Vol II Issue V Nov 2012

Impact Factor : 0.1870

ISSN No :2231-5063

Monthly Multidiciplinary Research Journal

Golden Research Thoughts

IMPACT FACTOR : 0.2105

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RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

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Golden Research Thoughts
Volume 2, Issue. 5, Nov. 2012
ISSN:-2231-5063
Available online at www.aygrt.net

ORIGINAL ARTICLE

HISTOCHEMICAL STUDIES ON MUCOSUBSTANCES IN THE PYLORIC STOMACH OF CHAMAELEON ZEYLANICUS LAURETI

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Abstract:

The histological architecture, localization and quality of mucosubstances in the pyloric stomach of Chameleon zeylanicus laureti is described. Pyloric stomach is a narrow tubular posterior part of stomach. The histological study revealed that the wall of pyloric stomach is composed of mucosa, submucosa, muscularis and serosa. Mucosal epithelium was single layered formed mainly of tall columnar cells and few goblet cells. Histochemical study showed the presence of moderate amount of neutral mucosubstances and poor quantities of sialomucins in the epithelial cells. Goblet cells contained moderate amount of sulfomucins. Three types of glands have been identified in pyloric stomach depending upon the type of mucosubstances secreted by the gland cells. Glands-Type I indicated the presence of only neutral mucosubstances (intense). Glands-Type II cells contained only sulfomucins (intense). In glands-Type III, two types of cells were observed, the type I and the type II cells. Type I cells in these glands contained only neutral mucosubstances (intense). Glands-type II cells contained only sulfomucins (intense).

KEYWORDS:,

Chamaeleon zeylanicus laureti, pyloric stomach, histology, histochemistry.

INTRODUCTION :

An insight into existing literature pointed out that very scanty information is available on histology of alimentary canal of reptiles and also on histochemistry of mucosubstances. It has been studied in lower vertebrates like fishes. Absence of glands was recorded in the pyloric stomach of M. cyprinoides by Pasha (1964). Mucosubstances in the pyloric stomach have been investigated by Reifel and Travill (1978) in eight species of fishes. They reported the presence of neutral mucosubstances, sulfomucins and both sialidase resistant and sialidase labile sialomucins at various sites. Jadhav (1985)showed the presence of numerous goblet cells in the pyloric epithelium of C. magur and columnar epithelial cells and goblet cells in T. mossambica. Histochemical results indicated the presence of goblet cells elaborating neutral mucosubstances in C. magur while in T. mossambia these cells elaborated both neutral mucosubstances and sulfomucins.

Zdravko Petrinec et. al. (2005) demonstrated superficial epithelial cells and simple tubular gastric glands in lamina propria of pike pyloric stomach. In cat fish gastric glands are arranged as tubular branched glands surrounded by connective tissue. Superficial epithelial cells indicated the presence of neutral mucosubstances and a small amount of acid mucosubstances. The acid mucosubstances are shown to be chiefly of the carboxylate and sialyated type. Sac like tubular gastric glands surrounded by connective tissue were observed in blue fin,tuna. The neck cells of gastric glands synthesize a mixture of neutral and acidic glycoproteins (Kozaric Z et.al. 2007).

Histochemical study by Norris (1959) indicated PAS reactive cells in the pyloric epithelium and

glands in R. pipiens. Presence of neutral mucosubstances in the surface goblet cells and pyloric glands have been reported in several amphibians (Suganuma et al., 1981a,Mutkekar 1982; Mangalware 1982 and Patil 1983).They further reported presence of trace quantities of sialmucins in the epithelial cells. Patil (1983) reported the distribution of neutral mucosubstances and sialomucins in the foveolar epithelial cells in the pyloric stomach of R. cyanophlyctis. Jadhav (1985) demonstrated the neutral mucosubstances in goblet cells. He reported the presence of only neutral mucosubstances in the goblet cells, glands and serosa; glycogen in muscularis and neutral mucosubstances and hyaluronic acid in the sumucosal connective tissue in the pyloric stomach of the fungoid frog. Giuseppa E Liquori et.al., (2003) observed that epithelial superficial cells of the pylorus were PAS positive and AB negative in Bufo viridis.

Moustafa Zaher et.al., (1981) observed the surface epithelial cells, gland pits and necks in pyloric stomach of Uromastyx aegeptiaca. The pyloric glands were simple tubular with longer necks. Neutral mucosubstances were abundantly found in the luminal poles of the mucal epithelium. Domenico Ferri et al., (1998) demonstrated neutral glycoprotein secreting cells in the pyloric glands of Chalcides chalcides.

In birds pyloric stomach is highly muscular and called as ventriculus or gizzard (Patt and Patt, 1069), in P. domensticus and C. splendes. Mogil naya and Bagatyr (1977) have reported the presence of neutral carbohydrates, sulfomucins and sialomucins in the gizzard of birds.

The histology of mammalian stomach has been described by Patt and Patt (1969), Forman et. al. (1979), Bhide (1979) and Deshmukh (1984), Birgele (1969) demonstrated that surface epithelial cells elaborated neutral mucosubstances in cow. Carvalho et.al. (1973) demonstrated that surface epithelial cells contain sulfated and carboxymucins in armadillo. Pyloric glands elaborating different kinds of mucosubstances have also been reported in mammals. The mucosubstances have been identified as neutral in ferret, Poddar and Jacob (1979) in dog, Tsujmura (1976); as neutral and acidic in cow (Birgele, 1969) and in armadillo (C. Carvalho et al., 1973). Nazan Keskin et al. (2011) indicated the presence of sulfated and acidic glycoconjugates in stomach of mouse.

MATERIALAND METHODS

Adult chameleons of both sexes were collected from Jakatwadi, Tal. Dist. Satara and brought to the laboratory. The live animals were sacrified by using chloroform and their pyloric stomach quickly removed. The pyloric stomach was then cut into smaller pieces measuring about 4 to 5 mm. in length and fixed immediately in cold (4oC) 2% calcium acetate in 10% neutral formalin.

After 24 hours of fixation the tissues were washed thoroughly in running tap water for 6 to 10 hours, dehydrated in ethanol grades, cleared in xylene and embedded in paraffin. The sections were cut at 5 – 6µ. After dewaxing and hydration some of the sections were stained with haematoxylin – Eosin (H – E) for histological observations and remaining sections were subjected to series of well established and recommended histochemical techniques for characterization of mucosubstances

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OBSERVATIONS-

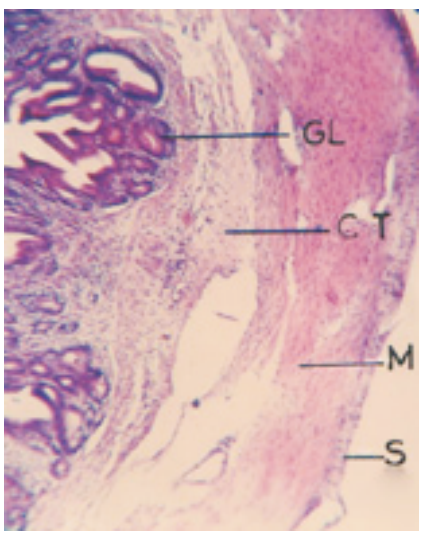

Fig. 1: T. S. of pyloric stomach of *C. zeylanicus laureti* H-E staining x 100

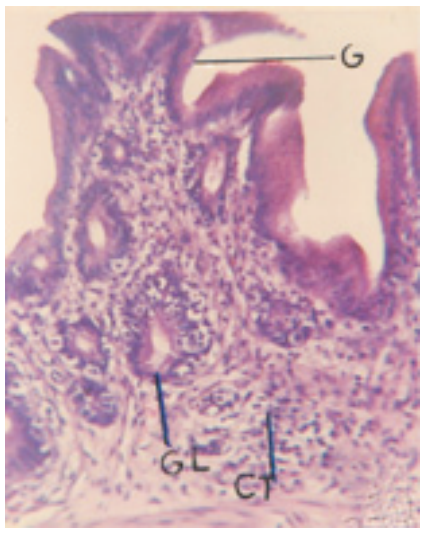

Fig. 2 :T. S. of pyloric stomach of *C.zeylanicus laureti* H-Estaining x 200

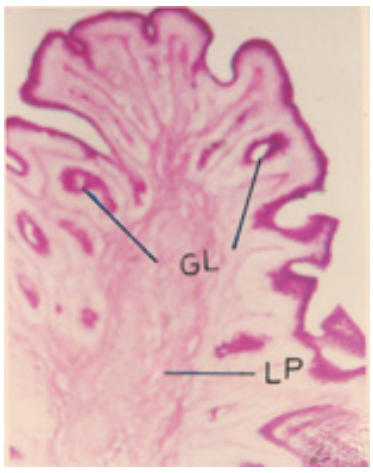

Fig. 3 : T. S. of pyloric stomach of *C. zeylanicus laureti* PAS staining x150

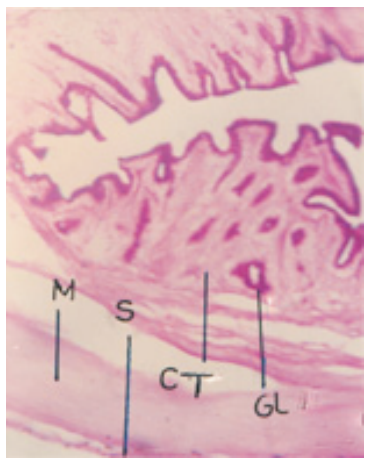

Fig. 4 : T. S. of pyloric stomach of *C. zeylanicus laureti* D-PAS x100

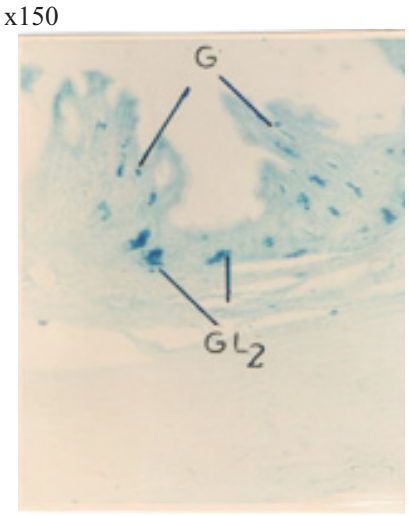

Fig. 5 : T. S. of pyloric stomach of *C. zeylanicus laureti* C.I. staining x 100

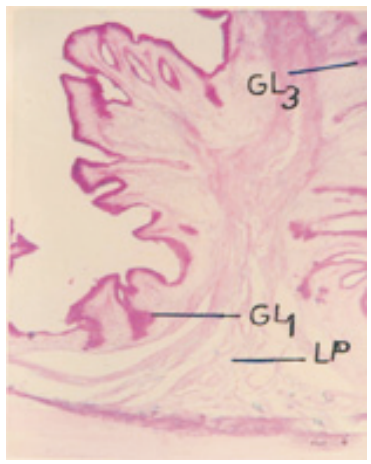

Fig. 6 :T. S. of pyloric stomach of *C. zeylanicus laureti* AB pH 1.0 – PAS staining x 100

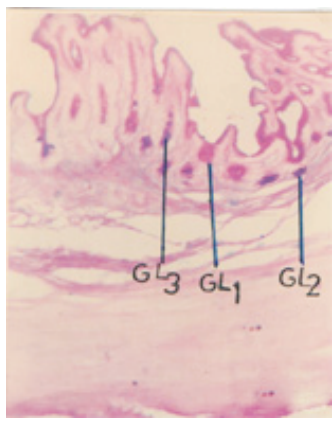

Fig. 7 : T. S. of pyloric stomach of *C. zeylanicus laureti* AB pH 2.5 – PAS staining x 100

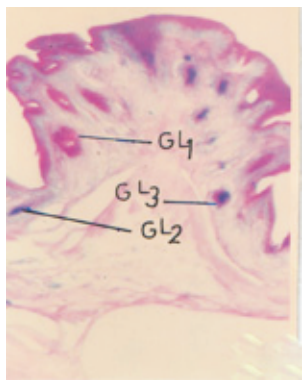

Fig. 8 :T. S. of pyloric stomach of *C. zeylanicus laureti* C. I. – PAS staining x 150

ABBREVIATIONS

G - Goblet Cells
S- Submucosa
CT- Connective tissue
M— Muscularis
LP- Lamina propria

GL - Glands
Gl1 - Glands – Type I
Gl2 - Glands – Type II
Gl3 - Glands – Type III

Table 1
Histochemical reactivities of mucosubstances in the pyloric stomach of C. zeylanicus laureti

Sr. No.	Histochemical Reaction	Mucosa						Submucosa	Muscularis	Serosa
		Ciliated epithelial cells	Goblet Cells	Glands Type – I	Glands Type – II	Glands Type – III				
						Type – I Cells	Type – II Cells			
1	PAS	++++P	+++P	+++P	++++P	+++P	++++P	+P	+P	+P
2	Ph -PAS	+P	+++P	-	++++P	-	++++P	± P	± P	± P
3	D-PAS	++++P	+++P	+++P	++++P	+++P	++++P	+P	±P	+P
4	AB pH 1.00	-	+++B	-	++++B	-	++++B	±B	±B	±B
5	AB pH 1.0 - PAS	++++P	+++P	+++P	++++B	+++P	++++B	+BP	+BP	+BP
6	AB pH 2.5	+ B	+++B	-	++++B	-	++++B	±B	±B	±B
7	AB pH 2.5 – PAS	++++BP	+++B	+++P	++++B	+++P	++++B	±BP	±BP	±BP
8	C.I.	+B	+++B	-	++++B	-	++++B	±B	±B	±B
9	C.I. PAS	++++BP	+++B	+++P	++++B	+++P	++++B	±BP	±BP	±BP

RESULTS

A)HISTOLOGICAL OBSERVATIONS

H-E stained sections revealed four coats such as mucosa, submucosa, muscularis and serosa (Figs.1 and 2).

i)MUCUSA : The folds were few and broad. The mucosal epithelium was single layered formed mainly of columnar cells and few goblet cells. The lamina propria was prominent. Three types of glands were observed. The differentiation of glands was very clear in histochemical observations particularly in combined histochemical procedures such as AB pH 1.0 – PAS (Fig. 6), AB pH 2.5 – PAS (Fig. 7) and C.I. – PAS (Fig. 8).

ii)SUBMUCOSA : The submucosal connective tissue layer was thin and projected into mucosal folds.
iii)MUSCULARIS : It was thick and distinguished into an inner circular and an outer longitudinal muscle layer.
iv)SEROSA : It was thin outermost layer surrounding the muscularis.

B)HISTOCHEMICAL OBSERVATIONS

The histochemical observations in the different histological sites of the pyloric stomach are recorded in Table No.1, according to staining intensities (++++intense, +++moderate, ++weak, +poor, ±trace and - negative). The distribution of mucosubstances are illustrated in photomicrographs (Figs. 3 – 8)

I)MUCOSA

i)EPITHELIAL CELLS

These cells exhibited intense PAS staining (Fig. 3) which could be blocked phenylhydrazine pretreatment indicated the presence of both the neutral mucosubstances and the acidic mucins. The PAS reactivity was not changed with diastase indicated absence of glycogen.

These cells exhibited negative staining with AB pH 1.0 and the alcianophilia appeared at AB pH 2.5 and C.I. These results indicated the presence of carboxymucins but absence of sulfomucins. The absence of sulfomucins and the presence of carboxymucins was also supported by only purple staining with AB pH 1.0 – PAS (Fig. 6).

The presence of neutral mucins in these cells was also inferred from pink staining with AB pH 1.0 – PAS (Fig. 6), blue purple staining with AB pH 2.5 – PAS (Fig. 7) and C. I. – PAS (Fig. 8) sequencing staining procedures. Thus, the aforementioned histochemical observations revealed the presence of neutral mucosubstances (moderate) and sialomucins (poor).

ii)GOBLET CELLS

These cells were few in number which exhibited moderate PAS reactivity. The PAS reactivity was resistant to phenylhydrazine pre-treatment and diastase digestion (Fig. 4) indicting the absence of neutral mucosubstances and glycogen.

These cells reacted moderately towards AB pH 1.0 and similar moderate blue staining was also seen in this layer with AB pH 2.5 and C.I. (Fig. 5). Moreover, these cells remained only moderately blue in sequential staining procedures such as AB pH 1.0 – PAS (Fig. 6), AB pH 2.5 – PAS (Fig. 7). Therefore, it was concluded that the goblet cells in these cells contain only sulfomucins. The aforementioned results therefore, indicated the presence of only sulfomucins in these cells of pyloric stomach.

GLANDS

Three types of glands have been identified in pyloric stomach depending upon the mucosubstances secreted by the gland cells, which were glands-type I, glands-type II and glands-type III.

a)GLANDS – TYPE I

The cells of this type of glands showed intense PAS reactivity which was completely blocked by phenylhydrazine pretreatment, however, the diastase digestion had no effect on the intensity of PAS in these cells. These initial results indicated the presence of only neutral mucosubstances while absence of glycogen.

The negative reaction of these cells with AB pH 1.0, AB pH 2.5, C.I. revealed absence of acidic mucosubstances. The presence of neutral mucosubstances in these cells was also substantiated by their only PAS reactivity in sequential staining procedures such as AB pH 1.0 – PAS (Fig. 6), AB pH 2.5 – PAS (fig. 7) and C.I. – PAS (Fig.8). Thus the aforementioned histochemical observations revealed the presence of only neutral mucosubstances (intense) in these cells of gland-type I.

b)GLANDS – TYPE II

The cells of this type of glands exhibited an intense PAS reactivity. The PAS reactivity was resistant to prior phenylhydrazine treatment and diastase digestions, indicating the absence of neutral

mucosubstances and glycogen.

These cells reacted intensely towards AB pH 1.0 and similar intense blue staining was also seen in these cells with AB pH 2.5 and C.I. (Fig. 7, 8). Moreover, these cells remained only intense blue in sequential staining procedures such as AB pH 1.0 – PAS (Fig. 6), AB pH 2.5 – PAS (Fig. 7). Therefore, it was concluded that these cells contained only sulfomucins.

c)GLANDS – TYPE III

In these glands two types of cells were observed; the type-I and the type-II cells.

i)Type-I cells :

These cells exhibited the histochemical results which were practically identical to the gland cells observed in glands type I. Type-I cells in these glands contained only neutral mucosubstances.

ii)Type-II cells :

These type of cells exhibited the results identical to the cells of glands type II. Therefore,it was concluded that these cells contained only sulfomucins (intense).

II)SUBMUCOSA

The connective tissue of submucosa showed poor staining with PAS which could partially be blocked by phenylhydrazine pretreatment but diastase digestion could not altered the PAS staining. These observations indicated that the connective tissue in this layer contained trace amount of both the neutral and acidic mucosubstances but the absence of glycogen.

The connective tissue showed trace alcianophilia at pH 2.5 and C.I. indicating the presence of only sialomucins (trace).

The fact that the connective tissue of submucosa also contained trace amount of neutral mucosubstances were inferred from blue-purple staining with AB pH 1.0 – PAS (Fig. 6), AB pH 2.5 – PAS (Fig. 7) and C.I. – PAS (Fig. 8). Therefore, it was concluded that the connective tissue in submucosa of pyloric stomach contained the neutral mucosubstances (trace) and sulfomucins (trae).

III)MUSCULARIS

The muscularis of pyloric stomach exhibited identical histochemical reactivities (Figs. 3-8) to that described for connective tissue of submucosa. The only difference was that this layer showed the presence of glycogen as the PAS reactivity get diminished with diastase digestion.

Therefore, it was concluded that this layer contained the neutral mucins (trace), sulfomucins (trae) and glycogen.

IV)SEROSA

This layer showed practically identical histochemical results which were observed in the connective tissue to the submucosa of this organ. Therefore, it was concluded that the serosa of pyloric stomach contained the mixture of neutral mucins (trace) and sulfomucins (race).

DISCUSSION

The wall of pyloric stomach is composed of mucosa, submucosa, muscularis and serosa as observed in other reptiles like Chalcides chalcides. Domenic Ferri et al., (1998), Uromastyx aegeptiaca, Mustafa Zaher et al., (1981). Mucosa is lined with epithelial cells and few goblet cells. The present investigation also reveal similar histology.

Jadhav (1985) stated that the mucosal epithelium consists of goblet cells in ground lizard and columnar cells in turtle. He also added that the short and alveolar glands are present in both these reptiles.

The remaining layers viz. submucosa, muscularis and serosa in chamaeleon resembled to the respective layers in the other reptilian stomach described by Patt and Patt (1967) in the skink described by Mandlik (1983), in the turtle and ground lizard as stated by Jadhav (1985) and as suggested by Patil 1987). Histological observations stated that there is no sexual dimorphism in the chamaeleon.

Regarding the distribution of mucosubstances in the pyloric stomach of the chamaeleon,, the present investigation showed the presence of moderate amount of neutral mucosubstances and poor quantities of sialomucins in the epithelial cells. However, among the other reptiles studied, Giroud et. al. (1979) reported only PAS reactive mucous cells in the entire mucosa of stomach in a lizard, T. scincoides, Ferri et al. (1975) identified neutral mucins in the pyloric cells of stomach of the snake, X. merremi,. Mandlik (1983) reported that in the pyloric stomach of skink present only the goblet cells which secrete only the neutral mucosubstances. Similarly, Jadhav (1985) and Patil (1987) also reported that the pyloric stomach contains only mucous cells secreting only neutral mucins in the reptiles they have studied.

In this way the present histochemical studies showed slightly different results for the epithelial cells than that have been reported earlier in other reptiles by previous authors.

In the present investigation three types of glands are observed. Histochemically these glands differ from each other. Pyloric glands in the present investigation showed some similarities and variations with the existing literature. In general the cells in the glands-type I under present investigation secrete only neutral mucins. The cells in the pyloric glands contain only neutral mucosubstances in Indian skink (Mandlik, 1983), in ground lizard (Jadhav 1985); in turtle, varanus and snake (Patil, 1987). The cells in the glands-type II in chamaeleon under present investigation secrete only sulfomucins while, the glands-type III contain two types of cells which secrete both neutral mucins and sulfomucins. Similar histochemical results have been stated by number of previous authors. Suganuma et al. (1981a) found some of the glands in the pyloric stomach of turtle containing neutral mucosubstances and sialomucins. Jadhav (1985) identified neutral sulfo and sialomucins in the pyloric glands of turtle. On the other hand Patil (1987) investigated two types of cells in the pyloric glands of turtle, some of them containing a mixture of neutral mucosubstances and sialomucins.

The connective tissue in the submucosa, muscularis and serosa of the pyloric stomach in the present investigation contain neutral (trace) sulfomucins (trace) besides the glycogen present only in muscularis. Similar histochemical results have also been obtained by Mandlik (1983) in Indian skink, M. carinata.

CONCLUSION:

The present investigation showed the presence of moderate amount of neutral mucosubstances ,poor quantities of sialomucins in the epithelial cells and moderate amount of sulfomucins in the goblet cells.Three types of glands have been identified.Glands- type I revealed the presence of only neutral mucosubstsnces (intense). Glands- type II contained only sulfomucins (intense). In glands- type III two types of cells were observed ,some elaborating only neutral mucosubstsnces (intense) and other contained only sulfomucins (intense).

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