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Histological Studies on the Caecum of Postnatal Guinea Pigs (*Cavia porcellus*)

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ABSTRACT

The present histological study was conducted on the caecum of guinea pigs at different postnatal age groups. Microscopically, the caecal wall was composed of four layers namely the tunica mucosa, tunica sub mucosa, tunica muscularis and tunica serosa. The shape of the mucosal folds in the epithelium was short and stout like in 0–2-week- old guinea pigs, whereas they appeared tongue shaped in remaining age groups. The Lamina propria was composed of loose connective tissue with Crypts of Lieberkuhn. The tunica submucosa was composed predominantly of collagen and reticular fibres, with few elastic fibres mainly around the walls of the blood vessels. The tunica muscularis com- prised an inner circular and an outer longitudinal muscular layer. The mean height and width of the mucosal folds, the height of the columnar epithelial cells and the thickness of the tunica muscularis increased progressively with advanc- ing age in all the age groups studied.

Key words: Caecum, guinea pig, mucosal folds, postnatal age.

INTRODUCTION

Guinea pigs are descendants of the wild cavy, which is one of the common rodents native to South America. They are characterized by a sticky body, short neck, short limbs and a tiny tail and are more closely related to porcupines than to mice and rats (Kunzl and Sachser, 1999 and North, 1999). This species is commonly used as an experimental model in biomedical research, as the immunological genes of guinea pigs were more similar to those of hu- mans than those of mice (Guo *et al*., 2012). The caecum is the first part of the large intestine con- nected to the ileum and colon (Raja *et al*., 2022). There is limited literature available on the caecum of guinea pigs at various postnatal age groups. Hence, the present study was undertaken to explore the histo-architectural features of the caecum of the guinea pigs in postnatal age groups.

MATERIALS AND METHODS

Histological studies on the caecum of the guinea pig (*Cavia porcellus*) were conducted in different post- natal age groups *viz.*, Preweaning (0-2 weeks), weaning (2-8 weeks), young (8-16 weeks) and adult (16-32 weeks)at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai. Guinea pigs were procured from the Department of Laboratory Animal Med icine, Madhavaram milk colony, Chennai. Approval was obtained from the Institutional Animal Ethics Committee (IAEC) TANUVAS, approval No. 1487/DFBC/IAEC/2018 dated 13.07.2018. These animals were euthanized by following the standard operating procedure using carbon dioxide asphyxiation as per CPCSEA norms. Samples from the guinea pigs of all postnatal age groups, were collected from the Proximal, mid and distal region of the caecum. They were washed in normal saline, fixed in 10 % neutral buffered formalin and tissues were dehydrated in the ascending grades of the alcohol, cleared in xy-lene and embedded in paraffin (58-60^{0}C). Sections of 4-5 µm thickness were cut and used for routine and special histological staining 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 demonstration of enterochromaffin cells were done. Microscopic im ages of stained tissue sections of the caecum of all postnatal age

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groups were captured at different magnifications. The various micrometrical parameters *viz.,* the height and width of the caecum, height of the columnar cells lining the mucosal fold and thickness of the tunica muscularis of the caecum were measured groupwise. The data were subject ed for one way ANOVA with arithmetic mean and standard error (Mean ± SE) were calculated as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The wall of the caecal segments was composed of four distinct layers, namely the tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa from inner to outer (Fig.1). There were no distinct differences in the micro anatomical features of the caecum between male and female guinea pigs in any of the postnatal age groups studied. In the present study, the tunica mucosa of the caecum in guinea pigs of all postnatal age groups consisted of a surface epithelium, lamina propria and muscularis mucosae. The surface epithelium of caecum was thrown into longitudinal folds along with the submucosa in many places. These findings agrees with the observations of Dellmann and Brown (1987) in domestic animals and Kadam *et al.* (2011) in sheep. Villi were absent in the caecum as noted by Calhoun (1954) in fowl, Trautmann and Fiebiger (1957) and Stinson and Calhoun(1981) in domestic animals. In all postnatal animals studied, the initial portion of the caecum near the ileo-caecal junction, the tunica mucosa was thrown into short folds without any plica and folds were crowdly placed (Figs.1,2), which agrees with the findings of Das and Biswal (1967) in domestic duck. In the middle and distal portions of the caecum, the mucosal folds were thrown into larger folds along with the submucosa in many places as plica circularis and the same was reported by Dellman and Brown (1987) and Pandit *et al.* (2018) in Uttara fowl. The number of plicae circularis increased with advancing age (Fig.2). The mean height and width of the mucosal folds of the caecum increased progressively from 0–2-week-old to16–32- week-old guinea pigs (Table.1).

The mucosal membrane of the caecum was lined by simple columnar epithelium along with goblet cells. The present finding is in agreement with the reports of Bevelander (1965) in human beings, Fitgerland (1969) in Japanese quails and Copenhaver *et al.* (1975) in mammals. The number of goblet cells exceeded that of columnar cells in the segments of the large intestine. The surface epithelium invaginated into the underlying lamina propria to form the intestinal glands orcrypts of Lieberkuhn (Fig.1).

Columnar cells

The columnar cells were the principal cells of the surface epithelium of the caecal mucosa. These cells were columnar in shape with a narrow base and a broad apex and their cell boundaries were distinct. The apical border of the columnar cells exhibited a well defined striated border, which appeared acidophilic when stained with H&E, as stated by Fitzgerald (1969) in Japanese quails, Frandson and Whitten (1981) in domestic animals and Ozdemir*et al.* (2004) in Badgers. The basophilic nuclei of these were oval to round in shape and were mostly located in the basal half of the cell. Chromatin material was distinct. The cytoplasm was acidophilic and homogenous with fine granules. The mean height of the columnar cells of the caecum was found to be increased with advancing age in all the postnatal age groups studied (Table.1).

Goblet cells

Goblet cells in the surface epithelium of the caecum were round to globular in shape. The nucleus was basophilic, flattened and located at the base of the cell. Goblet cells were intersparsed among the crypts of the caecum these findings were concurred with the reports of Trautmann and Fiebiger (1957). The goblet cells are with vacuolated cytoplasm distinct with H and E. The occurrence of the goblet cells was less in the surface epithelium and crypts of Lieberkuhn in 0–2-week-old guinea pigs and increased in number with advancing age.

Lamina propria

The histological features of the lamina pro pria of caecum resembled that of small intestine. It contained mostly the lymphocytes, fibrocytes,

fibroblasts, very few mast cells and eosinophils in all the postnatal age groups as stated by of Pandit *et al.* (2018) in Uttara fowl.Reticulin fibers were predominantly observed in the lamina propria.Very few collagen fibers were observed in all the postnatal age groups studied. Elastic fibers were observed only around the blood vessels of caecum. In general, the lamina propria of the caecum was not extensive in any of the postnatal age groups studied. The projection of the submucosa along with the muscularis mucosae into the core of the folds (plica circularis) and abutted the lamina propria along with the presence of more intestinal glands (Figs. 2 & 4). The occurrence of the crypts of Lieberkuhn was found to be more in the large intestine than in the small intestine, as recorded by Viguerus *et al.* (1999) in rats. The number of crypts of Lieberkuhn increased from 0-2 week to 16–32-week-old guinea pigs, in agreement with the findings of Snipes (1981) in mouse and Nzalak *et al*. (2011) in rats. Similarly, the number of the goblet cells within the crypts were found to be increased as the age advances from 0-2week to16–32-week-old guinea pigs.

Muscularis mucosa

In 0–2-week-old guinea pigs, the muscularis mucosae of the caecum was very thin and consisted of only a discrete layer of smooth muscle fibres (Fig.3). In other age groups, it was composed of a continuous circularly arranged smooth muscle fibers. The presence of inner circular and outer longitudinal layers of the muscularis mucosae in the large intestine of mammals as reported by Bevelander (1965) and Frandsonand Whitten (1981) were also observed in the present study.

Tunica submucosa

In all the postnatal age groups studied, the submucosa of the caecum was made up of loose connective tissue containing collagen and reticulin fibers (Figs.1,2). This observation in the present study agreed with the findings of Patt and Patt (1969) in mammals, Bergman and Afifi (1974) in human beings and Frandson and Whitten (1981) in domestic animals.Few elastic fibres were noticed around the blood vessels of the submucosa of the submucosa ,Adipose tissue was frequently noticed in the submucosa, which concurs with the reportings of the Nzalak *et al.* (2011) in African giant rat. In the present study, the submucosa was devoid of glands in all age groups. Meissner's plexuses were observed in all the postnatal age groups studied as noted by Frandson and Whitten (1981) in domestic animals.The mean thickness of the submucosa increased with advancing age, similar to the findings of Mohamed *et al*.(2018).

Tunica muscularis

The tunica muscularis of the caecum resembled that of the small intestine, as noted by Calhoun (1954) in fowl, Bevelander (1965) and Copenhaver *et al*.(1975) in human beings and Kadam *et al*. (2011) in sheep, cattle and goats. In addition to a thick inner circular and a thin outer longitudinal smooth muscle layer, obliquely arranged muscle fibres were also observed (Figs.1,2). Reticulin fibres were predominantly seen among the smooth muscle fibres of tunica muscularis. A few collagen and elastic fibres were also noticed in the tunica muscularis of guinea pigs of all postnatal age groups studied. Large blood vessels and well developed Auerbach's (myenteric) plexuses were observed, as recorded by Frandson and Whitten (1981) in domestic animals and Pandit *et al.* (2018) in Uttara fowl. The mean thickness of the tunica muscularis increased progressively with advancing age (Table.1). The tunica serosa lined by the mesothelial cells, was present in the caecum of guinea pigs in all the postnatal age groups studied. External to the tunica serosa, of loose connective tissue with adipose tissue and blood vessels was also observed as reported by Kadam *et al.* (2011) in sheep.

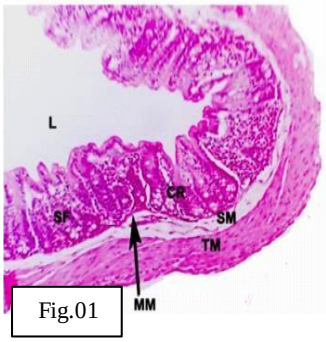

Fig.01

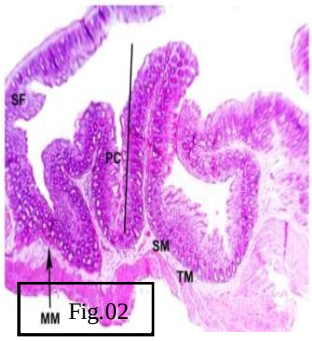

Fig.02

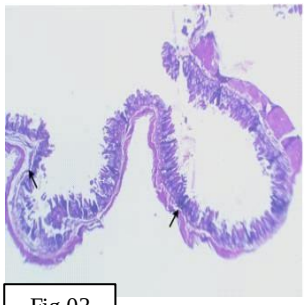
Fig.03

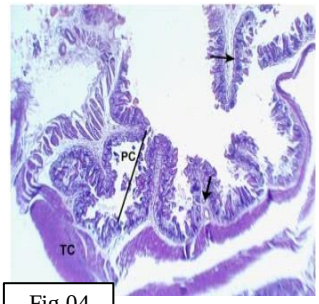

Fig.04

Fig.1 Photomicrograph of initial portion of the caecum of the 12-week-old guinea pig showing the shorter folds in the tunica mucosa.L-Lumen, SF-Shorter folds, TM –Tunica muscularis, SM- Submucosa, MM-Muscularis mucosa,CR-Crypts of Lieberkuhn. H&E X 100, **Fig.2** Photomicrograph of caecum of the 12-week-old guinea pig showing the plica circularis, (PC) in tunica mucosa. SF-Shorter folds, TM –Tunica muscularis, SM-Submucosa, MM-Muscularis mucosa. H&E X 100 , **Fig.3** Photomicrograph of initial portion of the caecum of one week-old guinea pig showing muscularis mucosa (arrows) was in the continuous thin band. H&E X 40, **Fig. 4** Photomicrograph of middle part of the caecum of one week-old guinea pig showing muscularis mucosa (arrows) was entering into the core of the fold/plica circularis (PC). TC-Taenia coli H&E X 100.

Table .1 Micrometry of the height and width of the mucosal fold, height of the columnar cells, height of the submucosa and tunica muscularis of the caecum in different age groups of guinea pigs (Mean ± S.E in µm).

S. No.	Age groups	Height of the mucosal folds caecum		Width of the mucosal folds of caecum		Height of the columnar cells of the caecum		Mean thickness Tunica submucosa of caecum		Mean thickness Tunica muscularis of caecum	
		Free wall	At-tachedWall	Free border	Attached border	Free border	Attached border	Free border	Attached border	Free border	Attached border
1	**0-2 week**	472.4±65.6	813.0±31.7	245.6±15.8	266.1±17.7	24.19±2.24	30.4±0.79	21.2±0.85	25.6±0.710	115.5±19.4	147.1±9.4
2	**2-8 week**	607.8±77.6	818.7±27.5	256.2±9.4	296.4±28.3	26.10±1.88	31.1±1.31	36.8±7.07	39.0±6.17	118.3±14.2	149.5±8.6
3	**8-16 week**	743.8±19.5	824.4±22.4	267.7±21.9	303.6±6.3	28.01±0.74	38.6±9.01	50.7±1.58	50.4±0.76	121.1±4.9	152.0±7.5
4	**16-32 week**	834.9±38.1	906.8±28.4	289.9±22.7	326.7±30.4	28.50±1.03	46.8±11.60	52.4±1.25	52.4±2.01	132.6±2.31	156.8±4.4
F-Value		**9.30****	**8.26****	**2.55***	**1.07***	**1.19***	**1.513***	**1.089***	**15.42****	**0.366**^{NS}	**0.287**^{NS}

Mean bearing different superscript differs significantly
* - Significant difference among groups (P≤0.05)
** - Highly significant difference among groups (P≤0.01)

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