

## THE STRUCTURE OF THE INTESTINAL VILLI OF THE CAMEL "CAMELUS DROMEDARIUS"

BY

E. M. ABDEL-MAGIED, A. A. M. TAHA and S. A. EL-MOUGY

Department of Veterinary Medicine, King Saud University.  
P. O. Box 1482 buraydah, kingdom of Saudi Arabia.

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### SUMMARY

The intestinal villi of the camel were examined with the scanning electron microscope, light microscope and transmission electron microscope. Scanning electron microscopy showed that the intestinal villi were short nodular or tongue-shaped in the initial parts of the duodenum. In the jejunum and ascending duodenum they were tall finger - or horn- shaped and were characterized by numerous irregular transverse furrows, which apparently increased the surface area of the intestinal mucosa. The intestinal villi in the ileum were short finger - shaped or dome - shaped. Light and transmission electron microscopy revealed that the villous epithelium contained three different cell types; these were the enterocytes, goblet cells and theliolymphocytes (intraepithelial lymphocytes). The enterocytes were tall columnar and characterized by a wide microvillar brush border, basal oval nuclei, apical mitochondria and apico-lateral junctional complexes. There were two types of enterocytes, dark and light enterocytes. The light enterocytes were few and mostly confined to the villous apex. Theliolymphocytes had pale round or undular nuclei and a pale cytoplasm that often contained large spherical dense bodies. The villar core lamina propria contained a central lacteal surrounded by bundles of smooth muscle fibres and an extensive network of subepithelial blood capillaries. The majority of the cellular elements of the lamina propria were lymphoid cells and unspecific immune system cells such as macrophages, granulocytes and mast cells.

### INTRODUCTION

Histological descriptions of the small intestine of normal farm animals are usually incorporated in reports on diarrhea to provide a basis for comparing the morphological changes induced by infective agents (Mebus, Newmann and Stair, 1975). In addition to the facilitation of easy absorption of nutritive materials, the intestinal tract also serves as an effective barrier against bacteria, viruses, toxins and different antigens (Pabst, 1987). The significance of the barrier function of the gut can be realized when the contact area of the small intestine is compared to that of the skin. According to Pabst (1987) the total surface area of the small intestine is 60X the total surface area of the skin. This enormous surface area is provided by a number of mucosal surface modifications such as mucosal folds and villi.

The camel is renowned for its ability to withstand drought and disease (Leese, 1927). Revealing the morphological characteristics of the small intestinal mucosa of the camel may throw light on its absorptive and defense mechanisms. The purpose of this study is to unveil the structural features of the intestinal villi of the camel.

### MATERIALS AND METHODS

Specimens were collected from the duodenum, jejunum and ileum of five camels (*Camelus dromedarius*) 6 months to 2 years old. Fixation was achieved by intraluminal perfusion of cold

5% glutaraldehyde (in cacodylate buffer at pH 7.2) into about 5 cm-long ligated segments of the small intestine. The intestinal segments were opened along their mesenteric attachment, fixed to flat pieces of polystyrene and further immersed in the fixative for 3 to 4 hours. For scanning electron microscopy (SEM) pieces of the small intestine about 1 cm X 1 cm were excised, dehydrated in ethanol, critical point dried with CO<sub>2</sub>, coated in a vacuum evaporator with 40 nm gold (Mebus *et al.*, 1975) and examined in a Joel 50 scanning electron microscope with an accelerating voltage of 10-15kV. Smaller tissue pieces about 0.3 cm X 0.3 cm were post-fixed for one hour in 2% osmium tetroxide, dehydrated in ethanol, cleared in acetone and embedded in epoxy resin (Robinson and Gray, 1990). Semi thin sections (about 0.7  $\mu$ m thick) were stained with 0.15 toluidine blue and examined with the light microscope. Ultrathin sections (60-90 nm thick) were stained with uranyl acetate and lead citrate (Robinson and Gray, 1990) and examined in a Zeiss EM 10c electron microscope.

## RESULTS



Fig. 1: Scanning electron micrograph of the luminal surface of the initial segment of the duodenum showing short villi (V) and orifices of crypts of Lieberkuhn (L). X 500.

Scanning electron microscopy has shown that the luminal surface of the small intestine was thrown into transverse folds and was characterized by numerous villi of different shapes. In the initial segment of the duodenum the villi were short, nodular, finger-shaped or tongue-shaped (Fig. 1). Fused villi were occasionally encountered in this region. In the ascending duodenum the vast majority of the villi were tall and finger-shaped (Fig. 2), whereas in the jejunum they were tall horn-shaped (Fig. 3) or finger-shaped. The villous surface was markedly wrinkled (Figs. 2,3) due to the presence of numerous transverse wavy furrows. In the ileum the villi were shorter and were mostly finger-shaped but a few were stout and dome-shaped. Under high magnifications goblet cells were easily identified being studded with small mucous globules (Fig. 4).

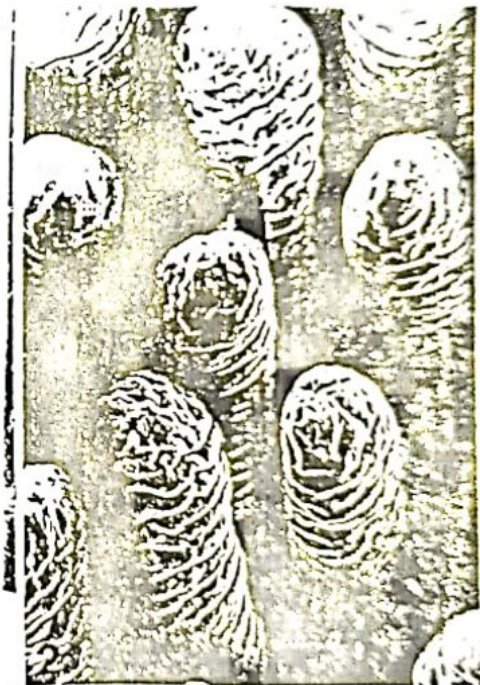


Fig. 2: Scanning electron micrograph showing the finger-shaped villi (V) of the ascending duodenum. Note the irregular villar transverse furrows. X 400.

Light microscopy has shown that the villous epithelium was of a high simple columnar type (Fig. 5). Its apical surface often showed deep furrows whereas the basal surface was usually undular. The epithelium was about 30 to 40  $\mu$ m thick. Most of the epithelial absorptive cells (enterocytes) were dark but a few were pale (light

cells). The number of light cells often increased towards the villous apex. The epithelium of the villous apex infrequently showed degenerate cells (Fig. 6). The nucleus of enterocytes was pale, oval, basally located and showed a few tiny heterochromatin granules. Intraepithelial lymphocytes (theliolymphocytes) were frequently encountered within the villous epithelium (Fig. 5). They were spherical and were characterized by dark spherical nuclei and a pale cytoplasm. The epithelium contained goblet cells that were more numerous in the ileum and jejunum than in the duodenum. Moreover, they were more numerous in the basal parts of the villous than in its apex. The lamina propria (villar core) was made up of a loose connective tissue rich in cellular elements that included fibroblasts, lymphocytes, plasma cells, mast cells, macrophages and eosinophils. In the centre of the villar core a wide lacteal was always present (Fig. 6). Many blood capillaries formed an extensive network just beneath the villous epithelium. They were comparatively wide (up to 30  $\mu$ m in diameter) and were lined by a thin endothelium. Bundles of smooth muscle fibers were usually present beneath the capillary network surrounding the lacteal (Fig. 5).



Fig. 3: Scanning electron micrograph showing the horn-shaped villi (V) of the jejunum. Note the deep villar furrows. X 400.



Fig. 4: High magnification of the tip of an intestinal villous showing two goblet cells (G). X1200.



Fig. 5: Light micrograph showing the tall columnar epithelium of a duodenal villous characterized by a distinct brush border (small arrows) and containing goblet cells (G) and a theliolymphocyte (large arrow). The lamina propria contains many subepithelial blood capillaries (B) and a lacteal (L) surrounded by smooth muscle fibres (M). The apical surface of the epithelium shows deep furrows while its basal surface is undular. X



Fig.6: The tip of a jejunal villous showing a central lacteal (L) and a network of blood capillaries (B). The epithelium contains dark (D) and Light (P) enterocytes. A degenerate enterocyte (large arrow) is seen close to the villous tip. X 600.

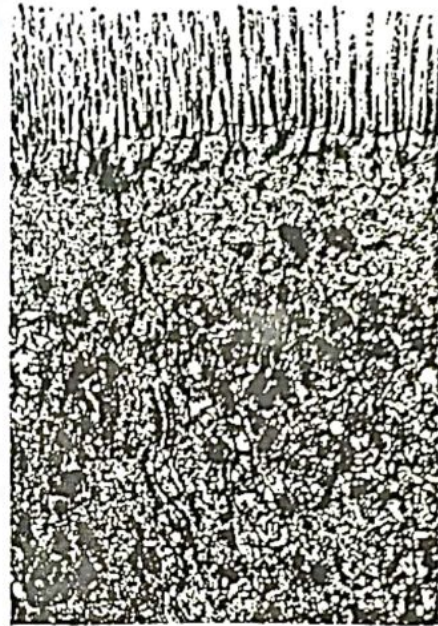


Fig. 8: Transmission electron micrograph showing the apical parts of two contiguous enterocytes characterized by tall microvilli (M) with distinct rootlets (R). The cytoplasm contains many mitochondria (m). J, Junctional complex. X. 13000.



Fig. 7: Transmission electron micrograph of the villous epithelium containing many enterocytes furnished with tall microvilli (M), a goblet cell (G) and many theliolymphocytes (T) . N, nucleus of enterocyte. X 7000.



Fig. 9: Transmission electron micrograph showing enterocytes (E) from the villar base characterized by numerous dark mitochondria. There is also a theliolymphocyte (T) containing many organelles including a dense body. N, nucleus of the theliolymphocyte. X 7000.

Transmission electron microscopy revealed three villar epithelial cell types; these were the goblet cells, enterocytes and theliolymphocytes. The goblet cell nucleus was irregularly oval or triangular (Fig. 7) and showed moderate to sparse peripheral heterochromatin condensations. The most prominent feature of goblet cells was the numerous large (0.5-1  $\mu$ m) apical mucous globules which were mildly electron dense. Infrequently the core of the globule was highly electron dense. The enterocytes (intestinal absorptive cells) were characterized by numerous apical microvilli (Figs. 7,8) with rootlets and terminal web. Rootlets and terminal web were more distinct in cells located towards the tips of the villi. The microvilli were uniform and tall, particularly towards the villar apex. A sparse glycocalyx was always present on top of the microvilli. The nucleus of enterocytes was often ovoid and basal with few peripheral heterochromatin condensations. The cytoplasm contained organelles that included mitochondria, polysomes, rough endoplasmic reticulum, lysosomes and Golgi complexes. The mitochondria were mostly confined to the apical regions of enterocytes (Fig. 9). They were dark elongate oval with transverse or oblique cristae. Their matrix often contained a few small electron dense granules. In addition to the mitochondria the apical cytoplasm contained a few polysomes and free ribosomes. Junctional complexes comprising zonulae occludens, zonulae adherens and desmosomes were invariably present between adjacent enterocytes.

Theliolymphocytes had a pale large round or undular nucleus which often showed dense peripheral heterochromatin. The cytoplasm was pale (Figs. 7, 9) and contained organelles that included a few small dark mitochondria, a few cisternae of rough endoplasmic reticulum, many free ribosomes and an occasional large spherical dense body.

The cellular elements of lamina propria namely fibroblast, lymphocytes, plasma cells, macrophages and mast cells showed their classical ultrastructural features.

## DISCUSSION

The results of this study have shown more or less marked differences in the SEM appearance of the villi between the different parts of the small intestine of the camel. The presence of very tall villi in the jejunum and ascending duodenum indicates that the surface area is largest in these regions of the small intestine. This is in line with the observations of Mebus, et al. (1975) and Landsverk (1979) in cattle. The large surface area created by the tall slender villi of the jejunum and ascending duodenum suggests that intestinal absorption is greatest in these regions. Obviously the deep furrows and wrinkles seen by SEM in the villous surface of the camel greatly increase the intestinal mucosal surface area and are probably a device that facilitates rapid absorption of water.

The LM and SEM appearance of the intestinal villi of the camel was in general similar to that of other mammalian species (Trautmann and Fiebiger, 1957; Mebus, et al., 1975; Shiner, 1983, Leeson, Lesson and Paparo, 1985). The epithelial height (30 to 40  $\mu$ m) was comparable to that of other mammals such as the cat (Henry and Al-Bagdadi, 1986), but the brush border was much wider in the camel (3  $\mu$ m) than in other mammals (1.5  $\mu$ m) such as man and cat (Lesson et al., 1985; Henry and El-Bagdadi, 1986). This suggests that the surface area per unit area of the intestinal epithelium is larger in the camel than in these other mammalian species. The presence of numerous mitochondria in the apical region of the camel enterocytes is suggestive of active digestion and absorption (Shiner, 1983). The presence of a few degenerate and many pale enterocytes in the apices of the intestinal villi of the camel is in accordance with the fact that enterocytes differentiate from stem cells at the base of the intestinal crypt and that they become mature and then degenerate as they migrate towards the villous apex (Shiner, 1983).

The theliolymphocytes (intraepithelial lymphocytes) of the camel intestine were similar to those noted in man (Dobbins, 1986; Pabst, 1987). The large spherical dense bodies present in their cytoplasm are comparable with the

azurophilic metachromatic granules seen by LM in intraepithelial lymphocytes of the human intestine (Pabst, 1987). Because of these granules and certain other morphologic characteristics, it has been proposed that theliolymphocytes are natural killer cells (Pabst, 1987). In man, however, intraepithelial lymphocytes are mainly T lymphocytes and more than 80% have the characteristics of suppressor cytotoxic lymphocytes whilst 10 to 20% express the T helper phenotype (Selby, Janossy, Bofill and Jewell, 1983; Pabst, 1987). Intraepithelial lymphocytes probably have a regulatory function in suppressing the systemic immune response to gut antigens (Dobins, 1986).

The profound network of blood capillaries present in the lamina propria of the intestinal villi of the camel and their location just beneath the epithelial lining, ensure rapid passage of water and digested food from the intestinal lumen into the blood circulation. The contraction of the smooth muscle fibres that surround the lacteals causes the villous to shorten and aids in pumping the lymph out of the lacteal into the lymphatic plexus below (Stinson and Calhoun, 1987). The vast majority of cellular elements of the villous lamina propria of the camel were lymphoid cells and unspecific immune system cells such as macrophages and granulocytes. It has been found in man that the majority of lymphocytes in the intestinal lamina propria are T helper lymphocytes whereas the vast majority of plasma cells are IgA positive, a few being IgM and IgG positive (Dhesi, Marsh, Kelly and Grove, 1984; Pabst, 1987). Intestinal mucosal macrophages have been reported in many mammalian species (Stinson and Calboun, 1987); Pabst, 1987). They are essential in degrading macromolecules and also initiate immune responses by presenting antigens to lymphoid cells (Le - Fevre, Hammer and Joel, 1979). It may then be concluded that the intestinal mucosa of the camel facilitates easy absorption of water and nutritive material by the extensive surface area provided by folds, villi, furrows and microvilli, and an extensive subepithelial network of blood capillaries. At the same time it acts as barrier against antigens due to the presence of junctional complexes, theliolymphocytes and propriat immune system cells.

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