ULTRASTRUCTURE OF THE MIDGUT EPITHELIUM OF *WIRENIA ARGENTEA* (MOLLUSCA: SOLENOGASTRES)

C. TODT AND L. VON SALVINI-PLAWEN

Institute of Zoology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

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ABSTRACT

The midgut epithelium of *Wirenia argentea* Odhner is composed of two cell types: dorsal ciliary cells and digestive cells. Ciliary cells bear simple locomotory cilia with a pair of rootlets and without an accessory centriole. Digestive cells are the site of intracellular digestion and are characterized by an extensive endosomal and lysosomal system. The midgut epithelium shows a plasticity in digestive cell size and contents, depending on the amount of absorbed food material. Additionally, digestive cells produce membrane-bound mineralized granules as well as glandular vesicles, which most probably contain extracellular digestive enzymes. Four stages within the digestive cycle are defined. Cell types and cell cycles are compared with those known for digestive epithelia of other molluscs.

INTRODUCTION

In contrast to all other molluscan classes, where the endodermal part of the digestive tract is divided either into a midgut-sac and midgut-duct or 'intestine' (Caudofoveata), or into even more compartments, such as stomach, midgut glands and intestine (Placophora, Tryblidia, Scaphopoda, Bivalvia, Gastropoda, Siphonopoda), the Solenogastres bear a simple, straight midgut lined by digestive and resorptive cells (Salvini-Plawen, 1981). The midgut occupies the whole body cavity with the exception of the mid-dorsal and mid-ventral spaces (dorsal gonad, ventral blood sinus). Because of the random serial arrangement of dorsoventral muscle bundles, the midgut often exhibits a latero-ventrally pouched configuration, especially in larger species. Generally, a ciliated mid-dorsal tract or fold is present, leading to the short, ciliated rectum that opens dorsally into the mantle cavity.

The midgut epithelium is considered to be the main site of absorption and intracellular digestion of food material in Solenogastres (Baba, 1940; Salvini-Plawen, 1967b, 1981, 1988a; Scheltema, Tscherkassky & Kuzirian, 1994). In contrast to a 'vertebrate-like' method of digestion characterized by a high degree of extracellular enzymatic decomposition of food material, digestion in molluscs includes the endocytosis of relatively large, extracellularly predigested food particles, followed by intracellular decomposition of material via the lysosomal pathway (see also Boucaud-Camou & Boucher-Rodoni, 1983). Consequently, digestive cells are able to absorb large amounts of liquid and particulate matter. This results in different cell morphologies correlated with food availability and with the respective stage within the digestive process. Therefore, discrimination of distinct cell types as opposed to merely different developmental stages of cells is difficult. This is reflected by a discordant terminology, especially in older literature, but ultrastructural studies can help to differentiate and define cell types by providing insight into cell functions.

The midgut of Solenogastres is lined by large, club-shaped cells that are reported to contain different kinds of mostly globular bodies which were termed ferment granules, food substances, pigment granules and oil droplets by Baba (1940), enzymatic granula and bodies by Salvini-Plawen (1988a), or a variety of inclusions by Scheltema *et al.* (1994). The preferred prey of Solenogastres are anthozoan (Alcyonaria, Gorgonaria, Zoantharia) or hydrozoan polyps (Salvini-Plawen, 1972, 1981). The discharge of nematocysts is prevented during ingestion, but they remain functional even within the midgut. Digestion of nematocysts is variable; many pass intact through the entire digestive tract (Salvini-Plawen, 1981). The presence of so-called pyramidal granula cells, similar to the granula cells of Caudofoveata (see Discussion), has occasionally been reported for Solenogastres (Thiele, 1894), but often regarded as a developmental stage of digestive cells (Salvini-Plawen, 1981, 1988a).

Baba (1940) studied digestion and absorption in *Epimenia babai* Salvini-Plawen, 1997 (as *E. verrucosa*), including physiological experiments on food absorption. Large pieces of prey tissue (Alcyonaria, Anthozoa) were found in the pharynx and predigestion within the foregut was postulated. Endocytosis of carmine particles by isolated midgut cells was observed, demonstrating that nutrients are absorbed by the cells of the midgut epithelium. Digestion was completed within the cells and old digestive cells became inactive with regard to secretion and endocytosis, but did store metabolic end-products. The cells were finally shed into the midgut lumen and defaecated. Thus, the midgut cells of Solenogastres and the digestive gland cells of higher molluscs share structural features indicating identical functions.

Here, we present the first data on the ultrastructure of the midgut epithelium of Solenogastres, represented by *Wirenia argentea*, including a description of the cell cycle during intracellular digestion.

MATERIAL AND METHODS

In August 2000, specimens of *Wirenia argentea* Odhner, 1921 (synonym *Aesthoherpia glandulosa* Salvini-Plawen, 1988b) (Pholidoskepia, Gymnomeniidae) (Fig. 1A) were sampled by means of a sledge-dredge in the Trondheimsfjord, Norway, at three different sites (Table 1). The sediment was washed with fresh seawater using 125-μm nets and sieves to remove clay and mud. The remaining, larger fractions, composed of inorganic material, detritus and living organisms, were kept in buckets and stored at 13°C overnight. Solenogastres were isolated, identified and stored at 13°C in clean Petri dishes for 3 h to 14 days until anaesthetization in 7% magnesium chloride solution isotonic with local seawater, followed by fixation.

Specimens were prefixed for several days in cold 5% glutaraldehyde in a 0.2 M sodium-cacodylate buffer pH 7.3 with 12% sucrose and 0.78% NaCl, and postfixed for 2.5 h in cold

Correspondence: C. Todt; e-mail: ChristianeTodt@gmx.net

0.25% osmium tetroxide in distilled water. After decalcification in ascorbic acid they were dehydrated twice for 10 min in DMP (2.2 dimethoxypropane) and twice for 5 min in 100% ethanol, and then embedded in Spurr's epoxy resin (Spurr, 1969). Five section series were produced on a Reichert Ultracut S using a Diatome Histo Jumbo Knife (Blumer *et al.*, 2002), whereby ultrathin (75 nm) sections alternated with semithin (1 µm) sections. Semithin sections were stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate before examination with a Zeiss EM 902 transmission electron microscope. Additionally, six semithin section series of *W. argentea* produced earlier by C. Handl (see also Handl & Salvini-Plawen, 2001), as well as eight azan-stained histological section series of *W. argentea*, were included in the study.

Photomicrographs were made with a Zeiss MC8 microscope camera on a Zeiss Axioplan microscope.

RESULTS

General structure and histology

With the radular apparatus retracted, the midgut forms a small anterodorsal caecum, then expands to occupy most of the body cavity (Fig. 1B). There is no midgut-muscularis, but laterally the thin basal lamina of the midgut epithelium lies adjacent to the muscle layers of the body wall, which is composed of proximal longitudinal muscle fibres and distal transverse and circular

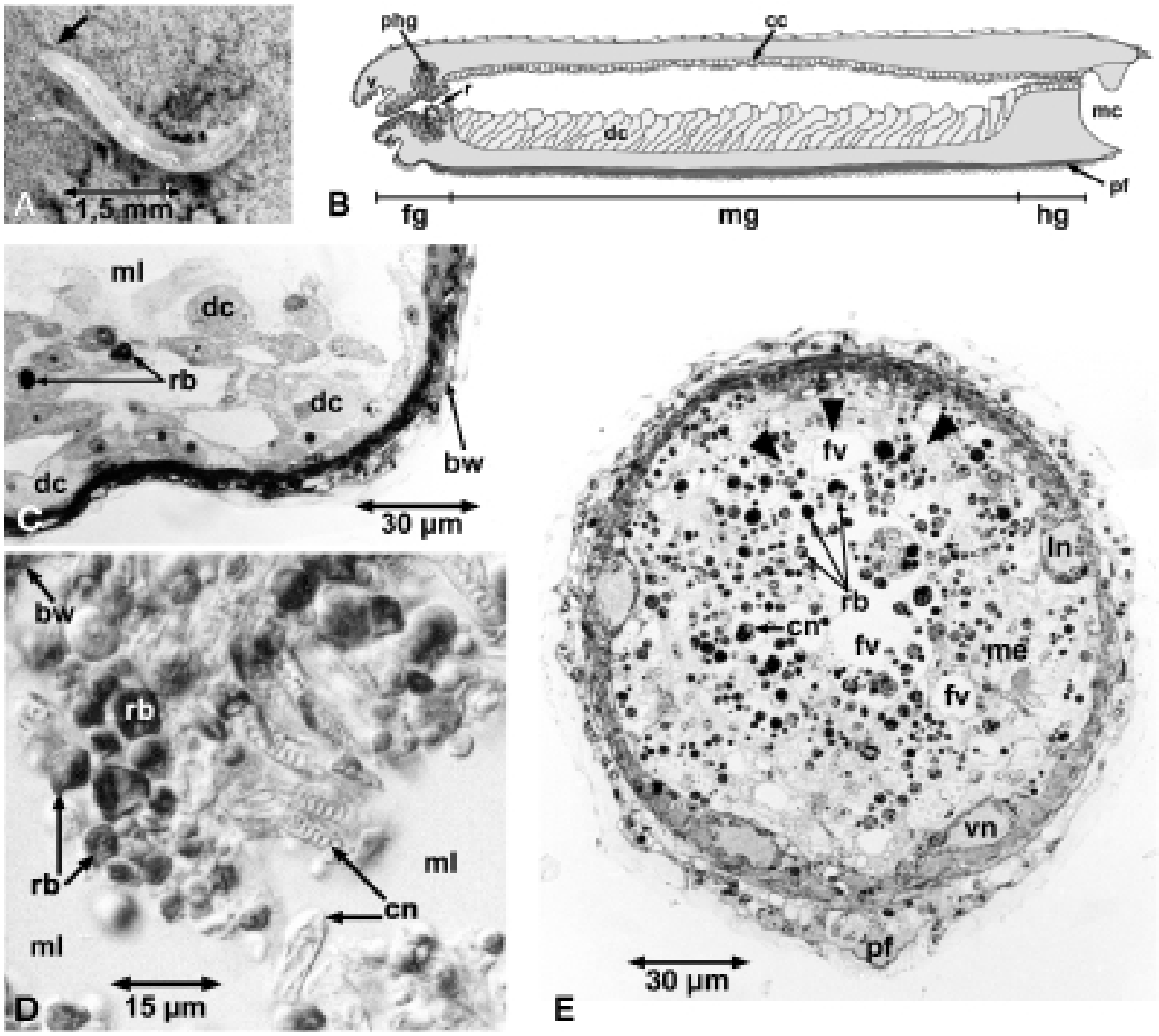


Figure 1. A. *Wirenia argentea*, living specimen on natural substrate, arrow marking anterior body end. **B.** Schematic drawing of the digestive tract of *W. argentea*. **C–E.** Semithin (**C**, **E**) and histological (**D**) cross-sections through the midgut epithelium. **C.** Midgut and body wall of a starved specimen with low digestive cells containing a few residual bodies, and a distinct midgut lumen. **D.** Midgut of a specimen fixed soon after removal from the natural substrate; numerous residual bodies and nematocysts are present, the midgut lumen is narrow. **E.** Cross-section of a well-fed specimen fixed soon after removal from the substrate; the midgut epithelium is high, the midgut lumen is restricted to a network of slit-like fissures between digestive cells, and dorsally between digestive cells and ciliary cells (arrowheads); the digestive cells contain numerous residual bodies, food vacuoles, and stenoteles. Abbreviations: bw, body wall; cc, ciliary cells; cn, nematocysts; dc, digestive cells; fg, foregut; fv, food vacuole; hg, hindgut; ln, lateral nerve chord; mc, mantle cavity; me, midgut epithelium; ml, midgut lumen; pf, pedal fold; phg, pharyngeal glands; rb, residual body; v, vestibulum; vn, ventral nerve chord.

fibres (Fig. 1C, E). Single transverse fibres of the dorso-ventral body muscles constrict the midgut and occasionally cause the epithelium to bulge slightly inwards. Dorsal and ventral to the midgut there are sinuses with many large haemocytes. Posteriorly, the dorsal sinus is displaced by the organs of the gonopericardial system. The midgut has a dorsal ciliary tract which is narrow and hardly discernible anteriorly, but becomes more prominent towards the transition to the hindgut.

Table 1. Sampling sites in the Trondheimsfjord, Norway: location, water depth and sediment characteristics.

Location	Longitude/latitude	Depth (m)	Sediment characteristics
Near Tautra, southwest of dumping ground	63°38′N/10°34′E	250	Clay
Flakkfjorden, north of Flakk	63°29′N/10°12′E	550	Clay, *Pennatula* sp.
South of Saeterbukta	63°33′N/10°24′E	330	Clay

Apart from the mid-dorsal ciliary cells, the midgut epithelium is composed of digestive cells with different morphology depending on the nutritional status of the animal. In some specimens most cells are roughly cuboidal and 15 μm to 30 μm high, with basal nuclei and only few vesicles and droplets, and the midgut lumen is wide (Fig. 1C). In others, the epithelial cells contain great numbers of vesicles with phagocytosed pieces of prey tissue in different stages of intracellular digestion; such cells are highly enlarged, restricting the midgut lumen, which may be indiscernible in histological sections (Fig. 1E). The cells are orientated at an oblique angle to the longitudinal body axis with the free cell apices pointing posteriorly. Therefore, the midgut epithelium appears spongy and multi-layered in cross-section. There is no significant difference in cellular status between the anterior and the posterior part of the midgut of each specimen. Phagocytosed pieces of prey tissue include whole nematocysts (Fig. 1D).

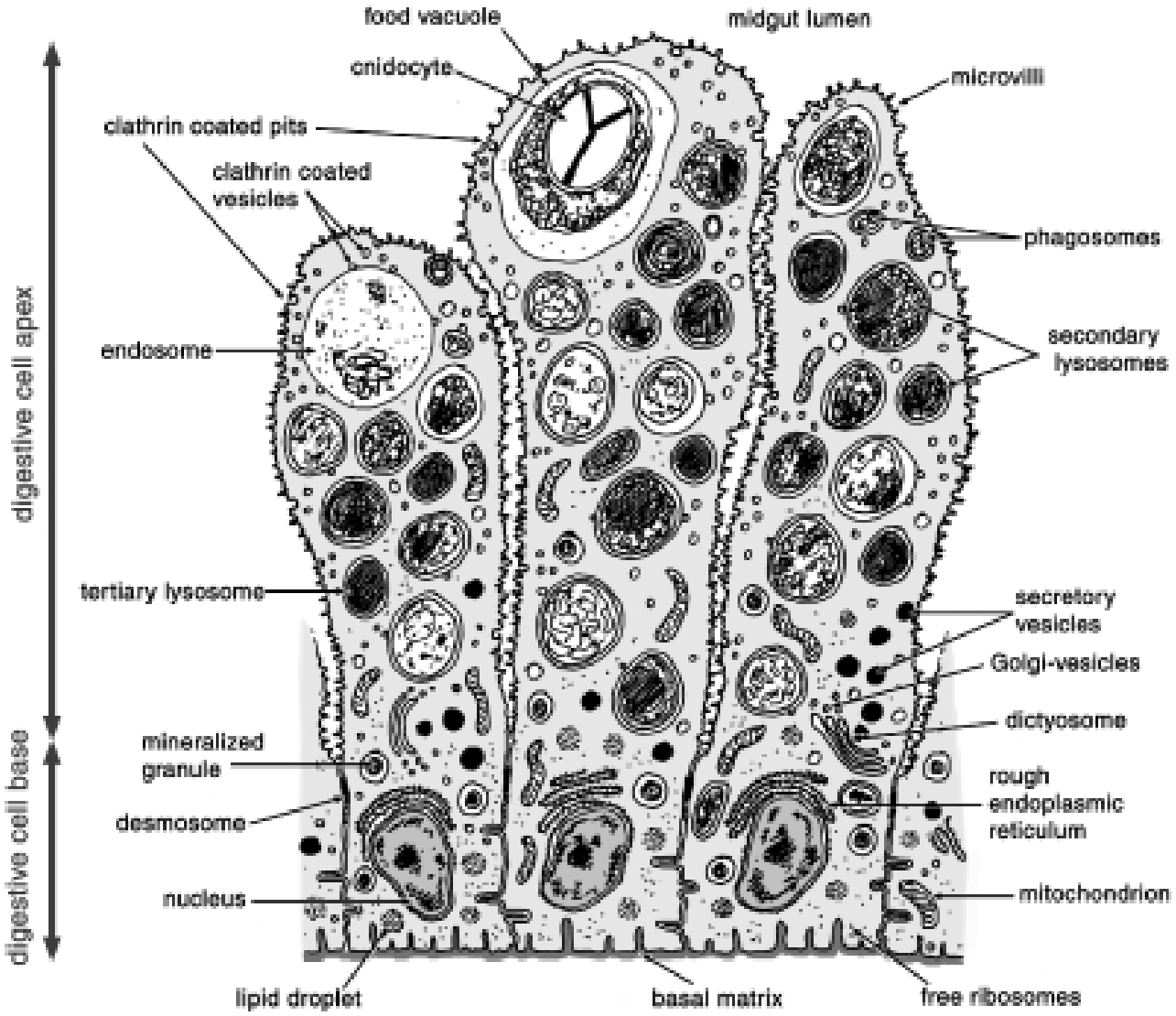
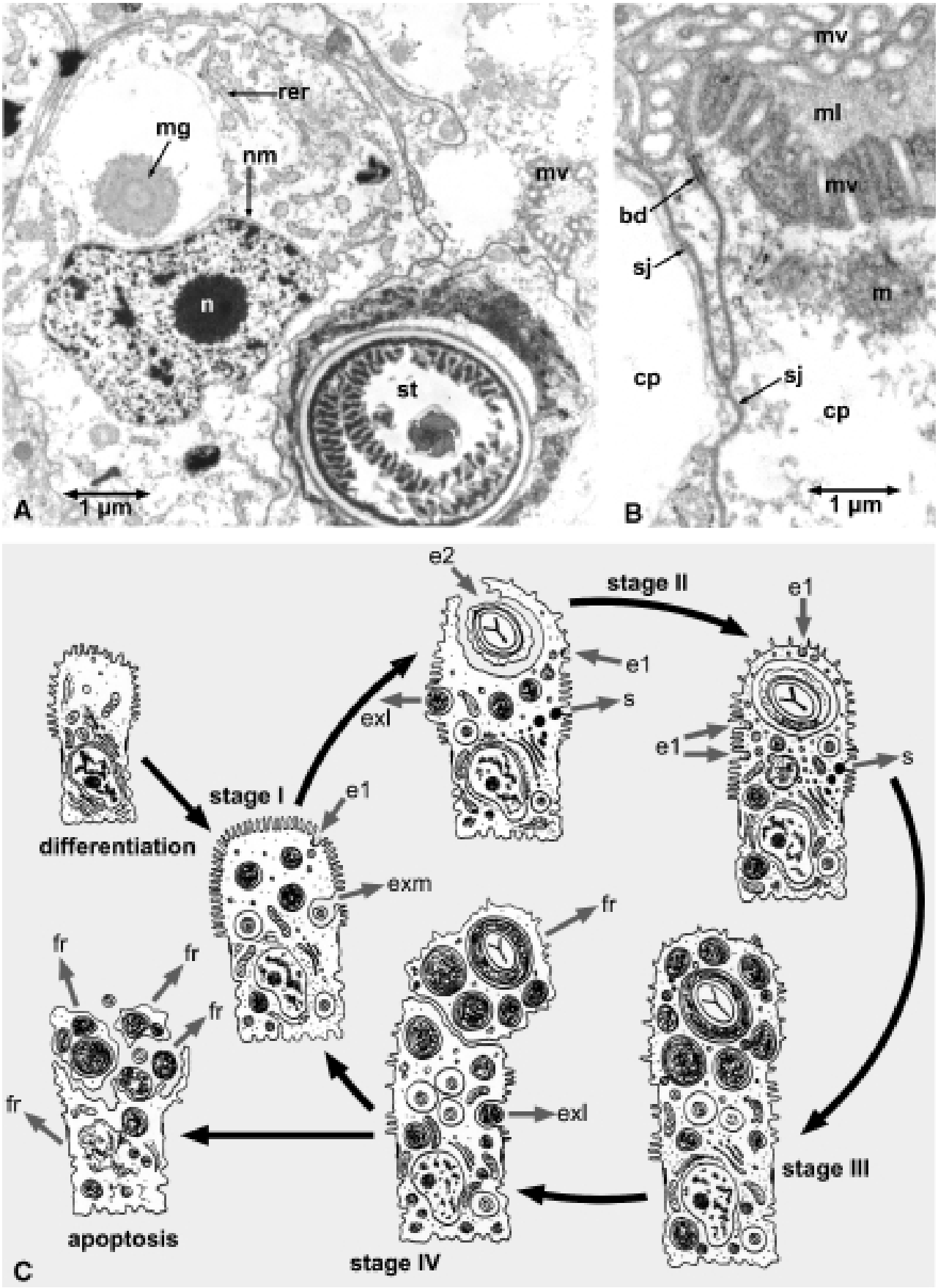


Figure 2. Schematic drawing of digestive cells with characteristic organelles. Note the extensive lysosomal system and the difference in size between the cell base and the cell apex, the latter defined as the cell-portion situated distal to the desmosome.



Ultrastructure

Digestive cells (Fig. 2) make up the majority of midgut cells. Generally, the basal cell membrane has undulations, but no basal labyrinth is developed; the lateral membranes show interdigitations (Fig. 3A). A single belt desmosome is located about 70–100 μm from the thin basal lamina, and is followed proximally by a septate junction (Fig. 3B). Distally, the cell bulges into the midgut lumen; this apical part may be 10 times larger than the basal part. The apical membrane bears microvilli (Fig. 3B). The nucleus is irregularly shaped and located in the region of the desmosome or proximal to it. The nuclear membrane bears ribosomes. Generally, the density of cell organelles, including elongate mitochondria, dictyosomes, peroxysomes, secretory vesicles and of lipid droplets is highest in the basal part of the cell. Digestive cells of all developmental stages contain electron-translucent granules with more or less distinct concentric striation, most likely representing mineralized concretions (Fig. 3A). Each granule is enclosed within a vacuole that is often much larger than the granule itself, probably an effect of shrinkage during fixation. Rough endoplasmic reticulum composed of swollen cisternae apparently takes part in the formation of the granules (Fig. 3A).

Digestive cell size and shape, as well as the abundance of cell organelles active in intracellular digestion, are highly variable. The presence of cells in transitional conditions, however, confirms that these are not different cell types, but rather developmental stages of a single cell type. Here, we define four stages within the cell cycle (Fig. 3C).

In starved animals, the digestive cells are low, with the apical part of the cell being smaller than the part proximal to the belt desmosome (stage I). The cytoplasm is electron-translucent with few membrane-bound mineralized granules, lipid droplets and residual bodies (Fig. 4A). The apical membrane bears numerous microvilli. When food is available, the cells enter the endocytotic stage (stage II), characterized by a high abundance of clathrin-coated pits at the surface and of clathrin-coated vesicles within the cell (Fig. 4B, C). The cell plasma appears electron dense with many free and membrane-bound ribosomes. It contains diverse small vesicles of varying electron density. These represent endosomes, formed by the fusion of clathrin-coated vesicles and primary and secondary lysosomes, as well as Golgi and transport vesicles (Fig. 4D). The Golgi apparatus is well developed and composed of highly active dictyosomes (Fig. 5A). Some produce electron-dense secretory vesicles (up to 0.6 μm in diameter) which are released into the midgut lumen by exocytosis (Fig. 5B); these probably contain enzymes that participate in extracellular digestion. The cytoplasm surrounding the dictyosomes is rich in microtubules (Fig. 5A). The dictyosomes are mostly located near the nucleus, which shows large patches of electron-dense heterochromatic areas and a well-defined nucleolus. In addition to the endocytosis of small particles and liquids (pinocytosis) via clathrin-coated vesicles, digestive cells may phagocytose larger food items, such as nematocytes containing fully developed nematocysts. These are enclosed within voluminous vacuoles, displacing the apical cell plasma to a narrow layer rich in small vesicles (Fig. 5C). In accordance with the amount of absorbed food material, the cell becomes larger and the apical part bulges far into the midgut lumen.

In the digestion stage (stage III) the apical cell membrane bears fewer microvilli and is free of clathrin-coated pits. Food vacuoles, endosomes and secondary lysosomes are abundant even in the most basal cell portion (Fig. 5D). The nucleus has only small heterochromatic areas. The cell plasma gradually appears more electron-lucent because rough endoplasmic reticulum, free ribosomes and vesicles become scarce, especially in the apical cell regions. The Golgi apparatus still produces secretory vesicles and primary lysosomes which fuse with endosomes and food vacuoles. The size of food vacuoles containing nematocytes apparently decreases with advancing degree of digestion until the indigestible remains are enclosed within secondary lysosomes. Endosomes and early lysosomes fuse to form large, globular secondary lysosomes. The internal structure of secondary lysosomes allows the definition of two different types. Type 1 is most common and contains a variety of small vesicles of varying electron density, stacks of membranes and areas with electron-dense, partly granular material. Typical features include entire stenoteles (Fig. 6A) or large fragments of electron-dense or electron-translucent material (Fig. 6B, H). These lysosomes attain large sizes, usually measuring 4–7 μm in sections, with a maximum diameter of 12 μm. Lysosomes, whose contents are less vesicular, and composed of areas of medium electron-transparency with distinct stacks of membranes and of small patches of electron-dense material (Fig. 6C, D), probably represent early stages of type 1 secondary lysosomes. Type 2 secondary lysosomes (Fig. 6E), on the other hand, are easily discernible from type 1 lysosomes. They are medium-sized (3–4 μm diameter in section) vesicles with tubule-like, occasionally branched contents, which measure about 0.1 μm in cross-section (Fig. 6F). This type of secondary lysosome occurs only sporadically. Small residual bodies that resemble lipofuschin-containing tertiary lysosomes (Fig. 6G) are encountered within digesting cells of stage III, but are relatively rare.

During the excretory stage (stage IV), the digestive cell expels late secondary and tertiary lysosomes (residual bodies) into the midgut lumen (Fig. 6H). While exocytosis of single residual bodies does occur, more typically a large portion of the apical part if the cell containing several lysosomes is tied off. This radically reduces the size of the cell. Excretory cells are often characterized by numerous mineralized granules, an electron-lucent cytoplasm and a nucleus with small clusters of heterochromatin.

Some digestive cells seem to undergo apoptosis during or after the excretory stage, as indicated by a degenerating nucleus. In others the nucleus remains intact, the cytoplasm is electron-translucent with few vesicles and a small Golgi apparatus, but the apical membrane bears abundant microvilli. These cells can probably enter another cell cycle if food becomes available, and thus represent stage I digestive cells.

Figure 3. A, B. Transmission-electron-microscopy (TEM) micrographs of digestive cells of *Wirenia argentea*. **A.** Section in region of the nucleus, nuclear membrane with ribosomes; rough endoplasmic reticulum consists of swollen cisternae near a vacuole containing a mineralized granule. The neighbouring cell bears a secondary lysosome with a stenotele in cross-section. **B.** Longitudinal section in the region of the belt desmosome and septate junctions. A mitochondrion is located near the apical cell membrane, which bears a brush border of microvilli towards the midgut lumen. **C.** Proposed cell cycle of digestive cells in *W. argentea*: The young cell differentiates to a stage I cell with a basic level of endocytotic (e1) and exocytotic activity, e.g. exocytosis of mineralized granules (exm). When food becomes available, secondary lysosomes are expelled (exl), and absorption starts by increased endocytosis of small food particles and liquids via clathrin-coated vesicles (e1) and phagocytosis of large particles such as nematocytes (e2). Thus, the cell grows to a stage II cell, which additionally secretes extracellular digestive enzymes (s). Intracellular digestion begins in stage II and is completed within stage III, where mineralized granules are also produced. In stage IV, indigestable residues, enclosed in secondary and tertiary lysosomes, are expelled via exocytosis (exl) or fragmentation of the cell apex (fr). Apoptosis of the entire cell may follow, or the cell reconstitutes to stage I. Abbreviations: bd, belt desmodome; bw, body wall; cp, cell plasma; m, mitochondion; mg, mineralized granule; ml, midgut lumen; mv, microvilli; n, nucleus; nm, nuclear membrane; rer, rough endoplasmic reticulum; sj, septate junctions; st, stenotele.

Ciliary cells (Fig. 7) are restricted to the dorsal part of the midgut and form a narrow ciliary tract, five to seven cells wide, between foregut and hindgut. Posteriorly, the tract broadens and is confluent with the ciliated hindgut epithelium. The polyciliated cells are 10–14 μm high and almost as wide. Basally and laterally, the cell membranes form numerous infoldings, interdigitating with the basal lamina and with one another. Neighbouring ciliary cells are linked to each other by an apical belt desmosome and a more proximally located septate junction. The apical cell membranes bear slender microvilli which

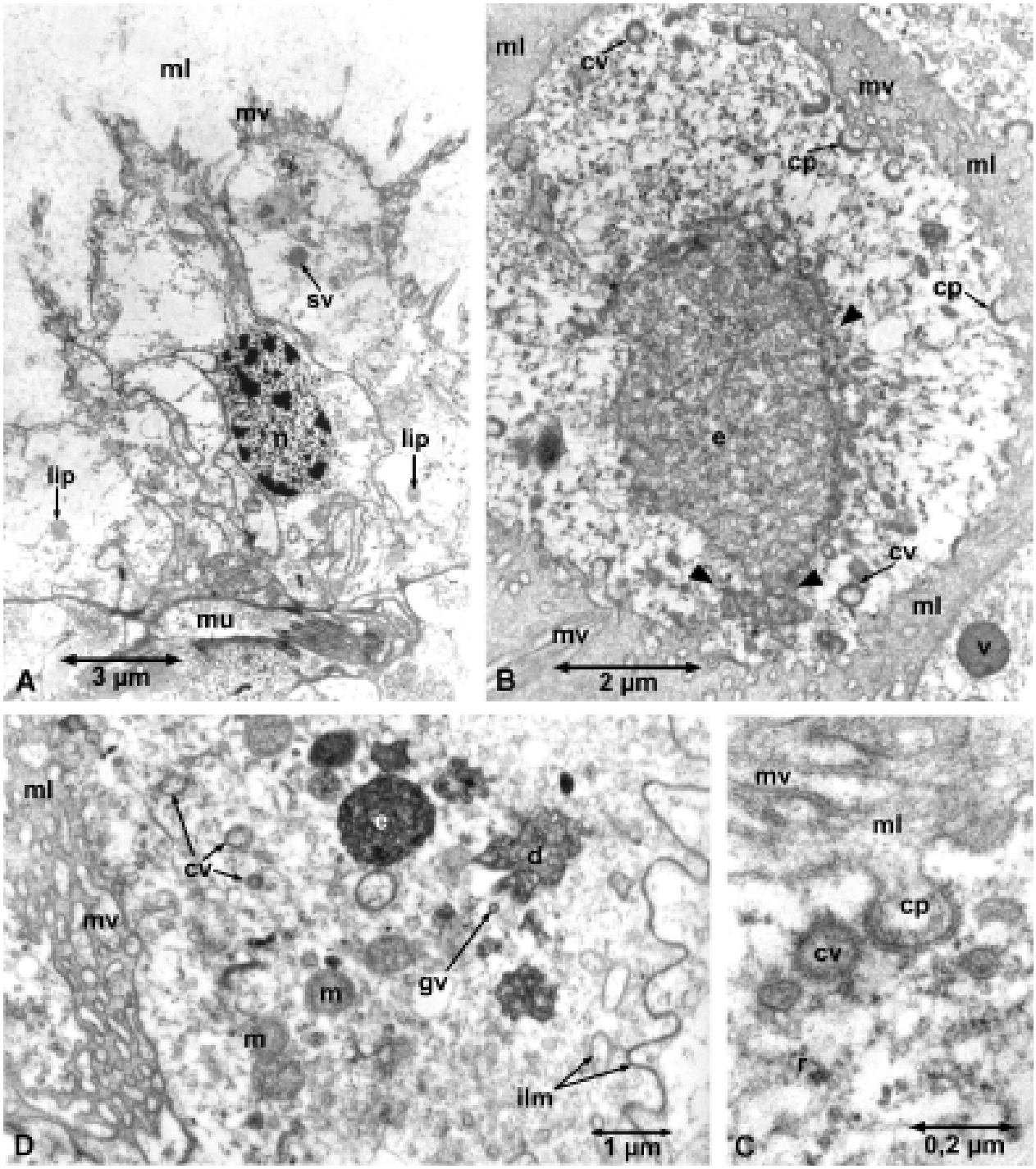


Figure 4. TEM micrographs of digestive cells of *Wirenia argentea*. **A.** Stage I digestive cell of a starved specimen with few lipid droplets basally and some secretory vesicles; the nucleus has distinct, electron-dense clusters of heterochromatin; the apical membrane bears densely set microvilli. **B.** Apex of a cell of early stage II with numerous clathrin-coated pits and clathrin-coated vesicles, some fusing (arrowheads) with a large central endosome. **C.** Detail of the apical cell membrane with a clathrin-coated pit, and a coated vesicle within the ribosome-rich cell plasma. **D.** Oblique section of a cell in stage II, cell partly bulging into the midgut lumen, partly bound to a neighbouring cell by interdigitations of the lateral cell membrane; it contains mitochondria, cisternae of dictyosomes in horizontal section, Golgi vesicles, clathrin-coated vesicles and endosomes. Abbreviations: cp, clathrin-coated pits; cv, clathrin-coated vesicles; d, dictyosomes, e, endosome; gv, Golgi vesicles; ilm, lateral cell membrane; lip, lipid droplets; m, mitochondia; ml, midgut lumen; mu, body-wall muscles; mv, microvilli; n, nucleus; r, ribosome; sv, secretory vesicles; v, vesicle.

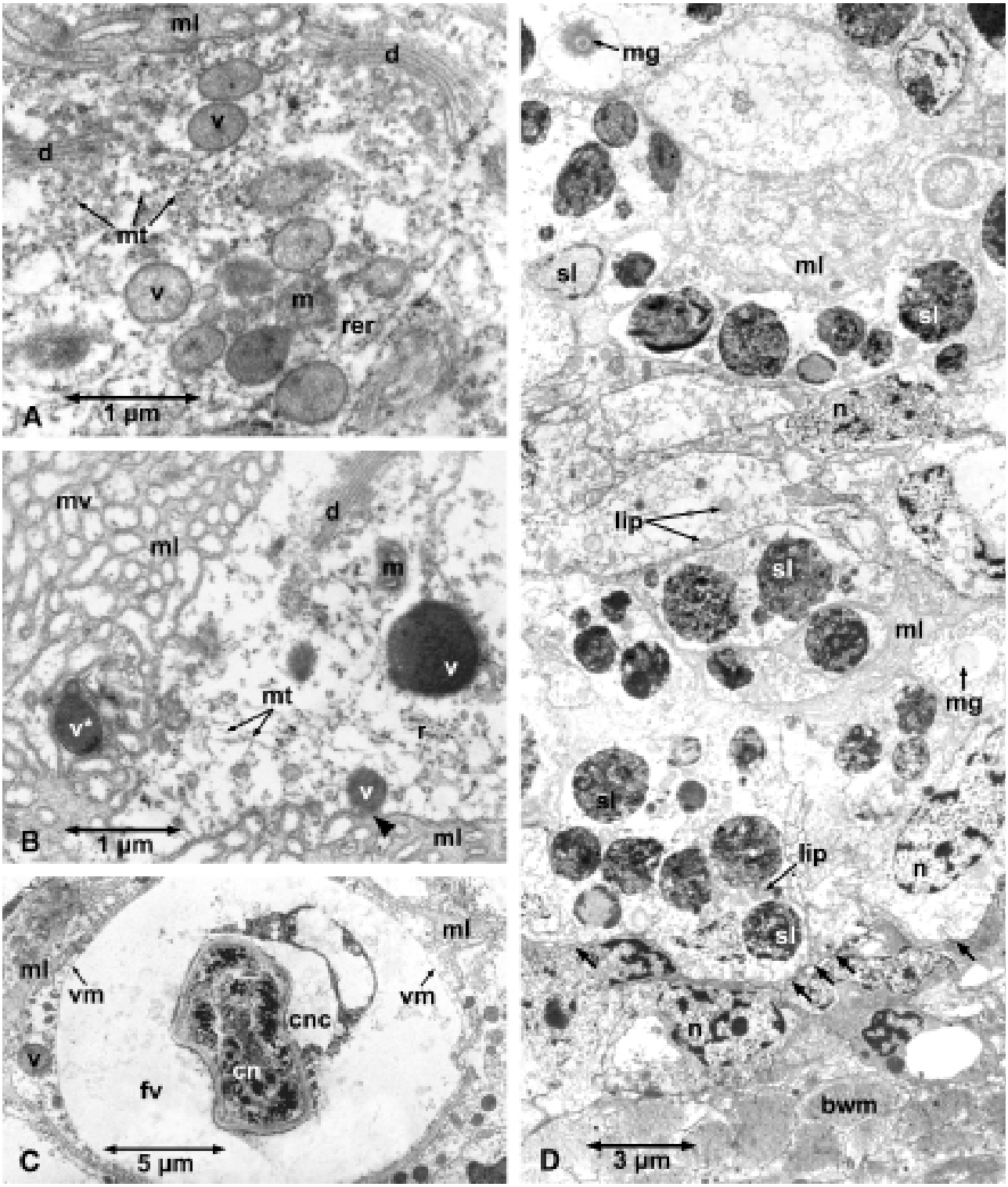


Figure 5. TEM micrographs of digestive cells of *Wirenia argentea*. **A.** Active dictyosomes in a digestive cell of late stage II, with a cluster of secretory vesicles. The cytoplasm is rich in rough endoplasmic reticulum, free ribosomes and microtubules, which are shown in cross-section. **B.** Lateral part of a digestive cell of stage III with an active dictyosome, and microtubules in longitudinal section. One secretory vesicle is fused with the cell membrane (arrowhead), a second vesicle (v*) is located within a digit-shaped protusion of the cell and is just prior to exocytosis. **C.** Apex of a midgut cell in stage II with a large central food vacuole containing a nematocyte with a nematocyst. The cell plasma is restricted to a thin layer confined proximally by the vacuole-membrane and distally by the cell-membrane, which adjoins the midgut lumen. **D.** Oblique section through the muscle layers of the body wall and the midgut epithelium with stage III digestive cells, which show slight undulations basally (arrows). Note that the lateral membranes of only the most basal cells and a few others are in contact. The interstitial space between cells is part of the midgut lumen. The digestive cells contain diverse secondary lysosomes, lipid droplets and membrane-bound mineralized granules. Abbreviations: bwm, muscle layers of the body wall; cn, nematocyst; cnc, nematocyte; d, dictyosomes; fv, food vacuole; m, mitochondrion; lip, lipid droplets; mg, mineralized granules; ml, midgut lumen; mv, microvilli; mt, microtubules; n, nucleus; rer, rough endoplasmic reticulum; sl, secondary lysosomes; v, secretory vesicles; vm, vacuole-membrane.

are densely set between the cilia. The cilia are up to 10 μm long and composed of nine sets of microtubule-duplets and a central duplet. There is a thick, electron-dense basal plate at the base of each cilium (Fig. 7A). The transitional zone between cilium and basal body is of medium electron density and about the same length as the basal body itself, which bears a pair of rootlets. The horizontal ciliary rootlet is ~15 μm long, the vertical rootlet about 4 μm. Both show a basal electron-translucent zone (Fig. 7B), and enclose an angle of ~120° (Fig. 7C). The ciliary base itself is thick-walled, not much higher than broad, and bears a basal foot located opposite to the horizontal rootlet but somewhat more distal (Fig. 7D). No accessory centriole was observed. Ciliary cells are rich in mitochondria and ribosomes, and contain lysosomes obviously representing autophagosomes.

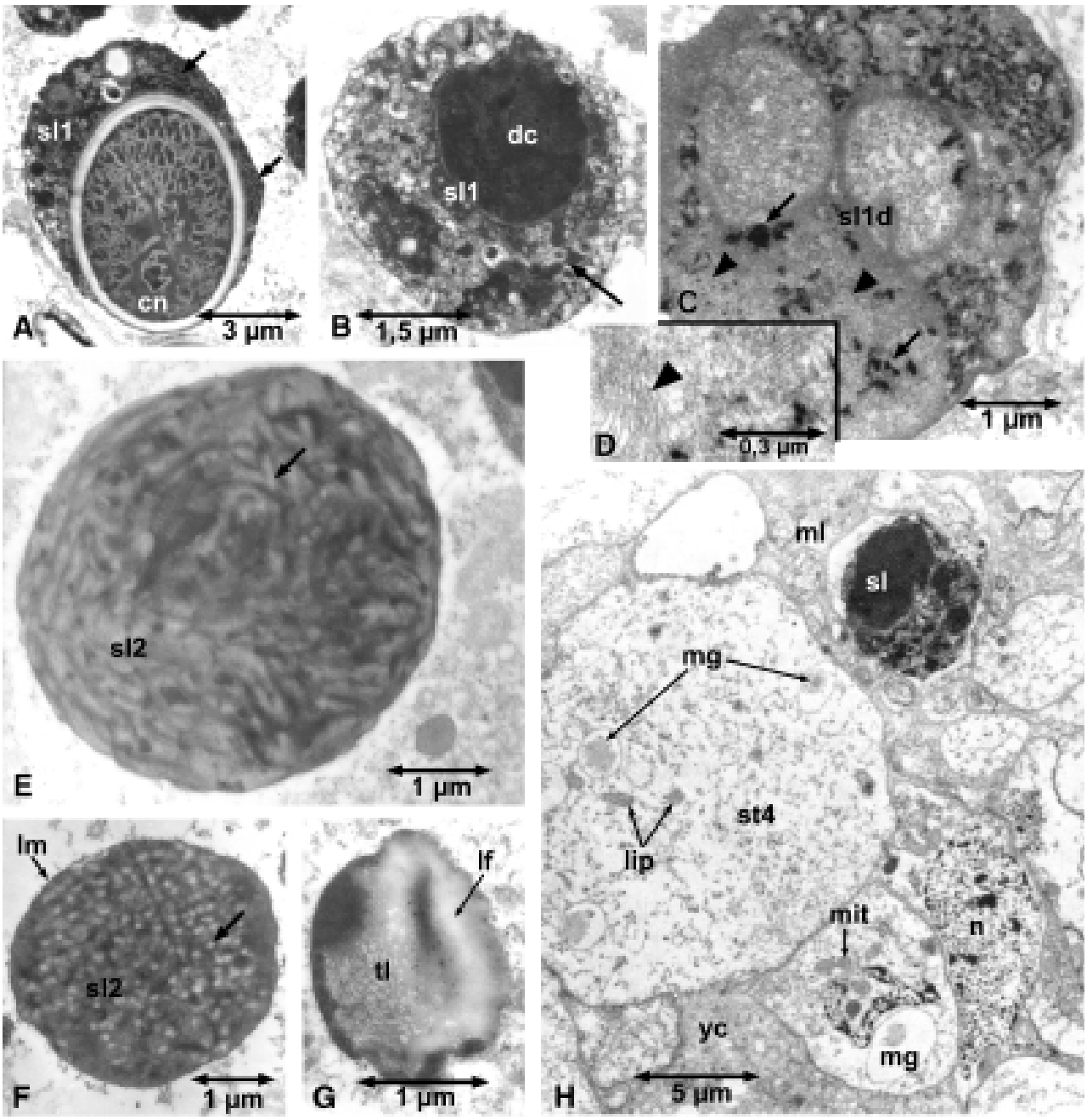


Figure 6. TEM micrographs of digestive cells of *Wirenia argentea*. **A.** Secondary lysosome of type 1 containing nematocyte. Note the stacks of tightly packed membranes with electron-dense material in between (arrows). **B**. Secondary lysosome of type 1 with an electron-dense core and vesicular contents of varying electron-density (arrow). **C, D.** Secondary lysosome, most probably representing an early developmental stage of type 1. **C.** Overview showing small patches of electron-dense material (arrows) and zones of medium electron-density composed of layers of membranes and small granules (arrowheads). **D.** Detail with loosely arranged layers of membranes (arrowhead). **E.** Secondary lysosome of type 2 with tubule-like, partly branched contents (arrow). **F.** Secondary lysosome of type 2 with tubule-like structures in cross-section (arrow). Note that the outer lysosome membrane is clearly defined, while the tubules do not show a membranous lining. **G.** 'Tertiary' lysosome with lipofuschin-like contents (lf). **H.** Digestive cells of late stage IV and young cell. Stage IV cells are characterized by an electron-translucent cytoplasm containing lipid-droplets and mineralized granules (mg). Cell apices show signs of fragmentation and one fragment contains a large secondary lysosome (sl). Abbreviations: cn, nematocyte; dc, secondary lysosome of type 1 with an electron-dense core; lip, lipid-droplets; lm, lysosome membrane; mg, mineralized granules, ml, midgut lumen; n, nucleus; sl1, secondary lysosome of type 1; st4, digestive cells of late stage IV; tl, 'tertiary' lysosome; yc, young cell.

DISCUSSION

The cytological features of the midgut epithelium of *W. argentea* described in the present study confirm earlier reports on the structure and function of the midgut of Solenogastres (e.g. Baba, 1940; Salvini-Plawen, 1981, 1988a; Scheltema *et al.*, 1994). The epithelium is single-layered and composed of large, club-shaped cells which are highly variable in size and shape in adaption to their absorptive and digestive functions.

In other molluscs, a variety of organs are involved in endocytosis and intracellular digestion: the anteror midgut of Caudofoveata (Salvini-Plawen, 1981; Scheltema *et al.*, 1994), the intestine of gastropods and bivalves (Morton, 1983; Bush, 1988; Boer & Kits, 1990; Franchini & Ottaviani, 1992), the crop and caecum of cephalopods (Boucaud-Camou & Boucher-Rodoni, 1983; Westermann & Schipp, 1998; Westermann *et al.*, 2000), and the so-called pancreatic appendages of cephalopods (Schipp & Boletzky, 1976; Boucaud-Camou & Boucher-Rodoni, 1983). However, the highest absorptive and digestive activities concerning large molecules and particulate matter throughout the digestive tract have been reported for the digestive glands (midgut glands, hepatopancreas) of bivalves (Morton, 1983), gastropods (Walker, 1972) and cephalopods (Boucaud-Camou & Yim, 1980; Westermann *et al.*, 2000). The midgut of Solenogastres is a simple tube which may bear surface enlargements (papilliform protusions and lateral pouches constricted by portions of dorso-lateral muscles), but never displays a complex, branched configuration like the digestive glands. Nevertheless, a comparison between the midgut epithelium of *W. argentea* and the tubule-epithelium of higher molluscs seems to be justified when discussing cytological characters and cell cycles.

Cell types of digestive epithelia

Digestive epithelia of molluscs consist mainly of cells characterized by high endocytotic activities and an extensive lysosomal system involved in the decomposition of predigested food material. The number and structure of additional cell types specialized, for example in secretion or in storage of certain metabolites, varies.

In *W. argentea*, the midgut epithelium is composed of two cell types: digestive cells characterized by extensive lysosomal compartments and ciliary cells that are restricted to a narrow mid-dorsal tract. There is no evidence of differentiation into more cell types as has been reported for some Solenogastres species. In the midgut epithelium of *Nematomenia banyulensis* (Thiele), for example, Thiele (1894) observed laterally situated low cells containing granules, club-shaped ventral cells with a dark nucleus and containing similar granules to the lateral cells, as well as dorsal ciliary cells. Van Lummel (1930) and Nierstrasz & Stork (1940) reported 'club-shaped cells' (German: Keulenzellen) and 'granula cells' (German: Körnerzellen) in certain species. In most Solenogastres, however, the midgut epithelium

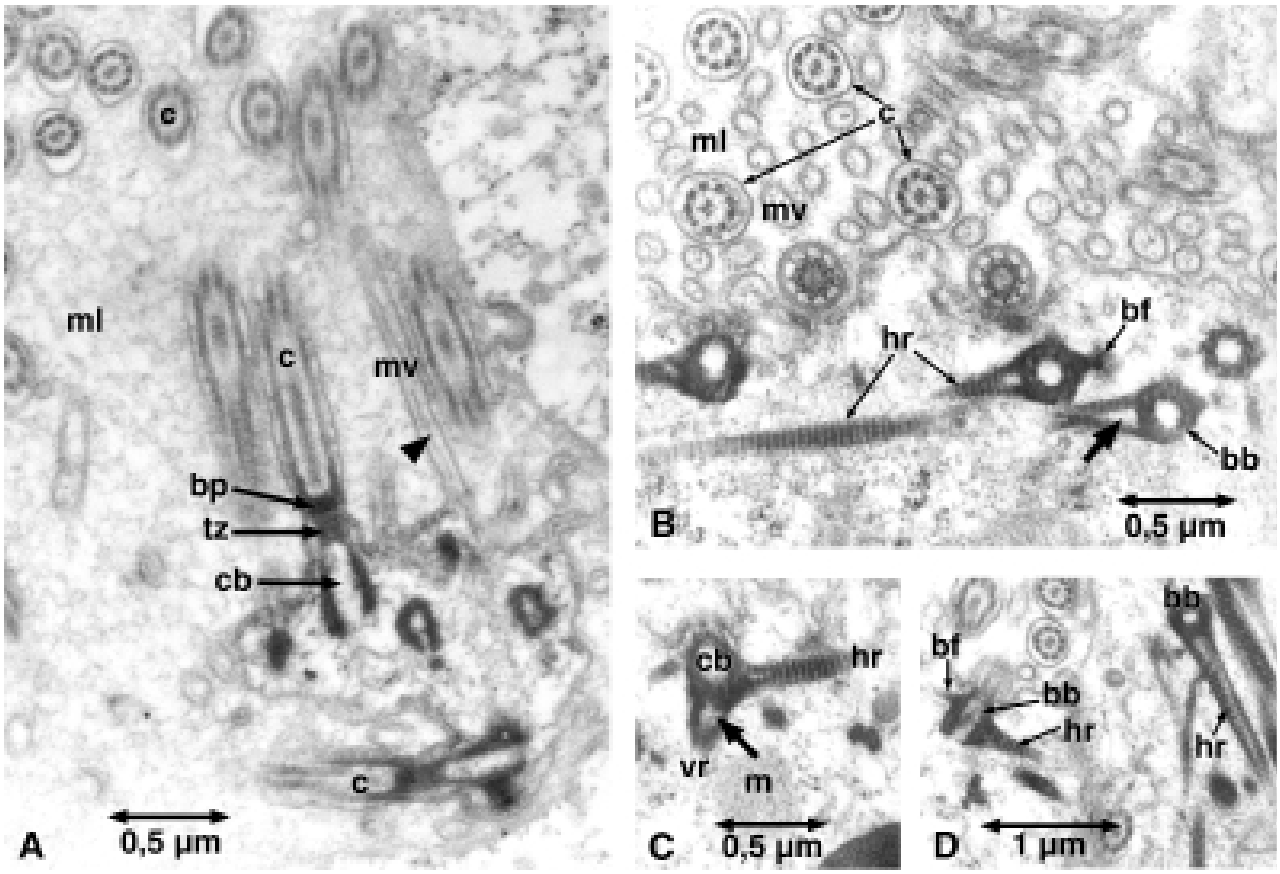


Figure 7. TEM micrographs of apices of ciliary midgut cells of *Wirenia argentea*. **A.** The apical membrane bears cilia and microvilli, one microvillus showing a longitudinally running microtubule (arrowhead). Longitudinal section of cilium clearly shows the electron-dense basal plate, the granulated transitional zone and the hollow ciliary basal body. **B.** Cross-section of cilia showing the different zones from the ciliary shaft (c), within the midgut lumen, to the transitional zone with centriolar triplets, and to the basal body, with the basal foot and horizontal rootlet (h) , which is hollow at its base (large arrow). **C.** Longitudinal section of a ciliary basal body with part of the horizontal and vertical rootlet which is hollow basally (arrow). **D.** Ciliary bases (in longitudinal section with basal foot and horizontal rootlet, and in oblique cross-section. Abbreviations: bb, basal body; bf, basal foot; bp, basal plate, c, cilia; cb, ciliary basal body; h, horizontal rootlet; m, mitochondrion; ml, midgut lumen; mv, microvilli; tz, transitional zone; vr, vertical rootlet.

is described as homogeneous with the exception of a single dorsal ciliary tract or a few longitudinal bands of ciliary cells. In *Genitoconia rosea* Salvini-Plawen (Pholidoskepia, Gymnomeniidae), on the other hand, the midgut lacks any ciliary cells (Salvini-Plawen, 1967a; C. Todt personal observation) but the structure of digestive cells is very similar to that of *W. argentea*, as confirmed by our semithin and ultrathin sections.

The digestive cells of *W. argentea* contain organelles involved in intracellular digestion, including pinocytotic vesicles, phagosomes, endosomes and lysosomes. Additionally, they store lipid and glycogen, produce secretory vesicles, and contain membrane-bound mineralized granules. Thus, the functions of intracellular digestion, storage, secretion and mineral accumulation are combined within each cell in the course of a cell cycle. These results are in accordance with the histological and histochemical findings of Baba (1940) on the midgut of the large solenogaster species *Epimenia babai*. As in *W. argentea*, there are dorsal ciliary cells and digestive cells ('villous cells'). He distinguished 'young cells' from 'flask-shaped gland cells', the latter displaying the structure of typical digestive cells. For these digestive cells he described distinct types of granules which, according to the cited structural and histochemical features, can easily be correlated with the cell organelles and structures we detected within the digestive cells of *W. argentea:* the 'ferment granules' correspond to residual bodies, and most probably also to secretory vesicles; the concentrically striated 'yellow globules', which are refractive in non-decalcified preparations, are membrane-bound mineralized granules; the 'pigment granules' apparently represent residual bodies containing pigments derived from food particles. Finally, both Baba and we detected lipid droplets in the basal part of the cells. As previously stated, nematocysts enter the midgut undischarged (Salvini-Plawen, 1967b, 1981, 1988a; Todt & Salvini-Plawen, 2004) and are partly digested, but stenoteles (even if absorbed by digestive cells and enclosed in lysosomes) stay intact until defaecation.

In *W. argentea* the ciliary cells, comparable to the duct cells of the digestive gland of bivalves (Morton, 1983), gastropods (Luchtel *et al.*, 1997) and cephalopods (Boucaud-Camou & Boucher-Rodoni, 1983), are restricted to a narrow dorsal zone. They contain only a few, small, secondary lysosomes and do not seem to be involved in digestion. No cilia were seen in the digestive cells of *W. argentea*, reflecting the situation in cephalopods. The digestive cells of some gastropods, on the other hand, bear cilia (Griebel, 1993; Rebecchi, Franchini & Bolpgnani Fantin, 1996; Muniain *et al.*, 2001), and in bivalves the basophilic cells of digestive tubule crypts develop cilia in certain phases of the digestive cycle (Morton, 1983). The cilia of the mid-dorsal ciliary tract of *W. argentea* are simple, with unspecialized tips and a pair of long rootlets. This is typical for locomotory cilia of Solenogastres, Caudofoveata and Placophora in contrast to those of all other molluscs, where only one ciliary rootlet is present (Lundin & Schander, 1999, 2001a, b). This ciliary structure and cell morphology imply that the mid-dorsal ciliary tract has no sensory function, but that the cilia help transport food particles and undigestible residues or distribute digestive enzymes within the midgut. Nonetheless, the overall effects of ciliary action on food transport, as a whole, are probably relatively low considering the small dimensions of the ciliary tract compared with the total midgut volume.

Little is known about the fine structure of the midgut and midgut-sac of Caudofoveata. In Chaetodermatidae, two types of cells were reported for the midgut-sac (Wirén, 1892; Salvini-Plawen, 1967b, 1981; Scheltema *et al.*, 1994): granular cells packed with membrane-bound mineralized granules restricted to the dorsal region ('Körnerzellen') and club-shaped cells with a central vacuole containing a glandular body ('Keulenzellen'). Absorption was not reported for these cells and therefore, in spite of conformities in terminology (Keulen- and Körnerzellen, see van Lummel, 1930; Nierstrasz & Stork, 1940), these cell types are obviously not the same as those in Solenogastres (Thiele, 1894). Detailed comparison, including also the midgut and midgut-duct epithelia of caudofoveates, however, remains difficult due to the lack of thorough ultrastructural studies.

The usually paired midgut gland of higher molluscs is a voluminous organ consisting of blind-ending tubules connected with the stomach through a branched system of primary and secondary ducts. In Placophora and Bivalvia it is composed of digestive and basophilic cells. Basophilic cells of Placophora bear mineralized granules and produce digestive enzymes (Eernisse & Reynolds, 1994). As was shown for *Acanthochitona crinita* (Pennant) and *Lepidochiton cinerea* (Linnaeus), digestive and basophilic cells contain distinct types of peroxisomes (Lobo-da-Cunha, 1997). The digestive tubules of bivalves also bear digestive and basophilic cells (for review, see Morton, 1983). The basophilic cells are clustered within the crypts of tubules and represent secretory cells as well as undifferentiated cells that laterally replace exhausted digestive cells and occasionally form new tubules (Owen, 1970). As was recently shown by Ibarrola *et al.* (2000), basophilic cells of *Cerastoderma edule* (Linnaeus) produce carboxylases.

In gastropods the variability of cell types within the digestive gland seems to be high, ranging from one type in *Viviparus ater* De Cristofori & Jan (Rebecchi *et al.*, 1996) to four in a *Runcina* species (Kress *et al.*, 1994), even if undifferentiated cells (thin cells) are excluded. The main cell type is the digestive cell. Cells with mineralized granules occur frequently in various gastropods and are often termed calcium cells or lime cells (e.g. Walker, 1970; Brooks & White, 1995; Taïb & Vicente, 1999; Dimitriadis & Konstantinidou, 2002). In *Lymnaea stagnalis* (Linnaeus), however, the mineralized granules are not composed of calcium (Arni, 1974), and in *Runcina* they consist of magnesium phosphate (Kress *et al.*, 1994). Uncertainties remain about the presence of so-called excretory cells. Some authors interpret them as a distinct type (e.g. Sumner, 1965; Arni, 1974; Brooks & White, 1995), others consider them as a late developmental stage of digestive cells (Walker, 1970; Dimitriadis & Konstantinidou, 2002). Taïb & Vicente (1999), on the other hand, describe them as a developmental stage of calcium cells. Distinct secretory cells producing extracellular digestive enzymes are reported for *Runcina* species (Kress *et al.*, 1994). Cnidophages known from the cerata of aeolid gastropods are specialized digestive cells which retain phagocytosed nematocysts and store them in a functional state (Graham, 1938; Greenwood & Mariscal, 1984; Greenwood, 1988; Kälker & Schmekel, 1976). Thus, the digestive gland of gastropods seems to show a trend towards diversification of distinct cell types for different cellular functions. Considering this high variability in gastropods, a differentiation into more than one cell type, correlated with functional partitioning (e.g. digestive *vs* secretory cells, or digestive *vs* mineralized-granule-containing cells), cannot be excluded for Solenogastres in general. This calls for detailed, ultrastructural studies of species reported to bear granular and club-shaped cells.

The digestive gland epithelium of cephalopods is composed of a single cell type (the 'boules' cell) which unifies the function of intracellular digestion with secretion of protein-containing substances, most probably digestive enzymes (for review, see Boucaud-Camou & Boucher-Rodoni, 1983). Thus, it shows similarities to the midgut epithelium of *W. argentea*. In most cephalopods, digestive enzymes are stored in large vesicles termed erythrophilic boules (Boucaud-Camou & Yim, 1980), but in loliginid squids such as *Sepioteuthis lessoniana* (Lesson, 1830), only small secretory vesicles are present (Semmens, 2001). The structure and size of the residual bodies termed heterolysosomes and boules b2 in cephalopods (Boucaud-Camou & Yim, 1979) also resemble those of *W. argentea*. We did

not, however, detect structures similar to the characteristic brown bodies of the cephalopod digestive gland, which are residual bodies containing distinct bundles of crystals. On the other hand, no mineralized granules have been reported in the digestive gland cells of cephalopods.

Cell cycles during digestion

The sequence of cytological changes within the digestive tubules over the course of a digestive cycle has been studied in cephalopods (Boucaud-Camou & Yim, 1979; Boucaud-Camou & Boucher-Rodoni, 1983, for review, see Semmens, 2001) and in gastropods (Walker, 1970; Taïb & Vicente, 1999; Taïb, 2000), but the earliest experimental studies were presented for bivalves (e.g. McQuiston, 1969; Morton, 1973; Langton, 1975) Robinson & Langton, 1980. Four phases are defined (for review, see Morton, 1983): a holding phase, a phase of food absorption, a phase of disintegration and excretion and a phase of reorganization. Under natural conditons, intracellular digestion in most bivalves is apparently continuous, although the rate is dependent on food availability. Ibarrola *et al.* (2000) showed experimentally that the digestive gland of *Cerastoderma edule* displays a high plasticity in relation to nutritional status. This includes modifications of the lysosome volume within the cells, of digestive epithelium thickness, of the proportion of basophilic *vs* digestive cells, and of the size of entire digestive tubules. As a short-term reaction to food exposure after starvation, residual bodies were expelled from the digestive cells while pinocytotic activity started. With ongoing absorption of food particles, the lysosomal volume increased within hours by the fusion of lysosomes with endosomes, and by enhanced synthesis of lysosomal constituents, causing an increase in protease activity. Similar differences in the thickness of the digestive epithelium and in the estimated volume of secondary lysosomes were observed in *W. argentea* specimens, killed and fixed after varying durations of starvation. The digestive cells of individuals fixed a few hous after being removed from the sediment were large and full of lysosomes; the midgut lumen was hardly detectable. In individuals kept for up to 14 days in clean Petri dishes, the midgut epithelium was low and contained few residual bodies. Thus, the midgut cells undergo a cycle from low cells (stage I cells; comparable to the 'holding cells' in Bivalvia), via a phase of high endocytotic activity (stage II), to large, club-shaped cells ('phase of absorption') that contain numerous lysosomes with food particles in varying degrees of decomposition (stage III). Finally, they are packed with residual bodies and the apical part is shed into the midgut lumen (stage IV; comparable to the 'phase of disintegration' in Bivalvia). No distinct phase of reconstitution was observed. These findings are in accordance with those of Baba (1940) for *Epimenia babai*, although the presence of uric acid crystals in old midgut cells could not be verified for *W. argentea*. Most of the midgut cells of a single specimen are in the same state of development, pointing to a cyclic change of cell status dependent on a previous feeding event. There is no significant difference in cellular status between the anterior and the posterior part of the midgut, which indicates that the ingested food material is dispersed over the whole midgut prior to phagocytosis.

Information on the duration of the digestive cycle of molluscs is scarce. The time span needed to complete the intracellular digestion of food particles is apparently highly variable, ranging from 4 h in the squid *Sepiotheutis lessoniana* (Semmens, 2002) to 54 h in the snail *Helix lucorum* (Dimitriadis & Konstantinidou, 2002). For bivalves, cyclic digestion processes have been reported repeatedly and the control mechanisms (endogenous *vs* exogenous) broadly discussed (Owen, 1970; Morton, 1973; Wilson & La Touche, 1978; Mathers, Smith & Collins, 1979). For *Mytilus edulis* from Whitsand Bay, England, the duration of each digestive cycle depends on the season, taking 3 h in June and 8 h in March (Hawkins, Bayne & Clarke, 1983). We do not know the exact time-span for *W. argentea* to completely absorb and digest food material. Nonetheless, the duration is >1 day, because specimens removed from the sediment, kept for ~24 h without food, and then fixed were still in the digesting phase.

The present work is a first attempt to understand digestive processes in Solenogastres. Further work is necessary to obtain a broader insight into the structure and function of the midgut, especially to evaluate the diversification of midgut cells into granular cells and club-shaped cells that has been reported for certain species.

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