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Ultrastructure of the Digestive Tract in *Acarus siro* (Acari: Acaridida)

Jan Šobotník,[1] Gerd Alberti,[2] František Weyda,[3] and Jan Hubert[4]*

[1]*Research Team of Infochemicals, Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, 166 10 Praha 6 Dejvice, Czech Republic*
[2]*Zoological Institute and Museum, University of Greifswald, D-17489 Greifswald, Germany*
[3]*Department of Physiology, Institute of Entomology, Biology Centre, Czech Academy of Sciences, 370 05 České Budějovice, Czech Republic and Faculty of Science, University of South Bohemia, 370 05 České Budějovice, Czech Republic*
[4]*Department of Stored Product Pest and Food Safety, Research Institute of Crop Production, 161 06 Praha 6 Ruzyně, Czech Republic*

ABSTRACT The gut of the mite *Acarus siro* is characterized on the ultrastructural level. It consists of the foregut (pharynx, esophagus), midgut (ventriculus, caeca, colon, intercolon, postcolonic diverticula, postcolon), and hindgut (anal atrium). The gut wall is formed by a single-layered epithelium; only regenerative cells are located basally and these have no contact with the lumen. Eight cell types form the whole gut: (i) simple epithelial cells forming fore- and hindgut; (ii) cells that probably produce the peritrophic membrane; (iii) regenerative cells occurring in the ventriculus, caeca, colon, and intercolon; (iv) spherite cells and (v) digestive cells forming the ventriculus and caeca; (vi) colonic cells and (vii) intercolonic cells; and (viii) cells forming the walls of postcolonic diverticula and postcolon. Spherite and digestive cells change in structure during secretory cycles, which are described and discussed. The cycle of spherite, colonic, and intercolonic cells is terminated by apoptosis. Ingested food is packed into a food bolus surrounded by a single homogeneous peritrophic membrane formed by addition of lamellae that subsequently fuse together. The postcolonic diverticula serve as a shelter for filamentous bacteria, which also are abundant in the intercolon. J. Morphol. 269:54–71, 2008.

KEY WORDS: peritrophic membrane; spherite cells; digestive cells; regenerative cells; postcolonic diverticula

Acaridid mites include serious pests of stored products and households in many countries (Sinha and Kawamoto, 1990). In the Czech Republic, for example, mites are the most dominant and frequent arthropod group in stored grain, infesting 65% of grain samples (Stejskal et al., 2003). Mites produce allergens and transmit mycotoxin-producing fungi (van Hage-Hamsten and Johansson, 1998; Hubert et al., 2003). *Acarus siro* (Acari: Acaridida: Acaridae) is the most serious mite pest in grain stores (Thind and Clarke, 2001; Stejskal et al., 2003).

The anatomy of *A. siro* (including the digestive tract) was first studied by Michael (1883), more recently by Hughes (1950), and the ultrastructure of the reproductive tract was studied by Witalinski et al. (1990). Some ultrastructural observations also have been made on other acaridid species (*Lis-trophorus leuckarti*: Wurst, 1993; *Psoroptes ovis*: Mathieson and Lehane, 2002; *Sarcoptes scabiei*: Desch et al., 1991), and the pyroglyphid mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Tongu et al., 1986). Since Acaridida currently are regarded as closely related to Oribatida, or even may represent a subgroup of Oribatida (e.g., Norton, 1998), comparison with the latter group of mites is also reasonable. However, exhaustive ultrastructural observations on the gut system are available only for a single oribatid species, *Archegozetes longisetosus* (Alberti et al., 2003). Many new observations and comparisons with older studies are summarized by Alberti and Coons (1999).

Our aim is to describe the complete gut system of *A. siro* at the ultrastructural level. Our work uncovered many surprising facts; e.g., we show here for the first time the presence of undifferentiated regenerative (RG) cells as well as the process of differentiation of some definite cell types. Postcolonic diverticula were found to shelter filamentous symbiotic bacteria.

MATERIALS AND METHODS

Specimens of *A. siro* Linnaeus, 1758 (Acari: Acaridida: Acaridae) originated from the laboratory culture of the Research

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*Correspondence to: Jan Hubert, Research Institute of Crop Production, Drnovská 507, 161 06 Praha 6 Ruzyně, Czech Republic. E-mail: hubert@vurv.cz

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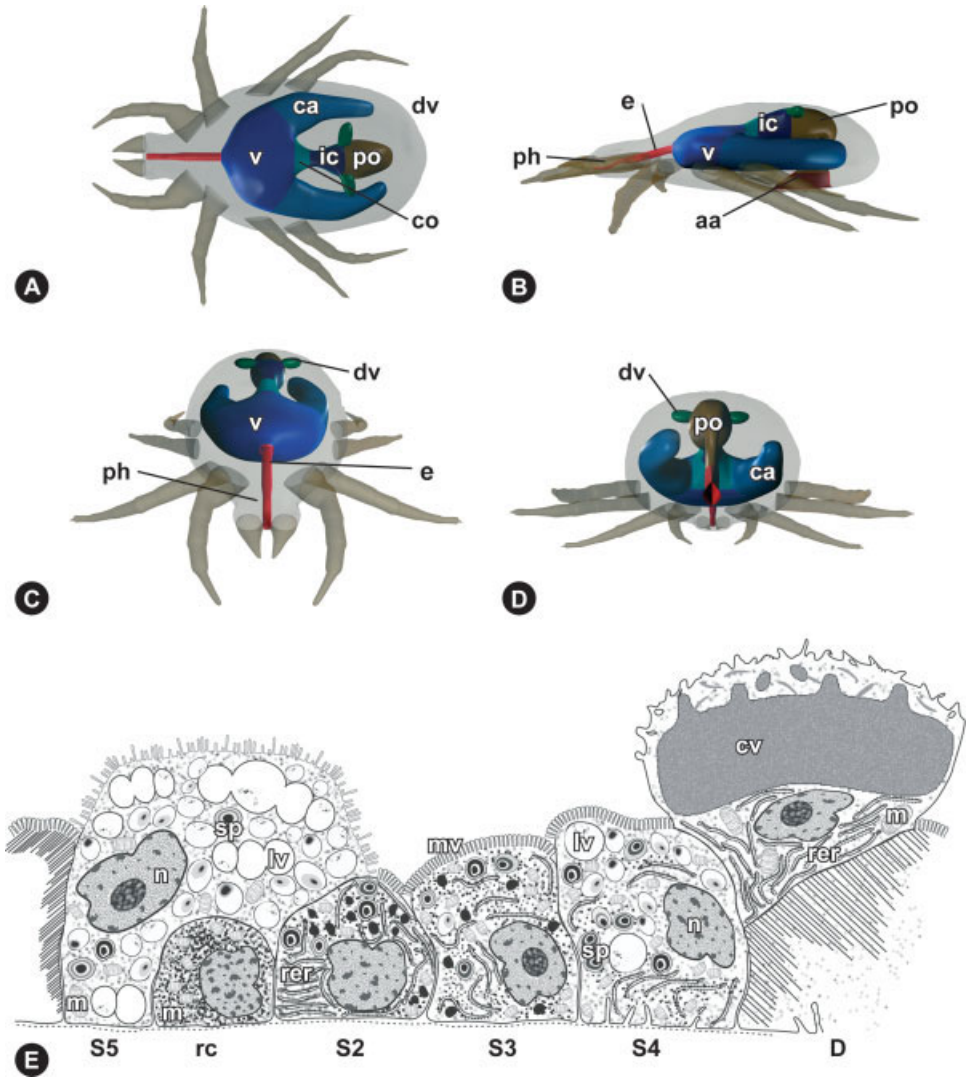

Fig. 1. **A:** 3D scheme of digestive tract of *Acarus siro*: dorsal view. **B:** Digestive tract of *A. siro*: lateral view 3D representation. **C:** Digestive tract of *A. siro*: frontal view 3D representation. **D:** Digestive tract of *A. siro*: ventral view 3D representation. **E:** Schematic drawing of basic features of regenerative, spherite, and digestive cells. The particular ultrastructures are not in scale. aa, anal atrium; ca, caecum; co, colon; cv, central vacuole; D, digestive cell; dv, postcolonic diverticula; e, esophagus; ic, intercolon; lv, lucent vacuole originated in spherite; m, mitochondrion; mv, microvilli; n, nucleus; ph, pharynx; rc, regenerative cell; rer, rough endoplasmic reticulum; S2, spherite cell in the stage S2; S3, spherite cell in the stage S3; S4, spherite cell in the stage S4; S5, spherite cell in the stage S5; sp, spherite; v, ventriculus.

Institute of Crop Production and were collected in Czech grain stores. They were mass-reared on wheat germ at 85% RH and 25°C, and were collected directly from the rearing chambers.

For microscopical observations, whole bodies of adult mites were fixed in a mixture of 2% glutaraldehyde and 2.5% formaldehyde (Polysciences, EM Grade) in 0.1 M phosphate buffer (pH = 7.2) at laboratory temperature for a day. Tissues were then washed in pure 0.1 M phosphate buffer and postfixed in 2% osmium tetroxide in the same buffer for 2 h. Subsequent washing in bidistilled water and dehydration in 50%, 75%, and pure ethanol was followed by embedding into Spurr resin (standard mixture). Semithin and ultrathin sections were made with a Reichert Ultracut ultramicrotome. Semithin sections were stained with Azur II and studied in a Zeiss Axioskop compound microscope (with Sony Cyber-shot digital camera). A 3D model of the gut was constructed based on 30 slides of semithin sections, oriented sagittally, vertically, and horizontally. The slides were edited in Adobe-Photoshop, and a model was constructed using Blender-3D software. For the outer shape of the body we adopted pictures of *A. siro* adults obtained with a scanning electron microscope (Jeol 6300). Ultrathin sections were stained with uranyl acetate and lead citrate (standard recipe) and studied using Jeol 1010 and Jeol 1011 transmission electron microscopes.

Denomination of particular cells and their components follow recent studies of Alberti and Coons (1999) and Alberti et al. (2003).

RESULTS

General Features

The gut starts with the mouth located at the end of preoral cavity and consists of the following parts (i) foregut: pharynx, esophagus; (ii) midgut: ventriculus, caeca, colon, intercolon, postcolonic diverticula, and postcolon; (iii) hindgut: anal atrium. (See Fig. 1A–D for model of the gut anatomy in *A. siro*.) The gut cells always form a single-layered epithelium (except areas with basally positioned RG cells, see later).

Food is cut by cheliceral action, swallowed into the pharynx, and passes through the esophagus into the ventriculus. The foregut lumen frequently contains electron-dense material (as do the ventriculus and caeca; see Fig. 2B,E), which also occurs in the preoral cavity. The connection of the esophagus and ventriculus is valve-like, with the posterior part of the esophagus projecting into the ventriculus (Fig. 2E). The ventriculus is a wide chamber that transforms into the colon posteriorly.

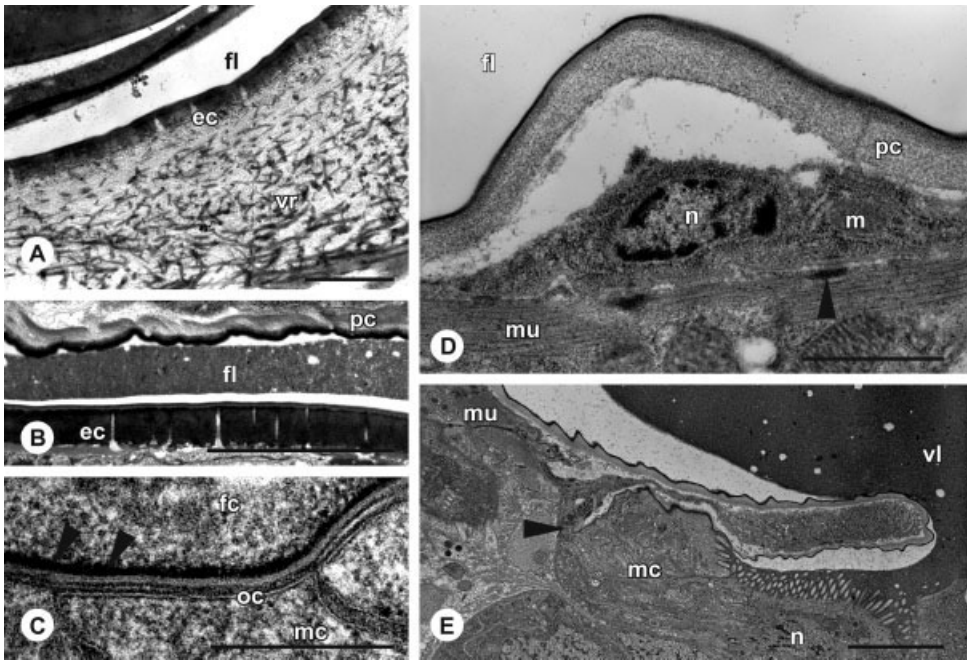

Fig. 2. Foregut of *Acarus siro*. **A:** Pharynx: ventral ridge. Sagittal section of the mouth (left side) and anterior pharynx (right side). Note rapid increase of ventral cuticle thickness at the preoral cavity–pharynx boundary. **B:** Posterior pharynx. Note the differences between dorsal and ventral cuticles of pharynx. **C:** Cuticle of posterior esophagus (esophageal valve) composed only of epicuticular layers. Arrowheads mark inner epicuticle. **D:** Esophagus. Esophageal wall, unmodified epidermal cell, and adhering cuticular intima. Arrowhead marks desmosome attaching muscle to the basement membrane of esophageal cells. **E:** Esophageal valve. Ventral part of esophageal valve, typical appearance of esophagus–ventriculus junction. Arrowhead marks the end of cuticular intima of the esophagus. ec, exocuticle; fc, foregut cell; fl, foregut lumen; m, mitochondrion; mc, midgut cell; mu, muscle; n, nucleus; oc, outer epicuticle; pc, procuticle; vr, ventral ridge; vl, ventriculus lumen. Scales: A = 1 μm, B = 4 μm, C = 250 nm, D = 1 μm, E = 4 μm.

Each food bolus is surrounded by a single homogeneous peritrophic membrane (PM), which is formed in the ventriculus. The paired lateral caeca are simple, sac-like structures; they are widely attached to the ventriculus close to the border between ventriculus and colon. Both colon and intercolon are wrinkled by folds that expand during passage of the food bolus. The postcolon is separated from the intercolon by an inconspicuous constriction; the openings of the postcolonic diverticula are located in this region. The postcolon is a long, wide chamber, continuing posteriorly into the anal atrium. The midgut is surrounded by two layers of muscles; the outer one is circular while the inner one is longitudinal. Both types of muscles are attached to the basement membrane by hemidesmosomes.

Preoral Cavity

The digestive tract starts with mouth at the simple, tube-like preoral cavity. The preoral cavity cuticle consists of a lucent procuticle and a dense epicuticle (not shown). The procuticle differs from that of the body in its lower density and lower number of pore canals. At the beginning of pharynx, the cuticle rapidly changes its structure.

Foregut

Pharynx. The pharynx is crescent-shaped in cross section. At the place of mouth, the thickness of the cuticle abruptly increases, in particular, ventrally because of the occurrence of a ventral ridge (Fig. 2A). The structure of ventral and dorsal cuticle differs (Fig. 2B). The ventral cuticle consists of (i) epicuticle formed by a thin outer epicuticle (alternating dense and lucent layers; thickness altogether about 15 nm) and thicker single-layered inner epicuticle (thickness about 50 nm; epicuticle of similar structure is shown in Fig. 2C); (ii) exocuticle (about 500-nm thick), which is heavily sclerotized and perforated by numerous pore canals (their diameter varies from 70 to 150 nm); and (iii) a basal ventral ridge (up to 3-μm thick) occurring only in the anterior part of the pharynx and being formed by lucent material containing a network of dense elements (Fig. 2A). The dorsal cuticle is simpler, formed by an epicuticle similar to the ventral one and a basal procuticle composed of two

ill-defined sublayers probably representing the exo- and endocuticle; its thickness varies from 150 to 500 nm. Pore canals are less numerous and are poorly defined within the dorsal cuticle.

Pharyngeal muscles are stretched between the roof of the pharynx and cervix (dorsal ones) and infracapitulum wall (ventral ones). All the observed muscles are attached to the cuticle via microtubule-associated junctions (not shown).

Esophagus. The esophagus is a simple tube passing through the synganglion. The esophagus ends in a valve-like connection with the midgut (Fig. 2E). Its lumen is slightly irregular in cross section and may differ among individuals according to the filling by regurgitated midgut secretion. Most often, three major folds were observed in cross sections.

The epicuticle consists of outer and inner epicuticle (similar to that of the pharynx, see Fig. 2C). The thickness of the outer epicuticle remains unchanged until its disappearance posteriorly. Thickness of the inner epicuticle decreases to 10 nm; the more basally placed layers gradually diminish and disappear before the end of the cuticle. The basal part of the cuticle consists of two ill-defined layers probably representing exocuticle (thick about 100 nm) and endocuticle (thick from 250 to 350 nm) sensu Alberti et al. (2003). Pore canals are absent from the cuticle of the esophagus. The esophageal cuticle ends at the base of the midgut cells in the valve (Fig. 2E). The esophagus is equipped only with circular muscles.

Foregut cells. Simple squamous cells form the foregut (Fig. 2D). They vary in thickness from 50 nm to 2 μm, usually being about 0.3 μm thick. All nuclei are irregular (from 1.75 to 3 μm in the largest dimension) with extensive heterochromatin aggregates. The basal lamina is from 30 to 35 nm thick and is formed by a compact electron-dense layer. Hemidesmosomes connect the basal lamina and the circular muscles. In the apical parts, the neighboring cells are always attached together by a belt desmosome. Septate junctions and gap junctions were observed infrequently. Apical parts of the cells usually adhere directly to the cuticle, but a space up to 2-μm wide may occur. Besides nuclei, scarce rough endoplasmic reticulum (RER), mitochondria, and some microtubules occur. Electron-dense granules were observed rarely; other organelles were not observed.

The esophagus usually contains regurgitated secretion that originates in the midgut (see later).

Midgut

Common characters of the whole midgut are as follows: cellular junctions comprise apical belt desmosomes followed by septate junctions, gap junctions occur in lower extent, cell borders are usually sinuate, and the basal lamina (about 35 nm thick) is formed by a single electron-dense layer.

Ventriculus and caeca. The ventriculus and caeca are formed by four basic cell types: cells that probably produce the peritrophic membrane (PM cells), RG cells, spherite cells (S cells), and digestive cells (D cells). The PM cells are localized laterally in the ventriculus, close to the caecal openings (Fig. 3A). For basic structure and position of S, D, and RG cells with respect to each other and to the basal lamina, see Figure 1E. Differentiation of RG cells into S cells was observed from the initial stages on, but only later stages of the differentiation of D cells have been seen.

PM cells. PM cells are cuboidal and from 2 to 5.5 μm thick (Fig. 3C). Their nuclei (up to 5 μm in diameter) are highly irregular with a prominent nucleolus. Heterochromatin aggregates are scarce. Basal parts of these cells are smooth; invaginations of fat body cells are sparse. The apical plasma membrane forms irregular microvilli with inconspicuous apical plaques. The microvilli vary strongly in dimensions, with length from 100 to 600 nm, and breadth from 150 to 300 nm. Locally, the apical membranes are smooth. Apical parts of PM cells are usually in close contact with a crude PM (see later). The main cytoplasmic organelle is tubular RER with electron-lucent inner contents and a large amount of free ribosomes. Mitochondria (from 0.4 to 1 μm in the largest dimension) are moderately abundant.

The cytoplasm contains variable amounts of lucent vacuoles (usually containing fine electron-dense particles), which are extruded at the microvillar bases. Smaller vacuoles arise directly from RER, while larger ones originate in dense biocrystals, in which lucent gaps start to occur and increase their extent until they change into lucent vacuoles. The size of the biocrystals ranges from 0.5 to 1 μm; they are formed by regular lamellar subunits about 7 nm in thickness. Other organelles were not observed.

Food, food boli, and PM. A PM surrounds most of the food material from the ventriculus onward. Food particles located freely in the lumen were sometimes observed inside the caeca, and rarely in the ventriculus as well, but not in more posterior gut regions.

During the formation of the PM, up to seven separate lamellae (from 150 to 300 nm thick) are secreted (Fig. 3D). Large amounts of other materials (dense secretion and/or even cell remnants) may appear between the lamellae. These materials gradually disappear and the PM in the intercolon is formed by a single lamella (from 0.4 to 1 μm thick). Bacteria were also observed between the lamellae; these bacteria remain alive within the PM and reveal no changes during transit of the food bolus.

The PM is a diffuse structure consisting of a network of dispersed electron-dense material

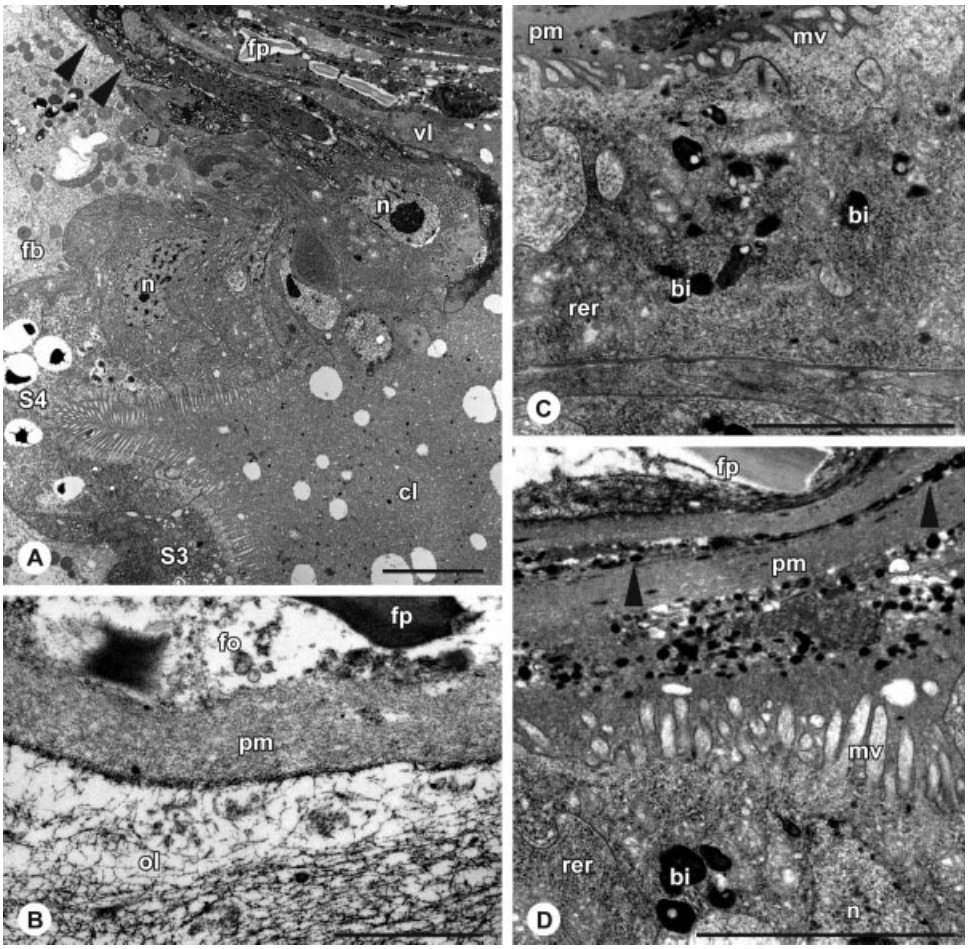

Fig. 3. PM and PM cells of *Acarus siro*. **A:** Localization of PM cells. Place of attachment of caecum to the ventriculus. PM cells are marked by arrowheads. **B:** Appearance of PM in the postcolon. The dense material originates from postcolonic cells. **C:** Overview of a single PM cell. **D:** Apical part of PM cell and newly produced PM lamellae between which a dense secretion from spherite cells (marked with arrowheads) is still present. bi, biocrystal; cl, caecum lumen; fb, fat body cell; fo, food bolus interior; fp, food particle; mv, microvilli; n, nucleus; ol, postcolon lumen; pm, peritrophic matrix; rer, rough endoplasmic reticulum; S3, spherite cell in the S3 stage; S4, spherite cell in the S4 stage; vl, ventriculus lumen. Scales: A = 4 μm, B = 1 μm, C = 4 μm, D = 4 μm.

(Fig. 3B). In general, the structure of the PM remains unchanged after its completion, but a thin outer layer (about 50 nm) occurs from the colon onward; its addition was not observed.

We presume that the PM is formed by action of what we designate as PM cells, because the outer lamella of the developing PM is usually in direct contact with their microvilli (Fig. 3C). No other cells were observed to contact the PM.

RG cells. Single RG cells were observed (Fig. 4B), but groups of two to seven cells occur more frequently in basal positions of the gut epithelium (Fig. 4A). They retain similar structures (prior to their differentiation, see later) and were observed in the ventriculus, caeca, colon, and intercolon. They are most common in the caeca. RG cells contain relatively large nuclei (from 3.5 to 4 μm) and little cytoplasm (Fig. 4B). The nuclei are rounded in shape, with extensive heterochromatin aggregates. The only observed organelles are large amounts of free ribosomes and some mitochondria. The RG cells often differentiate into other cell types; the first sign of this process is enlargement (see Fig. 4A) of the nucleus and reduction in number and size of chromatin aggregates (Fig. 4C). During further steps, the amount of cytoplasm and RER (only in the form of flat cisternae) increases. Mitotic division of RG cells was rarely observed.

S cells. The S cell is the most abundant cell type. Their height gradually decreases posteriorly; they are columnar in the anterior ventriculus while flat posteriorly; in the caeca, the S cells are rather cuboidal. Some S cells situated close to the esophageal valve have a differentiated apex presenting a region with only free ribosomes and bearing sparse or no microvilli (Fig. 5A).

The subcellular structure of S cells gradually changes, as related to aging and subsequent changes in production of two types of secretion; therefore, we have delineated five stages (**S1**–**S5**) to facilitate description of a continual transformation that is undergone by all S cells. Throughout aging of S cells, average density of the cells decreases.

The nuclei of S cells are irregular, about 6 μm in the largest dimension; chromatin is relatively dispersed, and heterochromatin aggregates are small. Mitochondria are moderately abundant in S cells. Invaginations of fat body cells vary from frequent to absent among particular S cells (compare

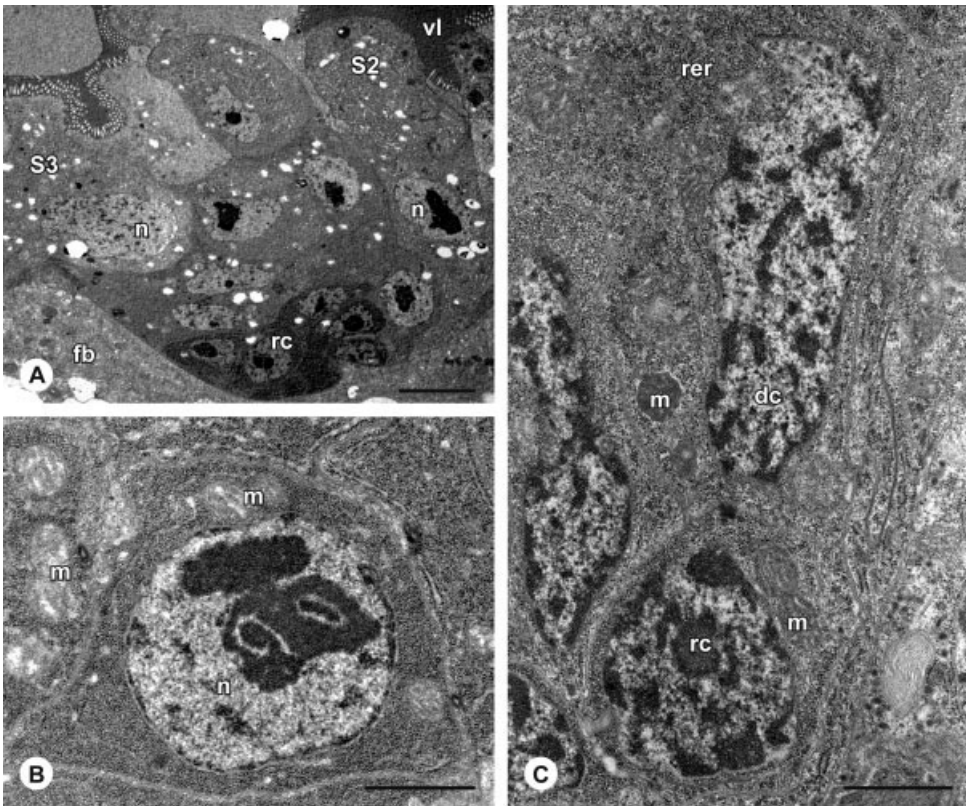

Fig. 4. Regenerative cells of *Acarus siro*. **A:** The wall of the anterior ventriculus with a group of regenerative (and differentiating) cells. **B:** Detailed view of a typical (inactive) regenerative cell. **C:** Regenerative and differentiating cells. Complete view of two cells: typical (inactive) regenerative cell and cell shortly after the initiation of differentiation. dc, cell shortly after the initiation of differentiation in regenerative cell; fb, fat body cell; m, mitochondria; n, nucleus; rc, regenerative cell; rer, rough endoplasmic reticulum; S2, spherite cell in the stage S2; S3, spherite cell in the stage S3; vl, ventriculus lumen. Scales: A = 4 μm, B = 1 μm, C = 1 μm.

Fig. 5B,F). A Golgi apparatus is rarely present and only in cell types S1–S3.

Spherites (0.8–1.4 μm in diameter) are formed by action of the RER (Fig. 5C); after their formation, ribosomes are detached but spherites remain enclosed in the membrane. Subsequent changes are manifested by enlargement of a lucent gap between the spherite and the membrane. Concurrently with the increase of lucent vacuole volume, spherites decrease their diameter while their electron density is increasing (aged spherites sensu Ludwig et al., 1994). During this process, spherites dissolve into large (up to 3 μm) lucent vacuoles, which may or may not contain a heavy dense remnant (from 0.25 to 1 μm in diameter). Spherites were infrequently observed to decompose into rod-like structures similar to those often present in the postcolonic diverticula and postcolon (Fig. 9B,C; see later).

Microvilli of S1 cells are sparse or absent (not shown). The cytoplasm of these cells contains predominantly RER of electron-dense inner content, which starts to produce small electron-dense vacuoles (about 0.5 μm in diameter). These vacuoles may be extruded at the apex.

The microvilli are fully developed at the stage S2 (Fig. 5D). Electron-dense vacuoles are frequent in the cytoplasm and commonly are extruded. Spherites start to be formed in the cytoplasm from this stage on, and may immediately change into lucent vacuoles. Particular cells of this category differ in proportion of spherites and both types of vacuoles (dense and lucent).

S3 cells possess well-developed microvilli, equipped with microtubules reaching to the electron-lucent apical parts of the cells. RER content is lucent. Tubular RER with a single electron-dense fiber inside the tubule was observed rarely at this stage (Fig. 5G). Lucent vacuoles are common in the cytoplasm and may be extruded (together with membrane coat, see Fig. 3A) at the apex from this stage onward. Electron-dense vacuoles are rare.

S4 cells start to accumulate electron-lucent vacuoles (particular vacuoles may fuse together) (Fig. 5A). As the amount of lucent vacuoles gradually increases, the cell is considered type S5. The S5 cell contains predominantly lucent secretion in one or a few vacuoles that are released by the S5 cell rupture (Fig. 5B). The amount of cytoplasm is limited, but it still contains RER and some mitochondria. Remnants of S cells (including mitochondria, RER, spherites, or lucent vacuoles) were infrequently observed in the gut lumen or inside the food boli.

Digestive cells. The D cells are very large and oval in shape (Fig. 6A,C). Their nuclei (up to 8 μm in the largest dimension) are irregular and basally situated, with large nucleoli and several heterochromatin aggregates. Contact of mature D cells (see later) with the basement lamina was not

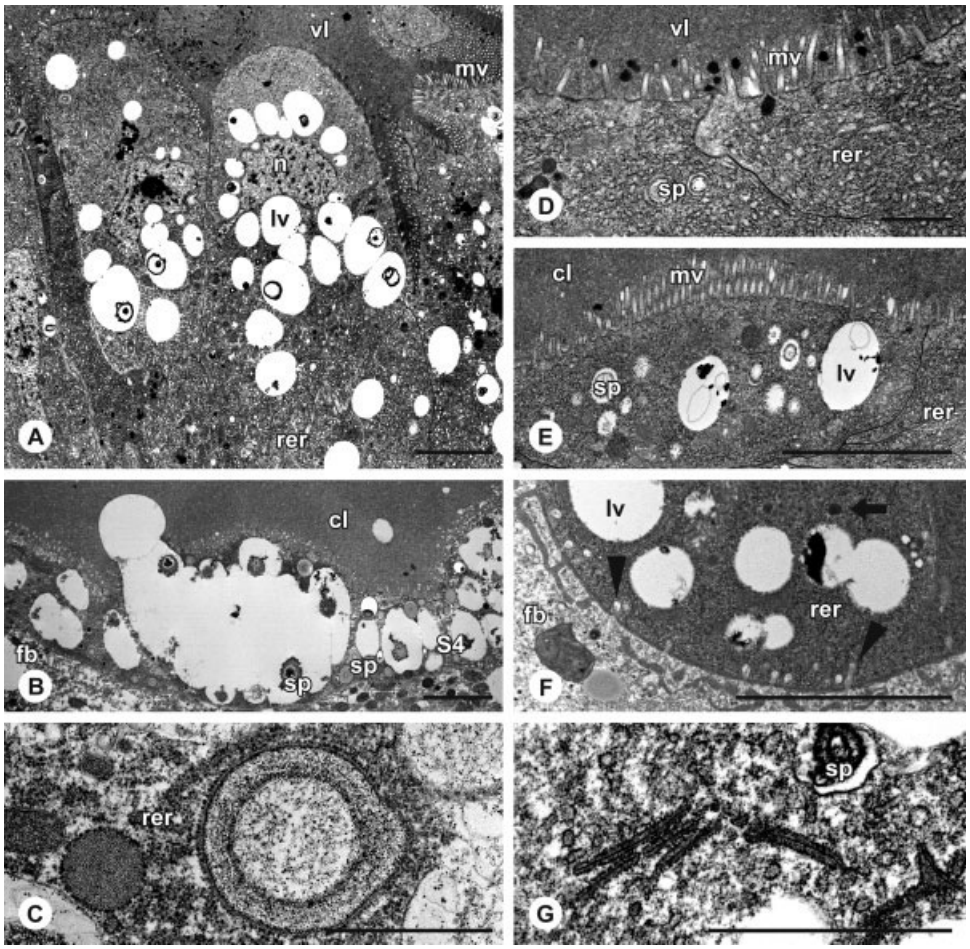

Fig. 5. Spherite cells of *Acarus siro*. **A:** Complete view of the anterior ventriculus gut wall formed by columnar spherite cells in the S3–S4 stage. **B:** Spherite cell in the S5 stage at the moment of rupture. **C:** Detailed view on two growing spherites inside the vacuolar RER. **D:** Spherite cell in the S2 stage showing release of electron-dense secretion. **E:** Spherite cell in the S3 stage. **F:** Invaginations (marked with arrowheads) of fat body cells into the spherite cell in the S3 stage. Note the electron-dense vacuole marked with arrow. **G:** Tubular RER producing single electron-dense fibers inside each tubule. cl, caecum lumen; fb, fat body cell; lv, lucent vacuole originated in spherite; mv, microvilli; n, nucleus; rer, rough endoplasmic reticulum; S4, spherite cell in the S4 stage; sp, spherite; vl, ventriculus lumen. Scales: A = 4 μm, B = 4 μm, C = 1 μm, D = 1 μm, E = 4 μm, F = 4 μm, G = 2 μm.

observed. Formation of pinocytotic vesicles occurs frequently at the apex of the D cells (Fig. 6B). The apical parts may form thin projections that include regular sets of ribosomes (Fig. 6B). Ribosomes may be attached directly to the apical plasma membrane as well (Fig. 6D). Tubular RER is very common, predominantly in the basal regions (Fig. 6C). Mitochondria are moderately abundant. Each D cell contains a single large central vacuole (Fig. 6A,C). Apical from the central vacuole is an extensive canalicular system (Fig. 6B). Canaliculi are of two types: (i) thin and (ii) thick. Thin canaliculi (diameter varies from 50 to 150 nm) may be derived from RER as ribosomes are infrequently attached to these canaliculi. Thin canaliculi are often interconnected. Thick canaliculi branch from the central vacuole (or fuse to form the large vacuole); their diameter varies from 350 to 600 nm.

The proportion of dense particles in the central vacuole of D cells varies considerably (see Fig. 6A,C), and so the appearance of the central vacuole varies from lucent to heavy dense (but is always heterogeneous). In contrast, the cytoplasm is very dense if the central vacuole is lucent and vice versa. This fact is caused by an increased amount of ribosomes, but probably by a higher density of cytoplasm as well. The inner space of the canaliculi has the same heterogeneous content that occurs in the central vacuole. Three basic subtypes of D cells were distinguished based on the density of central vacuole content (and cytoplasm); (i) D cells with lucent central vacuoles containing fine dense particles and relatively dense cytoplasm (Fig. 6A); (ii) D cells with heterogeneous central vacuoles and moderately lucent cytoplasm (Fig. 6A); (iii) D cells with heavy dense central vacuoles and relatively lucent cytoplasm (Fig. 6C).

Some D cells are relatively small and flat (Fig. 6E); they contain up to 15 large vacuoles (their diameter varies from 1.5 to 4 μm) filled with heavy dense secretion. We believe that such cells differentiate from RG cells, although neither direct differentiation nor apoptosis of D cells was observed. The single central vacuole of mature D cells probably develops by fusion of these electron-dense vacuoles. In contrast to mature D cells, squamous D cells were repeatedly observed to contact the basal lamina.

Colon. The vast majority of colonic cells belong to a single type. Nevertheless, RG cells and colonic cells at the beginning of autolysis were observed

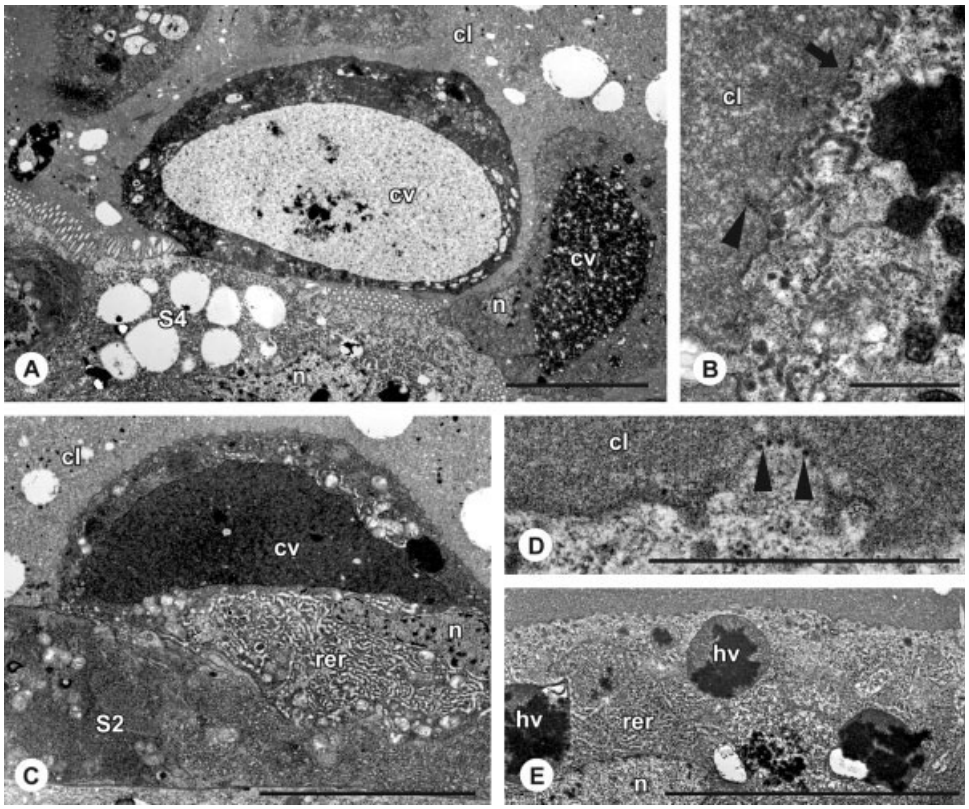

Fig. 6. Digestive cells of *Acarus siro*. **A:** Two digestive cells in different parts of their cycle as evidenced by different densities of central vacuoles. **B:** Apical part of digestive cell showing thick and thin canaliculi. Arrowhead marks set of ribosomes attached directly to the apical plasma membrane; arrow indicates formation of pinocytotic vesicle. **C:** Typical appearance of a digestive cell. Note that the cell does not reach the basement membrane. Note also the basally placed RER and nucleus, and a large electron-dense central vacuole. **D:** Apex of a digestive cell. Detailed view on the set of ribosomes attached directly to the apical plasma membrane marked by arrowheads. **E:** Digestive cell shortly after its differentiation as evidenced by the presence of several large electron-dense vacuoles. cl, caecal lumen; cv, central vacuole; hv, heavy electron-dense vacuole; n, nucleus; rer, rough endoplasmic reticulum; S2, spherite cell in the S2 stage; S4, spherite cell at the S4 stage. Scales: A = 8 μm, B = 1 μm, C = 8 μm, D = 1 μm, E = 8 μm.

as well (see later). We presume that all these cells represent various stages of a single cell type life cycle, of which other transitional stages were not observed.

Cells of the colon epithelium are of various shapes according to the presence or absence of a food bolus inside the lumen. The colon shows crypts (folds) (Figs. 7A and 8A), which become straightened during passing of a food bolus. Cells are up to 15 μm in the largest dimension, from 1.5 to 4 μm thick when the colon is expanded and the cells are flat (Fig. 7B).

The nuclei of typical colonic cells are irregular, about 6 μm in the largest dimension. The extent of the heterochromatin aggregates is moderate. The invaginations (always about 200 nm in diameter) of the fat body cells may be well developed but are always localized. Microvilli are short (length about 700 nm; diameter about 200 nm) and abundant (Fig. 7A,B). The cytoplasm of the microvilli and of the apical layer of cytoplasm is less dense than that in the rest of the cell (Fig. 7B). Mitochondria are moderately abundant. The dominant organelle is RER, which takes either a cisternal or a tubular form. The cisternal RER is often regularly ordered; the tubular RER is of variable diameter due to numerous dilations. The cytoplasm always contains many free ribosomes. The cells also contain some biocrystals enclosed in a plasma membrane to which many ribosomes are attached during biocrystal formation. Large lysosomes (about 3 μm) are frequently present in the colonic cells (Fig. 7C,D). They contain partially decomposed biocrystals, remnants of membranes, heterogeneous electron-dense contents, and sometimes also myelin figures. Multivesicular bodies occur commonly in some of the colonic cells (Fig. 7E).

At the colon base, RG cells were repeatedly observed although their transformation into colonic cells was not observed. The structure of RG cells is the same as described for those of the ventriculus and caeca.

Colonic cells at the beginning of autolysis differ from other cells only in the presence of numerous

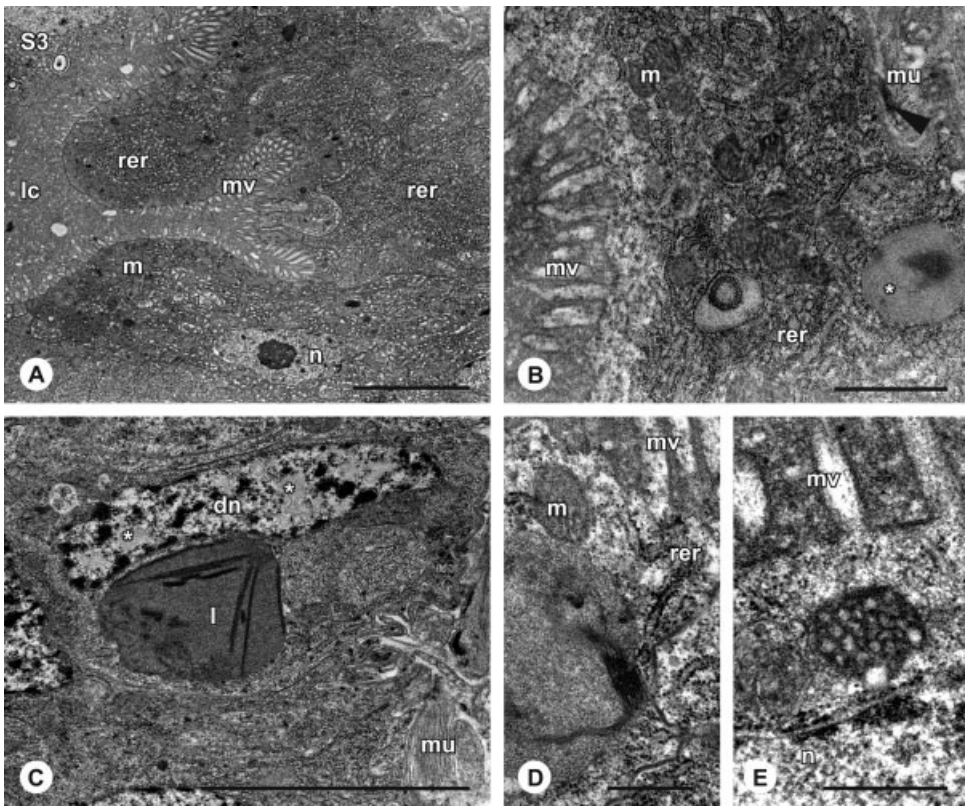

Fig. 7. Colon of *Acarus siro*. **A:** Overall view of colonic wall with a typical crypt. Note cell of posterior ventriculus in the upper part of figure. **B:** Cytoplasm of colonic cell showing RER, numerous mitochondria, and partially decomposed dense granule (asterisk). Arrowhead marks hemidesmosome attaching the muscle to the basement membrane of the gut. **C:** Colonic cell at the beginning of autolysis, as evidenced by numerous myelin figures (asterisks) inside the nucleus and a large lysosome adjoining the nucleus. **D:** Regular structures (resembling septate junctions) inside the decomposing dense granule. **E:** Multivesicular body in the apical part of a colonic cell. dn, degenerating nucleus; l, lysosome; lc, lumen of the colon; m, mitochondria; mu, muscle; mv, microvilli; n, nucleus; rer, rough endoplasmic reticulum; S3, spherite cell in the S3 stage. Scales: A = 4 μm, B = 1 μm, C = 4 μm, D = 0.5 μm, E = 0.5 μm.

myelin figures (from 150 nm to 1 μm in the largest dimension) inside the nuclei (Fig. 7C); other organelles are unchanged.

Intercolon. Intercolonic cells are all equivalent, but three phases of development were observed. These are RG cells (similar to those described earlier; see Fig. 8A), typical intercolonic cells, and degenerating (apoptotic) intercolonic cells (Fig. 8A,C; see later). The intercolon is formed by squamous, cuboidal, or columnar cells. The relative position of cells of particular shapes is not clear or varies among the studied individuals. Columnar cells are apparently situated in both anterior and posterior intercolon, at least in some individuals. Columnar cells form crypts. The largest dimension of intercolonic cells is about 10 μm. The intercolon is frequently inhabited by filamentous bacteria living freely in the lumen (outside the food bolus) (Fig. 8A,C,D).

A typical intercolonic cell possesses irregular nuclei, about 6 μm in the largest dimension (up to 9 μm when flat), with a prominent nucleolus (Fig. 8B). The extent of heterochromatin aggregates is moderate. The basal plasma membrane is simple; invaginations of the fat body cells occur only rarely. Cell borders are rather straight. Microvilli are irregular and abundant; their diameter decreases apically (length is from 1.5 to 2.5 μm; diameter at the base is about 350 nm; diameter at the end is about 80 nm). The apical layer of the cytoplasm (as well as microvilli) contains neither ribosomes nor other organelles; therefore, it is less dense compared to the rest of the cell. RER with a lucent inner space is the main organelle, and is present as cisternal or tubular RER. It produces lucent vesicles containing fine electron-dense particles. These vesicles are extruded at the apex. Lysosomes and biocrystals are frequent in intercolonic cells; their formation was not observed. A well-developed Golgi apparatus was observed in some of the intercolonic cells.

Various stages of apoptosis were frequently observed in the crypts (Fig. 8A,C,D), while neither degenerating nor RG cells were observed in the

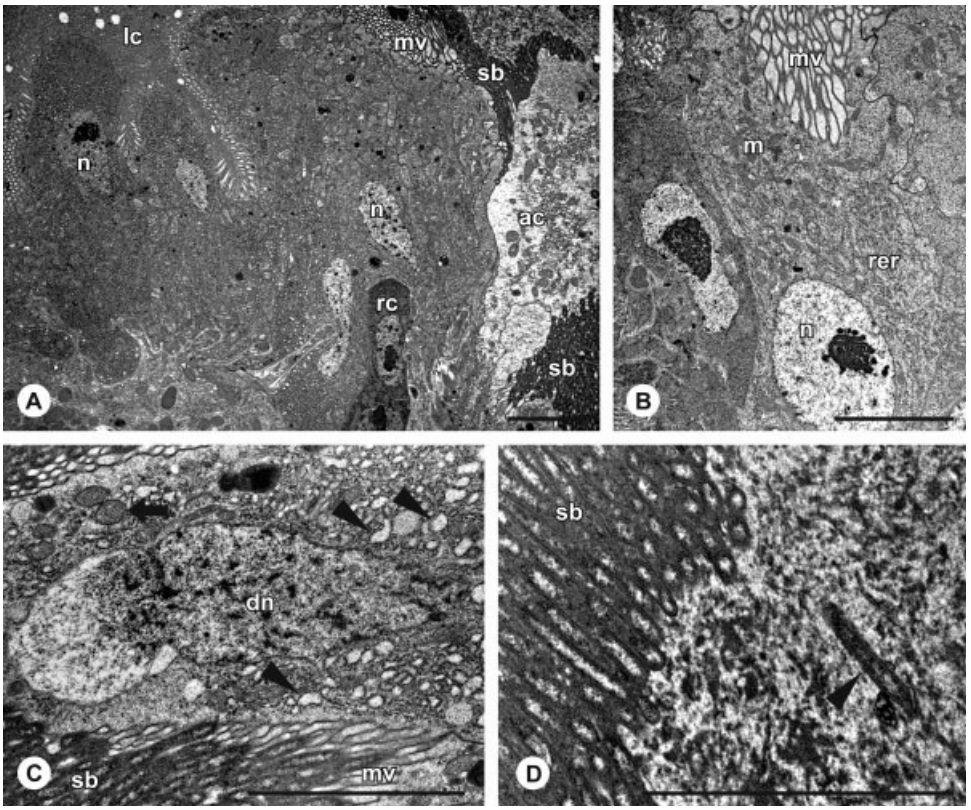

Fig. 8. Intercolon of *Acarus siro*. **A:** Overall view of the colon (left)—intercolonic boundary. **B:** Apical part of intercolonic cell with numerous mitochondria and RER. **C:** Intercolonic cell at the beginning of autolysis as evidenced by fusing of lucent vacuole with the nucleus and swelling of the RER (arrowheads) and mitochondria (arrow). **D:** Remnants of intercolonic cell entered by a filamentous bacterium (arrowhead). ac, apoptotic cell; dn, degenerating nucleus; lc, lumen of the colon; m, mitochondria; mv, microvilli; n, nucleus; rc, regenerative cell; rer, rough endoplasmic reticulum; sb, symbiotic bacteria. Scales: A = 4 μm, B = 4 μm, C = 4 μm, D = 4 μm.

parts composed of squamous cells. The first sign of degeneration of the intercolonic cell is fusing of the nucleus with the large lucent vacuole (see Fig. 8C) followed by dissolution of the nuclear material into a large vacuole of heterogeneous content. The characteristic pattern of heterochromatin condensations temporarily remains inside the vacuole. The cytoplasm of the degenerating cells is less dense than in typical intercolonic cells. RER and mitochondria swell (Fig. 8C) and the organelles disappear. The whole cell content becomes heterogeneous without an obvious subcellular structure (Fig. 8D). These areas of dead cells were also observed in postcolonic diverticula and between postcolonic microvilli and the food bolus. The highest concentrations of filamentous bacteria were observed in areas with a high frequency of degenerating cells; bacteria may even enter inside the cell remnants enclosed in membrane (Fig. 8D).

Postcolonic diverticula. The cells forming the postcolonic diverticula are cuboidal, about 4 μm from base to the apex (Fig. 9A). Nuclei are irregular, about 5 μm in the largest dimension. The extent of heterochromatin aggregates is moderate (Fig. 9B). Invaginations of fat body cells are in general moderately abundant; they occur in some cells more frequently than in others. The cytoplasm contains a dense network of microtubules. Some cells are equipped with sparse microvilli, but others have numerous microvilli without spacing. The length of microvilli ranges from 1 to 4 μm. Between the microvilli, large numbers of small rod-like structures occur (Fig. 9B) that are produced by the cells themselves (see later). Also, many filamentous bacteria similar to those occurring in the intercolon are present (Fig. 9A,B). These bacteria are attached to the microvilli by micelles (Fig. 9D).

Mitochondria are relatively abundant in the cells of postcolonic diverticula. The cytoplasm always contains a Golgi apparatus and many free ribosomes. The cells differ only in the amount of RER, which may be scarce or present in higher proportions (predominantly tubular RER). Biocrystals are infrequently present in the cytoplasm and may swell into lysosome-like vacuoles (from 1 to 2 μm in diameter). Two types of secretory inclusions were observed. These are (i) electron-dense granules and (ii) small vacuoles of heterogeneous content that originate from the Golgi apparatus. The electron-dense granules range in size from 0.3 to 1 μm. Their formation was not observed. The granules may change into myelin figures that are extruded at the cell apex (and temporarily remain among the microvilli; see Fig. 10B,D for a similar situation in the postcolon). Some of the dense granules dissolve and, during this process, irregular rod-like structures (from 50 to 100 nm in diameter) are formed (Fig. 9C). Small vacuoles (from 200 to 400 nm in diameter) contain a network of

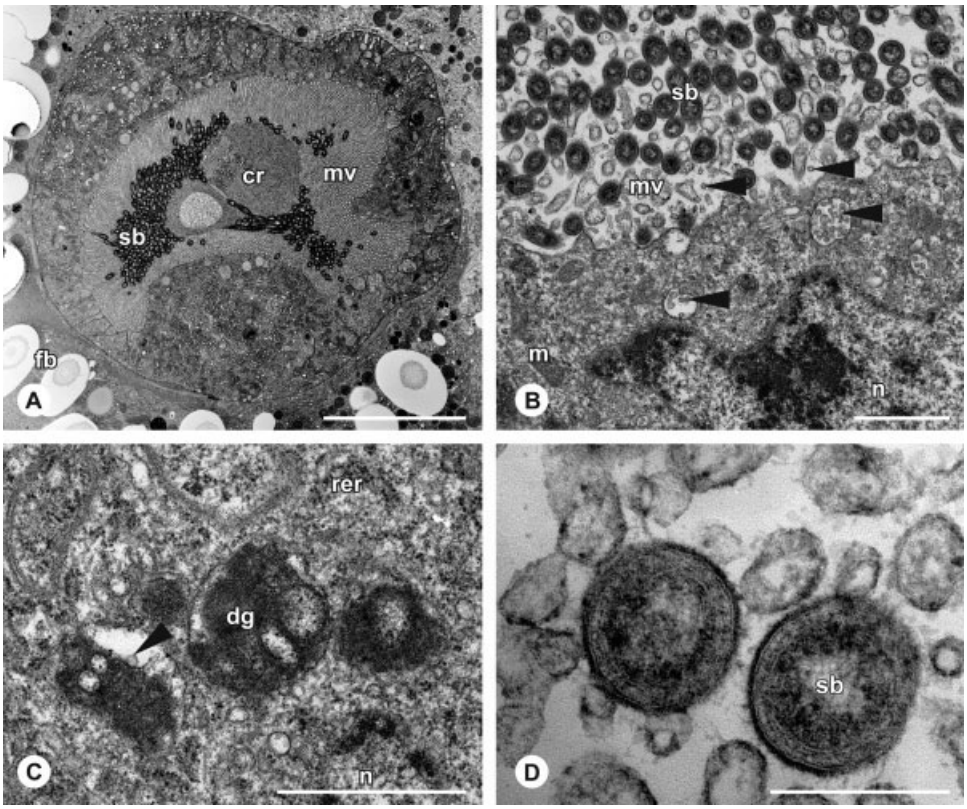

Fig. 9. Postcolonic diverticula ("Malpighian tubules") of *Acarus siro*. **A:** Overall view of section through postcolonic diverticulum. **B:** Apical part of postcolonic diverticulum cell. Arrowheads mark rod-like structures inside and outside the cell. **C:** Formation of irregular rod-like structures (arrowhead) during dissolving of electron-dense granule. **D:** Detailed view of attachment of symbiotic bacteria to the microvilli of postcolonic diverticulum cell. cr, remnants of apoptotic intercolonic cells; dg, electron-dense granule; fb, fat body cell; m, mitochondria; mv, microvilli; n, nucleus; rer, rough endoplasmic reticulum; sb, symbiotic bacteria. Scales: A = 8 μm, B = 1 μm, C = 1 μm, D = 250 nm.

dense material (probably an artifact during sample preparation). These vacuoles often fuse together and form large vacuoles (up to 1.5 μm in diameter) that are extruded at the apex (see Fig. 10C for a similar situation in the postcolon). Postcolonic diverticula contain neither food particles nor food boli. Remnants of intercolonic cells were observed in the lumen (see Fig. 9A).

Neither RG nor degenerating cells were observed in postcolonic diverticula.

Postcolon. The cells forming the postcolon (Fig. 10A–C) are similar to cells of the postcolonic diverticula. The following differences were observed: invaginations of fat body cells occur more frequently and run deeper into the cells (Fig. 10E), microvilli differ in shape and density (Fig. 10B–D), filamentous bacteria occur only rarely among microvilli, mitochondria are more numerous (especially in the posterior postcolon, see Fig. 10D), and other differences exist in the number and proportion of inclusions. Regarding the last point, biocrystals are abundant in postcolonic cells and frequently dissolve into lysosome-like vacuoles (Fig. 10B,G). The formation of biocrystals is due to RER action (Fig. 10F). Electron-dense granules changing into rod-like structures were rarely observed, and only in the anterior postcolon. Although the postcolonic cells are apparently of a single type, their ultrastructure gradually changes posteriorward. Anterior cells contain considerably higher amounts of inclusions (biocrystals, lysosomes, vacuoles), while in posterior cells mitochondria are much more abundant, especially in the apical part.

The microvilli are generally very long (up to 8 μm; see Fig. 10A); they are frequent but generally not closely packed. The diameter of microvilli decreases apically, from ~170 nm at the base of a microvillus to 120 nm at its apex. Cytoplasm filling the microvilli is less dense than that in the rest of the cell; some cells even possess an apical layer of lucent cytoplasm. A mesh of dense material (similar to the content of small vacuoles) was observed among the microvilli of some postcolonic cells and between the microvilli and the food bolus (see Fig. 3B).

In the space between the cells and the food bolus, remnants of intercolonic cells sometimes appear. RG cells were never observed in the postcolon.

Hindgut

At the connection between the postcolon and anal atrium, postcolonic cells overlap the cells of the anal atrium (Fig. 11A); the cuticle of the anal atrium originates at the base of the gut wall cells. Apical parts of the cells are simple; they usually adhere directly to the cuticle. The cuticle comprises epicuticular and procuticular layers. Four outer layers are very thin and represent probably

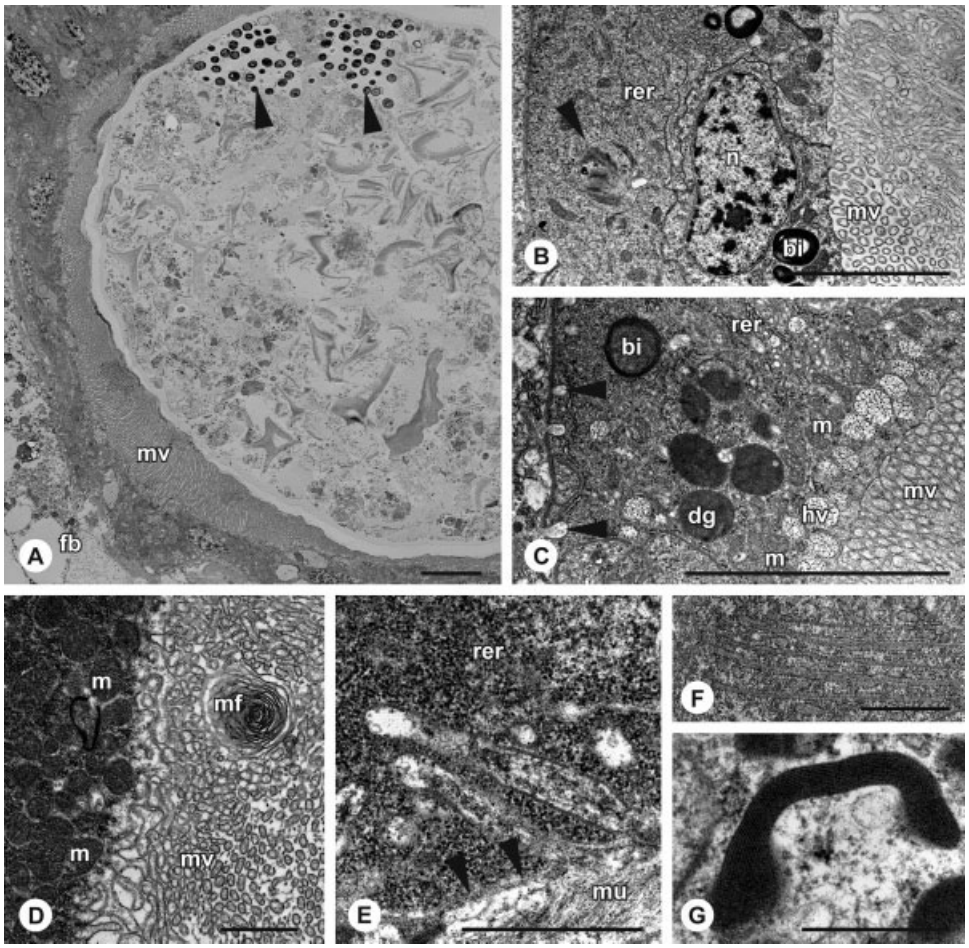

Fig. 10. Postcolon of *Acarus siro*. **A:** Overview on middle part of postcolon during passage of food bolus. Arrowheads mark living yeast cells inside the food bolus. **B:** Postcolonic cell showing dissolution of electron-dense granule into the lysosome-like vacuole (marked by arrowhead). **C:** Postcolonic cell showing massive release of heterogeneous vacuoles. Arrowheads mark invaginations of fat body cells into the postcolonic cell. **D:** Apex of posterior postcolonic cell. **E:** Detailed view of deep invaginations of fat body cell into the postcolonic cell. Arrowheads mark basement membrane. **F:** RER producing long threads of regular inner structure (biocrystals). **G:** Detail of regular structure of biocrystal (during its dissolution). bi, biocrystal; dg, electron-dense granule; fb, fat body cell; hv, heterogeneous vacuole; m, mitochondria; mf, myelin figure; mu, muscle; mv, microvilli; n, nucleus; rer, rough endoplasmic reticulum. Scales: A = 8 μm, B = 4 μm, C = 4 μm, D = 1 μm, E = 1 μm, F = 1 μm, G = 0.5 μm.

outer epicuticle; two inner layers represent probably inner epicuticle. The thickness of the whole epicuticle is about 40 nm. The procuticle varies in thickness from 0.8 to 2.5 μm and shows two ill-defined sublayers similar to those in the esophagus. The thickness of the procuticle increases toward the anus, while that of the epicuticle remains unchanged.

Proximally, the cells forming the anal atrium are cuboidal or slightly flattened (length varies about 7 μm, thickness about 5 μm; Fig. 11A), and their thickness gradually decreases such that distant cells are very flat (only 100 nm close to the anus; Fig. 11B,C). Their nuclei are irregular with extensive heterochromatin condensations. The basal lamina is about 25 nm thick; it is formed by a single compact electron-dense layer. Cellular connections consist of belt desmosomes that are always present at the apex; septate junctions or gap junctions were rarely observed. The cytoplasm of the cells forming the anal atrium contains RER and many free ribosomes as well as some mitochondria. Microtubules are abundant as well. Vacuoles (lucent or dense) were rarely observed. Other organelles were not observed.

The anus is clearly recognizable in sections, based on the rapid change in the structure of the cuticle (see Fig. 11B,C); the structure of adhering cells remains unchanged.

DISCUSSION

General Remarks

The gross structure of the gut of *A. siro* is similar to related acaridid mite species, i.e., *Caloglyphus mycophagus*, *C. berlesi*, *Carpoglyphus lactis*,

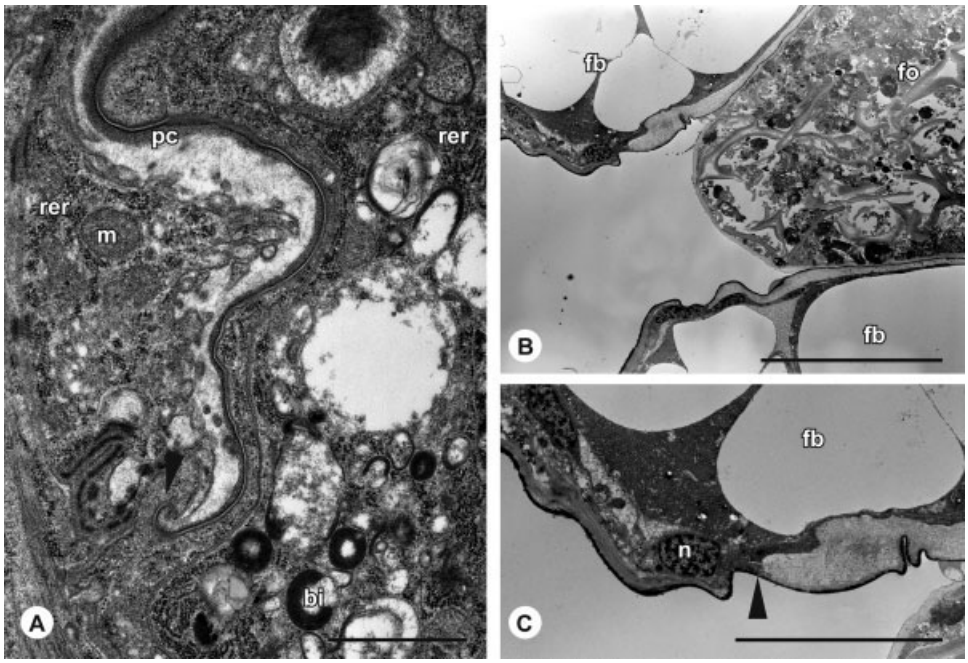

Fig. 11. Anal atrium of *Acarus siro*. **A:** Junction between postcolon (right) and anal atrium showing the rise of the anal atrium cuticle basally under the postcolonic cells (arrowhead). **B:** Total view on posterior hindgut during defecation. **C:** Detailed view on posterior hindgut. Arrowhead marks the exact place where rapid change in structure from gut (right) to body cuticle occurs. bi, biocrystal; fb, fat body cell; fo, food bolus interior; m, mitochondrion; n, nucleus; pc, procuticle; rer, rough endoplasmic reticulum. Scales: A = 1 μm, B = 8 μm, C = 4 μm.

Rhizoglyphus robini, *R. echinopus*, and *Tyrophagus infestans* (Prasse, 1967; Rohde and Oemick, 1967; Kuo and Nesbitt, 1970; Akimov, 1985; Baker and Krantz, 1985). The fore- and hindgut are equipped with a multilayered cuticle and are thus likely of ectodermal origin. The midgut originates from endodermal cells and lacks the cuticular intima (cf. Evans, 1992; Alberti and Coons, 1999; Alberti et al., 2003). The observed general structure of the gut cuticle corresponds well to previous observations (Klag, 1971); the structure of epicuticle is identical to that of the body cuticle. The procuticle is composed of two ill-defined sublayers that may correspond to endo- and exocuticle observed in the body cuticle, but the procuticle always lacks helicoidal structures. The ventral ridge (cuticular structure formed by a network of dense elements in the lucent matrix; see Fig. 2A) has no equivalent in the body cuticle.

Pharyngeal muscles are stretched between the roof of the pharynx and the cervix and intracapitulum walls, respectively. The esophagus possesses only circular muscles [similar to that described by Brody et al. 1972 from an acaridid mite and by Hoebel-Mävers 1967 and Alberti et al., 2003 from oribatid mites]. The midgut is surrounded by an irregular network of inner longitudinal and outer circular muscles. The same pattern was observed in the caeca and postcolonic diverticula as well. The inverse situation (longitudinal muscles as an outer layer) was described in *Steganacarus* sp. (Hoebel-Mävers, 1967) and several other oribatid mites (Bernini, 1973). No sphincter was observed around the anus; its opening is likely a consequence of the fecal pellet being forced to the exterior by the gut muscles, whereas its closure is probably due to cuticle properties.

Fore- and hindgut cells reveal a similar simple ultrastructure that does not differ from that of common epidermal cells. In contrast, the midgut comprises seven different specific cell types. These are (1) RG cells present in ventriculus, caeca, colon, and intercolon; (2) cells producing the PM, found laterally in the ventriculus close to the caecal attachment; (3) S and (4) D cells forming the epithelium of the ventriculus and caeca. Colon (5) and intercolon (6) are formed by separate cell types. Although cells forming postcolonic diverticula and postcolon are not quite the same, they may be classified as the same cell type (7). In addition to these cell types, various transitional stages occur in spherite, digestive, colonic, and intercolonic cells (see later).

Invaginations of fat body cells (also termed finger-like processes) into the adjacent basal parts of the gut epithelium are best developed in postcolonic cells where they are frequent and numerous. In the cells of postcolonic diverticula, the invaginations are moderately abundant and reach less deeply into the cells than in the postcolon. Invaginations are scarce in spherite, colonic, and intercolonic cells, and are absent in digestive and RG cells (as well as in the fore- and hindgut cells). Presence of the invaginations is a character common to many Arachnida (Ludwig and Alberti, 1990; Alberti et al., 2003). They probably serve for direct exchange (no pinocytosis) of certain chemicals between the fat body and gut cells.

Foregut

The structure of the pharynx (cuticle, epithelium, musculature) is similar to that of *D. farinae* (Brody et al., 1972) and the oribatid mite *A. longisetosus* (Alberti et al., 2003); no important differences were observed. Therefore, we expect that operation of pharynx is the same as described by Alberti et al. (2003).

The lumen of the esophagus in *A. siro* usually has three irregular longitudinal folds, but this trait differs among species. Five folds are present in *L. leuckarti* (Acaridida; Wurst, 1993); seven in *Euzetes globulus* (Oribatida; Hoebel-Mävers, 1967); eight folds in *D. farinae* (Acaridida; Brody et al., 1972) and *P. ovis* (Acaridida; Mathieson and Lehane, 2002); irregular folds are described in *A. longisetosus* (Oribatida; Alberti et al., 2003) and *S. scabiei* (Acaridida; Desch et al., 1991).

The composition of the cuticle of *A. siro* (outer and inner epicuticle, procuticle of two ill-defined layers) differs from that of both *A. longisetosus*, where the epicuticle is single-layered but the procuticle is formed by exo-, meso-, and endocuticle (Alberti et al., 2003), and *D. farinae* where the cuticle consists only of epi- and exocuticle (Brody et al., 1972).

The presence of an esophageal valve (and the immersion of cuticle at the esophagus–ventriculus border) seems to be a general feature of the acaridid and oribatid digestive tract. Its absence has been confirmed only in studied members of the oribatid family Damaeidae, and probably relates to gross structural modification of their digestive tract, i.e., the presence of a crop (Hoebel-Mävers, 1967).

Electron-dense material that frequently fills the esophageal lumen is similar to that filling the ventriculus and caeca; therefore, we expect that regurgitation of midgut secretion is a common feature accompanying the process of food ingestion. Such regurgitation was proven in *Histiogaster carpio* (Acaridida), in which alkaline phosphatase was found in the lumen of the esophagus (Baker, 1975). The function of the regurgitated material (together with saliva; Woodring and Cook, 1962) is probably food lubrication and its partial digestion. Salivary glands were observed to be well developed in *A. siro* (Hughes, 1950), but were not examined in our study.

Midgut

Ventriculus and caeca. In *A. siro*, as well as in some oribatid mite species (Woodring and Cook, 1962; Bernini, 1973), there are only superficial differences (consisting in cell shapes and not in subcellular structures) between the cells forming the ventriculus and caeca. On the other hand, considerable differences between the ultrastructure of the ventriculus and caeca were described in another oribatid (e.g., Alberti et al., 2003). The preventricular glands are lacking in some acaridid species, including *A. siro*, while they occur in other acaridids and in most oribatid species (Michael, 1883, 1884, 1901, 1903; Lönnfors, 1930; Woodring and Cook, 1962; Ludwig et al., 1991; Alberti et al., 2003).

Among cells forming the epithelia of the ventriculus and caeca in Arachnida, two types usually have been distinguished, based on a combination of light microscopy and TEM techniques (Ludwig and Alberti, 1990): D cells containing various structures related to food intake and spherites, and secretory cells containing numerous distinct dense granules. In oribatid and acaridid mites, typical secretory cells are not present. Instead, we found D cells (characterized by a conspicuous system of apical tubules and a large central vacuole) and S cells (characterized by the presence of spherical inclusions). S cells are widely distributed in all studied species of astigmatic and oribatid mites (Woodring and Cook, 1962; Akimov, 1985; Desch et al., 1991; Hubert and Šustr, 2001; Mathieson and Lehane, 2002; Alberti et al., 2003). Spherite-containing cells were also found in many other taxa (for a review see Alberti and Coons, 1999; or e.g., Hopkin, 1989; Ludwig and Alberti, 1990; Köhler, 2002). Of course, all these cells are not the same but the differences between cells of particular species are negligible, relative to their basic similarities, e.g., location within the gut, nature of their inclusion, RER as a dominant organelle. Therefore, we expect that S and D cells play similar roles in all species. In contrast, three cell types were distinguished by Kuo and Nesbitt (1970) in *C. mycophagus*; since their work was based on optical microscopy only, comparisons with our results are not feasible.

In spite of the similar ultrastructure of S cells in the whole ventriculus and caeca of *A. siro*, their size decreases posteriorly and their shape changes from columnar to squamous. A similar trend was described for the oribatid mite *Euzetes globulus* (Hoebel-Mävers, 1967). In addition, some of the anterior S cells (close to the esophageal valve) have differentiated apical parts that possess sparse or no microvilli and the apical cytoplasm contains only free ribosomes. This may indicate a slightly different function of these cells.

The whole cycle of S cell differentiation (S1–S5) is as follows: They originate from RG cells by increasing cellular and nuclear volume and by forming RER and apical microvilli (S1). The early stages of S cells (S1, S2) predominantly produce electron-dense vacuoles that are frequently extruded at the apex. From the S2 stage on, the cells start to produce spherites that are subsequently dissolved into large lucent vacuoles. This observation of the rather fast disappearance of

spherites (mineral stores) differs from observations on other taxa (e.g., Alberti and Coons, 1999; *Lepidoglyphus destructor* [Acaridida], Šobotník and Hubert, unpublished results), in which spherites are persistent and are accumulated by the midgut cells. S3 cells contain only spherites and lucent vacuoles (which may be extruded into the gut lumen) and S4 cells accumulate large amounts of spherites and lucent vacuoles. S5 cells undergo cell rupture and are largely discharged into the lumen, probably in a holocrine manner. Remnants of S cells were observed in the gut lumen as well as inside the food boli. Spherites in the gut cells are similar to those observed in the fat body cells, and the same is true in the acaridid species *L. destructor*, *Tyrophagus putrescentiae*, and *D. pteronyssinus* (Hubert and Šobotník, unpublished observation). In contrast, spherites are lacking in the fat body of *A. longisetosus* (Alberti et al., 2003). For a discussion on spherite occurrence and nature see, e.g., Alberti and Coons (1999) and Alberti et al. (2003).

The basic differences among D cells consist in number and size of large vacuoles: smaller D cells contain several large vacuoles but larger ones contain only a single extraordinarily large vacuole. This phenomenon may be related to aging of D cells, but the whole cycle, in particular, the differentiation from RG cells and disintegration of D cells, has never been observed. We assume that large D cells originate from smaller ones by fusing and swelling of heavy electron-dense vacuoles. In addition, differences in the density of the central vacuole (lucent, intermediary, electron dense) are recognizable among various mature D cells. The nature of these differences remains unclear. The cells of similar structure are present in other mites (see Alberti and Coons, 1999; Mathieson and Lehane, 2002; Alberti et al., 2003) and other Arachnida also (Ludwig and Alberti, 1990, 1992; Ludwig et al., 1994). We have not observed detachment of D cells and their change to free-floating cells, as happens in the acaridid mite *P. ovis* (Mathieson and Lehane, 2002). Remarkably, ribosomes attach directly to the apical membrane of D cells, which seems never to have been reported before.

RG cells are frequently present in the ventriculus, caeca, colon, and intercolon but were not observed in other gut parts. They are always of the same simple structure, predominantly containing free ribosomes and mitochondria. They were even observed to undergo mitotic division. We suggest that the RG cells occur in all those gut parts in which the cell cycle ends in the death of the cell. RG cells have never been reported in the literature on mite guts; RG cells were also repeatedly observed in other acaridid species, e.g., *L. destructor*, *T. putrescentiae*, and *D. pteronyssinus* (Hubert and Šobotník, unpublished). RG cells of similar ultrastructure have been frequently observed in the insect midgut wall (e.g., Del Bene et al., 1991; Lehane, 1998).

We interpret a regions of cells in the lateral walls of the ventriculus as producing the PM. PM cells are clearly distinct from all other gut cells of *A. siro*; their specific features are as follows: irregular microvilli, lucent vacuoles containing fine electron-dense particles, and absence of characters specific for spherite and D cells. PM cells are also the only gut cells that are in direct contact with the PM. PM cells are similar to epidermal cells producing the cuticle in insects, particularly with regard to the occurrence of RER, short irregular microvilli, and vacuoles carrying the material (Locke, 1961; Innocenti et al., 1997; Chapman, 1998; De Eguileor et al., 2001). One can expect, therefore, that the PM (at least in *A. siro*) also contains chitin, but this remains to be verified. The food is packed by the PM together with the electron-dense particles. We did not directly observe the formation of the food bolus, but we assume that this process generally runs as described for oribatid mites by Hoebel-Mävers (1967). An important difference lies in the frequent presence of food particles inside the caeca of *A. siro* in contrast to oribatid mites such as *Achipteria coleoptrata*, *Galumna elimata*, and *Scheloribates laevigatus* (Hubert et al., 2001). This distinction may be due to different gross morphology of the gut; the caeca of *A. siro* open widely into the ventriculus rather than having a narrow entrance.

The structure of the PM in *A. siro* strongly differs from that observed in *Phalangium* (Arachnida: Opiliones; Becker and Peters, 1985), and so it is not surprising that the structure of PM-producing cells differs as well. The structure of the PM is similar to that of *D. farinae* (Wharton and Brody, 1972). The denser outer layer of the PM (see Fig. 3B) was observed from the colon on, but its addition was not observed. A so-called mucoid membrane is present in all observed species of oribatid mites (Smrž, 1989; Smrž and Čatská, 1989; Hubert and Šustr, 2001), which is probably homologous to the PM of *A. siro* (cf. Alberti et al., 2003). The absence of a PM in several oribatids was reported by Bernini (1973), probably erroneously.

Colon and intercolon. The colonic cells correspond well to those described by Alberti et al. (2003) and differ only in containing larger numbers of mitochondria. Despite the presence of RG cells in the colon, we have not observed formation of new colonic cells. Apoptosis of colonic cells probably occurs only rarely; we have observed only its initial stages, indicated by the transformation of the material of the nucleus into several myelin figures.

As the main cytoplasmic organelle of the colonic cells is RER and the inclusions are predominantly electron-dense granules, we expect that colonic cells predominantly secrete proteinaceous

products. Nothing more is known about the function of the colon, except the observations Akimov (1973, 1985) indicating the start of protein hydrolysis inside the food bolus.

Although intercolonic cells of *A. siro* are of a single type, two forms were observed. These are squamous cells that never form crypts and columnar cells organized into crypts. We are not sure if these differences are interindividual or only regional. Intercolonic cells differ from colonic cells in the shape of microvilli and the nature of dense inclusions that are more often of a regular inner structure (biocrystals) and more often contain lysosomes. The intercolonic cells of *A. siro* differ from those of *A. longisetosus* (as described by Alberti et al., 2003) in the presence of invaginations of fat body cells and presence of Golgi bodies in some cells only.

RG and apoptotic cells are present only in intercolonic crypts. Apoptosis starts by fusing of a lucent vacuole with the nucleus followed by the dissolution of nuclear material into the large vacuole. Consequently, the organelles gradually dissolve and the whole cell contents become more and more heterogeneous. Such cell remnants detach from the gut epithelium and may be penetrated by filamentous bacteria. Cell remnants of the same appearance were also observed inside the postcolonic diverticula. Remnants of gut cells (but of different ultrastructure) were also described in *Dermatophagoides* spp. by Tongu et al. (1986).

Postcolonic diverticula and postcolon. Postcolonic diverticula (sometimes called "Malpighian tubules") are present in many species of acaridid mites (Hughes, 1950; Prasse, 1967; Baker, 1975), but are lacking in *D. farinae* (Brody et al., 1972) and *Lardoglyphus konoi* (Vijaymbika and John, 1974). All oribatid species observed to date lack distinct postcolonic diverticula (Woodring and Cook, 1962; Bücking, 2002; Alberti et al., 2003).

On the basis of the similar ultrastructure of cells forming postcolonic diverticula and the postcolon, we expect that postcolonic diverticula represent small diverticula of the anterior postcolon, and their function is basically similar to that of the postcolon. In addition, the postcolonic diverticula of *A. siro* serve as shelters for symbiotic bacteria, a function observed for the first time. Certain similarities between the cells forming postcolonic diverticula and postcolon were also found in *R. robini* (Baker and Krantz, 1985). The postcolonic diverticula of *A. siro* evidently differs in structure and probably function (symbiotic organ) from the Malpighian tubules of ticks and other Arachnida. If this is confirmed by future studies on other Acaridida with postcolonic diverticula, this may indicate that they evolved independently from Malpighian tubules (see also Alberti et al., 2003).

The remnants of cells frequently observed inside the postcolonic diverticula probably originate in degenerating intercolonic cells, as indicated by their identical structure. Neither degenerating nor RG cells were observed within postcolonic diverticula or the postcolon.

Postcolonic cells are similar to those described by Alberti et al. (2003) and differ only slightly in the heterogeneous content of the lucent vacuoles. The structural differences between the anterior and the posterior postcolon probably reflect different functions of the cells: anterior cells are likely secretory, and posterior cells probably resorb water. That the postcolon is a site of water resorption in acaridid and oribatid mites has been suggested by many authors (e.g., Woodring and Cook, 1962; Tarman, 1968; Hubert and Šustr, 2001).

Hindgut

As the hindgut is lined by cuticle, the underlying cells have a very simple ultrastructure with only sparse organelles. The thickness of the cells decreases posteriorly, as the thickness of the cuticle increases. The specific position of the anus is evidenced in sections by a rapid change of cuticle properties, but this location is inside the mite's body in a resting position. The infolded part evaginates during defecation. Muscles are attached to the hindgut cells only close to the postcolon and are completely lacking in the rest of the hindgut.

CONCLUSIONS

All oribatid and acaridid mites show a peculiar pharyngeal pump (crescent-shaped in cross section) differing from that of gamasid mites and ticks. The peculiar arrangement of pharyngeal muscles may also be a general feature, but this needs to be proven by future studies. However, it corresponds between Acaridida and Oribatida at least (Evans, 1992; or Alberti and Coons, 1999).

The division of the alimentary tract into the parts described earlier is a general feature of oribatid and acaridid mites, but may be modified considerably (see, e.g., Alberti and Coons, 1999).

Invaginations from the fat body into the midgut epithelium (so-called finger-like processes) have also been described from all examined acaridid and oribatid mites, but were never seen in gamasid mites and ticks (Alberti and Coons, 1999).

As far as we know, preventricular glands have never been reported except in Oribatida and Acaridida (Michael, 1883, 1884; Alberti and Coons, 1999; Alberti et al., 2003). However, their occurrence within these taxa is not completely known. Although they certainly do occur only in some Acaridida, their presence seems more general in Oribatida. But, they may be lacking in early derivative taxa, and they will be important to study with regard to this structure. This is especially of interest regarding the discussion about the

evolutionary relationships of these groups (Acaridida evolved from within-Oribatida hypothesis; see Norton, 1998).

The same is true with regard to postcolonic diverticula (Malpighian tubules), which are a common feature of ticks, showing a typical arrangement and fine structure. However, their existence in acaridid and oribatid mites as homologous structures may be doubted. Regarding the putative relationship between Oribatida and Acaridida, it is remarkable that distinct tubules starting from the border between colon and postcolon are only found in some Acaridida. According to our observations on *A. Siro*, they have a different fine structure and probable function (symbiotic organs) compared with typical Malpighian tubules (excretory function). It would be of much interest to know whether these tubules also function as symbiotic organs in other acaridid species or whether similar tubules occur in, e.g., early derivative oribatid mites.

Much uncertainty still exists concerning cell types in the midgut epithelia. For the first time we could show RG cells in the mite gut. Similar cells are only known from ticks (Coons and Alberti, 1999). Nothing is known about how gut epithelia are regenerated in other acarine taxa. We described a number of cell types from the various parts of the midgut. Some of these types have also been reported from other mites and seem to occur widely in Arachnida (e.g., S cells, D cells). However, the number and distribution of these cells in the midgut seem to vary considerably, and other cell types have only been described in specific taxa [e.g., secretory cells in predatory Arachnida (Ludwig and Alberti, 1990), ER-cells in *A. longisetosus* (Alberti et al., 2003), see also Coons and Alberti (1999) with regard to ticks]. A problem is that some of these cell types have obviously been interpreted in a different way with regard to function. Furthermore, there is still some uncertainty whether all these cell types represent separate cell lineages or are (at least in part) just phases of transformations during their development.

Finally, much work remains before we understand how these structures are related to peculiar forms of nutrition and/or life habits, i.e., how these peculiarities could be interpreted in terms of evolutionary biology.

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