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Histological, Histochemical and Ultrastructural Study of the Intestine of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: The histological and histochemical characteristics of the intestine and the ultrastructure of it's mucosa were studied in rainbow trout. The histological characterizations revealed that the wall of the intestine is composed of the tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Intestinal mucosa displays many villi and a simple columnar epithelium in associated with goblet cells and intraepithelial lymphocytes. Muscularis mucosae and mucosal tubular glands were not seen. Eosinophilic granular cells (mast cells) observed along the intestine mainly beneath the stratum compactum, an acidophilic longitudinal layer. Histochemical analysis showed that the goblet cells have acidic and neutral mucosubstances. At the ultrastructural level, the columnar cells of the intestine have microvilli toward the lumen and are joined together at the apical surfaces by the junctional complexes. Lysosomes, rough endoplasmic reticulum and polymorphic mitochondria are scattered in the supranuclear cytoplasm of enterocytes. Many pinocytotic vesicles were observed in the enterocytes of the middle part of the intestine.

Key words: Histology . histochemistry . ultrastructure . intestine . rainbow trout

INTRODUCTION

The anatomy, morphology and histology of the digestive tract of many teleostean fish species have been studied [1-7]. There are considerable differences in macroscopic and microscopic features and also functions of alimentary canal among fish species, although it showes some basic structural similarities. The overall gastrointestinal morphology is related to different feeding habits including the nature of the food and frequency of food intake, as well as to taxonomy, body size and shape [1, 8, 9].

Along with the general histological structure of the digestive tract, histochemistry and ultrastructure of the mucous layer also have been studied in some fish [2-5, 8-11]. These studies confirm that the main mucus secretors in the digestive tract are goblet cells, surface epithelium of intestine and some cells in intestinal glands, if present [1]. Histochemical characterization of mucosubstances secreted by teleost digestive tract vary among species and certain regions of the canal [1, 12-14].

The fundamental similarity between the ultrastructure of the intestinal epithelium in fish and other vertebrates has been noted [4]. In some studies it was demonstrated that there may be some differences in fine structure of intestinal epithelium in fish of various species [15].

The rainbow trout, *Oncorhynchus mykiss*, is one of the most economically important freshwater fish and is extensively cultured in Iran. There are a lot of cold freshwater sources in which to do rainbow trout culture and it is very easy to adapt to environment, breeding and also marketing [16].

Since knowledge of the structure of the intestine is essential to the understanding of physiological and also abnormal conditions, this research was undertaken to describe the intestinal histology, histochemistry and ultrastructure of rainbow trout.

MATERIALS AND METHODS

Ten adult rainbow trout fish weighing 1.5 to 2.5 kg and 30-40 cm in length, comprising both sexes, were collected from fish culture ponds, around Tabriz city. After being taken to the laboratory, fish were dissected and the entire digestive tracts of them were exposed. The samples of intestine (proximal, middle and distal Parts) were fixed in 10% formalin solution. After fixation, the samples were rinsed in water, dehydrated in graded ethanol solutions, cleared in xylene and embedded in paraffin. Dewaxed sections (5-6 μm) were stained for histological and histochemical purposes with haematoxylin and eosin (H&E), periodic-acid Schiff (PAS) and Alcian blue (PH 2.5) [17].

For transmission electron microscope investigations, small fragments from the anterior,

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middle and posterior parts of intestine were used. The materials were fixed in a mixture of glutaraldehyde (2.5%) and paraformaldehyde (4%) at low temperatures, buffered at phosphate and post-fixed in osmium tetroxide 2%. The pieces were washed in a graded series of ethanol. Embedding was performed using pure resin and ultrathin sections were stained with uranyl-acetate and lead citrate [18]. The sections were examined with a LEO 906 transmission electron microscope.

RESULTS

Histological findings showed that the basic organization of intestinal wall is similar to that in other vertebrates and is formed by tunica mucosa with a loose connective tissue lamina propria, tunica submucosa, tunica muscularis (inner circular and outer longitudinal smooth muscles) and tunica serosa layers (Fig. 1). Neither muscularis mucosae between the lamina propria and the submucosae, nor any mucosal tubular glands were observed in the tunica mucosa. A thick layer of densly packed acidophilic connective tissue, the stratum compactum, separates the mucosa from submucosa (Fig. 4). The mucosal surface has numerous progections (villi), decreasing in length towards the posterior intestine and they lined by simple epithelium comprised of the single-layered tall columnar cells with a basal nucleus containing a nucleolus, an apical brush border and acidophilic cytoplasm that are interspersed with goblet cells and intra-epithelial lymphocytes (IELs) (Fig. 2 and 3). The goblet cells exhibited a supranuclear region characterized by a swollen distal region that contained a trans lucent cytoplasm and a basal region with associated nuclei (Fig. 2).

IELs were small oval to round cells with deeply stained nuclei surrounded by small amounts of faintly stained cytoplasm (Fig. 3). Eosinophilic granular cells (mast cells) dominate in the submucosa and form a layer named stratum granulosum (Fig. 5), but can also be found in the mucosa.

Between two muscle layers of the tunica muscularis, there are many nerve plexuses. (Auerbach's plexuses) (Fig. 6). The tunica serosa is situated externally to the muscularis and consists of a thin layer of connective tissue that is covered by a single layer of squamous cells (Fig. 6). Except for lower length of villi and higher numbers of goblet cell in posterior intestine, no histological differences were observed between various intestinal regions.Histochemical analysis revealed that the goblet cells mucus contents included neutral (PAS positive) and acidic (Alcian blue positive) mucosubstances (Fig. 7 and 8).

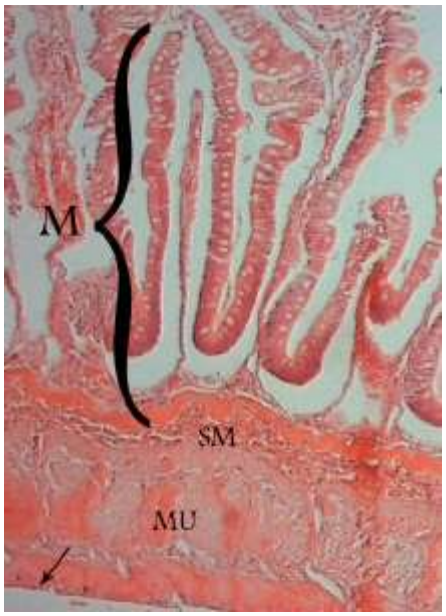

Fig. 1: Mucosa of the intestinal wall formed by tunica mucosa (M), tunica submucosa (SM), tunica muscularis (MU) and tunica serosa (arrow) (H&E, × 40)

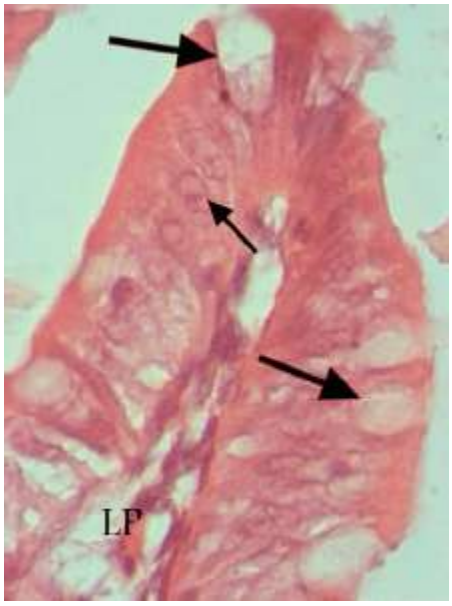

Fig. 2: Upper portion of a intestinal villus: Basally located nuclei of the enterocytes (small arrow), goblet cells (large arrows) and lamina propria (LP) (H&E, ×400)

Transmission electron microscope studies showed that columnar epithelial cells (enterocytes) are elongated and the apical part of them bears numerous microvilli toward the lumen, forming the brush border (Fig. 9). Within the microvilli many microfibrils are

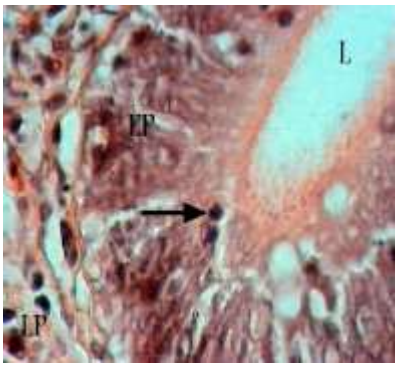

Fig. 3: Intraepithelial lymphocytes (arrow) are scattered between epithelium (EP). Note the lamina propria (LP) (H&E, ×400)

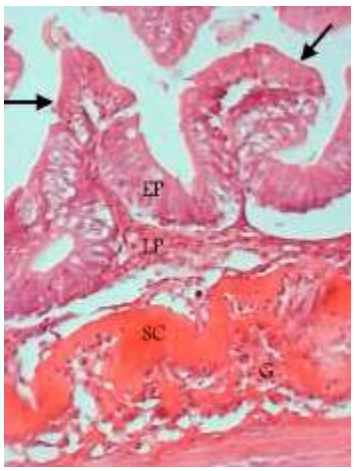

Fig. 4: Intestinal wall: mucosal villi (arrows), epithelium (EP), lamina propria (LP), stratum compactum (SC) and stratum granulosum (G) (H&E, ×100)

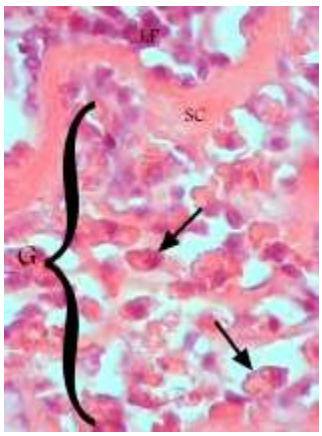

Fig. 5: Stratum granulosum (G) lies beneath the lamina propria (LP) and stratum compactum (SC) and contains many eosinophilic granular cells (arrows) (H&E, ×400)

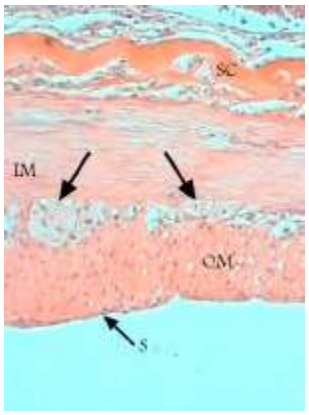

Fig. 6: Many nerve plexuses (Auerbach's plexuses) (arrows) lie between inner circular (IM) and outer longitudinal (OM) smooth muscles. Stratum compactum (SC) and tunica serosa (S) are seen (H&E, ×100)

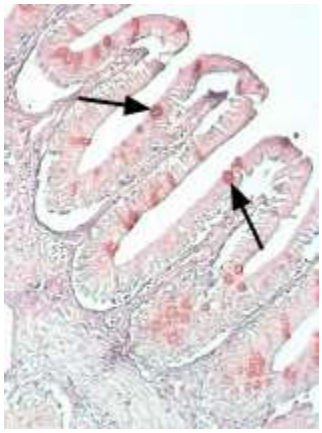

Fig. 7: The mucosa of intestine stained with periodic-acid Schiff's reaction, the goblet cells are positive (arrows) (PAS, ×100)

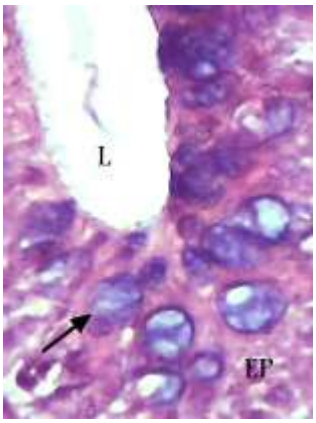

Fig. 8: Numerous goblet cells stained Alcian–blue positive (arrow). Epithelium (EP) and intestinal lumen re seen (Alcian-blue, ×400)

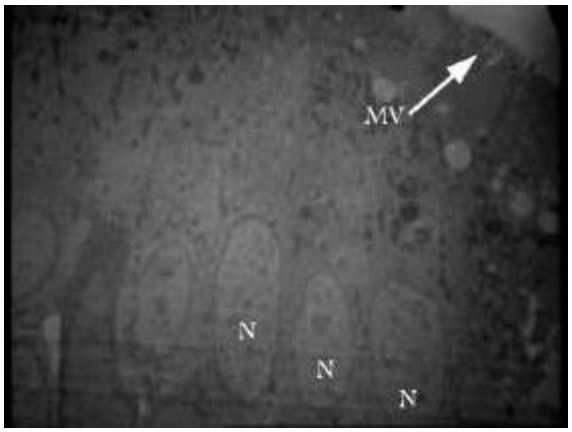

Fig. 9: Fine structure of the intestinal epithelium. Tall columnar cells have basally located nuclei (N) and many microvilli in their apexes (MV) (Scale bar: 7.2µm)

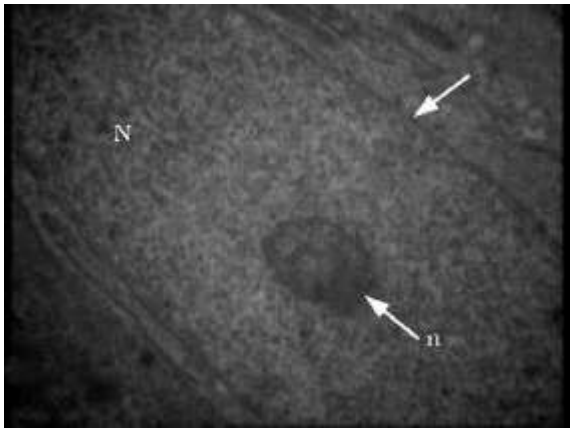

Fig. 10: Euchromatic nucleus of a enterocyte (N) with a nucleolus (n) and surronding nuclear envelope (arrow) (Scale bar: 1.56µm)

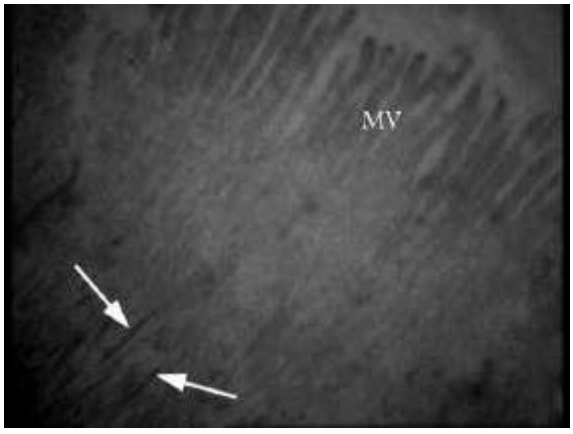

Fig. 11: Many microfibrils (arrows) in the apical cytoplasm and below the microvilli (MV) form terminal web (Scale bar: 1.2µm)

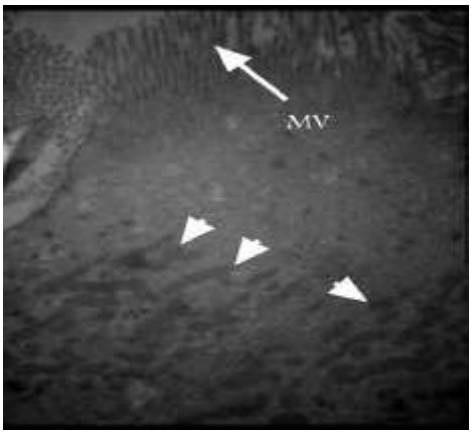

Fig. 12: Numerous polymorphic mitochondria (arrowheads) in enterocytes. Microvilli (MV) are seen (Scale bar: 2.6µm)

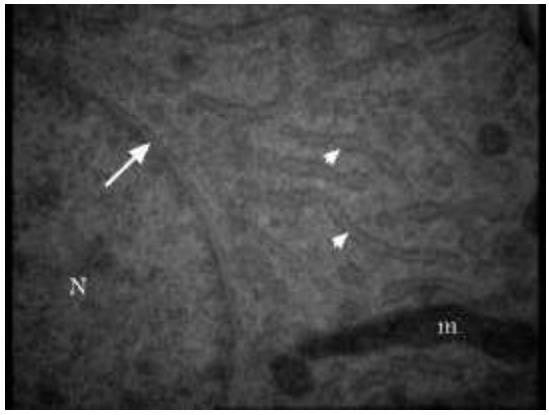

Fig. 13: Rough endoplasmic reticulumes (arrowheads), mitochondrion (m), nucleus (N) and nuclear envelope of an enterocyte (Scale bar: 0.93µm)

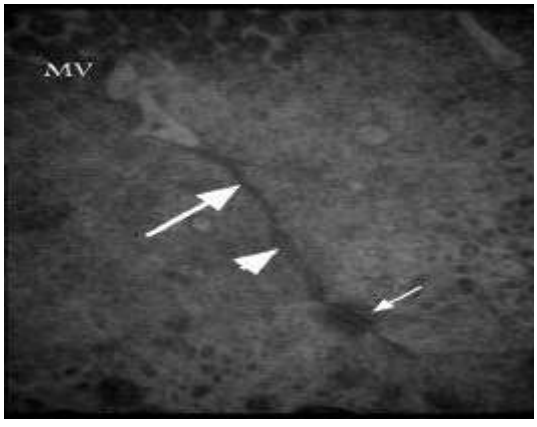

Fig. 14: Apical junctional complexes between columnar cells: tight junction (large arrow), adherens junction (arrowhead), desmosome (small arrow), microvilli (MV) are seen (Scale bar: 0.93µm)

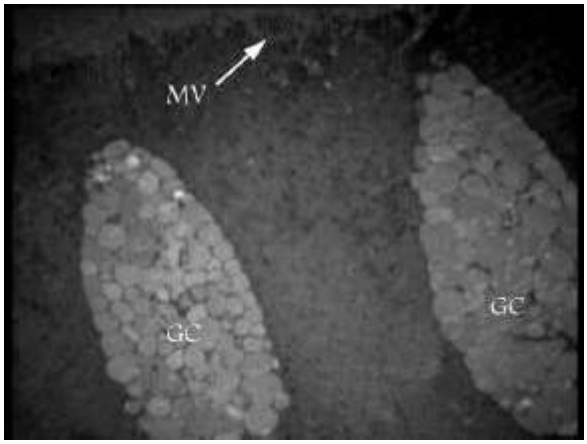

Fig. 15: Goblet cells (GC). Note that mucus granules with different sizes and densities occupy almost the whole cytoplasm. Microvilli (MV) of enterocytes are evident (Scale bar: 5.6µm)

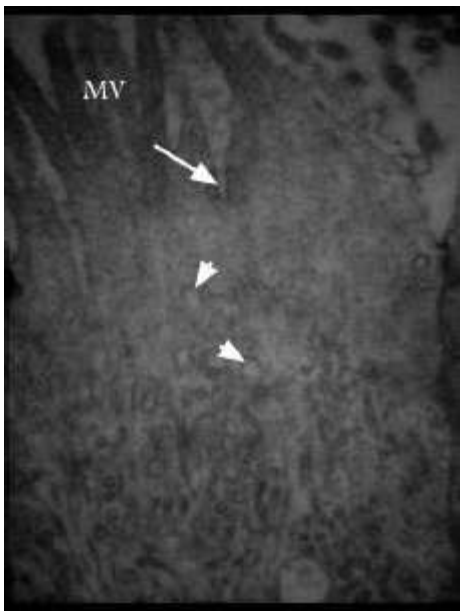

Fig. 16: Apical surface of an enterocyte in middle part of intestine: microvilli (MV), pinocytotic invagination (arrow) and many vesicles (arrowheads) (Scale bar: 0.93µm)

seen. The fibrilar threads project into the apical part of the cytoplasm where a terminal web is seen (Fig. 11). Oval nuclei with a nucleolus in the enterocytes are situated at the base of the cells (Fig. 9 and 10). The enterocytes are joind at the apical surfaces by the junctional complexes (Fig. 14). Numerous polymorphic mitochondria with a dense matrix (Fig. 12), lysosomes, vesicles and rough endoplasmic reticulum are evident in the upper cytoplasm of the cells (Fig. 13). Besides enterocytes, numerous large goblet cells are present so

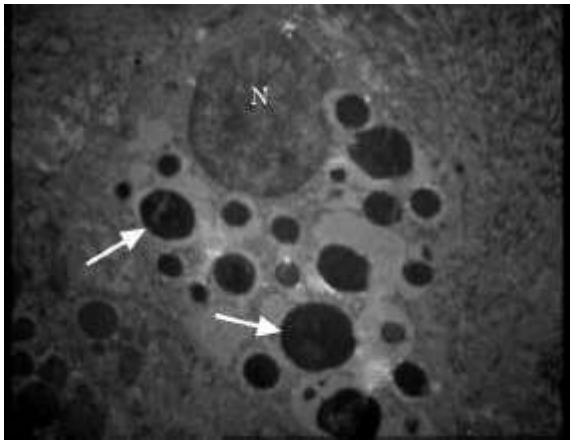

Fig. 17: Fine structure of a eosinophilic granular cells containing a round nucleus (N) and numerous electron dense granules (arrows) (Scale bar: 3.36µm)

that greater part of them are filled with mucous granules having variable sizes and electron densities that occupy almost the whole cytoplasm of the cells and the nuclei appearing basally (Fig. 15).

Eosinophilic granular cells that are predominantly located in the submucosa, show electron dense granules (Fig. 17). In the middle part of the intestine, enterocytes have many pinocytotic vesicles than other parts (Fig. 16).

DISCUSSION

Histologically, the overall structure of intestine in rainbow trout is similar to other fish, although few variations are distinguishable. In this study, villi showed progressively shorter length toward the posterior intestine. It has also reported that there were no mucosal differences between different regions in two related neotropical omnivorous fish except for villi lengths [19]. Ther are similar reslts in rice field eel [2]. These results accommodate with present study, but some researchers have observed distinct villi in the first two regions and not in the posterior intestine of flower fish [1]. The elevation of the mucosa into villi favores the absorptive role of it [9].

The increased density of goblet cells toward the posterior intestine observed in this study, has also reported in rice field eel and may imply the need for increased mucosa protection and lubrication for faecal expulsion [2, 9]. The presence of characteristic simple columnar epithelium in association with mucus producing goblet cells in the post – gastric intestinal mucosa have been observed in many fish and the first goblet cells can be determined early during the differentiation of the intestinal mucosa of fish [7, 9].

It has observed rare goblet cells on the intestinal villi of European catfish [7]. Intraepithelial lymphocytes (also called wandering or migratory lymphocytes) have also found in *Dentex dentex* [10], mud loach [6] and sea bass [20]. Leucocytes occur in all parts of the teleosts digestive system, most extensively in the intestine, where lymphocytes, plasma cells, granulocytes and macrophages are present in and under the epithelium. Although large lymphoid centers are lacking, many lymphoid cells, either scattered or in small groups were reported to be present in the epithelium and lamina propria, so that intraepithelial lymphocytes are necessary for a local or mucosal immune response [20].

In this study muscularis mucosae and mucosal tubular glands were not observed, but in pik's intestine there is muscularis mucosa and codfishes have deep glands in their intestine [21]. It is believed that the stratum compactum containing dense collagen layers function to strengthen and preserve the entirety of the gut wall [22].

The presence of mucus – secreting cells is a general character of teleosts, while secreted mucosubstances varies between species and different regions at digestive tract [23]. In this research it was clear that goblet cells have acidic and neutral mucosubstances. Similar findings have reported in northern pike and European catfish [7]. The acid mucosubstances are made by carboxylated and sulfated mucoconjugates. Some functions of mucus secreted by goblet cells are lubrication of undigested materials for unward progression and possible role in osmoregulation. Quality of gut mucosubstances is directly related to environmental conditions which in turn may directly affect the function of the alimentary tract. The presence of mucosubstances especially those sulfated in the intestine possibly regulates the transfer of proteins or a fragment of them as well as of ions and fluids [7]. Neutral mucosubstances combined with alkaline phosphatase assist in digestion and emulsification of food into chyme in vertebrates. Acidic mucins have been proposed to protect the intestinal epithelium against the degradative actions of glycosidases [10].

Intestinal ultrastructural features in this investigation were similar in different parts, with exception of pinocytotic vescicles that were present mostly in middle intestine and this can be related with high absorption rates in that part. Apparently in fish as in mammals, the surface of the intestine increases by 30-60 times due to microvilli in comparison with the smooth membrane. However in fish which vary in types of feeding, the degree of the increase in the digestive and transportive surface of the intestine due to the microvilli may differ [4]. It has reported that apical microvilli were a feature of epithelial cells from all regions of gastrointestinal tract in adult and juvenile Dover sole and cells from the distal regions showed invaginations and vacuolations of the apical cytoplanm [5].

Similar results with findings of this study regarding the presence of polymorphic mitochondria in the enterocytes, have been observed in pike, burbot and bream and it has also found that in mentioned fish, the goblet cells have granules with different sizes and densities. It has cleared that during the period of starvation, the number of cellular organells and their electron densities decrease. These data are evidence of the marked influence of feeding intensity on the ultrastructure of the intestinal mucosa in fish [4].

Eosinophilic granular cells (EGCs) are considered to be analogus to mammalian mast cells.It has characterized a series of rapid morphological EGCs responses to bacterial pathogens which may be involved in mediating inflammation in fish [24].

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