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Histological, Histochemical and Ultra Structural Studies of Ileum of Goat (*Capra hircus*)

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ABSTRACT

The tissues from ileum were collected from six young goats and processed for light microscopy, scanning and transmission electron microscopic studies. The villi of the ileum varied in shape and size. These were lined by simple columnar epithelium with few goblet cells. The intestinal glands were simple tubulo-acinar type having simple cuboidal to low columnar epithelium. Lamina muscularis mucosae was thin and interrupted. The submucosa was having loose irregular connective tissue and occupied by lymphoid nodules or Peyer's patches consisted of follicles of various shapes and size. Tunica muscularis had inner circular and outer longitudinal layers of smooth muscles. Histochemical studies presented presence of glycogen and acidic and neutral mucopolysaccharides in the intestinal glands. The scanning electron microscopy showed tongue shaped villi with distinctive corrugation on its surface. The transmission electron microscopy showed presence of simple columnar cells with microvilli in surface epithelium. The cryptal epithelium was having Paneth, enterochromaffin and goblet cells.

Keywords: Ileum, Histochemistry, Scanning electronmicroscopy, Transmission electron microscopy, Goat

Goat is a multi functional animal and its rearing is an enterprise which has been practiced by a large section of population in rural areas in India. Goats play an important role in the rural economy at national level. More than 70 per cent of the landless agricultural labourers, marginal and small farmers of the rural India rear them. The intestine being the important segment of the digestive tract is prone to the various parasites and pathological conditions. The ileum contains lymphoid tissue in the form of nodules within the mucosa as part of mucosa associated lymphoid tissue known as Peyer's patches which help to maintain the immunity (Forchielli and Walker, 2005). The endocrine cells play important role in maintaining gut motility along with enteric nervous system. The occurrence and distribution of different endocrine cell types in the gastrointestinal tract of large and small domestic animals have been studied (Ceccarelli *et al.,* 1995) and only few in ruminants (Kitamura *et al.,* 1985; Guilloteau *et al.,* 1997). The histology and histochemisrty of the ileum had been studied in buffalo (Barnwal and Yadava, 1975; Malik and Prakash, 1971), goat (Ramakrishna and Tiwari, 1979; Andleeb *et al.*, 2009) and in sheep (Kumar *et al.*, 2015). The scanning electron microscopy of small intestine has been studied in goat (Hassan and Moussa, 2015). The anatomical study of ileum will be helpful in understanding the mechanism of digestion and immunity.

MATERIALS AND METHODS

The small intestine containing ileum was collected from six goats (8-10 months age) immediately after their sacrifice from local slaughter house. The tissues from cranial, middle and caudal parts of the ileum were collected and fixed in 10% neutral buffered formalin and processed for light microscopy. The paraffin sections of 5-6 µ were stained by routine Harris haematoxylin and eosin stain (Luna, 1968). The sections were also stained by Crossman's trichrome for collagen fibres (Crossman, 1937), Gomori's method for reticular fibres, Weigert's method for elastic fibres, McManus' method for glycogen (PAS), PAS-Alcian blue method for mucosubstances, Alcian blue for mucosubstances (pH 2.5) (Luna, 1968) and

Fontana method (Humason, 1972) for enterochromaffin cells.

The fresh tissues from selected sites of ileum of six goats collected for electron microscopy were fixed in 2% glutaraldehyde solution for 6-8 hours after thorough washing with chilled 0.1M phosphate buffer (pH 7.4). The tissues were rewashed twice with 0.1M phosphate buffer and rest of the procedure was carried out at EM Lab., A.I.I.M.S, New Delhi. The tissues were dehydrated in grades of ethanol, critical point dried and sputter coated and the processed tissues were viewed in scanning electron microscope (Zeiss EVO-18) to record observations and photographs.

For transmission electron microscopy, tissues from selected sites of ileum of six goats were primarily fixed in 2.5% glutaraldehyde solution and secondarily fixed in 2% osmium tetraoxide for one hour. The rest of the procedure was carried out at EM Lab., A.I.I.M.S, New Delhi. The ultrathin sections (70-80 nm) were stained with uranyl acetate and lead citrate. The processed tissues were viewed in transmission electron microscope (Technai G^2) to record observations and photographs.

RESULTS AND DISCUSSION

The wall of the ileum was consisted of tunica mucosa, sub mucosa, muscularis and serosa. The tunica mucosa was having villi of different shapes and size which were lined by simple columnar epithelium having few goblet cells as observed in goat (Hassan and Moussa, 2015), sheep (Kumar *et al.,* 2015), pig (Sloss, 1954), buffalo (Barnwal and Yadava, 1975) and other domestic animals (Titkemeyer and Calhoun, 1955). The shape of villi varied from cranial to caudal portion. These were elongated towards cranial portion with broad base and narrower apical ends. These varied from club, conical, spatula to tongue shaped (Fig. 1a) whereas, these were mostly finger like and pointed in buffalo calves (Barnwal, and Yadava, 1975). The villi of dog and cat were much longer (Titkemeyer and Calhoun, 1955) and were short and broad at the base in pig (Sloss, 1954 and Talukdar, 1999) and piglets (Rajkhowa and Baishya, 2013). The height of the villi was gradually decreased towards the caudal part of the ileum as reported in goats (Hassan and Moussa, 2015) and sheep (Kumar *et al.,* 2015). The number of goblet cells was very few or at places absent in proximal half of the villi during present study. Their number was also decreased in goat (Hassan and Moussa, 2015) and sheep (Kumar *et al.,* 2015). In buffalo, their number was few or sometimes absent (Barnwal and Yadava, 1975).

The cytoplasm of columnar cell was slightly eosinophilic and granular however, the eosinophilia accentuated towards lumen. The nuclei were oval or rounded and situated at the base of the cells as reported in sheep (Kumar *et al.,* 2015) and buffalo calves (Barnwal and Yadava, 1975). The villi epithelium showed decrease amount of mucin in ileum as compared to other parts of the small intestine as observed in Gaddi goats (Andleeb *et al.,* 2009) and sheep (Kumar *et al.,* 2015). The luminal border of columnar cells showed mild reaction with PAS in ileum of Gaddi goat (Andleeb *et al.,* 2009), sheep (Kumar *et al.,* 2015) and presence of greater concentration of glycogen in epithelium in small intestine of goat foetii (Ramakrishna and Tiwari, 1979). The villi and the basement membrane of the epithelium showed weak reaction for Alcian blue stain (pH 2.5) as reported in Gaddi goat (Andleeb *et al.,* 2009). The striated border of columnar cells was PAS positive in small intestine of other mammals (Sheahan and Jarvis, 1976) along with positive reaction towards Alcian blue. The goblet cells within villi showed intense PAS positive reaction, and showed positive reaction with Alcian blue (pH 2.5) and also showed presence of more acidic than neutral polysaccharides with PAS-AB stain (Fig. 1b) as reported in sheep (Kumar *et al.,* 2015) and Gaddi goat (Andleeb *et al.,* 2009).

The lamina propria mucosae was having loose irregular connective tissue along with network of reticular, collagen and elastic fibres and a few lymphoid cells throughout the ileum. The lymphoid aggregates were also observed as reported in sheep (Kumar *et al.,* 2015), However, a large number of lymphocytes had been reported in addition to above structures in buffalo (Barnwal and Yadava, 1975). The intestinal glands were tubulo-alveolar lined with simple columnar epithelium and these were surrounded by small aggregates of lymphoid cells as reported in goat (Hassan and Moussa, 2015). However, these were coiled tubulo-alveolar in sheep (Kumar *et al.,* 2015) and pig (Talukdar, 1999). The strong PAS positive pattern for mucopolysaccharides especially in the goblet cells was observed in the glandular epithelium (Fig. 1c). However, the PAS-AB reaction in superficially placed intestinal glands was showing more concentration of acidic

mucopolysaccharides in goblet cells but the concentration of neutral polysaccharides increased towards basal part of the intestinal glands as reported in sheep (Kumar *et al.,* 2015) and Gaddi goat (Andleeb *et al.,* 2009). The Alcain blue (pH 2.5) reaction was strong in goblet cells of intestinal glands indicating presence of hyaluronic acid and sialomucins (Fig. 1d) as reported in sheep (Kumar *et al.,* 2015), whereas crypts of Lieberkuhn in Gaddi goats showed mild reaction with Alcian blue (Andleeb *et al.,* 2009).

The glandular epithelium was also having enterochromaffin cells (Fig. 2a) and Paneth cells located towards the basal portion of the crypts as observed in Gaddi goat (Andleeb *et al.,* 2009), pigs (Cadar, 2010), buffalo (Barnwal and Yadava, 1975), equines (Takehana *et al.,* 1998) and sheep (Ergun *et al.,* 2003).

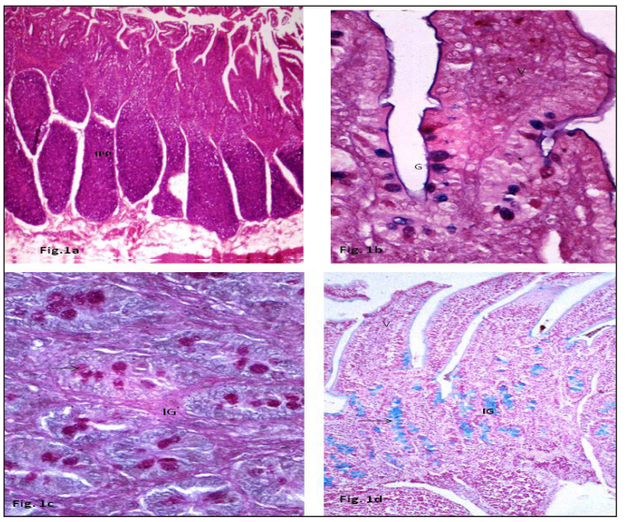

Fig. 1: (1a). Photomicrograph showing villi (V) and ileal Peyer's patches in ileum of goat. H&E 10X. **(1b)**. PAS-AB activity in goblet cells (G) of villi of ileum of goat. PAS-AB x400. **(1c)**. PAS activity in intestinal glands (IG) of ileum of goat. PAS × 400. **(1d)**. Alcianophilic activity in villi (V) and intestinal glands (IG) of ileum of goat. Alcian blue × 100

The lamina muscularis mucosae consisting of smooth muscles was interrupted at places due to invasion of Peyer's patches (Fig. 2b). Whereas, in buffalo, it was thick and made up of layer of smooth muscle fibers arranged in two rows and it was also interrupted at places (Barnwal and Yadava, 1975). However, it formed a thin continuous inner circular and outer longitudinal smooth muscle layer in pig (Sloss, 1954; Talukdar, 1999) and other domestic animals (Titkemeyer and Calhoun, 1955).

The submucosa occupied by lymphoid nodules or Peyer's patches was consisted of follicles of various shapes and size (Fig. 2b). Their shape varied from pear shape, elliptical, oval, round or triangular. Their size varied from large, medium and small in different regions of the ileum. They were localized towards the anti-mesenteric side of the ileum as reported in goats (Kumar *et al.,* 2015; Gautam *et al.,* 2013), pig (Sloss, 1954), buffalo (Barnwal and Yadava, 1975) and buffalo calves (Kapoor and Singh, 2015). Some lymphoid follicles had lightly stained germinal center and darkly stained peripheral zone called corona and follicles were separated by interfollicular regions (Fig. 2b).

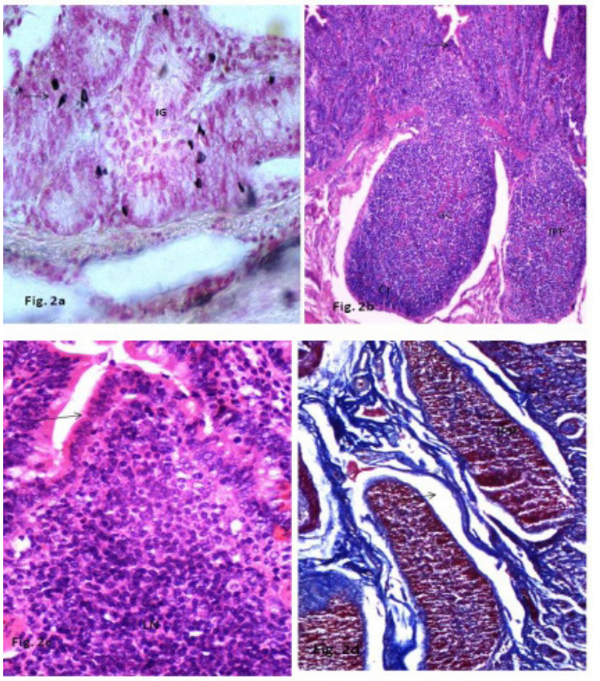

Fig. 2: (2a). Photomicrograph showing enterochromaffin cells (↑) in crypts (IG) of ileum of goat. Fontana x400. **(2b)**. showing FAE (↑), ileal Peyer's patches (IPP) having germinal centre (GC) and corona (C) of ileum of goat. H&E x100. **(2c)**. showing FAE (↑) and sub epithelial lymphoid nodule (LN) in ileum of goat. H&E x400. **(2d)**. showing ileal Peyer's patches (IPP) surrounded by collagen fibres (↑) in ileum of goat. Crossman's Trichrome x100

The germinal centre contained densely packed lymphocytes, lymphoblasts, plasma cells and macrophages

which were supported by reticular fibres. The corona contained compactly arranged lymphocytes which were deeply stained as compared to the germinal centre. Similar findings were reported in goat (Gautam *et. al.,* 2013), buffalo calves (Kapoor and Singh, 2015) and equines (Lowden and Heath, 1995).

The large number of blood capillaries of varying size and dimensions were observed especially towards the center of the follicles. The high endothelial venules were present towards the periphery of the lymphoid follicles. These venules also showed presence of lymphocytes passing in and out of these venules which was in agreement with findings in buffalo calves where abundance of blood vessels was present in the capsule of the follicles (Kapoor and Singh, 2015). Post-capillary venules were described in interfollicular region in goats, (Gautam *et al.,* 2013) whereas, high endothelial venules were localized in the intrafollicular region in one humped camel (Zidan and Pabst, 2008).

Some of the follicles reached up to lamina propria after piercing through the lamina muscularis mucosae and they were lined by a specialized epithelium i.e. Follicle associated epithelium (FAE) (Fig. 2b, 2c). The FAE present between normal absorptive epithelial cells was devoid of goblet cells as reported in sheep (Raju *et al.,* 2012), buffalo calves (Kapoor and Singh, 2015), Caspian pony (Asadi *et al.,* 2008), camel (Zidan and Pabst, 2008) and equines where goblet cells in FAE also reported (Lowden and Heath, 1995). The nodules were encircled by connective tissue capsule consisting of reticular, collagen (Fig. 2d) and elastic fibres as reported in goat (Gautam *et al.,* 2013) and buffalo calves (Kapoor and Singh, 2015).

Tunica muscularis was constituted by an inner circular and an outer longitudinal layer of smooth muscles. In between these layers, there were small blood vessels, nerve bundles and fatty tissue and at places myenteric plexus was also observed in sheep (Kumar *et al.,* 2015) whereas, in buffalo calves it was consisted of two well defined layers of smooth muscles fibres (Barnwal and Yadava, 1975).

Tunica serosa formed by loose irregular connective tissue had isolated collagen, elastic and reticular fibers along with varying amount of fatty tissue and few blood capillaries. A flat mesothelial cell layer was observed as reported in sheep (Kumar *et al.,* 2015).

Scanning electron microscopy

The ileal part of the intestine showed densely packed villi, which were gradually decreased in height as moved towards caudal segment of it. They were short but broader and mostly tongue shaped (Fig. 3a, 3b) as reported in goat (Hassan and Moussa, 2015). These were finger shaped in equines (Kotze and Soley, 1990), human (Marsh and Swift, 1969), jungle fowl (Kadhim *et al.,* 2010). These were flat long finger shaped in young pigs (Skrzypek *et al.,* 2005) and broadened and some fused villi of ileum observed in gnotobiotic dogs (Johnson *et al.,* 1986). Whereas in claves, these were observed as short and leaf shaped (Dubourguier *et al.,* 1978). The corrugations were very deep dividing the villi surface in to separate islands of tissue and were very prominent and pronounced (Fig. 3c, 3d).

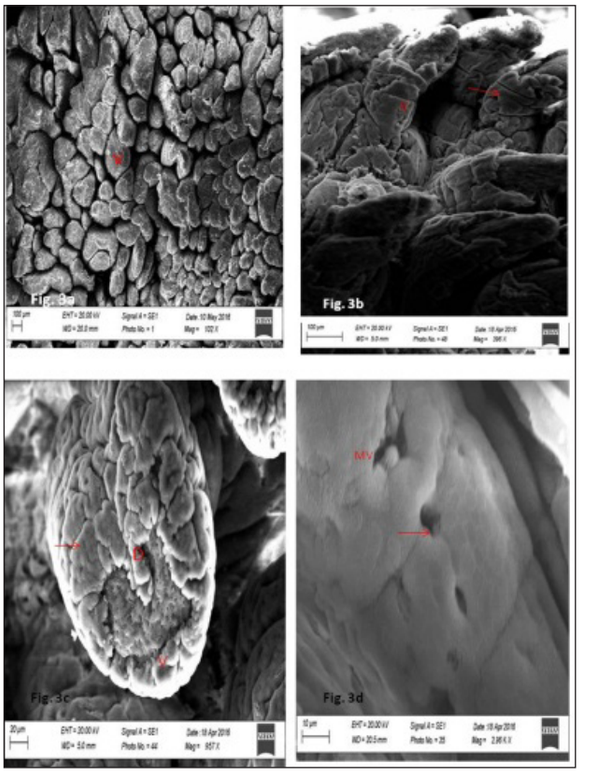

Fig. 3: (3a). Scanning electron micrograph showing ileal villi (V) of goat. x102 **(3b)**. Showing ileal villi (V) and corrugations (↑) on villi surface goat. x396 **(3c)**. Showing ileal villi (V) and corrugations (↑) and dome shaped islands (D) on villi surface of goat. x957 **(3d)**. Showing micro villi (MV) and goblet cell openings (↑) on villi surface of goat. x2.96K

The corrugations end abruptly and did not form a continuous system of clefts as in goat (Hassan and Moussa, 2015), equine (Kotze and Soley, 1990), gnotobiotic dogs (Johnson *et al.,* 1986), young pigs (Skrzypek *et al.,* 2005), humans (Marsh and swift, 1969). The surface of the villi at higher magnification showed many islands of dome shaped areas which were separated from each other by deep clefts which were representing the corrugations (Fig. 3c). Similar findings were reported in young pigs where they appeared as hexagonal cells in the form of enterocytes (Skrzypek *et al.,* 2005). The surface also had covering of dense mat of microvilli. The openings for the goblet cells (Fig. 3d) found to be less whereas these were numerous in young pigs (Skrzypek *et al.,* 2005). At places where villi were displaced, the crypts opening at the basal part of villi were observed in humans (Toner *et al.,* 1970).

Transmission electron microscopy

The surface epithelium of ileum was having simple columnar cells with goblet cells. The luminal surface was covered with microvilli (Fig. 4a, 4b) as reported in lambs (Gray *et al.,* 1980), gnotobiotic dogs (Johnson *et al.,* 1986) and human (Kelley, 1973). The regular microvilli contained core made up of fine filaments forming thick bundles which extended deeply in to the terminal web of the apical cytoplasm as observed in lambs (Gray *et al.,* 1980) and mice (Mukherjee and Williams, 1967). The columnar cells were joined at the apical surface by typical junctional complexes as reported in human (Kelley, 1973). The mitochondria of various shapes were abundant in number in the apical part of cytoplasm (Fig. 4b). The mitochondria were mainly supranuclear in location along with few were located below the nucleus also as reported in gnotobiotic dogs (Johnson *et al.,* 1986). The large euchromatic oval nuclei with prominent nucleoli were situated either centrally or towards the base of the cells, whereas in lambs nucleus was irregular in outline and basal in position (Gray *et al.,* 1980).

The crypt region or glandular epithelial region was consisted of columnar cells, along with Paneth, enterochromaffin, goblet, few plasma cells and lymphocytes. The microvilli appeared less numerous and short than those in superficial columnar cells as reported in gnotobiotic dogs (Johnson *et al.,* 1986). The Paneth cells were accumulated at the base of the crypts and their number decreased as reported in sheep (Ergun *et al.,* 2003). They were pyramidal shaped cells with their broad base resting on the basement membrane and narrowed towards the apical end. The irregular shaped nucleus with its prominent nucleolus occupied a third of the basal cytoplasm. The numerous osmeophillic granules which were located towards the apical portion the cytoplasm (Fig. 4c) as reported in sheep (Ergun *et al.,* 2003).

The enterochromaffin cells were detected in crypts but these were decreased in number during present study. These cells showed infranuclear electron dense osmiophillic granules in the cytoplasm (Fig. 4d) as observed in calves (Pearson and Logan, 1983) and other mammalian species (Dawson, 1970; Carvalheiar *et al.,* 1968) and equine small intestine (Sato *et al*.1976; Takehana *et. al.,* 1998).

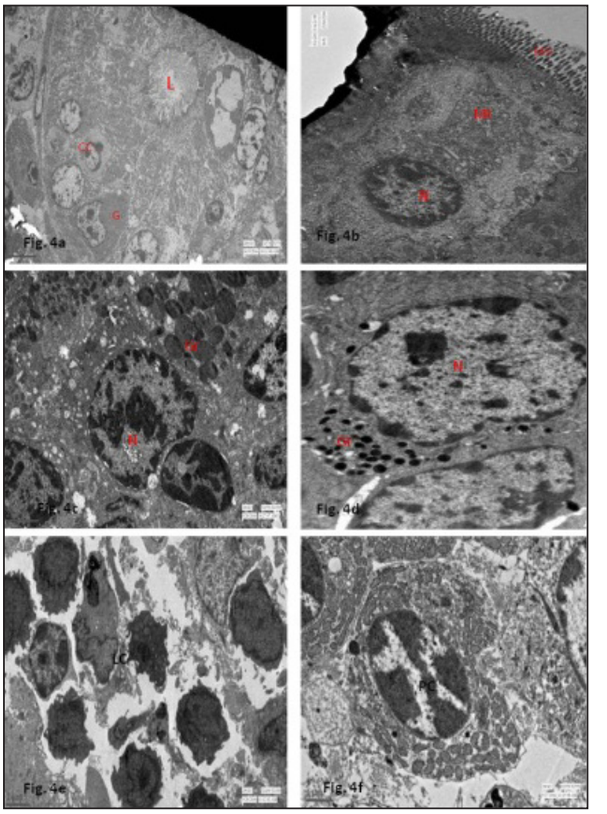

Fig. 4: (4a). Transmission electron micrograph showing lumen (L), columnar cells (CC) and goblet cell (G) in surface epithelium of ileum of goat x570. **(4b)**. Showing (N), microvilli (MV) and mitochondria in columnar cells of surface epithelium in ileum of goat x3100. **(4c)**. Showing nucleus (N) and osmeophilic granules (Gr) in cytoplasm of Paneth cell in cryptal epithelium of ileum

of goat x1550. **(4d)**. Transmission electron micrograph showing nucleus (N) and osmeophilic granules (Gr) in cytoplasm of enterochromaffin cell in cryptal epithelium of ileum of goat x1550. **(4e)**. Showing aggregations of lymphocytes (LC) in cryptal epithelium of ileum of goat x1100. **(4f)**. Showing plasma cells (PC) in cryptal epithelium of ileum of goat x2550.

A few goblets cells present in between columnar cells were distended with the mucous. The nuclei of mucus cells were located towards the basal surface. The cytoplasm between the nucleus and apical border was distended with mucus granules and these granules were enveloped by very fine membrane. The individual mucous granule with in cell varied in their electron opacity as reported in humans (Kelley, 1973) and mice (Mukherjee and Williams, 1967). There was presence of abundant of lymphocytes along with plasma cells in the propria of the ileum (Fig. 4e). The lymphocytes were having irregular large sized nucleus occupied most of the cytoplasm and cytoplasm was very less. The plasma cell was also observed showing round nucleus and chromatin material present in cart wheel like arrangement (Fig. 4f).

CONCLUSION

It may be concluded that the villi of the ileum varied in shape and size and lined by simple columnar epithelium with goblet cells. The intestinal glands were simple tubulo-acinar type. The submucosa was occupied by lymphoid nodules or Peyer's patches consisted of follicles of various shapes and size. The acidic and neutral mucopolysaccharides were present in the intestinal glands. The scanning electron microscopy showed tongue shaped villi with distinctive corrugation on surface. The transmission electron microscopy showed presence of simple columnar cells with dense mat of microvilli in the surface epithelium. The cryptal epithelium was having Paneth, enterochromaffin and goblet cells.

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