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Research article

The existence of argyrophilic endocrine cells in the digestive system of snake eels (*Pisodonophis boro*, Hamilton, 1822)

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Abstract

Observation of argyrophilic endocrine cells (AEC) in the digestive system of Rice-paddy eel (*Pisodonophis boro*, Hamilton, 1822), an economically important fish, was first examined. The existence of AEC was clearly detected along the digestive system, except for the posterior intestine by the Grumelius silver staining method. Two types of AEC (closed-type cell and open-type cell) were classified and observed within the mucosal layer. The closed-type cell was small and spherical in shape, whereas the open-type cell was triangular or elongated. The AECs in the stomach were detected in both the mucosal layer and the gastric gland, which were higher in abundance than that in the esophagus. The highest numbers of AEC were observed in the anterior intestine, whereas it was not observed in the posterior intestine. In addition, several granules in the hepatocyte and some cells in Langerhan's islets in the pancreas positively reacted with this method. The results indicated that the digestive tract and especially the anterior intestine may be the main site of AEC relating to the production of digestive hormones.

Keywords: Digestive system, Gastrointestinal endocrine cells, Snake eel, Thailand

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INTRODUCTION

The gastrointestinal endocrine cells (GECs) or gut endocrine cells are characteristically found in the digestive tract where they are exclusively detected in the mucosal layer and mucosal glands (Wang et al., 2010). Two types of GECs have been identified: open-type and close type cells. The open-type cell is the smaller cell of the two; it is in contact with the glandular lumen. The closed-type cell is larger and does not have a luminal connection (Solcia et al., 2000). Ultrastructural and biochemical studies have shown that the cytoplasm of the GECs contained enterohormones and secretory granules, which have been suggested to play a physiological role in the synthesis of a variety of enzymes and gastrointestinal (GI) hormones such as secretin, cholecystokinin and pancreozymin (Barrington and Dockray, 1972; Cetin, 1992; Ku et al., 2004; Rindi et al., 2004; Wang et al., 2010). Based on the presumptive importance of GECs, studies of their prevalence and characteristics in the GI system and corresponding functions have been warranted (El-Salhy and Sitohy, 2001).

Argyrophil endocrine cells (AEC), or secretory cells, are a key GEC component. The AEC plays a role in the amine precursor uptake and decarboxylation (APUD) system of the GI, and is crucial in the synthesis of GI hormones. AEC was first identified by Erspamer (1954). Although several studies have investigated the distribution, morphology and function of AEC in the digestive system of vertebrates (Fonseca et al., 2002; Kostiukevich, 2004; Rindi et al., 2004), very few studies have examined AEC in fish (Rombout, 1977; Mokhtar et al., 2015a). Many vertebrate studies have lent support to the important role of AEC in evolutionarily divergent animal lineages (Rombout, 1977; Fonseca et al., 2002; Kostiukevich, 2004; Rindi et al., 2004; Wang et al., 2010). Information from the earlier studies details the distribution of AEC throughout the digestive tract, with AEC prominently found in the esophagus, stomach and both the anterior and posterior intestine of many species (Rombout, 1977; Mokhtar et al., 2015a; 2015b). AEC have also been found in the liver and pancreas, although their functions, ultrastructure and prevalence are not yet well understood (Mokhtar, 2015).

AEC has not yet been clearly identified in the snake eel, *Pisodonophis boro* (Hamilton, 1822), which belongs to the family Ophichthidae. The snake eel has major economic value and potential for commercial fish markets in Thailand and throughout southeast Asia. In this study, we observed both the distribution and the number of AECs throughout the digestive system and in accessory organs (i.e., liver and pancreas) of *P. boro* using the Grumelius silver method. The Grumelius silver technique can easily identify the AEC from other components in histological sections. This technique is very widespread and robust for the identification and quantification of AEC, as recommended by several investigators (Hellerstrom and Hellman, 1960; Hellman and Hellerstrom, 1961; Grimelius, 1968a, 1968b).

MATERIALS and METHODS

Study site and fish collection

Female *P. boro* (n=10, average body length = 80 cm) were obtained during March to May 2015 from the Pranburi River estuary, Thailand (N 12°24'8.5" / E 99°59'0.2") and euthanized by a rapid cooling shock technique (Wilson et al., 2009). After dissecting fat and connective tissue, the digestive tract (esophagus to intestine) together with accessory organs (liver and pancreas) were fixed in Davidson's fixative for 36-48 hours at room temperature. The experimental protocol was approved by the Animal Care and Use Committee of the Faculty of Science, Chulalongkorn University (Protocol Review No. 1523005).

Histological observations to detect the existence of the argyrophil endocrine cell

The fixed tissues were processed by using standard histological techniques (Presnell and Schreibman, 1997; Suvarna et al., 2013). The tissue paraffin blocks were cut in 4 µm sections. The AEC were detected by using the Grumelius silver method (Grimelius and Wilander, 1980). Briefly, the sections were deparaffinized, hydrated with acidulated water for 5 minutes and treated with a 0.1% silver solution at 60°C for 1 hour. Finally, the sections were rinsed in tap water, dehydrated, cleared in xylene and covered with Permount. The distribution of AECs was investigated and photomicrograph with a Leica TE2000-U light microscope (Leica Camera AG, Wetzler, Germany).

Estimating the number of argyrophilic endocrine cells

The numbers of AECs were quantified from the histological sections. Three representative slides/regions of the digestive tract were examined. From each slide, all sections were viewed with ten randomized areas of the mucosal layer per section. The numbers of AECs were enumerated and averaged (mean±SD). One-way ANOVA was used to compare means of the number of AEC from different regions of the GI.

RESULTS and DISCUSSION

Overview

The present study demonstrated the distribution and qualitative data of argyrophilic endocrine cells (AEC) in the digestive system of the snake eel (*P. boro*, Figures 1- 3). The digestive tract examined in this species of fish included the esophagus, stomach, and anterior and posterior intestines. Each of these four components of the digestive tract consisted of four histologically distinct layers including the mucosa, submucosa, muscularis and serosa (Figure 1); importantly, AECs were observed only in the mucosa of *P. boro* (Figures 1-3), which is consistent with other fishes (Rombout, 1977; Mokhtar et al., 2015a). Two types of AEC cells were observed in different areas of the esophagus. AEC were

distributed among the mucosal epithelial cells (Figures 1A-1B), whereas some AECs were also detected beneath the goblet cells (Figure 1B). When viewed from histochemical sections of the stomach, a few of the AECs of two types were found and were basically located in the mucosal epithelial cells as well as in acinar gastric glands (Figure 1D). Unlike in higher vertebrates, only the close-type cells were found in the stomach regions (Ku et al., 2004; Wang et al., 2010).

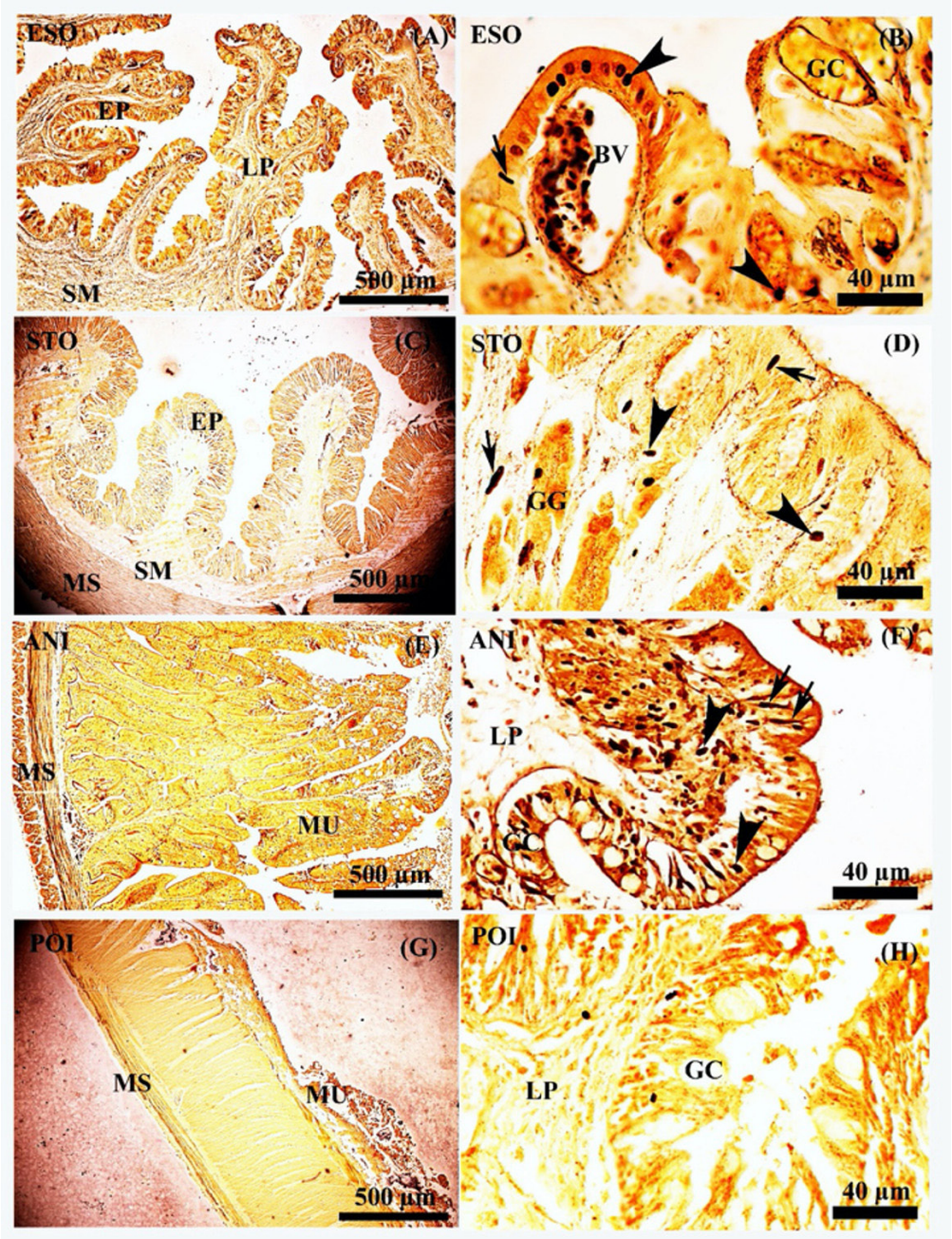

Figure 1 The detection of argyrophilic endocrine cells (AECs) in the digestive tract of *Pisodonophis boro* in the esophagus (ESO in A, B), the stomach (STO in C, D), the anterior intestine (ANI in E, F) and the posterior intestine (POI in G, H), respectively. Abbreviations and symbols are denoted as follows: arrowhead, close-type of AEC; arrow, open-type of AEC; BV, blood vessel; EP, epithelium; GC, goblet cell; GG, gastric gland; LP, laminar propria; MU, mucosa; MS, muscularis; SM, submucosa.

High magnification of histological sections in the digestive system of *P. boro* indicated that two types of AEC were present in the apical region of the mucosal layer; the distinction of these two types was based on cell locality and histological differences. The close-type cell with the small and round shape was prominently present (Figure 1B). In contrast, the open-type cell was triangular or elongated in shape with an apical cytoplasmic process (Figure 1F). The open-type cell was mostly located near blood vessels (Figure 1B), supporting the hypothesis that this cell type is involved with the release of digestive hormones into the blood, implying an endocrine function. Bülbring and Crema (1959) reported a similar pattern in which AECs were present close to blood vessels. It is likely that these AECs secrete hormones including secretin, cholecystokinin and pancreozymin (Barrington and Dockray, 1972; Cetin, 1992; Rindi et al., 2004).

Quantitative distribution of AEC in digestive tract

In the present study, the distribution and number of the two types of AECs in different areas of the digestive tract showed significantly higher levels in the intestinal area (Figure 2), but it was entirely absent in the posterior intestine (Figures 1G-1H). This is consistent with former observations in some fishes (Holmgren and Olsson, 2009; Mokhtar et al., 2015a) such as *Ctenopharyngodon idella* (Mokhtar et al., 2015a) and *Clarias gariepinus* (Mokhtar et al., 2015b). These showed that most of the enteroendocrine cells can be found in the anterior intestine, and in other vertebrates (Yamamoto, 1966; Ezeasor and Stokoe, 1981). High magnification revealed that two types of AECs were present in the mucosal intestine of this species (Figure 1F), an observation that is in contrast to several studies that observed only the open-type cell in the intestine where they are involved in the secretion of neuroendocrine substances used for digestion (Noaillac-Depeyre and Hollande, 1981; Arena et al., 1990; Pan et al., 2000). These digestive hormones include gastrin, gastric inhibitory peptide, glucagon, pancreatic polypeptide and secretin endocrine (Noaillac-Depeyre and Hollande, 1981; Arena et al., 1990; Pan et al., 2000).

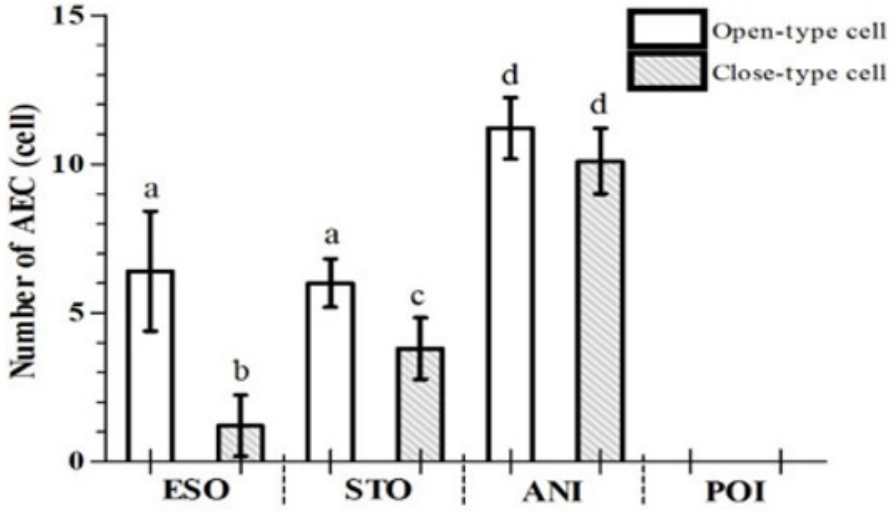

Figure 2 The distribution and number of close-type and open-type argyrophilic endocrine cells (AECs) in different regions of the digestive tract in *Pisodonphis boro*; data are presented as mean±SD, n=10. Different letters above each bar denote statistically significant differences (ANOVA, Bonferrani's Multiple Comparison test, P<0.0001). Abbreviations: ANI, anterior intestine; ESO, esophagus; POI, posterior intestine; STO, stomach.

The organization of the liver and pancreas were also histologically observed (Figure 3). The liver was composed of several hepatocytes (Figures 3A-3B). Several different types/sizes/shapes of granules within the hepatocyte were detected with the Grumelius silver method (Figures 3A-3B). However further research is needed to understand the importance of these silver-staining granules. The pancreas was divided into exocrine and endocrine portions (Figures 3C-3D). The exocrine pancreatic tissue was composed of scattered serous acini, which positively reacted with the silver stain (Figure 3C). The endocrine pancreatic tissue with the Langerhan's islets surrounded the exocrine acinar cells (Figure 3C). Each islet was surrounded by a thin capsule of connective tissue. Small cytoplasmic granules of AECs were scatted in these pancreases (Figure 3), as similar to a previous observation in the pancreas of *Ct. idella* (Mokhtar, 2015). However, the roles of these small cytoplasmic granules are unknown and should be explored.

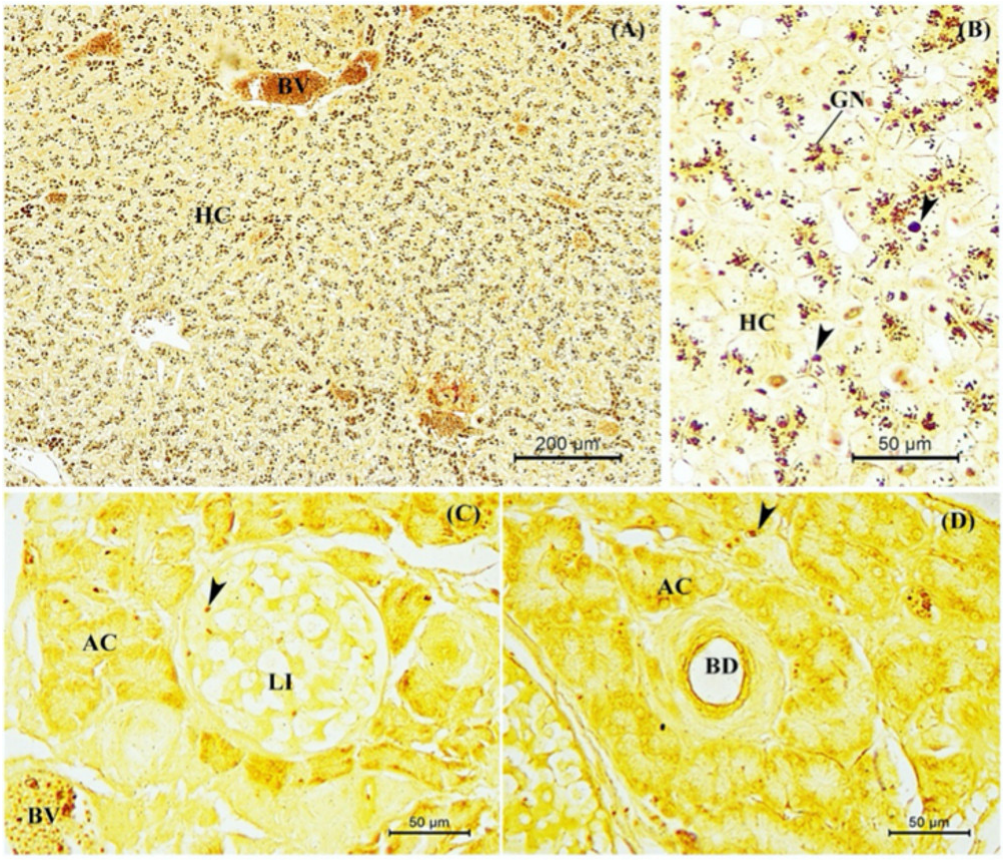

Figure 3 Light microscope images showing the presentation of the argyrophilic endocrine cells (AECs) in the liver (A, B) and the pancreas (C, D) of *Pisodonophis boro*. Abbreviations and symbols denoted as the following: arrowhead, AEC; AC, acini; BD, bile duct; BV, blood vessel; GN, granules; HC, hepatic cell; LI, Langerhan's islets.

CONCLUSION

Our observations were the first to document on the distribution and abundance of AEC in the digestive system of *P. boro*. The existence of AECs was clearly detected along the digestive system, with the exception of the posterior intestine by the Grumelius silver staining method. However, some aspects such as the absence of AECs in the posterior intestine area remain unknown and warrant further study.

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REFERENCES

Arena, P.C., Richardson, K.C., Yamada, J., 1990. An immunohistochemical study of endocrine cells of the alimentary tract of the King's skink (*Egernia kingii*). J. Anat.170, 73-85.

Barrington, E.J., Dockray, G.J. 1972. Cholecystokinin - pancreozymin - like activity in the eel (*Anguilla anguilla L.*). Gen. Comp. Endocrinol. 19, 80-87.

Bülbring, E., Crema, A. 1959. The action of 5-hydroxytryptamine, 5-hydroxytryptophan and reserpine on intestinal peristalsis in anaesthetized guinea-pigs. J. Physiol. 146, 29-53.

Cetin, Y. 1992. Chromogranin A immunoreactivity and Grimelius's argyrophilia. A correlative study in mammalian endocrine cells. Anat. Embryol. (Berl). 185, 207-215.

El-Salhy, M., Sitohy, B. 2001. Abnormal gastrointestinal endocrine cells in patients with diabetes type 1: relationship to gastric emptying and myoelectrical activity. Scand. J. Gastroenterol. 36, 1162-1169.

Erspamer, V. 1954. Pharmacology of indole-alkylamines. Pharmacol. Rev. 6, 425-487.

Ezeasor, D.N., Stokoe, W.M. 1981. Light and electron microscopic studies on the absorptive cells of the intestine, caeca and rectum of the adult rainbow trout, *Salmo gairdneri*, Rich. J. Fish Biol. 18, 527-544.

Fonseca, C.C., Nogueira, J.C., Barbosa, A.J. 2002. Argyrophilic and glucagon-immunoreactive cells in the ileum and colon of the developing opossum *Didelphis albiventris* (Marsupialia). Cells Tissues Res. 170, 29-33.

Grimelius, L. 1968a. A silver nitrate staining for alpha 2-cells in human pancreatic islets. Acta Soc. Med. Upsal. 73, 243-270.

Grimelius, L. 1968b. The argyrophil reaction in islet cells of adult human pancreas studies with a new silver nitrate procedure. Acta Soc. Med. Upsal. 73, 271-294.

Grimelius, L., Wilander, E., 1980. Silver stains in the study of endocrine cells of the gut and pancreas. Invest. Cell. Pathol. 3, 3-12.

Hellerstroem, C., Hellman, B. 1960. Some aspects of silver impregnation of the islets of Langerhans in the rat. Acta Endocrino. 35, 518-532.

Hellman, B., Hellerstrom, C. 1961. The specificity of the argyrophil reaction in the islets of Langerhans in man. Acta Endocrinol. (Copenh) 36, 22-30.

Holmgren, S., Olsson, C. 2009. Fish neuroendocrinology. In: Bernier, N.J., Van Der Kraak, G., Farrell, A.P., Brauner, C.J. (Eds.), The neuroendocrine regulation of gut function. (Fish Physiology Series). pp. 467-512.

Kostiukevich, S.V. 2004. Endocrine cells of mucosal epithelium in the distal part of the intestine of *Lacerta vivipara*. Tsitol. 46, 202-207.

Ku, S.K., Lee, H.S., Lee, J.H. 2004. Changes of gastrointestinal argyrophil endocrine cells in the osteoporotic SD rats induced by ovariectomy. J. Vet. Sci. 5, 183-188.

Mokhtar, D.M. 2015. Histological, histochemical and ultrastructural characterization of the pancreas of the grass carp (*Ctenopharyngodon idella*). Eur. J. Anat. 19, 145-153.

Mokhtar, D.M., Abd-Elhafez, E.A., Hassan, A.H.S. 2015a. A histological, histochemical and ultrastructural study on the fundic region of the stomach of Nile catfish (*Clarias gariepinus*). J. Cytol. Histol. 6, 341.

Mokhtar, D.M., Abd-Elhafez, E.A., Hassan A.H.S. 2015b. Light and scanning electron microscopic studies on the intestine of grass carp (*Ctenopharyngodon idella*): I-anterior intestine. J. Aqua. Res. Devel. 6, 374.

Noaillac-Depeyre, J., Hollande, E. 1981. Evidence for somatostatin, gastrin and pancreatic polypeptide-like substances in the mucosa cells of the gut in fishes with and without stomach. Cell Tissue Res. 216, 193-203.

Pan, Q.S., Fang, Z.P., Zhao, Y.X. 2000. Immunocytochemical identification and localization of APUD cells in the gut of seven stomachless teleost fishes. World J. Gastroenterol. 6, 96-101.

Presnell, J.K., Schreibman, M.P. 1997. Humason's Animal Tissue Techniques (5ed.). Johns Hopkins University Press, US.

Rindi, G., Leiter, A.B., Kopin, A.S., Bordi, C., Solcia, E. 2004. The "normal" endocrine cell of the gut: changing concepts and new evidences. Ann. N Y Acad. Sci. 1014, 1-12.

Rombout, J.H. 1977. Enteroendocrine cells in the digestive tract of *Barbus conchonius* (Teleostei: Cyprinidae). Cell Tissue Res. 185, 435-450.

Solcia, E., Rindi, G., Buffa, R., Fiocca, R., Capella, C. 2000. Gastric endocrine cells: types, function and growth. Regul. Pept. 93, 31–35.

Suvarna, K.S., Layton, C., Bancroft, J.D. 2013. Bancroft's theory and practice of histological techniques (7ed.). Elsevier, Canada.

Wang, J.X., Peng, K.M., Liu, H.Z., Song, H., Chen, X., Min, L. 2010. Distribution and morphology of argyrophilic cells in the digestive tract of the African ostrich. Tissue Cell 42, 65-68.

Wilson, J.M., Bunte, R.M., Carty, A.J. 2009. Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). J. Am. Assoc. Lab. Anim. Sci. 48, 785-789.

Yamamoto, T. 1966. An electron microscope study of the columnar epithelial cell in the intestine of fresh water teleosts: goldfish (*Carassius auratus*) and rainbow trout (*Salmo irideus*). Zeit. Zekkfirsch. 72, 66-87.

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