Zoomorphology (1994) 114:83–101

ORIGINAL ARTICLE

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Life-cycle and functional cytology of the hepatopancreatic cells of *Astacus astacus* (Crustacea, Decapoda)

Accepted 17 January 1994

Summary The hepatopancreas of the freshwater crayfish *Astacus astacus* was reinvestigated by means of light and electron microscopy using refined techniques of tissue preservation. The results contribute significantly to the solution of controversial problems of the decapod hepatopancreas such as cell genealogy, cellular interdependences, elimination of senescent cells and functional interpretation of the cell types. The three mature cell types of the organ, R-, F- and B-cells, are shown to originate independently from embryonic E-cells which are located at the blind-ending tips of the hepatopancreatic tubules. The less abundant M-cells are supposedly of non-hepatopancreatic origin since they are also found in other epithelia of the digestive tract. Differentiating cells can be assigned at an early stage to one of the three hepatopancreatic cell lines if the ultrastructural appearance and distribution pattern of their organelles are used as distinguishing features. The most sensitive markers are the Golgi bodies which have a cell-specific architecture and secretion product not only in mature cells but also in early differentiating stages. Later conversion of one cell type into another, as has often been proposed in literature, does not occur. Senescent cells are preferably expelled from the epithelium at the junction of neighbouring hepatopancreatic tubules and at the antechamber which links the hepatopancreas to the main digestive tract. Cellular discharge in the antechamber occurs by sliding of the oldest parts of the hepatopancreatic epithelium across a particular antechamber epithelium that was thus far unknown. New ultrastructural findings are described with respect to the absorptive apparatus of nutrient absorbing R-cells, the formation of Golgi vesicles and retrieval of membranes in digestive enzyme synthesizing F-cells, and the involvement of Golgi body and endoplasmic reticulum in the formation of heterophagic vacuoles in B-cells. The discovery of these ultrastructural features enables a more sophisticated functional interpretation of the hepatopancreatic cells of Decapoda.

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A. Introduction

The hepatopancreas of Decapoda is a complex tubular gland whose functions include absorption and storage of nutrients, synthesis of digestive enzymes or detoxification of xenobiotics (reviews: Gibson and Barker 1979; Dall and Moriarty 1983; Icely and Nott 1992). It is composed of hundreds of blind-ending tubules and adjoining collecting ducts which terminate in the antechamber. The antechamber links the hepatopancreas to both the pyloric stomach and the gut (Powell 1974). The entire tubular system is lined by a single-layered epithelium that is surrounded by a basal lamina, a close-meshed muscle network and haemolymph (Loizzi 1971).

Despite of the amount of data collected during almost 150 years, there are still considerable gaps in knowledge and discrepancies in interpretation even of very basic morphological aspects as discussed in detail by Icely and Nott (1992) and Vogt (1993). These gaps in knowledge include topics such as cell genealogy, the possible conversion of one cell type into another, discharge of senescent cells, the architecture of the junctions between the hepatopancreas and the stomach-gut system, and also the functional interpretation of the cell types. Thus far, these open questions could not be sufficiently answered due to limitations of tissue preservation and a rather high sensitivity of the organ to autolysis and mechanical damage. The latter problems hold particularly for the regions close to the stomach, such as the antechamber. In order to overcome these limitations, I have tested a wide variety of fixatives and processing techniques for light and electron microscopy. The most suitable procedures were then applied to reinvestigate the hepatopancreas of the freshwater crayfish *Astacus astacus* with particular respect to the open questions concerning this organ.

B. Materials and methods

Samples of hepatopancreas were taken from intermoult males of the European noble crayfish *Astacus astacus* (Linné, 1758) weighing approximately 50 g. The crayfish were kept in our laboratory for several weeks in aerated and filtered 120 × 80 × 20 cm plastic tanks at about 18° C. They were fed ad libitum with minced pork enriched with vitamins and minerals. For light microscopy the samples were fixed in Susa fixative (Böck 1989) for 24 h, dehydrated through a graded series of ethanol, transferred to methylbenzoate overnight and then embedded in paraffin. Sections of 5 µm were deparaffinized in xylene, transferred through graded ethanol and treated with 1% Lugol's iodine for 10 min and 0.25% aqueous sodium thiosulphate for 5 min to remove mercury precipitates. Thereafter, the slides were stained either with the routine stainings haematoxylin-erythrosin (HE) and Goldner, or with PAS and alcian blue-alcian yellow for mucosubstances (Böck 1989). A part of the sections for PAS was counterstained with haematoxylin and for alcian blue-alcian yellow with nuclear fast red. Micrographs were taken with a Leitz Aristoplan microscope.

For electron microscopy the samples were fixed in 2.5% glutaraldehyde in 0.1 M Sörensen's buffer (pH 7.3) for 2 h, rinsed in Sörensen's buffer, postfixed for another 2 h in 1% osmium ferrocyanide, rinsed again in Sörensen's buffer and 0.05 M maleate buffer (pH 5.2), en bloc-contrasted with 1% uranyl acetate in maleate buffer for 2 h, dehydrated in a graded series of ethanol and embedded in Spurr's resin. For orientation in the tissue, semithin sections, 0.5–1 µm thick, were prepared prior to ultrathin sectioning. These sections were stained with Richardson solution (methylene blue-azur II). Ultrathin sections were cut with glass knives on a Reichert-Jung OM 2 ultramicrotome and contrasted with 10% lead citrate for 2 min prior to examination with a Zeiss EM 9-S2 electron microscope.

For ultrastructural investigation of the digestive enzyme synthesizing F-cells, synthesis was stimulated by drainage of the gastric fluid from the cardiac stomach as described earlier (Vogt et al. 1989b). Specimens were sacrificed hourly between 0 and 6 h after stimulation. The samples of hepatopancreas were fixed and processed as described above.

C. Results

I. Morphology of the hepatopancreas

The morphology of the hepatopancreas of *A. astacus* is only roughly sketched since it follows principally the scheme given for other Decapoda (Icely and Nott 1992). It consists of two separate half-organs which are symmetrically arranged along both sides of the stomach. Each half is composed of several hundred blind-ending tubules that can be subdivided into distal, mid-, and proximal regions. The terms "distal" and "proximal" indicate the relative distance to the main digestive tract. The tubules then lead into numerous collecting ducts which finally open into an unpaired antechamber. The antechamber not only has luminal access to the filters of the pyloric stomach but also to the gut.

II. Histology (light microscopy)

The entire hepatopancreatic channel system is lined by a single-layered epithelium with a basal lamina facing the haemolymph space and a microvillous border facing the tubular lumen. The epithelium includes embryonic E-cells (Fig. 1) and three mature cell types, R (resorptive)-, F (fibrillar)- and B (blister-like)-cells (Fig. 2). These cell types are not evenly distributed throughout the organ as described in detail in the following chapters. In addition, the entire tubule system comprises a less abundant and small M (midget)-cell which always occurs alone at the haemolymph-oriented side of the epithelium (Fig. 2). M-cells are also found in the epithelia of the antechamber (Fig. 3) and the midgut.

1. Distal region of hepatopancreatic tubule

The distal region of the hepatopancreatic tubules is blind ended and can be subdivided into an embryonic zone and a differentiation zone. The embryonic zone at the blind tubular end comprises rather short and narrow, undifferentiated E-cells (Fig. 1a, b). Most E-cells have a PAS-positive vacuole in a supranuclear position (Fig. 1a–c). Mitotic stages are most often found a short distance away from the blind tubular end (Fig. 1b, c), but occasionally they can be located directly at a tubule's tip (Fig. 1a). Generally, cell division follows the normal scheme given for mitosis in animal cells and, hence, all stages of mitosis can be found (Fig. 1d–i, k). Mitotic cells are always located at the lumen-oriented half of the epithelium (Fig. 1b, c) and were never observed to reach the basal lamina. During cell division they maintain their microvillous border (Fig. 1c, e, h) and often also the supranuclear vacuole (Fig. 1h, k). PAS-haematoxylin turned out to be particularly suited to visualizing details of mitotic cells since it not only stains the condensed chromatin but also the cell membranes and the spindle apparatus (Fig. 1f).

The descendants of the E-cells are continuously pushed away from the embryonic zone by further mitotic pulses. Thus, a distinct age gradient is established along the hepatopancreatic tubule system with the youngest cells in the embryonic zone and the oldest in the antechamber. Of the differentiating cells, it is the F-cell that is recognized earliest under the light microscope. Differentiating F-cells are characterized by a large nucleus, basophilic cytoplasm and fibrillar cytoplasmic structures (Fig. 2a) which were identified as rough endoplasmic reticulum under the electron microscope. In contrast to F-cells, early differentiating R- and B-cells have acidophilic cytoplasm. Under the light microscope they can be distinguished from each other only as soon as the B-cells develop their typical apical complex. This PAS-positive, vesicle-bearing, apical cell structure is clearer on semithin resin sections than on paraffin sections. Distinction between R- and B-cells is also easy on paraffin sections when the central vacuole of the B-cells is being established (Fig. 2b). Further distinguishing features of differentiating R-, F- and B-cells are the appearance and staining patterns of their vacuoles (Fig. 2a, b).

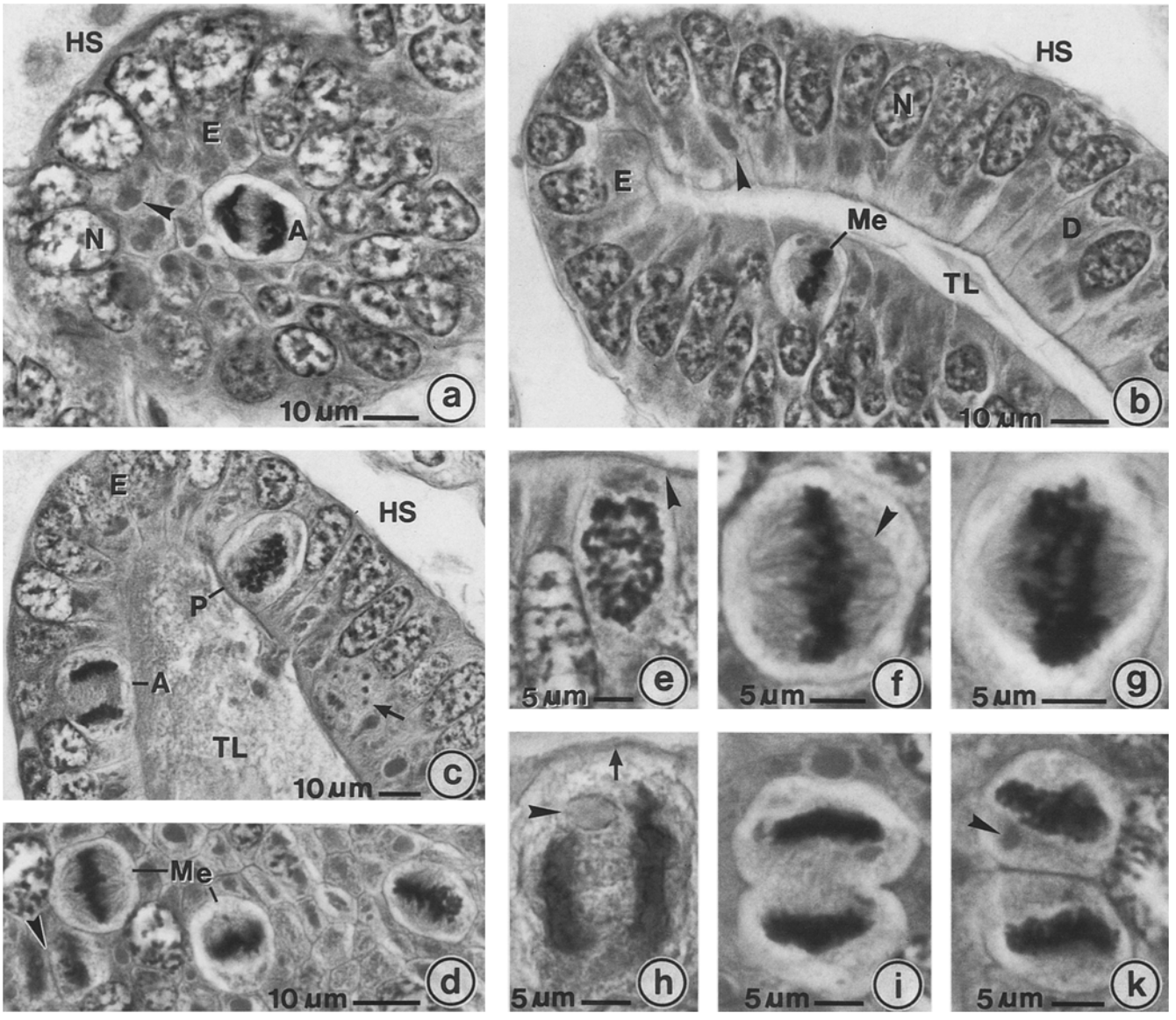


Fig. 1a–i, k Histology of embryonic zone of a hepatopancreatic tubule. Stainings: **a-g, i, k** PAS-haematoxylin, **h** Goldner. **a** Cross-section through closed tubular end displaying E-cells (*E*) and a central mitotic stage in anaphase (*A*). *Arrowhead* PAS-positive supranuclear vacuole, *HS* haemolymph space, *N* nucleus. **b** Longitudinal section of embryonic zone with E-cells, metaphase stage (*Me*) and differentiating cells (*D*). *Arrowhead* PAS-positive supranuclear vacuole, *TL* tubule lumen. **c** Embryonic zone with prophase (*P*) and anaphase (*A*) stages. Both cells have contact to the tubule lumen. *Arrow* anaphase stage viewed from a spindle pole. **d** High concentration of mitotic stages. *Arrowhead* stage with almost completed mitosis. **e** Prophase stage with microvillous border (*arrowhead*). **f** Metaphase stage. *Arrowhead* spindle apparatus. **g** Early anaphase stage. **h** Late anaphase stage with microvillous border (*arrow*) and supranuclear vacuole (*arrowhead*). **i** Telophase with cytokinesis starting. **k** Cytokinesis largely completed. *Arrowhead* supranuclear vacuole

2. Mid-region of hepatopancreatic tubule

The mid-region is traditionally defined by the presence of B-cells with distinct central vacuoles (Fig. 2c). Formation of the central vacuole is already initiated in the differentiation zone (Fig. 2b) but completed in the mid-region. Expansion of the vacuole is accompanied by displacement of the nucleus towards the cell base (Fig. 2b, c). Such displaced nuclei become crescent shaped and have a higher content of condensed chromatin (Fig. 2f). The majority of B-cells is discharged from the epithelium at the end of the mid-region by holocrine secretion (Fig. 2f). Occasionally, individual B-cells can persist in a differentiating stage to become mature later on. Aside from B-cells, the mid-region also includes F-cells with basophilic cytoplasm and R-cells with acidophilic cytoplasm (Fig. 2c, d). F-cells are preferably located in epithelial indentations, B-cells in bulges and R-cells in between (Fig. 2a–c, g). R-cells are easily recognized by the

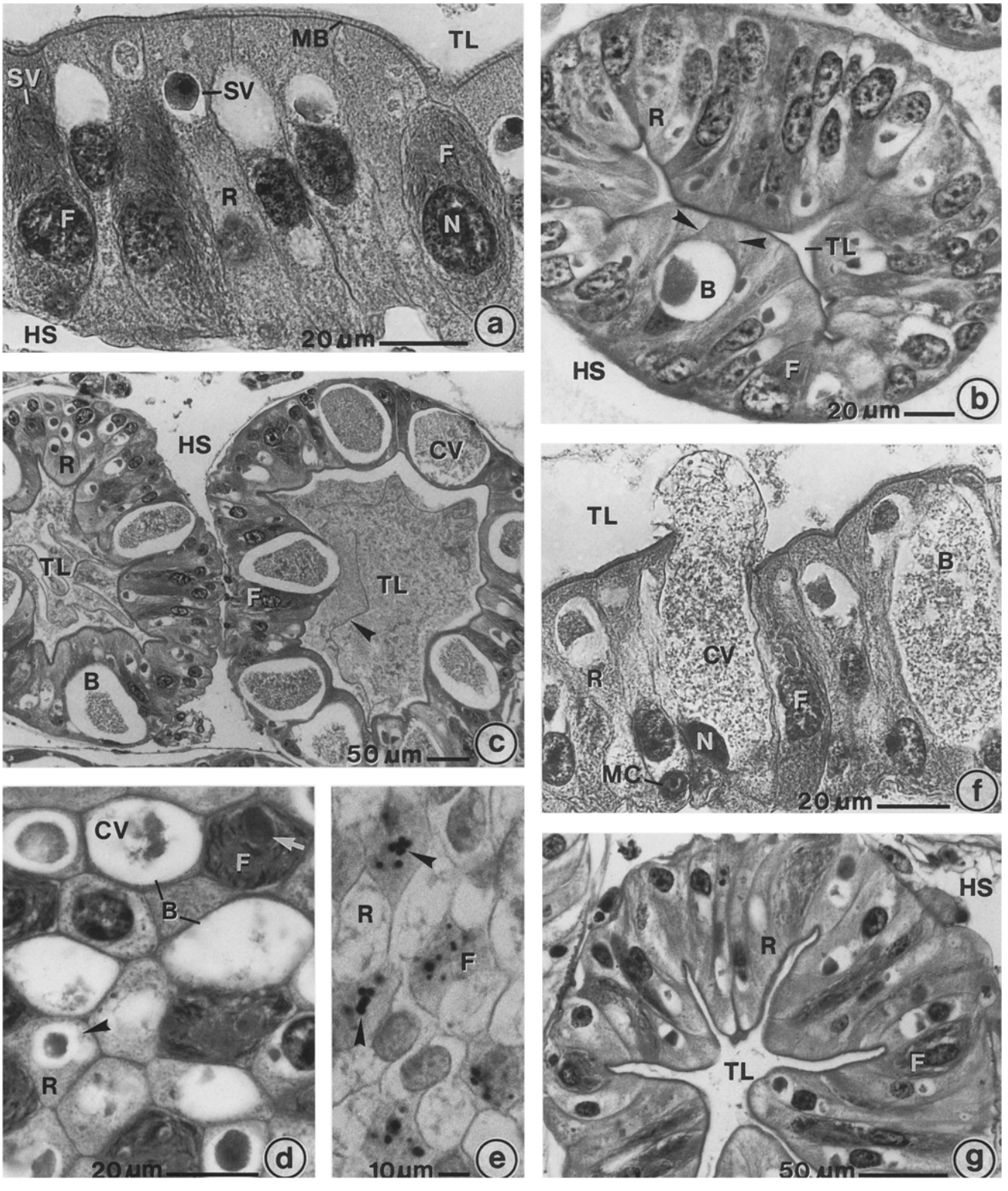


Fig. 2a–g Histology of differentiation zone, mid-region and proximal region of a hepatopancreatic tubule. Stainings: **a**, **f** Goldner, **b–d**, **g** PAS-haematoxylin, **e** alcian blue-alcian yellow/nuclear fast red. **a** Epithelium of differentiation zone close to the embryonic zone displaying R- (*R*) and F-cells (*F*). R-cells have light cytoplasm and supranuclear vacuoles (*SV*) with centrally compacted contents. F-cells have darker cytoplasm with fibrillar structures, large nuclei (*N*) and homogenously filled supranuclear vacuoles. *HS* haemolymph space, *MB* microvillous border, *TL* tubule lumen. **b** Cross-section through differentiation zone close to the mid-region displaying the typical tubular organization in bulges and indentations and a maturing B-cell (*B*) with developing central vacuole. *Arrowheads* apical complex. **c** Mid-region characterized by mature B-cells with PAS-positive central vacuoles (*CV*). *Arrowhead* peritrophic membrane-like structure. **d** Tangential section through mid-region with R-, F- and B-cells. Compare central vacuole of B-cells with supranuclear vacuoles of R- (*arrowhead*) and F-cells (*white arrow*). **e** Tangentially sectioned F-cells of mid-region with compartments rich in sulphated mucopolysaccharides (*arrowheads*). **f** Early phase of discharge of B-cell at the end of the mid-region. *MC* M-cell. **g** Proximal region with R- and F-cells only

presence of lipid droplets if the animals have been fed with a diet rich in lipids prior to tissue sampling.

Staining of the mid-region with PAS, alcian blue-alcian yellow and Goldner not only revealed some common patterns among the cell types but also differences. The microvillous border and the basal lamina stain generally red with PAS, strongly blue with alcian blue-alcian yellow and green with Goldner indicating the presence of acid mucopolysaccharides and neutral glycoproteins at those sites. The same staining pattern is observed for luminal structures which resemble peritrophic membranes (Fig. 2c). These structures can be found in all regions of the hepatopancreatic tubules but are not always present. Differences in the staining patterns concern the vacuoles and other smaller cell compartments. The central vacuole of B-cells, for instance, stains moderately red with PAS, blue-green with alcian blue-alcian yellow and yellowish-green with Goldner. In contrast, the supranuclear vacuoles of R- and F-cells stain intensely red with PAS, blue with alcian blue-alcian yellow and green with Goldner. F-cells include further PAS-positive and strongly alcian blue globules (Fig. 2e) which were identified as Golgi bodies by means of electron microscopy.

3. Proximal region of hepatopancreatic tubule and collecting duct

The proximal region of the hepatopancreatic tubules and the collecting ducts are histologically identical. Normally both segments include only R- and F-cells (Fig. 2g), but occasionally a few individual B-cells can be interspersed. All cell types display similar histological features and staining patterns as in the mid-region.

4. Antechamber

The unpaired antechamber is the most complex part of the hepatopancreas. It consists of three epithelia, hepatopancreatic epithelium, stomach epithelium and a particular antechamber epithelium. The hepatopancreatic epithelium is just a continuation of the collecting ducts and is therefore composed of R- and F-cells (Fig. 3a, b). It covers the lateral and posterior parts of the antechamber. Cuticle-bearing extensions of the pyloric stomach form the anterior roof and parts of the bottom. The anterior ventrolateral and lateral parts are lined by a particular antechamber epithelium that originates from short, blind-ending tubules with mitotic centres (Fig. 3a, inset). This epithelium is composed of one narrow cell type with elliptic nuclei (Fig. 3b) and has end-to-end contact with the hepatopancreatic epithelium (Fig. 3a). Interestingly, both epithelia have their mitotic centres on opposite sides, and consequently, their ends are pushed across each other at times of high mitotic activity (Fig. 3d, e). Such epithelial parts then break off and disintegrate within the antechamber lumen (Fig. 3c).

III. Cytology (electron microscopy)

This chapter focuses on the ultrastructural characterization of the hepatopancreatic cells. All cells depicted in Figs. 4–8 derive from the differentiation zone or the beginning of the mid-region, with the exception of E-cells and mitotic stages which are from the embryonic zone, and mature B-cells which are from the end of the mid-region. Respective cell types of the proximal region and the collecting ducts differ only in minor aspects.

1. E-cells and mitotic stages

E-cells are characterized by a high nucleus to cytoplasm ratio which is typical of embryonic cells (Fig. 4a). They have rather electron light cytoplasm (Fig. 4b) and have a microvillous border facing the tubule lumen (Fig. 4c) and a basal lamina (Fig. 4d) facing the haemolymph space. Compared to R-, F- and B-cells, they include only small amounts of cell organelles which are rather evenly distributed throughout the cell. Only the mitochondria display slight concentrations at the cell apex (Fig. 4c) and base (Fig. 4d). The rough and smooth endoplasmic reticulum (rER, sER) consist either of vesicles or short cisternae (Fig. 4b, c). The Golgi bodies are composed of one or two dilated cisternae at the cis side and several narrowly stacked cisternae of medium length at the trans side (Fig. 4e). They receive small transport vesicles from adjacent rER cisternae and release inconspicuous Golgi vesicles. The lysosomal system includes small primary lysosomes (Fig. 4b) and larger autophagosomes which are mostly located in a supranuclear position. The cell base comprises a weakly developed network of sER-like tubules (Fig. 4d). E-cells are mostly free of reserve deposits but occasionally they can contain one or two small lipid droplets (Fig. 4b) or a few glycogen particles.

During mitosis the nucleus and also the organelles of E-cells undergo considerable changes which are not described in detail in this paper. Instead, a prophase stage is depicted in Fig. 4f, g as an example. It is characterized by condensed chromatin, a fragmenting nuclear envelope, centrioles, small mitochondria and short ER cisternae. Neighbouring cells of such mitotic stages can already be assigned to one of the three mature hepatopancreatic cell types if the cell specific features listed below are hit on the section. The neighbouring cell of the prophase stage in Fig. 4g, for instance, can be identified as an R-cell on the basis of its Golgi body.

2. R-cells

The most characteristic features of R-cells are the nutrient reserves of lipid and glycogen, the Golgi bodies and the distinct polar organization into three areas: an apical area with microvillous border, sER tubules and plenty of mitochondria, a medial area with the nucleus,

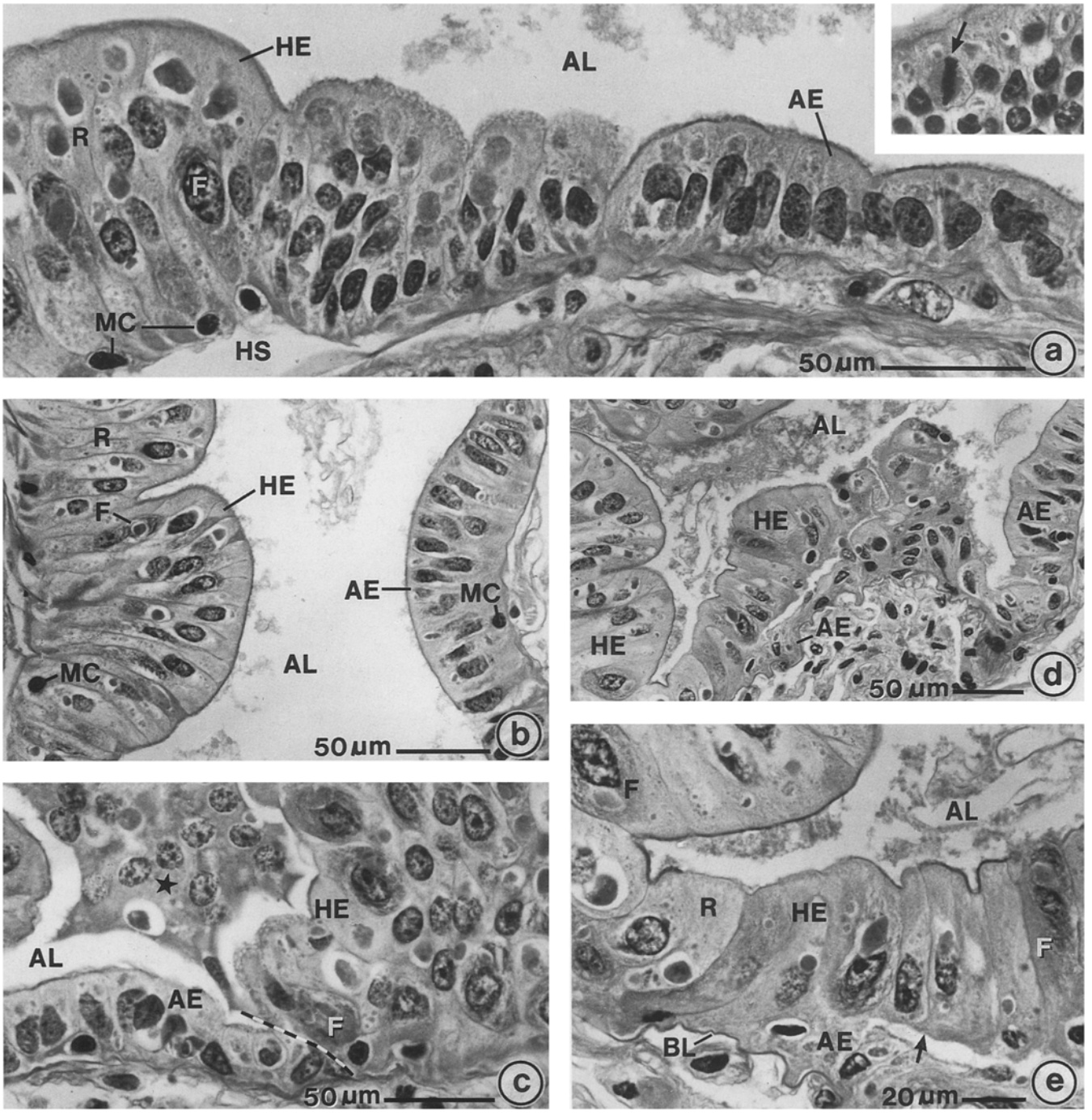


Fig. 3a–e Epithelia of antechamber and discharge of senescent cells. Stainings: **a**, **b**, **e** PAS-haematoxylin, **c**, **d** Goldner. **a** Contact zone of hepatopancreatic epithelium (*HE*) with F- (*F*) and R-cells (*R*) and antechamber epithelium (*AE*). *AL* antechamber lumen, *HS* haemolymph space, *MC* M-cell. *Inset* metaphase stage (*arrow*) in embryonic zone of the antechamber epithelium. **b** Sector of antechamber lined by hepatopancreatic epithelium (*left*) and antechamber epithelium (*right*). The antechamber epithelium is composed of a single narrow cell type. M-cells are present in both epithelia. **c** Junction of hepatopancreatic epithelium and antechamber epithelium (*broken line*) and discharged parts of the hepatopancreatic epithelium (*asterisk*) in the antechamber lumen. **d** Discharge of proximal end of the hepatopancreatic epithelium by sliding across the antechamber epithelium. **e** Close up of **d** showing that the discharging part of the hepatopancreatic epithelium is detached from the basal lamina (*BL*). *Arrow* cell bases without basal lamina

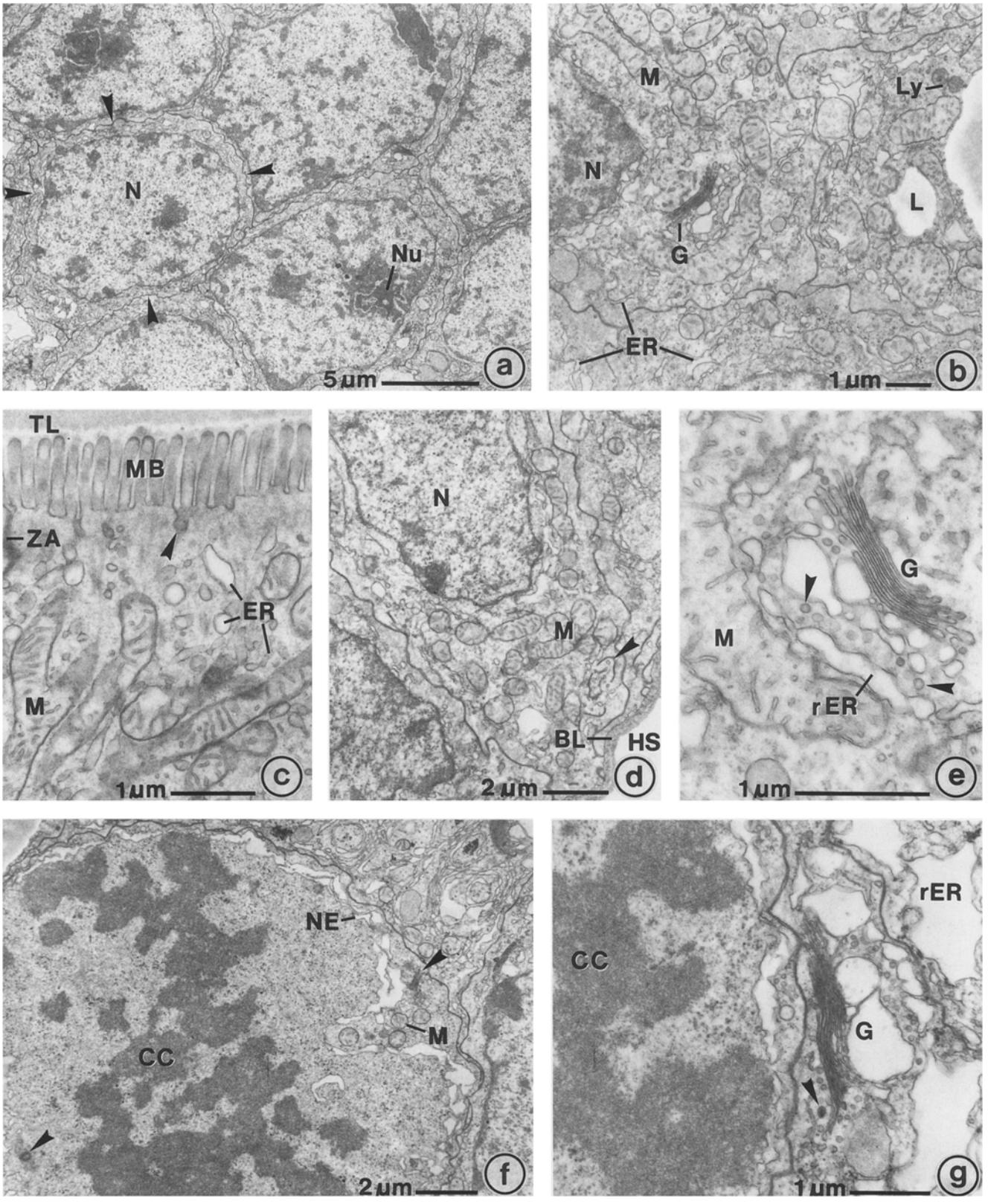


Fig. 4a–g Ultrastructure of E-cells and mitotic prophase stage. **a** Cross-section through blind end of a hepatopancreatic tubule illustrating the large nucleus to cytoplasm ratio of E-cells. *Arrowheads* mark limitation of one cell, *N* nucleus, *Nu* nucleolus. **b** Supranuclear area of E-cells with typical organelles: endoplasmic reticulum (*ER*), Golgi body (*G*), mitochondria (*M*) and lysosomes (*Ly*). Lipid inclusions (*L*) are rare in E-cells. **c** Apex of E-cell with microvillous border (*MB*) facing the tubule lumen (*TL*), lateral zonula adhaerens (*ZA*), pinocytotic invagination (*arrowhead*), mitochondria and ER. **d** Base of E-cell with nucleus, numerous mitochondria and a weakly developed sER-like tubule system (*arrowhead*). *BL* basal lamina, *HS* haemolymph space. **e** Typical Golgi body of E-cell. *Arrowheads* transport vesicles between rough endoplasmic reticulum (*rER*) and Golgi body. **f** Mitotic prophase stage with condensed chromatin (*CC*), fragmenting nuclear envelope (*NE*), mitochondria and centrioles (*arrowheads*). **g** Prophase stage (*left*) and neighbouring R-cell (*right*). The R-cell is identified by its Golgi body, particularly the appearance of the Golgi products (*arrowhead*)

nutrient reserves and most of the cell organelles, and a basal area dominated by an sER-like tubule system (Fig. 5).

The lumen-oriented surface of R-cells is greatly enlarged by plenty of well-ordered, elongated microvilli (Fig. 5a). Underneath there is a narrow strip with vertically arranged sER tubules which have a synapse-like contact to both the apical plasma membrane (Fig. 5b) and the mitochondria (Fig. 5c). Sometimes the lumen of the sER tubules even seems continuous with the intermembrane space of the mitochondria (Fig. 5c). The medial cell area includes the nucleus, branched sER tubules, stacks of short rER profiles, mitochondria, Golgi bodies, lysosomes and peroxisomes, as well as lipid and glycogen deposits (Fig. 5d–f). The lysosomal system includes small and homogenous primary lysosomes, large autophagosomes with membrane remnants and more electron dense residual bodies (Fig. 5d). The cell area basal from the nucleus is dominated by a well-developed, branched network of sER-like tubules (Fig. 5g). These tubules are often closely associated with rER profiles and mitochondria and open into the basolateral intracellular spaces and also into the space between the basal cell membrane and the basal lamina.

The most reliable markers to identify R-cells are the nutrient reserves of lipid and glycogen (Fig. 5e, f) and the Golgi bodies (Fig. 5h). Lipid droplets have very heterogenous sizes but include rather homogenous contents of moderate electron density (Fig. 5f). They are often associated with peroxisomes, sER and mitochondria. Glycogen is deposited as electron-dense, asterisk-like particles within glycogen fields (Fig. 5e, f). Both nutrient reserves are regularly found in R-cells of well-fed crayfish although their quantity can significantly vary among the cells. In differentiating cells they are less abundant than in mature cells and in poorly nourished crayfish they are lacking. The Golgi bodies have a similar appearance in both differentiating and mature cells. They are composed of one or two dilated cisternae at the cis face and several narrow cisternae of medium length at the trans face (Fig. 5h). The cis-most cisterna receives materials from adjacent rER cisternae by vesicular transport. At the trans face rather large Golgi vesicles are formed by sequestration of the coiled cisternal edges. They comprise coin-shaped, electron-dense material that appears either roundish (Fig. 5i). or stacked together like coins in a roll (Fig. 5h, k) depending on the angle of view. Synthesis of this material starts in the cis-most cisterna (Fig. 5i) and is continued in the medial cisternae where the products become more and more compacted (Fig. 5k). The fate of the Golgi vesicles after release from the Golgi body is not yet resolved. Golgi vesicles have never been found close to the apical, lateral or basal cell membranes and, therefore, exocytosis of the Golgi products seems unlikely. It is possible that they are discharged into the large autophagosomes.

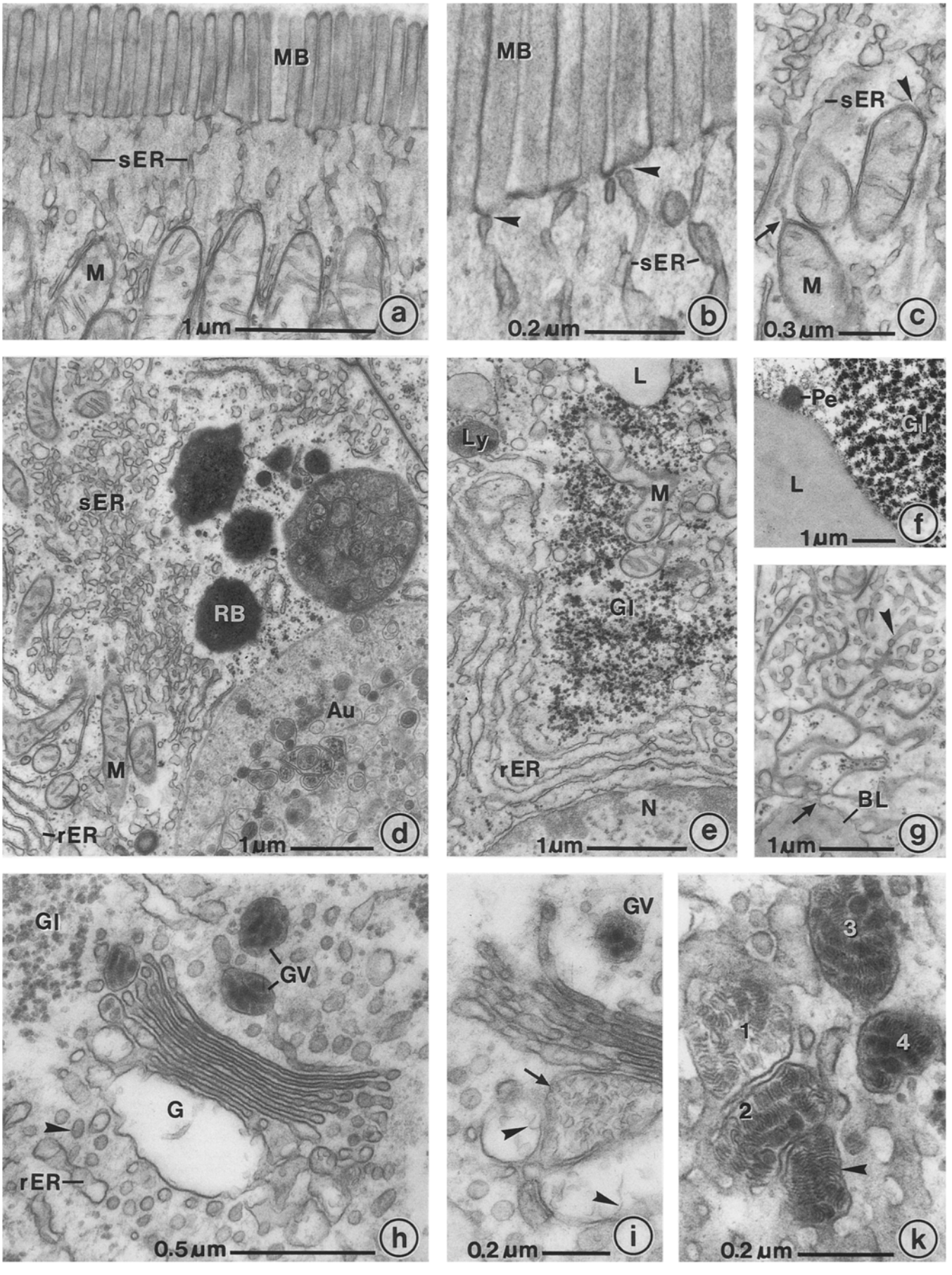
3. F-cells

F-cells are mainly characterized by plenty of short-chained rER and large Golgi bodies with heavily dilated cisternae. Their organelles are rather homogenously distributed within the cell and, hence, F-cells appear less polar than R-cells. Nutrient reserves are normally lacking (Fig. 6).

The cell apex is characterized by a regular microvillous border, bundles of vertically arranged microtubules and large, electron-transparent vesicles from the Golgi bodies (Fig. 6a) which release their contents into the tubule lumen by exocytosis. By means of immunohistochemistry they have earlier been shown to comprise digestive enzymes (Vogt et al. 1989b). They are particularly frequent two hours after artificial stimulation of enzyme synthesis by drainage of the gastric fluid from the cardiac stomach. Four hours after stimulation the apices harbour none or only a few of these Golgi vesicles but plenty of small vesicles instead (Fig. 6b, c). These small vesicles always occur together with endocytotic invaginations of the apical membrane and multivesicular bodies (Fig. 6b) and are, therefore, interpreted as part of a membrane retrieval system. Groups of endocytotic vesicles are often seen to initiate the formation of multivesicular bodies which are then converted or internalized into larger autophagosomes (Fig. 6d). There, the membranes seem to be degraded as indicated by the high amount of membrane remnants within the autophagosomes. The medial and basal cell areas are occupied by the nucleus, slightly dilated rER profiles, large Golgi bodies, mitochondria and lysosomes (Fig. 6e). Additionally, the cell base includes a weakly developed sER-like tubule system.

As in R-cells the most reliable markers of F-cells are the Golgi bodies. Compared to R-cells they are larger and have heavily dilated cisternae, particularly at the cis side (Fig. 6f). They receive material from the rER by

Fig. 5a–i, k Ultrastructure of R-cells. **a** Cell apex with microvillous border (*MB*), smooth endoplasmic reticulum (*sER*) and mitochondria (*M*). **b** Synapse-like contact of sER and apical cell membrane (*arrowheads*). **c** Close spatial relationship (*arrowhead*) and luminal continuity (*arrow*) of sER and mitochondria. **d** Cell organelles of supranuclear area including sER network, rough endoplasmic reticulum (*rER*), mitochondria, autophagosomes (*Au*) and residual bodies (*RB*). **e** Typical aspect of R-cell with nucleus (*N*), glycogen field (*Gl*), lipid droplet (*L*), rER cisternae, mitochondria and lysosomes (*Ly*). **f** Details of lipid and glycogen stores. *Pe* peroxisome. **g** Cell base with basal lamina (*BL*) and branched system of sER-like tubules (*arrowhead*). *Arrow* luminal continuity of tubule and extracellular space. **h** Typical Golgi body with transport vesicles from rER at cis face (*arrowhead*) and Golgi vesicles with electron-dense contents (*GV*) at trans face. **i** Synthesis of Golgi products in cis cisternae. *Arrowheads* filamentous products attached to the membrane of the cis-most cisterna, *arrow* higher concentration of products within the lumen of the following cisterna. **k** Coins in a roll-like compaction of Golgi products (*arrowhead*) in dilated edges of medial Golgi cisternae. Increasing grades of compaction are indicated by numbers 1–4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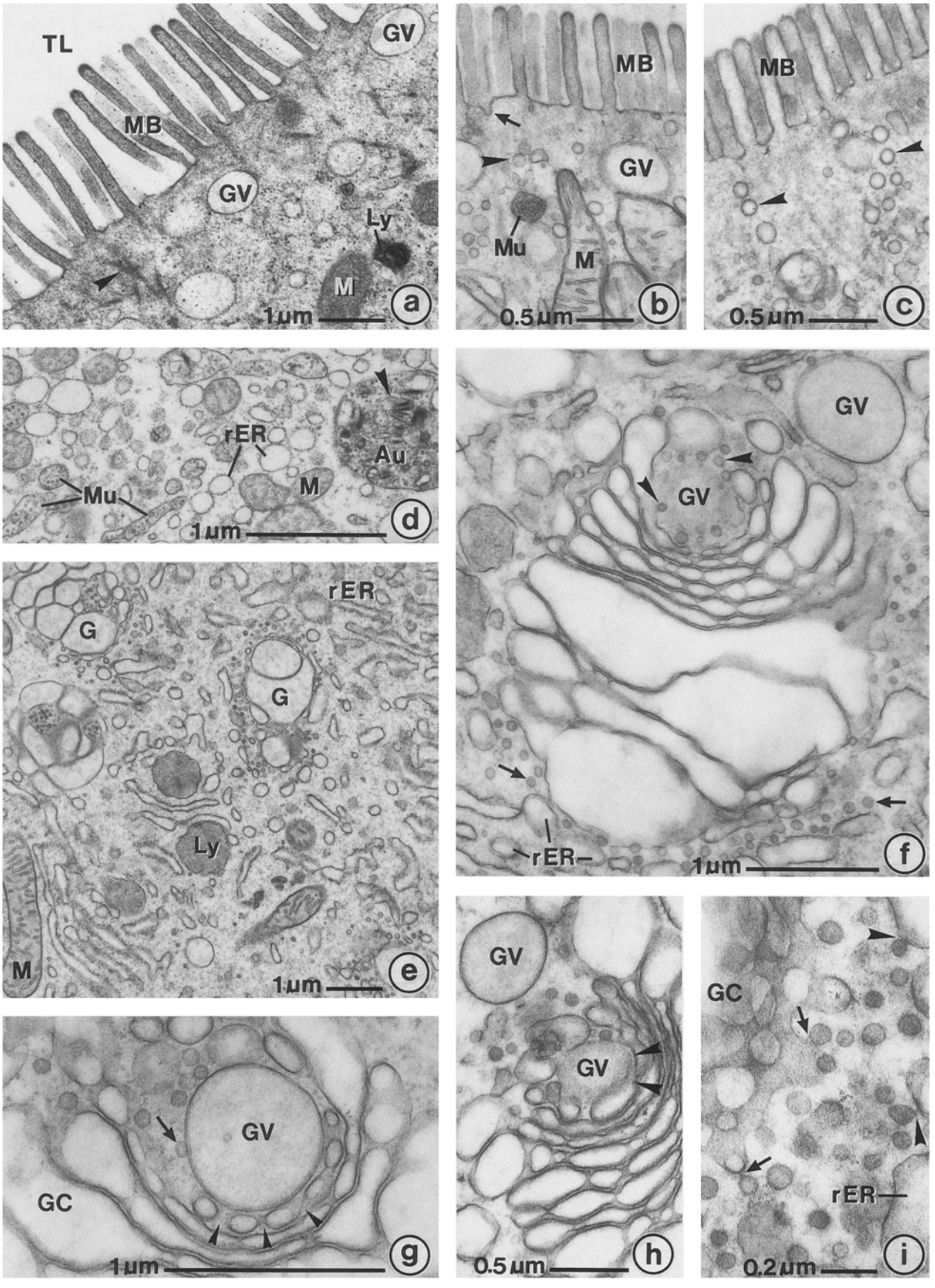
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transport vesicles that fuse with the cis-most Golgi cisterna. During phases of high transport activity this cisterna appears reticulate at its periphery due to the permanent addition of membrane material from fusing transport vesicles (Fig. 6i). The Golgi vesicles are formed in cup-shaped invaginations of the trans face. They originate from segments of the fragmented trans-most cisterna (Fig. 6h) and mature by incorporation of materials from small vesicles (Fig. 6f, g) which seem to derive from adjacent rER cisternae. Mature Golgi vesicles are released from the Golgi body and migrate to the microvillous border without any further morphological alteration. They are often found in close association with microtubules which may serve as a guide rail from the Golgi body to the site of exocytosis at the microvillous border.

4. *B-cells*

In contrast to R- and F-cells, which retain their typical ultrastructural features more or less from differentiation to death, the appearance of B-cells changes considerably during their life-cycle. The alterations include darkening of the cytoplasm, formation of the apical complex and the central vacuole, and displacement of the cell organelles towards the cell base during expansion of the central vacuole (Fig. 7).

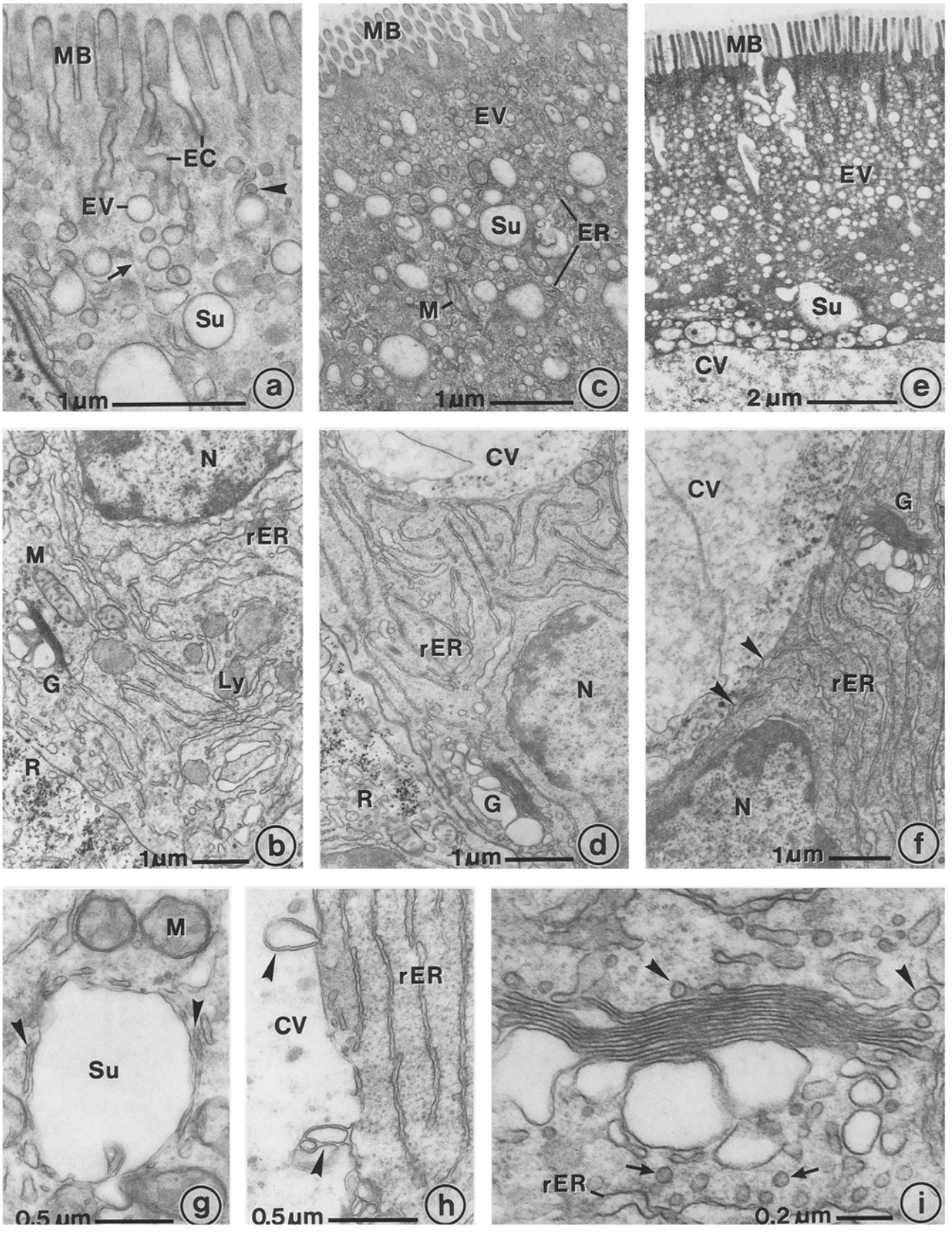
Differentiating B-cells can be clearly identified as soon as the apical complex starts to develop which already occurs in the upper part of the differentiation zone (Fig. 7a). Otherwise, they resemble differentiating R-cells but lack nutrient reserves and have different Golgi bodies (Fig. 7b). The apical complex is an assemblage of endocytotic channels, rather opaque endocytotic vesicles, smaller and denser Golgi vesicles, electron-transparent subapical vacuoles and microtubules (Fig. 7a). The endocytotic channels invaginate from the microvillous border deep into the cell and give rise to endocytotic vesicles which fuse with Golgi vesicles to create the subapical vacuoles (Fig. 7a). Such subapical vacuoles are then enlarged by further incorporation of endocytotic vesicles and Golgi vesicles. Aside from the apical complex, early differentiating B-cells include elongated rER cisternae, mitochondria, Golgi bodies, lysosomes and the nucleus (Fig. 7b). The Golgi bodies of both differentiating and mature B-cells are characterized by narrowly stacked, elongated cisternae (Fig. 7b, d, i). They receive materials from rER cisternae by vesicular transport and release small Golgi vesicles with moderately electron dense contents (Fig. 7i).

Maturation of B-cells is characterized by extension of the apical complex deep into the cell (Fig. 7c), darkening of the cytoplasm and formation of the central vacuole in a supranuclear position (Fig. 7d). This central vacuole originates from coalescence of subapical vacuoles and perhaps also autophagosomes and is enlarged by further internalization of both subapical vacuoles and Golgi vesicles. Subapical vacuoles and also the expanding central vacuole often have intimate contact with rER cisternae (Fig. 7f–h). Sometimes they are even limited by rER cisternae (Fig. 7h) suggesting that the rER is somehow involved in the formation of their membranous boundary. Expansion of the central vacuole is accompanied by displacement of the nucleus and the majority of cell organelles towards the cell base (Fig. 7d). In advanced stages of maturation a distinct zonation into three areas is established: an apical area including the microvillous border and apical complex with intermingled cell organelles (Fig. 7e), a medial area with the central vacuole and a small rim of cytoplasm, and a basal area with the nucleus and most of the cell organelles (Fig. 7f).

Fig. 6a–i Ultrastructure of F-cells. **a** Cell apex with regular microvillous border (*MB*), digestive enzyme-containing Golgi vesicles (*GV*), mitochondria (*M*) and bundles of microtubules (*arrowhead*). *Ly* lysosome, *TL* tubule lumen. **b** Cell apex with components of the membrane retrieval system: endocytotic invagination (*arrow*), endocytotic vesicles (*arrowhead*) and multivesicular body (*Mu*). **c** Cell apex with intense membrane retrieval. *Arrowheads* endocytotic vesicles. **d** Subapical area with numerous multivesicular bodies and an autophagosome (*Au*) comprising membrane remnants (*arrowhead*). *rER* rough endoplasmic reticulum. **e** Typical aspect of medial cell area with heavily dilated Golgi bodies (*G*), shortchained *rER*, mitochondria and lysosomes. **f** Golgi body with dilated cisternae, transport vesicles from rER (*arrows*) at cis face and Golgi vesicles (*GV*) at trans face. The tangentially sectioned Golgi vesicle is surrounded by numerous electron-dense vesicles (*arrowheads*). **g** Cup-shaped depression of trans face with almost mature Golgi vesicle fusing with small electron-dense vesicle (*arrow*). *Arrowheads* fragmenting trans-most cisterna, *GC* Golgi cisterna. **h** Derivation of primordial Golgi vesicle from segment of fragmenting transmost cisterna. *Arrowheads* indicate continuity between cisternal fragment and arising Golgi vesicle. **i** Tangentially sectioned cis face of Golgi body with transport vesicles between rER and reticulate cis-most cisterna. *Arrowheads* budding of vesicles from rER, *arrows* fusion of vesicles with cis-most cisterna

5. *M-cells*

M-cells are much less frequent than R-, F-, and B-cells. They are distributed throughout the entire hepatopancreatic tubule system and are also found in the epithelia of the antechamber and midgut. M-cells are always solitary, are exclusively located at the haemolymph side of the epithelium and have no microvillous border. They are characterized by a large lobulated nucleus and membrane-limited granules of different electron density (Fig. 8a, c). Histochemical stainings with PAS and alcian blue-alcian yellow indicate that the granules comprise neutral glycoproteins but lack acid mucopolysaccharides. The amount of granules can vary considerably among M-cells and, sometimes, granules are even absent (Fig. 8b). Their presence or absence is not related to the relative position of the cells in the hepatopancreatic tubule system. Aside from the granules, M-cells contain small mitochondria, short rER cisternae and medium-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sized Golgi bodies (Fig. 8d). The Golgi bodies receive material from the rER via transport vesicles and seem to give rise to the granules. M-cells can include smaller amounts of glycogen fields, particularly if granules are sparse or absent (Fig. 8b).

6. Peritrophic membrane-like structure

Occasionally, an extracellular ca. 0.2 µm-wide structure of filamentous composition is found within the lumen of the hepatopancreatic tubules (Fig. 8e). Some tubules even include two or three of these structures which resemble peritrophic membranes; they are often widened close to the apices of mature B-cells (Fig. 8f).

D. Discussion

Reinvestigation of the hepatopancreas of *A. astacus* with improved techniques of tissue fixation and processing yielded some new morphological data which shed more light on the life-cycle and functions of the hepatopancreatic cell types. In the following chapters these findings are discussed with respect to the literature in order to draw a more refined functional concept of the hepatopancreas of Decapoda.

I. Genealogy of hepatopancreatic cells

It is generally accepted that the hepatopancreatic epithelium which lines the tubules, collecting ducts and parts of the antechamber is regenerated from the blind-ending distal tips of the hepatopancreatic tubules since these are the only sites with mitotic stages. The particular antechamber epithelium, which is described for the first time in this paper, has its mitotic centres at the blind ends of very short tubules. These give rise to one cell line only. The regeneration of the stomach epithelium of the antechamber is not yet resolved.

The number of cell lines of the hepatopancreas and possible interdependences between the cell types have, until today, had conflicting interpretation (Icely and Nott 1992; Vogt 1993). The most favoured two-cell-line concept presumes that the embryonic E-cells give rise to only two cell types, R- and F-cells. B-cells are thought to originate later from a part of the F-cells (Dall and Moriarty 1983; Icely and Nott 1992). However, an F- into B-cell transition was never convincingly documented. Moreover, in the shrimp *Penaeus monodon* Fabricius, 1798 (see Vogt 1993) and now in *A. astacus* I was able to trail all of the three mature cell types back to the E-cells by using the appearance and distribution pattern of the cell organelles as distinguishing feature. The most reliable markers in both species for the identification of early differentiating cells are the Golgi bodies since in healthy intermoult specimens they have a cell-specific appearance throughout all life stages. These findings rather support a three-cell-line concept, as earlier proposed by Ogura (1959). Much of the confusion with the claimed interdependence between F- and B-cells was obviously caused by the basophilia of the cytoplasm in both cell types and the presence of a rather large supranuclear vacuole in F-cells which was mixed up with the primordial central vacuole of B-cells. However, both vacuoles differ with respect to origin and content. The supranuclear vacuole of F-cells is an autophagosome which serves for the digestion of damaged cell organelles, degradation of retrieved membranes from exocytosis of digestive enzymes and storage excretion of certain metals. In contrast, the central vacuole of B-cells is a heterophagosome which originates from the fusion of endocytotic vesicles and Golgi vesicles and which degrades materials from the tubule lumen.

II. Discharge of hepatopancreatic cells due to functional requirement or senescence

After origination in the embryonic zone, the young R-, F- and B-cells are gradually pushed down the hepatopancreatic tubule system by further mitotic pulses of the E-cells. During their migration to the antechamber they can be expelled at any site if damaged but normally they have specific areas of extrusion. B-cells are discharged by holocrine secretion at the end of the mid-region in a certain phase of digestion (Hopkin and Nott 1980; Vogt 1988). The functional significance of this discharge is not yet understood. R- and F-cells are only extruded from the epithelium if damaged or senescent. Their major sites of discharge are the junctions of neighbouring hepatopancreatic tubules or collecting ducts and the junction of hepatopancreatic epithelium and antechamber epithelium. This junction is also the site of discharge of the oldest cells of the antechamber epithelium. The architecture of the antechamber suggests that there should

Fig. 7a–i Ultrastructure of B-cells. **a** Apex of very young B-cell without central vacuole displaying microvillous border (*MB*) and apical complex composed of endocytotic channels (*EC*), endocytotic vesicles (*EV*), Golgi vesicles (*arrowhead*), subapical vacuoles (*Su*) and microtubules (*arrow*). **b** Medial and basal area of very young B-cell comprising nucleus (*N*), elongated rER cisternae (*rER*), mitochondria (*M*), lysosomes (*Ly*) and Golgi body (*G*). *R* R-cell. **c** Apex of maturing B-cell with darkened cytoplasm and proliferated apical complex. **d** Typical aspect of maturing B-cell with primordial central vacuole (*CV*), nucleus, Golgi body and elongated rER cisternae. **e** Apex of mature B-cell with extended apical complex and electron-dense cytoplasm. **f** Basolateral area of mature B-cell with large central vacuole, Golgi body and rER cisternae. Some rER cisternae seem to participate in the formation of the vacuole's boundary (*arrowheads*). **g** Close association of rER cisternae (*arrowheads*) and subapical vacuole in young B-cell. **h** Limitation of expanding central vacuole by rER cisternae which even protrude towards the vacuole's lumen (*arrowheads*). **i** Typical Golgi body of B-cell with transport vesicles between rER and cis cisterna (*arrows*) and narrowly stacked trans cisternae which release small Golgi vesicles (*arrowheads*)

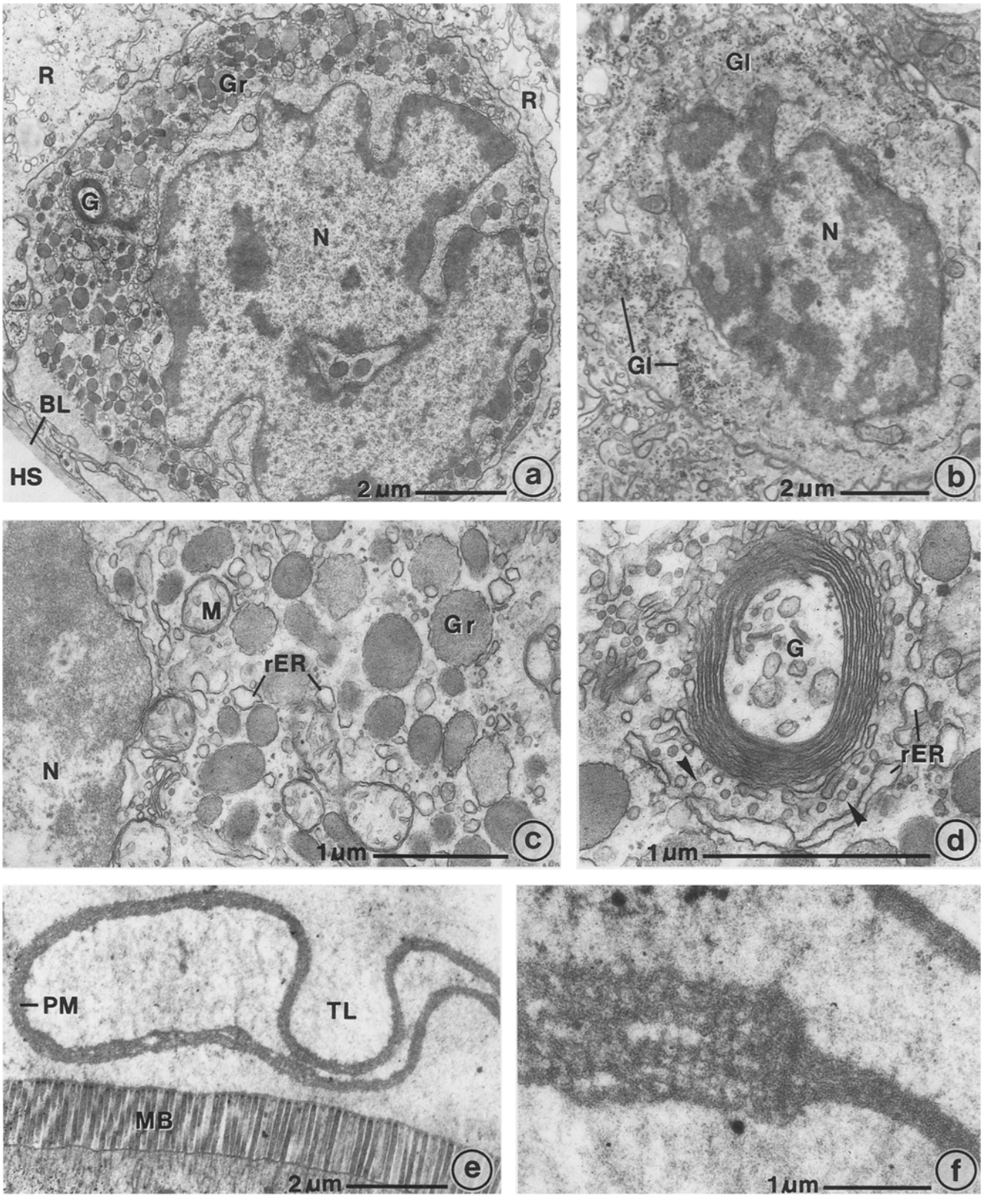

Fig. 8a–f Ultrastructure of M-cells and peritrophic membrane-like structure. **a** M-cell with lobulated nucleus (*N*) and plenty of membrane-limited granules (*Gr*). *BL* basal lamina, *G* Golgi body, *HS* haemolymph space, *R* R-cell. **b** M-cell without granules but with glycogen fields (*Gl*). **c** Higher magnification of granules and cell organelles. *M* mitochondria; *rER* rough endoplasmic reticulum. **d** Golgi body of M-cell with narrowly stacked cisternae and transport vesicles from rER (*arrowheads*). **e** Peritrophic membrane-like structure (*PM*) in tubule lumen (*TL*) close to the microvillous border (*MB*) of an R-cell. **f** Thicker and thinner segments of the peritrophic membrane-like structure

be a further site of cellular discharge at the junction between hepatopancreatic epithelium and stomach epithelium but morphological or experimental evidence is lacking thus far.

III. Ultrastructure and function of R-cells

R-cells execute the intestinal and hepatic functions of the hepatopancreas including absorption and catabolism of nutrients, delivery of metabolites to other organs, storage of reserves for periods of moulting, reproduction and starvation, and detoxification of heavy metals. Crayfish were shown to absorb about 90% of the nutrients of the feed in the hepatopancreas and only 10% in the main digestive tract (Speck and Urich 1970). All hepatopancreatic cells have the capacity principally to internalize nutrients via their microvillous borders which provide a greatly enlarged absorptive surface. But due to ultrastructural features discussed below, only the R-cells are thought to absorb nutrients for storage or export to other organs. The other hepatopancreatic cell types may absorb nutrients only for their own demand.

In Decapoda the food is ground in the gastric mill and filtered through the pyloric stomach. Normally, only fluid matter enters the hepatopancreas. The food molecules are thought to be already split extracellularly into small molecules by the exo- and endoproteases, amylases, lipases and nucleases of the gastric fluid (Zwilling and Neurath 1981; Dall and Moriarty 1983) and by further membrane-bound disaccharidases, peptidases, esterases and phosphatases of the microvillous border (Vogt 1988; Glass et al. 1989). As a consequence, nutrient absorption by the R-cells is expected to occur via transport of small molecules across the apical cell membrane. This idea is supported by the work of Ahearn and colleagues who have identified several carriers for amino acids and sugars (Ahearn 1987), and by the absence of ultrastructural evidence for endocytosis and intracellular digestion of particulate matter.

The complex of sER tubules and mitochondria underneath the microvillous border of R-cells is certainly involved in molecular absorption of the nutrients. In *A. astacus* it was particularly prominent two hours after feeding. At the same time, the microvillous border and the sER-containing apical cell area displayed heavy activity of the enzymes nonspecific esterases, acid phosphatase and alkaline phosphatase (Vogt 1988; Icely and Nott 1992). These activities decreased in the hour following and were absent thereafter with the exception of the microvillous border. Al-Mohanna and Nott (1987) also observed proliferation of an apical sER system in *Penaeus semisulcatus* De Haan, 1844, in the first two hours after feeding. This sER system persisted through the following hours but was again reduced after twelve hours. Interestingly, the apical sER tubules in *A. astacus* have synapse-like contact with the apical cell membrane, a feature that was previously only reported in the cephalocarid *Hutchinsoniella macracantha* (Elofsson et al. 1992). Elofsson and colleagues also interpret this feature as an ultrastructural correlate of molecular nutrient absorption. More basally the sER tubules of *A. astacus* have close contact or sometimes even luminal continuity with the intermembrane space of mitochondria. The functional significance of this close interrelation is not clear but it also seems to be related to nutrient absorption.

The medial cell area is dominated by the storage products, glycogen and lipid, and large amounts of all cell organelles, of which the autophagosomes are the most prominent. In Decapoda, lipid and glycogen reserves are deposited to compensate for periods of high energy demand or food deprivation since they are depleted during moulting (Johnson 1980; Al-Mohanna and Nott 1989), reproduction (Vogt et al. 1989a) and starvation (Storch and Anger 1983; Vogt et al. 1985). Glycogen fields can also be involved in the homeostasis of blood glucose (Sedlmeier 1987). Some Decapoda, such as *Penaeus monodon*, store only lipids in the R-cells and lack glycogen fields (Vogt 1985; Vogt et al. 1986). The supranuclear autophagosomes are interpreted as sites of intracellular waste deposition since they generally include plenty of membrane remnants from degraded cell organelles. Some Brachyura store plenty of calcium within such supranuclear vacuoles (Loret and Devos 1992). In specimens exposed to metal polluted water or feed, these vacuoles can additionally comprise large amounts of heavy metals (Lyon and Simkiss 1984; Andersen and Baatrup 1988; Vogt and Quinitio 1994). Smaller amounts of copper are also found in the autophagosomes of uncontaminated animals and, therefore, they are thought to detoxify copper from the catabolism of haemocyanin as well (Vogt and Quinitio 1994). The function of the Golgi bodies of R-cells is not yet clear. They synthesize a proteinaceous product as can be concluded from the transport of materials from the rER to the cis-most Golgi cisterna. Since the Golgi vesicles are neither discharged into the tubule lumen nor into the haemolymph space they may release their contents into the autophagosomes.

The basal tubule system of R-cells can be interpreted either as a basal labyrinth-like invagination of the basal and basolateral cell membranes (Sagristà and Durfort 1991) or as modified sER (Vogt 1985; Al-Mohanna and Nott 1987) which occasionally has open contact with the extracellular space. This tubule system is closely intermingled with rER and mitochondria. It seems to deliver nutrients to other organs via the haemolymph as indicated by the intense activity of nonspecific esterases in the cell base five hours after feeding (Vogt 1988; Icely and Nott 1992) and a high content of electron-dense particles during early phases of starvation (Vogt 1988) as well as during maturation of the ovaries (Vogt et al. 1989a). Such electron-dense particles were identified as lipoprotein complexes (Sagristà and Durfort 1991). In addition to these functions, the tubules may by involved in synthesis and export of blood proteins.

IV. Ultrastructure and function of F-cells

F-cells cover the pancreatic function of the decapod hepatopancreas, the synthesis of digestive enzymes (Vogt et al. 1989b). They are further involved in detoxification of certain metals, for instance iron and calcium, which are stored in the supranuclear vacuole (Lyon and Simkiss 1984). Moreover, they seem to detoxify organic xenobiotics as indicated by proliferation of the rER after intoxication with an insecticide (Vogt 1987).

F-cells of Decapoda are characterized by high amounts of rough endoplasmic reticulum and plenty of prominent Golgi bodies (Loizzi 1971; Al-Mohanna et al. 1985a; Vogt 1985) both of which are typical features of protein synthesizing and exporting cells. Due to these characteristics F-cells have often been regarded as the site of synthesis of digestive enzymes. According to a popular hypothesis, F-cells were thought to accumulate the enzymes within their supranuclear vacuole while transforming into B-cells. After gradual expansion of that vacuole the enzymes would be discharged by holocrine secretion of the mature F/B-cells (Gibson and Barker 1979). However, this hypothesis could not be verified by closer investigations. Immunohistochemistry with antibodies raised against the protease astacin from *A. astacus* revealed instead that the enzyme is synthesized in the Golgi bodies of F-cells and directly discharged into the lumen of the hepatopancreatic tubules (Vogt et al. 1989b). Malcoste et al. (1983) obtained similar results in *Palaemon serratus* Pennant with antibodies against α-amylase.

The mode of digestive enzyme synthesis in *A. astacus* is resolved for artificial stimulation of enzyme production by drainage of the gastric fluid from the cardiac stomach, but not yet for the normal digestive cycle (Vogt et al. 1989b). As shown earlier, complete refilling of the cardiac stomach after drainage of the gastric fluid takes about four hours. The cytological events in the F-cells throughout that period could only be reconstructed in detail after improvement of tissue fixation and processing. Two hours after drainage plenty of transport vesicles shuttle between rER cisternae and the Golgi bodies indicating an intense transport of enzyme precursors from the site of translation into the site of further processing. Formation of the Golgi vesicles is initiated by fragmentation of the trans-most Golgi cisterna into medium-sized vesicles which grow by further incorporation of small vesicles from adjacent rER cisternae. This mode of formation of Golgi vesicles is different from that of R- and B-cells where the Golgi vesicles are created by budding of the cisternal edges. After maturation at the trans face of the Golgi body, the digestive enzyme containing Golgi vesicles migrate to the microvillous border without further alterations, perhaps along microtubules, to release their contents into the tubule lumen by eccrine secretion. Interestingly, the Golgi bodies include plenty of sulphated mucopolysaccharides, particularly during phases of high synthetic activity. Such acidic mucopolysaccharides are also present in exocrine pancreatic cells of vertebrates (Tartakoff et al. 1974). They may be involved in concentration and precipitation of the digestive enzymes or protection of the organelles against self-digestion.

Retrieval of the membranes which have been incorporated into the apical plasma membrane by exocytosis of the digestive enzymes is at a maximum four hours after stimulation of enzyme synthesis (Vogt et al. 1989b). Membrane retrieval starts with the formation of endocytotic vesicles which form multivesicular bodies deeper in the cell. Multivesicular bodies are interpreted as a form of lysosome (Van Deurs et al. 1993) which are converted into or fuse with larger autophagosomes where the membranes are degraded. This mode of membrane retrieval and degradation in *A. astacus* is identical with the membrane retrieval in the exocrine pancreatic cells of vertebrates after discharge of the digestive enzymes (Geuze and Kramer 1974).

The mode of synthesis and storage of digestive enzymes in *A. astacus* is, in some major aspects, fundamentally different from vertebrates (Vogt et al. 1988; Vogt et al. 1989b). In vertebrates, the pancreatic enzymes are synthesized in advance for the next feeding-cycle and stored within the exocrine pancreatic cells as zymogen granules (Jamieson and Palade 1971). They are discharged in response to neuronal and hormonal impulses during digestion. In *A. astacus* the enzymes are also synthesized in advance but are stored extracellularly in an active form within the lumen of the cardiac stomach to await the next meal. Consequently, ultrastructural features which participate in the formation of the zymogen granules in vertebrates (Novikoff et al. 1978) are lacking in F-cells of *A. astacus* and also of other Decapoda. Interestingly, the enzymes of *A. astacus* are well adapted to this extracellular mode of storage since they are very stable (Zwilling and Neurath 1981; Stöcker et al. 1988) which is in contrast to the instability of the digestive enzymes of vertebrates. A further fundamental difference of digestion in Decapoda and vertebrates is the lack of acid and pepsin in Decapoda. This lack may be compensated for by the intense mechanical milling of the food in the chitinous, teeth-bearing cardiac stomach (Glass et al. 1989) and the existence of Decapoda-specific proteases with unusual cleavage specificity, such as astacin (Stöcker et al. 1993).

V. Ultrastructure and function of B-cells

The functional interpretation of the B-cell is the toughest nut to crack. B-cells are characterized by a pronounced endocytotic apical complex and a huge central vacuole. Similar cell types have been reported from the digestive tract of Copepoda (Hallberg and Hirche 1980), the hepatopancreas of Amphipoda (Storch and Burkhard 1984) and Gastropoda (Walker 1970), and also from the hindgut of goldfish (Iida et al. 1986) and the ileum of suckling rats (Wilson et al. 1991). As in Decapoda, the functions of these cell types is far

from being established. In suckling rats, such cells serve for the uptake and sorting of milk proteins. Hormones such as epidermal growth factor and prolactin are transported within vesicles through the cell into the blood, whereas other proteins are assumed to be degraded in the vacuole (Wilson et al. 1991).

The ultrastructure of B-cells, with the endosomal apical complex and the autophagosome-like central vacuole, clearly indicates an absorptive and degrading function. Above I have presented arguments and evidence for the R-cell as a nutrient-absorbing cell type. Moreover, the early loss of contact with the haemolymph space, the growth of the central vacuole as a consequence of endocytosis and the holocrine discharge of mature B-cells into the tubule lumen do not fit in with an interpretation of B-cells as nutrient-absorbing and metabolizing cells. So what kind of material is being absorbed and degraded? Experiments of Al-Mohanna and Nott (1986) with tracers revealed that inert particles from the feed are endocytosed, retained within the pinocytotic vesicles and then exclusively transported into the central vacuole, whereas biological material like ferritin is also found in the cytoplasm and even in the haemolymph space. These findings suggest that B-cells have a sorting mechanism in the apical complex that can distinguish between usable and waste material. The usable material is apparently degraded in the cytoplasm, probably to reutilize its energy and catabolites, whereas the nonusable material is deposited in the central vacuole.

In an earlier paper on the development of B-cells in *Penaeus monodon* (see Vogt 1993), I have proposed that B-cells might clear the hepatopancreatic tubules of remnants of digestion between the periods of nutrient absorption and secretion of digestive enzymes which seems necessary, due to the dead-end construction of the hepatopancreatic tubules. Further arguments for such a functional interpretation may be derived from preliminary immunohistochemical experiments with antisera against the complete gastric fluid of starved *A. astacus* and the purified gastric proteases trypsin, carboxypeptidase and astacin. Antiserum against the entire gastric fluid of starved specimens, which can comprise only antibodies against non-nutrient macromolecules such as digestive enzymes or perhaps fat emulsifiers, labels the apical complex intensely and the central vacuole moderately. The same is true for the antisera against the three purified proteases, suggesting that B-cells absorb the respective non-nutrient molecules to degrade them. If it is considered that 1 ml of gastric fluid of *A. astacus* comprises about 50 mg of pure protein (Zwilling and Neurath 1981), then reutilization of this energy source ideed appears worthwhile. Together with the exhausted digestive enzymes, B-cells may also absorb and degrade any other "waste" which remains in the hepatopancreatic tubules after absorption of nutrients by the R-cells.

Another topic to be discussed in relation to B-cells is the participation of rER cisternae in the formation of subapical vacuoles or the central vacuole. Such a mechanism seems strange at first glance but recently we have reported on the involvement of rER cisternae in the formation of autophagosome-like bacteria harbouring vacuoles in the hepatopancreas of the shrimp *Palaemon elegans* Rathke (see Vogt 1992). Moreover, Tsekos and Schnepf (1991) and Lawrence and Brown (1992) have shown that cisternae of the ER can form autophagosomes, in red algae and cultured rat hepatocytes respectively, by enclosure of a portion of cytoplasm. Hence, the involvement of rER in the formation or rapid enlargement of degrading cell compartments appears more widespread in eucaryotic cells than suspected.

VI. Ultrastructure and function of M-cells

M-cells are already known from several species of Decapoda (Icely and Nott 1992). They have been named basal cell (Johnson 1980), midget cell (Al-Mohanna et al. 1985b) or small cell (Vogt 1985). They always occur alone at the haemolymph side of the epithelium and are rather homogenously distributed along the tubule system. Since they are also found in other epithelia of the digestive tract they are supposedly of non-hepatopancreatic origin.

Their appearance is quite different in the various species. In *A. astacus* they include plenty of rather small granules, in the blue crab *Callinectes sapidus* Rathbun fewer but larger granules (Johnson 1980) and in the penaeid shrimps *Penaeus monodon* (see Vogt 1985) and *Penaeus semisulcatus* (see Al-Mohanna et al. 1985b) mostly one large inclusion. These inclusions can fill almost the entire cell or can be totally depleted. Al-Mohanna et al. (1985b) reported that the M-cells of *Penaeus semisulcatus* mature progressively towards the proximal end of the tubules. For *A. astacus* and *Penaeus monodon* I could not verify this observation. Therefore, I assume that the amount of granules is rather dependent on the physiological status of the organ than on the relative position of the cells in the tubule system. The distribution pattern of M-cells resembles the arrangement of endocrine cells in the digestive tract of vertebrates and their location at the haemolymph side suggests a close functional relationship to the blood. Therefore, M-cells might be interpreted as endocrine cells which regulate the activity of the epithelial cells or the muscle network around the hepatopancreatic tubules.

E. Conclusions

(1) The epithelium of the hepatopancreatic tubule system comprises three cell lines, R-, F- and B-cells which all originate directly from the embryonic E-cells at the distal tips of the hepatopancreatic tubules. The antechamber epithelium inludes only one cell line which derives from embryonic centres of other, very short tubules. M-cells are found in much smaller amounts in both epithelia.

(2) Differentiating cell types can be distinguished from each other by the cell-specific appearance and arrangement of their cell organelles, particularly the Golgi bodies.
(3) Mature B-cells are discharged from the epithelium by holocrine secretion in the mid-region of the hepatopancreatic tubules in a certain phase of the digestive cycle. R- and F-cells are discharged when senescent either at the junctions of neighbouring tubules or at the antechamber.
(4) R-cells display ultrastructural features that corroborate a nutrient-absorbing and metabolizing function. They have an absorptive complex inclusive of an unusually arranged sER for molecular absorption of nutrients, the nutrient stores of glycogen and lipid, and a basal sER-like tubule system for the export of metabolites to other organs.
(5) F-cells have the typical ultrastructural features of protein exporting cells. They synthesize digestive enzymes and discharge them directly into the hepatopancreatic lumen. The enzymes are stored extracellularly in the cardiac stomach in an active form, which is fundamentally different from vertebrates.
(6) B-cells are characterized by an apical endosome-lysosome complex and a large central vacuole. They are supposed to degrade exhausted digestive enzymes and any other waste products which remain in the hepatopancreatic tubules after absorption of the nutrients.
(7) M-cells resemble in some aspects endocrine cells and may have regulatory functions.

Acknowledgements Thanks to Marika Suhm for preparation of a part of the sections and Gisela Adam for reproduction of the micrographs.

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