

Research

Distribution of gastrin and secretin immunoreactive cells in the gut of an Indian Skipper frog, *Euphlyctis cyanophlyctis* (Schneider): An immunohistochemical study

Ritesh Kumar Shukla^{1,*} & N. Venkat Appa Rao¹

Abstract

The present investigation deals with the identification, localization, and mapping of gastrin and secretin-producing endocrine cells in different regions of the alimentary canal of *Euphlyctis cyanophlyctis* (Schneider) through the immunohistochemical (IHC) technique. G-IR (gastrin immunoreactive) cells of both the open and the closed types were present abundantly in the gastric mucosa of the pyloric region, in comparison to that of the fundic region. The open type of cells had long protoplasmic extensions reaching up to the lumen, suggesting the lumone concept of gastrin secretion. While the closed types were restricted towards the basal region of the mucosa and occurred in polymorphic forms ranging from rounded, oval, pyramidal, to amoeboid appearance. The closed type of G-IR cells was recorded in the duodenal mucosa, revealing the source of intestinal gastrin. Thus, suggesting a hormone-lumone-parahormone concept of gastrin secretion. The S-IR (secretin immunoreactive) cells were localized in both the duodenal and ileum regions of the alimentary canal. Comparatively, the number of S-IR cells was abundant in the duodenal mucosa, with respect to the ileum region. In the myenteric plexus of the ileum region, abundant secretin immunoreactivity was recorded, indicating that secretin not only acts as a hormone but also acts as a neuromodulator. All the S-IR cells were of the closed type, and no open type has been observed in the present study. The immunohistochemical study of amphibian gut endocrine cells provide valuable insights into gut health, gastrointestinal disorders, and the physiology and phylogeny of gut endocrine cells, thereby contributing to a deeper understanding and reframing of amphibian gut biology.

Received: 27 October 2025
Accepted: 01 December 2025
Published: 31 December 2025

¹Department of Zoology, St. Xavier's College, Ranchi, Jharkhand, India

*Correspondence:
riteshshukla@sxcran.org

Keywords

Immunohistochemistry;
Gut endocrine cells;
Indian Skipper frog;
Euphlyctis cyanophlyctis;
Gastrin;
Secretin

Introduction

The enteroendocrine cells, variously called Helle-Zellen cells or clear cells or agyrophylic cells (Feyrter, 1953) are one of the vital components of the enteric epithelial layer, involved in regulating the gastro-intestinal physiology, thus making the mechanically and chemically processed food ready for absorption. This results to the utilization of these nutrients for growth, energy production, maintenance and perpetuation by the different body cells. These enteroendocrine cells comprise about 1% of the total epithelial cells in the gastrointestinal tract, but still they are the essential regulators of digestion (May & Kaestner, 2010).

The specific endocrine cells are present in the different regions of the alimentary tract, uniquely diffused amidst the non-endocrine cell population of the gastro-enteric epithelium (Pearse, 1981). Till date hundreds of such different cells have been identified which through their specific secretions carry out many specific physiological actions in the specific regions of the digestive tract. The physiological

Citation

Shukla, R. K. & Rao, N. V. A. (2025). Distribution of gastrin and secretin immunoreactive cells in the gut of an Indian Skipper frog, *Euphlyctis cyanophlyctis* (Schneider): An immunohistochemical study. *T Appl Biol Chem J*, 6(4), e64001. DOI: [10.52679/tabcj.2025.64001](https://doi.org/10.52679/tabcj.2025.64001)

actions include the regulation of the secretions of enzyme and non-enzyme containing gastro-intestinal juices, regulating the mobility of the gastrointestinal tract (Track, 1980; Deveney & Wal, 1983), signaling the growth, repair and differentiation of enteric epithelial cells (Ekundayo et al., 1995) and even regulating the food intake and energy homeostasis through orexigenic effect (Gil-Campos et al., 2006). The above physiological actions are accomplished through autocrine, (Hoosein et al., 1990), paracrine/juxtacrine, luminocrine, endocrine manner (Larsson et al., 1979; Rehfeld, 1998; Hofer et al., 1999) and by release of gut hormones in the synaptic regions (Vizi et al., 1972; Dawson, 1978).

The gut endocrine cells have been classified as open, closed (Fujita & Kobayashi, 1974) and transitional type depending upon their morphology and mode of release of their secretory product. The open type of cells has long protoplasmic extensions reaching up to the lumen, thus releasing their content into the lumen and also involved sensing the gut content (Sternini et al., 2008), thereby get stimulated to secrete their hormones. Rao and Shukla, (2013) in their studies on *Lepidocephalus guntea*, have demonstrated the presence of open type of G – cells which are in the process of releasing their contents into the space between the intestinal villi, thereby suggesting the role of gastrin as lumone.

On the contrary the closed type of endocrine cells is devoid of such extensions and remains restricted to the basal lamina and release their products into the blood circulation (Fujita & Kobayashi, 1977). Shukla and Rao (2015) in the immunohistochemical (IHC) studies of gastrin producing cells in Indian Skipper frog *Euphyctis cyanophlyctis* have reported the third transitional types of cells with multiple protoplasmic processes are extending to variable directions indicating their possibility of transformation from one form to other depending upon their requirement.

The study of endocrinology started from the troika group of enteric hormones, secretin (Baylis & Starling, 1902), gastrin (Edkins, 1905) and cholecystokinin (Ivy & Oldberg, 1928). The identification, localization and distribution of the gut endocrine cells have been achieved through histochemical (Fange, 1962), ultrastructural (Vasallo et al., 1969), IHC, immunofluorescent (Coons et al., 1955) and IHC coupled with ultrastructural methods (Sun & Song, 2003). During the last few decades, the improvised IHC technique has been employed for the detection of more than one hundred gut hormones and putative hormone secreting cells including those of sorbin (Abou et al., 2000) ghrelin, (Zhao & Sakai, 2008), peptide YY (Le Roux et al., 2005), obestatin (Hassouna et al., 2010) and oxyntomodulin (Wynne et al., 2010).

Such studies carried out among different groups of vertebrates, help in understanding the digestive physiology in a much better way, its relation with feeding habit (Mortensen et al., 1979; Santos et al., 2008), clinical endocrinology, endocrine pathological conditions (De Lellis & Wolfe 1983; Hawkins et al., 1987; Ku et al., 2005) and even the phylogeny of gut endocrine cells (D'Este et al., 1994). Most of the studies have been carried out in amniote group of vertebrates (Bunnett, 1984; Arena et al., 1990; Kitamura et al., 1990; Huang & Wu, 2005; Nascimento et al., 2007; Tarakci et al., 2008) and less in anaminotes. Among anaminotes the amphibian group has been least investigated. Keeping this in mind and for filling the research gap the present investigators tried to identify, localize and map the gastrin and secretin producing endocrine cells in different regions of the alimentary canal of *Euphyctis cyanophlyctis* (Schneider) through immunohistochemical technique.

Material & Methods

For the present investigation, 10 adult live frogs, *Euphlyctis cyanophlyctis* (Schneider) were procured from their natural habitat (lentic freshwater body) of Ranchi (India) with average body weight of 20 ± 3 g and acclimatized to laboratory conditions for about fifteen days in a large aquarium (21X17X18 inch sized) containing muddy substratum, aquatic vegetation and pond water to mimic their natural habitat. They were maintained at 12h day/night cycle and fed with food ad libitum.

The acclimatized specimens were anaesthetized with the diethyl ether (Plate et al., 2005). Zamboni's fixative was injected into the mouth of the specimen with the help of the disposable syringe, to clear out the gut content. The entire gut was separated out from the dissected specimen and cut in pieces at several regions including those of oesophagus/ stomach junction, fundic and pyloric region of stomach, stomach/duodenum junction, duodenal, ileum and rectal region. These pieces were dipped in the same fixative overnight.

The tissues were washed under running tap water to remove the excessive fixative and were thereafter divided into two sets.

The tissues of set I were further processed for frozen block preparation and cryosectioning in the following manner:

- a) **Cryoprotection:** This was done by treating the tissues with sucrose gradient solutions, 10%, 20% for 2 hours each and 30% overnight.
- b) **Block preparation:** Next day CryO-Z-T (Product No. 27055, Ted Pella Inc., USA), a frozen embedding material was used to prepare the frozen blocks of the cryo-protected tissues.
- c) **Cryosectioning:** 12 μ m thick sections were cut with cryostat microtome (Model no: MSW 459), at -20°C and spread on Poly-L-lysine coated slides.

The spread slides were divided into three groups.

The slides of group 1 were subjected to antigen retrieval (AR). This was done by treating with 1 % sodium dodecyl sulphate prepared in 0.1 M PBS pH 7.4, for 5 min, (Robinson & Toshihiro, 2002) followed by standard immunocytochemistry (ICC) method (Hsu et al., 1981).

The slides of group 2 were subjected to IHC alone without AR.

The slides of group 3 were considered for Omission Control to affirm the presence of gastrin immune reactive cells and secretin immune reactive cells.

The tissues of set II were processed for paraffin block preparation and sectioning in the following manner:

- a) **Tissue dehydration:** This was done by treating the tissues in graded alcohol and cleared with xylene.
- b) **Wax penetration:** The dehydrated tissues were kept in molten wax for period of overnight in an incubator maintained at 60°C .
- c) **Block Preparation and sectioning:** On the next day paraffin blocks were prepared and serial sections at $6\mu\text{m}$ were cut and spread on albumin coated slides.

The spread slides were deparaffinized with xylene and divided into three groups.

Group 1: The sections were rehydrated in alcohol gradation followed by antigen retrieval with citrate buffer for forty minutes maintaining at 80°C in a water bath.

The slides of group 2 were subjected to IHC alone without AR.

The slides of group 3 were considered for omission control to affirm the presence of gastrin immune reactive cells and secretin immune reactive cells.

The antigen retrieved and non-antigen retrieved of both the cryosections and paraffin sections were subjected to the standardized IHC staining method in the following manner:

- 1) **Blocking of endogenous peroxidase** was done by washing the sections with 0.1M PBS (pH=7.4) followed by treatment with 3% hydrogen peroxide in methanol.
- 2) **Blocking of non-specific binding sites** with the help of blocking solution (1% Bovine Serum Albumin in 0.5% Triton X 100).
- 3) **Incubation with primary antibody:** One set of sections were now incubated with anti-gastrin (rabbit polyclonal antibody, Batch No: Z04509 and Z04518, ENZO SWISS) which worked best at 1:4000 dilutions, for two hours.

Similarly, another set of sections were incubated with anti-secretin (rabbit polyclonal antibody, Catalog No. ab8495 (250µL) Abcam, Cambridge, UK) which worked best at 1:20 dilution, for two hours.

Dilution test was performed for both the primary antibodies, to determine the dilution in which the best results can be obtained. Initially the different sections of the frog's gut were incubated with serial dilutions of the primary antibody. For localizing G-IR cells, 1:1000, 1:2000, 1:3000, 1:4000 and 1:5000 dilution of anti-gastrin was made and applied to different section of the gut, followed by the running the entire protocol to obtain the gastrin immunoreactivity. The best results were obtained in the slides which were incubated with 1:4000 dilutions. Similar steps of dilution test were performed for secretin immuno-localization using 1:10, 1:20, 1:30 and 1: 40 dilutions and the best results were obtained at 1:20 dilutions.

- 4) **Incubation with secondary antibody and avidin peroxidase:** After washing the tissue with PBS, the tissues were incubated with second antibody (biotinylated goat anti-rabbit IgG) and avidin peroxidase (Extra-3 Kit, Batch No: 100M4788, Sigma Aldrich, USA) for one hour each (1:20 dilution). All incubations were done in a humidity chamber.
- 5) **Application of chromogen:** Tissues were again washed with buffer and 3-amino-9-ethylcarbazole (AEC) chromogen (AEC 101 KIT, Batch 604 No. 090M4799, Sigma Aldrich, USA) was applied for 5–10 min, until reddish brown colour was visible. The colour so developed, was observed under a microscope after mounting with glycerol–gelatin mixture.
- 6) **Microphotography:** The photographs were taken through Olympus trinocular photomicroscope (Model No. CX21 with E420 digital camera attachment).
- 7) **Omission Control:** The omission control was performed for group-3 slides which were subjected to all steps except incubation with primary antibody to reaffirm the G- cells and S- cells.

Results

IHC study under the bright field light microscopic examination revealed the presence of G-IR cells only in the stomach and the duodenal region and the S-IR cells only in the intestinal region of the alimentary canal (Figure 1) of *Euphlyctis cyanophlyctis*. G-IR cells were abundantly present in the pyloric region of the frog's stomach (Figure 2). These G-IR cells were of varied shapes, ranging from oval, rounded and pyramidal shaped with distinct secretory granules (Figure 3 and Figure 4). The G-IR cells were also observed in the duodenum, but these were found restricted to the basal lamina region (Figure 5). Both the open and closed types of G-IR cells were recorded in the stomach regions, but those present in the duodenal region were of closed types.

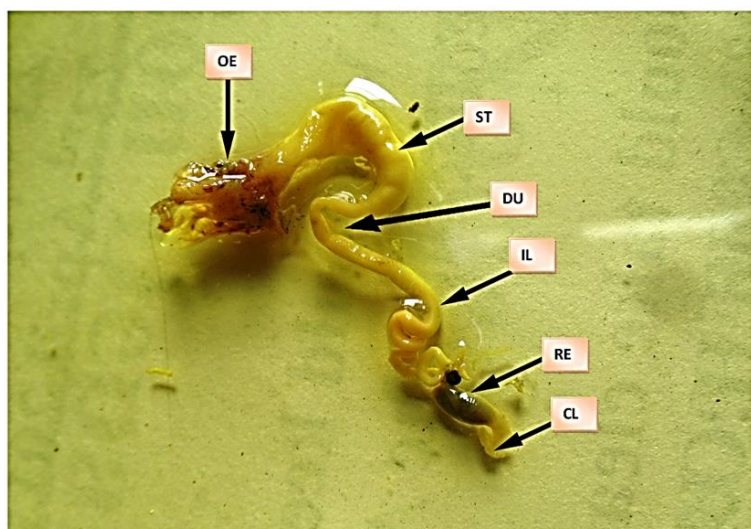


Figure 1. Alimentary canal of *Euphlyctis cyanophlyctis*. OE: oesophagus, ST: stomach, DU: duodenum, IL: ileum, RE: rectum and CL: cloaca.

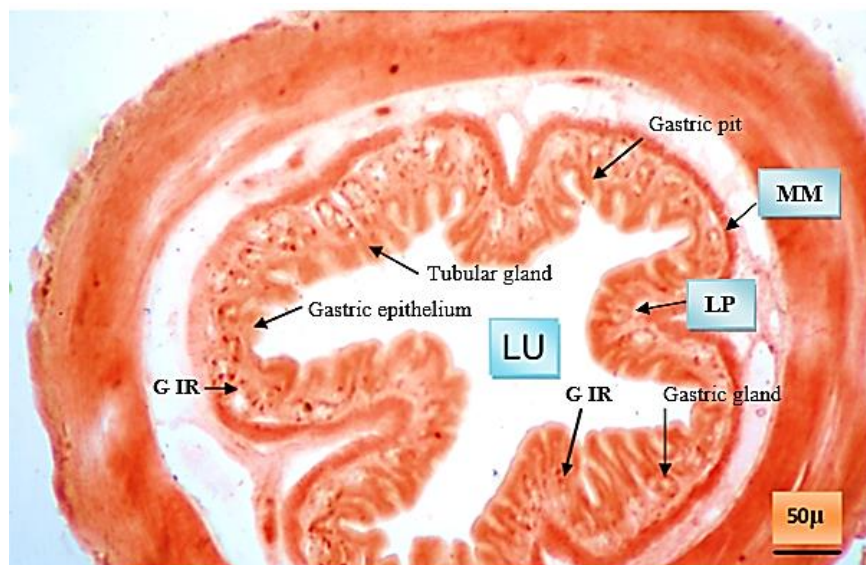


Figure 2. Photomicrograph of transverse section of stomach (pyloric region) of *Euphlyctis cyanophlyctis*, showing abundant G-IR cells in cryosection 12μ thick, without antigen retrieval method. LU: lumen, LP: lamina propria, MM: muscularis mucosa. 100X magnification.

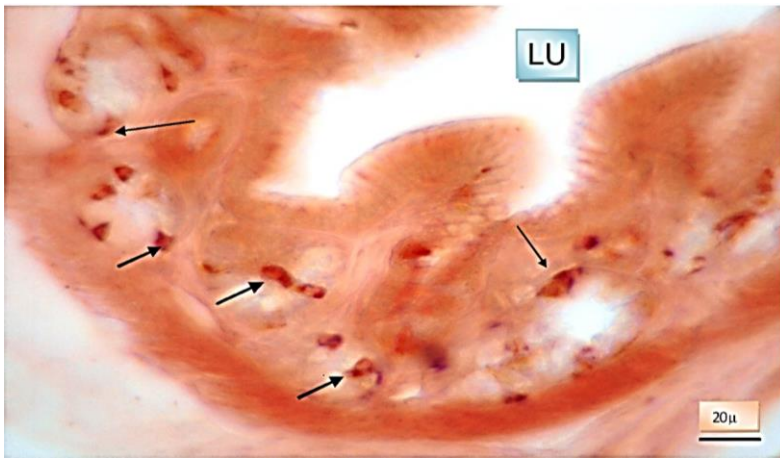


Figure 3. Magnified view of figure 2, showing varied shaped G-IR cells. LU: lumen. 400X magnification.

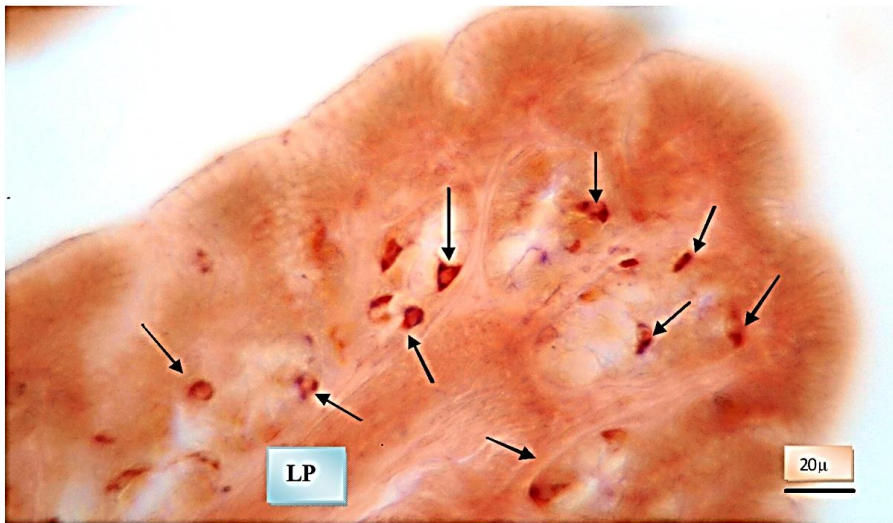


Figure 4. Photomicrograph of transverse section of stomach (pyloric region) of *Euphlyctis cyanophlyctis* showing pyramidal shaped, oval shaped and open type of G-IR cells with distinct secretory granules. LP: lamina propria. Cryosection 12μ thick, without antigen retrieval, 400X magnification.

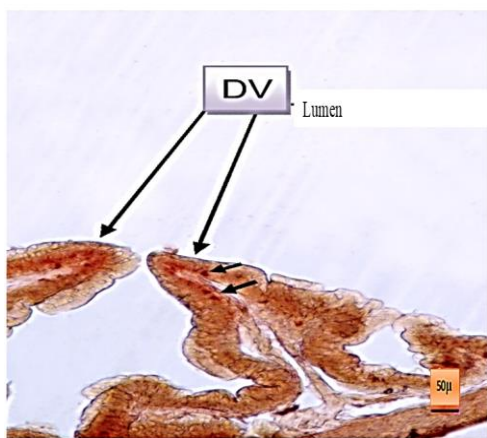


Figure 5. Microphotograph of transverse section of duodenum of *Euphlyctis cyanophlyctis* showing the presence of G-IR cells in the duodenal villi (DV). 100X magnification.

The S-IR cells were abundant in the duodenal mucosa and its frequency of occurrence was comparatively less in mucosa region of ileum part (Figure 6). Only closed type of S-IR cells were observed which were localized at the basal region of the mucosa (Figure 7). Abundance of secretin immunoreactivity was found in the muscle layers of ileum region (Figure 8). No gastrin and secretin immunoreactivity was observed in the histological slides in which omission control was performed (Figure 9 and Figure 10).

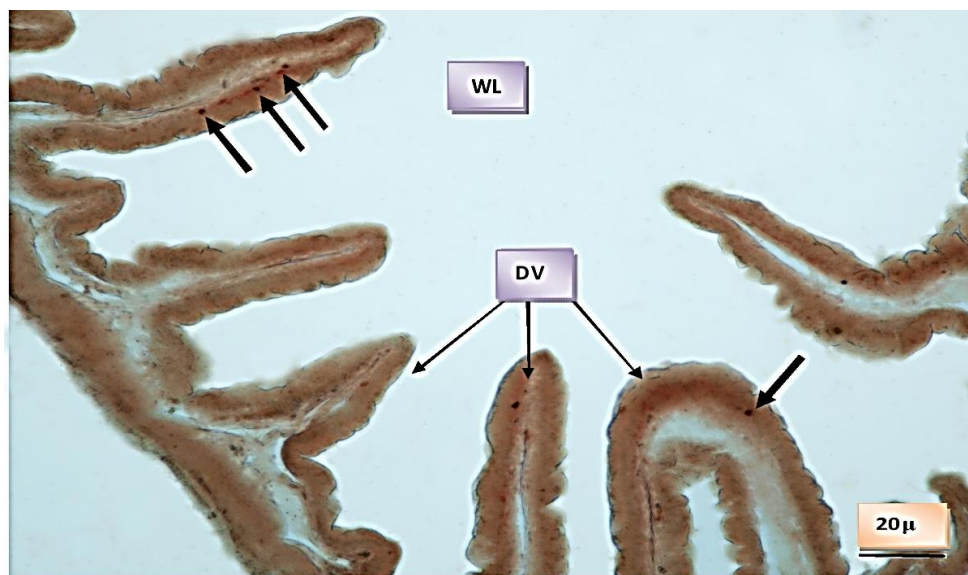


Figure 6. Photomicrograph of transverse section of duodenum of *Euphlyctis cyanophlyctis* showing the presence of S-IR cells in the duodenal villi (DV), marked by thick black arrows, WL: wide lumen. 100X magnification.

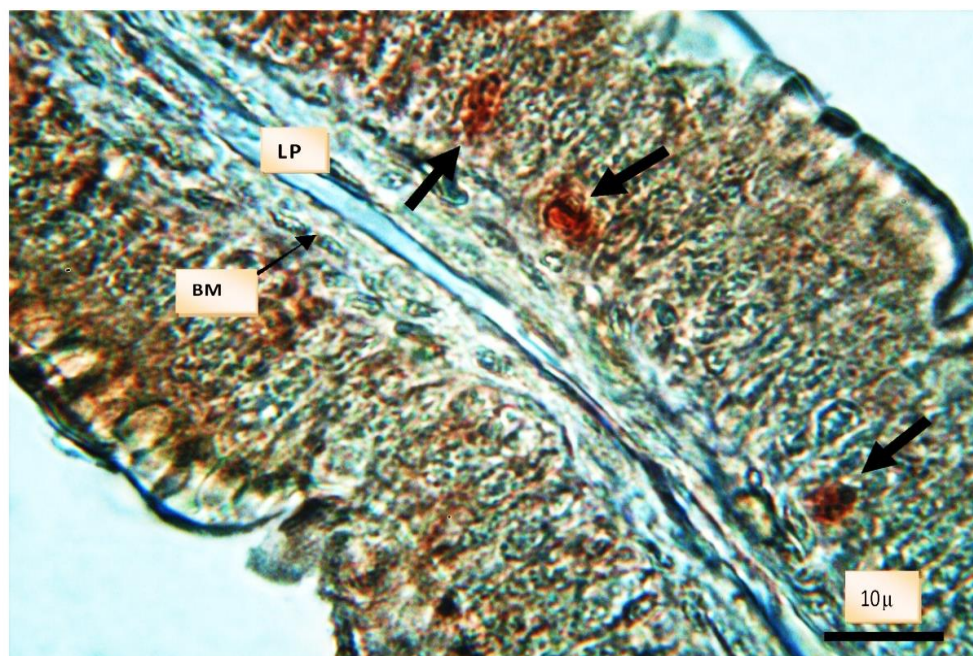


Figure 7. Photomicrograph of transverse section of duodenum of *Euphlyctis cyanophlyctis* showing a portion of villus with closed type S-IR cells localized towards the basal lamina. BM: basement membrane and LP: lamina propria. Cryosection 12μm thick, antigen retrieved, 1000X (Oil Immersion) magnification.

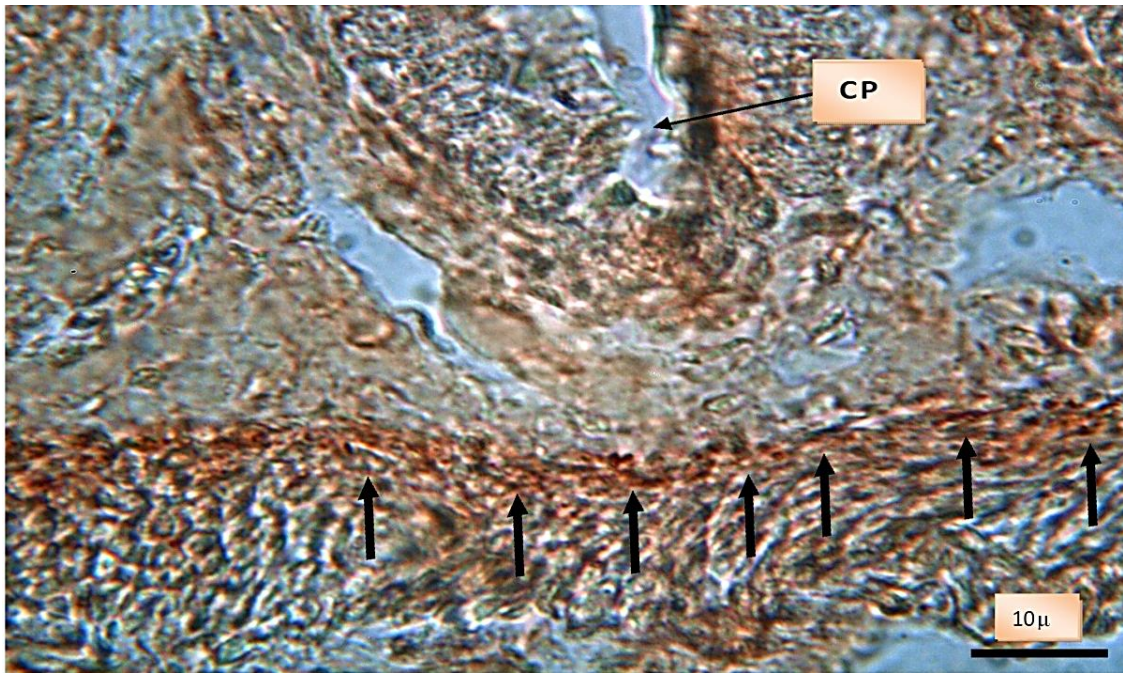


Figure 8. Photomicrograph of transverse section of ileum of *Euphlyctis cyanophlyctis* showing secretin immunoreactive areas localized between the muscle layers. Paraffin section 6μ thick, antigen retrieved, 1000X (Oil Immersion) magnification.

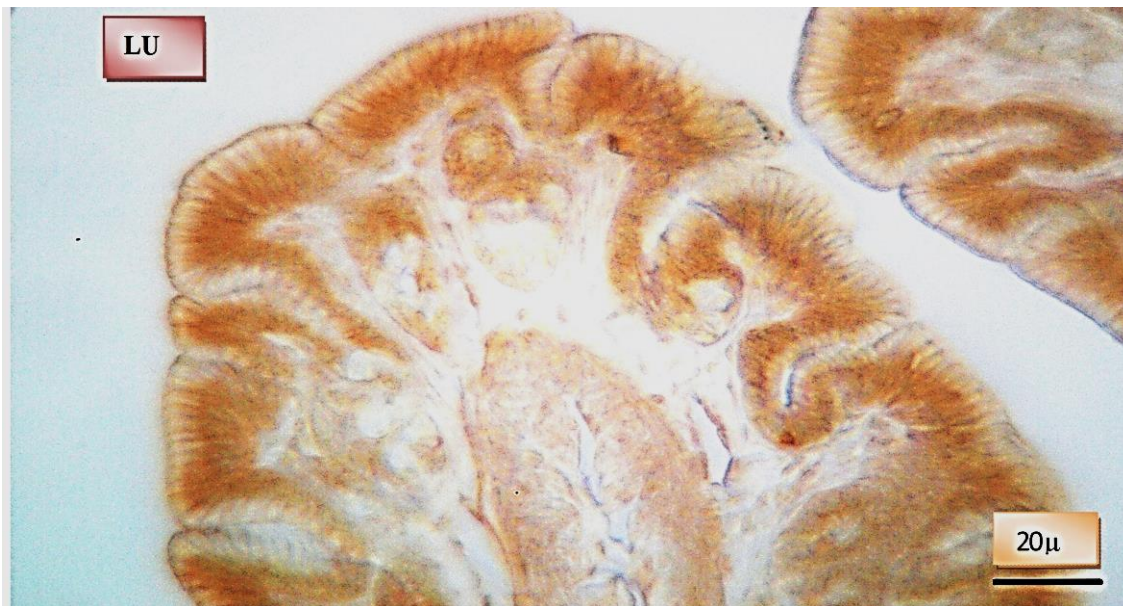


Figure 9. Photomicrograph of transverse section of stomach region of *Euphlyctis cyanophlyctis*, omission control slide, showing no immunoreactive cells are present. LU: lumen. Cryosection 12μm thick, 400X magnification.

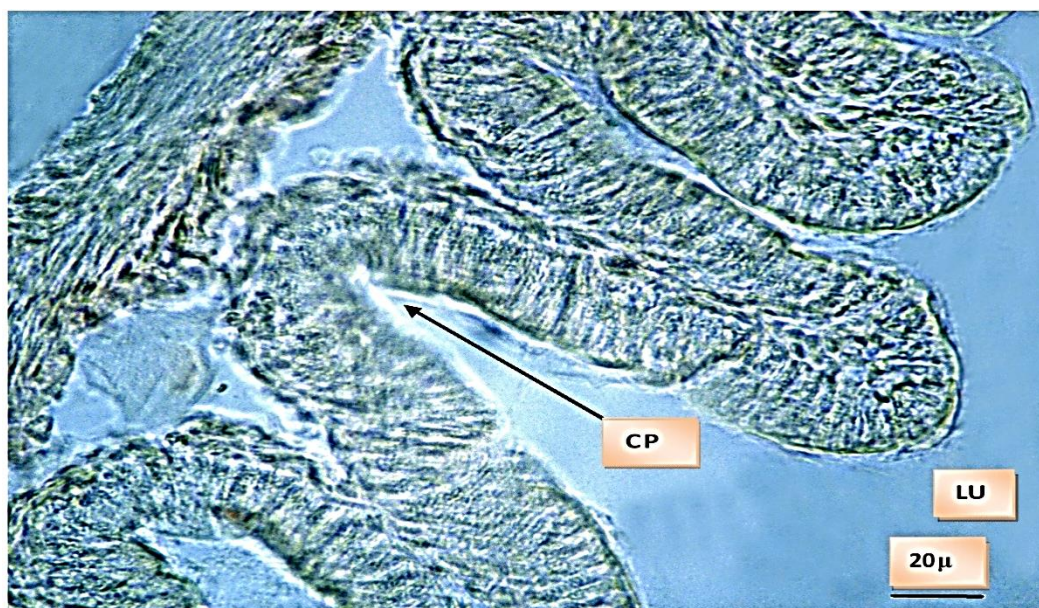


Figure 10. Photomicrograph of transverse section of ileum of *Euphlyctis cyanophlyctis*, omission control slide, showing no immunoreactive cells are present. CP: crypt, LU: lumen. Paraffin section 6μm thick, antigen retrieved, 400X magnification.

Discussion

The different morphotypes of gut hormone-producing cells in amphibians have been examined using immunohistochemical methods. Previous studies have reported a range of immunoreactive cell types in several species, including *Rana dybowskii* (Lee & Lee, 1996), *Rana temporaria* (Bodeges et al., 1997), *Rana pipiens* (Lechago et al., 1978), *Xenopus laevis* (Lee & Lee, 1992; 1997), *Rana esculenta* (Trandaburu & Nürnberger, 1995), *Bufo regularis* (El Salhy et al., 1981; 1982), *Rana catesbeiana* (Lee et al., 1998a; 1999), *Rana nigromaculata* (Lee et al., 1998b; Pan et al., 2009), *Bombina orientalis* (Ku et al., 2000a), *Hyla arborea japonica* (Ku et al., 2000b), *Rana rugosa* (Ku et al., 2003), and *Rana ridibunda* (Yaman et al., 2005). These studies used various mammalian-derived antisera to identify immunoreactive enteroendocrine cells.

Some of the researchers have coupled the IHC studies with different methods to explore the ultrastructural features of the amphibian gut endocrine cells. The immunocytochemical coupled with ultrastructural studies were performed in *Salamandra salamandra* (Buchan et al., 1980); in *Rana perezii* (Gallego-Huidobro & Pastor, 1996) and in *Rinella icterica* (Pelli-Martins et al., 2012).

De Rada et al. (1987) and Valverde et al. (1993) through immunocytochemical coupled with ultrastructural studies have identified the G-IR cells both amidst mucosa and around gastric glands in *Rana temporaria* and also measured the size of their secretory granules having a mean diameter of 190 nm.

Ku et al. (2000b) had reported the presence of six different kinds of IR cells in *Hyla arborea japonica* including spherical or spindle shaped gastrin IR cells, but didn't observe the secretin IR cells. Ku et al. (2003) had observed G-IR cells in *Rana rugosa*, which was reported to be restricted in gastric antral region with low frequency.

Yaman et al. (2005) in *Rana ridibunda*, could not report the presence of G-IR cells. However, in other species of *Rana*, Pan et al. (2009) have observed the frequency of gastrin immunoreactive cells, before and after fasting state. Buchan et al. (1980),

in their immunocytochemical coupled with ultra-structural studies using mammalian gut peptide antisera, identified immunoreactive gastrin in the nerve fibers in submucosa as well as in the myenteric plexus. Shukla and Rao (2012), through the comparative studies of cryosections and paraffin sections, studied the G-IR cells in *Rana cyanophlyctis*, reported the presence of G-IR cells in stomach as well as in small intestine, suggesting the occurrence of Intestinal gastrin.

The present investigation revealed that in *Euphlyctis cyanophlyctis* the G-IR cells occur in both mucosal region and around gastric glands, as also reported by De Rada et al. (1987). Moreover, the number of G-IR cells in the pyloric region is numerous while in fundic region these are scanty. In fundic region of the stomach the G-IR cells are localized in the vicinity of gastric glands. However, the presence of long protoplasmic extension of G-IR cells (open type) towards the lumen and the G-IR cells with small protoplasmic extensions, as well as spindle shaped cells in the close proximity of the gastric gland suggests a multiple mode of release of gastrin in either way, i.e., as a lumone or paracrine agent or a hormone. Furthermore, G-IR cells have also been localized in the duodenal region of intestine. In this region the cells are restricted to the basal lamina of enteric epithelium. Morphologically the intestinal G-IR cells are quite distinct from those found in stomach region. Here the G-IR cells (closed type) are rounded in shape and without any protoplasmic extensions. It is therefore, reasonable to assume that the occurrence of G-IR cells is in accordance with the need and their strategic location for secretion.

The IHC localization of secretin cells has been carried out by El-Salhy et al. (1981) reporting the presence of S-IR cells in both stomach and intestine of *Bufo regularis*; Ku et al. (2000a) observed S-IR cells in pylorus, duodenum and ileum regions of gut of a red-bellied frog *Bombina orientalis*. Lee et al. (2003) have identified the secretin IR cells in pancreas and reported them to be present in large numbers in the same species.

In the present study, secretin immunoreactivity is abundant in the duodenal mucosa of *Euphlyctis cyanophlyctis*. Majority of S-IR cells were located at the region of basal lamina of the enteric epithelium of the duodenal villi. Morphologically, all the S-IR cells are rounded or oval in shape and none of these S-IR cells have long protoplasmic extensions spanning the entire width of enteric epithelium. In the present findings the secretin immunoreactivity is seen in the ileum region both in its mucosa and in the myenteric nerve plexus areas. The number of S-IR cells in the mucosal region of ileum has been found to be less abundant in comparison to the number of the duodenal mucosa. The occurrence of S-IR cells in the mucosa of duodenum and ileum region was observed, but the S-IR cells were abundant in duodenum in comparison to ileum. Thus there is marked decrease in the number of mucosal S-IR cells from duodenum to ileum region of the alimentary canal. But in the myenteric nerve plexus much secretin immunoreactivity has been observed. So far the localization and morphology of mucosal S-IR cells are concerned; these are similar to those found in the duodenum. In both duodenum and ileum, the mucosal S-IR cells are restricted towards the basal lamina of the enteric epithelium and morphologically all are rounded or oval in appearance without any protoplasmic extensions. The physiological significance of the occurrence of closed type of S-IR implies that these cells are located at the basal region of the enteric epithelium and not directly exposed to the acidic chyme entering into the duodenum. These are activated indirectly via neural or hormonal signals and have synaptic associations with nerve endings, suggesting brain-gut communication required for neuro-hormonal control of digestion. The secretin exerts numerous roles in neurotransmission, neurogenesis and neural protection through neurocrine signaling as reported by Ng et al. (2002). Lu & Owyang (2009) have shown that secretin acts on the afferent vagal nerve to inhibit gastric mobility in rats.

The present finding also shows the presence of secretin immune-reactivity in the region between the muscle layers of the intestinal wall. This is an evidence of the fact that gastrointestinal hormones especially gastrin and secretin act as hormone-

parahormone-lumone molecules. In addition, these can also affect the nervous system thereby acting as neuro-modulators.

Future Implications of IHC study of endocrine gut cells in amphibian models will help to establish a baseline data for comparative and evolutionary origin of the gut endocrine cells, since amphibians hold a strategic position in evolutionary ladder above anaminote fishes and first true land vertebrates that is amniote reptiles. Moreover, the future study can also help in understanding how the development of gut endocrine cells alter during the transition phase of herbivorous tadpole larva metamorphosing into carnivore adult frog.

Conclusion

The present study reveals the IHC localization of closed and open type of G-IR cells and S-IR in different mucosa and myenteric regions of the alimentary canal of *Euphlyctis cyanophlyctis*. These findings greatly help in understanding the neuro-endocrinological regulation of digestive physiology in the anaminote member. Comparing with data from other anaminote and aminote members one can also picturize the phylogeny of these gut endocrine cells. The occurrence of abundant open type of G-IR cells in the pyloric region strongly supports the concept of gut luminal sensing property of these cells. Which are stimulated by sensing the luminal contents of the gut and then release their respective hormones either in the lumen or in the basal region, thus regulating the physiology of digestion through luminocrine, paracrine and endocrine signaling. The presence of closed type G-IR cells in the duodenal mucosa is the another regulatory pathway acting by the secretion of intestinal gastrin. The S-IR cells found were mainly of closed type in the present study, whose abundance were recorded in the duodenal mucosa and the myenteric region of the intestinal wall. These findings suggest the role of gut hormones as neuro-modulators establishing the brain-gut axis for regulation of digestive physiology. The IHC study of gut endocrine cells provide better understanding of comparative endocrinology, phylogeny of GIT, neuro-endocrine regulation of digestive physiology and even in gut pathology in amphibians.

Declarations

Acknowledgments: The authors are grateful to the authorities of St. Xavier's College, Ranchi for providing the research facilities and the UGC ERO, Kolkata for the financial support.

Author Contribution: Both the authors have equally contributed in conceiving the idea of research, design of the study, conduction of the research, analysis of the result and in writing the entire manuscript.

Conflict of Interest: The authors declare that no conflict of interest.

Funding: University Grants Commission Eastern Regional Office, Kolkata had funded the project as MRP (F.PSJ-011/10-11).

References

- Abou, F., Fadil-Nicol, E., Berger, F., Descroix-Vagne, M., & Pansu, D. (2000). Presence of sorbin in human digestive tract and endocrine digestive tumors. *Gut*, 46(2),182-190. [DOI] [PMID]
- 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. [PMID]
- Bayliss, W. M., & Starling, E. H. (1902). The mechanism of pancreatic secretion. *J Physiol*, 28, 325-353. [DOI] [PMID]
- Bodegas, M. E., Villaro, A. C., Burrell, M. A., Rovira, J., Valverde, E., Ortiz De Zarate, A., & Sesma, P. (1997). An immunocytochemical and ultrastructural study of the larval anterior intestine of the frog *Rana temporaria*, with especial reference to endocrine cells. *Tissue Cell*, 29, 549-559. [DOI]

- Buchan, A. M. J., Polak, J. M., & Pearse, A. G. E. (1980). Gut hormones in *Salamandra salamandra*: An immunocytochemical and electron microscopic investigation. *Cell Tiss Res*, 211(2), 331-343. [DOI] [PMID]
- Bunnett, N. W. (1984). The localization of gastrin-like immunoreactivity (GIR) in the alimentary tract of the sheep. *Q J Exp Physiol*, 69(3), 521-529. [DOI] [PMID]
- Coons, A. H., Leduc, E. H., & Connolly, J. M. (1955). Studies of antibody production. I. Method for the histochemical demonstration of specific antibody and its application to the study of the hyperimmune rabbit. *J Exp Med*, 102(1), 49-60. [DOI] [PMID]
- D'Este, L., Buffa, R., Pelagi, M., Siccardi, A. G., & Renda, T. (1994). Immunohistochemical localization of chromogranin A and B in the endocrine cells of the alimentary tract of the green frog, *Rana esculenta*. *Cell Tissue Res*, 277(2), 341-349. [DOI] [PMID]
- Dawson, I. M. P. (1978). Visualization of the diffused endocrine system. *J Clin Pathol*, 33, Suppl (Assoc Clin Pathol), 8, 7-11. [DOI] [PMID]
- De Lellis, R. A., & Wolfe, H. J. (1983). Contributions of immunohistochemistry to clinical endocrinology and endocrine pathology. *J Histochem Cytochem*, 31(1A Suppl), 187-192. [DOI] [PMID]
- De Rada, O. D., Sesma, P., & Vazquez, J. J. (1987). Endocrine cells of the gastric mucosa of *Rana temporaria*. *Histol Histopathol*, 2, 119-128. [PMID]
- Deveney, C. W., & Way, L. W. (1983). Regulatory peptides of gut. In Forsham, P. H. (Ed.), *Greenspan, Basic and Clinical Endocrinology* (pp. 479). Asian Edn., Singapore: Maruzen.
- Edkins, J. S. (1905). On the chemical mechanism of gastrin secretion. *Proceed Biol Sci*, 76(510), 376. [DOI]
- Ekundayo, A. A., Lee, C. Y., & Goodlad, R. A. (1995). Gastrin and the growth of the gastrointestinal tract. *Gut*, 36(2), 203-208. [DOI] [PMID]
- El-Salhy, M., Grimelius, L., Lundberg, J. M., Tatemoto, K., & Terenius, L. (1982). Immunocytochemical evidence for occurrence of PYY, a newly isolated gut polypeptide in endocrine cells in the gut of amphibians and reptiles. *Biomed Res*, 3, 303-306. [DOI]
- El-Salhy, M., Grimelius, L., Wilander, E., Abu-Sinna, G., & Lundqvist, G. (1981). Histological and immunohistochemical studies of the endocrine cells of the gastrointestinal mucosa of the toad (*Bufo regularis*). *Histochemistry*, 71, 53-66. [DOI] [PMID]
- Fänge, R. (1962). Pharmacology of poikilothermic vertebrates and invertebrates. *Pharmacol Rev*, 14, 281-316. [PMID]
- Feyrter, F. (1953). Über die peripheren endokrinen (parakrinen) Drüsen des Menschen. Wien; Düsseldorf: Wilhelm Maudrich, p. 27.
- Fujita, T., & Kobayashi, S. (1974). The cells and hormones of the GEP endocrine system. In Fujita, T. (Ed.) *Gastro-Entero-Pancreatic Endocrine System: A Cell Biological Approach* (pp. 1-16). Williams and Wilkins, Baltimore, Maryland.
- Fujita, T., & Kobayashi, S. (1977). Structure and function of gut endocrine cells. *Int Rev Cytology (Suppl.)*, 6, 187-233. [PMID]
- Gallego-Huidobro, J., & Pastor, L. M. (1996). Histology of mucosa of oesophagogastric junction and the stomach in adult *Rana perezi*. *J Anat*, 188(2), 439-444. [PMID]
- Gil-Campos, M., Aguilera, C. M., Cañete, R., & Gil, A. (2006). Ghrelin: a hormone regulating food intake and energy homeostasis. *Brit J Nutrition*, 96(2), 201-226. [DOI] [PMID]
- Hassouna, R., Zizzari, P., & Tolle, V. (2010). The ghrelin/obestatin balance in the physiological and pathological control of growth hormone secretion, body composition and food intake. *J Neuroendocrinol*, 22(7), 793-804. [DOI] [PMID]
- Hawkins, K. L., Summers, B. A., Kuhajda, F. P., & Smith, C. A. (1987). Immunocytochemistry of normal pancreatic islets and spontaneous islet cell tumors in dogs. *Vet Pathol*, 24(2), 170-179. [DOI] [PMID]

- Höfer, D., Asan, E., & Drenckhahn, D. (1998). Chemosensory perception in the gut. *News Physiol Sci*, 14, 18-23. [\[DOI\]](#) [\[PMID\]](#)
- Hoosein, N. M., Kiener, P. A., Curry, R. L., & Brattain, M. G. (1990). Evidence for autocrine growth stimulation of cultured colon, tumor cells by gastrin/ cholecystokinin-like peptide. *Exp Cell Res*, 186(1), 15-21. [\[DOI\]](#) [\[PMID\]](#)
- Hsu, S. M., Raine, L., & Fanger, H. (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *J Histochem Cytochem*, 29, 577-580. [\[DOI\]](#) [\[PMID\]](#)
- Huang, X.-G., & Wu, X.-B. (2005). Immunohistochemical study on gastrointestinal endocrine cells of four reptiles. *World J Gastroenterol*, 11(35), 5498-5505. [\[DOI\]](#) [\[PMID\]](#)
- Ivy, A. C., & Oldberg, E. (1928). A hormone mechanism for gallbladder contraction and evacuation. *Am J Physiol*, 86, 599-613. [\[DOI\]](#)
- Kitamura, N., Yamadal, J., Watanabe, T., & Yamashita, T. (1990). An immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the musk shrew, *Suncus murinus*. *Histol Histopathol*, 5(1), 83-88. [\[PMID\]](#)
- Ku, S.-K., Lee, H.-S., & Lee, J.-H. (2000a). An immunohistochemical study of endocrine cells in the alimentary tract of the red-bellied frog, *Bombina orientalis*. *J Vet Med Sci*, 62(6), 589-594. [\[DOI\]](#) [\[PMID\]](#)
- Ku, S.-K., Lee, H.-S., & Lee, J.-H. (2000b). Immunohistochemistry of endocrine cells in the alimentary tract of the tree frog, *Hyla arborea japonica*. *Korean J Biol Sci*, 4(1), 95-100. [\[DOI\]](#)
- Ku, S.-K., Lee, H.-S., Koh, J.-K., & Lee, J.-H. (2003). An immunohistochemical study on the neuropeptide-producing endocrine cells in the alimentary tract of wrinkled frog, *Rana rugosa* (Ranidae). *Gen Comp Endocrinol*, 131(1), 1-8. [\[DOI\]](#) [\[PMID\]](#)
- Ku, S.-K., Seong, S.-K., Kim, D.-Y., Lee, H.-S., Kim, J.-D., Choi, H.-Y., Seo, B.-I., & Lee, J.-H. (2005). Changes of the intestinal endocrine cells in the C57BL/6 mouse after implantation of murine lung carcinoma (3LL): An immunohistochemical quantitative study. *World J Gastroenterol*, 11(35), 5460-5467. [\[DOI\]](#) [\[PMID\]](#)
- Larsson, L. I., Goltermann, N., Magistris, L. D., Rehfeld, J. F. & Schwartz, T. W. (1979). Somatostatin cell processes as pathways for paracrine secretion. *Science*, 205(4413), 1393-1395. [\[DOI\]](#) [\[PMID\]](#)
- Lechago, J., Holmquist, A. L., Rosenquist, G. L., & Walsh, J. H. (1978). Localization of bombesin-like peptides in frog gastric mucosa. *Gen Comp Endocrinol*, 36, 553-558. [\[DOI\]](#) [\[PMID\]](#)
- Lee, H. S., & Lee, J. H. (1992). An immunohistochemical study of the endocrine cells on the gastro-entero-pancreatic system of the African clawed toad, *Xenopus laevis*. *Korean J Vet Res*, 32(4), 523-529.
- Lee, H. S., & Lee, J. H. (1996). Seasonal variations of the gastro-enteropancreatic endocrine cells of the frog, *Rana dybowskii*. *Korean J Vet Res*, 36, 11-21.
- Lee, H. S., & Lee, J. H. (1997). Immunohistochemical study of the endocrine cells of the gastrointestinal mucosa of the African clawed toad, *Xenopus laevis*. *Korean J Vet Res*, 37(1), 9-13.
- Lee, H. S., Ku, S. K., & Lee, J. H. (1998a). Ontogeny of gastrointestinal endocrine cells in the bullfrog, *Rana catesbeiana* during developmental stages. *Korean J Lab Anim Sci*, 14, 163-168.
- Lee, H. S., Ku, S. K., & Lee, J. H. (1998b). Immunohistochemical study of the gastrointestinal endocrine cells of the frog, *Rana nigromaculata*. *Korean J Lab Anim Sci*, 14, 157-162.
- Lee, H. S., Ku, S. K., & Lee, J. H. (1999). Changes in the serotonin, somatostatin and motilin-immunoreactive cells in the gastrointestinal tract of the bullfrog, *Rana catesbeiana*, at various developmental stages. *Korean J Biol Sci*, 3, 97-102. [\[DOI\]](#)
- Lee, J. H., Ku, S. K., Lee, H. S., & Kitagawa, H. (2003). An immunocytochemical study of endocrine cells in the pancreas of red-bellied frog (*Bombina orientalis*). *European J Histochem*, 47(2), 165-172. [\[DOI\]](#)

- Le Roux, C. W., Ghatei, M. A., Gibbs, J. S. R., & Bloom, S. R. (2005). The putative satiety hormone PYY is raised in cardiac cachexia associated with primary pulmonary hypertension. *Heart*, 91(2), 241-242. [DOI] [PMID]
- Lu, Y., & Owyang, C. (2009). Secretin-induced gastric relaxation is mediated by vasoactive intestinal polypeptide and prostaglandin pathways. *Neurogastroenterol Motility*, 21(7), 754–e47. [DOI] [PMID]
- May, C. L., & Kaestner, K. H. (2010). Gut endocrine cell development. *Mol Cell Endocrinol*, 323(1), 70-75. [DOI] [PMID]
- Mortensen, N. J. M., Morris, J. F., & Owens, C. (1979). Gastrin and ultrastructure of G cells in the fasting rat. *Gut*, 20(1), 41-50. [DOI] [PMID]
- Nascimento, A. A., Sales, A., Cardoso, T. R. D., Pinheiro, N. L., & Mendes, R. M. M. (2007). Immunocytochemical study of the distribution of endocrine cells in the pancreas of the Brazilian sparrow species *Zonotrichia capensis subtorquata* (Swainson, 1837). *Braz J Biol*, 67(4), 735-740. [DOI] [PMID]
- Ng, S. S., Yung, W. H., & Chow, B. K. C. (2002). Secretin as a neuropeptide. *Mol Neurobiol*, 26(1), 97–107. [DOI] [PMID]
- Pan, H., Chen, H., Zhu, L., Li, Y., Li, C., & Zhang, S. (2009). Adaptive changes of gastroenteropancreatic system endocrine cells in the black spotted pond frog, *Rana nigromaculata* after fasting. *Cur Zool*, 55(4), 301-308. [DOI]
- Pearse, A. G. E. (1981). The diffuse neuroendocrine system: falsification and verification of a concept. In Grossman, M. I., Brazier, M. A. b., & Lechago, J. (Eds.) *Cellular Basis of Chemical Messengers in Digestive System* (pp. 13-19). Academic Press, New York. [DOI]
- Pelli-Martins, A. A., Machado-Santos, C., Sales, A., & de Brito-Gitirana, L. (2012). Histochemical, immunohistochemical and ultrastructural observations of the esophagus morphology of *Rinella icterica* (Anuran, Bufonidae). *Acta Zoologica*, 93(4), 373-380. [DOI]
- Plate, A. Y. A., Crankshaw, D. L., & Gallaher, D. D. (2005). The effect of anesthesia by diethyl ether or isoflurane on activity of cytochrome P450 2E1 and P450 reductases in rat liver. *Anesthesia Analgesia*, 10(1), 1063-1064. [DOI] [PMID]
- Rao, N. V. A., & Shukla, R. K. (2013). Distribution of gastrin immune reactive cells in the alimentary canal of a stomachless hill stream loach, *Lepidocephalus guntea* (Hamilton): An immuno-histochemical study. *Proceed Nat Acad Sci India Sec B: Biolog Sci*, 83(4), 603-607. [DOI]
- Rehfeld, J. F. (1998). The new biology of gastrointestinal hormones. *Physiol Rev*, 78(4), 1087-1108. [DOI] [PMID]
- Robinson, J. M., & Toshihiro, T. (2002). Antigen Retrieval on ultrathin cryosections. *Acta Histochem Cytochem*, 35(5), 375-381. [DOI]
- Santos, C. M., Nascimento, A. A., Peracchi, A. L., Sales, A., Mikalauskas, J. S., & Gouveia, S. F. (2008). Immunocytochemical study of gastrointestinal endocrine cells in insectivorous bats (Mammalia: Chiroptera). *Braz J Biol*, 68(3), 663-669. [DOI] [PMID]
- Shukla, R. K., & Rao, N. V. A. (2012). A comparative study of cryo and paraffin sections in localizing gastrin cells (G-Cells) in the stomach of *Rana cyanophlyctis* (Schneider), using immunocytochemical technique. *Bioscan*, 7(1), 149-152.
- Shukla, R. K., Chakraborty, B., Rao, N. V. A., & Ahmad, M. F. (2015). Immunohistochemical localization of gastrin cells (G-cells) in the alimentary canal of a Skipper frog, *Euphlyctis cyanophlyctis*, Schiedner, (Anura; Ranidae). *Proceed Zool Soc*, 68(1), 45-51. [DOI]
- Sternini, C., Anselmi, L., & Rozengurt, E. (2008). Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing. *Curr Opin Endocrinol Diabetes Obes*, 15(1), 73-78. [DOI] [PMID]
- Sun, F. P., & Song, Y. G. (2003). G and D cells in rat antral mucosa: An immunoelectron microscopic study. *World J Gastroenterol*, 9(12), 2768-2771. [DOI] [PMID]

- Tarakci, B. G., Yaman, M., Bayrakdar, A., & Yaman, I. (2008). Immunohistochemical study on the endocrine cells in the gastro intestinal tract of the ostrich. *Medycyna Wet*, 64(1), 64-67.
- Track, S. N. (1980). The gastrointestinal endocrine system. *Can Med Assoc J*, 122(3), 287-292. [PMID]
- Trandaburu, T., & Nürnbergger, F. (1995). Somatostatin-immunoreactive cells in the gastrointestinal tract of the frog *Rana esculenta*. *Cell Tissue Res*, 279(2), 437-440. [DOI]
- Valverdae, E., de Rada, O. D, Burrell, M. A., Rovira, J., & Sesma, P. (1993). Immunocytochemical and ultrastructural characterization of endocrine cells and nerves in the intestine of *Rana temporaria*. *Tissue and Cell*, 25, 505-516. [DOI]
- Vassallo, G., Solcia, E., & Capello. C. (1969). Light and electron microscopic Identification of several types of endocrine cells in the gastrointestinal mucosa of the cat. *Z Zelforsch*, 98(3), 333-356. [DOI]
- Vizi, E. S., Bertaccini, G., Impicciatore, M., & Knoll, J. (1972). Acetylcholine-releasing effect of gastrin and related polypeptides. *Eur J Pharma*, 17(1),175-178. [DOI] [PMID]
- Wynne, K., Field, B. C., & Bloom, S. R. (2010). The mechanism of action for oxyntomodulin in the regulation of obesity. *Cur Opin Investig Drugs*, 11(10), 1151-1157. [PMID]
- Yaman, M., Tarakci, B. G., Bayrakdar, A., & Orsay, H. (2005). An immunohistochemical study on the endocrine cells in the gastrointestinal tract of the frog, *Rana ridibunda*, *J Animal Vet Adv*, 4(9), 771-774. [DOI]
- Zhao, Z., & Sakai, T. (2008). Characteristic features of ghrelin cells in the gastrointestinal tract and the regulation of stomach ghrelin expression and production. *World J Gastroenterol*, 14(41), 6306-6311. [DOI] [PMID]

Disclaimer/ Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of *The Applied Biology & Chemistry Journal* and/or the editor(s). *The Applied Biology & Chemistry Journal* and/or editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.