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Histological and Histochemical Observations on Brunner's Glands of Guinea pig

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Abstract:
Introduction: *Brunner's glands, located in the submucosa of the proximal duodenum, in general produce a mucous secretion and exist in all mammalian species.*
Aims and objectives*: The main purpose of present work is to histologically observe the brunner's glands of guinea pig and study different types of mucin secreted by them using various histochemical staining techniques.*
Materials and Methods: *Tissues were taken from the proximal part of duodenum of 10 guinea pigs and then subjected to various histochemical stains like AB at both pH 1 and 2.5, PAS, Diastase digestion, PAS- Phenyl hydrazine, AB pH 2.5- PAS and AB pH2.5- Saffranin.*
Result: *The Brunner's glands of guinea pig were composed of only mucous acini densely packed within the submucosa. The secretion produced is a mixture of both acid and neutral mucins.*
Conclusion: *Mucins have been classified into neutral and acidic types, the latter being subdivided into sulphomucins and sialomucins. In guinea pig the secreted acid mucin contain mixture of both sialomucin and sulphomucin with predominance of sialomucin.*
Keywords*: Brunner's glands, guinea pig, mucin, histochemistry*

I. Introduction

Brunner's glands are branched, tubuloalveolar glands whose secretory portions resemble mucous acini. The ducts of these glands penetrate the muscularis mucosae and usually pierce the base of the crypts of lieburkuhn to deliver their secretory product into the lumen of the duodenum (Gartner LP, Hiatt JL, 2001).[1] These glands are specific to mammals & have been observed in all the mammals (Krause,1988)[2]. In 1679, brunner's glands were discovered by John Jacob Wepter but named after the swiss anatomist Conard Brunner who first described these glands in 1687[3]. Following the work of Florey (1934),[4] it is accepted that main secretory product of these glands is mucin which protects the duodenal mucosa by neutralizing acidic chyme from stomach.(Grossman, 1958)[5.] Mucins have been referred to as mucopolysaccharide and glycosaminoglycans (Jeanloz 1960)[6], Spicer et al.1965[7] coined the word mucosubstances and then Reid & Clamp (1978)[8] have suggested the term glycoconjugates to replace the previous ones. Histochemically mucins were classified by Reid J, Clamp JR(1978)[8] into; Epithelial mucins (mucosustance) and Connective tissue mucins (mucopolysaccaride). Epithelial mucins are futher classified into: Acid mucins and Neutral mucins. Acid mucins are futher classified into sulfate containing mucins, Sulfomucins and sialic acid containing mucins, Sialomucins (Filipe 1979)[9].

Aims and objectives

The main purpose of present work is to histologically observe the brunner's glands of guinea pig and study different types of mucin secreted by them using various histochemical staining techniques.

II. Materials and Methods

Collection of specimens:
The following animal comprise the subject of study in present work.

- Order Rodentia - *Cavia Cobaya* (Guinea pig)

After ethical clearance specimen from proximal part of the duodenum of 10 guinea pigs were take from the Department of pharmacological , RIMS, Ranchi.

Tissue Processing;

- The samples were taken from proximal part of duodenum .
- 2% calcium in 10% formalin was used as fixative. It gives almost a neutral solution and is an ideal fixative both for histological and histochemical work.
- Tissues were fixed in solution for 24 hours.

- Then dehydrated through ascending grades of alcohol 50%, 70% ,90% then absolute alcohol.Tissues were dehydrated in each for 2minutes.
- Cleared in three changes of 1% solution of celloidin in methyl benzoate for 72 hours.
- Then passed through three changes in benzene for 24 hours.
- Wax bath was given for 2 hours with change in every half and hour at 60 degree centigrade for infiltration.
- Finally embedded in paraffin wax.

Sections:

Sections of all specimens were cut at a thickness of 6micron and were mounted on slides using egg albumin as adhesive. The mounted slides were incubated for 24 hours in the incubator.

For histochemical study , the following staining procedures were applied.

A. For highly sulphated acid mucin; Alcian blue pH 1.
B. For weakly sulphated acid mucin or sialomucin; Alcian blue pH 2.5
C. For neutral mucin: Periodic Acid Schiff (PAS) procedure.
D. For the presence of glycogen: PAS after diastase digestion.
E. For the confirmation of neutral mucin :PAS- Phenyl hydrazine procedure
F. For simultaneous demonstration of acid and neutral mucin ; Alcian blue pH 2.5 – PAS procedure.
G.For simultaneous demonstration of highly and weakly sulphated acid mucin; Alcian blue pH2.5 – Saffranin method.

A.Alcian blue pH 1

Solution – 1 gm of Alcian blue was dissolved in 100ml of 0.1 NHCL and filtered.

Method:

1. Section were dewaxed and brought to water through descending grades of alcohol.
2. Stained for 30 minutes in alcian blue solutions .
3. Blotted dry with filter paper.
4. Dehydrated quickly in two changes of absolute alcohol.
5. Cleared in two changes of xylene and mounted in D.P.X

B.Alcian blue pH 2.5

Solution - 1 gm of alcian blue was dissolved in 100ml of 3% glacial acetic acid and filtered.

Method:

1.Sections were dewaxed and brought to water.
2. Stained in alcian blue solution for 30 minutes.
3. Washed in running water for 5 minutes.
4. Dehydrated in 70% and 90% alcohol and two changes of absolute alcohol , cleared in two changes of xylene and mounted in D.P.X.

C.Periodic Acid Schiff (PAS)

Schiff reagent

Dissolve 1gm of basic fuchsin in 200 ml of boiling distilled water. Allow the solution to cool to 50 degree centigrade. Add 2gms of sodium metabisulphite. Add 2gm of activated charcoal and leave overnight in the dark at room temperature. Solution should be clear or pale yellow. Filter and store the solution at 0-4 degree centigrade.

Solutions;

•Periodic acid solution

Periodic acid 1g

Distilled water – 200ml

•Schiff reagent

Procedure:

1. Dewax and hydrate paraffin sections.
2. Treat with Periodic Acid solution for 5minutes
3. Rinse in tap and then in distilled water.
4. Place in Schiff's reagent for 15minutes.
5. Wash in running tap water for 5 to 10 minutes

6. Dehydrate in ascending grades of alcohol, clear in xylene and mount in DPX.

D. PAS after diastase digestion

Solution:

Diastase solution was prepared immediately before use by dissolving 100mg of diastase in 100ml of pH 6 buffer. pH 6 buffer was prepared by dissolving sodium chloride 8gm, disodium hydrogen phosphate 282mgm, sodium dihydrogen phosphate 1.97 gms in 1000ml of distilled water.

Method:

1. Sections were dewaxed and brought to water.
2. Digested in preheated diastase solution for 1 hour at 37 degree centigrade in a water bath.
3. Washed in water for 5 minutes.
4. Stained with Periodic Acid solution for 5minutes
5. Rinse in tap and then in distilled water.
6. Place in Schiff's reagent for 15minutes.
7. Wash in running tap water for 5 to 10 minutes
8. Dehydrate in ascending grades of alcohol , clear in xylene xand mount in DPX.

E.Periodic acid – Phenyl hydrazine Procedure

Solutions:
•Periodic acid – 1% aqueous
•Schiff's reagent
•Phenyl hydrazine -5% aqueous

Method:

1. Sections were dewaxed and brought to water through descending grades of alcohol.
2. Oxidised in 1% periodic acid for 10 minutes.
3. Washed in distilled water for 5 minutes.
4. Immersed in aqueous 0.5% phenyl hydrazine for 45 minutes at room temperature.
5. Washed in water for 10 minutes.
6. Place in Schiff's reagent for 15minutes.
7. Wash in running tap water for 5 to 10 minutes.
8. Dehydrate in ascending grades of alcohol, clear in xylene and mount in DPX.

F.Alcian blue 2.5 – PAS Procedure

• Acid and neutral mucins are clearly separated by this technique.
• The rationale is that by first staining all acid mucins with Alcian Blue, those acid mucins which are also PAS – positive will not react in the subsequent PAS reaction, only the neutral mucins will.

Solutions:

1.Alcian blue pH 2.5
2.1% aqueous periodic acid.
3.Schiff's reagent

Method:

1.Sections were dewaxed and brought to water through descending grades of alcohol.
2.Stained in alcian blue pH 2.5 for 30 minutes.
3.Washed in water for 5 minutes.
4.Oxidised in 1% periodic acid for 10 minutes.
5.Washed in water for 5 minutes.
6.Cover with Schiff's reagent for 15 minutes.
7.Wash in running tap water for 5 to 10 minutes
8.Dehydrate in ascending grades of alcohol, cleared in xyelene and mount in DPX.

G.Alcian blue pH 2.5 – Safranin Procedure

Solutions:

900 mgm of alcian blue, 45mgm of safranin and 1.2 gm of Ferric ammonium sulphate were dissolved in 250 ml of acetate buffer Ph 1.42. Acetate buffer was prepared by mixing 50 ml of N.Sodium acetate with 60 ml of N. HCL

Method :

1. Sections were dewaxed and brought to water .
2. Stained in alcian blue- safranin solution for 15 minutes.
3. Rinsed in tap water.
4. Dehydrated quickly in ascending grades of alcohol.
5. Cleared in xyelene and mounted in D.P.X.

III. Observations and Results

Figure No. : 1
Staining : H&E(Hematoxylin & Eosin)
Observation : Submucosal glands of Brunner projecting into the villi that consists of goblet cells and intestinal glands are seen.

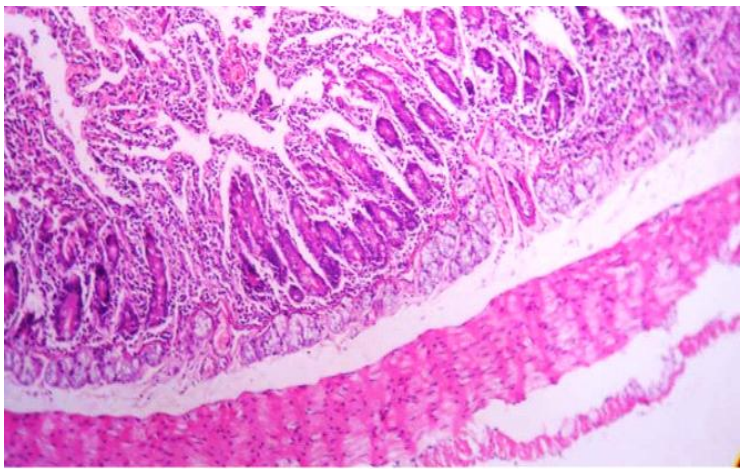

Fig.1: Guinea pig duodenum stained with H&E (Magnification 10X)

Figure No. : 2
Staining : H&E
Observation : Brunner's glands consisting of columnar cells with pale eosinophilic cytoplasm and basal flattened nuclei seen.

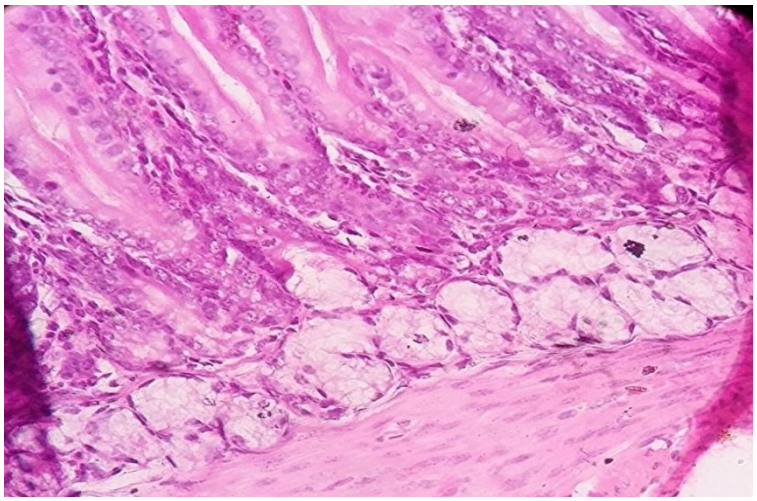

Fig.2: Guinea pig Brunner'glands stained with H&E(Magnification 40x)

Figure No. : 3
Staining : Alcian blue pH-1
Observation : Brunner's glands showed strong alcinophilia indicating the presence of highly sulphated acid mucin in substantial amount.

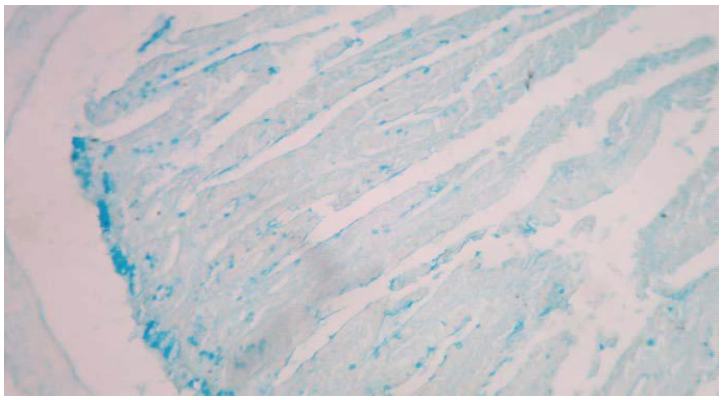

Fig.3 Guinea pig brunner's glands stained bright blue with Alcian blue pH 1 (Magnification 10X)

Figure No. : 4
Staining : Alcian blue pH-2.5
Observation: Guinea pig brunner's glands were stained blue with Alcian blue Ph 2.5 with high intensity. This indicated the presence of weakly sulphated acid mucin in substantial amount.

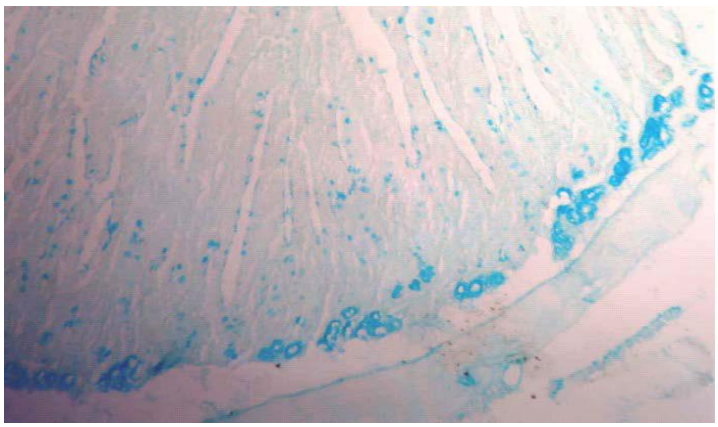

Fig.4: Guinea pig duodenum showing bright blue stained brunner's glands with alcian blue pH 2.5(Magnification 10X) .

Figure No. : 5
Staining : PAS
Observation: Brunner's glands were moderately stained magenta with PAS indicating the presence moderate amount of neutral mucin. There was no change in PAS staining after diastase digestion indicating absence of glycogen. Magenta stained brunner's glands with PAS got abolished after treatment with phenylhydrazine. This confirmed the presence of neutral mucin in substantial amount.

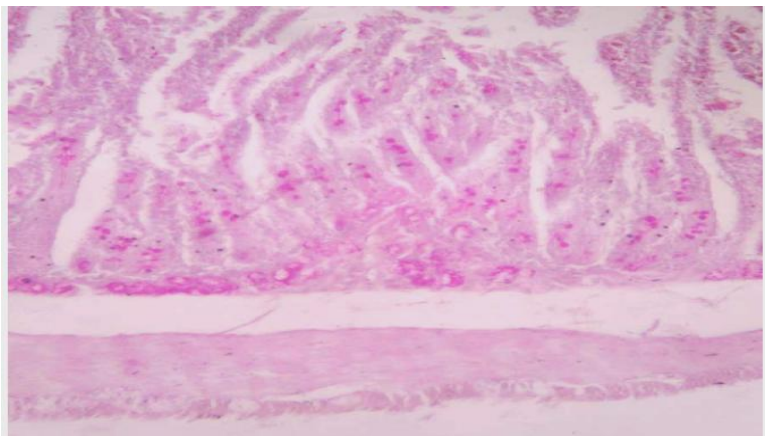

Fig.5: Guinea pig duodenum stained with PAS (Magnification 10X) showing bright magenta stained brunner's glands.

Figure No. : 6
Staining : Alcian blue pH 2.5 – PAS
Observation: Brunner's glands of guinea pig stained blue and purple with the combination stain of alcian blue ph 2.5-PAS indicating the presence of mixture of acid and neutral. More intensity of blue was due to higher amount of acid mucin and moderate neutral mucin.

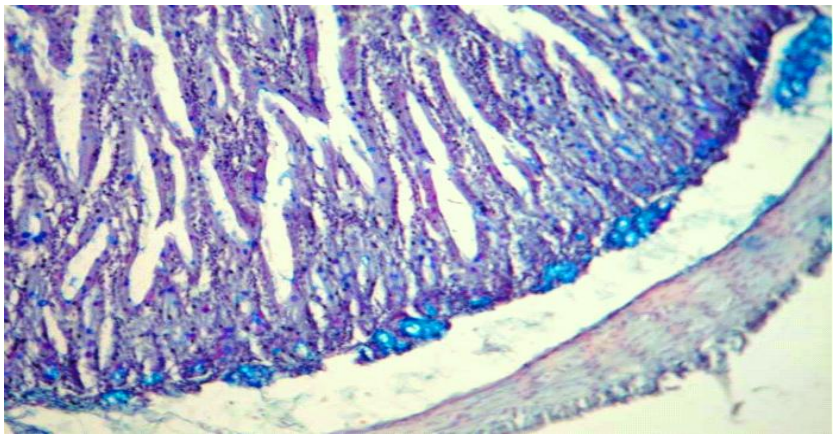

Fig.6: Guinea pig duodenum stained with AB pH 2.5- PAS(Magnification 10X) showing blue and purple stained brunner's glands.

Figure No. : 7
Staining : Alcian blue pH 2.5 – Saffranin
Observation: Blue purple stained cells of guinea pig brunner's glands indicated the presence of both highly and weakly sulphated acid mucin

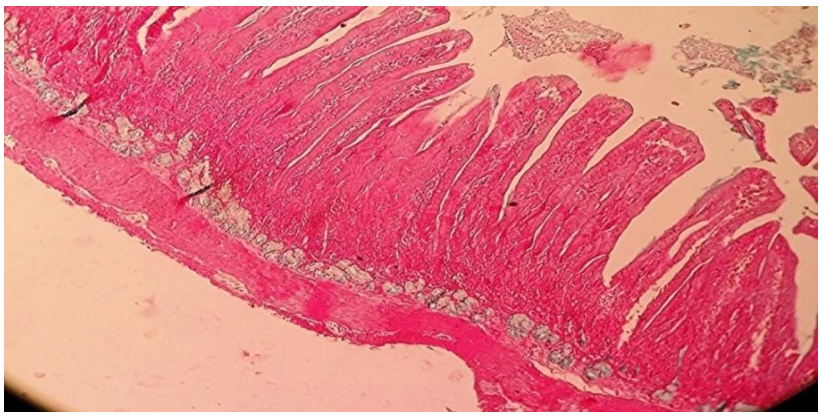

Fig.7: Guinea pig duodenum stained with Saffranin – AB pH 2.5(Magnification 10X) showing many blue and few light red stained cells of brunner's glands.

Table No.1 Results of histochemistry of brunner's glands of Guinea pig

SN.	Staining technique	BGs staining intensity
1.	AB pH 1	+++ B
2.	AB pH 2.5	+++ B
3.	PAS	++ M
4.	PAS after Diastase	++M
5.	PAS-Phenyl hydrazine	-ve M
6.	AB pH 2.5- PAS	+++ B, ++P
7	AB pH 2.5- Saffranin	+++B, ++R

Key to symbols in table;BGs= Brunner's glands, AB= Alcian blue, PAS=Periodic Acid Schiff, B= Blue, M= Magenta, P = Purple, R= Red -ve = Negative staining, ± = Weak or variable staining , + = Mild staining, ++ = Moderate staining, +++ = Strong staining .

IV. Discussion

Since the duodenal submcosal glands were discovered by Wepter in 1679, studies in many animal species were conducted to clarify the extent and the density of their distribution, the types of cells forming the glands by light and electron microscopy. However, there are only few histochemical studies on the composition of the secretion of this gland. Modern investigation on the secretions of Brunner's glands started with the work of Florey and his collaborators. Florey and Harding (1934)[4] reported staining of Brunner's glands with mucicarmine stain only, in the goat, pig, rabbit and rat. In the present study after H&E staining the brunner's glands of guinea pig were found to be compound, tubuloalveolar, composed only of mucous acini densely packed within the submucosa (Fig no.1 & 2). This finding correlates with Cochrane et al., (1964)[10] who studied the histochemistry and electronmicroscopy of the Brunner's glands in guinea pig and found that the component cells are mucous in type. Similar finding was given by Daniel G. Sheahan et al., (1976)[11], Mohammadpour, A. A.(2011)[12] .

The present study revealed that the brunner's glands of the guinea pig when treated with alcian blue pH 1 and 2.5 stained bright blue showing a positive staining reaction i.e., showed alcinophilia indicating the presence of substantial amount of acid mucins (Fig. no.3and 4). Cochrane et al., (1964)[10] obtained a similar positive staining reaction . Belanger LF (1963)[13] by applying Hale's and Alcian blue staining techniques reported positive reaction in the brunner's glands of the guinea pig, domestic pig, sheep and ox, but negative reaction in those of the rat and man. PAS staining revealed the presence of moderate amount of neutral mucin in the secretion of brunner's glands which stained magenta(Fig.5) PAS positive reaction similar to this was obtained by Cochrane et al., (1964)[10]. Daniel G. Sheahan and Helen R. Jervis,1976[11], found that in guinea pig, the deeper glands contain abundant sulfomucins with some neutral mucins. The superficial glands contain equal amounts of neutral and sialomucins. Schumacher et al.2004[14] obtained intense staining of guinea pig brunner's glands with PAS indicating the presence of substantial amount of neutral mucin .Mohammadpour, A. A., in 2011[12] reported the similar finding. When AB pH 2.5 – PAS technique was employed brunner's glands stained variably with blue and purple indicating presence of mixture of acid and neutral mucins. More intensity of blue indicates higher amount of acid mucin and moderate neutral mucin(Fig.6). This finding is similar to that of Daniel G. Sheahan et al., (1976)[11].

V. Conclusion

Brunner's glands of guinea pig were found to be compound, tubuloalveolar, composed only of mucous acini densely packed within the submucosa.They secreted mixture of both acid and neutral mucin where acid mucin was in substantial amount and neutral mucin in moderate amount. Combination of AB pH 2.5 – Saffranin staining technique was first time undertaken which revealed the presence of both highly sulphated and weakly sulphated or carboxylated acid mucin.This study might help in comparing different types of mucin secreted by brunner's glands of various other mammalian species.

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