

Available online at <http://www.ijims.com>

ISSN: 2348 – 0343

## **A Comparative Histochemical Study of Mucins Produced by Brunner's Glands of Guinea Pig, Rabbit and Goat**

Alka Rashmi Nag<sup>1\*</sup> and Renu Prasad<sup>2</sup>

<sup>1</sup>Junior Resident, Anatomy, Rajendra Institute Of Medical Sciences, Ranchi, India.

<sup>2</sup>Professor and HOD, Anatomy, Rajendra Institute Of Medical Sciences, Ranchi, India.

\*Corresponding author: Alka Rashmi Nag

### **Abstract**

Mucins are high-molecular weight glycoproteins that have gel-forming properties which are highly glycosylated and which in turn are responsible for their protective function. Normal distribution of mucin and its alteration in various inflammatory, benign and malignant lesions of gastrointestinal tract has aroused interest in the field of histochemistry. Brunner's glands, located in the submucosa of the proximal duodenum, in general produce a mucous secretion and exist in all mammalian species. For the present study, tissues were taken from the proximal part of duodenum of guinea pig, rabbit and goat then subjected to various histochemical stains like AB at both pH 1 and 2.5, PAS, Diastase digestion, PAS- Phenyl hydrazine and AB pH 2.5- PAS. It was found that the Brunner's glands of guinea pig secreted mixture of acid and neutral mucins. In rabbit mucous acini secreted moderate amount of acid mucin and serous acini secreted scanty amount of neutral mucin. Whereas in goat the brunner's glands secreted negligible amount of acid mucin but substantial amount of neutral mucin. It may be concluded that guinea pig, rabbit and goat were herbivore and had similar eating habits yet their brunner's glands secreted mucins differently. Hence dietary habits seemed to have no impact on the type of mucins secreted by the brunner's glands.

**Key words:** Brunner's glands, mucin, histochemistry, Histochemical Study.

### **Introduction**

A particular morphological feature of the duodenal submucosa is the presence of brunner's glands. These glands are specific to mammals & have been observed in all the mammals (Krause, 1988)<sup>1</sup>. 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<sup>2</sup>. Brunner's glands are branched, tubuloalveolar glands whose secretory portions resemble mucous acini. The ducts of these glands penetrate the muscularis mucosa and usually pierce the base of the crypts of lieburkuhn to deliver their secretory product into the lumen of the duodenum (Gasterner LP, Hiatt JL, 2001).<sup>3</sup>

Following the work of Florey (1934),<sup>4</sup> 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)<sup>5</sup>. In most species, by light microscopy the brunner's glands appear to be mucus secreting, although in the rabbit serous cells are also present (Schwalbe, 1872<sup>6</sup>; Martin, 1954<sup>7</sup>; Leeson & Leeson, 1967<sup>8</sup>. In the guinea-pig (Cochrane, Davies, Palfrey & Stockwell, 1964)<sup>9</sup> the component cells of brunner's glands by electron microscopy have been reported as typical mucous cells and same is for ruminant species like goat.

The mucus layer of the digestive tract have several functions such as, lubrication, digestion, absorption, hosting intestinal microflora and protecting the GIT against toxins and pathogens (Laux et al., 2005<sup>10</sup>; Bansil & Turner, 2006<sup>11</sup>; Rose & Voynow,2006<sup>12</sup>).

Mucins have been referred to as mucopolysaccharide and glycosaminoglycans (Jeanloz 1960 )<sup>13</sup>, Spicer et al.1965<sup>14</sup> coined the word mucosubstances and then Reid & Clamp (1978)<sup>15</sup> have suggested the term glycoconjugates to replace the previous ones. Histochemically mucins were classified by Reid J, Clamp JR' 1978<sup>15</sup> 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)<sup>16</sup>. Mucins are also classified according to their ability to form a gel, namely gel forming ,Secreted mucins or non-gel forming, membrane bound mucins (Devine & Mckenzie, 1992 )<sup>17</sup>.

As such in the present work an effort is being made to histochemically characterised the different types of mucins pesent in the brunner's glands and make a comparative study in few mammals. The histochemical information should make it possible to relate changes at cellular level with those which take place at molecular level.

## **Materials and Methods**

### **Collection of specimens:**

The following animals comprise the subject of study in present work.

- Order Rodentia - *Cavia Cobaya* (Guinea pig)
- Order Lagomorpha – *Lepus Ruficavidatus* (Rabbit)
- Order Artiodactyla - *Canis Indica* (Goat)

After ethical clearance specimen from proximal part of the duodenum was taken from the mammals like Guinea pig and Rabbit from Department of pharmacological , RIMS, Ranchi. Specimen from Goat was collected from slaughter house, bariatu, 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.

**A. Alcian blue pH 1**

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

**Methods-**

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 xylene and mount in DPX.

#### Observations:

- The present study revealed that when treated with alcian blue pH 1 the brunner's glands of the guinea pig stained bright blue (**Figure.1**), rabbit's mucous acini showed moderate alcinophilia (**Figure.2**) whereas goat showed very weak staining (**Figure.3**), indicating the presence of highly sulphated acid mucin in substantial amount in guinea pig, moderate in rabbit and negligible in goat.
- With alcian blue pH 2.5 the brunner's glands of guinea pig and mucous acini of rabbit stained bright blue indicating the presence of substantial amount of sialomucin but goat brunner's glands took weak staining indicating presence of scanty amount of sialomucin. In rabbit the serous acini remained unstained with alcian blue at both the pH indicating the absence of acid mucin in them.
- In guinea pig PAS staining revealed the presence of moderate amount of neutral mucin in the secretion of brunner's glands which stained magenta (**Figure.4**). The staining remained unaffected after diastase digestion, indicating the absence of glycogen whereas after treatment with phenyl hydrazine the stain got washed out, confirming the presence of neutral mucin. In rabbit, presence of neutral mucin was revealed in scanty amount. Serous acini were stained light magenta whereas mucous acini were unstained, indicating absence of neutral mucin in serous acini (**Figure.5**). Diastase digestion had no effect on PAS staining indicating the absence of glycogen but it became weak after phenyl hydrazine treatment which confirmed the presence of scanty amount of neutral mucin. A strong PAS reaction was observed in the brunner's glands of goat (**Figure.6**) indicating the presence of substantial amount of neutral mucin the staining was resistant to diastase and got abolished when treated with phenyl hydrazine indicating the absence of glycogen and confirming the presence neutral mucin.
- When AB pH 2.5 – PAS technique was employed brunner's glands of guinea pig stained variably with blue and purple (**Figure.7**) indicating presence of mixture of acid and neutral mucins. In rabbit many mucous acini took blue stain and few serous acini took light magenta stain (**Figure.8**) indicating the presence of acid mucin in mucous acini whereas neutral mucin was present in scanty amount in serous acini. In goat when the combination stain of AB PH 2.5- PAS was employed brunner's glands took more magenta and less purple stain (**Figure.9**) indicating the presence of both acid and neutral mucin but with higher amount of neutral mucin and negligible amount of acid mucin.

#### Discussion

Modern investigation on the secretions of Brunner's glands started with the work of Florey and his collaborators. Florey and Harding (1934)<sup>18</sup> reported staining of Brunner's glands with mucicarmine stain only, in the goat, pig, rabbit and rat. Bensley (1903)<sup>19</sup> reported positive staining for mucin in histological sections of 19 species, by using a modification of Mayer's mucicarmine stain. The present study revealed that the brunner's glands of the guinea pig when treated with alcian blue pH 1 and 2.5 showed alcinophilia indicating the presence of substantial amount of acid mucins. Cochrane et al., (1964)<sup>9</sup> obtained a similar positive staining reaction. Belanger LF (1963)<sup>20</sup> 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. Schumacher et al. 2004<sup>21</sup> also obtained strong staining of guinea pig brunner's glands with Alcian blue pH 1

and 2.5, revealing the presence of substantial amount of acid mucin also Mohammadpour, A. A.(2011)<sup>22</sup>, reported that the guinea pig brunner's glands reacted positively with Alcian blue pH 2.5.

In rabbit brunner's glands after staining with AB pH 1 the mucous acini showed moderate alcinophilia, whereas they were extensively stained with Alcian blue at pH 2.5, indicating presence of substantial amount of acid mucin. Serous acini remained unstained which indicated the absence of acid mucin. This finding correlates with the findings of Ergun et al(2010)<sup>23</sup> and Elnasharty M. A. et al.(2013)<sup>24</sup>. UDO Schumacher, 2004<sup>21</sup>, while studying the histochemistry of brunner's glands of cotton tailed rabbit and domestic rabbit concluded that Alcian blue pH 1.0 and 2.5, stained the brunner's glands of both species with the moderate intensity.

When Alcian blue pH 1 was applied to brunner's glands of goat they showed weak staining indicating the negligible presence of highly sulphated acid mucin. With Alcian blue pH 2.5 they showed weak alcinophilia indicating presence of sialomucin in scanty amount.UDO Schumacher, 2004<sup>21</sup> studied the brunner's glands of bison, a large ruminant and found them to be alcinophilic indicating presence of acid mucin.

In guinea pig PAS staining revealed the presence of moderate amount of neutral mucin in the secretion of brunner's glands. PAS positive reaction similar to this was obtained by Cochrane et al., (1964)<sup>9</sup>. Daniel G. Sheahan and Helen R. Jervis,1976<sup>25</sup>, 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<sup>21</sup> 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<sup>22</sup> reported the similar findings.

In rabbit, PAS staining revealed the presence of neutral mucin in scanty amount in serous acini.. Ergun et. al (2010)<sup>23</sup> while studying the histochemistry of brunner's glands of angora rabbit found that the mucous glands and secretory ducts did not react with PAS, while serous glands were weakly PAS-positive. His findings were similar to the present study. Elnasharty M. A. et al. (2013)<sup>24</sup> also gave the same conclusion. UDO Schumacher in 2004<sup>21</sup> reported that the brunner's glands of the cotton-tailed rabbit reacted more intensely with PAS than those of the domestic rabbit.Brunner's glands of goat were stained moderate to high magenta with PAS .This indicated the presence of substantial amount of neutral mucin. A strong PAS reaction was observed in the brunner's glands of goat, sheep and cattle by Ohwada and Suzuki in 1992<sup>26</sup>. UDO Schumacher, 2004<sup>21</sup> got the similar findings with bison brunner's glands.

In classic carbohydrate histochemistry, a positive PAS reaction indicates the presence of neutral carbohydrate, while positive Alcian blue reactions at pH 1.0 and 2.5 indicates the presence of acidic sulphated and acidic carboxylated residues, respectively. (Spicer & Schulte, 1992)<sup>27</sup>. In guinea pig when AB pH 2.5 – PAS technique was employed presence of mixture of acid and neutral mucins was revealed in the secretions of brunner's glands. Various studies dealing with the mucosubstance histochemistry of duodenal submucosal glands in large numbers of mammals show marked species variation and variation even within the species (Poddar & Jacob, 1979)<sup>28</sup>. Therefore, depending on species, duodenal submucosal glands in guinea pig secreted neutral and acidic carboxylated mucin.

In rabbit when AB pH 2.5- PAS staining technique was applied it was observed that many mucous acini took blue stain and few serous acini took light magenta stain indicating the presence of acid mucin in mucous acini whereas neutral mucin was present in scanty amount in serous acini. In goat brunner's glands took more magenta and less purple stain indicating the presence of both acid and neutral mucin with higher amount of neutral mucin and less of acid mucin.

Brunner's glands of guinea pig, rabbit and goat showed difference in mucin secretion in spite that these animals are herbivore having similar food habits. Earlier studies done on Brunner's glands in 27 species of bats that differed markedly in diet (insect-, fish-, blood-, nectar-, plant-, or animal-feeding species) were reported to produce only neutral mucins (Forman, 1971)<sup>29</sup>. UDO Schumacher, 2004, concluded that the histochemistry of Brunner's glands showed marked differences in the series of mammals. In bison, deer, guinea pigs, voles, and cotton-tailed and domestic rabbits, they contain acidic sulphated and carboxylated mucins whereas in humans, rhesus and Japanese macaques, cats, raccoons, rats, and opossums, they contain neutral mucins. This variation could not be attributed to either the order or diet of the mammals.

### Conclusions

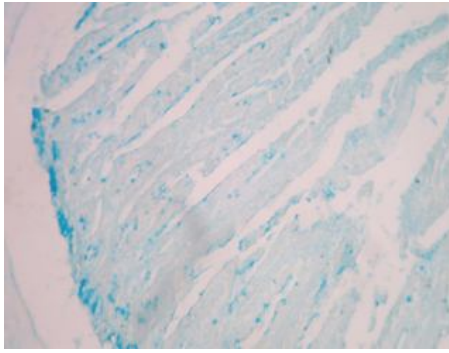
- The Brunner's glands of guinea pig secreted mixture of acid and neutral mucins. The secreted acid mucin contained mixture of sialomucin and sulphomucin with predominance of sialomucin. Acid mucin was present in substantial amount whereas neutral in moderate amount.
- In rabbit mucous acini secreted moderate amount of acid mucin which was mixture of both sulpho and sialomucin, with predominance of sialomucin. Acid mucin was absent in serous acini. However, serous acini secreted scanty amount of neutral mucin.
- In goat the Brunner's glands secreted negligible amount of acid mucin which was found to be the mixture of both sulpho and sialomucin. The remarkable finding was the presence of substantial amount of neutral mucin. Common finding in the secretion of Brunner's glands of these mammals was the absence of glycogen.
- Taking the dietary habit into consideration it was observed that rabbit, guinea pig and goat were herbivore and had similar eating habits yet their Brunner's glands secreted mucins differently. Neutral mucin was found in substantial amount in goat, moderate amount in guinea pig and negligible amount in rabbit whereas acid mucin was found in substantial amount in guinea pig, moderate in rabbit and negligible in goat.
- Hence dietary habits of the mammals seemed to have no impact on the type of mucins secreted by the Brunner's glands.

### References

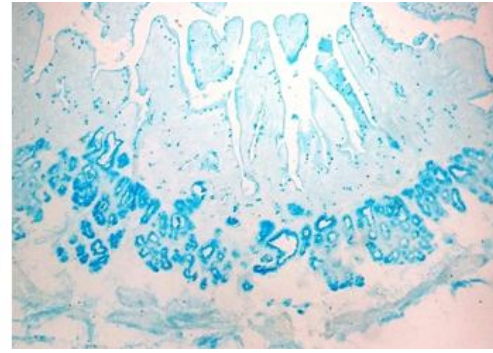
1. Krause WJ. 1988. Biology of duodenal (Brunner's) glands. In: Motta PM, Fujita H, editors. Ultrastructure of the digestive tract. Boston: Martinus Nijhoff Publishing. p 67-84.
2. Brunner JC. De glandulis in intestino duodeno hominis detectis. Dissertation, Heidelbergae 1688.
3. Gartner LP, Hiatt JL. Colour text book of histology. 2nd ed. Philadelphia: WB Saunders Company; 2001. p.399-400.
4. Florey HW, Harding HH. The functions of Brunner's glands and the pyloric end of the stomach. J Path Bact 1934;37:431-53.
5. Grossman MI. The glands of Brunner physiological reviews. 1958;38:675-690.
6. Schwalbe, G. (1872). Beitrag zur Kenntniss der Drusen in den Darmwandungen, insbesondere der Brunnenschen Drusen. Arch. mikrosk. Anat. EntwMech. 8, 92-140.
7. Martin, B. F. (1954). Serous cells in Brunner's gland of the rabbit. Nature, Lond. 174, 1195-1196.
8. Leeson, C. R. and Leeson, T. S: The fine structure of Brunner's glands in the rabbit. Anatomical Record, 1967: 159, 409-420.

9. Cochrane W, Davies DV, Palfrey AJ, Stockwell RA. The histochemistry and electron microscopy of Brunner's glands in the guinea pig. *J Anat* 1964;98:1-10.
10. Laux, D., Cohen, P., & Conway, T. (2005). Role of mucus layer in bacterial colonization of the intestine. In J. Nataro, P. Cohen, H. Mobley, & J. Weiser (Eds.), *Colonization of Mucosal Surfaces* (pp. 199-212). Washington, D. C.: ASM Press.
11. Bansil R, Turner BS. Mucin structure, aggregation, physiological functions and biomedical applications. *Curr Opin Colloid* 2006;11:164-70.
12. Rose, M., & Voynow, J. (2006). Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiological Reviews*, 80, 245-278.
13. Jeanloz, R.W: The nomenclature of mucopolysaccharides. *Arthritis & Rheumatism*, 1960, 3: 233-237.
14. Spicer SS, Lepp TJ, Stoward PJ. Suggestions for histochemical terminology of carbohydrate rich tissue components. *J Histochem Cytochem* 1965; 13:599-603.
15. Ried PE, Owen DA, Magee F, Park CM. Histochemical studies of intestinal goblet cell glycoproteins during the development of the human foetus. *Histochem J* 1990;22:81-6.
16. Filipe MI. Mucins in the human gastrointestinal epithelium: a review. *Invest Cell Pathol* 1979;2:195-216.
17. Devine PL, McKenzie IF. Mucins: structure, function, and associations with malignancy. *Bioessays* 1992;14:619-25.
18. Florey HW, Harding HH. The functions of Brunner's glands and the pyloric end of the stomach. *J Path Bact* 1934;37:431-53.
19. Bensley R.R. The structure of the glands of Brunner. *Univ Chicago Decennial Publ* 1903;10:279-329.
20. Belanger LF. Comparisons between different histochemical and histophysical technique as applied to mucus secreting cells. *Annals of the New York Academy of Sciences* 1963;106:364-78.
21. Schumacher U, Duker M, Katoh M, Jorns J, Krause WJ. Histochemical similarities of mucins produced by Brunner's glands and pyloric glands : A comparative study. *J Anat Rec Part A* 2004;278(A): 540-50.
22. A. A. Mohammadpour, Morphological and histochemical study of guinea pig duodenal submucosal glands, *Bulgarian Journal of Veterinary Medicine* (2011), 14, No 4, 201-208.
23. Ergun, E., Ergun, L., Ozen, A., Kurum, A. and Bayraktaroglu, A.G: Histomorphology of the Brunner's glands in the Angora rabbit. *J Anim*, 2010, Vet.9:887-891.
24. Elnasharty M. A., Abou-Ghanema I. I., Sayed-Ahmed A., and A. Abo Elnour Mucosal- Submucosal Changes in Rabbit Duodenum during Development *World Academy of Science, Engineering and Technology* Vol:7 2013-04-24.
25. Daniel G. Sheahan & H. R. Jervis 1976, *Comparative histochemistry of gastrointestinal mucosubstance* .Am. J. Anat. 146: 103-131.
26. Ohwada, S. and Suzuki, H. 1992. Lectin histochemistry on the Brunner's glands of domestic ruminants. *Tohoku Journal of Agricultural Research* 42: 3-4.
27. Spicer, S. S. & B. A. Schulte, 1992. Diversity of cell glucoconjugates shown histochemically: A perspective. *Journal of Histochemistry and Cytochemistry*, 40, 1-38
28. Poddar S, Jacob S. Mucosubstance histochemistry of Brunner's glands, pyloric glands and duodenal goblet cells in the Ferret. *J Histochem and Cell Biol* 1979;65:67-81.
29. Forman GL. Histochemical differences in gastric mucus of bats. *J Mammal*, 1971, 52:191-193.

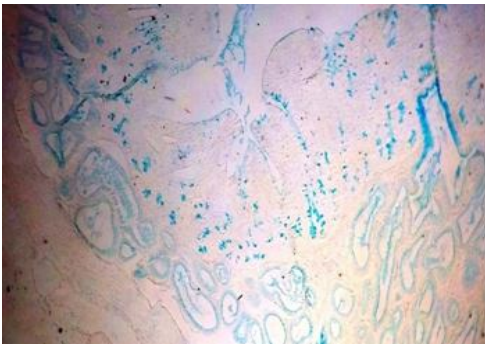




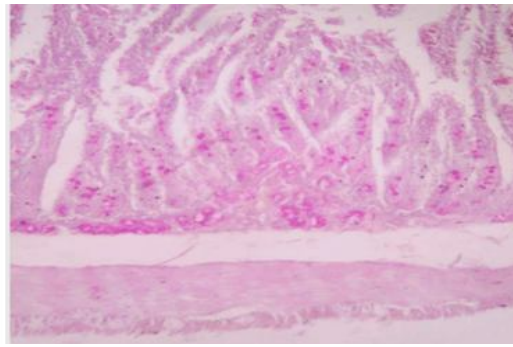
**Figure. 1:** Guinea pig brunner's glands stained bright blue with Alcian blue pH 1 (Magnification 10X).



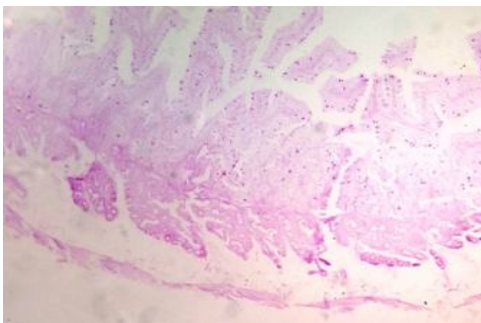
**Figure.2:** Rabbit brunner's gland stained with Alcian blue pH 1 (Magnification 10X), showing many blue stained acini and few unstained acini.



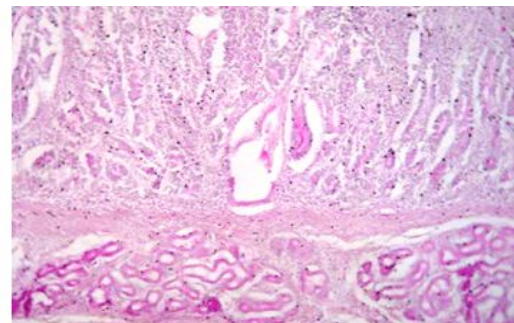
**Figure.3:** Goat duodenum stained with Alcian blue pH 1 (Magnification 20X) showing moderately stained goblet cells and weakly stained brunner's glands.



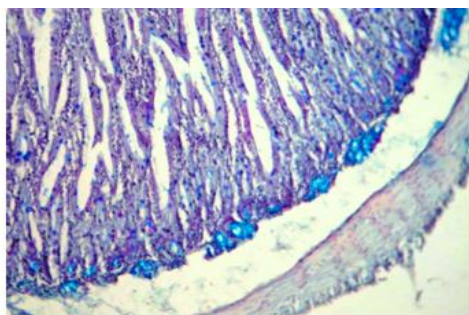
**Figure.4:** Guinea pig duodenum stained with PAS (Magnification 20X) showing bright magenta stained brunner's glands .



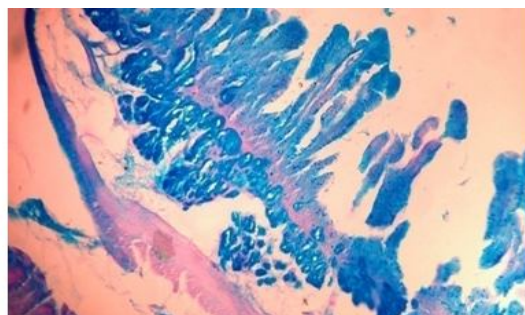
**Figure.5:** Rabbit brunner's glands stained variably with PAS (Magnification 10X) where mucous acini are unstained and serous acini are stained light magenta.



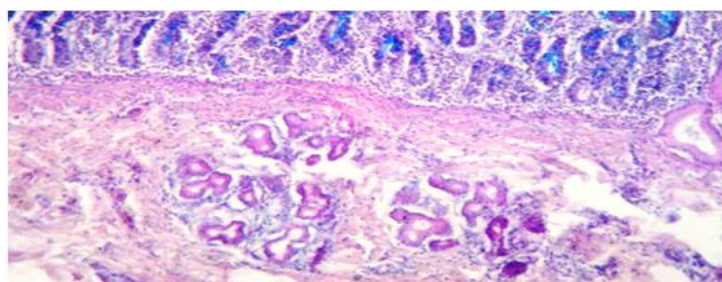
**Figure.6:** Goat duodenum stained with PAS (Magnification 20x) showing magenta stained brunner's glands.



**Figure.7:** Guinea pig duodenum stained with AB Ph 2.5- PAS(Magnification 20X) showing blue and purple stained brunner's glands.



**Figure.8:** Rabbit brunner's glands stained with AB 2.5- PAS(Magnification 20X) Showing many bright blue acini and few light magenta stained acini.



**Figure.9:** Goat duodenum stained with AB Ph 2.5 – PAS(Magnification 40X) showing Magenta purple stained brunner's glands.

**Table 1 : Results of histochemistry of brunner's glands**

SN.	Staining technique	Guinea pig BGs	Rabbit BGs	Goat BGs
1.	AB pH 1	+++ B	++ B ma -ve B sa	± B
2.	AB pH 2.5	+++ B	+++ B ma -ve B sa	± B
3.	PAS	++ M	-ve M ma + M sa	+++M
4.	PAS after Diastase	++M	-ve M ma + M sa	+++ M
5.	PAS-Phenyl hydrazine	-ve M	±+ M	-ve M
6.	AB pH 2.5- PAS	+++ B ++ P	+++ B ma + M sa	+++ M + P

Key to symbols in table:

BGs= Brunner's glands, AB= Alcian blue, PAS=Periodic Acid Schiff B= Blue, M= Magenta, P = Purple, Ma= mucous acini, Sa= Serous acini, -ve = Negative staining, ± = Weak or variable staining, + = Mild staining, ++ = Moderate staining, +++ = Strong staining.