Journal of Animal Research: v.13 n.04, p. 535-541. August 2023

DOI: 10.30954/2277-940X.04.2023.7

Histomorphological, Micrometrical Studies on the Ileum of the Guinea Pig (*Cavia porcellus*)

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Received: 11 July, 2023 **Revised:** 28 July, 2023 **Accepted:** 30 July, 2023

ABSTRACT

The present histological study was conducted on ileum of different postnatal age groups of guinea pig. Histologically the wall of the ileum composed of the four layers viz., tunica mucosa, tunica sub mucosa, tunica muscularis and tunica serosa. The shape of the villi in the epithelium was slender and finger like in the 0-2 week-old guinea pig where as tongue shaped in remaining age groups. Lamina propria composed of the loose connective tissue with Crypts of the Lieberkuhn. Tunica submucosa was made up of more number of collagen and reticular fibres and few elastic fibres in the wall of the blood vessels. Tunica muscularis was lined by the inner circular and outer longitudinal muscular layer. The mean height and width of the villi and height of the columnar cells and thickness of the tunica muscularis was increased as the age advanced in all the age groups studied.

HIGHLIGHTS

- Age related changes in the ileum of guinea pig.
- Micrometrical studies on the ileum in different age groups

Keywords: Guinea pig, histology, ileum, postnatal age, villi

Guinea pigs were commonly used as experimental model in biomedical research. Ileum was the part of the intestine next to the jejunum connected by the mesentery (Raja *et al.,* 2022). There is paucity of the literature on the histomorphological, micrometrical studies on the ileum of the guinea pig in the postnatal age groups. Hence, present study was conducted with aim to explore the histological features of the ileum of the guinea pigs in the postnatal age groups.

MATERIALS AND METHODS

Histological studies on the ileum of the guinea pig (*Cavia porcellus*) in postnatal age groups viz., Preweaning (0-2 weeks), weaning (2-8 weeks), young (8-16 weeks) and adult (16-32 weeks) were conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai. Guinea pigs were procured from the Department of laboratory animal medicine, Madhavaram milk colony, Chennai. Approval from the institutional animal ethics committee (IAEC) TANUVAS, approval No. 1487/DFBC/IAEC/2018 dated 13.07.2018 was taken. They were euthanized by the standard operating procedure by using carbon dioxide asphyxiation as per CPCSEA norms.

From the guinea pigs of postnatal age groups, samples from the mid part of the ileum were collected. They were washed in the normal saline and fixed in 10 % neutral buffered formalin and tissues were dehydrated in the

How to cite this article: Raja, K., Rajathi, S., Ushakumary, S., Kannan, T.A., Ramesh, G., Kumar, K. and Hemavathi, N. (2023). Histomorphological, Micrometrical Studies on the Ileum of the Guinea Pig (*Cavia porcellus*). *J. Anim. Res.,* **13**(04): 535-541.

Source of Support: None; **Conflict of Interest:** None

ascending grades of the alcohol, cleared in xylene and embedded in paraffin (58-60ºC). Sections of 4-5 µm thickness were cut and used for the routine and special histological staining techniques. The various histological techniques viz., Haemotoxylin and Eosin (H & E) method for the histological study (Bancroft and Gamble, 2003), Van Gieson's for collagen fibres, Masson's Trichrome for connective tissue fibres Gomori's silver method for reticulum (Luna, 1968), Weigert's method for elastic fibres (Humason, 1979) and Fontana Masson's Silver method for enterochromaffin cells were done. Microscopic images of stained tissue sections of the ileum of postnatal age groups were captured. The various parameters viz., height and width of the ileum, height of the columnar cells lining the villi and thickness of the tunica muscularis of the ileum were measured group wise and they were subjected for one way ANOVA with arithmetic mean and standard error (Mean ± SE) calculated as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Histologically the wall of the ileum of the guinea pig was made up of the four layers namely Viz., tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa from inner to outer side. In 0-2 week-old guinea pigs, ileum showed finger, tongue and spatula shaped villi (Fig 1). In other postnatal age groups studied, the ileum of the guinea pigs possesed spatula shaped villi which were almost uniform in height and closely arranged (Fig. 2). Height of the villi in ileum was shortest among the three segments of the small intestine. The surface epithelium was thrown into villi in the small intestine. In all postnatal age groups studied, the ileum of the guinea pigs possesed spatula shaped villi which were almost uniform in height and closely arranged which is in contrary to statement Bansal *et al.* (2000), since the finger like shape of villi of ileum was recorded in buffalo. However, Andleeb *et al.* (2016) observed that in ileum of Gaddi goat, the villi were few and broad at the base and narrow pointed towards the tip. Gahlot and Kumar (2018) reported that shape of the ileum varied in shape and size in goat. Mean height and width of villi of the ileum were found to be increased from 0-2 week-old to 16-32 week-old guinea pigs (Tables 1).

Villi of the ileum in guinea pigs were lined by simple columnar epithelium. Along with the tall columnar cells, goblet cells were also found in the surface epithelium (Fig. 3) as observed by Bacha and Bacha (2000) in mammals, Andleeb *et al*. (2016) in gaddi goat and Gahlot and Kumar (2018) in goat. In the present study, the number of goblet cells was found to be increased as the age advanced.In addition to these types of cells,a few globular leukocytes, intra epithelial lymphocytes and enterochromaffin cells were also observed in the surface epithelium which is agreeable with the findings of Vithya (2005) in sheep and Andleeb *et al*. (2016) in gaddi goat. Surface epithelium of the ileum invaginated into underlying lamina propria to form intestinal glands or crypts of Lieberkuhn in all the postnatal age groups of the guinea pigs (Fig. 4).

The columnar cells were the principle cells of the surface epithelium of villi of the small intestine. They were columnar in shape with narrow base and broad apex. The cell boundaries were distinct. The apical border of the columnar cells had a well marked striated border (Fig. 4) and was acidophilic when stained with H and E as stated by Ozdemir *et al.* (2004) in Badgers. The basophilic nuclei of these were oval to round in shape and mostly located at the basal half of the cell. The mean height of the columnar cells of the ileum was found to be increased as the age advanced in all the postnatal age groups studied.

Goblet cells in the surface epithelium ileum possesed round or globular shape. Nucleus was basophilic and flattened in shape (Fig. 3 & 4). It occupied the base of the cell. Vacuolated cytoplasm was distinct with H and E. The goblet cells were interspersed among the columnar cells lining the villi and the crypts in ileum. Occurrence of the goblet cells was found to be less in the surfaceepithelium and crypts of Lieberkuhn in 0-2 week old guinea pigs and increased in number as age advanced.

In the surface epithelium of ileum, very few globular leukocytes were observed. Globular leukocytes were observed as large round cells with acidophilic globular cytoplasm which in agreement with findings of Ergun *et al.* (2003) in ileum of Angora rabbit. A basophilic round nucleus was centrally placed and nucleolus was indistinct in the globular leukocytes.

Intra epithelial lymphocytes were present either in the apical or basal parts of the columnar cells of the villi, folds and crypts of Lieberkuhn as intra epithelial lymphocytes. These small lymphocytes were also present between the columnar cells of villi, folds and crypts of both small

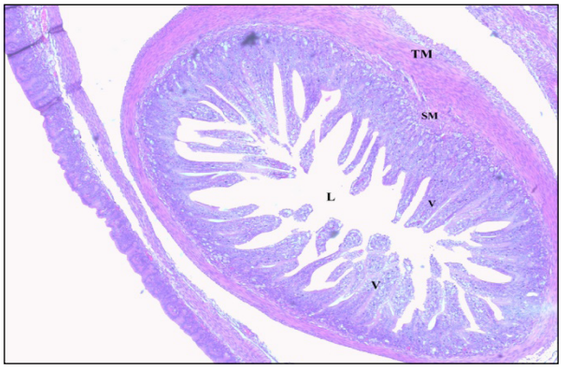

Fig. 1: Photomicrograph of ileum of one week old guinea pig showing the finger/slender shaped villi. V-Villi, L-Lumen, SM-Tunica submucosa, TM-Tunica muscularis. H&E × 40

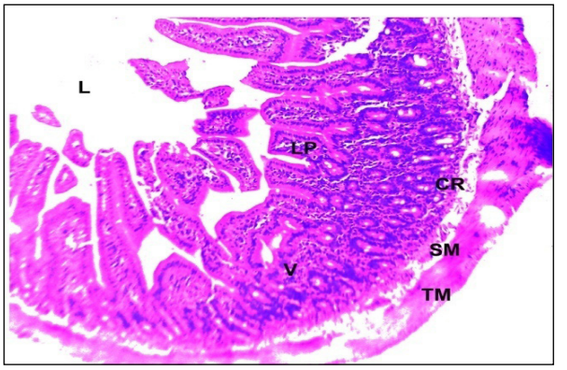

Fig. 2: Photomicrograph of ileum of fourteen week old guinea pig showing the villi with various shapes (tongue and finger like). V-Villi, L-Lumen, LP-Lamina propria, CR- Crypts of Lieberkuhn, SM-Tunica submucosa, TM-Tunica muscularis. H&E × 100

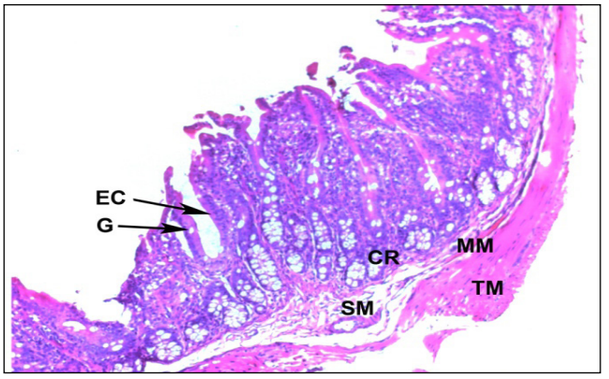

Fig. 3: Photomicrograph of ileum of fourteen week old guinea pig showing the tunica mucosa. V-Villi, CR-Cryts of Lieberkuhn, G-Goblet cells, SM-Submucosa, MM-Muscularis mucosa, EC-Enterocytes, TM-Tunica muscularis. H&E × 100

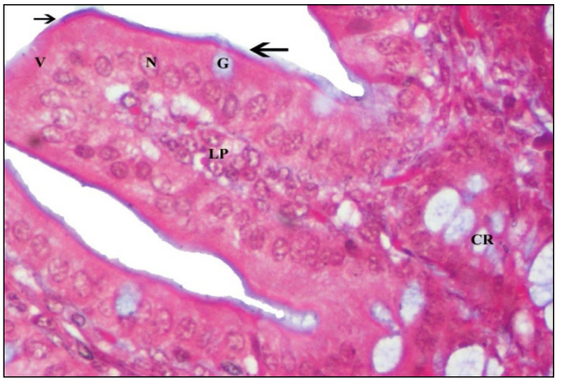

Fig. 4: Photomicrograph of ileum of fourteen week old guinea pig showing the tunica mucosa showing the brush border (arrow). V-Villi, N-Nucleus, G-Goblet cells, LP-Lamina propria, CR-Crypts of Lieberkuhn Masson's Trichrome stain × 400

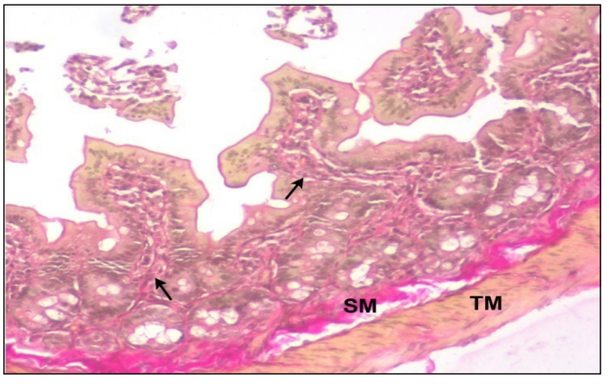

Fig. 5: Photomicrograph of ileum of six week-old guinea pig showing collagen fibres in the lamina propria (arrows). SM-Tunica submucosa, TM-Tunica muscularis, Van Gieson's Method × 100

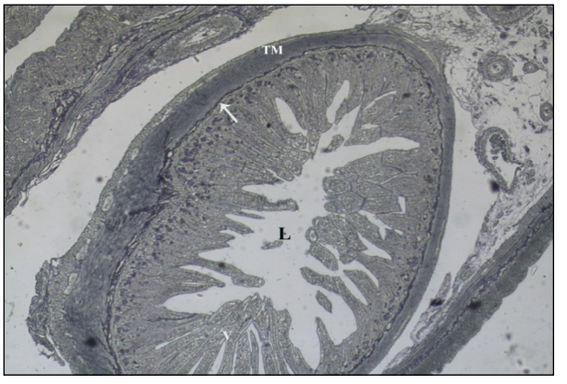

Fig. 6: Photomicrograph of the ileum of the one week-old guinea pig showing the presence of Reticular fibres (white arrows). CR-Crypts of Lieberkuhn, TM-Tunic muscularis. Gomori's Reticulum method × 100

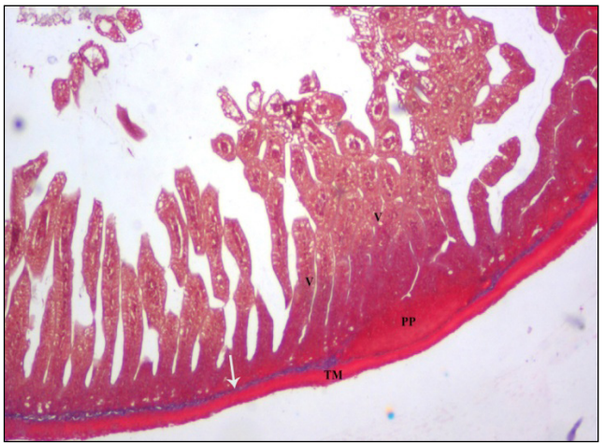

Fig. 7: Photomicrograph of ileum of one week old guinea pig showing the Peyer's Patches (PP). V-Villi, TM-Tunic muscularis. Masson's Trichrome stain × 12.5

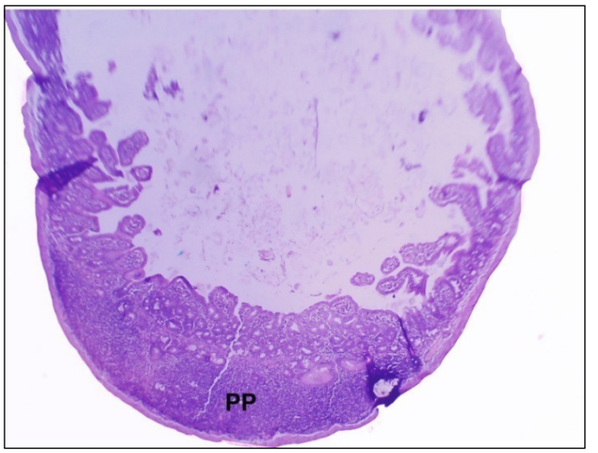

Fig. 8: Photomicrograph of ileum of eight week old guinea pig showing peyer's patches (PP) in the submucosa. H&E × 12.5

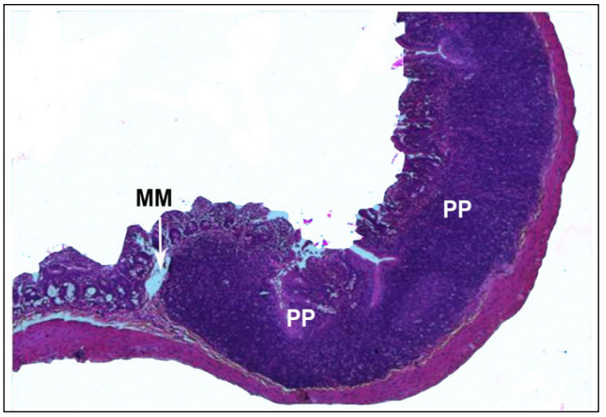

Fig. 9: Photomicrograph of ileum of 30 week-old guinea pig showing well developed peyer's patches (PP) in the submucosa. H&E × 40

Fig. 10: Photomicrograph of the ileum of the one week-old guinea pig showing tunica serosa (Black arrow). Masson's Trichrome stain × 100

intestine these finding are is agreeable with findings of Vithya (2005) in the small intestine of sheep. Occurrence of intra epithelial lymphocytes was more in the sections of the intestine where the lymphocytic infiltration was heavy in the lamina propria.

Lamina propria

Lamina propria of ileum of guinea pig was made up of loose connective tissue which in turn had highly cellular components along with connective tissue fibres. Intestinal glands or crypts of Lieberkuhn were noticed in the lamina propria (Fig. 5) of ileum of guinea pigs of all postnatal age groups studied and this isin accordance with the findings of Menaha (2003) in guinea fowl, Vithya (2005) in sheep, Gulbinowicz *et al.* (2004) in mice, Andleeb *et al.* (2016) in gaddi goat and Gahlot and Kumar (2018) in goat. The lining epithelium of crypts of Lieberkuhn of ileum of guinea pig was made up of simple columnar epithelium along with more number of goblet cells as noted by Gahlot and Kumar (2018) in the ileum of goat. Lacteals and blood vessels were noticed in the lamina propria of the intestines of guinea pig. The highly cellular connective tissue formed the lamina propria of the mucousmembrane of small intestine. It occupied the core of the villi and inter glandular spaces in the ileum. Cells of lamina

Table 1: Micrometry of the height and width of the villi, height of the columnar cells and tunica muscularis of the ileum different age groups of guinea pigs (Mean ± S.E in µm)

Sl. No.	Age groups	Height of the villi of the Ileum		Width of the villi of Ileum		Height of the columnar cells of the Ileum		Mean thickness Tunica muscularis of Ileum	
		Free border	Attached border	Free border	Attached border	Free border	Attached border	Free border	Attached border
1	0-2 week	268.4±16.9	215.3±19.6	56.2±4.8	61.5±3.82	24.3±1.71	27.3±072	59.1±7.46	80.8±18.15
2	2-8 week	329.6±46.9	306.3±25.2	60.3±7.02	64.7±3.95	26.7±1.34	29.06±1.00	67.4±1.6	110.8±6.88
3	8-16 week	346.6±9.08	362.7±51.9	64.3±2.26	67.8±5.02	27.1±0.28	29.06±1.1	84.0±5.76	186.2±6.28
4	16-32 week	192.5±10.7	243.1±23.8	66.4±4.51	69.2±1.01	27.5±0.40	29.8±0.56	118.2±21.9	189.4±15.9
F-Value		8.61^{**}	7.56^{**}	0.84^{NS}	0.82^{NS}	1.625^{*}	1.379^{*}	4.781^{**}	17.794^{**}

Mean bearing different superscript differs significantly.

* - Significant difference among groups (P≤0.05).

** - Highly significant difference among groups (P≤0.01).

NS–No significant difference between width of the Intestinal villi of ileumin free and attached border.

propria included fibrocytes, fibroblasts, lymphocytes, mast cells and eosinophils. Lymphocytes of various sizes were predominantly seen which agreed with findings of Andleeb *et al.* (2016) in gaddi goat. Lamina propria had a framework of the connective tissue fibers. Reticular fibers predominated in the lamina propria of ileum (Fig. 6) and Collagen fibres (Fig. 5) were less in their occurrence. Elastic fibers were few and could be seen around the blood vessels. The intestinal glands or crypts of Lieberkuhn were observed throughout the lamina propria. Reticulin and collagen fibers were noticed around intestinal glands or crypts of Lieberkuhn in all the postnatal age groups studied which is in agreement with findings of Gahlot and Kumar (2018) in goat. The number of intestinal glandsin duodenum, jejunum and ileum appeared to increased from 0-2 week-old to 16-32 week-old postnatal age groups studied. Further, intestinal glands appeared to beincreased in ileum of all the postnatal age groups studied as noted by of Menaha (2003) in guinea fowl, Vithya (2005) in sheep and Andleeb *et al.* (2016) gaddi goats. Similarly, the number of goblet cells lining the crypts was observed to be increased in ileum of all the postnatal age groups. Moreover, as the age advanced. In the base of some of the crypts, few pyramidal shaped cells i.e Paneth cells with a broad base and narrow apex were noticed in between the regular cells of lining epithelium of crypts of Lieberkuhn of ileum as described by Andleeb *et al*. (2016) in gaddi goat. In the present study, basally located basophilic nucleus of Paneth cells was large round in shapewith indistinct nucleolus. Cytoplasmic granules were coarse and unevenly distributed and were stained by Masson's trichrome method. Coarse granules took bright red colour in Masson's trichrome staining method as recorded by Zanuzzi *et al.* (2008) and Saffar and Haaik (2016) in rabbits

Muscularis mucosa

In guinea pigs of all the postnatal age groups studied, the muscularis mucosa of ileum of postnatal age groups was thin and consisted of only circularly arranged smooth muscle fibers (Fig. 3) as noted by Menaha (2003) in small intestine of guinea fowl and Vithya (2005) in small intestine of sheep.

In ileum of guinea pigs of 0-2 week-old age groups, the muscle fibers of this layer were closely adhered to the inner circular layer of tunica muscularis except where submucosa was distinct. In the present study, few fibrils from the muscularis mucosa extended into the core of villi in ileum as observed by Menaha (2003) in guinea fowl. The thickness of the muscularis mucosa of ileum was apparently increased as the age advanced.

Tunica submucosa

The submucosa was distinct ileum of all thepostnatal age groups studied. However, the submucosal layer was very thin in 0-2 week-old guinea pigs. It was made up of loose connective tissue fibers mainly collagen fibers (Fig. 5)

and reticulin fibers (Fig. 6) in all the postnatal age groups. Few elastic fibers were noticed around the blood vessels of submucosa as recorded by Menaha (2003) in guinea fowl. Submucosa demarcated the inner circular layer of the muscular tunic from that of the muscularis mucosa.

The submucosa of ileum of guinea pigs showed the presence of lymphoid nodules known as Peyer's patches in all the postnatal age groups studied. This finding is similar tothe observation of Gahlot and Kumar (2018) in goat. However, lymphoid aggregation / nodules were also noticed in some places of submucosa of jejunum of guinea pigs.

Peyer's patches

Peyer's patches were observed as lymphoid nodules/ solitary nodules in the submucosa of the ileum of guinea pig all the postnatal age groups as recorded by Nzalak, (2010) in rats and Gahlot and Kumar (2018) in goats. Peyer's patches was found to be smaller in 0-2 week-old guinea pigs (Fig. 7 & 8). In few sections of ileum of all the postnatal age groups, due to extreme development of Peyer's patches, submucosa became wide and abutted the lamina propria (Fig. 9). In those places, villi became reduced in height and muscularis mucosa became less evident. Peyer's patches were surrounded by the collagen and reticular fibers as noted by Gahlot and Kumar (2018) in goats.

In guinea pig, the muscular tunic of ileum wascomposed of compactly arranged thick inner circular and thin outer longitudinal smooth muscle layers which is agreeable with the findings of Vithya (2005) and Gahlot and Kumar (2018) in goat, Andleeb *et al.* (2016) in gaddi goat. Reticulin fibres were seen abundantly among the smooth muscle fibres of tunica muscularis. A few collagen and elastic fibres were also noticed in the tunica muscularis of all postnatal age groups studied. Large blood vessels and well developed Auerbach's plexuses or myenteric plexuses were noticed in the inter muscular connective tissue as observed by Fekete *et al.* (2000) in humans, Maifrino *et al.* (2007) in mouse and Singh *et al.* (2017) in rats. The mean thickness of the tunica muscularis of ileum was increased as the age advanced.

Tunica serosa

In postnatal guinea pigs, tunica serosa was distinct. Tunica serosa was lined by mesothelium (Fig. 10). Appreciable amountof loose connective tissue was found between the tunica muscularis and tunica serosa as reported by Vithya (2005) in sheep and Menaha (2003) in guinea fowl.

CONCLUSION

The shape of the villi in the epithelium of the ileum was various slender and finger like in the 0-2 week-old guinea pig where as tongue shaped in remaining age groups. Tunica submucosa was made up of more number of collagen and reticular fibres and few elastic fibres in the wall of the blood vessels and showing the peyer's patches. The mean height and width of the villi and height of the columnar cells and thickness of the tunica muscularis was increased as the age advanced in all the age groups studied.

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