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Research Article

HISTOCHEMICAL STUDIES ON THE GASTRO-INTESTINAL TRACT OF RABBIT

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Abstract: The present study was undertaken to reveal the histochemical moiety of the digestive tract of rabbit using different staining techniques. The degree of keratinization of esophageal epithelium increased significantly from cervical to abdominal course which protected the underlying mucosa from the course food particles. The fibrous components of gastro-intestinal wall were positive for acid mucopolyssacharides while the epithelium, underlying basement membrane and muscular layer was PAS positive. The goblet cells, cryptal cells and the Brunner's gland were strongly alcinophilic and believed to protect the duodenal mucosa as well as the caecotrophes from the gastric hydrochloric acid.

Keywords: Histochemistry, Gastro-intestinal tract and Rabbit

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Introduction

Rabbits are herbivores, hind-gut fermenters and specifically caecotrophic in nature. Caecotrophy implies to the behaviour of directly feeding own soft faeces during night time. The distinctiveness lies in the dual function of the proximal colon. Muscular contractions of the colon divert fine, non-fibrous particles into the caecum and only pass non-fibrous particles larger than 3 mm, which are formed into hard faeces. The material sent to the caecum is subjected to several hours of microbial fermentation. During the early morning hours, the remaining caecal contents entering the colon are formed into small 5mm pellets by the colon wall contractions. The pellets are composed of digesta that has been exposed to bacterial digestion, as well as considerable quantities of bacteria, all from the caecum. The colon wall secretes a mucous layer over the pellets, which protects the pellets from the stomach acids following ingestion where they are stored for several hours. These caecotrophes are directly swallowed from the anus. These caecotrophes are a good source of proteins, vitamins and minerals [1]. The histochemical moiety of the GIT wall thus plays an important role in maintaining the digestive physiology in rabbits. Hence the present study was undertaken to reveal the qualitative histochemical contents of digestive tract of rabbit.

Materials and Methods

The present study was carried out in the Department of Veterinary Anatomy and Histology, West Bengal University of Animal and Fishery Sciences, Kolkata. All the animal experiments complied with the guidelines promulgated by the Institutional Animal Ethics Committee (IAEC) of the university. Adult rabbits (n=12) between body weights of 1.0 to 1.5 kg were reared under standard procedures, euthanized and tissue samples from gastro-intestinal tract (esophagus, stomach, small intestine and large intestine) were collected. From esophagus, the samples were collected from cervical, thoracic and abdominal course. From stomach the samples were collected from cardiac, fundic and pyloric region. In small intestine the tissues were collected from duodenum, jejunum and ileum and from the large intestine the tissue samples were taken from caecum, colon and rectum. The samples collected were trimmed and fixed in 10% NBF and Bouin's fixatives for 24-48 hours. After that they were subjected to the standard procedures of processing before proceeding for sectioning [2]. All sections were cut at 5µm thickness with the help of Leica 2125 DM rotary microtome. The sections were taken on clean grease free albumenized glass slides for demonstration of neutral and acid mucopolysaccharides by PAS-AB staining method (pH 2.5) and demonstration of keratin and pre-keratin in stratified epithelium of esophagus by Ayoub-Shklar staining method [2]. Leica Qwin Image Analyser software in Lecia DM 2000 Microscope was used for taking micrometrical data and desired photographs from the stained sections.

Results and Discussion

Keratin and pre-keratin protein

The present observation revealed that the esophageal epithelium had a highly positive stratum corneum for keratin proteins throughout the course of the esophagus whereas pre-keratin in the underlining strata gradually decreased from cervical to abdominal course [Fig-1]. The average thickness of keratin layer in cervical, thoracic and abdominal portions of esophagus was 6.6±0.704 µm, 7.86±0.547 µm and 11.16±1.137 µm respectively that increased significantly from cervical to abdominal region [Table-1]. Eurell and Frappier, (2006) [3] had reported that the degree of keratinization varied with the species; being non-keratinized in carnivores, slightly keratinized in pigs, horses and highly keratinized in ruminants. This varying degree of keratinization depended on the feeding habit with herbivores like rabbit and guinea pig having keratinized squamous epithelium; however, the African giant rat, being an omnivore, had non-keratinized esophageal epithelium [4].

Neutral and Acid mucopolysaccharides

Esophagus

In the present observation, the esophageal epithelium and basement membrane showed a moderate PAS positive reaction while the keratin layer showed a strong mixed reaction for neutral and acid mucopolysaccharides. The muscular layers and the wall of blood vessels also showed a positive reaction for neutral mucopolysaccharides, while all other histological layers rich in fibrous components were strongly positive for acid mucopolysaccharides [Fig-2]. In contrary to the above findings, Oliver, *et al*., (2010) [4] reported that the esophagus of the African

Table-1 Mean ± S.E. of micrometrical observations of different regions of esophagus (µm).

Esophagus	Cell layers in epithelium	Epithelial thickness (µm)	Keratin layer thickness((µm)	Tunica mucosa (µm)	Tunica sub mucosa (µm)	Tunica muscularis (µm)	Tunica adventitia/ serosa (µm)
Cervical	11.7±0.396^{a}	129.36±9.783^{a}	6.6±0.704^{a}	182.43±12.053^{a}	113.11±34.892^{a}	363.82±15.965^{a}	22.29±3.535^{ab}
Thoracic	11.9±0.378^{a}	130.24±8.466^{a}	7.86±0.547^{a}	185.74±9.554^{a}	67.56±23.123^{a}	345.96±18.489^{a}	17.53±1.79a
Abdominal	12.2±0.554^{a}	132.56±9.181^{a}	11.16±1.137^{b}	210.27±10.485^{a}	64.54±9.431^{a}	472.48±19.147^{b}	29.87±2.226^{b}

N.B.-Mean Values bearing different superscripts in a column differ significantly, where $P<0.05$

Table-2 Goblet cell count/10,000 µm^{2} area in different segments 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 bearing different superscripts in a row differ significantly, where $P<0.05$

giant rat was PAS-AB negative. He postulated that the mucous produced by the salivary glands might be helping in protecting the mucosal surface of the esophagus from sharp objects since, the mucous barrier was also an important factor in the protection of the esophagus from damage. This contradictory observation might be due to the different feeding behavior of these two species. Rabbit being an herbivore needs better mucosal protection in the esophagus to prevent any mechanical injury from the coarse feed particles; hence PAS-AB reactions of varying intensity was observed in the present study.

Stomach

In all the three different regions of stomach, the columnar epithelium, basement membrane, muscular layers and perivascular tissue showed a moderate PAS-positive reaction and all the fibrous components *viz*., lamina propria, tunica submucosa and tunica serosa were alcinophilic [Fig-3]. The parietal cells had PAS-positive granules while the chief and mucous cells were alcinophilic [Fig-3]. The present observations were in accordance with the reports of Khalel and Ghafi, (2012) [5] for the cardiac and pyloric regions of rabbit's stomach but not for the fundic region. Fundic region of the gastric mucosa is the major site for enzymatic digestion, hence varying intensity of staining might be observed that may range from negative to moderate reactions.

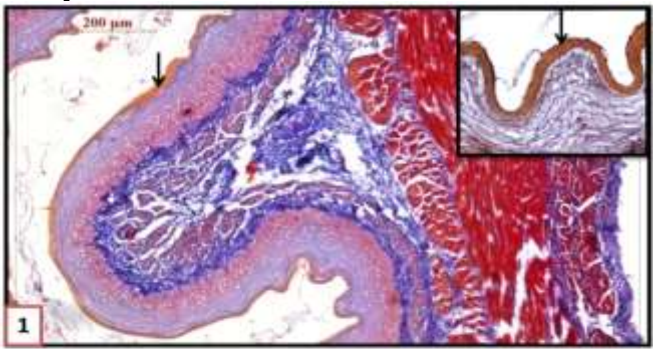

Fig-1 Microphotograph of abdominal region of esophagus showing deposition of keratin protein (Black arrow) in the stratum corneum of stratified squamous epithelium. Ayoub Shklar method 10X [Inset: Deposition of keratin protein in the stratum corneum. 40X]

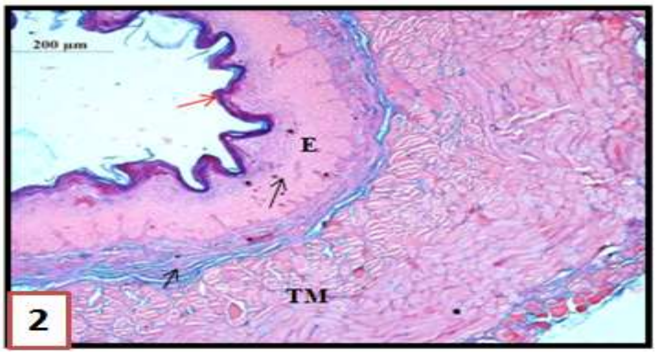

Fig-2 Microphotograph of rabbit esophagus showing PAS positive epithelium (E), Tunica muscularis (TM) and alcinophilic fibrous component (arrow). The keratin layer (red arrow) shows strong mixed reaction. PAS-AB 10X.

Small Intestine

Throughout the length of small intestine, the structures having fibres *viz*., tunica serosa, submucosa and lamina propria were alcinophilic and the muscular layers were PAS positive. Within the tunica submucosa, the Brunner's gland in duodenum was strongly alcinophilic indicating the presence of acid mucins [Fig-4]. Krause, (2000) [6] and Verdiglione, *et al*., (2002) [7] also reported that depending on species, Brunner's glands contained neutral or acidic mucin glycoproteins or the combinations of both types of mucin. Generally, the duodenal glands are believed to protect the duodenal mucosa from the gastric hydrochloric acid. Within the tunica mucosa of duodenum, jejunum and ileum; the crypts of lieberkuhn was positive for neutral mucopolysaccharides with presence of alcinophilic goblet cells. The goblet cells within the villi of small intestine were strongly alcinophilic and were filled with acid mucins [Fig-4] and [Fig-5]. The present observations were in accordance with Al-Kennany, *et al*., (2012) [8] and Ergün, *et al*., (2010) [9] who also reported that the goblet cells were strongly alcinophilic. The villi, lining epithelium and the basement membrane throughout the small intestine were PAS positive [Fig-4] and [Fig-5]. The glycocalyx layer over the epithelium was moderately alcinophilic. The reaction in the jejunum was same as that of duodenum. The lymphatic aggregation within the ileum was alcinophilic with few PAS positive granules especially in the medulla region. In the sacculus rotundus, the germinal centres of the lymphatic nodules had PAS positive granules. This was in conformity with the findings of Kumar, *et al*., (2013) [10] who also reported that in sheep intestine the villi, basement membrane, crypts and luminal border of the columnar epithelial cells were moderately positive for PAS. In the small intestine the number of goblet cells/ 10,000 µm2 increased from the oral to aboral direction. Within the crypts and mucosal epithelium, the goblet cell number was significantly highest in the ileum than that of the duodenum and jejunum [Table-2]. That might be due to the reasons that the principal function of the goblet cells and cryptal glands are protection of the intestinal mucosa against the erosive effects of the gastric juice by virtue of the mucoid nature of its secretion, its alkalinity, and possibly by the buffering capacity of its bicarbonate content.

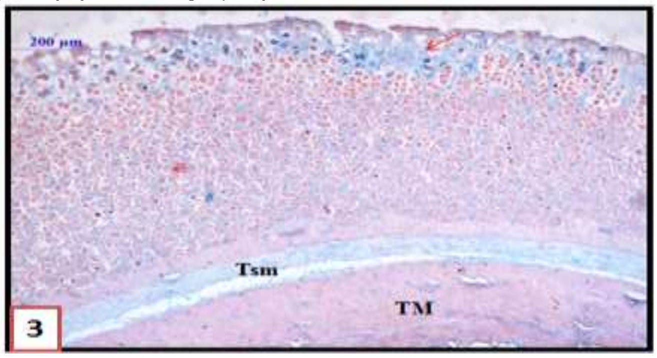

Fig-3 Microphotograph of section of stomach showing alcinophilic mucous cells at the neck of the gland (red arrow), PAS positive tunica muscularis (TM) and alcinophilic tunica submucosa (Tsm). PAS-AB 10X.

Large Intestine

Throughout the length of large intestine, the fibrous structures *viz*., tunica serosa, submucosa and lamina propria were alcinophilic and the muscular layers were PAS positive [Fig-6] and [Fig-7].

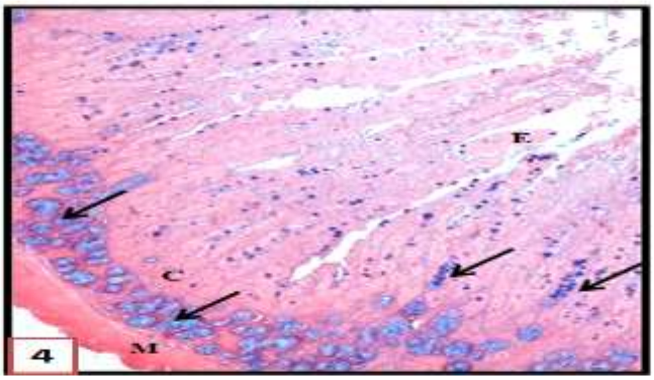

Fig-4 Photomicrograph of duodenum showing PAS +ve muscularis (M), Cryptal glands (C), Epithelium (E) and alcinophilic Brunner's gland and goblet cells (arrow). PAS-AB 4X

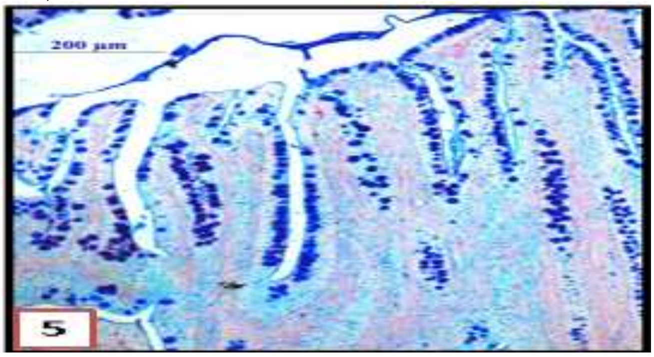

Fig-5 Section showing the intense alcinophilic goblet cells in the villi of ileum. PAS-AB 10X.

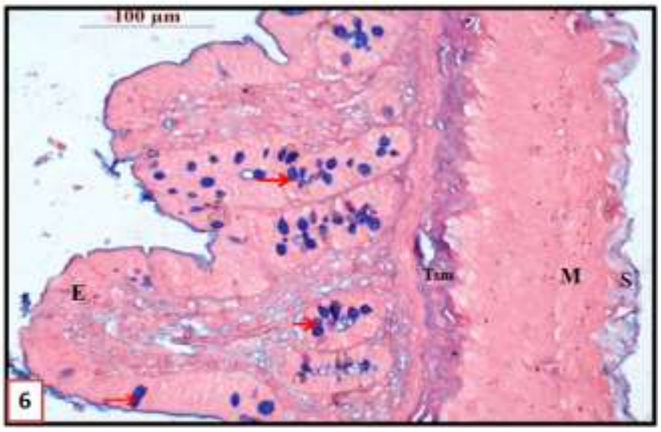

Fig.-6 Photomicrograph of caecum showing strongly alcinophilic goblet cells (arrow) and PAS^{+ve} muscularis (M) and epithelium (E). Glycocalyx and serosa are AB+ve. PAS-AB 10X.

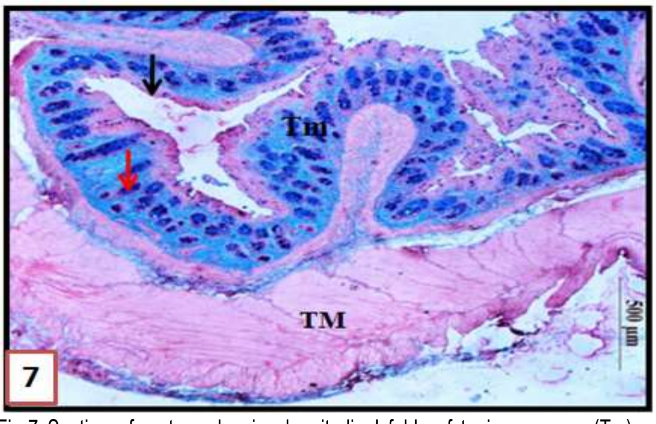

Fig-7 Section of rectum showing longitudinal folds of tunica mucosa (Tm) and alcinophilic short cryptal glands (red arrow), numerous AB^{+ve} goblet cells (black arrow), PAS positive Tunica muscularis(TM) and alcinophilic fibrous layers. PAS-AB 4X.

The villi, lining epithelium and the basement membrane throughout the length of large intestine were PAS positive. The glycocalyx layer over the epithelium was moderately alcinophilic [Fig-6]. The germinal centres of the lymphatic nodules in vermiform appendix had PAS positive granules. The crypts had moderate content for neutral muco-polysaccharides with numerous strongly alcinophilic goblet cells. Grant and Specian, (2001) [11] also reported that in rabbit the goblet cells present in the caecum and proximal colon indicated the presence of acidic mucins. The goblet cell number increased from caecum towards rectum and was even more than that of small intestine. Within the mucosal epithelium the goblet cell count did not varied significantly while in the crypts the number of goblet cells in the colon was significantly high as compared to caecum and rectum [Table-2]. Oliver, *et al.,* (2011) [12] also reported that the different segments of the colon of the African giant rat had abundant goblet cells and intestinal glands. An increase in number of goblet cells in the colon indicated the need for increased mucosal protection and lubrication for fecal expulsion. The presence of large number of mucous secreting cells provided a mucous layer around the fecal pellets, facilitating its release and protecting the epithelium. Keskin, *et al.,* (2012) [13] reported the same in the large intestine of mouse. In case of rabbits, Halls, (2008) [1] had reported the caecotrophic nature of feeding 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. This might be the reason that the goblet cell concentration was significantly high in the colon as observed in the present study.

Conclusion

The investigation of various histochemical moieties in different region of gastro-intestinal tract of rabbit and in different histological layers of these regions revealed that the mucous plays an important role in protecting the caecotrophes from gastric pH. These caecotrophes further are a good source of nutrients to the rabbits. The goblet cell count was significantly high in colon which further aids in mucosal coating of the caecotrophes. The ileal goblet cell secretions prepare the food particles to undergo microbial fermentation in caecum. The keratinization of the esophageal epithelium prevents the underlying layers from any mechanical injury caused due to coarse feed particles.

Application of research: Rabbit is the most commonly used laboratory animal like for drug testing, antibodies production, nutritional studies, etc. Hence knowledge of rabbit anatomy, especially GIT is of utmost important.

Research Category: Veterinary Anatomy

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Abbreviations:
PAS- Periodic acid Schiff, AB- Alcian Blue, GIT- Gastro-intestinal tract

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Conflict of Interest: None declared

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Ethical Committee Approval Number: Pharma/IAEC/138, dated: 30.06.2014

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