A HISTOLOGICAL STUDY ON THE DIGESTIVE TRACT OF EEL (ANGUILLA ANGUILLA L.) WITH SPECIAL REFERENCE TO ULTRASTRUCTURE OF GASTRIC GLANDULAR CELLS

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ABSTRACT

The digestive tracts were obtained from six healthy adult male and female eels, a migrating teleost, the tract was differentiated into oesophagus, stomach and intestine. Specimens from each were processed for light microscopy. Small pieces from gastric mucosa were processed for electron (transmission) microscopic examination of gastric glands. The results revealed a wide structural regional variations in the oesophagus. While, the mucosal lining of its anterior region was formed of stratified squamous epithelium containing taste buds, mucous cells and club (alarm substance) cells, it was replaced by simple columnar secretory epithelium forming serrated outlines at its posterior region. On the other hand, the tunica musculosa was formed of thick striated muscle bundles at the anterior region, which replaced by thick inner circular and thin outer longitudinal smooth muscle bundles at the posterior region. The structure of the gastric wall not showed regional differentiation. It was lined by high columnar cells. The gastric ducts were lined by mucous neck cells. The gastric glands were tubular in shape and lined by dominant cuboidal cell type. On the basis of ultrastructural features, the gastric glandular cells could be differentiated into oxynticopeptic cells, endocrine cells and basal cells. The intestinal tract showed an excellent regional differentiation. The anterior (respiratory) region was characterized by diminished low cuboidal lining epithelium followed by highly vascular lamina propria containing fine capillary bed well branched and extend to invest the epithelial lining. These features were associated with reduction of tunica musculosa and well -developed vascular serosa. The intestine at the mid-region and at the posterior region showed long mucosal folds and lined by high columnar epithelium containing goblet cells in between. The goblet cells were increased through the lining epithelium of the posterior region (rectum).

It can be concluded that, the digestive tract of eel may exert compound functions: taste, digestion as well as respiration (intestinal air breather teleost) to be adapted to its habitat.

INTRODUCTION

The alimentary tract of teleosts has attracted a considerable interest because of its diversity of form which has been related to diet (**Anderson, 1986**). Histological studies of the gut has been useful to assess disease problems (**Molnar, 1989**), nutritional stress (**Domeneghini et al., 2002**) and environmental toxicity (**Crespo et al., 1986**) as well as physiological adaptations to temperature changes (**Lee and Cossins, 1988**).

The eel, Anguilla anguilla L. is a carnivorous fish (**Landau, 1992**), lives most of its life span in fresh water. It migrates to sea water in spawning (**Sumich, 1999**). Its high commercial value makes its culture economically important in Japan, Taiwan and recently in Egypt (**El-Dosoky, 2001**). Little reports can be obtained in literatures on the histological structure of the eel digestive tract. The present investigation aimed to put a light spot on the microscopical structure of the eel digestive tract in the Egyptian sea water to be a guide for studying pathological or physiological alterations, either related to infectious or artificial diets.

MATERIAL AND METHODS

Six adult healthy male and female eels were used in this study. Their body lengths ranged from 50-55 cm. The fish were caught from Manzalla lake (Egyptian sea water) in March. The digestive tracts were dissected and photographed. For light microscopic examination, specimens from anterior, middle and posterior regions of oesophagus, stomach and intestine were prepared for paraffin sections (5-6µ), stained with haematoxylin and eosin (H & E), Crossmon's trichrome, periodic acid Schiff reagent and alcian blue (pH 2.5). The procedures were adopted according to **Bancroft and Stevens (1990)**. For transmission electron microscopic examination of gastric glands, small pieces of stomach mucosa were immediately fixed in 2.5% cold glutaraldehyde in phosphate buffer (pH 7.2) for 24 hours. Then post fixed in 1% cold osmium tetroxide in phosphate buffer (pH 7.2) for 4 hours. The specimens then were dehydrated in graded alcohol and embedded in araldite resin (**Hayat, 1989**). Semi-thin sections (1 µ in thickness) were stained with 1% toluidine blue to select the field for ultrathin sections, mounted in grids, stained with uranyl acetate and lead citrate and examined by JEOL 100 CX transmission electron microscope.

RESULTS

Gross anatomy :

The length of the alimentary tract of eel was relatively short, approximately 40% of the whole body length. The oesophagus was generally short. While its anterior region was wide and funnel shaped, its posterior region was tubular in shape. The oesophagus was followed by direct connection with the stomach. The stomach was long, simple tube-like with blinded end (have no any pyloric caeci). The stomach connected the intestine at the gastrointestinal sphincter. The intestine was relatively short, it became narrower toward its distal region, then it widened to form a rectum (Fig. 1).

Microscopic structure :

The digestive tract of eel in general showed the structural arrangement that is typical of tubular organs. The epithelial mucosal lining was underlain by lamina propria / submucosa of fibroelastic connective tissue and a tunica musculosa composed of inner circular and outer longitudinal layers of smooth muscle fibers. The tract was covered externally by serosal layer.

Oesophagus :

The anterior oesophagus displayed large number of primary, high longitudinal mucosal folds (Fig. 2). The mucosal folds were covered by stratified squamous epithelium (Figs. 2 & 3). It included taste buds, mucous cells and club (alarm substance) cells in between its strata (Figs. 3 & 4). The taste buds were long, pear shaped located in the epithelium of the mucosal fold apices. Their structure was represented by light and dark staining cells alternating with each other (Fig. 3). The mucous cells were large, spherical in shape with foamy cytoplasm reacted positively to PAS. They were located in the superficial cell layers. The club-shaped (alarm substance) cell were large polyhedral, located at different levels of epithelial strata (Fig. 4). The lamina propria/ submucosa was formed of fibrous layer of connective tissue formed mainly of collagenic fibers, which extended to fill the core of mucosal folds (Fig. 2). The tunica musculosa was formed of thick layer of longitudinally arranged striated muscle bundles (Fig. 2). The anterior oesophagus was covered externally by adventitia.

The posterior oesophagus displayed broad and long mucosal folds alternating with short triangular ones. The mucosal folds were covered by columnar cells. These cells formed serrated margin (Fig. 5). However, there were no goblet cells were located in between the lining epithelium, the apical border of the columnar lining epithelium was reacted positively to periodic acid schiff-reagent (secretory columnar epithelium). (Fig. 6). The lamina propria / submucosa was

formed of densely packed connective tissue which extended to fill the core of the mucosal folds (Fig. 5). The tunica musculosa was formed of very thick inner circular and thin outer longitudinally arranged smooth muscle fibers (Figs. 5 & 6). The posterior oesophagus was covered externally by serosa (Fig. 5).

Stomach:

The stomach structure appeared to be undifferentiated into regions along its length except at the sphincter area (gastrointestinal sphincter). The mucosal layer was thrown up into long, broad pyramidal folds (Fig. 7). The stomach was entirely lined by tall columnar cells (Figs. 7 & 8). The lamina propria / submucosa was formed of densely packed fibroelastic connective tissue which extended to fill the core of mucosal folds (Fig. 7). It contained a continuous layer of subepithelial gastric glands. They were simple branched tubular type opened directly to the surface by short ducts (Figs. 7 & 8). The gastric ducts were lined by columnar mucous neck cells. The glandular epithelium was formed of dominant type of cuboidal cells (Fig. 8) which have no reaction to alcian blue or PAS. Tunica musculosa was composed of thick inner circular and thin outer longitudinally arranged smooth muscle layers. The stomach was covered externally by serosa.

Ultrastructure of the gastric glandular cells :

Transmission electron microscopy demonstrated three types of cells lining the gastric glands, viz. oxynticopeptic cells, endocrine cells and basal cells.

The oxynticopeptic cells were cuboidal in shape with centrally located spherical nuclei. The texture of the nuclear material was uniformly granular. The nuclei in general were regularly contoured, however, those may occasionally showed some surface indentations (Figs. 9, 10), also prominent nucleolus with electronoptically dense nature were evident (Fig. 10). The lateral cell membranes were straight (Figs. 9 & 11) but were elaborately interdigitated greatly to form basal interdigitated whorls at the basal cell membranes (Fig. 10). Widely distributed mitochondria were generally located all over the cytoplasm (Figs. 9 & 10). Many of their cristae were seen to transverse the full width of the mitochondira (Figs. 9 & 10 and 11). Well developed intracytoplasmic tubular network of smooth endoplasmic reticulum were notable (Figs. 9 & 10). Notable groups of rough endoplasmic reticulum tended to be oriented more or less parallel (Fig. 9). Spherical electron dense granules with smoothed surfaced membranes were distributed into the cytoplasm (Figs. 9 & 11).

The endocrine cells were lodged between the oxynticopeptic cells. They were characterized by electron lucent cytoplasm and few electron dense granules. Their cohesion with oxynticopeptic cells was ensured by numerous desmosomes (Fig. 11). The endocrine cells have large ovoid nuclei with well prominent nucleoli. The nuclei had clumps of sparsely located heterochromatin

which attached peripherally to inner surface of the nuclear membrane (Figs. 11 & 12). Their electron lucent cytoplasm contained numerous cisternae of smooth endoplasmic reticulum. Few mitochondria were occasionally located around the nuclear region. The cytoplasmic dense granules were small and distributed all over the cytoplasm (Fig. 11).

The basal cells were small polyhedral in shape. They have large rounded nuclei with well prominent nucleoli. The basal cells showed lateral interdigitations with each others. Their apical membranes form the interdigitated whorls with the basal membranes of oxynticopeptic cells (Fig. 12). The cytoplasm of the basal cells appeared dark contained few cytoplasmic organelles.

The gastro-intestinal sphincter :

The stomach was terminated and the intestine was initiated, both regions were sheathed by heavy circularly arranged smooth muscle fibers forming gastro-intestinal sphincter (Figs. 13 & 14). The stomach at the sphincter area was composed of mucosa, submucosa and musculosa. The mucosa was thrown into long, highly branched mucosal folds. The lamina epithelialis was formed of simple columnar cells having well prominent basely situated nuclei and apical acidophilic cytoplasm. The lamina propria was formed of fibroelastic connective tissue extended to fill the core of the mucosal folds. The submucosa was formed of loose connective tissue. The absence of gastric glands in this region was the most characteristic feature of this gastric region. Tunica musculosa was represented by heavy circularly arranged smooth muscle fibers (muscle sphincter) (Fig. 13). The intestine at the sphincter area was appeared as several pouches which separated by connective tissue septa. The intestinal pouches were lined by high columnar epithelium with basally situated prominent nuclei. All the intestinal pouches were surrounded by inner heavy circularly arranged smooth muscle bundles (muscle sphincter) (Fig. 14), and outer thin longitudinally arranged smooth muscle bindles. The sphincter was covered externally by serosa.

Intestine:

The intestinal structure appeared to be differentiated into three regions. Firstly, anterior intestine (respiratory). Its mucosa was thrown into broad long folds. They were flappy in shape leaving a wide lumen in between (Figs. 15 & 16). The lamina epithelialis was diminished to flat or low cuboidal cells (Figs. 16 & 17). It contains pale staining goblet like cells. Subepithelial lymphocytic infiltrations were occasionally located (Fig. 17). Lamina propria / submucosa was formed of loose fibroelastic connective tissue containing subepithelial capillary bed. The blood venules which were filled with ellipsoidal blood cells and lined by thin endothelium were branched and connected beneath the lining epithelial cells to form subepithelial capillaries (Figs. 16 & 17). The tunica musculosa showed a marked reduction in thickness at this region. It was

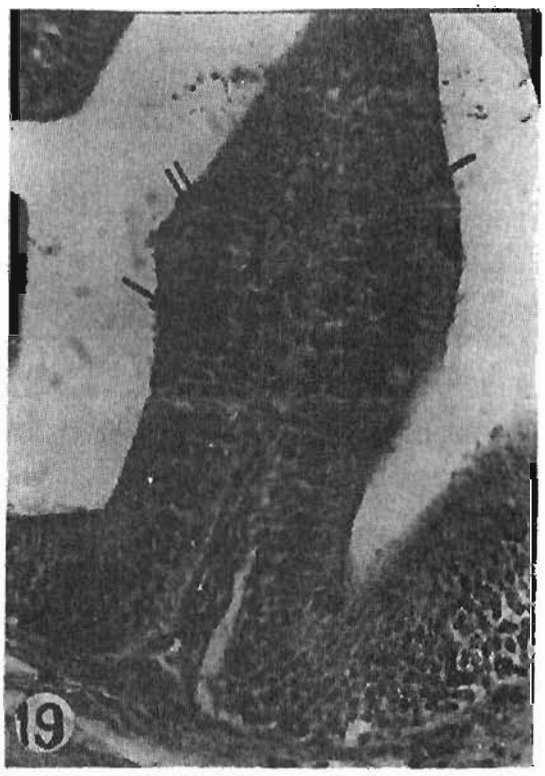

Fig. (19) : A photomicrograph of a section of the mid-intestinal region of eel showing, long mucosal fold (F), covered by high columnar epithelium (arrows) containing few goblet cells (double arrows). The propria / submucosa was formed of thin layer of fibroelastic connective tissue (P) extended to form the core of mucosal folds containing high subepithelial lymphocytic infiltrations (L). (Alcian blue, x400).

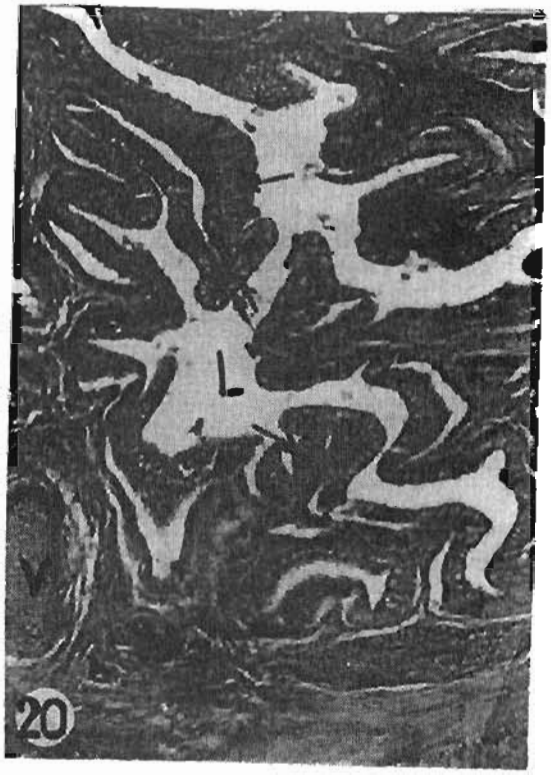

Fig. (20) : A photomicrograph of longitudinal section of the distal intestinal region (rectum) of eel showing, long wavy mucosal folds (arrows), leaving a wide lumen in between (L). Lamina epithelialis was formed of columnar cells containing numerous goblet cells in between (double arrows). Lamina propria / submucosa is formed of connective tissue mainly collagenic fibers. Notice the rectal valve which formed of circularly arranged smooth muscle bundles (v). (Crossmon's trichromic st., x40).

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الملخص العربى

دراسة هستولوجية على القناة الهضمية فى أسماك الثعابين بالإشارة إلى التركيب الدقيق لخلايا غدد المعدة

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لقد أستخدم فى هذا البحث عدد ست قنوات هضمية لإناث وذكور أسماك الثعابين البالغة (أسماك عظمية مهاجرة) وقد تألفت القناة الهضمية من مرىء ومعدة وأمعاء، جهزت عينات من كل منها للفحص بالميكروسكوب الضوئى، كما جهزت أجزاء صغيرة من الغشاء المخاطى المبطن للمعدد وذلك لفحص غدد المعدة بالميكروسكوب الإلكترونى (النافذ) وقد أظهرت النتائج مايلى :-

أظهر المرىء إختلافات واسعة فى تركيب أجزائه المختلفة، فبينما كان الغشاء الطلائى المبطن للجزء الأمامى مكوناً من غشاء مصفف حرشفى محتوى على براعم تذوقية وخلايا مخاطية وأخرى منبهه للخطورة إلا أنه تحول فى الجزء الخلفى إلى نسيج طلائى مكون من طبقة واحدة من الخلايا المفرزة العمادية الطويلة وكانت ذات حافة مستنة، أما الطبقة العضلية فكانت مكونة من حزم عضلية مخططة وسمكية فى الجزء الأمامى ثم تحولت إلى طبقتين داخلية سمكية ودائرية الترتيب وأخرى خارجية رفيعة وطولية الترتيب فى الجزء الخلفى.

لم تظهر المعدة أى إختلافات فى تركيبها النسجى تجعلها مقسمة إلى مناطق فقد كانت على مدى إمتدادها مبطنة بنوع الخلايا العمادية الطويلة أما قنوات غدد المعدة فكانت من نوع الخلايا العنقية المخاطية، كما كانت الغدد المعدية أنبوبية الشكل وهى مبطنة بنوع سائد من الخلايا المكعبة، أظهر التركيب الدقيق لها أنها مؤلفة من خلايا مفرزة للحامض والبيبسين بينها خلايا صماء وأخرى قاعدية.

أما الأمعاء فكانت واضحة فى تميزها إلى مناطق، فالجزء الأمامى منها (التنفسى) فيها تضاءلت خلايا الطبقة المبطنة إلى النوع المكعب المنخفض وكانت مصاحبة لها وسادة من التعيرات الدموية المتفرعة فى الطبقة اللبادية لتمتد فروع منها تحت خلايا الطبقة الطلائية وبالإضافة إلى ذلك ظهر تصاؤلاً فى الطبقة العضلية أما الطبقة الليفية الخارجية فكانت نامية ووعائية، أما فى الجزء الأوسط والجزء الخلفى من الأمعاء فقد أظهرت الطبقة المخاطية ثنايا طولية عالية أما الغشاء المبطن لهما كان مكوناً من نوع الخلايا العمادية العالية متضمنة فيما بينها خلايا كأسية مخاطية بزداد عددها فى الجزء الخلفى من الأمعاء (المستقيم).

والخلاصة : أن القناة الهضمية فى أسماك الثعابين لها وظائف مركبة منها التذوق والهضم وكذلك التنفس (أسماك عظمية ذات تنفس هوائى معوى).