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Research Article

GROSS, HISTOLOGICAL AND MORPHOMETRICAL STUDIES ON THE SMALL INTESTINE OF RABBIT (*ORYCTOLAGUS CUNICULUS*)

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ABSTRACT

The present study was undertaken to explore the gross and histo-morphological details of the small intestine and was carried out on twelve adult rabbits between body weights of 1.0 to 1.5 Kg. The tissue samples were processed, sectioned and subjected to specific stains to reveal histological details. The general architecture was similar to other mammals with few peculiarities: the shape of the villi varied from leaf-like in the duodenum to conical in jejunum and club-shaped in ileum. Brunner's gland was present in the initial part of the duodenum only. Peyer's patch was present only at the termination of the ileum. The sacculus rotundus was a unique structure in rabbit and was distributed with numerous lymph nodules which took part in gut associated immune response in rabbit. The mean thickness of tunica muscularis in the ileum was significantly high.

Keywords: Gross, Histology, Morphometry, Rabbit, Small intestine

The morphology and gross disposition of the digestive tract has been extensively studied and reported in various species of mammals like sheep, cattle, pigs, horses, dogs (Sisson and Grossman, 1953), man (Harold, 1992) and birds (Devyn *et al.*, 2000). Rabbits are extensively used in the laboratories as a classical experimental animal model for various experimentations including the nutritional studies (McNitt *et al.*, 2011). Rabbit meat is high in protein and low in fat (Holmes *et al.*, 1984). Sodium and caloric content of the meat is also low (McNitt *et al.*, 2011). Hence, it is a good source of protein for children, sick, aged and health conscious people. Hence, the present work was focused on anatomical details of small intestine of rabbits which play a major role in digestion of food particles and absorption of nutrients.

MATERIALS AND METHODS

The present study was carried out on twelve adult rabbits between body weights of 1.0 to 1.5 Kg after approval of Institutional Animal Ethics Committee (IAEC). After topographic study, gross parameters for length and diameters were recorded for different components of small intestine. Tissue specimens from every 10 cm intervals were taken from different segments of intestine i.e., duodenum, jejunum and ileum and fixed in 10% Neutral buffered formalin (10% NBF) and Bouin's fixatives. These samples were processed by acetone-benzene schedule and these 5-6 µm thick paraffin sections were stained with Haematoxylin and eosin (H&E) for routine histoarchitecture, Masson's trichrome method for Collagen fibers, Gridley's method for reticular fibers and Weigert's method for elastic fibers (Luna, 1968). Micrometry was done by using Leica Qwin Image Analyzer software in Leica DM 2000 microscope. The data collected were analyzed statistically (Snedecor and Cochran, 1994).

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RESULTS AND DISCUSSION

Gross Anatomy

The small intestine comprised of three segments; duodenum, jejunum and ileum. The ileum had a dilated terminal end, the Sacculus Rotundus (SR).

Duodenum: In the present study, after its origin from the pylorus, the duodenum was found to progress caudally along the descending part of colon below the right lateral lobe of liver and medial to the right lateral abdominal wall (Fig. 1). It presented a descending part, a transverse part and an ascending part from oral to aboral direction. The descending part extended posteriorly up to the level of pelvic inlet, and then it continued as transverse part and proceeded cranially as ascending part. The ascending part reached the level of last thoracic vertebra, passed downward and forward and finally backward, reached the right lateral abdominal region and continued as jejunum. Similar observations have been reported by Prasad (1971) in rabbits and Perez *et al.* (2011) in chinchilla.

Jejunum: The jejunum appeared as a highly convoluted portion of small intestine. It occupied its place in right lateral abdominal region between the stomach and the caecum. The whole tube consisted of numerous "U" shaped loops and was found to be attached at the margin of mesentery. It was 167.17±11.04 cm in length and 5.86±0.34 mm in diameter. However, Ali *et al.* (2008) reported that in African giant rat, the jejunum occupied the abdominal floor between the stomach cranially and the urinary bladder caudally.

Ileum: The terminal portion of small intestine, i.e. ileum was devoid of any loops, it was 28.42 ± 0.811 cm long and

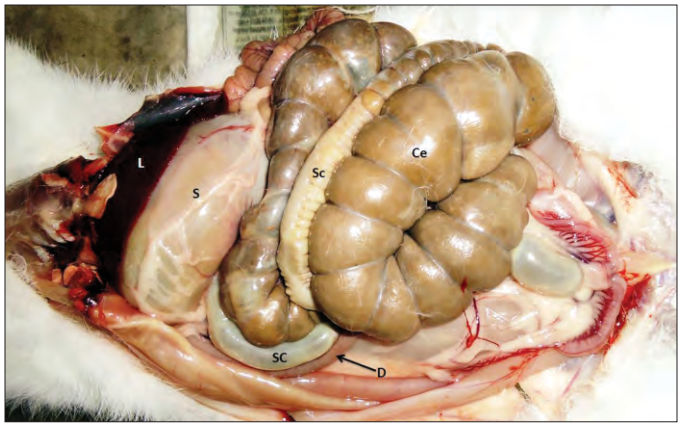

Fig. 1. Photograph showing the descending duodenum (D) between smooth colon medially (SC) and abdominal wall laterally. Other structures are caecum (Ce), Sacculated colon (Sc) and cranially placed stomach (S) and liver (L).

Fig. 2. Photograph showing the ileum (IL) terminating into the Sacculus rotundus (SR), opening dorsally on the second gyrus fo Caecum (Ce) and initiation of Sacculated colon (Sc) from the Caecum, arrow indicating caeco-colic junction.

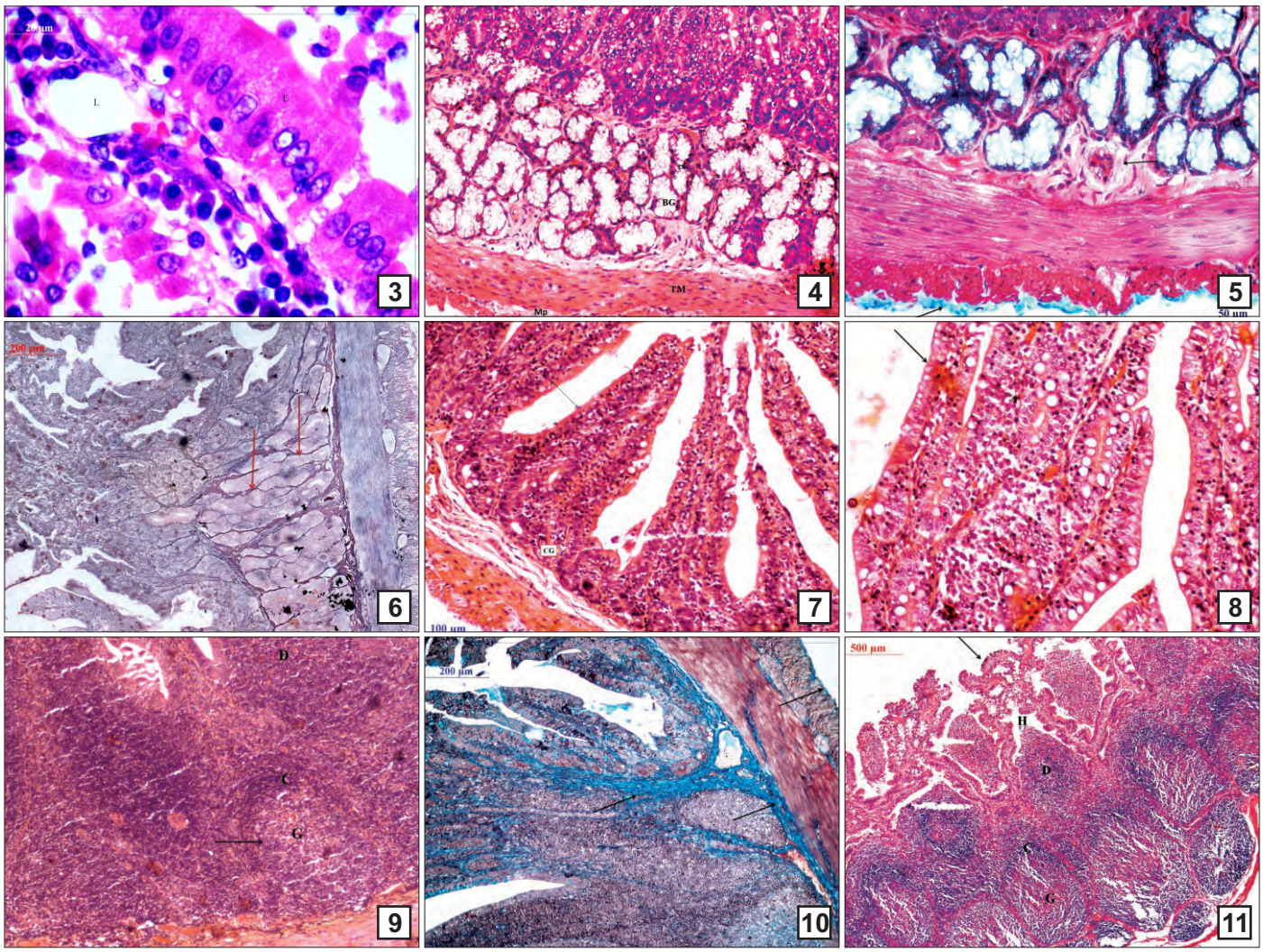

Fig. 3. Microphotograph showing the leaf shaped villi of duodenum with simple columnar epithelium (E) and lamina propria having lacteals (L). H&E 100X; **Fig. 4.** Microphotograph showing the tunics of duodenum viz., Tunica mucosa containing cryptal glands (CG), Tunica submucosa containing Brunner's glands (BG), Tunica muscularis (TM) with myenteric plexus (Mp) and Tunica serosa (TS). H&E 20X; **Fig. 5.** Photomicrograph showing distribution of collagen fibers in serosa and submucosa of duodenum of rabbit (arrow). Masson's trichrome 40X; **Fig. 6.** Photomicrograph showing distribution of reticular fibers around Brunner's gland and cryptal gland (arrow) in duodenum of rabbit. Gridley's 10X; **Fig. 7.** Microphotograph of jejunum showing conical shaped villi (arrow) with simple columnar epithelium and cryptal glands (CG). H&E 20X; **Fig. 8.** Section of ilcum showing clubbed shaped villi with simple columnar epithelium and goblet cells (arrow). H&E 20X; **Fig. 9.** Section showing the first appearance of peyer's patch (arrow) at the termination of the ileum having Corona (C), Dome (D) and germinal center (G). H&E 10X; **Fig. 10.** Section showing the distribution of collagen fibers in submucosa around the lymphatic nodules, lamina propria and scrosa in ilcum (arrow). MT 10X; **Fig. 11.** Section of sacculus rotundus showing mushroom shaped tunica mucosa (black arrow) and lymph nodules in submucosa having germinal centre (G), Corona (C) and Done (D) and Crescent shaped hollows (H). H&E 4X.

4.71±0.33 mm in diameter. It was located almost in the median plane of the abdominal cavity. It then passed to the right crossing the mid line ventral to the vermiform appendix and dorsal to the sacculated colon. The organ then turned caudally and ran in a spiral manner for a short distance to form an ampullary dilatation, the Sacculus rotundus (Fig. 2). The light rose colored sacculus rotundus (SR) appeared as a thick-walled, cylindrical structure on the caudal part of ileum that opened dorsally to the second gyrus of the caecum. (Fig. 2).

Besoluk *et al.* (2006) also reported similar observations in the gross disposition of ileum in Angora rabbits.

HISTO-MORPHOLOGY

The histological observation revealed the presence of numerous villi lined by simple columnar epithelium with numerous goblet cells in all the three segments of small intestine.

Duodenum: The duodenal mucosa was observed to have numerous villi, throughout its length, lined by simple columnar epithelium with striated free borders (Fig. 3). The villous was slender leaf shaped. The mean length of villi was more in length to that of the villi of jejunum and ileum but did not varied significantly with the other two segments of small intestine (Table 1). Similar observations had been reported by Neogy (2000) in rabbits.

In the present investigation, goblet cells were found interspersed between columnar absorptive epithelial cells (Fig. 3). The average goblet cell population was same throughout the length of the duodenum (Table 1), however, Kumar *et al.* (2013) reported the varying number of goblet cells ranging from few to moderate in caudal portion of the descending duodenum of sheep. The lamina propria beneath the epithelium formed the core of the villi and consisted of collagen and reticular fibers, fibrocytes, numerous lymphocytes, few plasma cells and mast cells. Small blood capillaries were also evident. The centre of the lamina propria within the villi contained clear spaces, the lacteals, a lymphatic capillary (Fig. 3). The lamina muscularis consisted of smooth muscle fibers separating the tunica mucosa from the underlying submucosa. Similar observations had been reported by Neogy (2000) in rabbits and Eurell and Frappier (2006) in other mammals.

The mucosal glands (crypts of Lieberkuhn) in the lamina propria were found to open between the bases of the villi. These simple branched tubular glands lined by cuboidal to low columnar epithelium with basal nucleus and acidophilic cytoplasm were present throughout the duodenum (Fig. 4). The cryptal depth in duodenum was significantly more than the jejunum and ileum (Table 1). Similar observations had been reported by Kumar *et al.* (2013) in sheep and Eurell and Frappier (2006) in other domestic animals.

Tunica submucosa consisted of collagen and reticular fibers, fibrocytes, lymphocytes and blood vessels (Fig. 5 & 6). The elastic fibers were well evident in the inner and outer elastic lamina of large blood vessels. A large area of submucosa was accommodated by Brunner's gland that was specifically present in the duodenum (Fig. 4). Eurell and Frappier (2006) in domestic animals and Kumar et al. (2013) in sheep also reported the presence of loose irregular connective tissue cells and fibers in the duodenal submucosa that was mainly occupied by submucosal or Brunner's glands.

The average thickness of submucosal layer was significantly more than that of the jejunum and ileum (Table 1) that might be due to the presence of Brunner's glands in the tunica submucosa of duodenum while it was absent in jejunum and ileum. The glandular epithelium was cuboidal to pyramidal. The Brunner's glands were present in the submucosa upto approximately 40 cm from the pylorus, rest part was devoid of these glands. The findings of Kumar *et al.* (2013) was in partial confirmation with the present observations in terms of glandular epithelium.

The tunica muscularis consisted of thick inner circular and thin outer longitudinal smooth muscle layers (Fig. 4, Table 1). In between the two muscle layers,

Table 1

Mean ± S.E. of micrometrical observations of different regions of Small Intestine (µm)

Small Intestine	Villous length	Cryptal depth	Tunica submucosa	Tunica muscularis	Tunica serosa	Goblet cell count/ 10,000 µm^2 area	
						Cryptal epithelium	Mucosal epithelium
Duodenum	577.03±15.55^a	188.13±22.36^b	177.14±7.96^c	85.88±5.57^a	13.55±1.19^b	5.10±0.64^a	6.00±0.58^a
Jejunum	557.27±35.29^a	95.69±8.60^a	24.31±1.55^a	71.77±3.56^a	6.94±0.84^a	3.35±0.33^a	7.30±0.60^a
Ileum	546.01±33.03^a	103.08±9.88^a	44.76±5.55^b	218.91±15.95^b	11.53±0.86^b	12.45±0.96^c	10.80±1.04^b

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

myenteric plexus was present (Fig. 4). The tunica serosa comprising of collagen and few elastic fibers invested the organ throughout its length. (Fig. 5). Similar observations were recorded by Kumar et al. (2013) in sheep and Eurell and Frappier (2006) in other mammals.

Jejunum: In agreement with the findings of Neogy (2000) in rabbits, the lining epithelium was simple columnar having striated free borders with goblet cells like that of the duodenum. The lamina propria formed the core of the villi and consisted of collagen and reticular fibers, fibrocytes, numerous lymphocytes, few plasma cells and small blood capillaries similar to that of the duodenum. The lamina muscularis consisted of smooth muscle fibers and separated the tunica mucosa from the underlying submucosa. Byanet *et al.* (2011) also reported similar observations in the jejunum of the grass-cutter, a wild herbivore rodent.

The mucosal glands (crypts of Lieberkuhn) were similar in structure to those observed in the duodenum and were present throughout the length of the jejunum but the number of goblet cells within the crypts increased significantly (Fig. 7, Table 1).The same was reported by Byanet *et al.* (2011) in grass-cutter These glands extended down to the muscularis mucosa in rabbits.

The tunica submucosa was similar in structure to that of the duodenum except the Brunner's glands that were absent in the jejunum (Fig. 7). The tunica muscularis consisted of a thick inner circular and a thin outer longitudinal smooth muscle layer that had non-significant variation with that of the duodenum but was significantly less than that of the ileum (Table 1). In between the two muscle layers, myenteric plexus was present (Fig. 7). The tunica serosa was a outer most connective tissue layer comprising of collagen fibers.

Ileum: The histological orientation in terms of different layers of ileum was found to be almost similar to that of the jejunum with some characteristic differences. The villi were more clubbed shaped as compared to the leaf shaped villi of duodenum and conical villi of jejunum (Fig. 8). The mean length of villi varied non-significantly with other two segments of small intestine (Table 1). The lining epithelium was simple columnar with numerous goblet cells (Fig. 8). The cryptal depth was significantly less than the duodenum but almost similar with the jejunum that might be due to the more superficial arrangement of mucosal glands in both the jejunum and ileum but not in case of duodenum where the glands were located in several layers (Table 1). Besoluk *et al.* (2006) reported that villi were spindle shaped in ileum of Angora rabbits.

The thickness of tunica submucosa increased significantly than that of the jejunum because of the presence of aggregation of lymphocytes within this tunica. The first appearance of the lymphocytic aggregation was observed at about 164 cm from the pylorus i.e. at the jejuno-ileal junction. These aggregated lymphocytes were absent throughout the length of ileum but again appeared at the terminal part of the ileum (Fig. 9) which were more defined in the form of nodules (Peyer's patch). Some plasma cells were also present. The nodules were separated by a wide inter-nodular area containing lymphocytes and blood capillaries, the nodules were encapsulated by collagen fibers (Fig. 10) and numerous reticular fibers in the submucosa and lamina propria. Eurell and Frappier (2006) also reported the presence of large aggregated lymphatic nodules (Peyer's patch) in the tunica submucosa of ileum in other animals.

The tunica muscularis and tunica serosa contained the same pattern of smooth muscle fibers distribution as in duodenum and jejunum. The micrometrical observations revealed a significant increase in its thickness than that of the duodenum and jejunum (Table 1). This might be reason that the ileum act as a temporary halt zone for the chymes so that it could make ready the intestinal contents for further caecal digestion.

Sacculus Rotundus (SR): The present histological observations revealed that tunica mucosa of SR formed a mushroom-shaped structure over the lymphatic nodules present within the submucosa. The outermost covering (lamina epithelialis) of the mushroom structure was lined by simple columnar epithelium with few goblet cells (Fig. 11). The underlying lamina propria was scanty. Crescent shaped hollows were observed between the lamina propria and the underlying apical part of lymph nodules (Fig.11). The lymphatic nodules were encapsulated by thick collagen fibers. The inter-follicular space was absent. Within the nodule, the centrally positioned germinal center was well differentiated from the peripheral darker zone. The peripheral darker zone, the corona of the superficial lymph node was further covered by dome, the apical part of the lymph nodule. The apical margin of the nodule (dome) was lined by follicle associated epithelium that was cuboidal to low columnar with numerous infiltrated lymphocytes but lacked the goblet cells. But the dome was absent in the lymph nodes which were below the superficial layer (Fig. 11). The entire nodule was filled with small and medium sized lymphocytes and some plasma cells. Alboghobeish and Zabiehy (1996) and Besoluk *et al.* (2006) cited similar observation in rabbit. Besoluk *et al.* (2006) reported the presence of infrequent goblet cells within the simple columnar epithelium of mushroom shaped tunica mucosa in rabbits which was in accordance with the present findings.

CONCLUSION

The present study revealed significant gross and histological differences between the three segments of the small intestine of rabbit which signifies its functional aspects. Moreover, the histological architecture of sacculus rotundus marked its significant role as a primary organ for immune response.

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