

Morphological and histochemical investigation of the camel (*Camelus dromedarius*) abomasal mucous membrane by light and scanning electron microscopy (SEM)

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Summary

Morphological and histochemical study of the abomasal epithelium in camel (*Camelus dromedarius*) was carried out by light and scanning electron microscopes. The lining of the abomasum was divided into four regions, i.e. cardiac, pseudocardiac, fundic and pyloric. Our investigation revealed that the cardiac and pseudocardiac regions occupy a wide part of the abomasum in camel and it reaches approximately the third-fourth of the abomasum. Our gross anatomical observation showed small diverticulae in the Fundic region, this part was covered with thick mucosal folds that have been separated by deep branching furrows. In the histological study we observed that, the mucosa has extensive gastric folds and small invaginations or gastric pits, which were in continuation with the gastric glands. The mucosal surface was covered with simple columnar epithelial. Our histochemical revealed that the surface epithelium in abomasum was negative to AB and positive to PAS staining, whereas in the gastric pit cells it was positive to AB and PAS staining, but gastric gland cells were negative to PAS and positive to AB staining. In the SEM study, after complete removal of mucin from the surface of the mucosa, simple columnar epithelial cells with a mean length of 20 μm were observed, and some epithelial cells have been arranged as flower body (FB). Also, we observed hexagonal structures on the surface of the abomasums that resembled honeycomb structure (HC). Mean diameter of these HC structures was 30-40 μm . For the first time our investigation revealed these FB and HC structures in the abomasal mucosa of camel.

Key words: Histomorphology, Histochemistry, Abomasum, Camel, SEM

Introduction

Digestive system of camel is identical to ruminants in several aspects, which include regurgitation of ingesta, and active microbial fermentation in the stomach (Frandsen *et al.*, 2003). In the typical ruminant, the first three compartments (rumen, reticulum and omasum) are non-glandular whereas the fourth part abomasums, as glandular stomach, contains typical cardiac, fundic and pyloric glands (Eurell and Frappler, 2006; Samuelson, 2007). However, in contrast to the compound stomach of typical ruminants camel has only three compartments (Vallenas *et al.*, 1971; Dyce *et al.*, 1995; William and Bacha, 2002). Stomach of the Bactrian camel was divided into three ventricles, they regarded the first and second

compartments as one stomach that differs from the typical rumen and reticulum and referred to the third one as the abomasum that was divided into four, (i.e.) cardiac, pseudocardiac, fundic and pyloric (regions) (Wang *et al.*, 2000; Abdel-Magied and Taha, 2003). This study will constitute the morphological and histochemical abomasal mucous membrane of the camel.

Materials and Methods

Tissue sampling

Tissue samples were collected from abomasal mucosa of 20 apparently healthy 1-2-year-old male camels. For LM, samples (about 0.5 \times 0.5 cm) were taken from four regions of abomasum including cardiac, pseudocardiac, fundic and pyloric regions.

Immediately after the slaughter of the animals, the specimens were collected and put in buffered neutral formalin (10%) for fixation. Samples for SEM study were washed thoroughly with phosphate buffer solution, by carefully rubbing the mucosal surface with gloved finger, then the samples were pre-fixed in 2.5% glutaraldehyde buffer pH = 7.2 for 24 h. and post-fixed with 1% osmium tetroxide solution for 1 h, dehydrated using graded ethanol series and then all samples were dried at room temperature and coated with Palladium and Gold by sputter coating (SC 7620) (Bozzola and Russell, 1999).

Preparation of specimens for histomorphological studies

For LM after fixation, samples were soak rinsed several times in absolute alcohol, and then embedded in paraffin. Serial 6- μ m transverse sections were cut on a microtome (Leica RM 2145) and placed on glass slide. The slides were then routinely stained with haematoxylin and eosin, van gieson (VG), varo (V), alcian blue (AB), periodic acid schiff (PAS) and PAS-VG (Luna, 1968; Raji and Norouzi, 2010). The mounted specimens were observed by SEM (LEO 1450 VP) at accelerating voltage of 35.0 KV.

Neutral and mucin staining

Determination of neutral mucin was by staining 6 μ m sections with PAS. Briefly, the procedure is:

- 1) Deparaffinize and hydrate to water to eliminate the contribution of sialic acid residues before periodic acid schiff (PAS) staining
- 2) Oxidization in 0.5% periodic acid solution for 15 min
- 3) Rinse in distilled water
- 4) Immerse in Schiff reagent for 30 min (sections become a light pink color during this step)
- 5) Wash in lukewarm tap water for 10 min (immediately sections turn a dark pink color)
- 6) Dehydrate and mount in glass slide

Determination of acid mucin was by staining 6 μ m sections with AB pH = 2.5. In brief, the method is:

- 1) Dissolving dye in 3% acetic acid to provide a solution with pH = 2.5
- 2) Bring sections to distilled water
- 3) Staining in the alcian blue solution pH = 2.5 for 15 min
- 4) Wash well in running tap water 5 min
- 5) Rinse in distilled water
- 6) Counter stain with neutral red stain 1 min
- 7) Rapidly dehydrate in absolute alcohol, clear and mount

Results

The camel stomach was over 1 meter long and comprised three regions. The third region was tubular in shape with its distal part slightly distended. The abomasum in camel was divided to 4 parts consisting of: cardiac, pseudocardiac, fundic and pyloric regions. The cardiac and pseudocardiac regions occupy a wide part of abomasum in this camel and comprise approximately third-fourth of this organ. Gross anatomical study showed small diverticulae in the fundic region, and this part was covered by thick longitudinal folds that were separated by deep branching furrows.

In histological study we observed numerous gastric folds and small invaginations called gastric pits, which are continuous with the gastric glands. The mucosal surfaces, and the gastric pits, were lined with tall simple columnar epithelium (Fig. 1).

Our histochemical study showed that the surface epithelium in abomasum was negative to AB and positive to PAS stainings but in the gastric pit the epithelium was positive to AB and PAS staining, and the gastric gland cells were negative to PAS and positive to AB staining (Fig. 2). Our observations revealed that, four structurally and functionally distinct cells types constitute the secretory epithelium of gastric glands in the abomasum of camel, i.e. mucosal neck cells, chief cells, parietal cells and endocrine cells.

The pyloric glands were branched, coiled, and relatively shorter than the other gastric glands.

The luminal surface of the abomasum in camel was completely covered by mucous. After removing this mucosal layer by

rubbing the mucosal surface with gloved finger we observed that the abomasum in camel was covered by simple tall columnar cells (Fig. 3). The average length of epithelial cell was 20 μm . The epithelial surface was covered with small invaginations called gastric pits that were related to the surface of lumen by foramen (Fig. 4). Many epithelial cells together created continuous crest structures like flower (FB) (Fig. 5). These structures (FB) with mucosa between them were observed as six-sided buildings similar to honeycomb (HC) (Fig. 5). Average diameter of a honeycomb structure was 30-40 μm in the fundic region. In SEM of fundic and pyloric regions we observed a large number of small (637 nm) and large (6263 μm) bacteria (Fig. 5).

In this investigation, for the first time flower body (FB) and honeycomb (HC) structures were identified and explained in

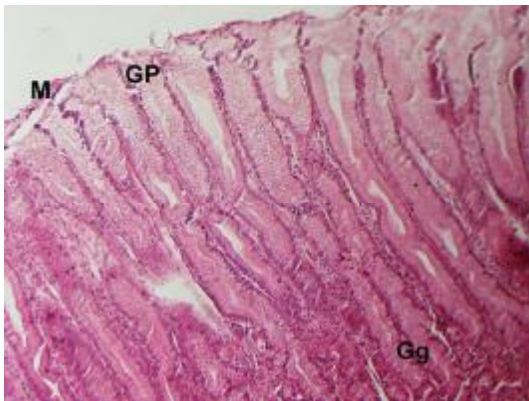


Fig. 1: Mucosal surface of pseudocardiac region, mucous (M), gastric pit (Gp), gastric gland (Gg), (H&E, $\times 160$)

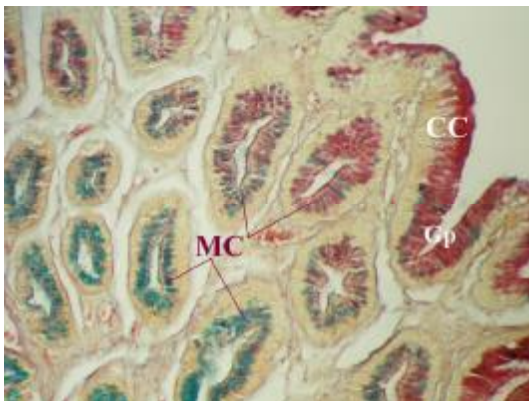


Fig. 2: Mucosal surface of fundic region, columnar cell (CC), gastric pit (Gp), mucous neck cell (Mc), (PAS&AB, $\times 320$)

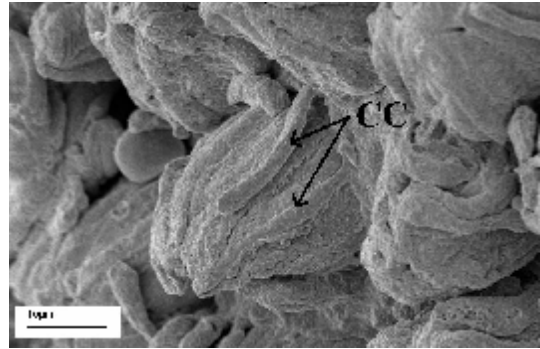


Fig. 3: SEM of cardiac region, columnar cell (CC) of epithelium in cardiac region, ($\times 3000$)

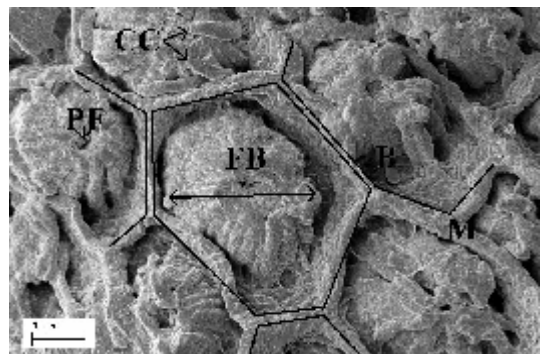


Fig. 4: SEM of fundic region, columnar cell (CC), pit foramen (PF), flower body (FB) and mucous (M), honeycomb (five-sided shape), ($\times 2000$)

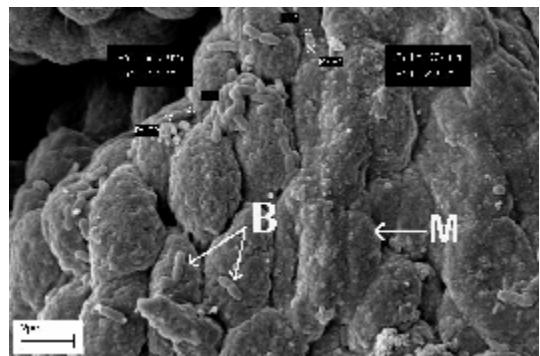


Fig. 5: SEM of fundic region, mucous (M), bacteria (B), ($\times 3000$)

the mucosal surface of abomasum in camel by scanning electron microscopy.

Discussion

The camel stomach is comprised of three regions and its structure differs in some aspects with ruminant. The fundic region of abomasum in camel has diverticulæ which are typical of this animal and are not found in other domestic animals. Histological

study revealed that this part has large gastric glands, and more likely this may indicate that, the camel stomach is more capable of chemical digestion and less so for fermentative digestion as compared to cattle and sheep (Abdel-Magied and Taha, 2003).

The fundic region occupies over half of the stomach in carnivores, over one third in the horse, approximately one quarter in the pig and two thirds of abomasum in ruminant (Eurell and Frappler, 2006). As in the other domestic mammals the surface epithelium in the abomasums of the camel are tall columnar. Our histochemical observation indicated that in the fundic region the neck cells are similar to those of other mammals and produce acid mucins.

Acid mucins secreted by neck cells protect the mucosa from the hydrolytic activities of proteases produced by chief cells and hydrolytic acid produced by parietal cells (Samuelson, 2007).

The cardiac gland region of the mucosa occupies a narrow strip at the junction of the glandular and nonglandular mucosa in camel, which is similar to other domestic mammals except pigs, in which it covers nearly half the stomach, including most of the diverticulum ventricle (Eurell and Frappler, 2006).

Pseudocardiac and fundic gland regions in the camel are simple, branched straight tubular glands that extend to the lamina muscularis and pyloric glands are simple, branched, coiled tubular glands that are relatively short compared to the other gland.

The pyloric region in camel makes up approximately half of the abomasum but in carnivores half, and in horses and ruminants one third of the stomach (Eurell and Frappler, 2006).

Our investigations by SEM in camel revealed that, the same as in cattle, (Kressin and Sommer, 1996), feline (AL-Tikriti *et al.*, 1986), humans (Siew and Goldstein, 1981; Saito *et al.*, 2002) grey, white and black Karakul lambs (Groenewald, 1992) the abomasums are completely covered by mucous.

In this research, for the first time flower body (FB) and honeycomb (HC) structure were observed in the mucosal epithelium by scanning electron microscope. This structure more likely creates enhanced strength in

mucosal epithelium of abomasum in camel.

In SEM study we observed many bacteria in fundic and pyloric regions of abomasum in camel that have probably returned back to fundic and pyloric regions from duodenum. We recommend this method of SEM observation for histological, microbiological and histopathological study of abomasum in other mammals.

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