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

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ABSTRACT
The objective of the present study was to identify the gastrin immunoreactive (G IR) cells in the stomach of *Rana cyanophlyctis* (Schneider). For the present study both cryosections and paraffin sections of the stomach were used for immunocytochemistry. Both open and closed types of cells have been observed. The occurrence of G IR cells was found to be amidst gastric mucosal epithelium, around gastric glands with prominent secretory granules and also in muscularis region. Such presence was assumed to be their strategic position for secretion. The cryosections were more convenient for study in comparison to paraffin sections. Counter staining for paraffin sections is suggested.

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 endocrine cells are implicated in the regulation of secretions, motility, digestion, absorption and cell proliferation of gastro-intestinal tract (Holst and Schmidt, 1994) and pancreas (Guilloteau, 2006). These endocrinocytes are either open type or closed type in nature (Fujita and Kobayashi, 1974). The open types of cells are those which extend their protoplasmic extensions towards the lumen while closed type extends towards the basal lamina (Fujita and Kobayashi, 1977).

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 deparaffinzed 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.

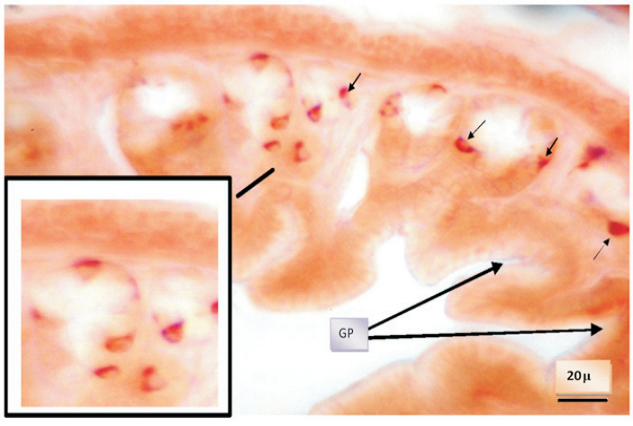

Figure 1: Photomicrograph of T.S. of stomach (pyloric region) of *Rana cyanophlyctis,* showing G IR cells by arrows. The cells are present around gastric glands marked by the presence of secretory granules at the base of G IR cells. GP: Gastric pits. [Cryosection of 12μ thick, 10x40]. (A magnified part of section shown in the box).

RESULTS

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

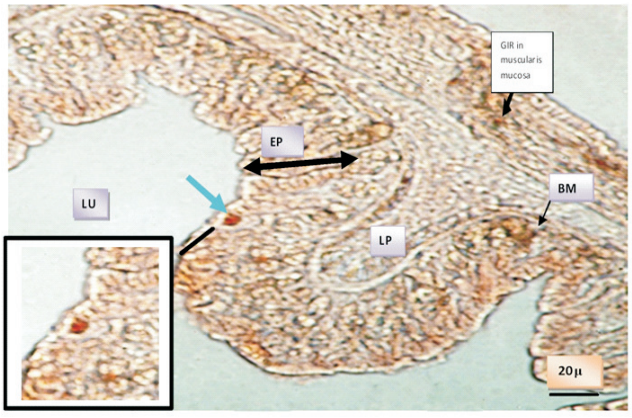

Figure 2: Photomicrograph of T.S. of stomach (pyloric region) of *Rana cyanophlyctis,* showing G IR cells by arrows. BM: Basement membrane, EP: Entric epithelium, LP: Lamina propria and LU: Lumen. [Paraffin sections of 6μ thickm 10x40, Antigen retrieval with tri-Sodium Citrate buffer]. (A magnified part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

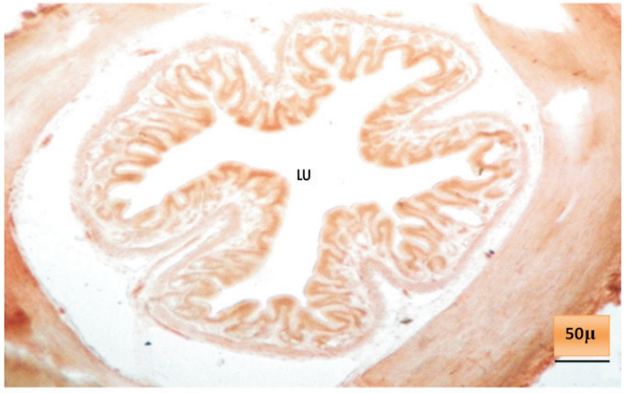

Figure 3: Photomicrograph of T. S. of fundic region of stomach of *Rana cyanophlytis,* not showing the presence of G IR cells. LU: Lumen. Cryosection of 12μ thick, Omission Control (10x10)

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, nearby 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 entero endocrinocytes 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 later 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 haemotoxylin. 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 utrastructural studies are more appropriate.

Bussolati and Pearse (1970) performed immunoflourescent technique to identify the gastrin secreting cells in cat pyloric mucosa. The immunohistochemical and immunocyto-chemical localization of gastrin was reported by Mc Guigan (1968); Vaillant *et al*. (1979); Bunnett, (1984); Sun and Song (2003) and Nisa *et al*. (2005) in different mammals. Among amphibians, Rada *et al*. (1987a) and Vilvarde *et al*. (1993) have performed immunocytochemical coupled with ultrastructural studies to identify the gastrin immunoreactive (G IR) cells in *Rana temporaria* and also measured the size of secretory granules with a mean diameter of 190 nm. Ku *et al*. (2000) have observed G IR cells in *Hyla arborea* in pyloric region only, which are spherical or spindle shaped. Ku *et al*. (2003) found that in *Rana rugosa* G IR cells are closed type and restricted to antral region only with low frequency and no G IR cells around gastric glands. Pan *et al*. (2009) observed G IR cells in *Rana nigromaculata* between feeding and fasting stages and observed more G IR cells in pyloric region, less in cardiac region during feeding phase while the number decreased in both the regions during fasting phase.

Our present study on *Rana cyanophlyctis* reveals the presence of G IR cells in both mucosal region and around gastric glands contradicting the observation of Ku *et al*. (2003) and corroborating those of Rada *et al*. (1987a,b). We are of the opinion that such occurrence of G IR cells is species specific and distant related species have more variation while closely related have more similarity. Furthermore, the presence of immunoreactivity is more around gastric glands. 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. Through the comparative studies of cryosections and paraffin sections, it is quite evident that the cryosections are more advantageous for study. However, the present authors also believe that the counter staining with haemotoxylin to paraffin sections may provide an equal opportunity for such kind of study.

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