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ORIGINAL ARTICLE

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Development of the digestive tract of sea bass (*Dicentrarchus labrax* L). Light and electron microscopic studies

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Abstract The developing gut of sea bass was studied by light and electron microscopy, four phases being established. Phase I, from hatching to the opening of the mouth, was a lecitotrophic period, in which the gut appeared as a straight undifferentiated tube lined by a simple epithelium that became stratified in the most caudal region. The epithelial cells increased in length towards the caudal zone, as did the number and height of the apical microvilli and the magnitude of the lamellar structures in their basal region. Cilia were more numerous in the caudal region than in the rest of the gut. Signs of lipid but not of protein absorption were found in the epithelial cells at this phase. Phase II, from the opening of the mouth to the complete resorption of the yolk sac, was a lecitoexotrophic period in which an esophagus, a gastric region, an intestine and a rectum, the last two separated by a valve, were present. During this phase the differentiation of the gut started at the esophagus and the rectum. In the esophagus, the epithelium became stratified and goblet cells containing acid mucosubstances, including sulphomucins, appeared. In the epithelial cells of the rectum, supranuclear vacuoles and an incipient endocytotic apparatus that seemed to be involved in the absorption and digestion of proteins were found. In both regions the mucosa was folded. Phase III, from the complete resorption of the yolk sac to the appearance of the first gastric glands, initiated the exclusively exotrophic period. During this phase the intestine formed the mucosa folds, while the first pyloric caeca and the epithelial cells acquired the ultrastructural features of mature absorptive cells with many lipid inclusions. Goblet cells containing neutral mucosubstances appeared and increased in number in both the intestine and the rectum. Neutral mucosubstances were also present in the cells lining the gastric region. During phase IV, from the appearance of the first gastric glands onwards, the intestinal absorptive surface increased with the formation of new pyloric caeca and two intestinal loops. The stomach acquired its definitive anatomy and histology with the development of the caecal and pyloric regions alongside differentiated gastric glands. The glandular cells had the ultrastructural features of the cells that secrete both pepsinogen and hydrochloride acid in the adult teleost stomach.

Keywords Gut · Differentiation · Ultrastructure · Mucosubstances · *Dicentrarchus labrax* (Teleostei)

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Introduction

Developmental patterns vary greatly among teleost fish. In some, such as the salmonids, the embryos have large supplies of endogenous food and develop directly to become juveniles with the organs of adults. However, most teleosts, after the embryonic period of endogenous feeding, undergo a larval period of exogenous feeding, even though their definitive organs are not yet fully differentiated (Balon 1985). The alimentary canal of fish larvae is morphologically, histologically and physiologically less elaborate than that of adult fish, and it has been suggested that digestion in teleost larvae differs from the digestion of adult specimens (Tanaka 1969). One of the difficulties involved in rearing teleost larvae is the selection of appropriate food for each of the developmental stages. Furthermore, there is an increasing tendency in aquaculture to replace natural food with synthetic inert microdiets as early as possible, so that a knowledge of the digestive system and its functional abilities during development is of great interest.

Although the developing gut has been investigated in some species (Iwai 1967, 1969; Iwai and Tanaka 1968; Tanaka 1971, 1972; Stroband et al. 1979; Govoni 1980; Stroband and Kroon 1981; Albertini-Berhaut 1987, 1988; Loewe and Eckmann 1988; Boulhic and Gavaudan 1992; Sarasquete et al. 1995; Calzada et al. 1998; Ribeiro et al. 1999), we only have partial knowledge of

the ontogeny of the digestive tract of teleosts. Most studies carried out in recent years deal with specific problems of the rearing techniques in the commercial production of larvae (Yúfera et al. 1996; Fontagné et al. 1998; Navarro and Sarasquete 1998).

The sea bass, *Dicentrarchus labrax*, is a teleost, serranidae, which is widely reared in extensive aquacultural production facilities in countries bordering the Mediterranean sea. There are a few investigations on the morphology of the developing gut of sea bass larvae, one at the light microscopic level (Tan Tue 1976) and others that study only the intestine and/or rectum by electron microscopy at certain developmental stages (Connes and Benhalima 1984; Deplano et al. 1991a, b). The occurrence of mucosubstances and of digestive enzyme activities at certain larval stages (Tan Tue 1980, 1983) and the influence of diet on the enzyme activities (Zambonino Infante and Cahu 1994; Cahu and Zambonino Infante 1994) have also been reported.

In a previous investigation four developmental stages were established in sea bass ontogeny according to the principal morphological changes that take place in the gut and pancreas (García Hernández and Agulleiro 1992). The aim of the present study was a structural and ultrastructural study of the digestive tract of sea bass larvae during development to determine its functionality.

Materials and methods

Sea bass, *Dicentrarchus labrax*, ranging in age from hatching to 61 days, were provided by the Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, Spain, where they had been reared at 18–19°C, with 42‰ salinity and a natural photoperiod and fed with rotifers and fitoplancton from the opening of the mouth to 25–30 days after hatching and then with *Artemia* naupli until 45–50 days after hatching. From this time they were fed with *Artemia* metanaupli. A total of fifty-four and sixty specimens were used for the light- and electron-microscopic studies, respectively.

Light microscopy

Larvae were fixed by immersion for 24 h in Bouin's fluid, embedded in Paraplast Plus (Sherwood, Athy, Ireland) and sectioned at 4 µm. After dewaxing and rehydration, consecutive sections were stained with haematoxylin-eosin and Mallory's trichrome. Periodic acid Schiff reagent (PAS) and Alcian blue at pH 2.5 were used to reveal neutral and acid mucosubstances, respectively, and Alcian blue at pH 1 was used to detect sulphomucins.

Electron microscopy

Specimens ranging from hatching to 9 days were processed whole, while from this age onwards they were cut transversely into two (12- to 20-day-old larvae), three (25- to 34-day-old larvae) or four (39- to 61-day-old larvae) pieces. The samples were fixed for 4 h at 4°C in 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), then post-fixed for 2 h at 4°C in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2) and embedded in Epon. The samples were sectioned with a Reichert-Jung ultramicrotome. Semithin sections were stained with toluidine blue and examined by light microscopy. The ultrathin sections were stained with uranyl acetate and lead citrate and examined by Zeiss EM 10 C and EM 109 electron microscopes.

Results

Phase I (from newly hatched to 6–7-day-old larvae, 3–5 mm length)

Newly hatched larvae (Fig. 1) had a large yolk sac containing an eosinophilic material that extended along half of the total body length and progressively diminished during this phase. The gut consisted of a straight tube with a smooth lumen (Figs. 1, 2a) reaching the most caudal zone, which was curved (Fig. 2b). The anus and the mouth opened during and at the end of this phase, respectively. The gut had an epithelium, whose cells varied in height, lined by a layer of squamous cells at hatching (Fig. 1) and, later on, by numerous mesenchymal cells (Fig. 2). The epithelial cells had a basal, euchromatinic nucleus with one or two nucleoli.

Despite its undifferentiated aspect, some ultrastructural variations were observed in the epithelial cells of the anterior, medium, and posterior gut zones.

The narrow lumen of the anterior region was lined by cubic cells (Fig. 2a) that had a smooth apical surface and small junctional complexes, numerous free ribosomes, short dilated cisternae of rough endoplasmic reticulum (rer), small stacks of Golgi complex cisternae, and numerous round or ovoid mitochondria with a clear matrix and irregular crests (Fig. 3). Large, round or irregular vesicles with a medium electron-dense content, and

Fig. 1 Sagittal section of a newly hatched larva showing the posterior gut region (*g*; *N* notochord, *YS* yolk sac, *M* striated muscle cells). Toluidine blue. ×320 ▶

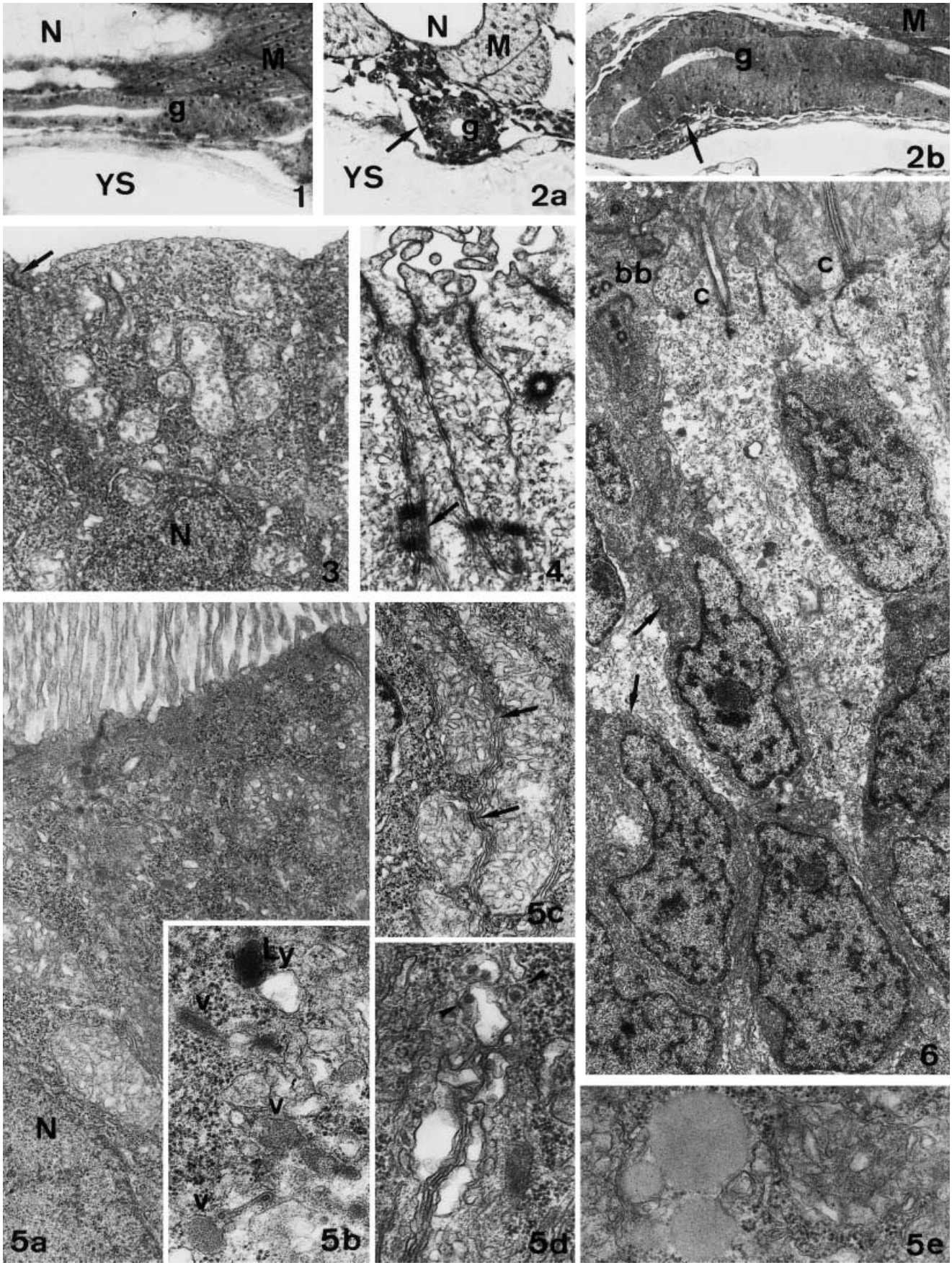
Fig. 2a, b Transversal (**a**) and sagittal (**b**) sections of a 5-day-old larva showing the anterior region of the gut (*g*) lined by short columnar cells (**a**) and the curved caudal region of the gut with a pseudostratified epithelium (**b**; *arrow* mesenchymal cells). **a** Mallory's trichrome. ×300; **b** Toluidine blue. ×480

Fig. 3 Epithelial cells of the anterior region of the digestive tract of a newly hatched larva. Note the smooth lumen, the homogeneous cytoplasm with numerous free ribosomes and mitochondria, and the small junction complexes (*arrow*; *N* nucleus). ×7,500

Fig. 4 Apical region of the epithelial cells of the medium gut of a newly hatched larva showing developed junction complexes and a basal body (*arrow* microfilaments). ×12,000

Fig. 5a–e Epithelial cells of the posterior gut of a 5-day-old larva. **a** Apical region with long microvilli, large mitochondria and numerous ribosomes. **b** Detail of the juxtanuclear region with bead-like vesicles of medium electron density (*v*) and a lysosome (*Ly*). **c** Basal region with lamellar structures (*arrow*) associated with mitochondria. **d** Golgi complex with dense lipid particles in some cisternae (*arrowhead*). **e** Lipid droplets (*N* nucleus). **a** ×13,000; **b, d, e** ×24,000; **c** ×14,000

Fig. 6 Caudal region of the gut of a 5-day-old larva with a pseudostratified epithelium. Note the basal bodies (*bb*) and cilia (*c*), and the cytoplasm of the dark cells (*arrows*) mostly occupied by free ribosomes. ×5,000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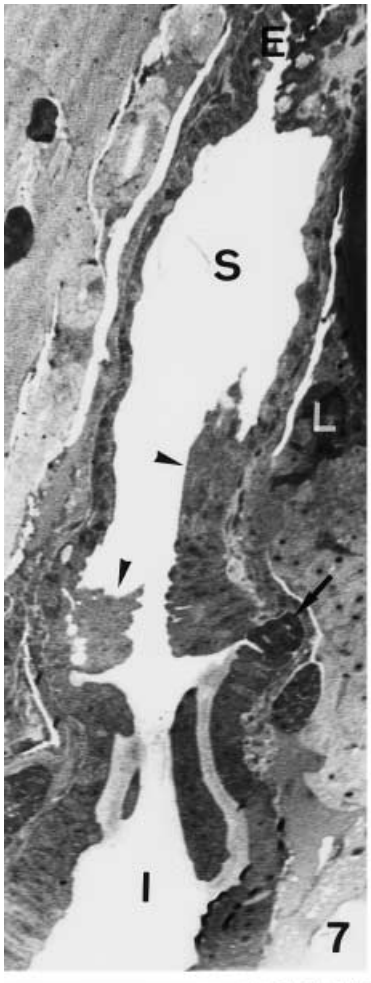
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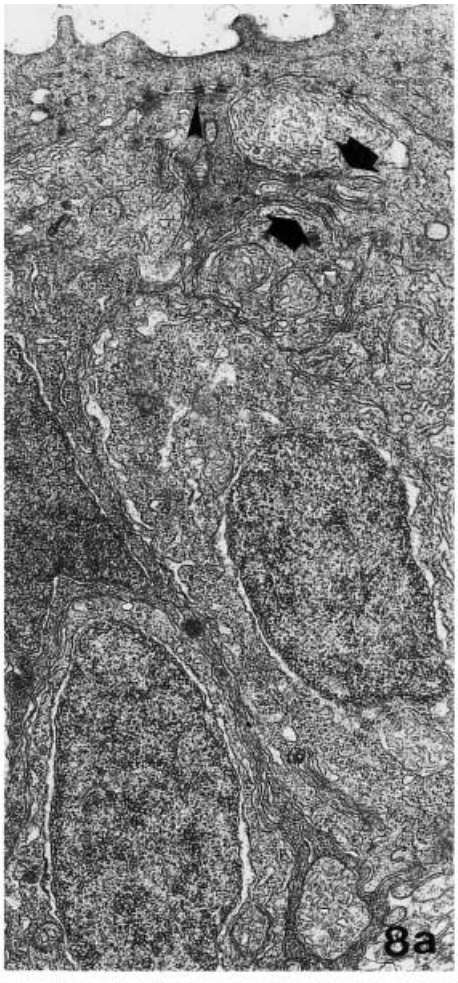
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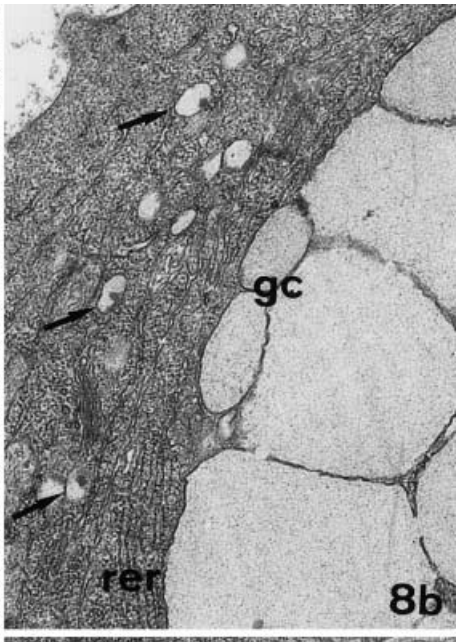
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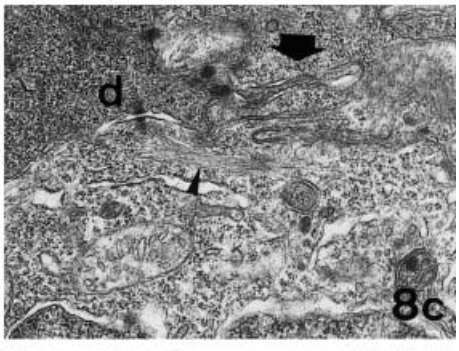
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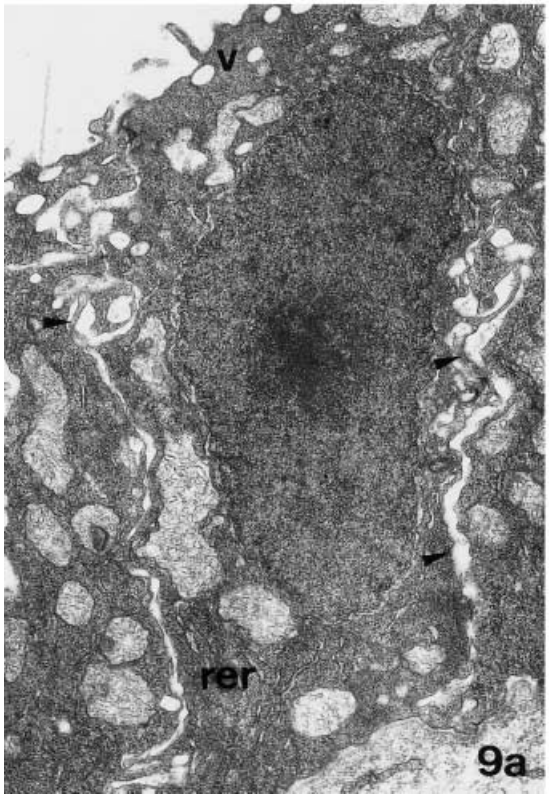
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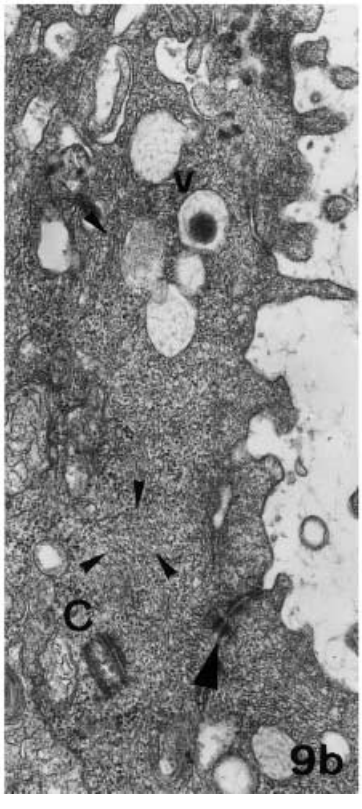
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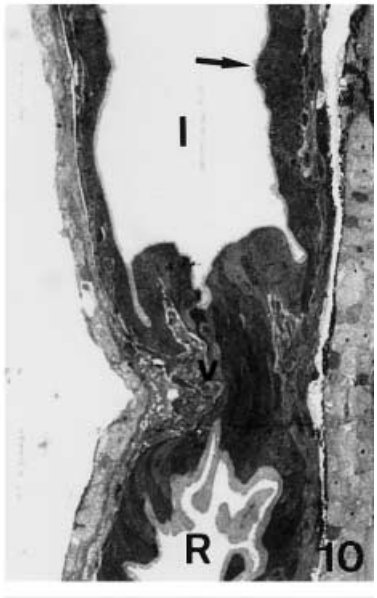
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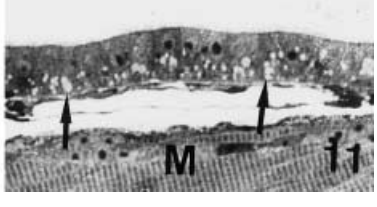
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some small microfilament bundles near the lateral cell membranes were also observed.

The medium region of the gut was very short and lined by epithelial cells that showed scarce irregular microvilli (Fig. 4). These epithelial cells were higher and had more developed junctional complexes and microfilament bundles than those in the anterior region. Small presumed primary lysosomes were found near the Golgi complexes and in the apical zone, where clear vesicles and occasional small multivesicular bodies and cytolysosomes were observed. The basal bodies were frequent (Fig. 4) and intercellular or apical cilia could be observed.

The posterior gut zone had high columnar epithelial cells with a developed striated border of long microvilli (Fig. 5a). These cells showed bead-like or tubular vesicles with a medium or high electron-dense content that were grouped (Fig. 5b) and often close to the Golgi cisternae, and lamellar structures consisting of long membranes parallel to the lateral cell membranes in association with filamentous mitochondria (Fig. 5c). Isolated or grouped dense lipid particles surrounded by membrane or inside the Golgi cisternae (Fig. 5d) and small groups of lipid droplets of varying size in the basal cell region (Fig. 5e) were seen.

The most caudal zone of the posterior gut region had a pseudostratified epithelium consisting of clear and dark cells that bordered an irregular lumen and showed cilia and ultrastructural features similar to those seen in the medium gut region (Fig. 6). Dark cells had numerous free ribosomes (Fig. 6).

◀ **Fig. 7** Frontal section of a 12-day-old larva showing the esophagus with goblet cells (*E*), the gastric region (*S*) and the initial part of the intestine (*I*), which is indicated by the junction with the pancreatic and biliary ducts (*arrow*). Note that the epithelium of the gastric region is higher in the caudal region (*arrowheads*; *L* liver). Toluidine blue. ×480

Fig. 8a–c Epithelium of the esophagus of a 12-day-old larva. **a** Electron-dense cells with lateral interdigitations (*large arrows*), junction complexes (*large arrowhead*) and short microvilli. **b** Detail of the apical region showing an electron-dense cell with small, clear vacuoles (*small arrows*) with or without an osmiophilic core and a goblet cell (*gc*). **c** Detail of a clear cell and an electron-dense one joined by a desmosome (*d*) and interdigitations (*small arrowhead* microfilaments, *rer* rough endoplasmic reticulum). **a, c** ×9,000; **b** ×12,500

Fig. 9a, b Epithelial cells of the gastric region of a 9-day-old larva. **a** Cells with short irregular microvilli, tortuous and dilated intercellular spaces (*small arrowheads*), long basal rough endoplasmic reticulum cisternae (*rer*), numerous free ribosomes, small apical vacuoles (*v*), and dense homogeneous nucleus with a large nucleole. Note the large, clear mitochondria. **b** Detail of the apical region with small vacuoles (*v*), some with an osmiophilic core, and junction complexes (*large arrowhead*; *C* centriole, *small arrowheads* microfilaments). **a** ×7,500; **b** ×14,000

Fig. 10 Sagittal section of a 12-day-old larva with the valvular structure (*v*) separating the intestine (*I*) with the epithelium thickened (*arrow*) and the rectum (*R*). Note the developed striated border of the epithelial cells, the rectal ones in particular. Toluidine blue, ×320

Fig. 11 Intestinal epithelium of a 12-day-old larva with basal lipid vacuoles (*arrows*; *M* striated muscle cells). Toluidine blue, ×800

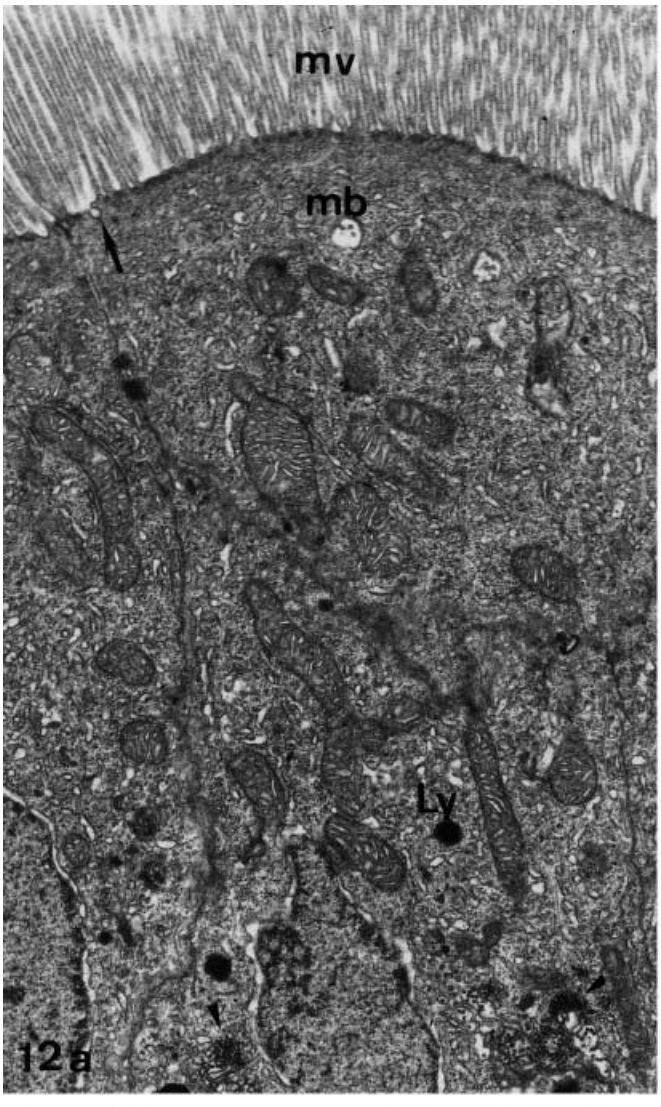
Epithelial cells in different mitotic phases were seen through the whole gut.

Phase II (from 6–7- to 13–15-day-old larvae, 5–8 mm length)

A small yolk sac, which progressively decreased in size and disappeared at the end of this phase, was observed. From this phase onwards, esophagus, gastric region, intestine (Fig. 7), and rectum were distinguishable.

The epithelium of the esophagus showed long longitudinal folds. A loose connective tissue made up the fold axis and the thin layer that surrounded the epithelium. An external circular layer of striated muscle cells completed the esophagus wall. Cubic cells gradually appeared in the epithelium, which became stratified. The lumen was lined by cells with short regular microvilli (Fig. 8a). Three cell types were distinguished: (1) Electron-dense cells (Fig. 8a) whose lateral membranes showed some interdigitations, as well as junction complexes in a short, apical zone. These cells had a homogeneously granular dense nucleus with one or two nucleoli, numerous free ribosomes and some microfilaments. The mitochondria, with a clear matrix and irregular crests, were pleomorphic. The rer formed long cisternae parallel to the plasma membrane in the lateral zones, and short cisternae scattered among the mitochondria. Various dictyosomes were juxtanuclear. Small clear vacuoles with an occasional osmiophilic content were near the cell surface (Fig. 8b). Some small lysosomes and centrioles were also observed. (2) Clear cells were spread through the epithelium, some reaching the lumen. They were joined to the adjacent electron-dense cells by desmosomes (Fig. 8c). These cells had a euchromatinic nucleus, scattered, occasionally dilated cisternae of rer, a developed Golgi complex, some microfilament boundles and fewer ribosomes than the dark cells (Fig. 8c). (3) Goblet cells appeared among the dark and clear cells (Fig. 8b). The immature goblet cells, which had few mucus granules, were irregularly shaped and located in the basal zone of the epithelium. They had a well-developed Golgi complex and rer cisternae. As they moved up to the apical region of the epithelium, the cytoplasm was progressively occupied by low or medium electron- dense mucus secretory granules. The mature goblet cells had short apical microvilli, basal nuclei and the cytoplasm was completely occupied by mucus granules. Goblet cells contained acid mucosubstances, including sulphomucins, but not neutral mucosubstances.

The esophagus was continuous with a dilated gastric region, which had longitudinal folds (Fig. 7) and an undulated lumen. This gastric region consisted of a cephalic zone lined by a short-columnar epithelium and a caudal zone, with a central constriction and a ventral flexion, lined by a high-columnar epithelium (Fig. 7). The layer of connective tissue and some circular bundles of smooth muscle cells surrounding the epithelium were thickened at the level of the constriction. The epithelial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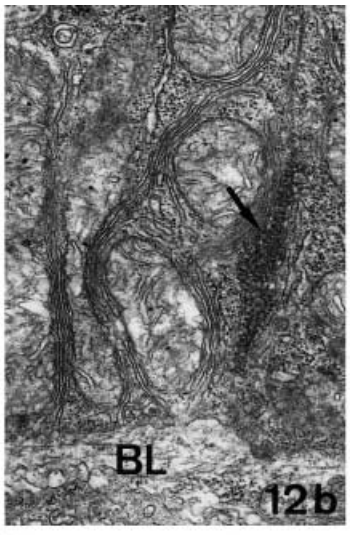
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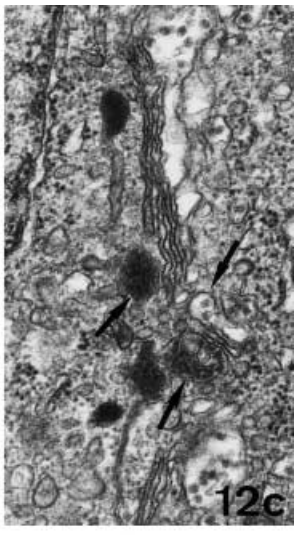
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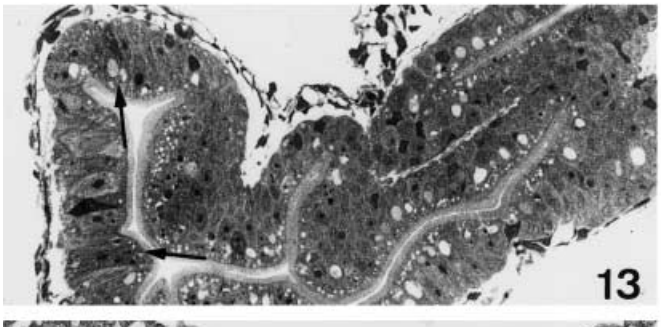
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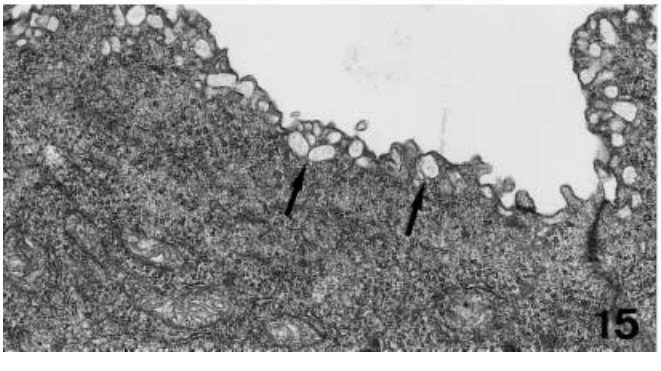
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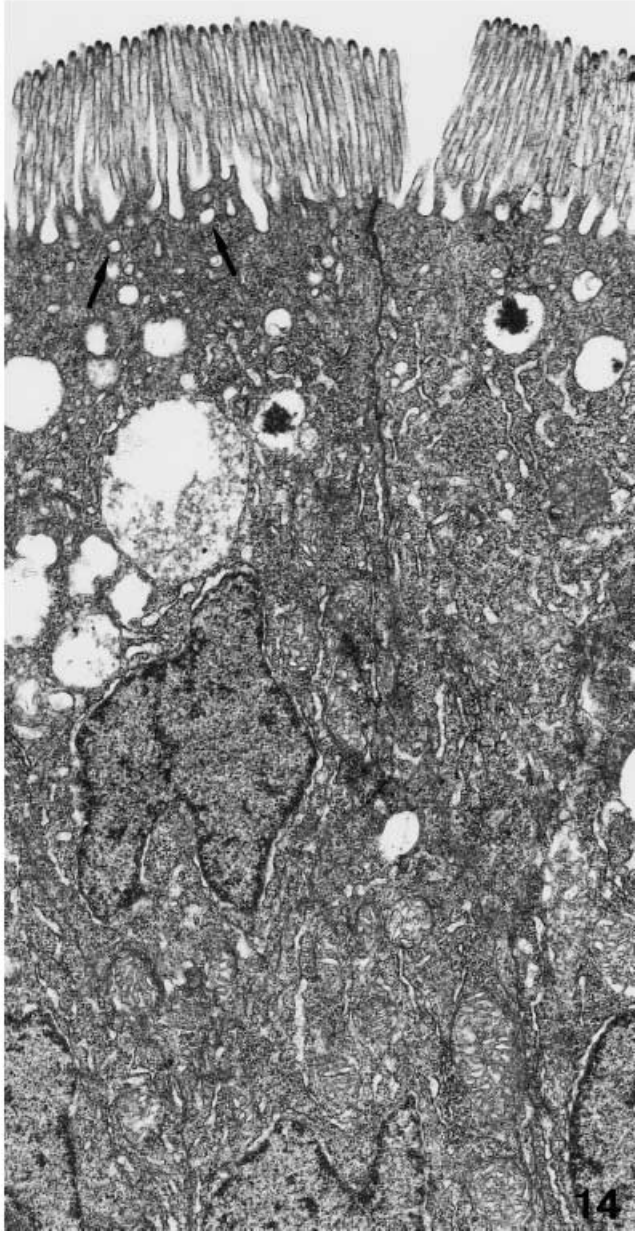
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cells of the gastric region had ultrastructural features similar to those of esophagic dark cells, but they had scarce short irregular microvilli (Fig. 9a). Their lateral membranes were very interdigitated and showed tortuous dilated intercellular spaces, conspicuous junctional complexes in a short, apical zone, and some aligned desmosomes (Fig. 9b). The microfilament bundles were abundant, especially in the apical and lateral areas (Fig. 9b). The numerous pleomorphic mitochondria had a clear matrix and irregular crests (Fig. 9a). Small clear vacuoles with an occasional osmiophilic content were also present in these cells (Fig. 9).

The intestine was the longest and widest region of the gut, its onset being determined by the sudden variation of the epithelium from that described above to an unfolded simple columnar epithelium and the junction with the biliar and pancreatic ducts (Fig. 7). The epithelium was thickened at several points, resulting in an undulating surface (Fig. 10). The epithelial cells had a well-developed striated border and a central nucleus with a large nucleolus (Figs. 10, 11). The basal region of these cells in some larvae showed numerous lipid vacuoles of varying size, especially in the caudal region (Fig. 11). The epithelial cells showed numerous long, regular microvilli (Fig. 12a) and free ribosomes, and large pleomorphic mitochondria that, in the basal region, were associated to lamellar structures (Fig. 12b). Dispersed long cisternae of rer, lysosomes of varying size, small multivesicular bodies and occasional small apical endocytotic vesicles were found. Small dense lipid particles appeared in groups and enclosed by a membrane (Fig. 12a), inside Golgi saccules and vesicles (Fig. 12c) and in the basal intercellular spaces (Fig. 12b).

The intestine was separated from the rectum by a valve formed of connective tissue surrounded by a columnar epithelium (Fig. 10). The rectum consisted of a simple columnar epithelium and two layers of squamous cells. At the beginning of this phase, the epihelium showed points of thickening. These progressively became mucosa folds with an axis of loose connective tissue. The epithelial cells were very high, with a well-developed striated border (Fig. 13) of long microvilli (Fig. 14), a basal nucleus with one or two nucleoli, dispersed cisternae of rer, (Fig. 14), some lysosomes, and sporadic cilia. The epithelial cells had small endocytotic vesicles and some microfilaments in the apical region. Large supranuclear vacuoles, increasing in size towards the nucleus, were seen; some of them contained a material that was clear and finely granular or dark and amorphous after staining with toluidine blue (Fig. 13). This material was scarce and osmiophilic or loose and moderately electron dense (Fig. 14). The lamellar structures were less developed than those in the intestine. Lipid droplets were observed in some larvae.

In the epithelial cells of the rectum, the microvilli, the endocytotic vesicles and the supranuclear vacuoles diminished in size and number towards the last zone of the gut, the preanal region (Fig. 15). This zone showed a pseudostratified epithelium with scarce or no microvilli, some apical small clear vacuoles, similar to mucus granules (Fig. 15), and fewer mitochondria and cisternae of rer than the cells that lined the rest of the rectum.

Although mitotic cells were seen throughout the gut, they were more frequent in the rectum.

Phase III (from 13–15- to 55-day-old larvae, 8–21 mm length)

No considerable variations were observed in the gut during the first 10 days of this stage, although a quite differentiated aspect was seen at the end of this phase. In the esophagus, the mucosa folds became deep, the surrounding layer of connective tissue thickened, and the number of goblet cells increased in comparison with the previous stage. No significant changes were observed at the ultrastructural level, except for the appearance of clear cells that were characterized by a developed Golgi complex, numerous tubules and vesicles and large mitochondria of clear matrix and tubular crests (Fig. 16).

The gastric region consisted of a long anterior and short posterior zone with longitudinal and transversal mucosa folds, respectively, that were separated by the constriction that appeared during the previous phase (Fig. 17a). A second constriction that affected only the dorsal wall of the gut, determined the limits between the gastric and intestinal regions (Fig. 17b). During this phase, the mucosa folds increased in length and number in the anterior gastric region, while the caudal gastric region grew gradually. The underlying connective tissue layer, more developed than in the previous phase, was surrounded by a circular layer of striated muscle cells, which was conntinuous with that of the esophagus. Smooth muscle fibres replaced the striated ones at the level of the first constriction. In the posterior gastric region, the underlying connective tissue and the layer of smooth muscle cells were more developed at the two constrictions.

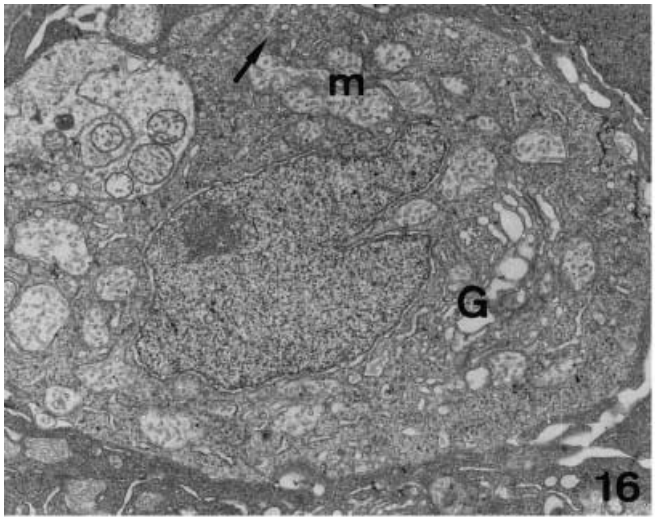
The epithelial cells of both gastric regions contained neutral mucosubstances and showed the same ultrastructural features. These cells were columnar and had scarce,

◀ **Fig. 12a–c** Intestinal epithelial cells of the intestine of a 12-day-old larva. **a** Cells with long microvilli (*mv*), dispersed cisternae of rer, scarce endocytotic vesicles (*arrow*), multivesicular bodies (*mb*) and small lysosomes (*Ly*). Note the grouped dense particles enclosed by a membrane (*arrowheads*). **b** Detail of the basal region of a cell with lamellar structures, mitochondria, and small dense lipid particles grouped in an intercellular space (*arrow*). **c** Detail of a cell showing small dense lipid particles surrounded by a membrane close to and inside Golgi cisternae (*arrows*; *BL* basal lamina). **a** ×7,500; **b** ×15,000; **c** ×23,000

Fig. 13 Rectum of a 12-day-old larva with a developed striated border and supranuclear vacuoles (*arrows*). Toluidine blue, ×640

Fig. 14 Epithelial cells of the rectum of a 9-day-old larva with microvilli and endocytotic vesicles (*arrows*). Note the supranuclear vacuoles of varying size and content. ×7,500

Fig. 15 Detail of the apical region of the epithelial cells of the preanal zone of a 9-day-old larva with small clear vacuoles similar to mucus granules (*arrows*). ×8,750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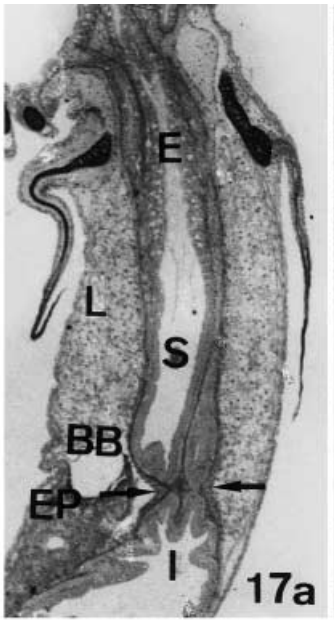
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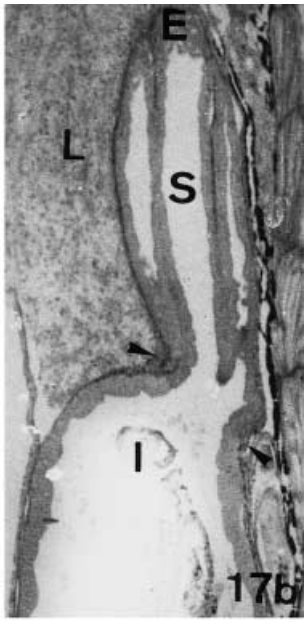
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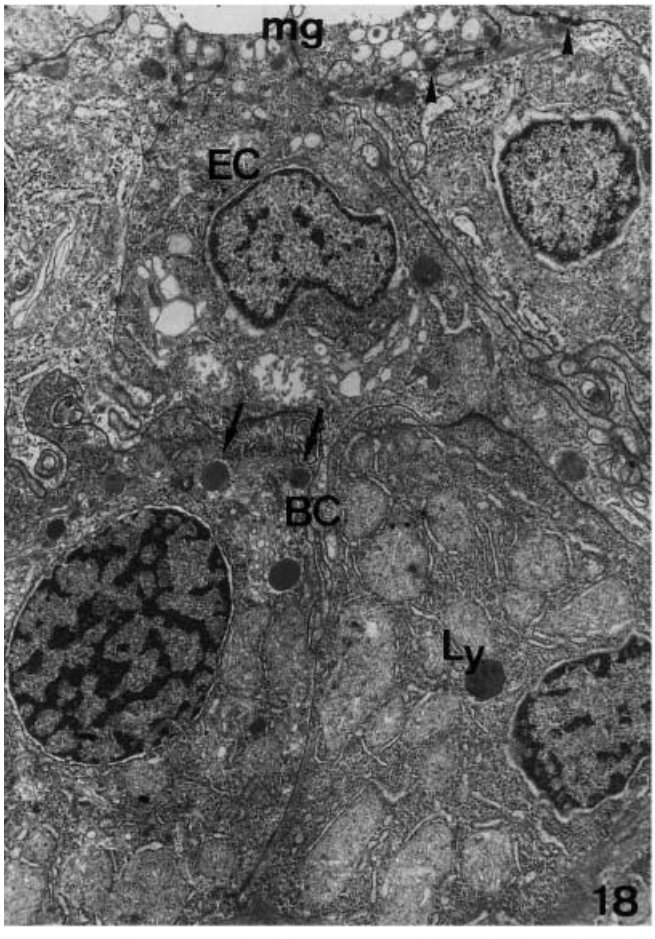
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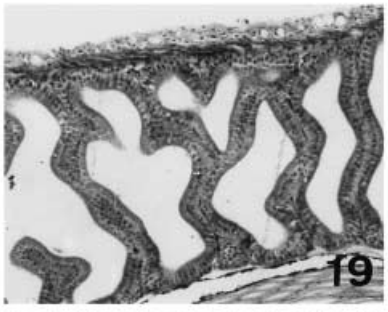
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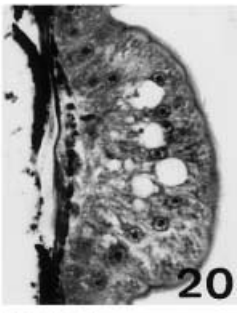
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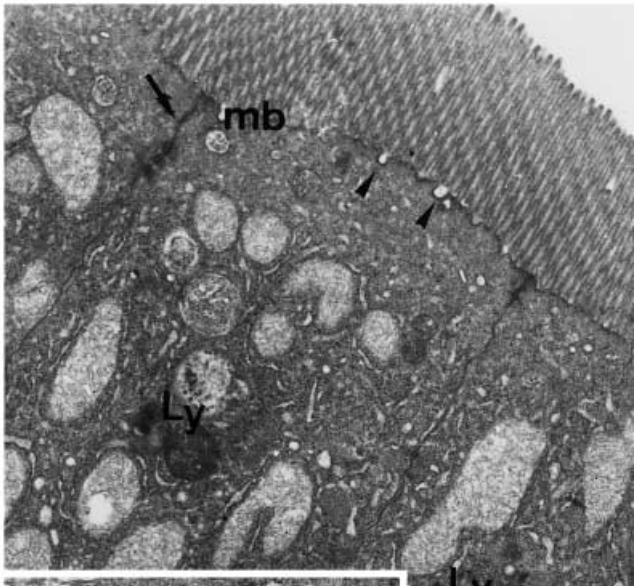
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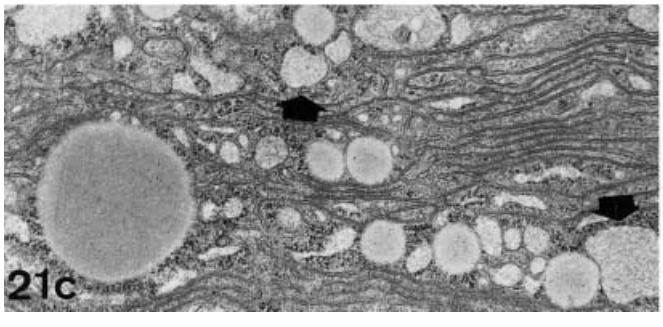
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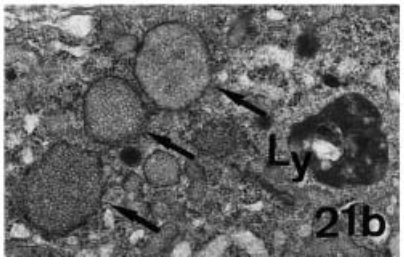
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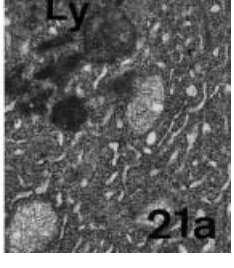
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short microvilli, interdigitations in the lateral membranes, and junctional complexes that were more patent than in the previous phase (Fig. 18). The dense basal nucleus was homogeneous, with a large nucleolus. The dense cytoplasm contained numerous free ribosomes and microfilament bundles. The supranuclear area showed many polymorphic mitochondria with clear matrix and irregular crests, and cisternae of rer that were often dilated. Numerous small lysosomes with a homogeneous content were grouped in the medium region. Small apical clear mucus granules were also seen (Fig. 18). In 39-day-old larvae, small, triangular or ovoid grouped cells were sometimes seen in the basal region of the epithelium. They had numerous free ribosomes, large mitochondria, dispersed long cisternae of rer, some round granules with a medium electron-dense homogeneous content, similar to the zymogen granules, and lysosomes. Some of these cells showed dense cromatin strands, suggesting a mitotic stage (Fig. 18).

In the intestine, the underlying connective tissue entered the thickened points of the epithelium seen in the previous phase to form true mucosa folds, which grew in size and ramified during this phase (Fig. 19). In some larvae, large lipid droplets were found in the epithelial cells, especially in the caudal zone of the intestine (Fig. 20). The epithelial cells had a terminal web, numerous ribosomes, some endocytotic vesicles and scarce multivesicular bodies among the microfilaments (Fig. 21a).

◀ **Fig. 16** Clear cell with large mitochondria of clear matrix and tubular crests (*m*), some tubules and vesicles (*arrow*) and a well-developed Golgi complex (*G*) in the esophagus of a 40-day-old larva. ×6,000

Fig. 17 Frontal (**a**) and sagittal (**b**) sections of a 25-day-old larvae showing the esophagus (*E*), gastric region (*S*) and intestine (*I*). Note the long longitudinal esophageal folds that are continuous with those of the gastric region and the constrictions separating the anterior and posterior gastric regions (**a**, *arrows*), and the gastric region and the intestine (**b**, *arrowheads*), respectively (*L* liver, *BB* goll bladder, *EP* exocrine pancreas). Hematoxilin-eosin. **a** ×200; **b** ×300

Fig. 18 Gastric region of a 39-day-old larva showing lining epithelial cells (*EC*) with mucus-secretory granules (*mg*) and basal triangular cells (*BC*) showing numerous mitochondria, rer, and zymogen-like granules (*arrows*). Note the nucleus with condensed chromatin strands (*Ly* lysosome, arrowheads junction complexes). ×7,000

Fig. 19 Longitudinal section of a 40-day-old larva showing the mucosa folds of the intestine. Haematoxilin-eosin. ×300

Fig. 20 Detail of the intestine of a 25-day-old larva with large lipid vacuoles. Mallory's trichrome. ×640

Fig. 21a–c Intestinal epithelial cells of a 40-day-old larva. **a** Cells with numerous clear mitochondria, dispersed cisternae of rough endoplasmic reticulum and grouped lysosomes (*Ly*). **b** Detail of the supranuclear cytoplasm showing lipid inclusions consisting of small clear particles enclosed by membrane (*arrows*) and a large secondary lysosome. **c** Detail of the basal cytoplasm showing well-developed lamellar structures, varying sized lipid droplets and dilated cisternae of endoplasmic reticulum with a granular, presumable lipidic content (*large arrows*; *arrowheads* endocytotic vesicles, *mb* multivesicular bodies, *small arrow* junction complex). **a** ×7,500; **b** ×10,000; **c** ×15,000

The rer was more developed than in previous stages (Fig. 21a). Small presumably primary lysosomes were seen next to the Golgi complex. Large secondary lysosomes and multivesicular bodies were located more apically (Fig. 21a). In some larvae, the epithelial cells contained grouped, small lipid particles enclosed in a membrane (Fig. 21b). In the basal region, the lamellar structures associated with mitochondria were more developed than in the previous phase (Fig. 21c). Basal lipid droplets of varying size and electron density were found (Fig. 21c). Occasionally, lipid droplets were very large and numerous and occupied almost the whole cytoplasm; the endoplasmic reticulum then appeared dilated and with a granular, low electron-dense material of presumably lipid nature (Fig. 21c). Goblet cells appeared on about the 25th day, their number increasing as the larvae aged. The ultrastructure of these cells was similar to those in the esophagus but they contained neutral mucosubstances.

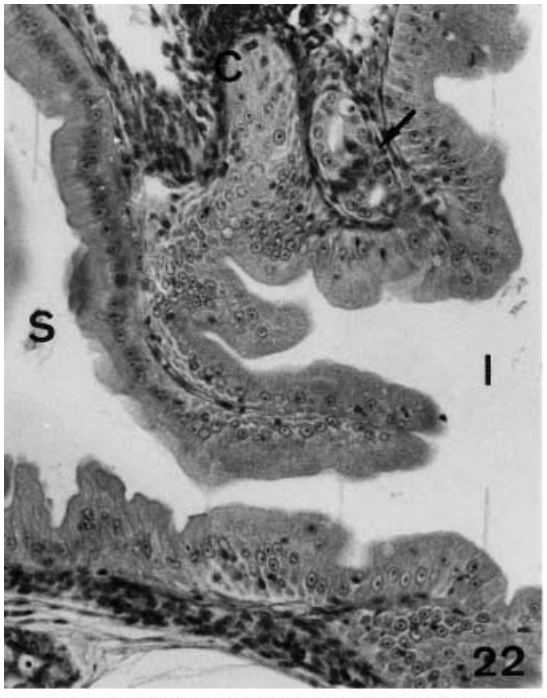
At the junction of the stomach and the intestine, the epithelium thickened at the beginning of this phase and progressively formed continuous folds that divided the lumen. The connective tissue and the muscle layer entered the folds, giving rise to evaginations which resulted in the pyloric caeca (Fig. 22). On the 46th day, two or three pyloric caeca were seen. These extended towards the stomach, their lumen diminishing progressively. They had a simple columnar epithelium, which was lightly folded in the initial zone and undulated in the distal zone. They showed numerous mitosis. The epithelial cells were similar to those of the intestine.

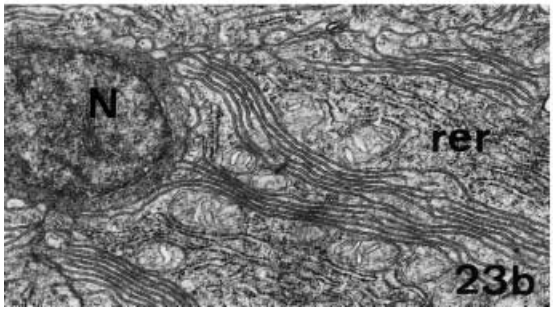
In the rectum, the length of the mucosa folds and number and size of the supranuclear vacuoles increased in comparison with those in the previous stage; the epithelial cells had more numerous endocytotic vesicles than in previous stages, coated vesicles, and numerous microfilament bundles. Some supranuclear vacuoles contacted or fused with each other or with primary lysosomes of varying size, which were numerous nearby. Large vacuoles with a diffuse content and enormous secondary lysosomes were seen near the nucleus (Fig. 23a). The lamellar structures were more developed than in the previous phase (Fig. 23b). Lipid droplets were occasionally found. Goblet cells appeared in this region 25 days after hatching and increased in number during this phase. They contained neutral mucosubstances.

Phase IV (from 55-day-old larvae onwards, 21 mm length onwards)

During this phase the development of the gut was completed with the differentiation of the stomach, which reached its definitive morphology and structure, and the formation of two intestinal loops.

The constriction that partially separated the gastric region from the intestine in the previous phase extended to the whole gut wall and became more apparent. Moreover, the anterior gastric region developed caudally and





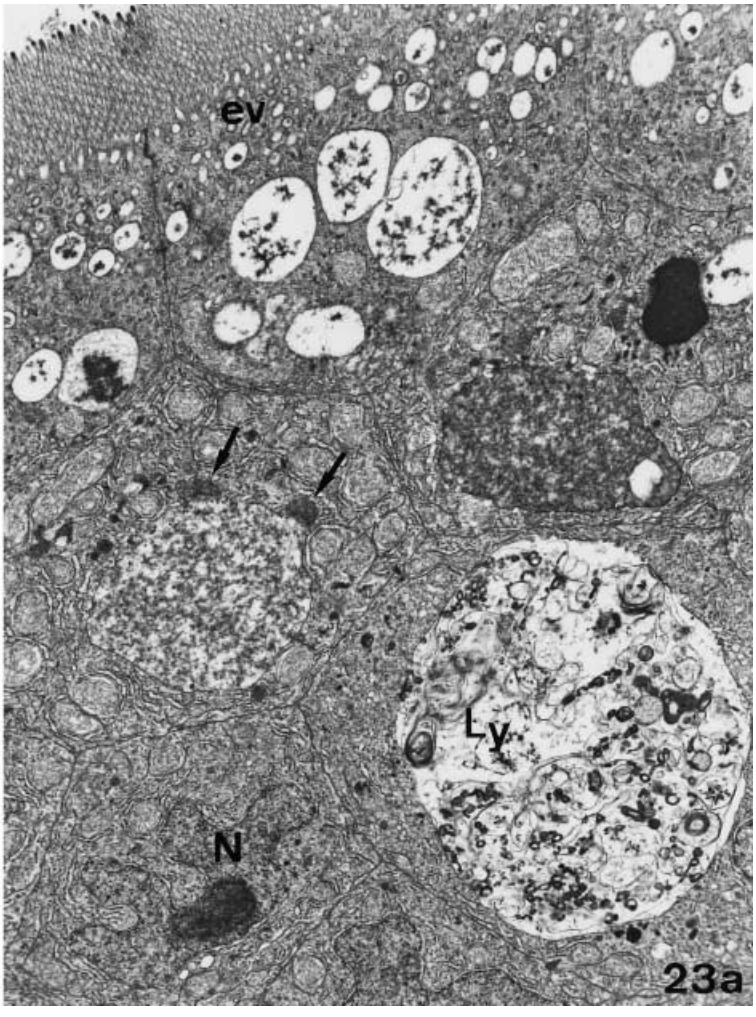


Fig. 22 Longitudinal section of a 46-day-old larva showing the gastric region (*S*) and the intestine (*I*) junction. Note the developing pyloric caecum (*C*) and the bile and pancreatic ducts (*arrow*). ×640

Fig. 23a, b Epithelial cells of the rectum of a 25-day-old larva. **a** Cells with numerous endocytotic vesicles (*ev*), supranuclear vacuoles of varying size and content, large secondary lysosome (*Ly*) and small lysosomes fusing with some vacuoles (*arrows*). **b** Detail of the basal region with long lamellar structures (*N* nucleus, *rer* rough endoplasmic reticulum). **a** ×5,000; **b** ×6,000

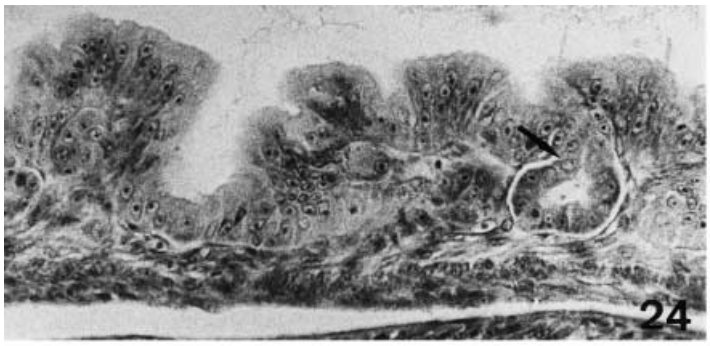
surpassed the posterior one, producing a blind sac, dorsal to the intestine. The lining epithelium of the anterior region folded transversally and formed the gastric pits, where the first gastric glands, which were seen at about the 55th day, opened (Fig. 24). These simple tubular glands proliferated fast and invaded the underlying connective tissue, a thick lamina propria or corium becoming apparent. The short cephalic zone of the anterior region was lined by short epithelial cells and some goblet cells, and had a thin corium without gastric glands and short, longitudinal mucosa folds, which were continuous with those in the esophagus. In the posterior gastric region, no glands were differentiated, the folds being deeper and the epithelial cells higher than in the previous

Fig. 24 Detail of the stomach of a 60-day-old larva. Note the gastric glands opening at the gastric pits (*arrow*). Haematoxilin-eosin. ×640 ▶

Fig. 25a, b Lining epithelial cells of the stomach of a 60-day-old larva. **a** Cells with lateral membrane interdigitations and microfilament bundles (*arrows*). Note the clear mucus granules with a medium electron-dense core in the apical zone. **b** Detail of the apical zone with grouped medium electron-dense mucus granules and very conspicuous junction complexes and microfilament boundles (*Ly* lysosome). **a** ×7,500; **b** ×5,000

Fig. 26 Detail of a gastric gland with a narrow lumen, the cells show large mitochondria (*m*), and numerous clear vesicles and microvilli in the apical region in a 55-day-old larva. Note the nuclei with dense chromatin strands and a large nucleoli (*d* Golgi complex). ×6,000

Fig. 27a, b Detail of a gastric gland with a wide and irregular lumen of a 61-day-old larva. **a** Cells showing mitochondria with a dense matrix, dispersed cisternae of rer, and tubules and clear vesicles and zimogen granules (*zg*) in the apical region. Note the irregular cell surface with cell processes and the clear nucleus. **b** Detail of the apical zone of glandular cells with coated pits and vesicles (*arrowheads*), numerous tubules and vesicles and mitochondria with tubular crests. **a** ×8,750; **b** ×12,500



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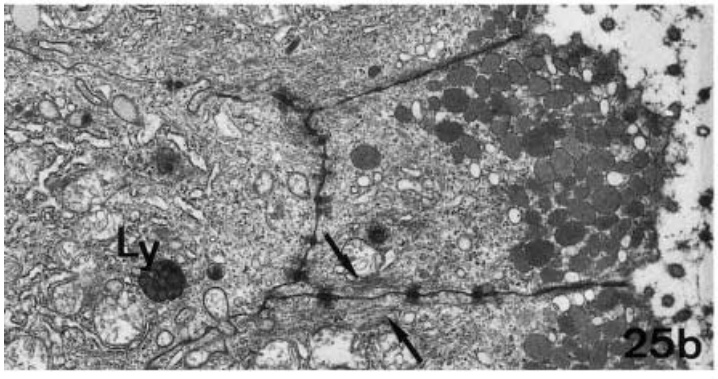

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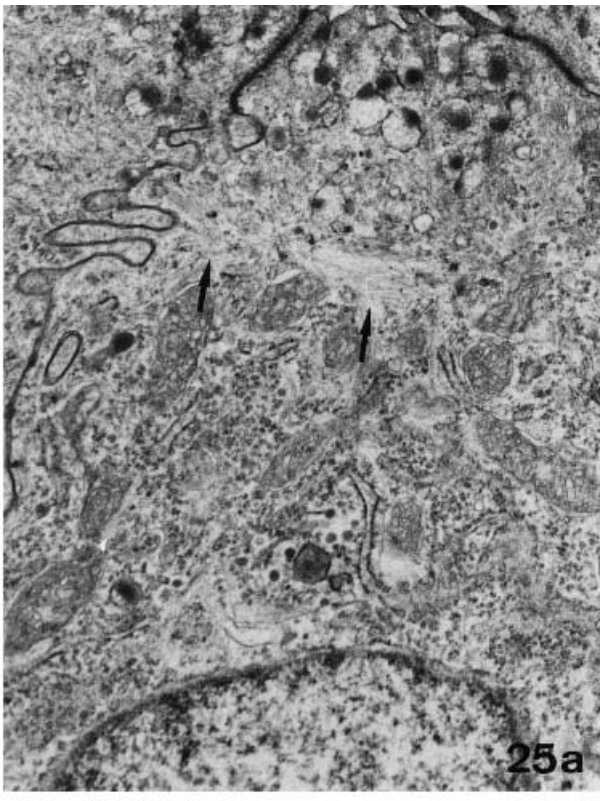

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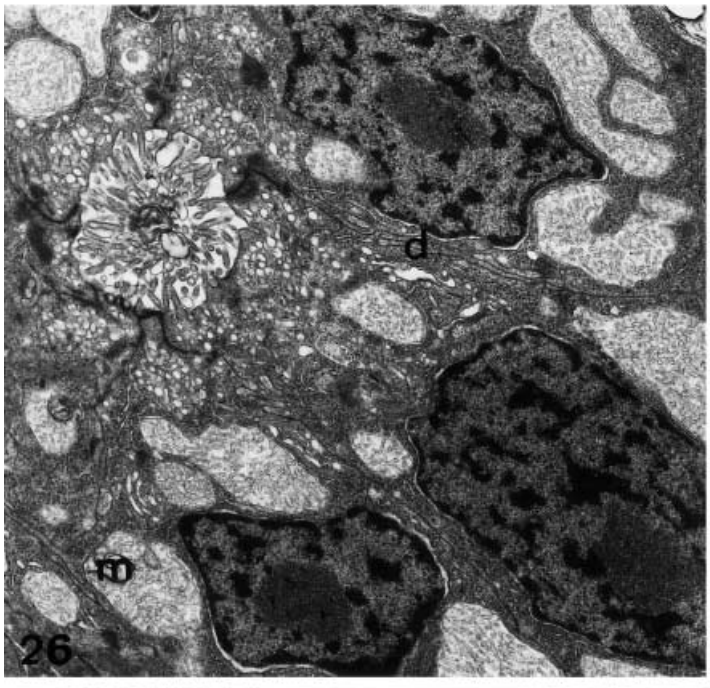

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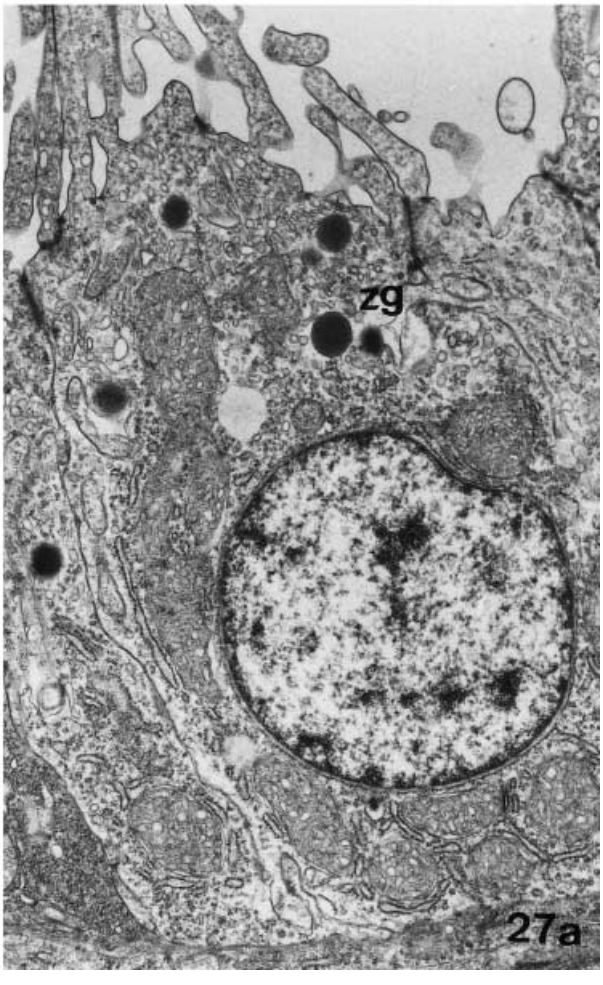

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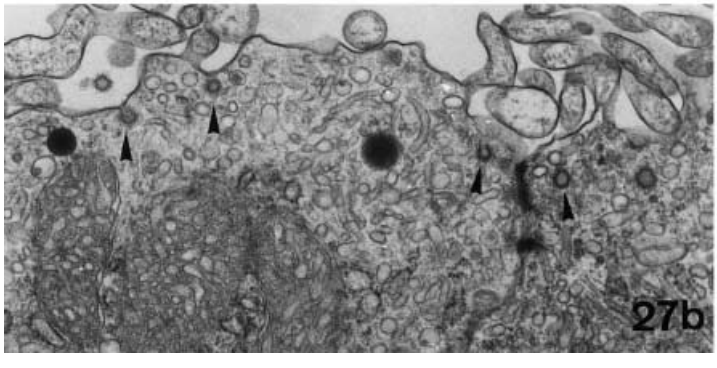

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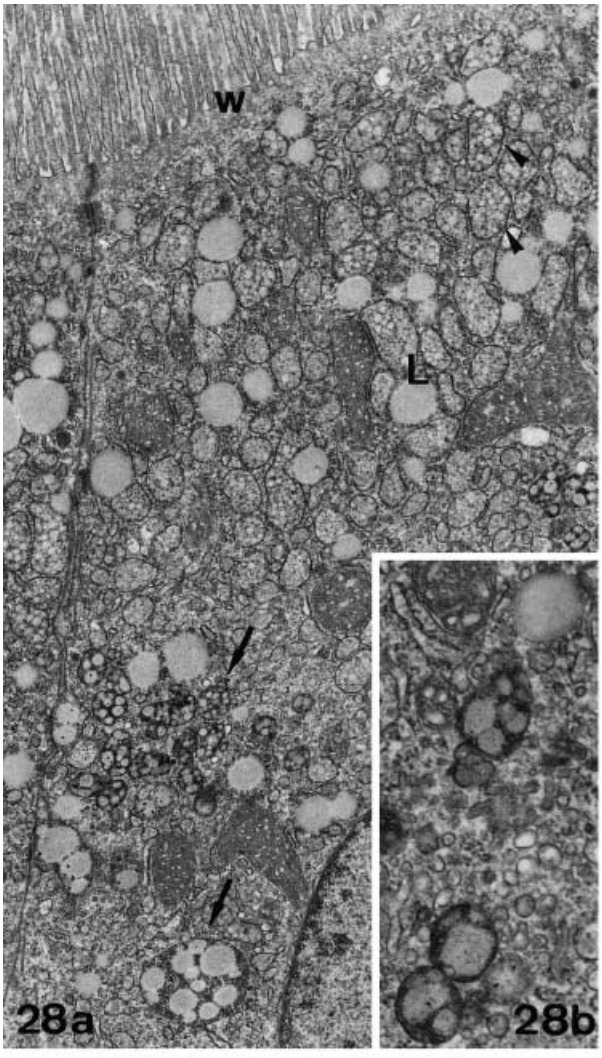

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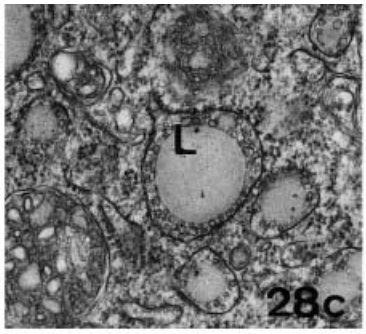

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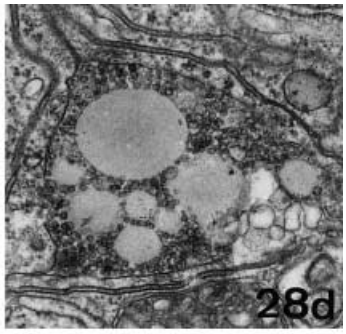

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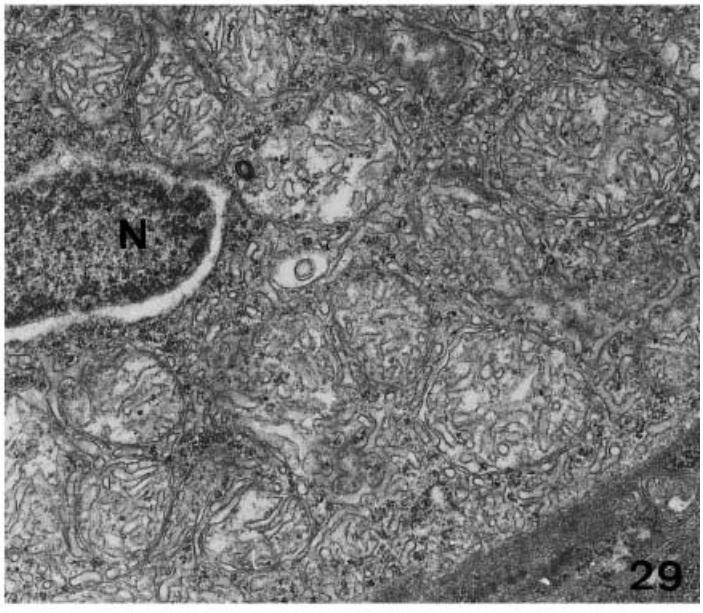

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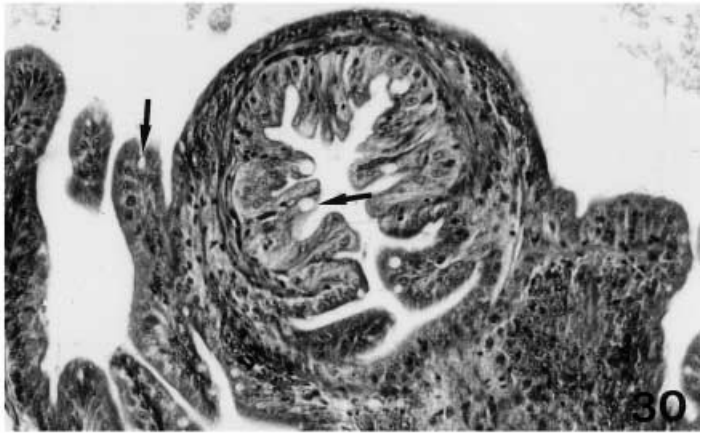

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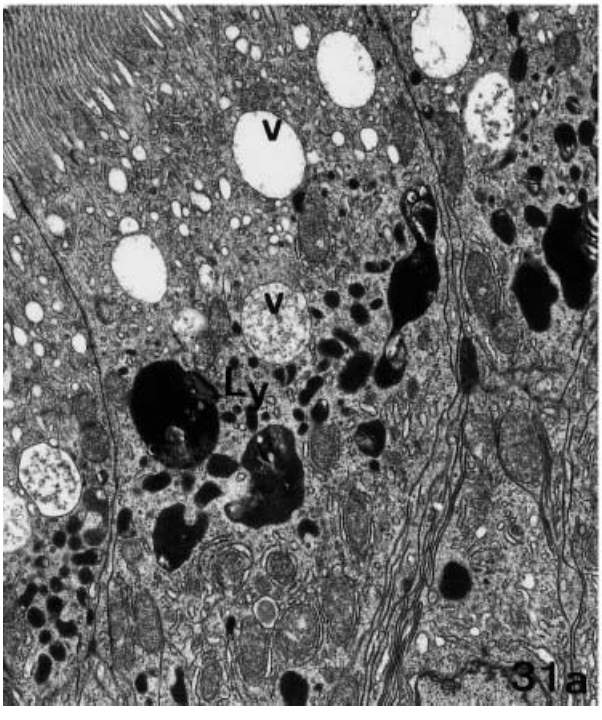

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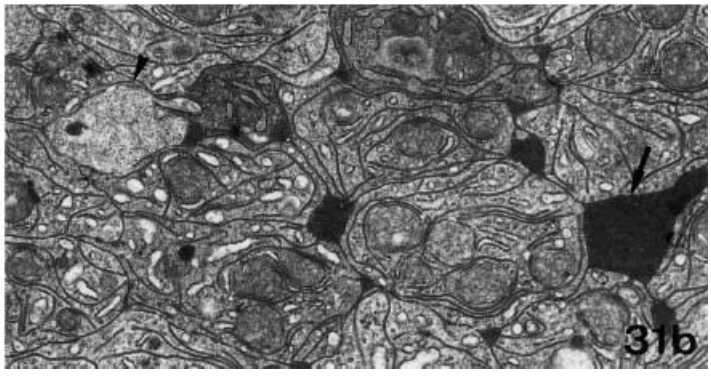

31b

stage. The surrounding connective tissue and muscular layers became very thick. The completely differentiated stomach consisted of a glandular, descending branch, and a nonglandular ascendant or pyloric one, thus corresponding to the caecal type (Grassé 1958). The surrounding muscular layer consisted of striated muscle cells in the first part of the descending branch up to the beginning of the blind sac. The rest of the stomach, like the intestine and the rectum, was surrounded by smooth muscle cells.

Compared with in previous stages, the gastric epithelial cells were higher and had more developed junction complexes and lateral membrane interdigitations (Fig. 25a); the microfilaments were more numerous and tended to organize in the cell periphery or delimited zones with grouped mitochondria and ribosomes; the mitochondria were smaller and had a dense matrix (Fig. 25a). Cisternae of rer, dictyosomes and some homogeneous lysosomes were observed in the perinuclear cytoplasm. The mucus granules were spread throughout the supranuclear region and gathered in the apical zone. They were low or medium electron-dense (Fig. 25b) or clear with dense areas (Fig. 25a). Lipid doplets were rarely observed. The gastric glands were formed by polyhedric cells lining a lumen of variable width. At ultrastructural level, two types of glands were distinguished. The undifferentiated glands found in some 55-day-old larvae had a narrow lumen limited by cells that showed apical irregular microvilli, and a central nucleus with clumps of heterochromatin and a central or eccentric nucleolus (Fig. 26). The cytoplasm had numerous free ribosomes and was very dense except in the apical region, where numerous small clear vesicles were accumulated (Fig. 26). Large, polymorphic mitochondria with clear matrix and tubular crests, and scarce cisternae of rer were observed in the supranuclear region. Small, lateral dictyosomes also appeared (Fig. 26). The differentiated glands observed in some 55- and in 60-day-old larvae had a wide lumen limited by cubic cells with large, irregular apical cell processes. These cells showed numerous mitochondria with a matrix denser than that in the glandular cells described above, rer cisternae in the basal region and osmiophilic granules, similar to zymogen granules, in the supranuclear and apical regions (Fig. 27a). In the latter zone, a complex system of tubules and vesicles was observed (Fig. 27b).

The intestine was folded to form two intestinal loops. The epithelial folds increased in length in the anterior intestinal zone. The goblet cells were more numerous than in the previous phase and increased in number towards the caudal zone. The epithelial cells showed a well-developed terminal web (Fig. 28a). The mitochondria were smaller and had a more electron-dense matrix than in the previous phase (Fig. 28a). In some larvae, lipid inclusions were very numerous (Fig. 28a); besides those seen in the previous phases, inclusions consisting of grouped large clear and small, osmiophilic or clear lipid particles enclosed in a membrane, in the hyaloplasm (Fig. 28b), inside the dilated endoplasmic reticulum (Fig. 28c) and in the intercellular spaces (Fig. 28d) were found. The four or five pyloric caeca increased in diameter, and their epithelium, in which the first goblet cells containing neutral mucosubstances appeared, folded progressively.

Morphological changes were minor in the esophagus and rectum. In the esophagus, the clear cells were more differentiated, showing numerous tubules and vesicles and large mitochondria (Fig. 29). In the rectum, besides the increased size of the mucosa folds and number of goblet cells, which also appeared in the epithelium lining the valve (Fig. 30), the occurrence of numerous primary and secondary lyososmes of variable size close to the vacuoles in the apical region (Fig. 31a) was of note, as were the well-developed lateral and basal lamellar structures, and the intercellular spaces that were very conspicuous and which often had an osmiophilic content (Fig. 31b). The underlying connective and muscular layers of the intestine and rectum were more developed than in the previous phase.

Tables 1 and 2 summarize the main morphological changes and the appearance of mucosubstances, respectively, during the different developmental stages.

◀ **Fig. 28a–d** Epithelial cells of the intestine of 60-day-old larvae. **a** Cells with numerous lipid inclusions consisting of lipid droplets (*L*), grouped small, osmiophilic and large, clear lipid particles surrounded by a membrane (*arrows*), and small clear lipid particles inside the endoplasmic reticulum (*arrowheads*). Note the terminal web (*w*) and the mitochondria with dense matrix. **b–d** Details of the cytoplasm showing a lipid droplet and grouped small, osmiophilic and large, clear lipid particles surrounded by a membrane (**b**), large (*L*) and small osmiophilic or clear lipid particles inside the endoplasmic reticulum (**c**) and in the intercellular spaces (**d**). **a** ×5,000; **b–d** ×16,000

Fig. 29 Clear cell of the esophagus of a 61-day-old larva showing many mitochondria with tubular crests and a complex system of tubules and vesicles (*N* nucleus). ×15,000

Fig. 30 Transverse section of the valvular structure separating the intestine and the rectum in a 60-day-old larva. Note the mucosa folds and the goblet cells (*arrows*). Haematoxilin-eosin. ×640

Fig. 31a, b Details of the supranuclear (**a**) and basal (**b**) regions of epithelial cells of the rectum of a 60-day-old larva showing microvilli, grouped dense lysosomes of varying size (*Ly*) near the vacuoles (*v*), and pinocytotic vesicles in the former (**a**), and numerous lamellar structures, and intercellular spaces with clear (*arrowhead*) or osmiophilic (*arrow*) material, in the latter (**b**). **a** ×5,000; **b** ×6,250

Discussion

Four significant phases have been established in the ontogeny of sea bass by reference to some of the morphological changes that take place in both pancreas and gut and in the first appearance of the different peptide-like immunoreactivities in the endocrine pancreas (García Hernández and Agulleiro 1992). These phases can also be applied to the development of the digestive tract, as showed by the present study. The onset of these phases coincides with important events in the development of the sea bass gut. Thus, hatching, opening of the

Table 1 Main morphological changes during the different developmental stages of sea bass

Phase	Onset (age)	Gut region	Morphological and functional features
I	At hatching		Straight undifferentiated gut Start of lipid absorption in the midgut
II	Opening of the mouth (5–9-day-old)	Esophagus	Appearance of mucosa folds and goblet cells
		Gastric region	
		Intestine	Increased lipid absorption
		Rectum	Start of protein absorption Appearance of mucosa folds
III	Total resorption of the yolk sac (13–15-day-old)	Esophagus	Appearance of "chloride" cells
		Gastric region	Identification of neutral mucosubstances in the lining epithelial cells
		Intestine	Appearance of two pyloric caeca and goblet cells
		Rectum	Enhanced protein absorption Appearance of goblet cells
IV	Appearance of the first gastric glands (55–61-day-old)	Esophagus	
		Stomach	Differentiation of caecal and pyloric regions in the stomach Differentiation of gastric glands
		Intestine	Intestinal loops
		Rectum	

Table 2 Mucus cells in the developing gut of sea bass (*Pz* presumptive zones, –absence of mucus cells, +presence of some mucus cells, ++numerous mucus cells, +++very numerous cells)

Developmental stage	Gut region	Neutral mucosubstances	Acid mucosubstances including sulphomucins
Phase I	Esophagus (Pz)	–	–
	Stomach (Pz)	–	–
	Intestine	–	–
	Rectum	–	–
Phase II	Esophagus	–	+
	Stomach (Pz)	–	–
	Intestine	–	–
	Rectum	–	–
Phase III	Esophagus	+	++
	Stomach	+	–
	Intestine	+	–
	Rectum	+	–
Phase IV	Esophagus	++	+++
	Stomach	++	–
	Intestine	++	–
	Rectum	++	–

mouth (and, therefore, the start of exogenous feeding) (6–7 days after hatching), the complete resorption of the yolk sac (13–15 days after hatching), and the appearance of the first gastric glands (55 days after hatching), initiate phases I, II, III, and IV, respectively. Although differently designated, these phases are comparable with the developing periods established for other teleosts (see Boulhic and Gabaudan 1992) and are in accordance with those described previously in sea bass (Tan Tue 1976). Although the start of the exogenous feeding agrees with a previous report (Deplano et al. 1991b), there are differences in the time when the events defining the phase onset occur. Thus, the start of the exogenous feeding has also been reported to occur 3–4 days after hatching (Tan Tue 1976) and the resorption of the yolk sac, 9–10 (Tan Tue 1976) and 20 (Deplano et al. 1991b) days after hatching, respectively, with the appearance of gastric glands being described 30 days after hatching (Tan Tue 1976). These differences probably reflect variations in the factors affecting larval development, such as egg size, incubation temperature, breeding conditions or genetic origin (Tan Tue 1976; Blaxter 1988).

A presumptive gut with no lumen, appearing 2 or 3 days after hatching (Rombout et al. 1978; Stroband et al. 1979) or a straight and undifferentiated rudimentary gut (O'Connell 1981; Govoni et al. 1986; Segner et al. 1994; Calzada et al. 1998; Ribeiro et al. 1999) have been

reported in newly hatched larvae of teleost fish that pass through a larval stage. In sea bass, an undifferentiated digestive tract lined by a simple epithelium, except in the most caudal region, where there was a pseudostratified epithelium, was found in newly hatched larvae and during phase I, a lecitotrophic or endogenous-feeding period. In a previous study, the whole sea bass gut was described as being lined by a simple cell layer for 2 days after hatching and by a stratified epithelium for several days preceding the opening of the mouth (Tan Tue 1976). An undifferentiated digestive tract has also been reported in some developing teleosts before the start of exogenous feeding (*Solea solea*, Boulhic and Gabaudan 1992; *Sparus aurata*, Sarasquete et al. 1995). However, in other species, such as *Scophthalmus maximus* (Segner et al. 1994) and *Solea senegalensis* (Ribeiro et al. 1999), the intestinal anatomy is already differentiated during the period from hatching to the start of the exogenous feeding.

Ciliated cells have been reported in the presumptive intestine, except in the caudal rectum (Calzada et al. 1998) and in the posterior gut region or in the rectum (Iwai 1967; Loewe and Eckmann 1988) during the early developmental stages of some fish larvae, disappearing in later stages. In sea bass, ciliated cells lined the caudal gut and were occasionally found in the rest of the gut during phase I. In a previous study on the developing gut of this species, ciliated cells were only observed in the rectum of 2-day-old larvae (Connes and Benhalima 1984). The caudal region with a ciliated pseudostratified epithelium described in the present study has not been reported previously.

The lamellar structures have been widely reported in the larva gut after the start of exogenous feeding, especially in the intestine (Iwai 1968; Albertini-Berhaut 1988) or the rectum (Stroband 1977; Stroband and Kroon 1981; Rombout et al. 1984). These features have been related to solute-linked water transport and are characteristic of cells involved in fluid transport (see De Ruiter et al. 1985). In sea bass larvae, the epithelial cells of the gut during phase I, despite appearing to be undifferentiated, already show the lateral or basal lamellar structures associated with mitochondria, except in the most anterior region. This suggests that the gut is involved in osmoregulation before the start of exogenous feeding, although it is in the rectum of the oldest larvae that these structures reach their greatest development.

Signs of lipid digestion were also found in the cells of the digestive tract of sea bass larvae during phase I. Both lipid droplets and dense particles, similar to those described as lipoproteins in embryos and adult specimens of *Salmo gairdneri* (Sire and Vernier 1979, 1981), were observed. These lipoproteins might correspond to very low density lipoproteins (VLDL) synthesized from endogenous lipids, as has been proposed for trout (Sire and Vernier 1979), since the epithelial cells of the intestine of the sea bass larvae seem to be capable of synthesizing and transporting small lipoprotein particles before the start of exogenous feeding (Deplano et al. 1991a), as found in sea bream (Calzada et al. 1998). In other species, such as turbot, no signs of lipid absorption are evident in the gut epithelial cells of pre-feeding larvae (Segner et al. 1994). The epithelial cells of the posterior gut region of sea bass larvae showed no signs of protein absoption before the start of exogenous feeding, which agrees with the suggestion that lipids rather than proteins are used as energy source during early development (Ostrowski and Divakaran 1991).

The opening of the mouth and therefore the start of exogenous feeding determines regional gut differentiation in most teleost species (Iwai 1968; O`Connell 1981; Rombout et al. 1978, 1984; Stroband et al. 1979; Stroband and Kroon 1981; Albertini-Berhaut 1987; Boulhic and Gabaudan 1992; Sarasquete et al. 1995). In sea bass, four distinct regions: esophagus, gastric region, intestine and rectum were found at the start of exogenous feeding (phase II). These results are in accordance with a previous study on this species (Tan Tue 1976), although two intestinal regions and a rectum, with the posterior intestine and the rectum being ultrastructurally identical, have also been described (Connes and Benhalima 1984). Some morphological variations observed during this phase, such as the appearance of goblet cells in the esophagus, a valvular structure indicating the beginning of the rectum, as well as mucosa folds in both the intestine and the rectum, are in agreement with previous reports (Tan Tue 1976). However, the caudal gut zone lined by cells with apical vesicles similar to mucus secretory granules has not been described in sea bass or in other teleost larvae, except in *Barbus conchonius* (Rombout et al. 1984). The appearance of an incipient valve separating the intestine from the rectum or posterior intestine has also been described in other teleost larvae shortly after exogenous feeding begins (Boulhic and Gabaudan 1992; Sarasquete et al. 1995; Luizi et al. 1999; Ribeiro et al. 1999).

The start of exogenous feeding also determines the morphofunctional differentiation of the gut epithelial cells. In most teleosts, the first intestinal segment seems to be involved in the absorption of lipids. Except in some species (Albertini-Berhaut 1988), the anterior region of the intestine of teleost larvae is characterized by lipid inclusions in the epithelial cells (Iwai 1968; Stroband 1977; Stroband and Kroon 1981; Rombout et al. 1984; Kjorsvik et al. 1991). Groups of small lipid particles surrounded by a membrane, and considered as lipoproteins or chylomicrons, have been found associated with the Golgi complex, inside the endoplasmic reticulum, in the hyaloplasm and/or in the intercellular spaces (Iwai 1968; Stroband 1977; Stroband and Kroon 1981; Rombout et al. 1984; Kjorsvik et al. 1991; Segner et al. 1994). Large lipid droplets consisting of triglycerides (Bergot 1981), have also been described in these cells (Stroband 1977; Stroband and Kroon 1981; Rombout et al. 1984; Kjorsvik et al. 1991; Boulhic and Gabaudan 1992; Sarasquete et al. 1995; Calzada et al. 1998; Ribeiro et al. 1999). Chylomicrons or VLDL are the form of exportation of absorbed fatty acids from fish enterocytes (Bergot

1981; Sheridan 1988), while lipid droplets seem to be a form of temporary storage of the re-esterified fatty acids that accumulate when fatty acid uptake exceeds the enterocyte exporting capacities (Bergot 1981; Sheridan 1988) or because an inability to metabolize lipids (Loewe and Eckmann 1988; Kjorsvik et al. 1991). In sea bass larvae, lipoproteins were abundant shortly after the start of exogenous feeding and were found not only inside the vesicles of the Golgi complex (as in the previous phase) but also grouped in the hyaloplasm, and, as has been described previously (Deplano et al. 1991a), in the intercellular spaces. Thus, the abundance of lipid droplets of varying size in the intestinal epithelial cells of these larvae does not seem to be the result of a default in the lipoprotein synthesis mechanism, as suggested by Deplano et al. (1991a), but a mechanism of energy storage, as proposed for other species (Watanabe and Sawada 1985). According to Deplano et al. (1991a) lipid droplets decrease as lipoprotein synthesis increases from the start of exogenous feeding to 25 days after hatching. In our study, although lipoprotein synthesis increases, the lipid droplets were still abundant, even in the oldest larvae (61-day-old). This does not seem to be related with the diet, since in both cases larvae were feed with *Artemia*, although the larvae used in the present study were initially feed with rotifers. In common carp, the accumulation of lipid droplets in the epithelial cells of the anterior intestine was associated with a dietary deficiency of phospholipids, but were not observed in *Artemia*-fed larvae (Fontagné et al. 1998). However, in *Hippoglossus hippoglossus* the lack of such lipid vacuoles in the intestine of *Artemia*-fed larvae was interpreted as a sign of reduced intestinal lipid digestion due to the rapid passage of *Artemia* through the alimentary canal of the larvae (Luizi et al. 1999).

The intestinal epithelial cells of some sea bass larvae during phase III, an exclusively exothrophic period, were almost totally occupied by lipid droplets, some of them very large. These larvae also showed a material of a probable lipid nature inside the dilated endoplasmic reticulum and seemed to be actively synthesizing lipoproteins, which agrees with the extensive capacity for effectively synthesizing lipoproteins reported for this species between 18 and 25 days after hatching (Deplano et al. 1991a). Lipids were also abundant in the intestinal epithelial cells of some larvae during phase IV; large lipid particles, similar to lipid droplets were frequent inside the dilated endoplasmic reticulum cisternae. These large lipid inclusions may be large lipoproteins, since it has been considered that the triglyceride droplets never appear inside the endoplasmic reticulum in the intestinal epithelial cells of teleosts (Bergot 1981). The large size of these lipoproteins might be due to deficient protein synthesis, as in mammals (Zilversmit 1978).

The epithelial cells of the rectum also showed lipid droplets in all the phases studied, although less frequently than those in the intestine, which is in agreement with other studies in this species (Deplano et al. 1991b). In Atlantic halibut larvae, it is in this region that the lipid droplets were found (Luizi et al. 1999); and in adult turbot, the posterior region of the digestive tract has been shown to be an active area for the uptake of lipid digestion products (Koven et al. 1994).

After the start of the exogenous feeding, the epithelial cells lining either the posterior or the second intestinal region of larvae that have two or three intestinal segments, respectively, show acidophilic supranuclear inclusions (Iwai 1968, 1969; Iwai and Tanaka 1968; Tanaka 1971, 1972; Gauthier and Landis 1972; Stroband et al. 1979; Govoni 1980; O'Connell 1981; Albertini-Berhaut 1987; Boulhic and Gabaudan 1992; Sarasquete et al. 1995; Luizi et al. 1999; Ribeiro et al. 1999). These inclusions are the result of protein pinocytosis, as demonstrated using peroxidase (Iwai and Tanaka 1968; Stroband et al. 1979; Georgopoulou et al. 1986; Govoni et al. 1986). These inclusions are absent from starving larvae (Theilacker 1978; Yúfera et al. 1993). At the ultrastructural level, considerable pinocytotic activity is evidenciated by supranuclear vacuoles of varying size and content, primary and secondary lysosomes and dense and multivesicular bodies (Iwai 1968; Iwai and Tanaka 1968; Stroband 1977; Stroband and Kroon 1981; Rombout et al. 1984; Georgopoulou et al. 1986; Albertini-Berhaut 1988; Segner et al. 1994; Calzada et al. 1998). This endocytotic apparatus is involved in the intracellular digestion of absorbed proteins (Iwai 1968; Iwai and Tanaka 1968) and seems to have an important nutritive function in sea bass larvae, in which the gastric glands are not developed and the pepsin and trypsin-chemotrypsin activities (and, hence, protein digestion) occurs in the middle and posterior intestine (Tan Tue 1983). In some teleosts, such as Dover sole and turbot, proteins are absorbed shortly after first feeding (see Boulhic and Gabaudan 1992). In sea bass larvae, although an endocytotic apparatus was observed in the epithelial cells of the posterior gut region just after exogenous feeding started, it was only after the resorption of the yolk sac that it became considerably developed. It has been suggested that these cells have a much greater capacity for incorporating proteins than the cells of adults, and is comparable with that of other teleost larvae, adult stomachless teleosts and adult and newborn mammals (Tan Tue, 1980; Deplano et al. 1991b).

In some teleosts, the supranuclear inclusions of the epithelial cells of the posterior intestine disappear when the stomach becomes functional, and the gastric glands appear (Tanaka 1972; Luizi et al. 1999). However, the epithelial cells of the sea bass rectum, contrary to that reported in a previous study (Tan Tue 1980), showed, during phase IV, an endocytotic apparatus that was as developed, or even more so, than in the larvae of the previous phase, although the gastric glands were already differentiated. These results agree with the findings reported for other teleosts (Tanaka 1972) and suggest that the incorporation of proteic macromolecules is not a consequence of the absence of a functional stomach. Similar conclusions resulted from studies on the larvae of other stomach-owner (Iwai 1968, 1969; Stroband and Kroon 1981) and stomachless (Noaillac-Depeyre and Gas 1974, 1976;

Stroband and Debets 1978) teleosts. In adult teleosts, this endocytotic apparatus was reported to be involved in the transport of antigens from the intestinal lumen, through the epithelial cells, to intra-epithelial lymphoid cells or macrophages (Rombout et al. 1985; McLean and Ash 1987). The immunological relevance of this intestinal segment has been taken into account for developing oral vaccines for fish (Quental and Vigneulle 1997).

Differentiation of the intestine is completed with the appearance of the mucosal folds, goblet cells and the first pyloric caeca during phase III, when the larvae only feed exogenously. These events are in accordance with those reported previously, except that the pyloric caeca were observed at the beginning of phase IV (Tan Tue 1976).

Mucins are known to play an important role in protecting the mucosa against bacterial attack and physical and chemical damage (Allen et al. 1986). The appearance of mucus or goblet cells in the teleost digestive tract varies among species. In some freshwater teleosts, the goblet cells appear before the first exogenous feed intake, while in other species these cells are first found shortly after the start of exogenous feeding (Tanaka 1971; Boulhic and Gabaudan 1992; Ribeiro et al. 1999) or in more advanced developmental stages (Albertini-Berhaut 1987; Sarasquete et al. 1995; Calzada et al. 1998). In sea bass, goblet cells were first observed during phase II, in the esophagus and later on, in phase III, in the intestine and rectum, which agrees with the studies mentioned above and with a previous study on this species (Tan Tue 1980). The mucus cells of the esophagus contained, from their very first appearance, acid mucosubstances, as has been seen in other teleost larvae (Boulhic and Gabaudan 1992; Ribeiro et al. 1999), although in other species both acid and neutral mucosubstances were found (Sarasquete et al. 1995). The mucus produced by the goblet cells of the intestine and the rectum of sea bass larvae was rich in neutral mucosubstances, as in *Solea senegalensis* (Ribeiro et al. 1999), which does not agree with the results obtained by Tan Tue (1980), who found that most mucosubstances of the goblet cells of the intestine and rectum of sea bass larvae were acid. In other larvae both acid and neutral mucosubstances were found in these cells (Boulhic and Gabaudan 1992; Segner et al. 1994; Sarasquete et al. 1995). Inter- and intraspecific differences in the content of mucosubstances in the digestive goblet cells could be related to different feeding habits (see Sarasquete et al. 1995). In the esophagus of sea bass larvae, the only region in which two types of carbohydrates coexist, the acid ones appear before the neutral ones, which does not support the suggestion that the appearance of different carbohydrate types in the epithelium could indicate different degrees of maturation in the mucus secretion, thus goblet cells first synthesizing neutral glycoprotein that are subsequently carboxyled and sulphated (see Elbal and Agulleiro 1986). The presence of neutral mucins in the digestive tract has been related to the absorption of easily digested substances, such as disaccharides and short-chain fatty acids (see Ribeiro et al. 1999).

Cells containing many large mitochondria and a complex system of tubules and vesicles appeared in the esophagus of sea bass larvae from phase III onwards. These cells were ultrastructurally similar to the chloride cells that appear in the teleostean branchial epithelium (Meseguer et al. 1982) and are key to the osmoregulatory processes of these fish (see Shiraishi et al. 1997). This suggests that the esophagus of sea bass larvae plays a role in osmoregulation. Although active ion transport has been described in the epithelial cells of the esophagus of adult teleosts (see Cataldi et al. 1993), the occurrence of chloride cells in the teleost esophagus has not been reported before in adults or in larvae; in the latter, where osmoregulatory organs are absent or not fully developed, chloride cells have been seen in the epithelia covering the body and in the yolk sac membrane (see Shiraishi et al. 1997). In the adult teleost esophagus, a type of columnar cell rich in mitochondria and with prominent lateral intercellular spaces and interdigitations has been reported to be involved in osmorregulation (Yamamoto and Hirano 1978; Elbal and Agulleiro 1986). These cells are ultrastructurally similar to the cells that lined the gastric region of sea bass larvae before the appearance of the gastric glands, which suggests that these gut region has a role in osmoregulation before it acquires its definitive function with the differentiation of gastric glands.

The development of the stomach and of pyloric caeca as the last step in sea bass gut differentiation is in accordance with the general findings of in other teleosts (O'Connell 1981; Stroband and Kroon 1981; Govoni et al. 1986; Pedersen and Falk-Petersen 1992; Segner et al. 1994). Although a stomach anlage, similar to the gastric undifferentiated region described in sea bass, appears in other first-feeding larvae (Pedersen and Falk-Petersen 1992; Segner et al. 1994), the differentiation of the stomach and pyloric caeca has been related to the transition from larva to juvenile (Tanaka 1971; Govoni et al. 1986). Thus, the appearance of gastric glands has been described during (Pedersen and Falk-Petersen 1992; Segner et al. 1994), or after (Boulhic and Gabaudan 1992) metamorphosis. In sea bass larvae, development of the caecal and the pyloric regions of the stomach was contemporaneous with the differentiation of the gastric glands. However, the caecal region has been described as appearing much earlier than the gastric glands in a previous study (Tan Tue 1976). In other species, the gastric glands appear shortly after the commencement of stomach development and before the development of the pyloric caeca (Tanaka 1971).

A decrease in the number of free ribosomes and an increase in the amount of rer and mucus-secretory granules characterized the differentiation process of the epithelial cells lining the gastric region of sea bass larvae, which agrees with that reported for *Clarias lazera* (Stroband and Kroon 1981). These cells contained neutral mucosubstances from phase III (before the appearance of the first gastric glands) onwards, as observed in *Sparus aurata*, in which sulphomucins were also detected (Sarasquete et al. 1995). Glandular cell precursors simi-

lar to those found in the present study, in 39-day-old larvae, have not been described previously in teleost larvae. The glandular cells initially had numerous free ribosomes and clear vesicles in the apical zone. As they differentiated, an apical tubule-vesicular network, a very developed endoplasmic reticulum and zymogen granules appeared, as has been described for *Clarias lacera* (Stroband and Kroon 1981). These glandular cells were similar to the oxynticopeptic cells of the adult teleost stomach, which, as those of other bony fish, amphibians and birds (see Rebolledo and Vidal 1979), secrete both hydrochloric acid and pepsinogen (Noaillac-Depeyre and Gas 1978; see Elbal and Agulleiro 1986). However, no light and dark cells suggested to produce mainly pepsinogen or acid, respectively, as those described by Elbal and Agulleiro (1986) in *Sparus aurata*, were found in sea bass larvae. In these all gastric gland cells had both a tubulo-vesicular system, that it is known to participate in the production of hydrochloric acid, and zymogen-like granules.

In sea bass larvae, the structure and ultrastructure of the mucosa of the pyloric caeca were similar to that of the intestine. This agrees with the belief that the development of the pyloric caeca is an adaptation to increase the intestinal surface area and has no specific role in digestion other than to enlarge the surface area (Lie and Lambertsen 1985; Buddington and Diamond 1987).

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