A COMPARATIVE STUDY OF CRYO AND PARAFFIN SECTIONS IN LOCALIZING GASTRIN CELLS (G-CELLS) IN THE STOMACH OF RANA CYANOPHLYCTIS (SCHNEIDER) USING IMMUNOCYTOCHEMICAL TECHNIQUE

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INTRODUCTION
The gastrointestinal tract is the first physiological state of animals involved in the transformation of diet into nutrients and their absorption across intestinal mucosa, the phenomenon of digestion. This entire episode is regulated by the cumulative actions of two unique components of alimentary canal i.e., enteric nervous and endocrine components (Guyton and Hall, 2008). The enteric endocrinocytes among vertebrates exist as diffused cells amidst mucosal cell population (Pearse, 1981). It is supposed that more than hundred regulatory substances including both hormones and putative hormones are secreted by the gastrointestinal mucosa (Hadley and Levine, 2009), hence gastrointestinal tract is also called as ‘largest endocrine gland in the body’ (Thompson et al., 1987; Ahlman and Nilsson, 2001).

Feyrter (1938, 1953) described the enteric endocrinocytes are helle-Zellen cells or clear cells or agyrophilic cells and suggested their action in paracrine mode. Pearse (1968) described these cells as Amine Precursor Uptake and Decarboxylation (APUD) cells. The secretions of enteric endocrinocytes among vertebrates exist as diffused cells amidst mucosal cell population (Pearse, 1981). It is supposed that more than hundred regulatory substances including both hormones and putative hormones are secreted by the gastrointestinal mucosa (Hadley and Levine, 2009), hence gastrointestinal tract is also called as ‘largest endocrine gland in the body’ (Thompson et al., 1987; Ahlman and Nilsson, 2001).

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Gastrin is one such regulatory peptide identified by Edkins (1905) and the cells secreting gastrin are abbreviated as ‘G-cells’ (Solcia et al., 1967), involved in stimulating the secretion of gastric juice especially acid secretion from gastric glands (Edkins, 1905). The gastrin is released into the circulation as hormone and as parahormone. In addition it is also listed as lumone, as it is released into the lumen of gut (Jordan and Yip, 1972; Uvnas-Wallensten, 1977). Hence, the hormone-parahormone-lumone concept of gastrin exists now-a-days. The distribution and localization of gastrin cells in gastrointestinal tract were identified through various histochemical (Gremelius, 1968; Solcia et al., 1969), ultrastructural (Frossman and Orci, 1969), immunoflourescent (Mc Guigan 1968; Bussolati and Pearse, 1970) and immunohistochemical (Bunnett, 1984; Ito et al., 1987; Nisa et al., 2005; Santos et al., 2008) methods. However, much of the work so far reported is related to mammals. In non-mammalian vertebrates specially in amphibians, published work has been restricted to the identification of ‘G-cells’ through immunohistochemistry in Rana temporaria (Rada et al., 1987a, b; Vilvarde et al., 1993), Rana perezi (Gallego-Huidobro and Pastor, 1996), Hyla arborea (Ku et al., 2000), Rana rugosa (Ku et al., 2003) and in Rana nigromaculata (Pan et al., 2009).
The present work is taken up by us for two reasons, firstly, so far there is no such identification of ‘G -cells’ in *Rana cyanophlyctis* through immunocytochemistry and secondly there is no comparative studies between cryosections and paraffin sections, for the optimization of method to identify the enteroendocrine cells.

**MATERIALS AND METHODS**

Fifteen adult live frogs, *Rana cyanophlyctis* (Schneider) were procured from their natural habitat of Ranchi (India) with an average weight of 20 ± 3g and acclimated to laboratory conditions for about fifteen days keeping them in large aquaria. They were maintained at 12h day/night cycle and fed with food *ad libitum*.

The animals were anaesthetized with diethyl ether. Zamboin’s fixative was perfused into alimentary canal and was kept in the same fixative overnight. The alimentary canal was then cut into pieces, washed with running tap water to remove excess of fixative then processed as follows:

a) For cryosections, the tissue was treated with gradient sucrose solution for cryoprotection. 10%, 20% for two hours each and 30% overnight. Tissue Cry O Z T blocks were prepared and serial sections were cut in 12μ at -20ºC and spread on poly-L-lysine coated slides.

b) For paraffin sections, the tissue was dehydrated with graded alcohol, cleared with xylene, followed by wax penetration overnight. On the next day paraffin blocks were prepared, serial sections at 5μ were cut and spread on albumin coated slides. The spread slides were deparaffinized with xylene, rehydrated followed by antigen retrieval with citrate buffer for forty minutes maintaining at 80ºC in a water bath.

Both cryosections and deparaffinized sections were treated as follows:

i) Washed with Phosphate Buffer Saline (PBS) two times.

ii) Endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol.

iii) Non specific binding was inhibited with blocking solution (1% Bovine Serum Albumin in 0.5% Triton X 100).

iv) The tissue was incubated with primary antibody, [Enzo –Swiss] (antigastrin worked best at 1: 4000 dilution) for two hours.

v) Washed with PBS two times.

vi) The tissue was then incubated with biotinylated second antibody and avidin peroxidase [Sigma Aldrich, USA] (one hour each)

vii) Washed with PBS buffer twice

viii) 3-Amino-9-ethyl carbazole (AEC) chromogen was applied for 5-10 minutes which resulted to the appearance of reddish brown colour.

c) Parallel to this, omission control was also done, in which slides containing tissue sections were treated with all except primary antibody.

**RESULTS**

The microscopic examination reveals the gastrin immunoreactivity in both paraffin and cryosections except in

![Figure 1: Photomicrograph of T.S. of stomach (pyloric region) of *Rana cyanophlyctis*, showing G IR cells by arrows. BM: Basement membrane, EP: Entric epithelium, LP: Lamina propria and LU: Lumen. [Paraffin sections of 6μ thickm 10x40, Antigen retrieval with tri-Sodium Citrate buffer]. (A magnified part of section shown in the box).](image)

![Figure 2: Photomicrograph of T.S. of stomach (pyloric region) of *Rana cyanophlyctis*, showing G IR cells by arrows. BM: Basement membrane, EP: Entric epithelium, LP: Lamina propria and LU: Lumen. [Paraffin sections of 6μ thickm 10x40, Antigen retrieval with tri-Sodium Citrate buffer]. (A magnified part of section shown in the box).](image)

![Figure 3: Photomicrograph of T. S. of fundic region of stomach of *Rana cyanophlyctis*, not showing the presence of G-IR cells. LU: Lumen, Cryosection of 12μ thick, Omission Control (10x10).](image)
the omission control sections (Fig. 1, 2 and 3). The gastrin immunoreactive cells (G IR) in the stomach region of *Rana cyanophlyctis* were localized amidst mucosal epithelium, nearly the gastric glands/pits and also at the base of epithelium (Fig. 1). The G IR cells were of variable shapes. Some are flask or bottle shaped while others were irregular or amoeboid. The storage product appears to be in the basal region of the cell (Fig. 1). Some spindle shaped cells have also been observed (Fig. 2). The frequency of G IR cells seems to be more in cryosections than in paraffin sections (Fig. 1 and 2). Both open and closed types of G IR cells have been found.

**DISCUSSION**

The endocrine cells of the gut located amidst mucosal epithelium appear in variable shapes. These may appear as pear shaped or flask shaped or triangular with broad base towards the basement membrane and the narrow part towards lumen often bearing microvilli. These cells are designated as open type. Some of the enteroendocrinocytes are also irregular in shape with pseudopodia like extensions/projections pointing towards submucosa as well towards mucosa and are embedded in deep. Such cells are named as closed type of cells. The former type of cells act via paracrine agent or act as lumone while latter type beside acting as paracrine agent also secrete their content into the synaptic area (Vizi et al., 1972; Dawson, 1978).

Solcia et al. (1969) identified the enteroendocrine cells using lead haematoxylin. However, Dawson (1970) observed that in human, gastrin producing cells exhibit positive Grimelius silver stain but are negative to diazo stain. Solcia et al. (1973) studied different types of enteroendocrine cells at ultrastructural level based on the size and amount of secretory granules but could not differentiate different types of cells chemically. They suggested that histochemical coupled with ultrastructural studies are more appropriate.

Bussolati and Pearse (1970) performed immunofluorescent ultrastructural studies are more appropriate.

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**REFERENCES**


Guyton, A. C. and Hall, J. E. 2008. Gastro Intestinal physiology. *In:


