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A Study of the Anatomy, Histology and Ultrastructure of the Digestive Tract of *Orthrias angorae* Steindachner, 1897

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The anatomy, histology and ultrastructure of the digestive tract of *Orthrias angorae* (Steindachner, 1897) were investigated using light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The histological structure consists of four layers: mucosa, submucosa, muscularis and serosa. The esophageal mucosa consists of undifferentiated basal epithelial cells, mucous cells and surface epithelial cells. It was observed that the J-shaped stomach had a meshwork of folds in the cardiac region, and longitudinal folds in the fundic and pyloric regions. A single layer of columnar cells, PAS positive only in their apical portions, forms the epithelium. The convoluted tube-shape intestine is lined by simple columnar epithelial cells, which have microvilli at the apical surface. The wall of the esophagus and stomach are thicker than that of the intestine because of the thick muscle layer. There were numerous goblet cells in the intestine. There were numerous gastric glands in the submucosa layer of the cardiac stomach, but none were present in the pyloric region of the stomach. There were no pyloric caeca between the stomach and intestine. The enterocytes with microvilli contained rough endoplasmic reticulum, ribosomes and rounded bodies, and the gastric cells contained a well-developed Golgi apparatus.

Key words: *Orthrias angorae*, digestive tract, ultrastructure, anatomy and histology.

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The histology and anatomy of fish digestive tracts have been well documented in the literature using light microscopy as well as scanning and transmission electron microscopy (MARTIN & BLABER 1984; MORRISON & WRIGHT 1999; SARİEYYÜBOĞLU *et al*. 2000; PARK & KIM 2001; SIS *et al*. 1979; CLARKE & WITCOMB 1980; CATALDI *et al*. 1987; GRAU *et al*. 1992). The esophagus is lined with squamous epithelium and numerous PAS/AB positive goblet cells. The tunica muscularis consists of two layers of striated muscle: an outer circular and an inner longitudinal layer (SIS *et al*. 1979; MORRISON & WRIGHT 1999; ALBRECHT *et al*. 2001). HIRJI (1983), reported that the contents of the mucous cells stain strongly with PAS/AB. Histologically, the stomach is composed of four basic layers; mucosa, submucosa, tunica muscularis and serosa. The simple columnar epithelium of the stomach contains neutral mucopolysaccharides in the apical portions of the cells. The lamina propria of the cardiac and fundus regions possesses gastric glands absent in the pyloric region (SIS *et al*. 1979; GRAU *et al*. 1992). There is a general relationship between the length of the intestine and feeding habits. The intestine in carnivorous fish is shorter than that of omnivores and herbivores (SIS *et al*. 1979; KURU 2001). CLARKE and WITCOMB (1980), noted that the intestinal surface was in the form of zigzag folds under scanning electron microscopy. However, regions in the intestine are not differentiated macroscopically (SCOCCO *et al*. 1997; GARGIULO *et al*. 1998). The intestinal mucosa of omnivorous fishes is a single-layered columnar epithelium with a well-developed brush border (ALBRECHT *et al*. 2001). The columnar cells form the major part of the mucosa being elongated with a large nucleus; they contain neutral mucopolysaccharides and have a possible absorbtive function (SIS *et al*. 1979; CLARKE & WITCOMB 1980; ALBRECHT *et al*. 2001). KUPERMAN and KUZMINA (1994), observed by transmission electron microscopy that the apical part of the enterocyte plasmalemma bears numerous microvilli, forming the brush border, the cytoplasm of the enterocytes contains numerous cisternae of rough endoplasmic reticulum, ribosomes, dense rounded bodies, well-developed Golgi apparatus (posterior intestine), and that apical parts of the epithelial cells are linked with one another by various types of intermediated junctions and desmosomes. These findings were similar to those recorded by KAYANJA *et al.* (1975) and ELBAL and AGULLEIRO (1986).

However, no reports could be found in the literature on the morphology, histology and ultrastructure of the digestive tract of *Orthrias angorae*. Therefore, the purpose of this study is to examine the morphology, histology and ultrastructure of the digestive tract using light, scanning electron and transmission electron microscopy. These observations will provide a basis for future studies on the diet and disease in *Orthrias angorae*.

Material and Methods

Light microscopy

Specimens were collected from the Yeşilırmak River. Sixteen *Orthrias angorae*, 7cm length were used in the investigation. The body cavity was opened and the digestive tract was immediately fixed in 10 % neutral buffered formaldehyde and Bouins' solution, embedded in wax, 5μm sections were deparaffinized and stained with haematoxylin and eosin, Giemsa stain (DRURY *et al*. 1967) and periodic acid schiff (PAS) (VACCA 1985) and viewed under a binocular microscope.

Scanning electron microscopy (Jeol 5400)

For scanning electron microscopy, tissues were fixed in 3% gluteraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) for 4 h, dehydrated through an ethanol series, Critical point dried with liquid CO_2, coated with gold and viewed in a Jeol 5400 scanning electron microscope.

Transmission electron microscopy (Jeol 100 CX2 electron microscope)

Small fragments of the digestive tract were placed in 3% gluteraldehyde in 0.1 M phosphate buffer at pH 7.2 for 3 h at room temperature. After rinsing in phosphate buffer, the specimens were postfixed in 1% buffered osmium tetroxide at pH 7.2 for 3 h at 4^{o} C. They were then dehydrated and embedded in araldite. Thin sections were stained with uranyl acetate and lead citrate (GLAUERT 1975) and examined using a Jeol 100 CX2 electron microscope.

Results

Light microscopy

The digestive tract of *Orthrias angorae* is composed of the esophagus, stomach and intestine, and the length was approximately 3-4 times the standard body length. The short esophagus is followed by a J-shaped stomach. The intestine was convoluted and had the same thickness throughout.

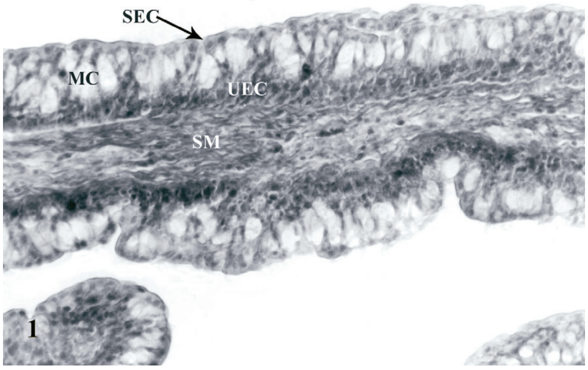
Histologically, the digestive tract was made up of four distinct layers: mucosa, submucosa, muscularis and serosa. The mucosa of the esophagus consists of surface epithelial cells, mucous cells and undifferentiated basal epithelial cells (Fig. 1). Some goblet cells stained PAS-positive because of neutral mucopolysaccharides, present in the area immediately above the undifferentiated basal epithelial cells. The muscularis was made up of two layers, a thicker outer circular and an inner longitudinal layer of striated muscle. The serosa consists of mesothelial cells, small blood vessels, blood cells and loose connective tissue. The mucosa of the stomach was composed of a single layer of columnar cells which were PAS-positive only in their apical portion. The columnar cells are tall and cylindrical with an oval nucleus situated at the base of the cells. The tunica muscularis consists of smooth muscle: an inner circular and an outer longitudinal layer (Fig. 2). There are many gastric glands in the cardiac stomach, but not in the fundic and pyloric stomach. There are no pyloric caeca between the stomach and intestine. Goblet cells were also found in the intestine (Fig. 3). The intestinal wall is composed of a mucosa, thin submucosa, tunica muscularis and serosa. The mucosa has elongated columnar cells with basal nuclei and microvilli. A number of goblet cells are situated among epithelial cells. Many of these cells densely stain PAS-positive because of neutral mucopolysaccharides (Fig. 4).

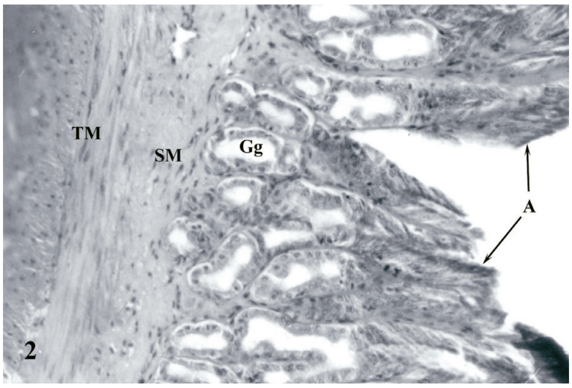
Scanning electron microscopy

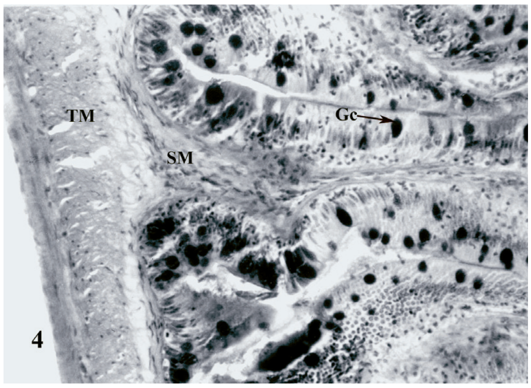
The esophageal mucosa showed primary longitudinal folds and it was observed that the cardiac stomach had a meshwork of primary folds (Fig. 5). The luminal surface of columnar cells was pentagonal in the cardiac stomach (Fig. 6). In the fundic and pyloric stomach, there were many deep regular longitudinal folds, and transverse secondary folds (Fig. 7). Zigzag-shaped longitudinal secondary folds were observed on the intestinal surface (Fig. 8).

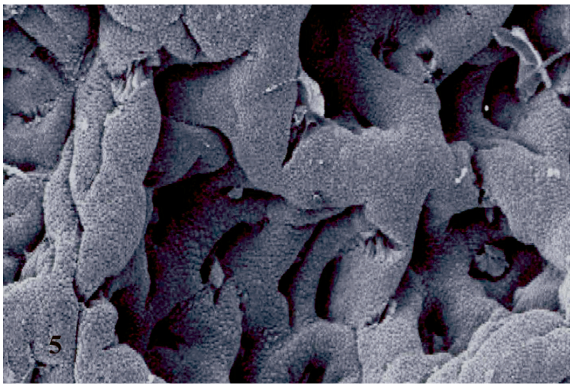
Transmission electron microscopy

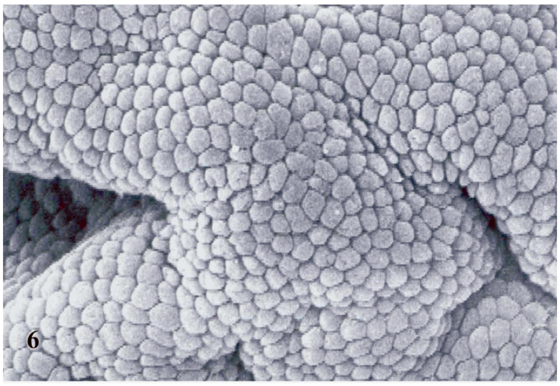
The large goblet cells were filled with numerous mucous droplets of low electron-density and high electron-density between surface epithelial cells

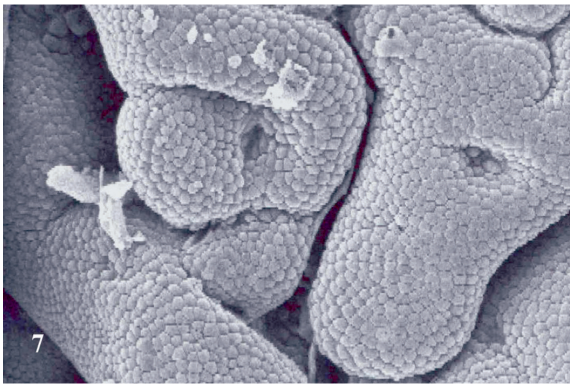












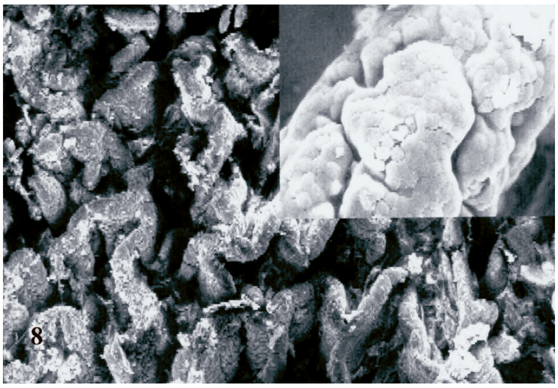
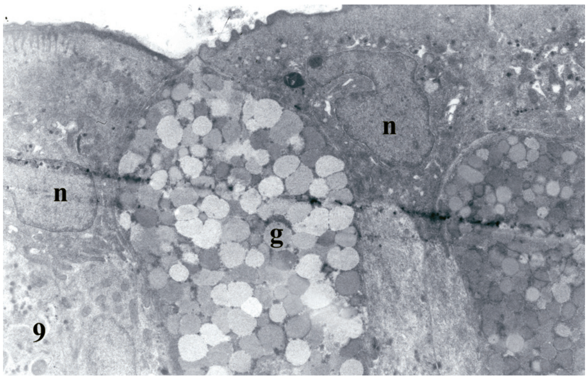
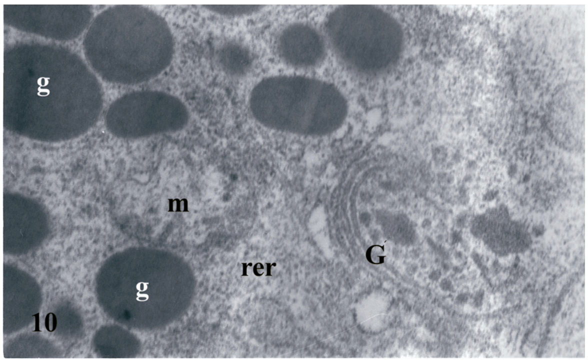


Fig. 1. Oesophageal fold. MC – mucous cells; SEC – surface epithelial cells; SM – submucosa; UEC – undifferentiated epithelial cells. H&E, x 100. Fig. 2. Longitudinal section of stomach. A – epithelium with PAS positive apical cells; TM – tunica muscularis; Gg – gastric glands; SM – submucosa; PAS, x 100. Fig. 3. Pyloric sphincter (between stomach and intestine). A – epithelium with PAS positive apical cells; Gc – goblet cell; I – intestine; S – stomach. PAS, x 50. Fig. 4. Intestine. Gc – goblet cell; TM – tunica muscularis. PAS, x 100. Fig. 5. Meshwork form folds in the cardiac region of the stomach (SEM), x 200. Fig. 6. Pentagonal columnar cells in stomach (SEM), x 1000. Fig. 7. Transverse secondary folds in fundic and pyloric stomach (SEM), x 500. Fig. 8. Intestine. Zigzag form longitudinal folds (SEM), x 50; Insert, x 500.





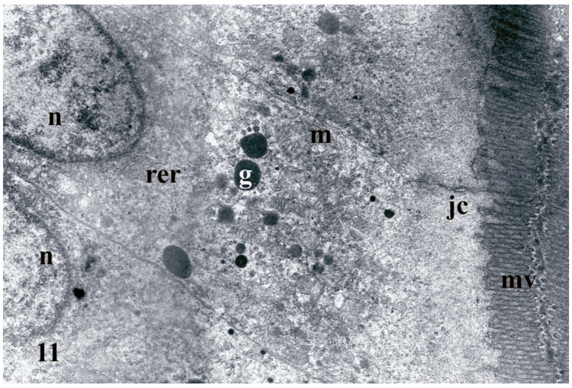


Fig. 9. Oesophagus. g – secretory granules; n – nucleus, x 4000. Fig. 10. Gastric cells in the stomach. G – Golgi apparatus; g – secretory granules; M – mitochondria; rer – rough endoplasmic reticulum, x 29000. Fig. 11. Intestinal epithelium. g – secretory granules; jc – junctional complexes; mv – microvilli; n – nucleus; rer – rough endoplasmic reticulum, x 5000.

and undifferentiated epithelial cells in the esophagus. Surface epithelial cells and undifferentiated epithelial cells had a sparse endoplasmic reticulum and numerous mitochondria scattered in their cytoplasm (Fig. 9). The most notable feature of the gastric cells was that they contained a well-developed Golgi apparatus, large mitochondria and spherical secretory granules containing a homogeneous material. The Golgi apparatus consisted of several parallel arrays of flat cisternae and numerous small vesicles (Fig. 10).

The highly folded intestinal mucosa had a simple epithelium consisting mainly of columnar cells with a striated border and goblet cells. The striated border consisted of many homogeneously distributed microvilli. The large oval nucleus, located in the cell base, and numerous mitochondria were scattered throughout the cytoplasm. In addition, these cells had a moderate amount of rough endoplasmic reticulum. The epithelial cells (enterocytes) were joined laterally near the free surface by junctional complexes. Goblet cells were found scattered throughout the intestinal epithelium. These cells had homogeneous electron-dense granules (Fig. 11).

Discussion

The anatomy of the esophagus of *Orthrias angorae* resembles that of other teleosts (SIS *et al*. 1979; CLARKE & WITCOMB 1980; HIRJI 1983; FERRARIS *et al*. 1987; CATALDI *et al*. 1987; MORRISON & WRIGHT 1999). However, taste buds were not found in the esophagus of the *O. angorae*. The mucosal folds of the esophagus, described by SIS *et al*. (1979), CLARKE and WITCOMB (1980), CATALDI *et al*. (1987) were similar to the esophagus of *O. angorae*. One of the most remarkable features in the digestive tract of the *O. angorae* was the existence of a thick striated muscle layer in the esophageal region. The muscle layers had outer circular and inner longitudinal layers. These results were the same as those described by SIS *et al*. (1979), FERRARIS *et al*. (1987), GRAU *et al*. (1992), MORRISON and WRIGHT (1999) and ALBRECHT *et al*. (2001). The mucosa of the esophagus was similar to that described by CLARKE and WITCOMB (1980) and VERIGINA and ZHOLDASOVA (1986).

The J-shaped stomach consists of three regions; the cardiac, fundus and pylorus. GRAU *et al*. (1992) found that the stomach displayed a large number of primary longitudinal folds which contained secondary folds. But the cardiac surface of

O. angorae is a meshwork of primary folds; the fundic and pyloric surfacse have many deep regular longitudinal folds with transverse secondary folds. As reported by ALBRECHT *et al*. (2001), the epithelium is formed by a single layer of columnar cells, PAS-positive in their apical portions. According to MARTIN and BLABER (1984), gastric glands were packed beneath the epithelial layer, but the epithelium did not have goblet cells. The muscular layer of *O. angorae* was similar to that described by CLARKE and WITCOMB (1980), GRAU *et al*. (1992), VERIGINA & ZHOLDASOVA (1986), MORRISON and WRIGHT (1999), and serosa as reported by CLARKE and WITCOMB (1980).

WILLIAMS and NICKOL (1989), GRAU *et al*. (1992) and ALBRECHT *et al*. (2001) reported that pyloric caeca were located in the anteriormost portion of the intestine, however they were not present in the same region in *Orthrias angorae*. Scanning electron microscopy showed that the intestinal surface had the same zigzag longitudinal folds as reported by CLARKE and WITCOMB (1980). The columnar epithelium of the intestinal mucosa may have an absorptive function as reported by SIS *et al*. (1979), CLARKE and WITCOMB (1980), and ALBRECHT *et al*. (2001). These researchers also reported that numerous mucous-secreting goblet cells were present among the columnar cells in *O. angorae*. These goblet cells stained PAS-positive and were observed because they contained neutral mucopolysaccharides. There were two kinds of Goblet cells in the esophagus; immature goblet cells and mature goblet cells. While the mature goblet cells were filled with numerous mucous droplets of low electron density, immature goblet cells were filled with numerous mucous droplets of high electron density. These findings for the oesophagus are in agreement with those in the literature (GARGIULO *et al*. 1997; ELBAL & AGULLEIRO 1986).

The gastric gland cells of *O. angorae* contained a well-developed Golgi apparatus, large mitochondria and many spherical secretory granules as in most bony fishes, amphibians and birds (LING & TAN 1975; REBOLLEDO & VIAL 1979).

The columnar cells of the cranial intestine of *O. angorae* were similar to those described by Kayanja *et al*. (1975), who observed tubules of smooth endoplasmic reticulum amongst the mitochondria, supranuclear cytoplasm containing elements of the Golgi complex, and rough endoplasmic reticulum that was particularly plentiful in the cytoplasm around the nuclei. The enterocytes were joined laterally near the free surface by junctional complexes; each of these was composed of an occluding junction, a desmosome and a gup junction (CACECI & HRUBEC 1990).

The mucus-secreting cells in the oesophageal epithelium of *O. angorae* are important along the entire esophagus for even lubrication of food particles during swallowing. The fibrils in the connective tissue of the esophagus and stomach provide increased elasticity, allowing this species to swallow larger items and store more food. The secretions of the gastric glands probably protect the mucosa from the acidic contents of the stomach. In our opinion, the existence of blood cells, especially lymphocytes, occurring along the entire gut, is an adaptation to the turbid and volatile benthic habitat of this organism.

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