

Short Paper

The study of microanatomy of intestinal epithelium in the Chinese soft-shelled turtle (*Pelodiscus sinensis*)

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Summary

The microanatomy of the intestinal epithelium in the Chinese soft-shelled turtle (CST) was studied by light and transmission electron microscopy (TEM). The small intestinal epithelium (SIE) was single layered or pseudostratified. The enterocytes contained mitochondria or mitochondria and lipid droplets. The enterocytes were arranged tightly in the apical parts of epithelium and connected by desmosomes and interdigitations. The large intestinal epithelium (LIE) was pseudostratified and the enterocytes did not contain lipid droplets. Enterocytes were arranged compactly in the apical part, forming spaces in the middle and basal parts of epithelium. Numerous mucous cells were scattered in the epithelium and there were intraepithelial lymphocytes (IELs) with their pseudopodia extended into the intestinal lumen. This study provides detailed features of intestinal epithelium in the *Pelodiscus sinensis* that could be related to function. In addition, these findings are discussed in relation to other vertebrates.

Key words: Anatomy, Chinese soft-shelled turtle, Intestinal epithelium, Microanatomy

Introduction

The Chinese soft-shelled turtle (CST) is included in the list of endangered species by the International Union for Conservation of Nature. Conservation includes physiological, clinical and anatomical tasks (Kraut *et al.*, 2013; Liu *et al.*, 2015). However, only a few instances have studied the anatomy and histology in CST (Bao *et al.*, 2011; Bian *et al.*, 2013; Xu *et al.*, 2013).

The anatomy of organs such as the intestine is important due to its role in digestion, absorption and immunity. Several reports have investigated the gut-associated lymphoid tissue (Solas and Zapata, 1980) in reptiles, nevertheless, an histologic examination of the CST intestinal epithelium has not yet been performed using light and transmission electron microscopy (TEM). Therefore, a careful examination of the structure of the intestinal epithelium of the CST could provide a basis for future studies on its intestinal function. Thus, the aim of this study was to describe the microanatomy of intestinal epithelium in the normal CST (*Pelodiscus sinensis*), using light and TEM.

Materials and Methods

Animals and tissue blocks preparation

CST from ten adults (5 males and 5 females) was collected from a breeding (Jiangsu, China). All turtles

were fed with fishes and shrimps. Eight days later, they were anesthetized with sodium pentobarbital (1-2 ml/kg) and euthanized following the protocols of the Ethical Chinese Committee.

Light and transmission electron microscopy studies

After necropsy, the intestine was fixed in formalin and embedded in paraffin wax. Forty sections (5 µm thick) were processed for staining with haematoxylin and eosin for light microscopic observation.

For TEM studies, pieces (2-3 mm long) from intestine were immersed in 2.5% glutaraldehyde fixative in phosphate buffered saline (4°C, pH = 7.4, 0.1 M) and postfixed with 1% osmium tetroxide. Semi-thin sections (1 µm thick) were cut and stained with toluidine blue. Ultrathin sections were contrasted with lead citrate and uranyl acetate and examined with a JEM-1200EX microscope.

Counting procedure and statistics

The numbers of intraepithelial lymphocytes (IELs) per 1000 enterocytes in the epithelium were counted in 50 slides from each turtle. Statistical procedures were performed using SPSS (Ver. 14.0).

Results

The intestinal epithelium of the CST was composed

by enterocytes, mucous cells, channels (C), IELs and plasma cells.

Enterocytes

The small intestinal epithelium (SIE) consisted of a single layered or pseudostratified columnar epithelium (Figs. 1-2). In its apical part, enterocytes were connected by tight and intermediate junctions and desmosomes (Fig. 3). The middle and basal parts of epithelium showed desmosomes and interdigitations between neighboring enterocytes (Fig. 4). The majority of enterocytes had oval nucleus and spherical mitochondria. Within their nuclei, the heterochromatin became rhabdoid shaped (Fig. 5). In contrast, only a few enterocytes contained several mitochondria and lipid droplets (Fig. 6).

Large intestinal epithelium (LIE) was pseudostratified. In its apical part, enterocytes were fastened by tight junction and interdigitations (Fig. 7). In the middle and basal parts, enterocytes were not as densely packed as in the small intestine, forming obvious intercellular spaces (Fig. 8). In addition, their nuclei were elliptical or fusiform and did not contain lipid droplets.

Mucous cells

There were two types of mucous cells scattered throughout the intestinal epithelium. One type had numerous mucous granules of low electron density (Fig. 9), whereas the other showed granules of high electron

density. On the surface of mucous cells, there were small microvilli (Fig. 10). The morphology of these cells was similar in all epithelium, with higher number in large intestine. Occasionally, a mucous cell was found in the gut lumen. The intestinal epithelium surrounding the free mucous cell was complete (Fig. 11).

Channels

Channels were seen between the enterocytes in the entire epithelium. They ranged from 1 to 7 μm , showing obvious limits and tight junctions with surrounding enterocytes (Figs. 12-13). In large intestine, there were not junctions between the C and surrounding enterocytes. Mucous granules and cytoplasmic fragments were also observed in C (Fig. 14). Occasionally, a lymphocyte (2 μm in diameter) containing several mitochondria was seen into C (Fig. 15).

Intraepithelial lymphocytes

IELs were divided into three layers. One parallel to the enterocyte's nuclei was regarded as nuclear layer, another under the enterocyte's nuclei called basal layer, and a third over the enterocyte's nuclei named as apical layer (Fig. 2). The number of IELs in each 1000 enterocytes was calculated in all the layers (Table 1). IELs showed condensed chromatin (Fig. 5) and nuclei with irregular shape (Fig. 16). The cytoplasm of some IELs contained mitochondria with vacuolization (Fig. 17). IELs measured 4-9 μm ; showing 1-2 pseudopodium that were extended to the lumen (Fig. 18). Some IELs

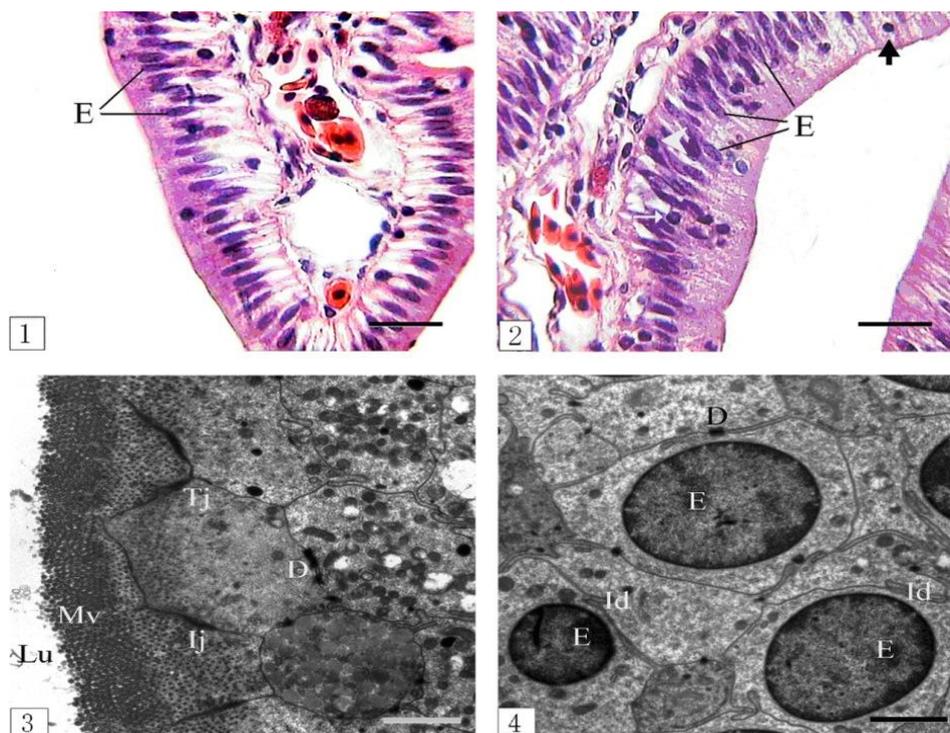


Fig. 1: Light micrograph of small intestinal epithelium. Enterocytes (E) arranged as a single layered columnar epithelium, (H&E, bar=25 μm). **Fig. 2:** SIE. E arranged as typical pseudostratified epithelium. IELs were seen in the apical (\blacktriangleright), nuclear (\blacktriangleright), and basal layers (\blacktriangleright) of epithelium, (H&E, Bar=25 μm). **Fig. 3:** SIE. Tight (Tj), and intermediated junction (Ij), and desmosome (D) are seen at the apical parts of the epithelium. Microvilli (Mv), and gut lumen (Lu), (TEM, Bar=2 μm). **Fig. 4:** SIE. Desmosome (D) and interdigitate (Id) along the lateral plasma membranes of two adjacent enterocytes (E), (TEM, Bar=2 μm)

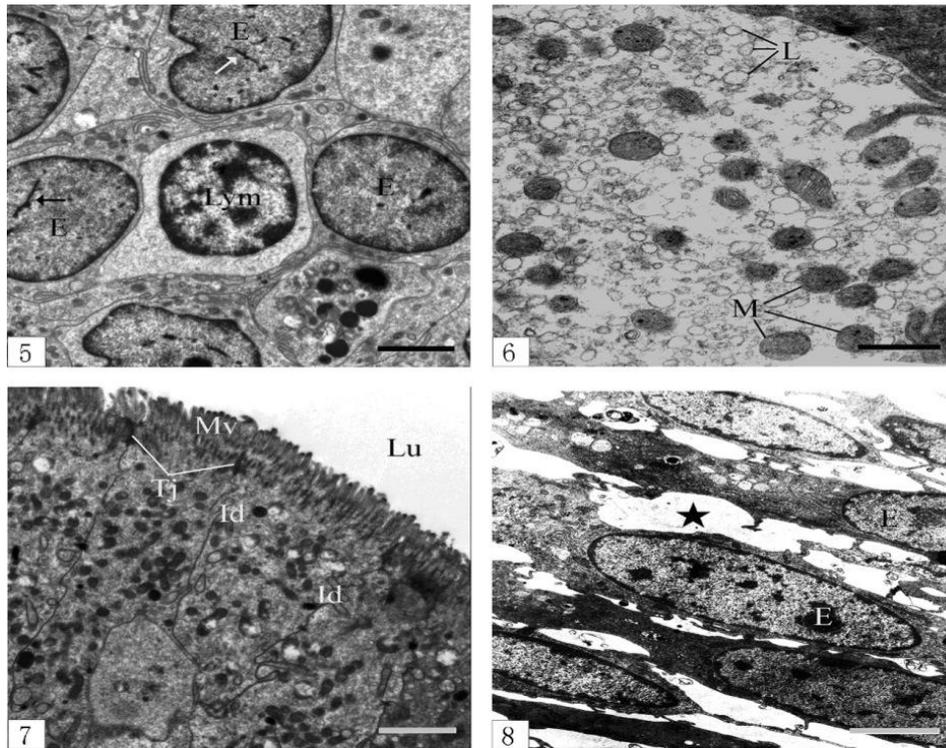


Fig. 5: E and IEL. IEL located between the enterocytes, it had a high nuclear cytoplasmic ratio. Heterochromatin (→) within the enterocyte's nucleus, (TEM, Bar=2 μm). **Fig. 6:** Enterocytes with mitochondria (M) and lipid droplets (L), (TEM, Bar=500 nm). **Fig. 7:** Large intestinal epithelium. The tight junction (Tj) and interdigitate (Id) are visible at the apical parts of epithelium. Microvilli (Mv), and gut lumen (Lu), (TEM, Bar=2 μm). **Fig. 8:** LIE. There are obvious intercellular spaces (★) between the enterocytes (E) in the basal parts of epithelium, (TEM, Bar=2 μm)

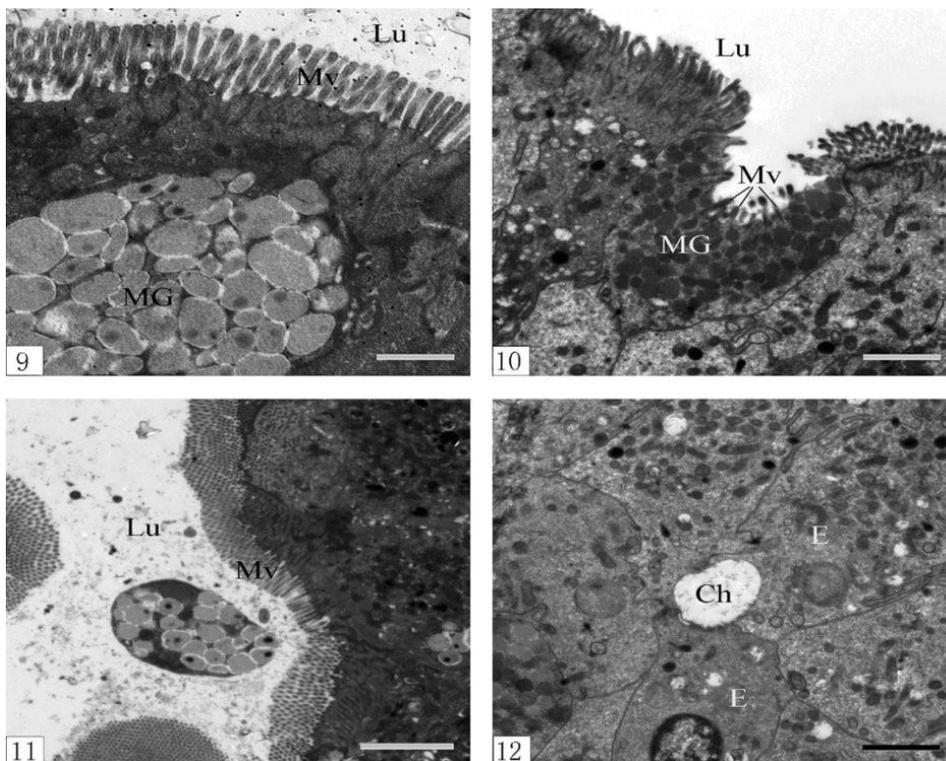


Fig. 9: Mucous cell with mucous granules (MG) of low electron density. Microvilli (Mv), and gut lumen (Lu), (TEM, Bar=1 μm). **Fig. 10:** Mucous cell with mucous granules (MG) of high electron density. Microvilli (Mv), and gut lumen (Lu), (TEM, Bar=2 μm). **Fig. 11:** A complete mucous cell is seen in the gut lumen (Lu). Microvilli (Mv), (TEM, Bar=3 μm). **Fig. 12:** Channel (C) between the enterocytes (E), (TEM, Bar=2 μm)

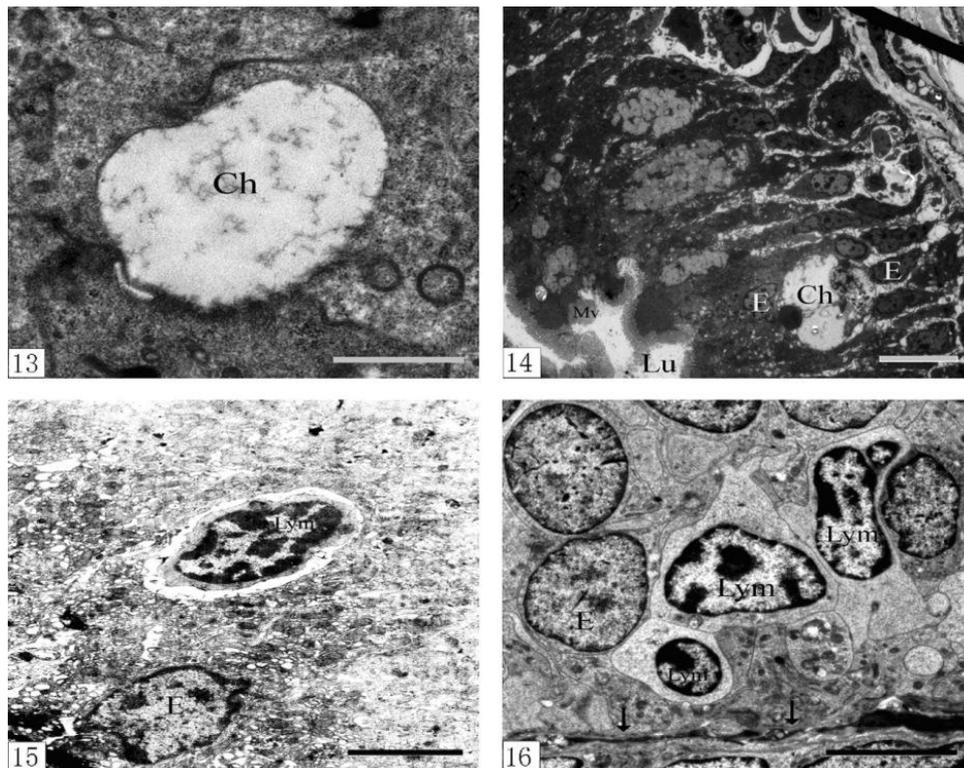


Fig. 13: High magnification of Fig. 12, (TEM, Bar=1 μ m). **Fig. 14:** Some mucous granules and cytoplasmic fragments are observed in the C. Enterocyte (E), microvilli (Mv), and gut lumen (Lu), (TEM, Bar=10 μ m). **Fig. 15:** A lymphocyte is located in the channel. Enterocyte (E), (TEM, Bar=2 μ m). **Fig. 16:** IELs (Lym) with irregularly appearance are located in the basal layer of epithelium. Basement membrane (\rightarrow), and enterocyte (E), (TEM, Bar=5 μ m)

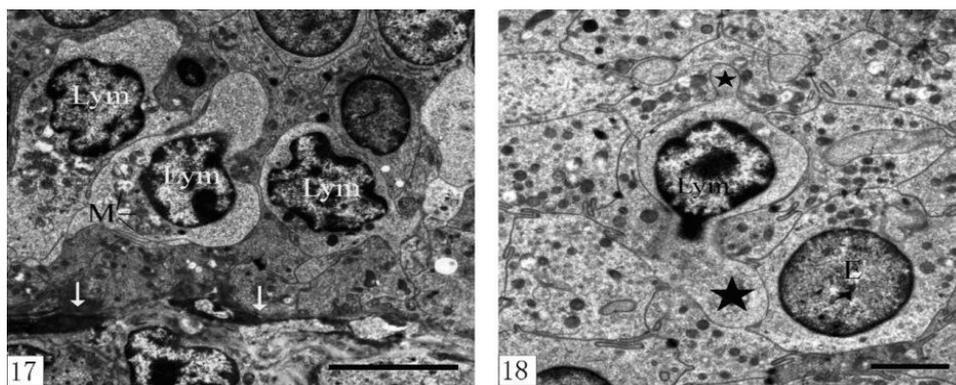


Fig. 17: IELs (Lym) with some mitochondria (M) in the cytoplasm. Basement membrane (\rightarrow), (TEM, Bar=5 μ m). **Fig. 18:** IEL with pseudopodium (\star). Enterocyte (E), (TEM, Bar=2 μ m)

Table 1: Numbers of IEL in the three layers of turtle intestinal epithelium ($\bar{x} \pm SD$)

	Apical layer	Nuclear layer	Basal layer
IEL number	19.3 \pm 4.26	176 \pm 3.05	246 \pm 6.18

contained avariable number of cytoplasmic azurophil granules with length between 0.3-0.6 μ m. In addition, an intact IEL of 5 μ m was found in the gut lumen.

Plasma cells

Plasma cells were located in the middle and basal layers of SIE and LIE. Some cells had dilated rough endoplasmic reticulum. Their nuclei were oval-shaped and eccentrically placed. They had an armillary space

with surrounding enterocytes. Nevertheless, other plasma cells had rough endoplasmic reticulum in parallel arrangement and well-developed Golgi apparatus.

Discussion

This study showed that CST intestine had single layered or pseudostratified columnar epithelium. Some enterocytes had small lipid droplets, and others only contained several mitochondria. Previous studies on reptiles showed similar differences during feeding and fasting conditions (Starck *et al.*, 2007). Despite all of the turtles being fed in similar conditions, they showed different structure of intestinal epithelium and

enterocytes. Therefore, additional studies in larger series of turtles should be conducted to better understand these differences and their physiological effects.

There is a considerable variation in the presence of intercellular spaces in relation to the nutritional status of reptiles. During feeding these spaces are present; but in fasting they are reduced in number or absent (Lignot *et al.*, 2005). The location of these spaces was similar to the Cs found in the CST, although the morphology of channel and intercellular spaces was different. Therefore, a well-defined limit and tight junctions were present between the channel and surrounding enterocytes. In contrast, there were no cell junctions between intercellular spaces and neighbouring enterocytes. In addition, Cs were seen in the intestinal epithelium and their size and shape were suitable for the lymphocyte they were located in, suggesting that C were the route used for lymphocyte to reach the intestinal epithelium and gut lumen.

The ratio of IELs in the apical, nuclear and basal layers of the CST intestinal epithelium was different from that reported in other species (Xie *et al.*, 1997; Vega-López *et al.*, 2001), suggesting that distribution of IELs could be related to animal species. Most of the IELs observed in the epithelium and gut lumen of the CST showed irregular pseudopodia, suggesting that IELs could migrate due to specific antigen stimulation or discharge of the epithelium (Komoto *et al.*, 2005).

Some studies identified plasma cells containing dilated rough endoplasmic reticulum in the intestine (Veazey *et al.*, 1997). Similar structures were seen in the plasma cells of CST, although some of them showed rough endoplasmic reticulum in parallel arrangement. Some studies differentiated these cells from B lymphocytes, which provided humoral immunity (Von Allmen *et al.*, 2008). In mammals and birds, these antibodies migrate from the lamina propria, via epithelium basement membrane, until the gut lumen (Veazey *et al.*, 1997; Bai and Che, 1999). However, in the CST, these cells were found in the intestinal epithelium and only crossed the epithelium to reach the gut lumen, suggesting better immune response compared to mammalian and birds.

In conclusion, this is the first study that describes a detailed morphology of intestinal epithelium in the *P. sinensis*. Our ultrastructure analysis found a similar cytological and anatomical organization as that for other species, but the presence of lymphocytes into the C could be related to immunological function. Further studies are necessary to compare microanatomy of the intestinal epithelium of Trionychidae with other chelonians.

Acknowledgements

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Conflict of interest

We declare that there is no conflict of interest.

References

- Bai, XY and Che, FX (1999). Advances on mucosal immune. Foreign Medical Sciences (Immunology Section). 22: 255-259.
- Bao, HJ; Liu, Y; Qin, JH; Xu, CS; Hei, NN; Jaber, JR and Chen, QS (2011). An immunohistochemical study of S-100 protein in the intestinal tract of Chinese soft-shelled turtle. Res. Vet. Sci., 91: 16-24.
- Bian, X; Zhang, L; Yang, L; Yang, P; Ullah, S; Zhang, Q and Chen, Q (2013). Ultrastructure of epididymal epithelium and its interaction with the sperm in the soft-shelled turtle *Pelodiscus sinensis*. Micron. 54-55: 65-74.
- Komoto, S; Miura, S; Koseki, S; Goto, M; Hachimura, S; Fujimori, H; Hokari, R; Hara, Y; Ogino, T; Watanabe, C; Nagata, H; Kaminogawa, S; Hibi, T and Ishii, H (2005). Effect of specific antigen stimulation on intraepithelial lymphocyte migration to small intestinal mucosa. Clin. Exp. Immunol., 140: 249-257.
- Kraut, S; Fischer, D; Heuser, W and Lierz, M (2013). Laser therapy in a soft-shelled turtle for the treatment of skin and shell ulceration. Tierarztl. Prax. Ausg. K. Kleintiere Heimtiere. 41: 261-266.
- Lignot, JH; Helmstetter, C and Secor, SM (2005). Postprandial morphological response of the intestinal epithelium of the Burmese python. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 141: 280-291.
- Liu, L; Cao, Z; Lin, F; Ye, XP and Xu, Y (2015). Partial sequence of a novel virus isolated from *Pelodiscus sinensis* hemorrhagic disease. Intervirology. 58: 197-204.
- Solas, MT and Zapata, A (1980). Gut-associated lymphoid tissue in reptiles: intraepithelial cells. Dev. Comp. Immunol., 4: 87-97.
- Starck, JM; Cruz-Neto, AP and Abe, AS (2007). Physiological and morphological responses to feeding in broad-nosed caiman (*Caiman latirostris*). J. Exp. Biol., 210: 2033-2045.
- Veazey, RS; Rosenzweig, M; Shvetz, DE; Pauley, DR; DeMaria, M; Chalifoux, LV; Jonson, RP and Lackner, AA (1997). Characterization of gut-associated lymphoid tissue (GALT) of normal rhesus macaques. Clin. Immunol. Immunopathol., 82: 230-242.
- Vega-López, MA; Arenas-Contreras, G; Bailey, M; González-Pozos, S; Stokes, CR; Ortega, MG and Mondragón-Flores, R (2001). Development of intraepithelial cells in the porcine small intestine. Dev. Immunol., 8: 147-158.
- Von Allmen, CE; Bauer, M; Dietmeier, K; Buser, RB; Gwerder, M; Muntwiler, S; Utzinger, S; Saudan, P; Bachmann, MF and Beerli, RR (2008). Identification of Ly-6K as a novel marker for mouse plasma cells. Mol. Immunol., 45: 2727-2733.
- Xie, ZJ; Liu, WQ; He, YC; Zhang, ZB and Zhao, Y (1997). Morphology studies on intestinal intraepithelial lymphocytes of mice in mucosal immune response. Acta Anatomica Sinica. 28: 309-313.
- Xu, CS; Yang, P; Bao, HJ; Bian, XG and Chen, QS (2013). Ultrastructure of nephron in the *Pelodiscus sinensis*. Micron. 44: 451-462.