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Research Article

HISTOMORPHOLOGICAL STUDY OF ENDOCRINE CELLS IN VARIOUS REGION OF DIGESTIVE TRACT OF BUFFALO *(BUBALUS BUBALIS)*

S. Dehury, R.K. Das, U.K. Mishra, G.R. Sahoo* , R. Patra

ABSTRACT: The histomorphology of enteroendocrine cells were studied in different segments of gastrointestinal tract of six adult buffalo. Depending upon the histomorphological study, the enteroendocrine cells were divided into 7 different types *viz.* oval cell (type I), pyriform cell (type II), spherical or rounded cell (type III), elongated cell (type IV), pyramidal cell (typeV), spindle cell (type VI) and large oblong cell (type VII). Further the granulation pattern of the cell types were noted as basal granulation, dense granulation, peripheral granulation and diffuse granulation.The endocrine cells revealed differential staining character with each of the stain (Masson-Haemperl argentaffin reaction, Grimelius silver technique, Ferric ferricyanide reduction reaction, Lead haematoxylin) employed in the present study depending upon their physiological status.

Key words: Entero-endocrine cells, Gastrointestinal tract, Argentaffin reaction.

INTRODUCTION

Specialized endocrine cells present in the gastrointestinal tract (GIT) and pancreas are known as enteroendocrine cells (Lorenz and Gordon 1993; Gordon and Hermiston 1994). They secret gastrointestinal hormones and various peptides (Skipper and Lewis 2000) into the blood stream in response to various stimuli to produce systemic effect, diffuse them as a local messenger and control the secretion, motility of GIT and transmit them to the enteric nervous system to activate nervous responses (Solcia *et al.,* 1981; Rehfeld *et al.,* 1998). Enteroendocrine cells reported to be densely distributed in various region of GIT (Ahlman *et al.,* 2001; Schonhoff *et al.,* 2004 and Moran *et al.,* 2008). About nine types of endocrine cells were identified in the digestive tract of buffalo depending upon the arrangement of secretion granules within the cells and degree of argentaffinity by Mishra and Das (1999) in the gastro-intestinal mucosa of Indian buffalo. Certain endocrine cells get affected in a particular disorder of gastrointestinal tract

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(Fujita and Kobayashi 1977). Loss of enteroendocrine cells in mice alters lipid absorption and glucose homeostasis and impairs postnatal survival in mice (Mellitzer *et al.*, 2010). Ileal interposition surgery in rat revealed an increase in number of enteroendocrine cells which resulted in delays of the type 2 diabetes (Hansen *et al.*, 2014). Very scanty literatures are available on histomorphology and histomorphometry of enteroendocrine cell. Here a detail study on histomorphology and histomorphometry of gut endocrine cells in buffalo was undertaken so that by knowing the normal characters, deviation can easily be detected as occurring in certain gastrointestinal pathological condition. In the present study, histomorphology and histomorphometry of the endocrine cells of the buffalo gastrointestinal mucosa were investigated.

MATERIALS AND METHODS

To investigate the histomorphology of enteroendocrine cells, tissue samples from eight segments of alimentary canal (cardia, fundus, pylorus, duodenum, jejunum, ileum, caecum and colon) were collected from 6 adult buffalo aged 1.5 to 2 year from the slaughter house of Nandankanan national zoological park. The tissues were immediately fixed in 10% buffered neutral formalin (BNF) for 72 hours and routinely processed to obtain 7-8µ thick serial paraffin sections. Tissue slides were stained with Masson-Haemperl argentaffin reaction, Grimelius silver technique, Ferric ferricyanide reduction reaction and Lead haematoxylin staining method. The staining procedures were followed from the book "Theory and practice of histological techniques" by Bancroft and Stevens (1996). The histomorphometrical study was conducted by using a research microscope with the help of a software *i.e.* Leica application suite software (LAS). The statistical analysis was made from the observed data and the mean and standard deviation were calculated. (Snedecor and Cochran 1976).

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RESULTS AND DISCUSSION

Depending on the histomorphological character, the enteroendocrine cells were categorized into 7 different types *viz.*

(1) Oval cells (type I): were oval in outline (Fig. 1). The secretory granules distributed in various patterns such as basally, densely, peripherally or diffusely. The cells were located in isolated manner in the villus. In the crypt the cells were found either in clusters or in an isolated manner. Most of them were opened into the lumen (open type) and few had no luminal contact (close type). In the villi areas most of the cells were of closed type. The nucleus was vesicular and centrally located.The size of the oval cell type ranges from 8.25±2.48µm to 6.05±0.76µm in diameter and that of the nucleus range from 2.96±0.99µm to 1.02±0.66µm.

(ii) Pyriform cells (type II): were of almond shaped (Fig.1). They had basal, peripheral, dense or diffuse granulation pattern.The maximum diameter of this cell type ranges from 8.86±1.19µm to 6.16±0.31µm.The nucleus was not clearly seen. However in some cells a vesicular, elongated and eccentric nucleus was apparent. The diameter of the nucleus varies from1.09±0.05µm to 2.95±0.22µm.

(iii) Spherical or rounded cells (type III): Cells were round in shape (Fig. 2, 4) with nearly equal diameter from all sides. The cells were mostly found in crypt region (close type) but not in the villi. The granulation pattern was either of the above sub types. The diameter

Table 1: Mean±sd of diameter (µm) of argentaffin endocrine cells and their nuclei in the digestive tract of adult buffalo (Bubalus bubalis): n=6, n=number of observations.

	TYPE- I	TYPE- II	TYPE-III	TYPE-IV	TYPE- V	TYPE – VI	TYPE - VII
CARDIAC	7.68±1.35* 5.45±1.61** 1.72±1.5***	7.33±1.01* 4.33±0.89** 2.12±0.24***	5.91±1.69* 5.92±1.58** 2.22±0.97***	8.29±1.25* 4.56±1.36** 1.28±0.59***	7.45±1.94* 5.23±1.78** 1.56±0.48***	7.65±1.26* 5.26±1.54** 1.96±0.43***	10.02±2.41* 6.28±1.62** 1.96±0.28***
FUNDIC	6.816±1.02* 4.72±1.38** 1.23±0.65***	6.59±1.56* 4.26±1.22** 1.69±0.59***	6.64±1.45* 6.45±1.26** 1.02±0.98***	8.42±1.96* 5.56±1.38** 1.56±0.95***	7.25±2.23* 5.41±1.54** 1.56±0.62***	7.29±1.54* 5.64±1.29** 1.59±0.52***	9.56±2.25* 6.24±1.54** 2.23±0.61***
PYLORIC	6.05±0.76* 4.87±0.62** 1.33±0.21***	7.22±0.35* 6.13±1.76** 2.02±0.6***	6.16±0.74* 6.36±0.83** 1.85±0.27***	9.58±2.24* 4.25±1.56** 1.22±0.28***	8.25±1.56* 4.29±1.24** 1.22±0.53***	8.21±1.96* 5.22±1.41** 1.69±0.52***	9.95±2.84* 5.09±1.43** 2.02±0.86***
DUODENUM	7.7±0.73* 4.51±0.81** 1.62±0.56***	7.08±1.2* 4.08±0.69** 1.66±1.15***	5.44±1.18* 5.31±0.85** 2.4±0.76***	9.53±2.56* 4.51±1.48** 1.52±0.69***	8.72±2.25* 5.26±1.49** 2.22±0.51***	8.95±2.07* 5.27±1.47** 1.72±0.21***	9.62±2.03* 5.21±1.59** 1.04±0.14***
JEJUNUM	6.95±1.32* 4.77±1** 1.47±0.32***	6.81±1.21* 4.29±1.21** 1.39±0.28***	6.62±0.52* 6.51±0.84** 1.56±0.39***	8.74±2.58* 4.01±1.36** 1.11±0.22***	7.88±2.21* 5.56±1.24** 1.25±0.89***	7.63±2.17* 5.21±1.54** 1.85±0.64***	–
ILEUM	6.32±0.6* 4.83±1.67** 2.72±0.49***	7.97±0.58* 6.02±1.09** 2.13±1.03***	5.84±0.84* 5.71±0.61** 1.13±0.16***	–	7.53±2.29* 4.52±1.52** 1.54±0.84***	–	–
CAECUM	6.27±0.3* 4.62±0.88** 1.29±0.26***	6.16±0.31* 4.56±0.49** 1.22±0.12***	7.82±1.02* 7.66±0.76** 2.3±0.19***	9.32±1.55* 5.02±1.09** 1.65±0.78***	7.22±2.18* 4.51±1.05** 1.84±0.74***	–	–
COLON	6.48±1.1* 5.07±1.12** 1.23±0.21***	7.79±0.97* 4.44±0.2** 1.32±0.25***	6.9±0.38* 6.57±0.29** 1.31±0.25***	–	–	–	–

(*) indicate maximum diameter/ height of cell,(**)indicate minimum diameter/ width of cell, (***) indicate diameter of nucleus

Table 2: Mean±sd of diameter (µm) of argyrophilic endocrine cells and their nuclei in the digestive tract of adult buffalo (Bubalus bubalis): n=6, n=number of observations.

REGION	TYPE- I	TYPE- II	TYPE-III	TYPE-IV	TYPE- V	TYPE – VI	TYPE - VII
CARDIAC	6.1±0.36* 4.23±0.57** 1.27±0.69***	6.78±1.44* 4.6±0.88** 1.44±0.54***	5.69±0.76* 5.34±0.6** 1.93±0.48***	8.39±0.73* 3.99±0.59** 2.03±1.09***	7.98±0.57* 6.66±0.71** 1.93±1.53***	6.14±0.68* 4.03±0.6** 1.38±0.33***	—
FUNDIC	6.16±0.43* 5.12±0.32** 2.38±0.12***	6.87±0.42* 5.12±0.48** 1.74±0.55***	6.44±1.63* 6.11±1.61** 2.31±0.98***	8.25±0.71* 3.14±0.71** 2.1±0.35***	7.44±1.27* 5.23±0.61** 2.31±0.34***	7.79±0.85* 5.64±1.25** 1.9±0.6***	—
PYLORIC	6.35±0.67* 4.88±0.76** 1.58±0.32***	7.29±0.82* 4.61±1.26** 1.36±0.35***	6.31±0.63* 6.02±0.77** 1.5±0.35***	—	—	7.13±0.53* 5.06±0.94** 1.49±0.41***	—
DUODENUM	6.93±2.6* 6.28±2.02** 2.79±1.03***	7.15±0.26* 5.83±0.63** 2.47±0.88***	5.03±0.99* 5.77±0.88** 2.24±0.34***	8.46±2.38* 3.89±1.84** 1.3±0.4***	7.09±2.69* 6.13±2.14** 2.18±0.58***	7.26±0.71* 5.16±0.73** 1.39±0.26***	10.46±2.71* 7.04±1.43** 3.14±1.26***
JEJUNUM	6.3±1.42* 5.32±0.72** 1.31±0.28***	7.07±0.73* 5.3±1.04** 2.5±0.47***	—	8.49±1.19* 3.73±0.86** 1.55±0.48***	6.72±2.72* 6.04±2.88** 1.49±0.26***	—	—
ILEUM	7.93±2.5* 5.84±2.23** 1.82±0.8***	6.52±1.65* 5.52±2.4** 2.95±0.22***	—	—	—	—	—
CAECUM	7.63±1.4* 4.51±0.37** 1.5±0.43***	7.12±0.58* 5.12±1.11** 1.8±0.3***	—	—	—	6.88±0.28* 4.52±0.55** 1.29±0.36***	
COLON	6.35±0.84* 5.58±1.25** 1.15±0.21***	7.74±0.54* 5.04±0.66** 2.01±0.26***	—	9.02±0.97* 4.55±0.42** 1.67±0.34***	—	—	—

(*) indicate maximum diameter/height of cell, (**) indicate minimumdiameter / width cell
(***) Indicate diameter of nucleus.

Table 3: Mean±sd of diameter (µm) of Ferric freeicyanide reduction reacting endocrine cells and their nuclei in the digestive tract of adult buffalo (Bubalus bubalis): n=6, n=number of observations.

REGION	TYPE-I	TYPE-II	TYPE-III	TYPE-IV	TYPE-V	TYPE-VI	TYPE-VII
CARDIAC	6.96±1.28* 4.9±0.59** 2.08±123***	7.99±0.51* 5.44±1.19** 1.83±1.01***	5.93±0.33* 5.64±0.24** 1.32±0.14***	9.18±3.93* 3.86±0.56** 2.18±1.08***	8.27±1.44* 6.71±1.25** 2.32±1.92***	9.90±0.53* 6.56±1.59** 1.17±0.22***	10.14±0.21* 4.58±0.85** 2.41±0.91***
FUNDIC	6.32±1.39* 4.15±0.56** 1.02±0.66***	7.04±1.32* 5.59±1.24** 1.26±0.59***	6.69±0.88* 6.53±1.2** 1.39±0.64***	8.59±1.22* 4.26±1.32** 1.22±0.68***	7.85±1.56* 5.23±1.49** 0.96±.033***	7.48±1.23* 5.76±1.33** 1.25±0.85***	–
PYLORIC	8.64±0.26* 4.30±0.59** 1.93±0.45***	8.86±1.19* 4.38±1.01** 2.12±0.41***	–	–	7.51±1.48* 3.86±0.64** 1.10±0.17***	–	–
DUODENUM	8.52±1.09* 5.54±0.98** 2.96±0.99***	7.00±0.51* 5.07±0.51** 2.29±0.43***	5.53±0.85* 5.70±1.02** 1.13±0.13***	6.71±2.23* 5.54±1.27** 1.24±0.28***	6.70±2.23* 5.53±1.26** 1.24±0.27***	6.14±0.49* 3.90±0.41** 1.13±0.13***	11.18±1.13* 7.29±1.66** 2.61±0.54***
JEJUNUM	7.24±1.87* 5.37±0.56** 2.15±0.41***	–	4.83±1.03* 4.78±1.35** 1.24±0.3***	8.67±1.02* 3.24±0.35** 0.96±0.20***	7.44±1.83* 4.84±1.56** 1.39±0.28***	–	8.08±0.51* 5.24±0.60** 1.03±0.11***
ILEUM	7.10±.1.03* 6.51±1.42** 1.9±0.23***	7.53±0.32* 3.48±0.40** 1.55±0.43***	6.67±1.18* 6.33±0.80** 2.07±0.14***	–	—	–	–
CAECUM	7.57±0.69* 6.37±3.15** 1.35±0.21***	7.74±0.44* 5.49±0.89** 1.29±0.24***	6.51±0.22* 6.36±0.26** 1.74±0.36***	–	–	8.05±0.49* 5.12±0.49** 1.46±0.20***	
COLON	7.47±0.69* 5.74±1.5** 1.25±0.13***	7.31±0.9* 4.51±0.75** 1.61±0.49***	6.66±0.81* 6.51±0.75** 1.31±0.22***	8.42±0.69* 5.19±0.32** 1.04±0.48***	–	–	–

(*) indicate maximum diameter/ height of cell, (**) indicate minimum diameter/ width of cell,(***) indicate diameter of nucleus.

Table 4 : Mean±sd of diameter (µm) of Lead haematoxylin reacting APUD cells and their nuclei in the digestive tract of adult buffalo (Bubalus bubalis): n=6, n=number of observations.

REGION	TYPE-I	TYPE-II	TYPE-III	TYPE-IV	TYPE-V	TYPE-VI	TYPE-VII
CARDIA	7.32± 2.18* 5.21± 1.54** 2.23±0.55***	8.21±3.12* 5.21±2.12** 2.56±1.03***	6.48±2.21* 6.83±1.56** 2.16±0.56***	8.49±1.19* 3.73±0.86** 1.55±0.48***	7.45±1.94* 5.23±1.78** 1.56±0.48***	7.29±1.54* 5.64±1.29** 1.59±0.52***	9.56±2.25* 6.24±1.54** 2.23±0.61***
FUNDUS	8.25±2.48* 5.14±2.01** 2.14±1.01***	8.29±3.54* 6.85±2.2** 2.85±0.63***	6.56±2.48* 6.25±1.25** 2.69±0.54***	9.02±0.97* 4.55±0.42** 1.67±0.34***	8.25±1.56* 4.29±1.24** 1.22±0.53***	8.95±2.07* 5.27±1.47** 1.72±0.21***	11.18±1.13* 7.29±1.66** 2.61±0.54***
PYLORUS	7.25±2.14* 5.21±2.05** 2.12±1.11***	7.84±2.64* 5.32±1.54** 2.16±0.75***	6.47±2.56* 6.58±2.48** 1.16±0.54***	8.39±0.73* 3.99±0.59** 2.03±1.09***	7.98±0.57* 6.66±0.71** 1.93±1.53***	7.26±0.71* 5.16±0.73** 1.39±0.26***	9.62±2.03* 5.21±1.59** 1.04±0.14***
DUODENUM	8.02±3.12* 6.21±2.04** 2.41±0.95***	8.56±3.21* 6.87±2.14** 2.69±0.54***	5.03±0.99* 5.77±0.88** 2.24±0.34***	8.42±1.96* 5.56±1.38** 1.56±0.95***	6.72±2.72* 6.04±2.88** 1.49±0.26***	7.79±0.85* 5.64±1.25** 1.9±0.6***	10.02±2.41* 6.28±1.62** 1.96±0.28***
JEJUNUM	8.15±3.41* 6.45±2.06** 2.25±1.14***	7.28±2.16* 6.58±1.14** 1.09±0.05***	6.69±0.88* 6.53±1.2** 1.39±0.64***	8.46±2.38* 3.89±1.84** 1.3±0.4***	7.88±2.21* 5.56±1.24** 1.25±0.89***	6.14±0.49* 3.90±0.41** 1.13±0.13***	9.62±2.03* 5.21±1.59** 1.04±0.14***
ILEUM	7.84±2.13* 6.25±1.18** 2.16±1.05***	8.65±3.25* 5.95±1.15** 1.85±0.36***	6.62±0.52* 6.51±0.84** 1.56±0.39***	–	6.72±2.72* 6.04±2.88** 1.49±0.26***	–	–
CAECUM	7.98±3.21* 5.12±2.15** 2.85±1.22***	7.45±2.12* 5.41±2.58** 2.23±0.65***	–	9.53±2.56* 4.51±1.48** 1.52±0.69***	8.59±1.22* 4.26±1.32** 1.22±0.68***	7.48±1.23* 5.76±1.33** 1.25±0.85***	–
COLON	8.21±3.18* 6.48±2.15** 2.11±1.09***	7.69±2.65* 5.36±1.16** 1.09±0.54***	5.91±1.69* 5.92±1.58** 2.22±0.97***	8.74±2.58* 4.01±1.36** 1.11±0.22***	6.70±2.23* 5.53±1.26** 1.24±0.27***	–	–

(*) indicate maximum diameter/ height of cell,(**) indicate minimum diameter/ width of cell,(***) indicate diameter of nucleus

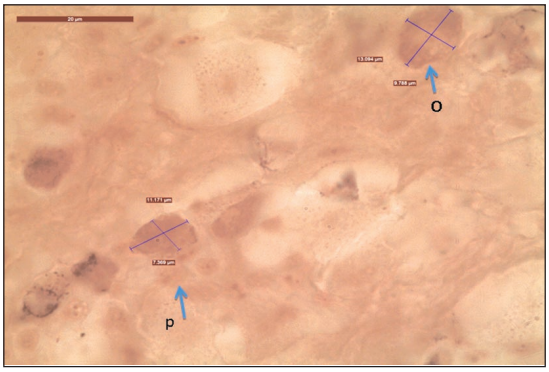

Fig. 1: Photomicrograph of cardia of buffalo showing the distribution of secretion granules in a pyriform cell (p), oval cell (o). Griemilus silver X 1000.

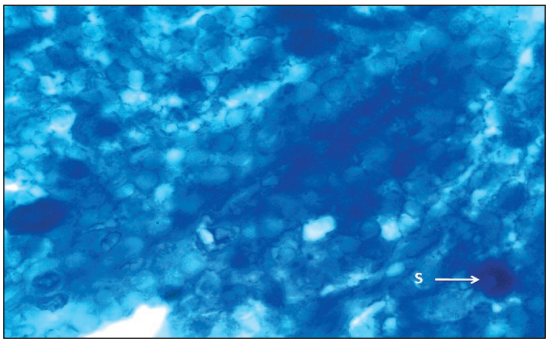

Fig. 2: Photomicrograph of jejunum of buffalo showing the distribution of secretion granules in a spherical cell (S). Ferric ferricyanide reduction reaction X 1000.

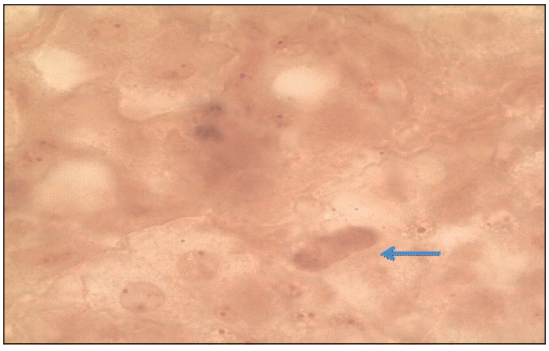

Fig. 3: Photomicrograph of cardia of buffalo showing the distribution of secretion granules in elongated cell. Griemilus silver X 1000.

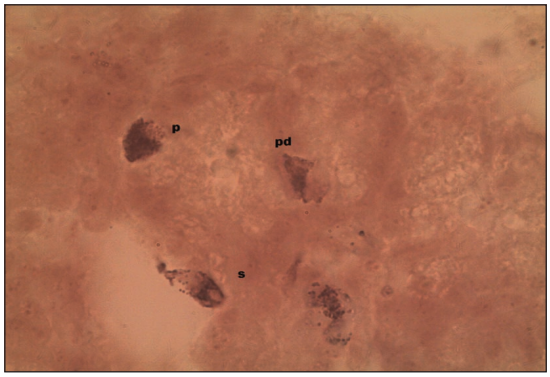

Fig. 4:Photomicrograph of duodenum of buffalo showing the distribution of secretion granules in a pyriform cell (p), pyramidal cell (pd), spindle shaped cell (s). Griemilus silver X 1000.

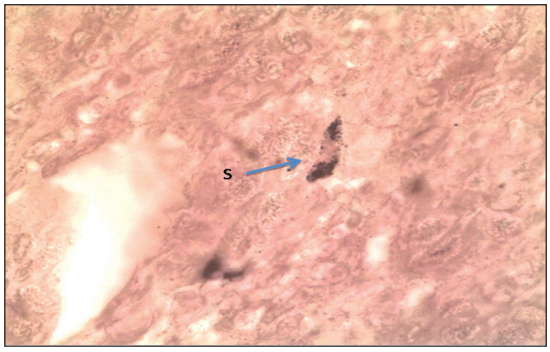

Fig. 5: Photomicrograph of jejunum of buffalo showing the distribution of secretion granules in spindle shaped cell (s). Griemilus silver X 1000.

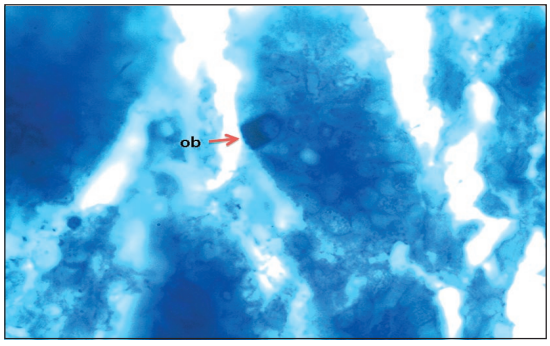

Fig. 6: Photomicrograph of jejunum of buffalo showing the distribution of secretion granules in a large oblong cell (ob). Ferric ferricyanide reduction reaction X 1000.

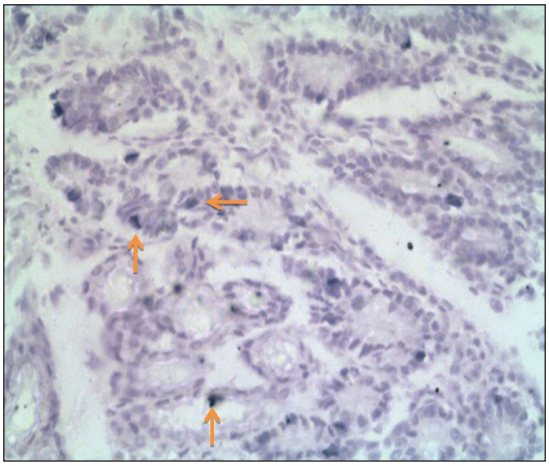

Fig. 7: Photomicrograph of ascending duodenum of buffalo showing scanty distribution of enteroendocrine cells in the middle part of the crypt. Lead Haematoxylin reaction X 400.

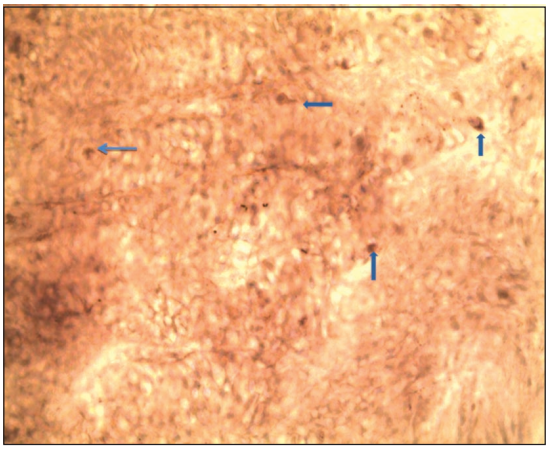

Fig. 8: Photomicrograph of descending duodenum of buffalo showing the scanty distribution of argentaffin cells in the wall of duodenal gland. Note the cells have very weak affinity for Argentaffin reaction. Masson-Hamperlargentaffin reaction X 400.

ranges from 7.82±1.02µm to 4.78±1.35µm. Nucleus of this cell type was spherical or centrally located with diameter ranges from 2.69±0.54µm to 1.02±0.98µm.

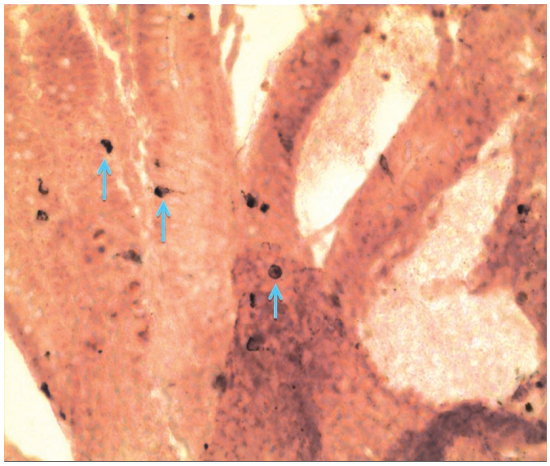

Fig.9: Photomicrograph of descending duodenum of buffalo showing the histomorphology of Argyrophilic cells stained with Griemilus silver X 400.

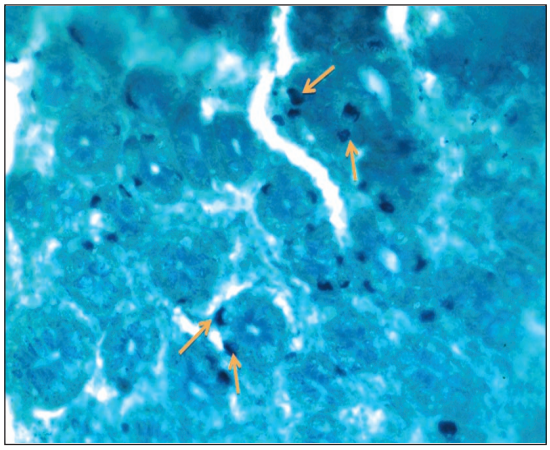

Fig.10: Photomicrograph of descending duodenum of buffalo showing the histomorphology of enteroendocrine cells having Ferric ferricyanide reduction reaction of X 400.

(iv) Elongated cells (type IV): Cells were elongated with dense granulation (Fig. 3). They were larger in size and often associated with cell processes. Cells were few in the crypt region and rare in villi zone. Most of the cells were of open type while some were of close

type.The length of the cell varies from 9.58±2.24µm to 6.71±2.23µm. Nucleus (2.1±0.35µm to0.96±.033µm in diameter) was central and vesicular.

(v) Pyramidal cells (type V): Cells were pyramidal in shape (Fig. 4) with closed type and present in few numbers in the neck of the gland and villi. The height of the cell ranges from 9.58±2.24µm to 8.25±0.71µm.The nucleus was circular and eccentric with its diameter ranged from 2.32±1.92µm to 0.96±.033.

(vi) Spindle cells (type VI): Cells were spindle shaped (Fig. 4, 5) narrow at both ends and wide centrally. Located in isolated manner (open type) in crypt and were not seen in villi. They had mostly bipolar granulation pattern. The length of the cell varies from 8.95±2.07µm to 6.14±0.49µm.The nucleus (1.96±0.43µm-1.13±0.13µm in diameter) was vesicular, rounded and centrally placed. In some cells, an elongated nucleus was seen.

(vii) Large oblong cells (type VII): were characterized by large size, oblong shape (Fig. 6) with fine granulation pattern. Cells appeared in isolated manner being related to the basal lamina and were of closed type. The diameter of the cells range from 11.18±1.13µm to 8.08±0.51µm. The nucleus was circular and eccentric with diameter ranges from 3.14±1.26µm to 1.03±0.11µm.

The morphology of these seven types of cell as measured under different stains (Masson-Haemperl argentaffin reaction, Grimelius silver technique, Ferric ferricyanide reduction reaction and Lead haematoxylin staining methods) used in this present study is presented in table (1), (2), (3), (4) respectively.

In contrast to the seven number of cells as observed in the present study only two categories of cells were reported by Das (1984) *i.e.* argentaffin cell and chromaffin cell and nine categories of cells were identified by Mishra and Das (1999) depending on granulation pattern and shape of cell *i.e.* basally granulated cells (type-I), peripherally granulated cells (type-II), densely granulated cells (type-III), diffusely granulated cells (type-IV), small dense elongated cells (type-V), pyramidal cells (type-VI), light stained granular cells (type-VII), large oblong argentaffin cells (type-VIII) and oval non-argentaffin chromaffin cells (type-IX). In agreement to these present findings Mishra (1990) reported seven types of cells in sheep, goat and cattle. The morphological basis of classification is supported by the reports of Rezzotti *et al.* (1979). In the ovine abomasum, 8 types of endocrine cells were classified at ultrastructural level. The gastric-type endocrine (EC) cells contained oval and pleomorphic granules with high electron density. The intestinal-type EC cells were filled with oval, irregular and highly dense granules. Endocrine like (ECL) cells contained irregular granules with high density and wide clear spaces. D cells were filled with round granules showing low to moderate density and finely granular matrix. D1 cells contained round or oval granules with variable, low to moderate density and finely granular content. G cells showed round and oval granules with moderate density, densely packed or flocculent content. F cells were filled with oval or elliptic granules showing low density with a finely granular and flocculent matrix. X cells contained round granules with high density and homogeneous material (Oomori 1983). Using immunohistochemistry today several cell categories have been characterized in digestive mucosa of different animals, birds including human beings. These cells include the

somatostatin, gastrin, glucagon, cholecystokinin, grehlin secreting cells and so on depending on the hormone content of the secretion granules (Kitamura *et al.*, 1984; Furness *et al.*, 2013). 14 different enteroendocrine cell types by Rindi *et al.*, (2004) and 10 cell types (Mellitzer *et al.*, 2010) have been identified in GIT of mice basing on their hormone production.

Out of four histochemical staining methods employed for demonstration of endocrine cells in gut mucosa wall, the Grimelius silver technique (Fig.1,3,4,5,9) was found to be effective for demonstrating the entero-endocrine cells followed by Ferric Ferricynide reduction (Fig.2,6,10). Both the methods stained a large population of endocrine cells with their distinct morphology along with their nucleus. The cell processes if present could be clearly visualized by these staining techniques. By employing Masson-Haemperl argentaffin reaction (Fig. 8) plenty of endocrine cells were found in gut mucosa wall but the morphology of the cells were not very clear. Out of the four staining technique lead haematoxylin (Fig.7) stained least number of cells with indistinct cell morphology.

CONCLUSION

On the basis of shape and size of the enteroendocrine cells, they were classified into oval cell, pyriform cell, spherical cell, elongated cell, pyramidal cell, spindle cell, large oblong cell. For the demonstration of the enteroendocrine cell morphology Grimelius silver technique is most suitable one among the 4 above staining methods.

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