Histol Histopathol (2000) **15: 429-439**

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

Ultrastructural features of the gut in the white sturgeon, *Acipenser transmontanus*

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Summary. Electron-microscopic examinations of the sturgeon gut were performed. Oesophageal goblet cells were abundant in the stratified epithelium. The ultrastructural features of the secretory granules of the oesophageal and intestinal goblet cells were quite similar to those of other vertebrates. Lobules of multilocular adipose tissue were observed in the deep tunica propria-submucosa of the oesophagus, in close association with vasculature and large fibre bundles of myelinated and unmyelinated axons. Similarly composed nerve fibre bundles were observed in the cardiac stomach. too. The presence of myelinated axons is an unusual feature in the vertebrate enteric nervous system. Cardiac and fundic zones of the stomach showed an epithelium with columnar ciliated and non-ciliated cells, the latter equipped with fuzzy microvilli. Cells lining the tubular gastric proper glands were markedly granulated. Intestinal superficial epithelium was columnar and contained ciliated, as well as non-ciliated and goblet cells. In the tunica propria all over the intestine, the presence and ultrastructure of granulated cells was in addition described. Intraepithelial granulated leukocytes were seen throughout the alimentary canal. Various types of endocrine cells were seen both in the stomach and in the intestine, the size of their granules was measured and their ultrastructure described and compared to that of mammalian cell types.

Key words: Sturgeon, Gut, Morphology, Ultrastructure, Neuroendocrine system

Introduction

Macro- and microscopic anatomy of the gut in osteichthyes, basically similar in many morphological features, shows several species-specific differences not always related to taxonomic criteria. In general, gastrointestinal morphology can be influenced by feeding habits and frequency of food intake, as well as by body size and shape (Kapoor et al., 1975; Smith, 1989; Domeneghini, 1995; Buddington et al., 1997). Acipenseriformes are a very ancient, poorly known group of osteichthyes. Previous data on the white sturgeon gut (Domeneghini et al., 1999a,b) evidenced some morphological peculiarities not completely understood from a functional point of view, also because of the almost total lack of bibliographic references on gastroenteric morphology in acipenseridae (Buddington and Doroshov, 1986a,b; Gawlicka et al., 1995) and other chondrostei (Weisel, 1973, 1979). An electron microscopical research into some aspects of the sturgeon gut will help to get a deeper insight into its morphological peculiarities, probably correlated to specific functional attitudes of the gastrointestinal apparatus in this and related species. This study and the previous were prompted by considering that those of acipenseriformes are endangered species, and with the aim to optimize the management of a species subject to aquaculture. It may be that in future years, only the aquaculture conditions will save the biodiversity and help the survival of endangered species.

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Materials and methods

Adult ***Acipenser transmonfanus*** were used for this study (body weight ranging between 8 and 10 Kg; body length 80-110 cm). Specimens were collected at slaughtering at Agroittica Lombarda fish hatchery, Calvisano, Italy, at the same time of day (about 12 a.m.), in two different months, November and June. Several samples of the oesophagus (proximal, medium, distal), stomach (in its different glandular zones: cardiac, gastric proper, pyloric) and intestine (pyloric caeca, proximal intestine, medium intestine with the spiral valve, distal intestine or rectum) were collected.

For the ultrastructural analysis, small fragments were fixed in 4% paraformaldehyde and 2.5% glutaraldehyde in 0.2M cacodylate buffer, pH 7.4, for 3 h at 4 "C, then post-fixed or not for 90 min in 1% OsO_4 in the same cacodylate buffer, dehydrated and embedded in

Epon-Araldite. Ultrathin sections (60-70 nm), collected on copper grids, were counterstained with uranyl acetate and lead citrate, then observed and photographed through a Hitachi 7000 electron microscope.

Part of the specimens were fixed in 4% paraformaldehyde in phosphate-buffered saline for 5 h at 4 °C, dehydrated and paraffin-embedded. The following silver reactions were performed on dewaxed sections, 4-6 μm-thick: Grimelius' (1968) reaction for evaluation of argyrophily of endocrine cell secretory granules and Masson-Singh's reaction (Singh, 1964) for evaluation of argentaffinity, linked to the presence of reducing amines.

Relative percentages of different endocrine cell types described were evaluated. Measurements of the diameters of the secretory granules of endocrine cells were taken on randomly-sorted micrographs of each cell type at suitable magnification. Only nucleated cells were considered.

Results

A detailed light microscope description of white sturgeon gut was given in Domeneghini et al. (1999a). The most peculiar aspect of the oesophagus was the presence of large longitudinally-oriented myelinated nerve fibre bundles in the lamina propria-submucosa in close relationship with numerous lobules of multilocular adipose tissue, supplied by an extensive vasculature. These lobules were very abundant in the specimens collected in November, much smaller and fewer in the warm season. Electron microscope observations (Fig. 1) evidenced superficial epithelial cells furnished with short microvilli (Table 1), intermingled with large goblet cells whose secretory mucous granules had ultrastructural features quite similar to those of other vertebrates (Fig. 1a,b). "Immature" goblet cells were also seen, characterized by their smaller size and the presence of small, irregular and heavily electron-dense cytoplasmic granules. Additional features of the surface cells were the presence of a large euchromatic nucleus, frequently indented, and of numerous differently sized vesicles, especially crowded in the cytoplasm between the nucleus and the lumen (Fig. 1a).

Nerve fibre bundles were made up of either unmyelinated (Fig 1c) or myelinated (Fig 1d) fibres, the latter larger than the former. Nerve fibres were joined together by a conspicuous endoneurium. Lobules of adipose tissue were composed of typical multilocular adipose cells, having a shrunk, almost central nucleus surrounded by differently sized fat globules lacking a limiting membrane (Fig. 1e). Capillaries were often seen in close proximity to the adipose cells (Fig. 1f).

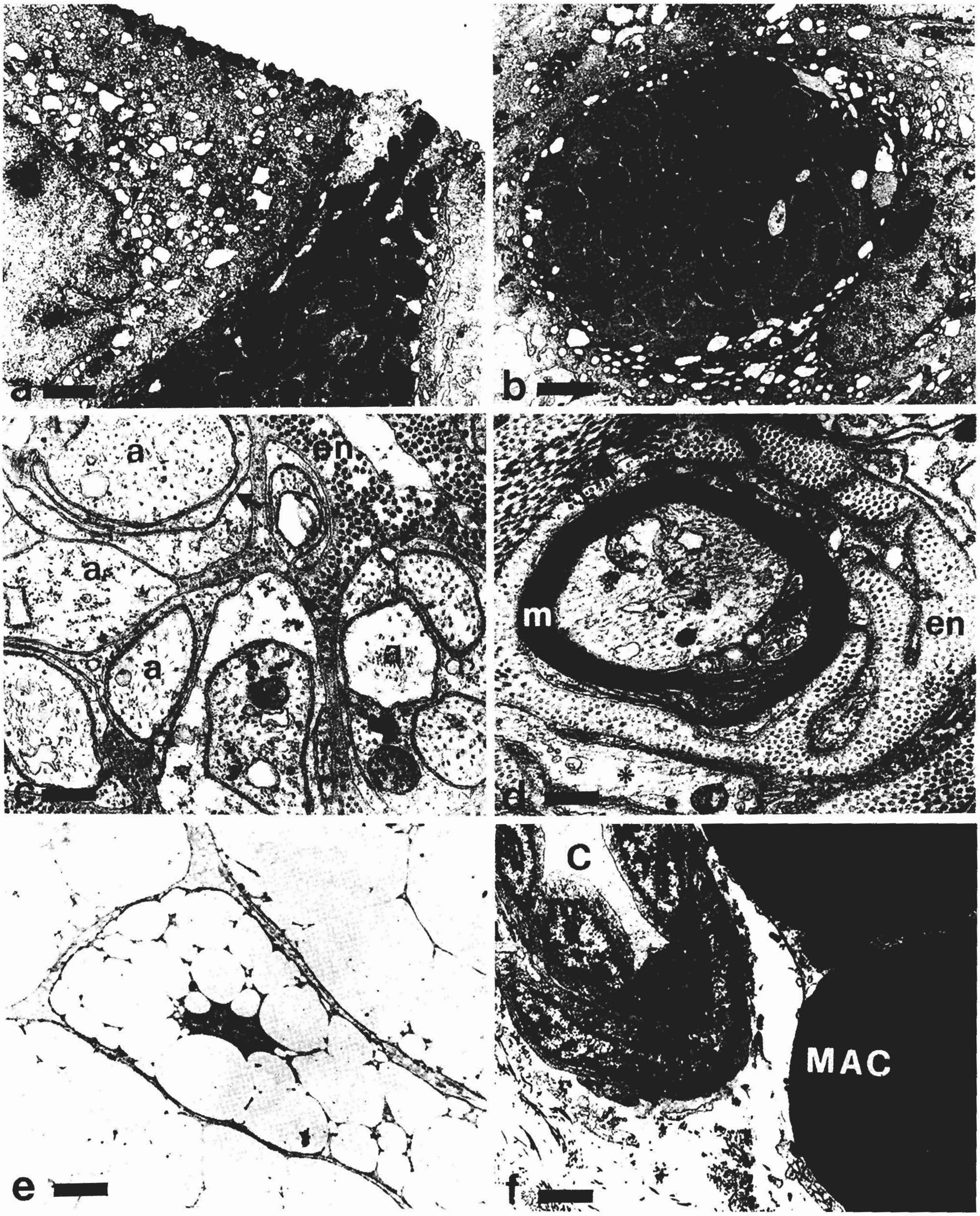
The stomach mucosa was lined by a simple columnar epithelium, which was ciliated in cardiac and gastric proper gland zones, and non-ciliated in the pyloric one. The superficial and gastric pit columnar cells synthesized neutral glycoconjugates. Tubular glands of the tunica propria, limited to the cardiac and gastric proper gland zones, were lined by cells showing a markedly granular texture, possibly related to the synthesis of both pepsinogen and acid, as in most fish. In the cardiac stomach voluminous nerve bundles similar to those described in the oesophagus were also present in the deep tunica propria-submucosa (Domeneghini et al., 1999a). Electron microscope observations (Fig. 2) evidenced columnar cells bearing motile cilia in the superficial epithelium of the cardiac and gastric proper gland zones, alterning with columnar secretory cells, whose secretory glycoconjugates formed a thick superficial film all over the epithelium (adherent mucous gel) (Fig. 2a). The cilia of ciliated cells (Fig. 2b), intermingled with fuzzy microvilli, had the usual microtubular pattern and prominent basal bodies. Ciliary rodlets were sometimes evident. Mitochondria were numerous in the cytoplasmic zone just beneath. The secretory cells of the tubular glands (Fig. 2c) were pyramidal and contained large amounts of intensely osmiophilic roundish granules, as well as many mitochondria and well developed synthetic machinery. Their nucleus was large and euchromatic. In the pyloric gastric zone the superficial epithelium was composed by a tall secretory cell type only, provided with short microvilli (Table 1) and numerous mucous granules crowding the adluminal cytoplasm (Fig. 2d). The voluminous nerve bundles observed in the cardiac stomach were ultrastructurally similar to those described in the oesophagus.

A special light microscope feature of the intestinal tracts was the spiral valve, occupying the majority of the lumen of the medium intestine. It showed a folded mucosa with numerous goblet cells containing almost

Table 1. Length of microvilli of superficial epithelial cells in the different gastrointestinal tracts.

GASTROINTESTINAL TRACT	LENGTH OF MICROVILLI (μm)
Oesophagus	0.1
Pyloric stomach	0.1
Pyloric caeca	0.5
Proximal intestine	1
Medium intestine	0.6
Rectum	0.1

Fig. 1. Oesophagus. **a.** In the superficial epithelium cells furnished with short microvilli intermingled with goblet cells (GC) can be seen. Epithelial cells contain numerous variously-sized vesicles (arrows). Scale bar: 1.10 μm. **b.** A goblet cell (GC) with secretory mucous granules having various electron-density is shown. Scale bar: 1.5 μm. **c.** Tunica propria. A bundle of unmyelinated nerve fibres with endoneurium (en) can be seen. a: axons; arrows indicate mesaxons. Scale bar: 0.4 μm. **d.** Tunica propria. A myelinated nerve fibre can be seen. Unmyelinated terminals can also be seen (asterisks). a: axon; m: myelinic sheath; en: endonevrium. Scale bar: 0.45 μm. **e.** Tunica propria. Multilocular adipose tissue cells. The cytoplasm is filled with differently sized fat globules lacking a limiting membrane, the nucleus is central and shrunk. Non-osmicated sample. Scale bar: 2 μm. **f.** Tunica propria. Close relationship between a capillary (c) and a multilocular adipose cell (MAC). Osmicated sample. Scale bar: 1.8 μm.


a
b
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MAC

exclusively sulphated glycoconjugates, and a tunica propria-submucosa rich in lymphatic diffuse tissue and solitary lymphatic follicles (Domeneghini et al., 1999a). Electron microscope observations of the intestinal mucosa (Fig. 3) of the proximal tract permitted the observation of columnar ciliated cells alterning with absorptive and goblet cells, each of them having well differentiated characteristics. The ciliated cells (Fig. 3a b) had few, short cilia intermingled with microvilli showing a peculiar aspect: they originated as a large bud from the superficial plasmalemma, and in the outer part separated into a tuft of three-four short, slender microvilli. Ciliary microtubular pattern was typical (Fig. 3b). Energetic supply for ciliary movements was furnished by a large group of mitochondria located in the cytoplasm just below. The columnar absorptive cells were equipped with microvilli, whose measured height was different in different tracts (see length in Table 1). The columnar cells of the proximal intestine had long microvilli and an absorptive apparatus beyond, comprising a lot of vacuoles, multivesicular bodies and lysosomes with a varied aspect. Large vacuoles containing absorbed matter likely to be lipoproteins were often present in the supranuclear cytoplasm (Fig. 3a). Goblet cells were filled with differently sized mucous granules, showing a pale to intense osmiophily and in some places containing an electron-dense core. In the distal intestinal tract (rectum) ciliated cells were no longer observed and absorptive cells had shorter microvilli (Table 1), but an even more extended absorving apparatus of caveolae, canaliculi and vesicles (Fig. 3c).

All along the gut, the epithelial cells equipped with microvilli often showed a peculiarity in that their mitochondria possessed dense matrix granules well discernible in a heavy electron-dense matrix (Fig. 2d, 3b,c). These cells were usually seen near other epithelial absorptive cells whose mitochondria showed a clear matrix, devoid of dense granules (Fig. 3b).

As regards the histochemistry of the diffuse endocrine system, either Masson-Singh's or Grimelius' reactions failed to demonstrate endocrine cells with argentaffin and argyrophilic secretory granules. Electron microscope observations, however, evidenced more than one type of endocrine cells dispersed among gastric and intestinal epithelial cells (Fig. 4). These cells were often seen accompanying intraepithelial granulated leukocytes (IGLs), whose characteristics will be described below. The shape of endocrine cells was round or ovoid, the nucleus was euchromatic, oval and often indented or even lobated (Fig. 4d). Never were endocrine cells observed to open to the luminal surface, and so they were classified as "closed" type, according to Fujita and Kobayashi (1973). The cytoplasm of endocrine cells was electron-lucent and contained a variable number of perinuclear secretory granules. These were highly characteristic and showed different size, texture and osmiophily in such a way that it was possible to distinguish five cellular types. Table 2 summarizes granule morphology and diameter measurements (min-max) for each cellular type, as well as percentages of the different cell types.

Type I cells had numerous, round, ovoid or elongated secretory granules (Fig. 4 a). A membrane was always present surrounding the granules, and an electron-lucent halo was sometimes evident. Their content was usually electron-dense. This cell type was the most numerous and was observed both in gastric and intestinal mucosa.

Type II cells had secretory granules, the most part of which were round and homogeneously osmiophilic (Fig. 4b). Typically, granules were intermingled with numerous irregularly-shaped vesicles, and usually one large vesicle was present in the cytoplasm. This cell type was only present in the gastric mucosa.

Type III cells had uniformly-sized, round secretory granules, which often showed a thin halo between the limiting membrane and content, which was uniformly and heavily electron dense (Fig. 4c). This cell type was present in the stomach.

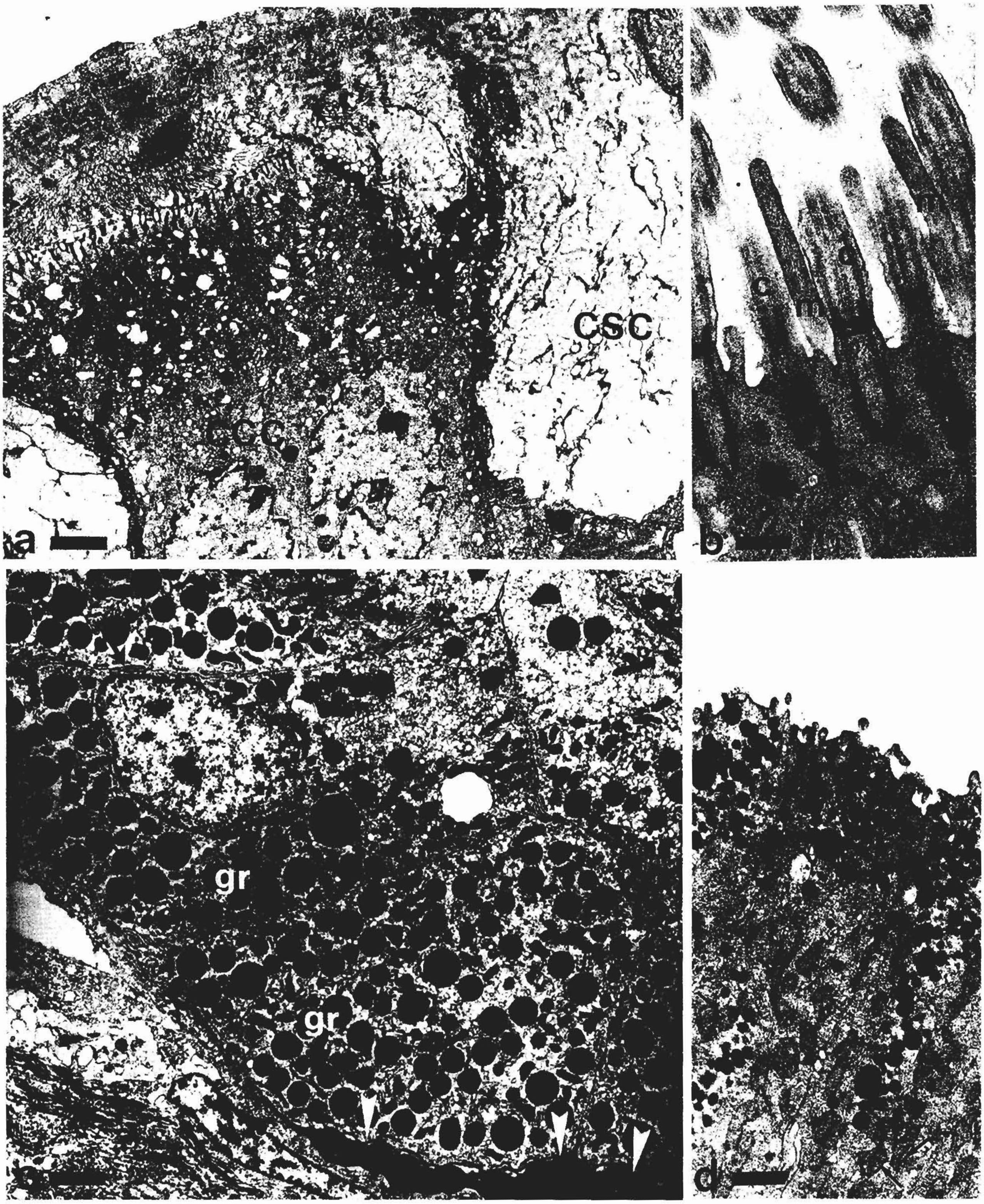
Type IV cells contained numerous round secretory granules with a fine granular, moderately osmiophilic content (Fig. 4d). This cell type was present in the stomach and intestine.

Type V cells had few and small secretory granules sparsely distributed in the electron lucent cytoplasm (Fig. 4e). Cells of this type were very scarce.

Table 2. Percentages of different endocrine cell types, morphology of granules and measurements of their diameters.

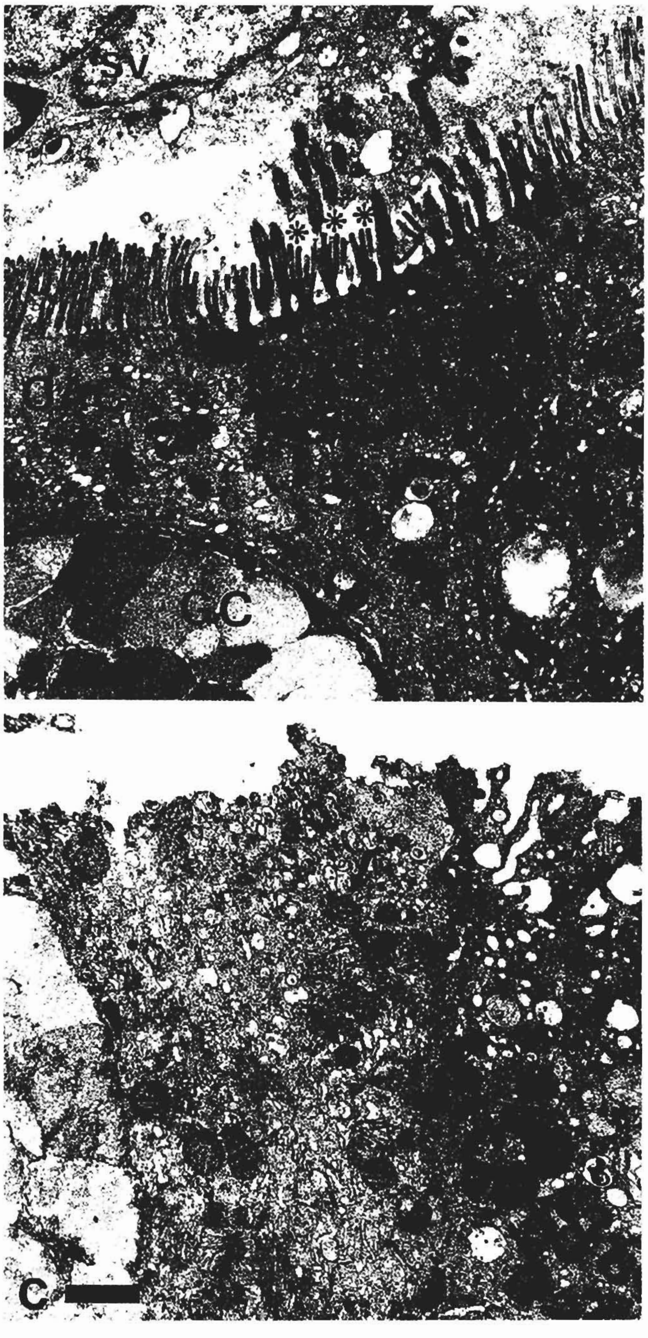
Cell type	Percentage	Granule morphology	Granule diameter (µm)
type I	50		0.06-0.11
type II	12.5		0.06-0.1
type III	12.5		0.05-0.08
type IV	18.75		0.045-0.067
type V	6.25		0.025-0.05

Fig. 2. Stomach. **a.** Superficial epithelium of the gastric proper zone. Columnar ciliated cells can be seen (CCC), near to columnar secreting cells (CSC). The adherent mucous gel is also seen (asterisks). Scale bar:1.4 µm. **b.** Detail of a columnar ciliated cell of the stomach superficial epithelium. Cilia (c) having the usual axonema and prominent basal bodies are intermingled with fuzzy microvilli (m). A ciliary rodlet is arrowed. Scale bar: 0.41 µm. **c.** Secretory cells of the tubular glands in the gastric proper gland zone. Their cytoplasm is full of intensely osmiophilic roundish granules (gr). Many mitochondria and synthetic organelles are present in the supranuclear cytoplasm. Lateral membranes show junctional devices (arrows). At the bottom right of the picture, part of a granulated cell of the lamina propria is arrowed. Scale bar: 1.8 µm. **d.** Superficial epithelium of the pyloric zone. Columnar secretory cells are furnished with very short microvilli and contain large amounts of intensely osmiophilic granules in the adluminal cytoplasm. Mitochondria and other organelles are present beneath. Note the presence of dense intramitochondrial matrix granules (arrows). Scale bar: 0.55 µm.


a
CSC
b
gr
gr
d

Intraepithelial granulated leukocytes were seen throughout the alimentary canal. They usually showed a roundish profile, and were often observed "embedded" in the cytoplasm of an endocrine cell (Fig. 4d). Nuclei were heterochromatic, with one or more indentations. The cytoplasm was pale and with few organelles in perinuclear position. A constant finding in IGLs was the presence of generally numerous electron-dense, membrane-bound roundish granules, whose diameter ranged between 0.17 and 0.23 mm. The granules very often had a well discernible halo between the core and limiting membrane.

A further, granulated cell type was finally identified throughout the alimentary canal in its lamina propria, just beneath the epithelium (Fig. 4f). This cell type, perhaps identifiable as the eosinophilic granule cell (EGC) of the light microscope observations (Domeneghini et al., 1999a), contained numerous



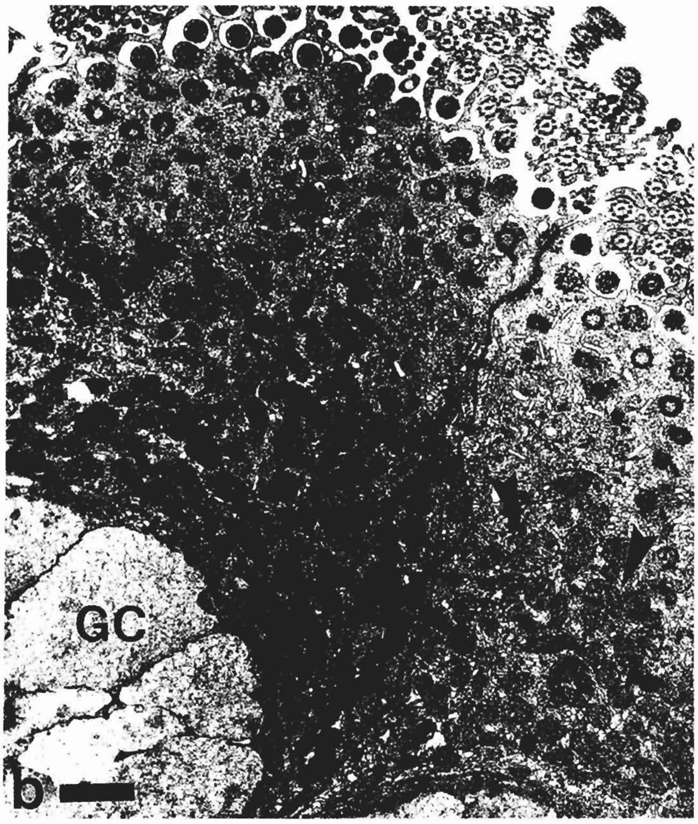


Fig. 3. Intestine. **a.** In the proximal-medium intestine columnar ciliated cells (CCC) can be seen alternating with goblet (GC) and columnar absorptive cells (CAC). In the ciliated cell, few short cilia are intermingled with tufts of microvilli (asterisks). Many mitochondria (m) are located in the adluminal cytoplasm. Absorptive cells have long microvilli, a lot of vacuoles and multivesicular bodies with varying aspect, and large vacuoles of absorbed lipid matter (arrow). In the lumen, part of the spiral valve (sv) is discernible. Scale bar: 0.87 μm. **b.** Apical portion of two neighbouring ciliated cells in the proximal intestine. Cross-sectioned cilia show the typical microtubular and basal body patterns. Mitochondria of the cell on the left show a dense matrix in which dense granules are evident (arrows). Mitochondria of the cell on the right are devoid of dense granules (arrowheads). At the bottom left corner, part of a goblet cell (GC) can also be seen. Scale bar: 0.62 μm. **c.** In the rectum columnar absorptive cells have very short microvilli and an extended absorbing apparatus of caveolae, canaliculi, vesicles and multivesicular bodies. In this picture intramitochondrial dense matrix granules can also be seen (arrows). Ciliated cells are absent. Scale bar: 0.67 μm.

roundish or ovoidal granules whose electron-density varied from light to heavy, and whose content, often showing a heavier core, was evidently encircled by a membrane-surrounded halo. Nucleus and organelles were pushed apart by the abundance of such a granule population.

Discussion

The ultrastructural study of the sturgeon gut was undertaken to clarify some peculiarities shown by our previous light microscopic morphological and histochemical studies (Domeneghini et al., 1999a,b). Special ultrastructural patterns possibly evidence peculiar functional attitudes of the gut, in particular in its proximal tract, which reside in a specially organized and composed intramural innervation. The diffuse endocrine system could also be better understood by means of the electron microscope, since different cell types were found and described, besides the only one previously identified by immunohistochemistry. Moreover, this study further evidences some special aspects of the gut in *Acipenser transmontanus* in comparison with other fish (Noaillac-Depeyre and Gas, 1979; Ezeasor and Stokoe, 1981; Elbal and Agulleiro, 1986; Elbal et al., 1988; Kuperman and Kuz'mina, 1994; Visus et al., 1996; Satora, 1998). These features had been described only in part in previous works on the same species (Buddington and Doroshov, 1986b) and other chondrostei (Weisel, 1973, 1979).

The ultrastructural examination of the oesophageal and intestinal goblet cells did not show noteworthy peculiarities compared to other osteichthyes and vertebrates in general (Specian and Oliver, 1991; Tibbetts, 1997). Histochemical reactivity related to the glycoconjugate composition of the sturgeon goblet cells (Domeneghini et al., 1999a) was different if compared with other osteichthyes (Scocco et al., 1997; Tibbetts, 1997; Domeneghini et al., 1998), probably in relation to different feeding habits and environmental characteristics, which in turn may affect the alimentary canal and its mucosubstances. It is possible that the presence of an electron-dense core within the secretory granules of the goblet cells in the distal intestine is related to the synthesis of sulpho-glycoconjugates. This hypothesis needs further studies, possibly upon cytochemical grounds.

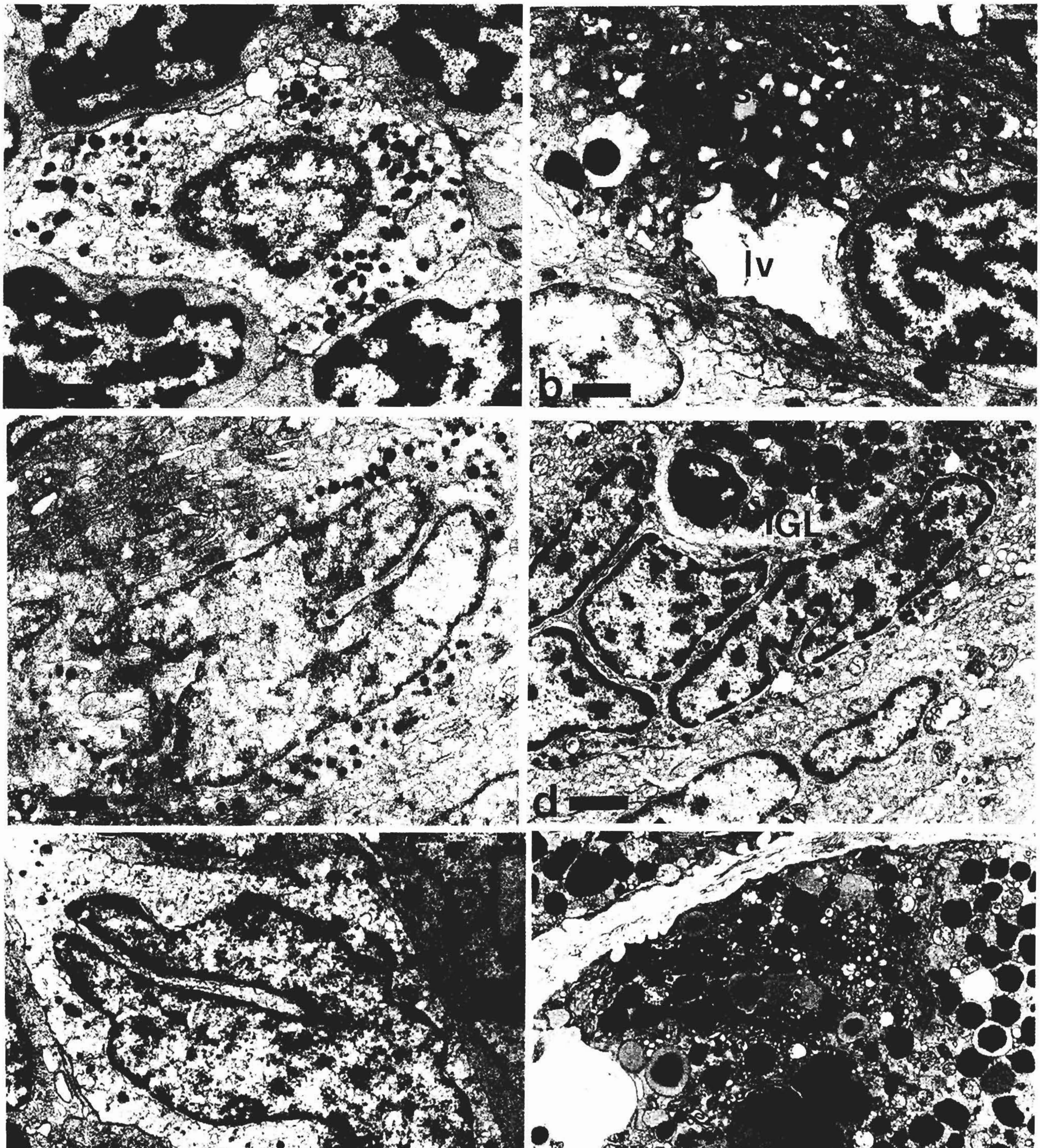
The oesophageal surface cells, with their short microvilli and vesicles crowded in the adluminal cytoplasm, are surely related with some resorptive activities on luminal fluids, as is usual for an organ which in fish may show "osmotic" functions (Smith, 1989; Loretz, 1995).

The unusual presence of numerous lobules of multilocular adipose tissue was an intriguing peculiarity of the oesophagus propria-submucosa, with the exception of the proximal tract. Structure of fat lobules was better clarified by electron micrographs, and their close relationship with vasculature and voluminous longitudinal bundles of nerve fibres, too. Ultrastructurally, the adipose tissue lobules showed the presence of typical multilocular cells. Nerve fibre bundles were shown to contain both myelinated and smaller unmyelinated nerves, joined together by a conspicuous endoneurium. The association of fat lobules, bundles of nerve fibres and vasculature suggests an active metabolism of adipocytes, perhaps towards cold temperature acclimation, considering that multilocular adipose cells are present, and the lobules are well vascularized and innervated, mostly during the late autumn-winter, whereas they are almost entirely absent during summer. A demonstration exists that visceral lipid tissues are highly mobilizable in the white sturgeon (Hung et al., 1997).

The presence of myelinated nerves within the enteric nervous system of vertebrates is not a usual finding (Gabella, 1979; Domeneghini et al., 1997) and it may be related to an extremely rapid response of the target structures (oesophageal, as well as proximal gastric) to nerve inputs. We recently hypothesized upon immunohistochemical grounds (Domeneghini et al., 1999b) that the myelinated nerves may be adrenergic in nature, and we know that the adrenergic inputs of the gut cephalic autonomic nervous system are in most fish excitatory in significance (Burnstock, 1969).

In the gastric mucosa, electron microscopic observations confirmed that the epithelial layer of the cardiac and gastric proper gland zones is ciliated. In addition, columnar ciliated and secretory cells are intermingled. In our opinion, the ciliated epithelium of the adult sturgeon, far from being a primitive aspect, may co-operate with the peristaltic activity and sustain movements of fluids coming from the oesophageal mucosa, or secreted by the gastric superficial and pit

Fig. 4. Granulated cells. **a.** A type I endocrine cell in the pyloric zone of the stomach. The secretory granules are electron-dense, roundish or elongated. Scale bar: 0.76 μm. **b.** A type II endocrine cell in the pyloric zone of the stomach. The secretory granules, round and osmiophilic, are intermingled with small vesicles (sv). A large vesicle is also visible (lv). Scale bar: 0.95 μm. **c.** A type III endocrine cell in the cardiac gland zone of the stomach. Round secretory granules, often having a thin halo between the limiting membrane and the heavily electron dense content can be seen. The euchromatic nucleus appears deeply indented. Scale bar: 0.67 μm. **d.** A type IV endocrine cell in the proximal intestine mucosa. It contains numerous round granules with fine granular, moderately osmiophilic content. Nucleus is lobated. The endocrine cell envelops an intraepithelial granular leukocyte (IGL). The latter cell contains a heterochromatic nucleus and round cytoplasmic granules which are electron dense, and show a well discernible halo between the core and limiting membrane. No junctional devices can be seen between the intraepithelial leukocyte and endocrine cell. Scale bar: 0.87 μm. **e.** A type V endocrine cell in the intestine. Its secretory granules are round and scarce. Nucleus is deeply indented. Scale bar: 0.76 μm. **f.** In the proximal intestinal lamina propria, granulated cells are present, containing large, heterogeneous granules having different osmiophily and texture, often encircled by a membrane-surrounded halo. Nucleus and organelles are pushed apart by the abundance of this granule population. Scale bar: 1.5 μm.


b
lv
c
IGL
d
f

epithelium itself, or by the tubular glands. In fact, a thick film is present in the form of adherent mucous gel overlaying the epithelial surface, as electron micrographs well demonstrate. This ultrastructural finding agrees well with the previous observation of a large abundance of neutral glycoconjugate in the secretory activities of the gastric mucosa (Domeneghini et al., 1999a), which, being fluid, may be thrown out by ciliary strokes.

The cells of the tubular glands in the cardiac and gastric proper gland zones had a strongly granular appearance, likely to be the aspect of protein-secreting cells rather than acid-producing ones. Actually, it has been shown that stomach pH in *Acipenser transmontanus* is never lower than 4 (Buddington and Doroshow, 1986b). It is generally thought that fish gastric glandular cells synthesize both pepsinogen and acid (Buddington and Doroshow, 1986a; Smith, 1989), but chloride secretion may be weak and without a morphological counterpart similar to that of mammals. The functional significance of these observations may be better understood if we consider that in most fish the digestion of the proteic component of food is performed in the distal, or medium-distal intestine, and thus the gastric step of the protein hydrolysis may not be fully developed as in other vertebrates.

The non-ciliated pyloric zone was lined by a unique type of columnar cells, whose adluminal cytoplasm was full of secretory granules hanging over a large synthetic apparatus. This gastric zone was previously shown to synthesize a large amount of neutral glycoconjugates, a minor quantity of sialoglycoconjugates at the apex of the columnar cells and a small quantity of sulphoglycoconjugates by the deep gastric pits (Domeneghini et al., 1999a).

The epithelium of the intestinal tunica mucosa showed a large number of goblet cells. Their glycoconjugate secretions are rich in the sulphated component, above all distally, and the mucosal secretions are viscous. In addition, the epithelium was composed by columnar ciliated and non-ciliated cells. The ciliated cells showed a peculiar feature of tufts of microvilli and cilia, whereas the non-ciliated cells showed variously heightened microvilli forming the brush border. The measured height of microvilli, which reaches its maximum in the proximal intestine, is evidently related to differently aimed absorptive activities. Thus, we can hypothesize that columnar absorptive cells of the proximal intestine show the maximum capacity of absorption. In the proximal intestine, the cytoplasm beneath the adluminal zone frequently shows the presence of vesicles with a lipoprotein content. This is possibly the morphological counterpart of the lipid absorption which is described in this intestinal tract in numerous fish species (Segner et al., 1994). The intestinal structure simplifies in the distal intestine (rectum), where the mucosa flattens and lacks ciliated cells, the absorbing cells becoming prevalent and conspicuously equipped with absorbing organelles. It is known that the distal part of the gut in most fish is capable of ingesting proteins via a pinocytotic pathway and of digesting them intracellularly (Segner et al., 1994). It was demonstrated that the distal part of the sturgeon gut shows the maximum of total nutrient uptake capacity (Buddington and Doroshow, 1986a,b). A significant role of the columnar absorptive cells towards the luminal content is further underlined if we consider that mitochondria of some of them show a dense matrix in which dense granules are present. This aspect is in some species (Bunton et al., 1987) related to the presence in excess of metals like copper in the water, and thus the examination of mitochondrial population of gut absorptive cells may be useful in testing water pollution and its reflexes upon species in aquaculture as well as natural life conditions.

In the subepithelial tunica propria the noteworthy presence of peculiar granulated cells was confirmed by electron photographs. Cells having a coarse granular aspect and stainable by the method of Clark (1979) were previously shown by light microscopy in the sturgeon distal intestine (Domeneghini et al., 1999a) and it was hypothesized that they could correspond to the eosinophilic granule cells (EGCs) of other fish, namely salmonids. This is a cell type related to defence mechanisms, alternatively likened to the mammalian eosinophil granulocyte (Sire and Vernier, 1995), then to the Paneth cell as it contains lysozyme (Sveinbjørnsson et al., 1996), ultimately to the mammalian mast cell and globule leukocyte (Ezeasor and Stokoe, 1980; Reite, 1996, 1998). By electron microscopy, these granulated cells were seen in a larger number, also in other tracts of the sturgeon gut, and their cytoplasmic granules are highly characteristic in their electron-density and heterogeneity. The different number of EGCs seen by light microscopy vs electron microscopy observations can be explained according to Sharp et al. (1989) who reported that EGCs are infrequently discernible with light microscope due to degranulation. Actually, EGCs detected in the sturgeon gut by electron microscope had the morphological aspect described by Powell et al. (1993) as Class IV, and were interpreted to be partially degranulated. To our knowledge reports of EGCs in fish different from salmonids are rare, and this is the first in acipenseriformes.

Previous immunohistochemical studies (Domeneghini et al., 1999b) identified an infrequent somatostatin-like immunoreactive endocrine cell type in the stomach mucosa. These endocrine cells were small and poorly granulated. Other endocrine cell types had not been observed, possibly due to an insufficient specificity of the antisera used, directed towards mammalian antigens. In addition, in the present study either Masson-Singh's or Grimelius' reactions failed to demonstrate argentaffin and argyrophilic secretory granules within the endocrine cells. Exactly the same negative results were obtained by applying silver reactions on the gut of other fish species (our unpublished observations). Evidently, the gut endocrine cells of fish do not contain the lipoprotein components whose presence allows to histochemically evidence mammalian gut endocrine cell in their secretory granules. In contrast, electron microscope observations enabled us to identify five different endocrine cell types, based on the morphology of the secretory granules and other cytoplasmic details. Their functional roles in producing peptides and other chemical messengers may only be hypothesized, considering the resemblance of these cell types to mammalian endocrine cell types. And so, type I endocrine cells recall mammalian EC cells, related to the synthesis and release of the biogenic amine serotonin, and type II endocrine cells, limited to the gastric mucosa, are similar to mammalian G cells, which produce gastrin. Type III and IV endocrine cells are similar to mammalian A-like and respectively D cells, the latter of which are related to the synthesis of somatostatin peptides. Type V endocrine cells may perhaps be similar to mammalian D1 cells, but, on the whole, these attributions need further studies.

All over the mucosal epithelia of the alimentary canal intraepithelial granulated leukocytes were seen. Their ultrastructure is similar to that described in another fish organ, the liver (Speilberg et al., 1994) and in hepatic and extrahepatic organs in mammals (Kaneda and Wake, 1985) and other vertebrates, interpreted as being large granular lymphocytes. In the mammalian liver, cells with a similar aspect are better known as "pitt cells", and their natural killer function is now proven (Wisse et al., 1997). In the sturgeon, their defence function in organs directly exposed to exogenous matter is guessable, as a component of gut-associated lymphoid tissue (GALT) (Abelli et al., 1997; Picchietti et al., 1997). Their frequent, close relationship with endocrine cells is noteworthy, and this may signify a mutual relationship between these two cell types possibly aimed at neuroendocrine modulation of immune functions (Blalock, 1994).

In conclusion, electron microscopic examinations of the sturgeon gut better clarified some light microscopic peculiarities observed in previous studies and permitted us to link these special features to peculiar functional attitudes of the gut in this species. In our opinion, ultrastructural features of the proximal tract have to be related primarily to fat metabolism which varied throughout the year. Ciliated epithelium of the cardiac and fundic zones of the stomach, unusual to be evidenced in adult fish, must be referred to movements of fluids coming from the oesophagus and secreted by the gastric superficial epithelium, pits and glands. Strongly granulated secretory cells lining the tubular gastric proper glands are most probably related to the synthesis of digestive enzymes, and acid to a lesser extent. Intestinal superficial epithelium, containing scattered ciliated cells, showed absorptive capacities all over, possibly directed towards different components of the food in the different tracts. Ultrastructural description of (eosinophilic) granulated cells observed in the tunica propria all along the intestine permitted us to better discuss their possible nature in comparison with similar cells of other fish species. Various types of endocrine cells were described ultrastructurally both in the stomach and intestine, and their frequent relationship with intraepithelial granulated lymphocytes was also underlined.

Acknowledgements. This work was supported by the Italian Ministero Università Ricerca Scientifica Tecnologica (MURST 60% and 40%). Authors wish to thank Agroittica Lombarda fish hatchery (Calvisano, Italy) for kindly providing the animals, and Mr Giovanni Caporale for his valuable technical collaboration.

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Accepted November 9, 1999