occurs at the interface between the stomodeal region and the more posterior midgut epithelium (Fig. 4c and 5d). Certain cells at this interface are specialized for secretion (Fig. 5d–f) and possibly are endocrine cells. These cells at the posterior edge of the stomodeal valve were the only cells of the midgut observed to contain conspicuous secretory granules.

Fig. 4. The stomodeal valve represents an intussusception of posterior foregut epithelium (fe) into anterior midgut epithelium (me). (a) The folded foregut epithelium is surrounded by the anterior midgut. Anterior is to the right. (b, c) Longitudinal sections show inner lumen cuticle (ic) and outer lumen cuticle (oc) of the foregut. Between these two cuticles lie two foregut epithelial layers and an enteric muscle layer (m). Midgut epithelium is the outermost layer of the valve. (c) Represents the region delimited by the rectangle in (b). The morphological discontinuity is indicated by the arrow. The peritrophic membrane (p) lies in the lumen separating foregut and midgut. (d) The concentric arrangement of three epithelia, two lumina and one muscle layer is shown in this transverse section of the valve. From periphery to center: (1) midgut epithelium with microvilli lining the lumen in which the peritrophic membrane forms; (2) foregut epithelium faces this lumen and (3) enteric muscles (m) occupy the space between this foregut epithelium and the foregut epithelium facing the innermost lumen. Scale bars: (a, b, d) 50 μm; (c) 20 μm.
Fig. 5. At higher resolution, longitudinal sections of the stomodeal valve reveal details of peritrophic membrane formation and the presence of special secretory cells. (a) At the interface between the foregut epithelium and midgut epithelium lies a confluence of muscle (m), foregut epithelium covered by cuticle (fg) and secretory microvilli (arrows) of midgut epithelium. Anterior is at the bottom. (b, c) Cephas secretion of peritrophic membrane material (*) occurs from the microvilli of midgut epithelial cells at the anterior end of the stomodeal valve. Note the high density of mitochondria in the adjacent midgut cells. In (b) the newly formed peritrophic membrane (arrow) lies between the foregut (fg) cuticle and the tips of the microvilli. (d) Distinctive secretory cells (arrow) lie within the midgut epithelium of the stomodeal valve at the border between midgut cells with microvilli and midgut cells without obvious microvilli (See Fig. 4c). The gut lumen is at upper left. (e, f) Close-ups of the secretory cell showing the nucleus (n), rough endoplasmic reticulum (arrowhead) and the high density of secretory granules (*). Scale bars: (a) 10 μm; (b, c) 2 μm; (d) 5 μm; (e) 1 μm; and (f) 0.2 μm.
Posterior to the stomodeal valve, a striking gradient of microvillar length occurs along the antero-posterior (AP) axis of the midgut epithelium (Fig. 6), with short (~0.1 to 0.2 μm) microvilli occupying anterior regions of the midgut and extremely long, straight and densely packed (~10 μm, Figs. 7 and 9) microvilli occupying posterior regions of the midgut. This morphological gradient is evident in longitudinal sections of the midgut (Fig. 6a–c) as well as the series of transverse sections from different locations along the AP axis of the Belgica gut (Figs. 6d–f and 7–9).

Underlying the antero-posterior topography revealed by the microvilli of the midgut is a parallel topography, at the base of the microvilli, reflected by contours of the apical surfaces of the midgut cells. These surfaces are most convoluted in regions of the midgut with the shortest microvilli and are least convoluted in regions of the midgut with the longest microvilli (Fig. 6d–f).

Rough endoplasmic reticulum (RER) is present throughout all regions of the midgut (Figs. 7d, 8d, and 9d). Stacks of large flattened sacs of endoplasmic reticulum are especially evident in the posterior region of the midgut epithelium. Some smooth endoplasmic reticulum (SER) is interspersed among the RER of the anterior third of the midgut, with little if any SER is observed in the middle third or the posterior third of the midgut. However, clearly defined Golgi complexes are not evident in any of the midgut cells.

The lumen of the middle third of the Belgica midgut is lined with microvilli of intermediate length (~1 μm) that are associated with electron-dense particles of uniform size (~0.05 μm). These particles lie within the ectoperitrophic space and show a strong affinity for the microvilli (Fig. 8a–c). Within the cytoplasm of the underlying midgut cells, electron-dense particles of identical size are concentrated in autophagic vacuoles, each delimited by a plasma membrane, and are presumed to enter these epithelial cells by endocytosis. Numerous coated vesicles at the base of microvilli on the luminal surfaces of these midgut cells (Fig. 8c) offer an obvious route for the cellular uptake of these electron-dense particles from the ectoperitrophic space of the gut lumen.

Regenerative cells are scattered throughout the larval midgut epithelia and presumably represent imaginal stem cells that replace the larval epithelium at metamorphosis. These cells are located basally in the larval epithelium and are densely packed with ribosomes and endoplasmic reticulum (Fig. 10).

3.5. Contents of the midgut lumen

3.5.1. Endoperitrophic space

Within the gut lumen, the peritrophic membrane separates an inner endoperitrophic space from an outer ectoperitrophic space (Terra and Ferreira, 1994; Fig. 6). The anterior endoperitrophic space of the midgut is packed with relatively intact multicellular microbial organisms. Gradual digestion of microbes is reflected in the disappearance of pigmentation from the gut lumen in the posterior third of the midgut (Fig. 1a). Within the confines of the midgut’s peritrophic membrane, microbial cells arranged singly or in various aggregates can be readily discerned. These cells can be identified as predominantly nonbacterial microbes – i.e., algae, fungi, lichens, protists (Fig. 11a–c).

3.5.2. Ectoperitrophic space

The surrounding ectoperitrophic space is lined by midgut epithelium with microvilli whose lengths are graded along the...
antero-posterior axis. Within one of the 10 whole mounts of larval guts prepared, gregarines with distinctive appendages were found in this midgut zone (between the peritrophic matrix and midgut epithelial surface) (Fig. 11d and e). Considering the isolation of these gregarines from other related host species, these protists most likely represent a distinct species of dipteran parasite.

3.6. Spatial differentiation of the hindgut

3.6.1. Ring of undifferentiated, presumptive adult hindgut epithelium

In the images of the Belgica gut labeled with DAPI (Fig. 12a), a discontinuity in nuclear labeling is observed at the midgut–hindgut boundary. At the anterior-most region of the larval hindgut, an imaginal ring of undifferentiated presumptive adult hindgut cells appears in whole mounts as a zone of cells with small nuclei among the polyploid nuclei in cells of the larval hindgut epithelium.

Immediately posterior to the imaginal ring of epithelial cells, the anterior third of the hindgut is lined by a smooth cuticle approximately 0.25 μm thick. This hindgut cuticle, like the foregut cuticle, is contiguous with the exoskeleton. Also, like the foregut cuticle, the hindgut cuticle secretes a well-delineated, electron-dense outermost cuticular layer. The arrangement of these different layers secreted by hindgut epithelial cells is distinct from the arrangement of the cuticular layers secreted by epidermal epithelia (Fig. 3b). The anterior hindgut cuticle is secreted by attenuated, highly folded extensions of the apical surfaces of the hindgut epithelium containing conspicuous mitochondria and separated by large vacuoles that lack electron-density (Fig. 12b and c).
While apical ends of epithelial cells in the anterior third of the hindgut have conspicuous large vacuoles, the central region of the hindgut is occupied by epithelial cells that lack vacuoles but that have extremely convoluted apical membranes characteristic of epithelial cells involved in active transport of ions and water (Fig. 12d and e). Also in the central region of the hindgut, the presence of the electron-dense bacteria observed in high-resolution images is reflected in the pigmentation of the central portion of the hindgut as viewed in whole mounts of larval guts (Fig. 1a).

In the posterior third of the hindgut or rectum, the highly convoluted cuticle lining the lumen mirrors the structure of the foregut. The apical surfaces of the epithelial cells of the rectum lack infolded membranes associated with mitochondria and are apparently not specialized for transport of ions and water. The basal surface of this region, by contrast, is highly infolded. Association of this basal surface with numerous muscles suggests this posterior-most hindgut epithelium serves a mechanical function. In this most posterior portion of the hindgut, the lumen is devoid of both resident microbes as well as ingested microbes (Fig. 12f).

### 3.7. Muscles associated with the gut epithelia

The basal surface of the hindgut epithelium, like the foregut epithelium, is closely apposed to a uniformly thick layer of circumferential muscles (~10 μm) (Figs. 3c, 12e, f). By contrast, muscles associated with the midgut epithelium are sparsely but regularly distributed over the midgut’s basal surface (Figs. 6–9). Relatively widely dispersed muscle cells lie on the basal surface of the midgut epithelium and are embedded in the matrix of the epithelial basal lamina. These represent the longitudinal muscles of the gut. Sparsely distributed circumferential muscles are also present.
4. Discussion

The study of insect diversity and evolution has been advanced by extensive surveys of molecular phylogeny and morphology of external integuments; however, knowledge and appreciation of insect diversity remain incomplete without adequate knowledge of the diversity of internal anatomy/physiology of insects and how this diversity is influenced by environment.

With the paucity of information on the cellular architecture of insect guts, however, associating particular gut epithelial structures with adaptation to particular diets and/or environments remains in a rudimentary state. Conventional descriptions of gut epithelial diversity (Lehane, 1998; Noble-Nesbitt, 1998; Terra et al., 1988) clearly do not consider the marked regional differentiation of microvilli on midgut cells of *B. antarctica* to be a common feature of epithelial cells of insect guts. Establishing how general or how unique internal features are among insects, however, awaits additional structural studies on other related insect species.

4.1. Differentiation of foregut–midgut boundary: structure of the peritrophic membrane and stomodeal valve

A specialized luminal region at the foregut–midgut boundary can be visualized even in whole mounts of the alimentary canal (Fig. 1a). In cross-sections and longitudinal sections of the alimentary canal at this boundary region, an inner concentric ring of folded foregut epithelium and associated muscle layers lies within the anterior midgut epithelium (Figs. 4, 5). Among the Diptera, the degree of specialization of foregut and midgut epithelial cells varies among the suborders. The most complex specialization of the foregut–midgut interface is found among the muscoid flies, in which specialized anterior midgut...

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**Fig. 9.** In a–c, the midgut lumen is to the left. Different magnifications of the posterior region of the midgut are illustrated. (a) Note numerous clear vacuoles (arrows) that lie between the luminal microvilli and the basal lamina (lower right). (b) The long microvilli are densely packed and extend approximately 10 μm into the lumen. (c) The basal membranes of cells in this posterior region are highly folded. Basal lamina is indicated with arrowheads. Muscle — m. (d) Perinuclear region of midgut cell. Arrowheads indicate rough endoplasmic reticulum; arrows point to mitochondria. Scale bars: (a) 10 μm; (b) 5 μm; (c) 2 μm; and (d) 1 μm.
epithelium is closely apposed to the stomodeal valve to form the distinctive cardia (Eisemann et al., 2001; Binnington, 1988); but the simplest specialization of the foregut–midgut interface is observed in the suborder Nematocera, of which Belgica is a member. In these flies, the foregut has been described as forming a short intussusception into the anterior midgut referred to as the stomodeal valve (King, 1991; Wigglesworth, 1930). For Belgica, however, this intussusception of foregut as a percentage of total foregut surrounded by midgut is higher than that reported by Volf et al. (2004) for four other nematoceran Diptera in the families Culicidae (Culex pipiens) and Psychodidae (Lutzomyia longipalpis, Phlebotomus duboscqi, Phlebotomus papatasi).

Peritrophic structures for many insect species have often been described as chitinous, reticulated membranes with chitin microfibrils in a hexagonal or orthogonal arrangement (Lehane, 1998). These peritrophic structures consist of chitin networks embedded in protein–carbohydrate matrices (Wang and Granados, 2001; Tellam et al., 1999).

The origin and consistency of peritrophic membranes, gels and matrices differ among the insects (Terra, 2001; Binnington et al., 1998). The peritrophic structures of some insects, such as lepidopteran larvae, have traditionally been described as arising from cells along the length of the midgut epithelium (type I peritrophic matrix). Recent studies involving labeling of lepidopteran peritrophic proteins have indicated that while one peritrophic protein (i.e., invertebrate intestinal mucin) is secreted by epithelial cells throughout the length of the midgut (Harper and Granados, 1999), certain peritrophic proteins recognized by an antibody raised against the peritrophic membrane of Heliothis virescens are produced by specialized cells near the foregut–midgut interface (Ryerse et al., 1992).

At the junction of foregut and midgut epithelia in Diptera, the peritrophic structure (type II) arises from microvilli of midgut epithelia (Eisemann et al., 2001) and lines the lumen of the more posterior midgut epithelia. In Belgica, the midgut epithelial cells of the stomodeal valve are the only cells observed to produce a copious secretion associated with a newly formed structure that represents a type II peritrophic membrane.

4.2. Regional differentiation of midgut epithelium

At least in some insects, the midgut is differentiated both structurally and functionally along its length (Lehane, 1998; Marana et al., 1997; Ferreira et al., 1990; Terra et al., 1988; Dow, 1981). The marked and graded differences in microvillar lengths observed for Belgica midgut epithelial cells, however, represent an extreme example of such regional differentiation (Fig. 6).

Extensive infolding of the basal epithelial surface of midgut and hindgut cells, however, as frequently observed in other insects, is only evident in certain regions (anterior third and posterior third) of the Belgica midgut and the posterior third (rectum) of its hindgut (Villaro et al., 1999; Lehane, 1998; Marana et al., 1997). See Figs. 7c, 8e, 9c and 12f.

The high concentrations of electron-dense particles that occupy the ectoperitrophic space of the central region of the midgut are not observed elsewhere in the alimentary canal. The presence of comparable particles in autophagic vacuoles of these midgut cells suggests that these particles are taken up by cells rather than secreted by gut cells. The high concentration of particles in the gut lumen also implies that their movement proceeds toward the low concentration of particles observed within the midgut epithelial cells.

Although regenerative cells have not been observed in midgut epithelia of certain immature arthropods such as larval
Musca domestica (Terra et al., 1988) and immature Allacma fusca (Rost-Roszkowska et al., 2007a), regenerative cells (presumptive imaginal stem cells) are observed scattered throughout the monolayer of the Belgica midgut epithelium as they are in the gut epithelia of many other insects (Rost-Roszkowska et al., 2007a,b; Rost, 2006; Ohlstein and Spradling, 2005; Evangelista and Leite, 2003; Lehane, 1998; Hecker, 1977). In addition to high-resolution images of Belgica regenerative cells (Fig. 10), the dark cell on the basal surface of the anterior midgut epithelium in Fig. 7a, probably also represents such a regenerative cell.

4.3. Differentiation of hindgut epithelium

A discontinuity in nuclear size at the midgut–hindgut interface is evident in whole mounts of Belgica guts (Fig. 12a). A ring of epithelial cells with nuclei that are smaller than those nuclei located both anterior and posterior to the ring presumably represents the stem cells or presumptive imaginal cells for the adult hindgut (Takashima et al., 2008; Murakami and Shiotsuki, 2001). The rectal pads and/or papillae of many terrestrial insects have been shown to be involved in uptake of water and ions. Rectal epithelial cells (posterior hindgut) of these terrestrial insects have conspicuous folding of their apical plasma membranes. However, the ilea or anterior hindguts of aquatic insects such as Cenocorixa and Ephydrella as well as some terrestrial insects such as Formica, are adapted for ion and/or water transport (Villaro et al., 1999; Marshall and Wright, 1974; Jarial and Scudder, 1970). Insects living in aquatic or semi-aquatic environments presumably have different absorptive activities, being more concerned with absorption of inorganic ions rather than with water absorption.

Based on examination of other insect hindguts (Noble-Nesbitt, 1998; Jarial and Scudder, 1970), numerous vacuoles are correlated with an absorptive function for these epithelial cells. The anterior two-thirds of the Belgica hindgut or ileum seems to be adapted for absorption of ions and water in the larva’s semi-aquatic environment (Murakami and Shiotsuki, 2001; Heuser et al., 1993). Unlike the rectal pads or papillae of terrestrial insects, the posterior third of the hindgut or rectum epithelium of Belgica shows no special adaptations on its apical surface for transport of ions and water;
Fig. 12. Images of the hindgut epithelium are presented as DAPI-labeled whole mounts (a) and thin sections (b–f). Fig. 10a shows the midgut (mg)–hindgut interface of the larval gut that has been labeled with the specific DNA stain DAPI. At its anterior-most region, the nuclei of the hindgut epithelial cells are dwarfed by the polyplloid nuclei of the Malpighian tubules (arrows) and more posterior hindgut cells (*). (b) The anterior third of the hindgut consists of cells with polyplloid nuclei (n) whose apical ends are occupied by numerous vesicles and covered by a thin cuticle (arrow). A thin basal lamina (bl) is located on its basal surface. (c) Convolutet membranes, electron-lucent vesicles and mitochondria are concentrated at apical surfaces of this anterior third of the hindgut epithelium. The cuticle is indicated with an arrow. (d, e) Bacteria inhabit the lumen of the middle third of the hindgut. The apical ends of these epithelial cells are covered by a thin cuticle and have extremely convoluted membranes (arrow). Basally the epithelium has a thick muscle layer (m). (f) The cuticle lining the lumen of the posterior third of the hindgut is convoluted (arrow). The basal lamina of epithelial cells has conspicuous infoldings (arrowheads); m = muscles. Scale bars: (a) 50 μm; (b) 2 μm; (c) 1.0 μm; (d) 2 μm; (e) 20 μm; and (f) 2 μm.
this portion of the hindgut is surrounded by a thick layer of muscles and apparently has a mechanical rather than a transport function (Fig. 12c–f).

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