



Morphological Studies on the Postnatal Development of the Gut-associated Lymphoid Tissues of the Rabbit Cecum

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Abstract

The macroscopic, morphometric, light and scanning electron microscopic structure of gut-associated lymphoid tissue (GALT) of cecum were studied in the rabbits aged from birth to 16 weeks. The GALT were formed of lymph follicles covered by low columnar epithelium containing intraepithelial lymphocytes and leukocytes. They were concentrated at the ileocecal entrance (ileocecal patch) and in the blind end of the cecum vermiform appendix. In the ileocecal patch, GALT were in direct contact with the lumen, while those of the appendix were covered by the interval intestinal villi in young rabbits and mucosal folds in the adult rabbits. The lymphoid follicles of the ileocecal patch were composed of dome region and germinal center and were separated by narrow inter-follicular areas. Whereas, the lymphoid follicles of the appendix were composed dome region and germinal center in the newly born rabbits and up to the 2nd week of age, the follicles became composed of four different sites: dome region, germinal center, coronal area, and a wide interfollicular area between neighboring follicles. Morphometrically; the dimensions of the lymphoid follicles of the cecal GALT increased in size with the advancement of the age. By SEM the lymphoid structures covered with special epithelium consisted of two types of cell absorptive enterocytes and M cells. The M cells in the cecal patch were microvilliated and present on the tips and sides of the dome lymphoid regions while in the appendix were non-microvilliated and present only on the sides of the dome regions.

Keywords: Cecum; Rabbit; GALT; M cells; appendix; ileocecal patch

Introduction

Rabbits are true non-ruminant herbivores and considered as hind gut fermenters. They have a large cecum that can hold up to 40% of the intestinal contents and enables them to eat a primarily fibrous diet (Delaney-Johnson, 2006). The cecum and the vermiform appendix are the places where early lymphoid defense was provided from 30th days of gestation (Dorier *et al.*, 1989). The rabbit contains better developed GALT in comparison with other mammalian species (Cesta, 2006). The GALT in the rabbit are commonly considered to constitute Peyer's patches, sacculus rotundus, appendix, and cecal patches (Haley, 2003); therefore, most of the studies regarding the lymphoid structure in the rabbit focus on these structures (Gebert and Bartels 1991; Gebert *et al.*, 1992; Jepson *et al.*, 1992, 1993; Regoli *et al.*, 1995). While, in other animals, as in ruminants, pigs, and dogs, the GALT are present in the Peyer's patches region (Haley 2003; Yasuda *et al.*, 2006). There were many studies on the rabbit

cecum and its lymphoid structure but the studies on the postnatal development were few so this study aimed to clarify the morphological and morphometrical changes in the cecal GALT during the postnatal stages.

Materials and methods

Thirty five apparent healthy rabbits of both sexes ranging from 1 day old to 16 weeks old were used in this study. For the light microscope, specimens from the ileocecal patch and appendix were collected and fixed in Bouin's fixative and processed for paraffin embedding. Paraffin sections of 5 μ m thicknesses were stained with H&E, alcian blue and PAS stains used for histological structures and micromorphometrical measurements. For scanning electron microscopy, representative tissue samples from different parts of the cecum and appendix were taken, fixed in glutaraldehyde 5% solution in 0.1M phosphate buffer for 18 hours at 4°C. After washing in the same buffer, the specimens were post-fixed in osmium tetroxide 1% in phosphate buffer for 2 hours followed by washing in the same

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buffer. The samples were then dehydrated in ascending grades of ethanol followed by critical point drying in carbon dioxide then sputter-coated with gold and examined and photographed with a JEOL-5400LV scanning electron microscope.

Results

The rabbit cecum consisted of wide proximal base, narrower body and appendix twisted around itself forming three cecal turns. In all studied ages, the GALT were demonstrated at the ileocecal entrance "ileocecal patch" and in the blind end of the cecum "vermiform appendix" (Fig. 1). The ileocecal patch was irregularly oval-shaped and continued with the sacculus rotundus at the end of the ilium.

It measured about 0.3, 0.5, 1.2, 1.5, 2.5 cm in diameter in the 1, 2, 4, 8 and 16 weeks old rabbits respectively. While, the cecal appendix was large and formed terminal blind end of the rabbit cecum. It was bright in color and characterized by thick wall and. It measured about 2.1, 3, 3.6, 4.2, 5.2 and 8.5 cm in 1, 2, 4, 8 and 16 weeks old rabbits respectively. The lymphoid follicles extended around the whole intestinal circumference of appendix (Table 1). In the rest parts of the cecum, the lymphoid follicles were not observed grossly.

Histologically, the gut associated lymphoid tissues were composed of many repeated lymphoid follicles, occupied the whole lamina propria and extended to the submucosa and separated from each other by narrow interfollicular spaces.

In the ileocecal patch, the GALT (Figs. 2a, b & c) consisted of dome-shaped lymphoid follicles separated from each other by low mucosal folds. The follicles were separated from the mucosal folds

by clefts. The tips of these follicles became wide and flat with advancement of the age. In the newly born rabbit, they were consisted of small dome region and the ill-developed germinal center and separated from the adjacent by a wide interfollicular areas. With advanced age, the follicles increased in size and became composed of well defined dome region and germinal center up 2nd week of the age, while the interfollicular areas decreased. The dome regions were in direct contact with the lumen. They were covered with special epithelium which characterized by pale cells without well-defined brushed border, numerous intraepithelial lymph cells, a few enterocytes and the lack of goblet cells (Figs. 2d & e).



Fig. 1. The rabbit cecum consisted of base (B), body (Bo) and appendix (ap). The cecal patch (cp) situated at the ileocecal junction continued with the sacculus rotundus (sr): (As) ascending colon, (Inf) intestinal folds.

In the appendix (Figs. 3a, b & c), the GALT were in the form of small lymph follicles in the newly born rabbits and increased rapid in size with age to become pyramidal lymph follicles at 15 days old. And up to 30 days old, these follicles enlarged and became barrel-shaped with round mucosal tip

Table 1. Showing the measurements of the cecal patch and appendix

	One day	Two weeks	Four weeks	Eight weeks	Adult
Cecal patch					
Diameter (cm)	0.3 ± 0.08	0.5 ± 0.09	1.2 ± 0.1	1.5 ± 0.15	2.5 ± 0.3
Wall thickness (µm)	333.40 ± 16	364.50 ± 18.4	549.20 ± 36.2	609.6 ± 42.5	712.50 ± 25.9
Height of the lymph follicle (µm)	173.5 ± 30.3	231.30 ± 16.4	342.40 ± 29.7	423.1 ± 32.9	512.60 ± 36.2
Width of the lymph follicle (µm)	50 ± 36.6	146.20 ± 90.6	185.50 ± 13.1	285.1 ± 12.2	246.20 ± 12.6
Vermiform appendix					
Length (cm)	2.10 ± 0.43	3.00 ± 1.30	3.60 ± 0.14	5.20 ± 0.20	9.48 ± 0.95
Diameter (cm)	0.13 ± 0.06	0.19 ± 0.08	0.25 ± 0.03	0.45 ± 0.12	0.61 ± 0.14
Wall thickness (µm)	469.50 ± 36.5	546.70 ± 23.2	985.62 ± 56.9	1382.60 ± 85.1	2215.1 ± 73.5
Height of the lymph follicle (µm)	350.40 ± 36.3	403.50 ± 12.3	846.60 ± 35.6	1645.30 ± 65.5	1957.50 ± 96.3
Width of the lymph follicle (µm)	135.40 ± 8.1.8	178.30 ± 16.8	236.30 ± 25.5	242.30 ± 24.3	365.10 ± 36.8

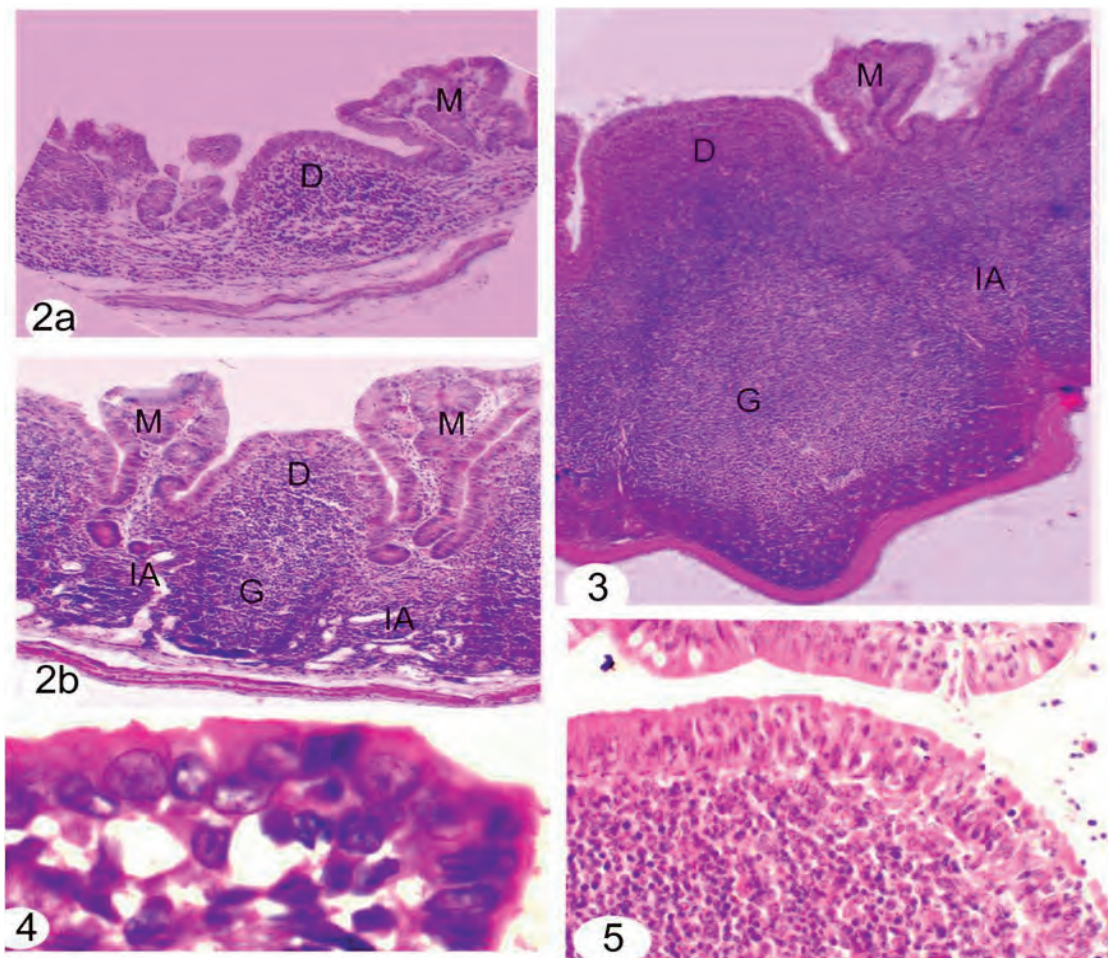


Fig. 2 (a - e). Photomicrographs of rabbit cecal patch showing the shape and structures of the lymphoid follicles at one day (2a), two weeks (2b) and 16 weeks old (2c). Dome region (D), germinal center (G), interfollicular areas (IA) and mucosal ridges (M). H&E, x40. 3c): H&E, (x12.5). 2d): The lymphoid follicles of the ileocecal patch covered with cuboidal epithelium with few intraepithelial lymphocytes in the newly born rabbits H&E, (x 400). 2e) Photomicrograph of the 16 weeks old rabbit ileocecal patch showing covering epithelium of the lymphoid follicles was columnar epithelium with many intraepithelial lymphocytes. H&E, x 400.

and fused with each other under the epithelial invagination. In the newly born rabbit, each lymphoid follicle consisted of dome region and germinal center, while up to the 2nd week of age the follicles presented four different regions: dome region, germinal center, coronal area and a wide interfollicular area between neighboring follicles. The covering mucosa of the appendix formed tongue shaped mucosal folds in the newly born rabbit. With the advancement of the age, these folds increased in size became elongated projections with wide luminal tips and appeared as umbrella like structures over the tips of the dome lymphoid follicles. Their covering epithelium (Figs. 3d & e) was cuboidal or short columnar contained many intraepithelial lymphocytes and lacked the goblet cells. The tips of the lymphoid follicles were covered with columnar epithelium while the sides covered with cuboidal epithelium with interepithelial lymphocytes.

Morphometrically (Table 1), the dimensions of the lymphoid follicles of the cecal GALT increased in size with the advancement of the age. But the increase differed from the cecal patch to the appendix and also in the dimension of the same structure. The height of the lymphoid follicles of the cecal patch in the adult rabbit was three folds that in the newly born rabbit, while their width at the former rabbits was 5 folds at the later ones. The height of the lymphoid follicles of the appendix in the adult rabbits was 6 folds that in newly born ones, while their width in the former was 3 folds that in the newly born rabbits.

By Scanning electron microscope, the mucosal surface of the ileocecal patch presented dome shaped lymphoid follicles surrounded by mucosal ridges separated by deep grooves. In the newly born rabbits (Fig. 4a), the lymphoid structures were small irregular oval or rounded in shape and not separated completely from the surrounding ridges.

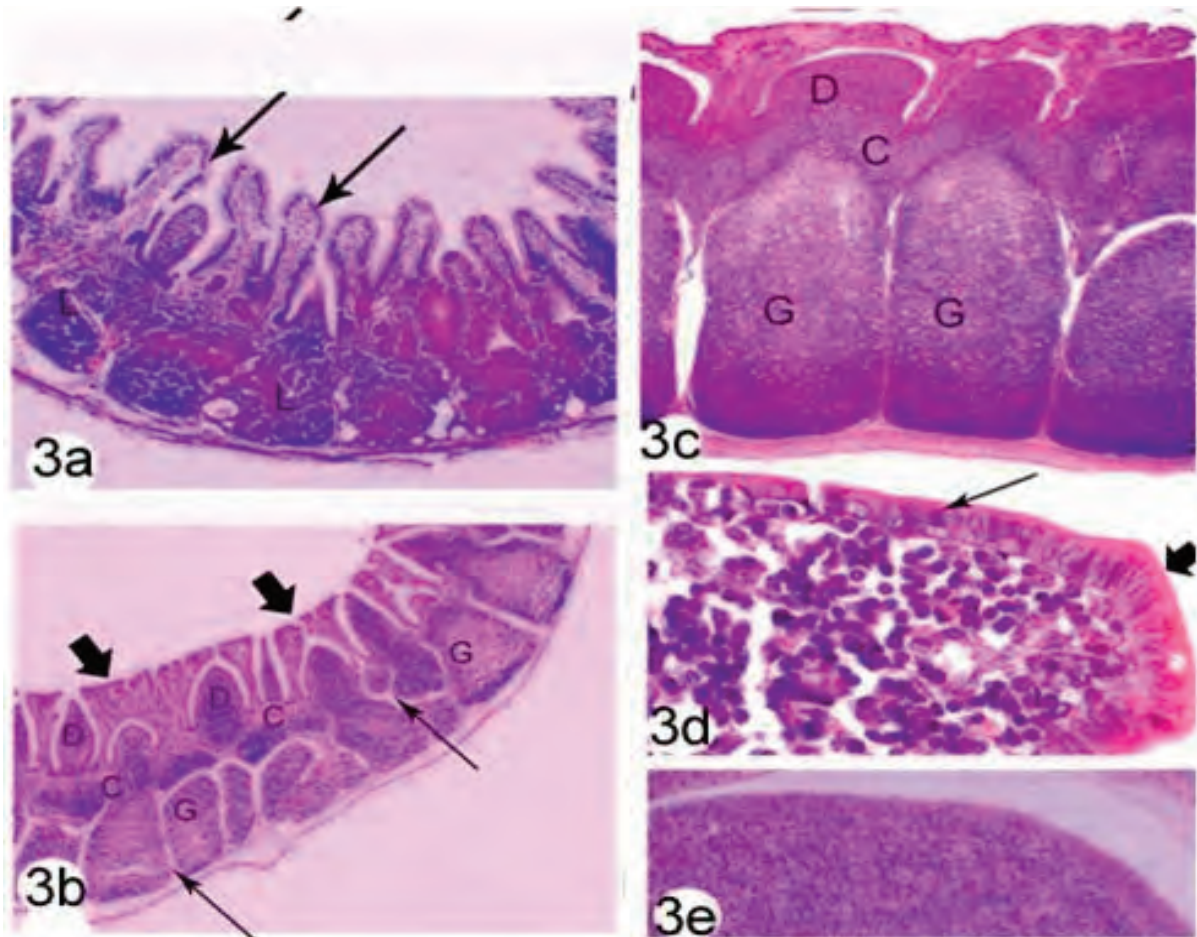


Fig 3 (a - e). 3a): Photomicrograph showing that the mucosa of the appendix presented short tongue shaped villi in the newly-born rabbits (Arrows). The lymphoid follicles (L) consisted of dome region and germinal center and occupies the greater part of the wall thickness. H&E, (x100). 3b) in the two old rabbits, the mucosal villi of appendix appeared as umbrella like structure (Arrowheads) over the dome shaped lymphoid follicles. The lymphoid follicles were large and consisted of well developed dome region (D), coronal (C), germinal center (G) and separated by well defined interfollicular areas (IA). H&E, (x40). 3c): The lymph follicles of the rabbit appendix were very large barrel-shaped with round dome regions (D), fused each other at the coronal areas (C) under the epithelial invaginations and separated by well defined interfollicular areas. (G) germinal center. H&E, (x 12.5). 3d) Photomicrograph of two weeks old rabbit appendix showing that the surface epithelium covering the lymphoid follicles was cuboidal epithelium with interepithelial lymphocytes at the side of dome region (Arrow) and tall columnar epithelium at its tip (arrowhead). H&E, (x 400). 3e) In adult rabbit, the covering epithelium of the lymphoid follicles was tall columnar epithelium with many interepithelial lymphocyte on the sides and tips of the dome region. H&E, x 200.

With the advancement of age, they increased gradually in size and became irregular rounded or dome shaped at two weeks old (Fig. 4b) and appeared as button like structures which completely separated from the surrounding ridges up to 4 weeks old (Fig. 4c). In all studied ages, the covering epithelium of the lymphoid structures consisted of cells with variable sizes, shapes, and surface structure (Fig. 4d & e). It consisted of large polygonal cells with dense, uniformly long microvilli (enteroabsorptive cells), smaller cells with less dense, thicker microvilli of irregular length (M cells). Goblet cells could not be observed on the surface of the lymphoid follicles. The epithelium of mucosal ridges and the intestinal crypts were consisted of large polygonal enteroabsorptive cells with uniformly long microvilli and

goblet cells in addition to many round openings of the intestinal crypts. From the cut wall, the dome areas and lymphoid follicles showed a similar appearance, with tightly packed lymphoid-like cells generally with a smooth surface, but we were unable to find differences between germinal centers and mantle areas of the follicles.

At the appendix, the surface mucosa appeared in form of irregular mucosal folds separated by deep grooves contained many dome-shaped lymphoid structures (Figs. 5a, b, c & d). The surface epithelium of the mucosa folds consisted of microvilliated cells and goblet cells and showed many round openings of the intestinal glands in all studied ages (Fig. 5h). While that of the lymphoid structures consisted of irregular polygonal mi-

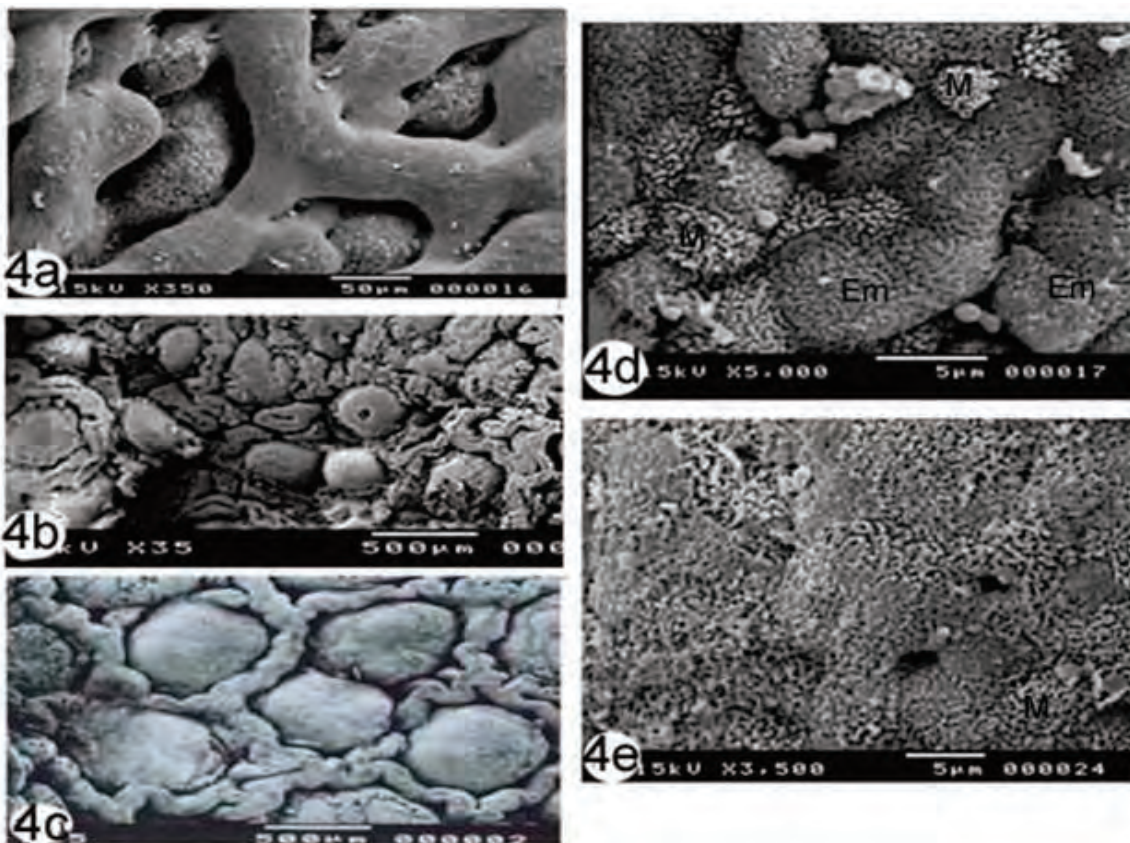


Fig. 4 (a - e). 4a): SEM of cecal patch in one day old rabbits, showing the lymphoid follicles were small irregular oval or rounded in shape and not separated completely from the surrounding ridges. 4b & c) SEM of the cecal patch in rabbits, the lymphoid follicles were irregular rounded or dome shaped at two weeks old and appeared button like structures and completely separated from the surrounding ridges by deep clefts at 16 week old rabbits. 4d & e): SEM showing the surface epithelium of the cecal patch of newly born rabbit (4d) and adult rabbit (4e) respectively. It consisted of enteroabsorptive cells (Em) and M cells (M).

crovillated cells at their tips and consisted of two types of cells at their sides: small polygonal cells with dense, uniformly short microvilli and large irregular with micro-folded surface and lack the microvilli (Figs. 5e, f & g). The microvillated cells were similar to that of deep structures of the intestinal crypts.

Discussion

Gut associated lymphoid tissues were presented throughout the gastrointestinal tract in mammals and consisted of aggregated and solitary lymph nodes (Macdonald 2003; Newberry 2008). The rabbits presented better developed GALT in comparison with other mammalian species (Cesta, 2006). They were commonly considered to constitute Peyer's patches, sacculus rotundus, appendix, and ileocecal patches (Haley, 2003). Whereas, cecum of the ruminants, pigs, and dogs, the GALT presented only in the ileocecal patches (Haley, 2003; Yasuda *et al.*, 2006). In the human, the GALT were found in the ileocecal patches and appendix

(Gebert and Bartels, 1991; Gebert *et al.*, 1992; Jepson *et al.*, 1992, 1993; Regoli *et al.*, 1995). In the present study, GALT were macroscopically observed through both mucosal and serosal surfaces of the cecum at the ileocecal patch and at the blind end vermiform appendix. The appearance and organization of these GALT were consistent with previous reports regarding classical Peyer's patches (Haley, 2003; Cesta, 2006).

The ileocecal lymphoid tissue were composed of numerous aggregated lymph follicles, each of them have dome region, germinal center and separated by narrow inter-follicular areas in different studied ages. While, in the appendix, the lymphoid structures were composed of one lymphoid follicle with germinal center in the newly born rabbit, but up to the 2nd week of age the follicles present four different sites: a dome region, a germinal center, a coronal area, and a wide interfollicular area between neighboring follicles.

The lymph follicles were covered by low columnar epithelium containing intraepithelial lymphocytes and leukocytes, the same results have

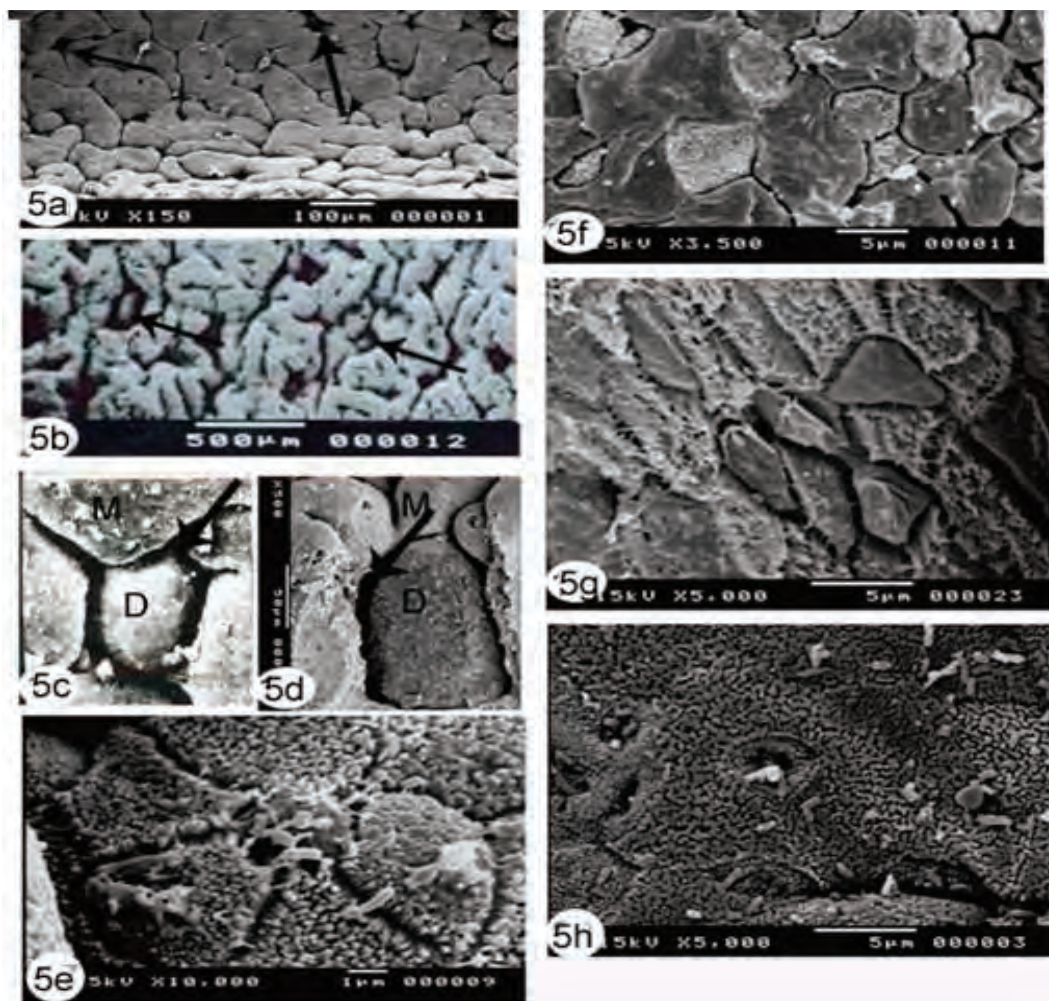


Fig. 5 (a - h). 5a & b): SEM of the appendix one week old day old and 16 weeks old rabbits respectively showing that the mucosa appeared in form of irregular mucosal folds separated by deep grooves contained many dome-shape lymphoid structures (Arrows). 5c & d): SEM of appendix showing the tip and sides of the dome-shaped lymphoid tissue. between irregular ridges. dome-shaped (D) structure, mucosal ridges (M), deep grooves (Arrow). 5e): SEM showing that the surface epithelium of the lymphoid structure consisted of polygonal microvillated cells at their tips. 5f): The side of the dome-shaped lymphoid covered with two types of cells: small polygonal cells with dense, uniformly short microvilli and large irregular with micro-folded surface and lack the microvilli. 5g): SEM of 16 weeks old rabbit appendix showing that the side of the dome-shaped lymphoid covered with two types of cells: polygonal cells with dense, uniformly long microvilli and large irregular with micro-folded surface and lack the microvilli. 5h): SEM of 16 weeks old rabbit appendix showing the epithelium of mucosal ridges consisted of large polygonal enteroabsorptive cells with uniformly long microvilli and goblet cells.

been reported by Snipes (1978) and Macdonald (2003) and Newberry (2008). The ileocecal lymphoid follicles were in direct contact with the lumen, while those of the appendix were covered by the interval mucosal folds. The dome epithelium provided both a protective barrier over lymphoid follicles and a route for antigen uptake from the gut, develops in rabbit caecum during the first week of neonatal life (Roy and Varvayanis, 1987). By SEM, the epithelium covered the lymphoid follicles was cuboidal or short columnar with two types of cells; enterocytes with homogeneous and tightly packed microvilli and another cell type, of reduced number with thicker and longer microvilli, the so-called M cells. These result are similar to that de-

scribed in Pyre's patches of the pig (Torres-Medina, 1981 and Jose *et al.*, 1990). So we can concluded that the ileocecal lymphoid patches are important in the immunity of the region. Where they were well situated functionally in that they are exposed both to unfermented ingesta from the ileum, and to the fermenting contents of the caecum and proximal colon. They seem to imply some effect on luminal flow which may create changes in the microenvironment. Although the functional significance of the recesses is not known, their structure would seem

Morphometrically; the lymphoid follicles of the cecal GALT increased in size with the advancement of the age. The height of the lymphoid folli-

cles of the cecal patch in the adult rabbit reach three folds that in the newly born rabbit, while their width at the former rabbits was 5 folds at the later ones. On other hand, the height of the lymphoid follicles of the appendix in the adult rabbits was 6 folds that in newly born rabbits, while their width in the former was 3 folds that in the newly born rabbits.

In the appendix, the mucosa presented two types of epithelium: a short columnar with goblet cells form tongue shaped mucosal ridges in the newly born rabbits. They increased in size and differ in shape with advancement of the age and became elongated projections (umbrella like villi) up to the 4th week of ages. Another short cuboid, almost smooth, at the level of the short conic projections or dome. The villus epithelium may have digestive functions of absorption and secretion, while the second, with smooth cells, similar to "M" cells, described for Peyer's patches, could be antigen receiver and presenter cells (Shreedhar *et al.*, 1996; Montcourter *et al.*, 2001). The specialized follicular associated epithelium containing M cells might represent induction sites for the mucosal immune system in (Pabst *et al.*, 2005). Similar findings has been established in rabbit (Keren *et al.*, 1978; Dasso *et al.*, 2000; Iwatsuki *et al.*, 2002) mice (Hamada *et al.*, 2002), guinea pig (Rosner and Keren 1984), human (Moghaddami *et al.*, 1998), and. Also the dome epithelium provides both a protective barrier over lymphoid follicles and a route for antigen uptake from the gut, develops in rabbit cecum during the first week of neonatal life (Roy and Varvayanis, 1987).

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