Haryana Veterinarian (June, 2026) **65(1),** 42-46 ISSN 0033-4359 (P) **Research Article**

HISTOMORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON GOBLET CELLS, MUCOSAL AND SUBMUCOSAL GLANDS OF INTESTINE OF RABBIT

RAJESH RANJAN* and PARTHA DAS[1]
Department of Veterinary Anatomy, Co.V.Sc. & A.H., Rewa, N.D.V.S.U., 486001 India
[1]Department of Veterinary Anatomy, Faculty of Veterinary Science, WBUAFSc, Kolkata-700037, India

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ABSTRACT

The present research work was undertaken to elucidate histomorphological and histochemical characters of goblet cells, mucosal glands and submucosal glands within the rabbit intestine as these structures collectively play a role in protecting the intestinal mucosa from acidic gastric pH by secreting mucous. Since, the rabbit intestine has anatomical similarities with that of human intestine; hence, this present study was carried out in rabbits. Samples from different segments of intestine were collected, fixed, processed and stained with specific stains as per the standard protocols. The results revealed that the goblet cells and mucosal glands were present throughout the intestinal length with varying anatomical features; the submucosal Brunner's glands were limited to the duodenum only. The goblet cells and Brunner's glands were highly alcinophilic while on the other hand; the mucosal glands (Crypts of Lieberkühn) were intensely PAS-positive with scattered alcinophilic goblet cells within its secretory epithelium.

Keywords: Brunner's glands, Crypts of Lieberkühn, Goblet cells, Histochemistry, Histomorphology, Rabbit intestine

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The intestinal epithelium is protected from the adverse effects of gastric acid and enzymes by a protective mucous layer. In intestine, this mucous is secreted by goblet cells and submucosal Brunner's gland. Goblet cells are present throughout the lining epithelium of the intestine as well as mucosal glands (Crypts of Lieberkühn). The mucosal glands are present within the tunica mucosa and open at the villous base. These are simple tubular glands having goblet cells within their secretory epithelium. Brunner's gland is present specifically in the submucosa of duodenal segment of small intestine of all the mammals (Verdiglione and Montesi, 2019). They are branched tubulo-alveolar glands and have functional importance owing to mucous secretions in order to neutralize the acidic ingesta entering the small intestine from stomach and lubricating the intestinal mucosa. So, before the chyme proceeds further down, it acidity gets balanced (Zheng *et al.*, 2009). Furthermore, the rabbits are caecotrophic in nature, i.e. during the night time; they directly feed their own soft faeces. Hence, the re-ingested faeces need further protective mucous coating to protect the microbes from acidic pH of the stomach (Halls, 2008). Mucous is a gel like substance comprising of water, mucin and some electrolytes. This mucin is a high molecular weight glycoprotein that has gel forming properties which is responsible for its protective functions (Schumacher *et al.*, 2004).

Hence, this research work was designed to investigate the histological details of goblet cells, mucosal glands and Brunner's gland in rabbit, as the rabbit intestine has anatomical similarities with that of human intestine (Amiry *et al.*, 2019) that can provide the basic information for further work focused on intestinal diseases using the rabbit model for pre-clinical studies.

*Corresponding author: rajesh.ranjan837@gmail.com

MATERIALS AND METHODS

The present research work was carried out on twelve adult rabbits between body weights of 1.0 to 1.5 kg as per IAEC guidelines. The animals were euthanized and the tissue samples were collected from every 10 cm interval of intestine and quickly fixed in 10% Neutral buffered formalin (10% NBF) and Bouin's fixatives after properly washing in 0.9% normal saline solution. Processing of tissue samples was done by acetone-benzene schedule and 5-6 μm thick sections were obtained on clean glass slides. The sections were stained with Haematoxylin and eosin (H&E) for routine morphology, Masson's trichrome method for collagen fibers, Gridley's method for reticular fibers, Weigert's method for elastic fibers and PAS-AB (pH 2.5) for mucopolysaccharides contents (Luna, 1968). Micrometrical observations were recorded using Leica Qwin Image Analyzer software and microscopic photographs of stained slides were taken in Leica DM 2000 microscope. The data analysis was done using SPSS statistical software.

RESULTS AND DISCUSSION

Goblet cells

In between the columnar epithelial cells of intestine and within the mucosal glands (crypts of Lieberkühn), goblet cells were interspersed which were histologically characterized by clear apical cytoplasm with basal nuclei (Fig. 1) which accorded with the reports of Mohammad *et al.* (2020) in sheep and rabbit. Special staining with PAS-AB (pH 2.5) indicated presence of strong alcinophilic mucinous granules within the cytoplasm of goblet cells (Fig. 2) which was in accordance with the reports of Schumacher *et al.* (2004) in cotton-tailed and domestic rabbits, Ergün *et al.* (2010) in Angora rabbits and Al-Kennany *et al.* (2012) in mice.

The average goblet cell population in the small intestine increased from oral to aboral direction which was similar with the reports of Jawad *et al.* (2019) in rabbit and rats. Within the crypts and mucosal epithelium, there was significantly highest number of goblet cells in the ileum as compared to duodenum and jejunum. The goblet cell count further increased in large intestine; however, within the mucosal epithelium the goblet cell number did not varied significantly while within the crypts of Lieberkühn, the goblet cell count in the colon was significantly high as compared to caecum and rectum (Table 1). Oliver *et al.* (2011) also reported the presence of abundant goblet cells within the colon of the African giant rat that indicated its role in increased mucosal protection and lubrication for fecal expulsion. Keskin *et al.* (2012) reported similar observation in mouse. The caecotrophic activity of rabbit has been reported by Halls (2008) which include re-ingestion of soft faeces passed out at night time. The mucous coating over the soft faeces would further prevent the caecal microbes adhered and mixed with the faeces from acidic gastric pH.

Mucosal glands (Crypts of Lieberkühn)

The mucosal glands (crypts of Lieberkühn) were present within the lamina propria of the entire length of intestine that opened between the villous bases. These glands were arranged in different layers within the lamina propria (Fig. 3). These simple branched tubular glands were lined by cuboidal to low columnar epithelium having basal spherical to oval nuclei and acidophilic cytoplasm (Fig. 4) which was in accordance with the reports of Kumar *et al.* (2013) in sheep and Mohammad *et al.* (2020) in sheep and rabbit. Special staining with PAS-AB (pH 2.5) revealed that the crypts were positive for neutral mucopolysaccharides with presence of alcinophilic goblet cells within their secretory epithelium (Fig. 2) which was similar with the reports of Al-Samawy *et al.* (2020) in rats and rabbits.

The cryptal depth in duodenum (188.13±22.361 μm) was significantly higher than that of the jejunum (95.69±8.596 μm) and ileum (103.08±9.877μm). That might be due to the reason that the crypts of Lieberkühn were present in various superficial and deeper parts in the lamina propria of duodenum (Fig. 3) and more superficially arranged in jejunum and ileum. In the large intestine, the cryptal glands within the lamina propria of caecum and rectum were relatively short and straight while, the lamina propria of colon had long and straight mucosal glands (Fig. 5) that significantly increased the mean thickness of tunica mucosa of the colon (600.84±16.066 μm) as compared to caecum (247.35±17.536 μm) and rectum (481.83±30.938 μm). Presence of numerous goblet cells and intestinal glands in colon of the African giant had also been observed by Oliver *et al.* (2011).

Submucosal glands (Brunner's gland)

Brunner's glands were observed specifically in the submucosal layer of the duodenum (Fig. 3) only, while the remaining segments of the small intestine were completely devoid of it. Within the duodenum also, the cranial $3/4^{th}$ of the total length presented densely packed Brunner's gland within its submucosa while the rest of the caudal duodenal length showed a decreasing concentration and was almost absent in the last few sections which was in accordance with the reports of Al-Samawy *et al.* (2020) in rats and rabbits.

These glands were observed to be branched tubulo-alveolar, densely packed within the submucosal connective tissue and opened at the base of the intestinal mucosal glands (crypts of Lieberkühn), due to which, the average thickness of submucosal layer of duodenum (177.14±7.960 μm) was significantly more than that of the jejunum (24.31±1.545 μm) and ileum (44.76±5.550 μm) which accorded with the reports of Ergün *et al.* (2010) in Angora rabbit.

In routine histological staining with H & E, it was observed that the lining epithelium of glands were cuboidal to pyramidal. The nuclei of the glandular epithelium were flattened to oval, heterochromatic, basally located and strongly basophilic. The cytoplasm

Table 1. Number of Goblet cell /10,000 μm^2 area of stained slides of different parts of intestine.

Structure	Duodenum	Jejunum	Ileum	Caecum	Colon	Rectum
Cryptal epithelium	5.1 ± 0.64^{a}	3.35 ± 0.334^{a}	12.45 ± 0.958^{c}	9.55 ± 1.243^{b}	21.4 ± 0.916^{d}	12.9 ± 0.817^{c}
Mucosal epithelium	6.0 ± 0.580^{a}	7.3 ± 0.598^{a}	10.8 ± 1.037^{b}	6.2 ± 0.484^{a}	7.6 ± 0.654^{a}	6.4 ± 0.373^{a}

N.B. – Mean Values having different superscripts in a row differ significantly, where P<0.05

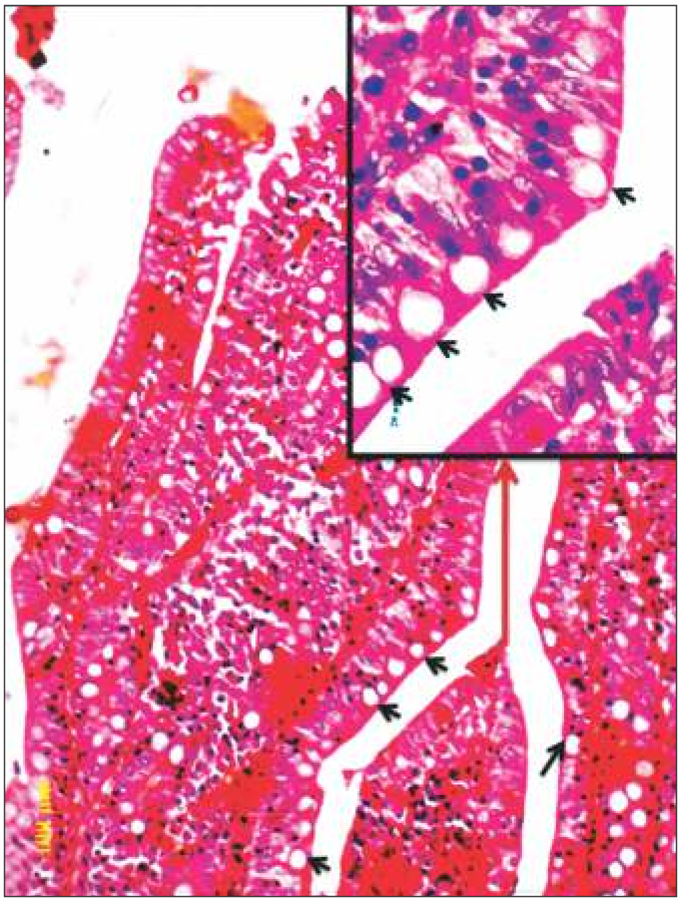

Fig. 1. Photomicrograph of rabbit ileum showing numerous goblet cells with clear apical cytoplasm (arrow) within the lining epithelium. H&E 20x. [Inset: Goblet cells. H&E 100x]

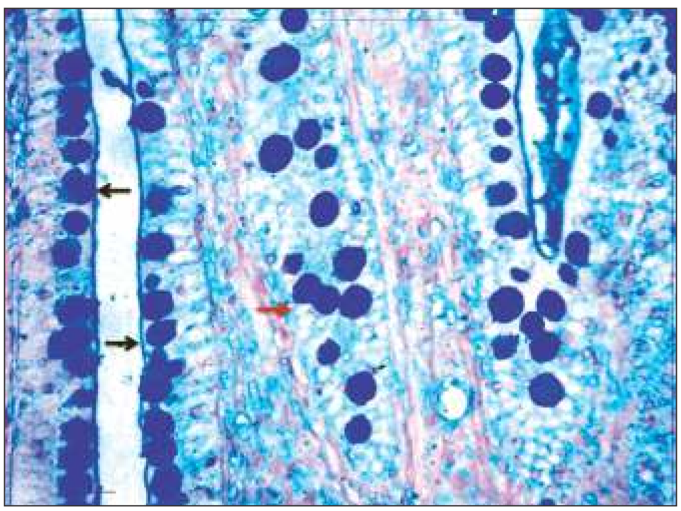

Fig. 2. Photomicrograph of rabbit ileum showing numerous alcinophilic goblet cells within the lining epithelium (black arrow) and within the crypts of Lieberkühn (brown arrow). PAS-AB 40x.

was finely granular and eosinophilic (Fig. 6) which was in accordance with the reports of Verdiglione and Montesi (2019) in bovines. Kumar *et al.* (2013) also reported varying shapes and dimensions of the acini of Brunner's glands in sheep duodenum with simple columnar glandular epithelium. The nucleus was strongly

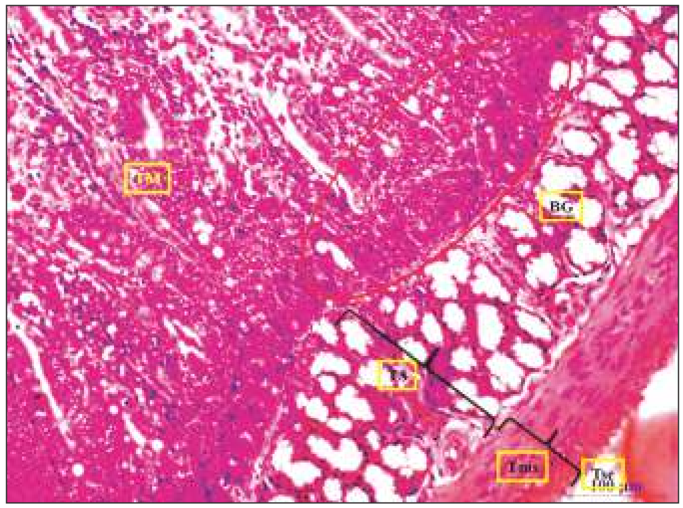

Fig. 3. Photomicrograph of rabbit duodenum showing all the tunics viz; Tunica mucosa (TM), Tunica submucosa (TS), Tunica muscularis (Tms) and Tunica Serosa (Tse). The tunica mucosa has numerous crypts of Lieberkühn (red circle) arranged in several layers. The tunica submucosa is filled with numerous Brunner's gland (BG). H&E 20x.

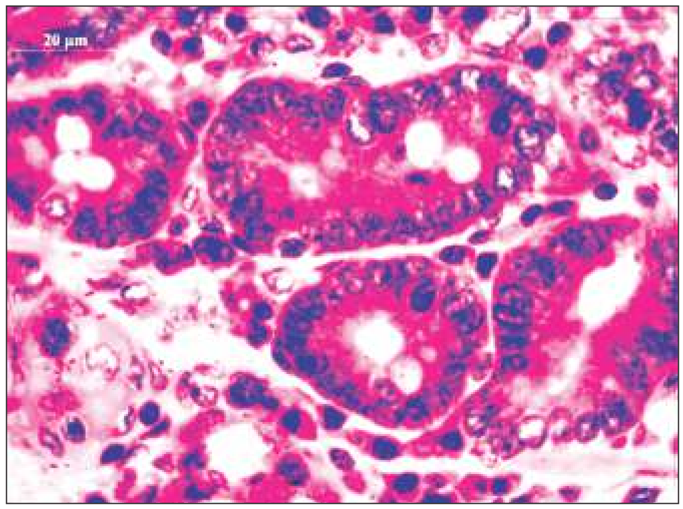

Fig. 4. Photomicrograph showing cross-sections of crypts of Lieberkühn (mucosal glands) within the lamina propria of intestine lined by cuboidal to low columnar epithelium with basal spherical to oval nucleus and acidophilic cytoplasm. H&E 100x.

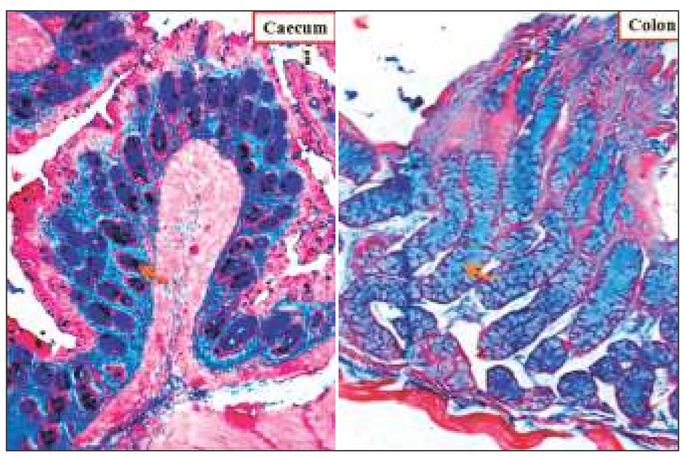

Fig. 5. Photomicrograph showing cross-sections of crypts of Lieberkühn (mucosal glands) within the lamin a propria of caecum and colon in rabbit. The cryptal glands in caecum are relatively short and straight while, very long and straight cryptal glands are present in colon. PAS-AB 10x.

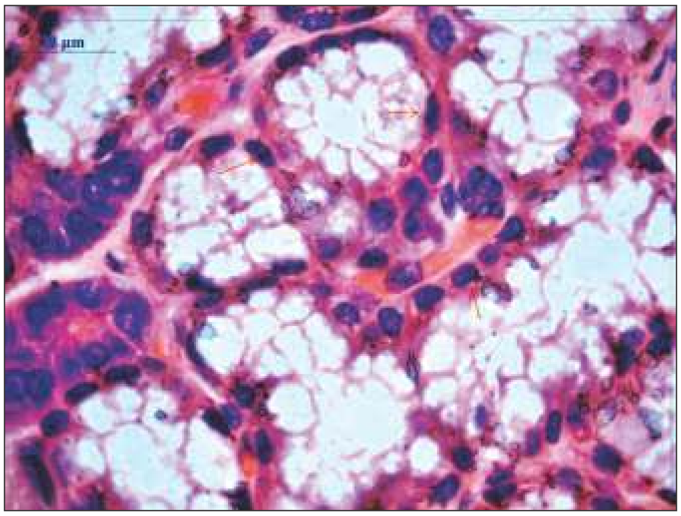

Fig. 6. Photomicrograph showing cross-sections of Brunner's glands (Submucosal glands) within the tunica submucosa of duodenum in rabbit. The nuclei of the glandular epithelium is flattened to oval, basally located and strongly basophilic (arrow). The cytoplasm is finely granular and eosinophilic. H&E 100x.

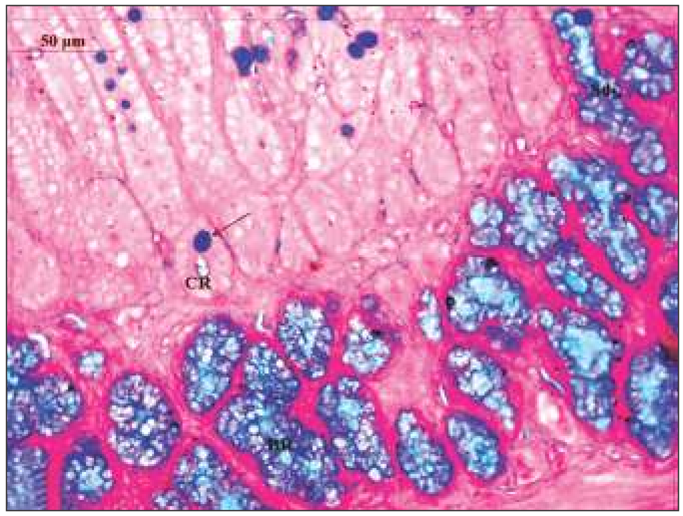

Fig. 7. Photomicrograph showing cross-sections of Brunner's glands (BR; Submucosal glands) within the tunica submucosa and crypts (CR; mucosal glands) within the lamina propria of duodenum in rabbit. The Brunner's glands and secretory duct (Sd) opening at the base of crypt are highly alcinophilic, while the crypts are positive for PAS with alcinophilic goblet cells (arrow). PAS-AB 40x.

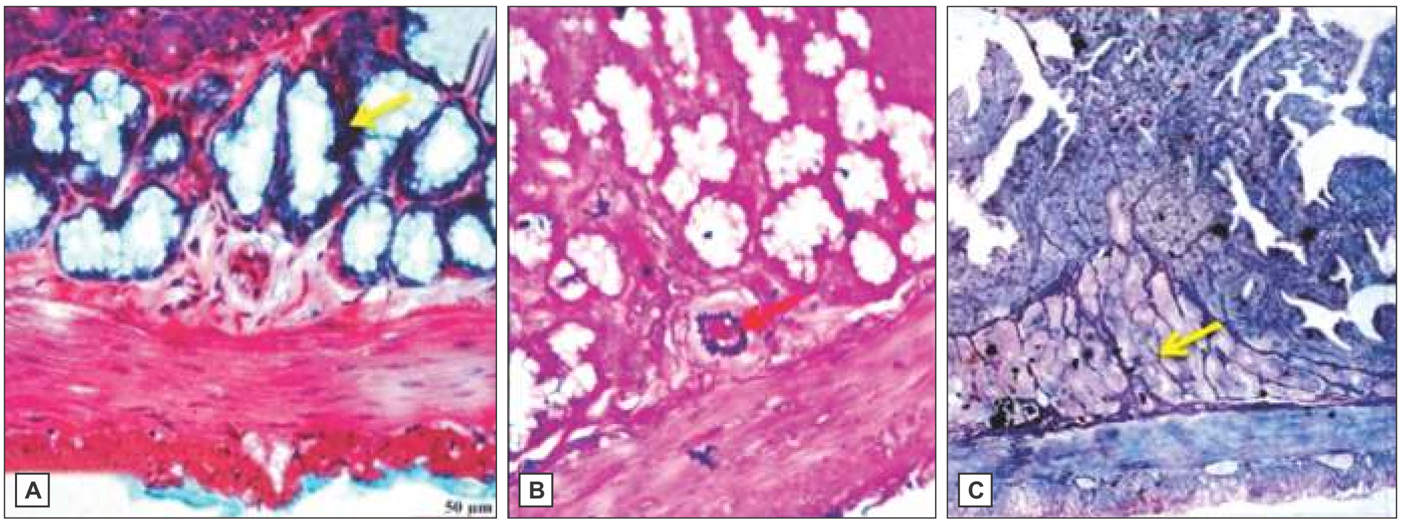

Fig. 8. Photomicrograph showing very sprase distribution of collagen fibers around Brunner's glands [arrow; (A)] elastic fibers are present in blood vessels only [arrow; (B)] and reticular fibers are present as thick bands around Brunner's glands [arrow; (C)].

basophilic, elongated and pushed towards the base. The gland concentration reduced towards the caudal part of the descending duodenum.

After specific staining with PAS-AB (pH 2.5), it was observed that these Brunner's gland consisted of strong alcinophilic mucous cells. The secretory granules within the mucous cells and the excretory duct did not react for PAS but was strongly positive for AB at pH 2.5 (Fig. 7). Very few serous cells present within the mucous acini were weakly positive for PAS. Thus it can be inferred that the mucous cells of the Brunner's gland contained acid mucopolysaccharides in a very high concentration. Krause (2000) and Verdiglione *et al.* (2002) also reported a species variation regarding mucin content of the Brunner's glands.

Upon staining for connective tissue fibers, it was evident that the submucosal layer was rich in collagen and reticular fibers along with connective tissue cells, but very sparse collagen fibers were evident around the Brunner's gland which was in conformity with the reports of Burgisser *et al.* (2024) in rabbit who also observed the presence of weak collagen staining around Brunner's gland. Elastic fibers were evident within the wall of blood vessels. Individual alveolar masses were distinctly separated from each other by thick bands of reticular fibers (Fig. 8).

CONCLUSION

The present research findings revealed a reverse relation between the goblet cell count, mucosal gland concentration and Brunner's gland concentration in different segments of rabbit intestine. The average goblet cell population in the intestine increased from the oral to aboral direction with significantly higher count in ileum (small intestine) and colon (large intestine), while on the other hand, the submucosal Brunner's glands were present only in the duodenum, the remaining segments intestine were devoid of Brunner's gland. The mucosal glands were also present throughout the length of intestine with varying anatomical features. Within the duodenum also, the cranial 3/4th of the total length presented densely packed Brunner's gland within its submucosa while the rest of the caudal duodenal length showed a decreasing concentration and was almost absent in the last few sections. Regarding functional aspects, both the goblet cells and Brunner's glands were highly alcinophilic while, the mucosal glands (Crypts of Lieberkühn) were intensely PAS-positive with scattered alcinophilic goblet cells within its secretory epithelium indicating their role in synthesis and secretions of mucous that play definite protective role in neutralizing the acidic pH of ingesta passing from stomach to intestine, thus preventing the destruction of intestinal mucosal lining from corrosive effects of acidic pH of ingesta and other proteolytic enzymes.

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