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SCANNING ELECTRON AND LIGHT MICROSCOPY OF THE MUCOSA OF THE EQUINE ILEOCAECAL JUNCTION

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ABSTRACT

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The ileocaecal junction mucosal surface morphology of 5 horses and 1 donkey was examined using scanning electron microscopy and light microscopy. Based on differences in surface morphology, the junction could be divided into 4 distinct regions. The distal extremity of the ileum was characterized by the presence of typical finger-like villi followed by a narrow zone displaying short, stubby villi which gradually merged into a 3rd region representing the true transitional zone between the small and large intestine. In this region the villi fused, forming low ridges arranged in circular, semi-circular or S-shaped patterns. Many of the fused villi were indented at the tips giving these structures a doughnut-like appearance. The caecal portion of the ileocaecal junction formed the 4th region and was characterized by the absence of villi and the occurrence of regularly spaced crypt openings each surrounded by a slightly raised circular mound.

INTRODUCTION

Scanning electron microscopy (SEM) has been widely used in studies of the surface morphology of the intestinal mucosa of man and domestic animals (De Ocampo, Nolasco & Bundac, 1983; Roberts & Hill, 1974; Wille, 1975). The effects of hypoxia on the small intestinal mucosa in ponies has also been investigated using this technique (White, Moore & Trim, 1980; Moore, White, Trim & Garner, 1980).

Changes in surface morphology that occur between the small and large intestine at the ileocaecal junction of the equine have not been studied. The purpose of this paper is to describe the surface features of the transitional region in normal horses and a donkey as revealed by SEM and light microscopy (LM).

MATERIALS AND METHODS

Animals

Tissues were collected from 5, clinically healthy, 4–7 year old thoroughbred horses of either sex and a 5 year old female donkey. They were anaesthetised with 20 % chloral hydrate and exsanguinated after the left common carotid artery had been catheterised. The ileocaecal junction together with the adjacent regions of the ileum and caecum of each animal was removed immediately after death and opened longitudinally along the mesenterial border.

Light microscopy

Longitudinal strips, 5 mm wide and 8 mm long of the ileocaecal junction, including the very distal portion of the ileum, the junction and the caecum immediately adjacent to the junction were fixed for 8 h in Bouin's solution. The specimens were rinsed in 70 % alcohol, routinely processed, cut at a thickness of 5 µm, and stained with haematoxylin and eosin.

Scanning electron microscopy

Specimens were collected from the animals as described for light microscopical examination, after which the mucosa was carefully dissected from the underlying muscle layers and washed in a jet of physiological saline to remove blood, mucus and attached debris. Specimens were fixed by immersion in 4 % gluteraldehyde in Millonig's phosphate buffer. To prevent folding of the tissue and to facilitate trimming of excess material, specimens were temporarily pinned on a wax sheet in a pool of fixative. After 20 min fixation, the tissue samples were further fixed for 24 h at 4 °C in glass bottles containing fresh fixative. In an attempt to remove mucus from the surface of the tissue, some of the specimens were subsequently treated with a 0,2 M sucrose solution in 0,1 M phosphate buffer for a further 24 h (Ludwig, Patek, Nilsson, Metzger & Hafez, 1975).

Specimens were dehydrated through an ascending ethanol series and transferred to the chamber of a Polaron E 3000 critical point drying apparatus. Samples were critical point dried using carbon dioxide as the transitional fluid, mounted on aluminium stubs using double-sided tape, earthed to the stub with colloidal silver, and coated with carbon and gold in a sputter coater prior to examination in a Hitachi S450 electron microscope operated at 15 KV.

RESULTS

On SEM and light microscopy, 4 distinct regions reflecting differences in mucosal surface morphology, were observed along the length of the ileocaecal junctions examined.

The distal part of the ileum adjacent to the ileocaecal junction displayed long finger-like villi which tapered slightly towards the tips (Fig. 1a & 3a). The surface of the villi presented a corrugated appearance due to horizontal or transverse clefts which did not completely encircle the villi (Fig. 1a).

This region was followed by a narrow zone (measuring between 1 – 2,2 mm in length) where the villi abruptly became shorter, stubbier, and slightly flattened in cross section, with the tips of the villi having a definite rounded appearance on SEM (Fig. 1b). On LM these villi were shorter and wider compared to villi in the distal part of the ileum (Fig. 3a & 3b).

This area gradually changed into a 3rd region which seemed to represent the true transitional zone between the small and large intestine. It varied in length from 17,5 – 30,1 mm in the horses, and 0,8 – 1,2 mm in the donkey. The villi were shorter than those in the 2nd region and fused to form low ridges in a circular, semi-circular or S-shaped fashion (Fig. 2b). The tips of the fused villi were often indented to form conspicuous pits which caused the villi to have a doughnut-like appearance (Fig. 2a, 2c, 2d & 2e). Sagittal sections of this region revealed that these pits did not extend to any great depth (Fig. 2d, 2e; Fig. 3b, 3c).

The transitional zone continued into the caecal portion of the ileocaecal junction, where the villi disappeared completely (Fig. 3d), and the mucosa

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FIG. 1a Ileal part of the ileocaecal junction showing typically long, finger-like villi having a corruguated appearance due to irregular horizontal clefts or furrows (arrow).

FIG. 1b Shorter, stubbier and slightly flattened appearance of villi adjacent to the true junction of ileum to caecum.

FIG. 1c Caecal portion of the junction, showing a crypt opening (single arrow), surrounded by a slightly elevated mound (double arrows), and circular groove (triple arrows).

FIG. 1d A low magnification of the caecal mucosa adjacent to the junction, showing the evenly spaced crypt openings (one of which is marked with an arrow), and the inter-cryptal areas forming slight circular mounds around each opening. The folded appearance of the mucosa may be due to muscular contraction or the effect of fixation.

formed well developed folds (Fig. 1d). Openings of the crypts of Lieberkuhn were visible on the surface at regular intervals (Fig. 1c & d) and the inter-cryptal areas were slightly raised in a circular fashion around each crypt opening (Fig. 1d). On higher magnification, these raised areas seemed pushed together in some places, forming a groove around each mound (Fig. 1c).

DISCUSSION

Three major types of villi have been described in the small intestine of humans and other species, namely, finger-shaped, tongue-shaped and leaf-like villi (De Ocampo *et al.*, 1983; Marsh & Swift, 1969).

Long, finger-like villi identified in the ileal part of the ileocaecal junction in this study are similar to the

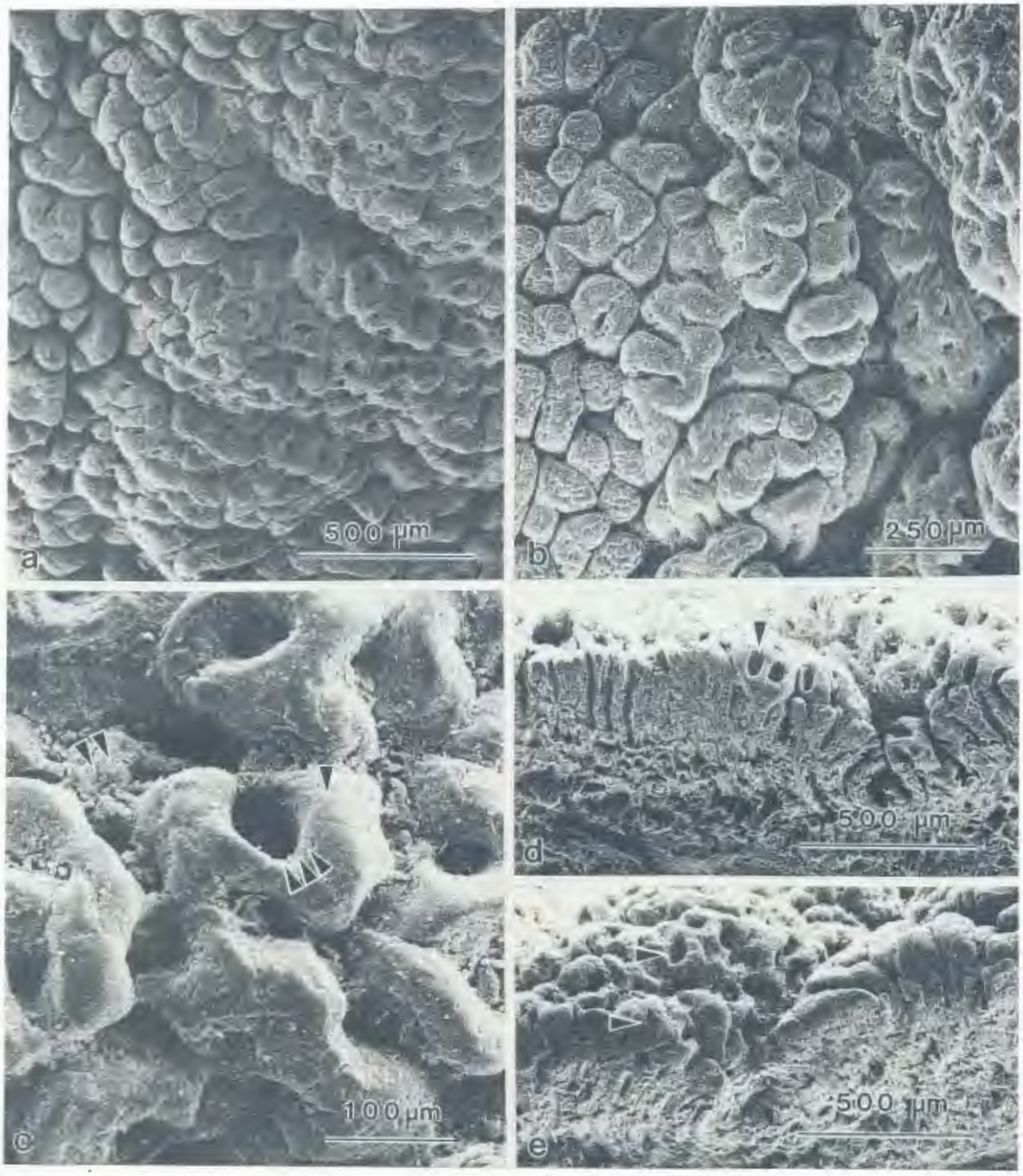

FIG. 2a The true ileocaecal junctional area, showing circular, semi-circular and S-shaped, ridge-like villi on the left, and characteristic doughnut-shaped villi on the right.

FIG. 2b A slightly higher magnification of the ridge-like villi found in the junctional area. The region of short, stubby villi is visible in the left margin of the micrograph.

FIG. 2c The true junctional area, showing doughnut-shaped appearance of the villi (arrow), caused by their indented tips (tripple arrow), and the openings of the crypts of Lieberkuhn covered by debris (double arrow).

FIG. 2d & e Sagittal views of the true junctional area showing doughnut-like villi (arrows).

villi observed in the small intestine of the horse (Roberts & Hill 1974), man (Leeson, Leeson & Paparo, 1988), calf (Mebus, Newman & Stair, 1975) and dog (Hoskins, Henk & Abdelbaki, 1982). Stubby, flattened villi detected in the second region of the ileocaecal junction can be compared with a variation of the finger-shaped villi noticed in the human small intestine (Marsh & Swift, 1969). Irregular transverse furrows on the villous surfaces noted in this study have also been reported the small intestine of clinically normal horses (Roberts & Hill, 1974) and ponies (Moore *et al.*, 1980; White *et al.*, 1980). Similar furrows have also been reported in the human small intestine (Marsh & Swift, 1969) and are due to the contraction of smooth muscle elements in the villous core (Neutra & Padycula, 1983).

Low circular, semi-circular and S-shaped ridges, formed by the fused villi in the transitional area between the ileum and caecum, seen in this study, can be compared to the ridge-like villi observed in the small intestine of the Philippine swamp buffalo (De Ocampo *et al.*, 1983) and in pigs (Mouwen, 1971). In addition, bridged and convoluted villi have been de-

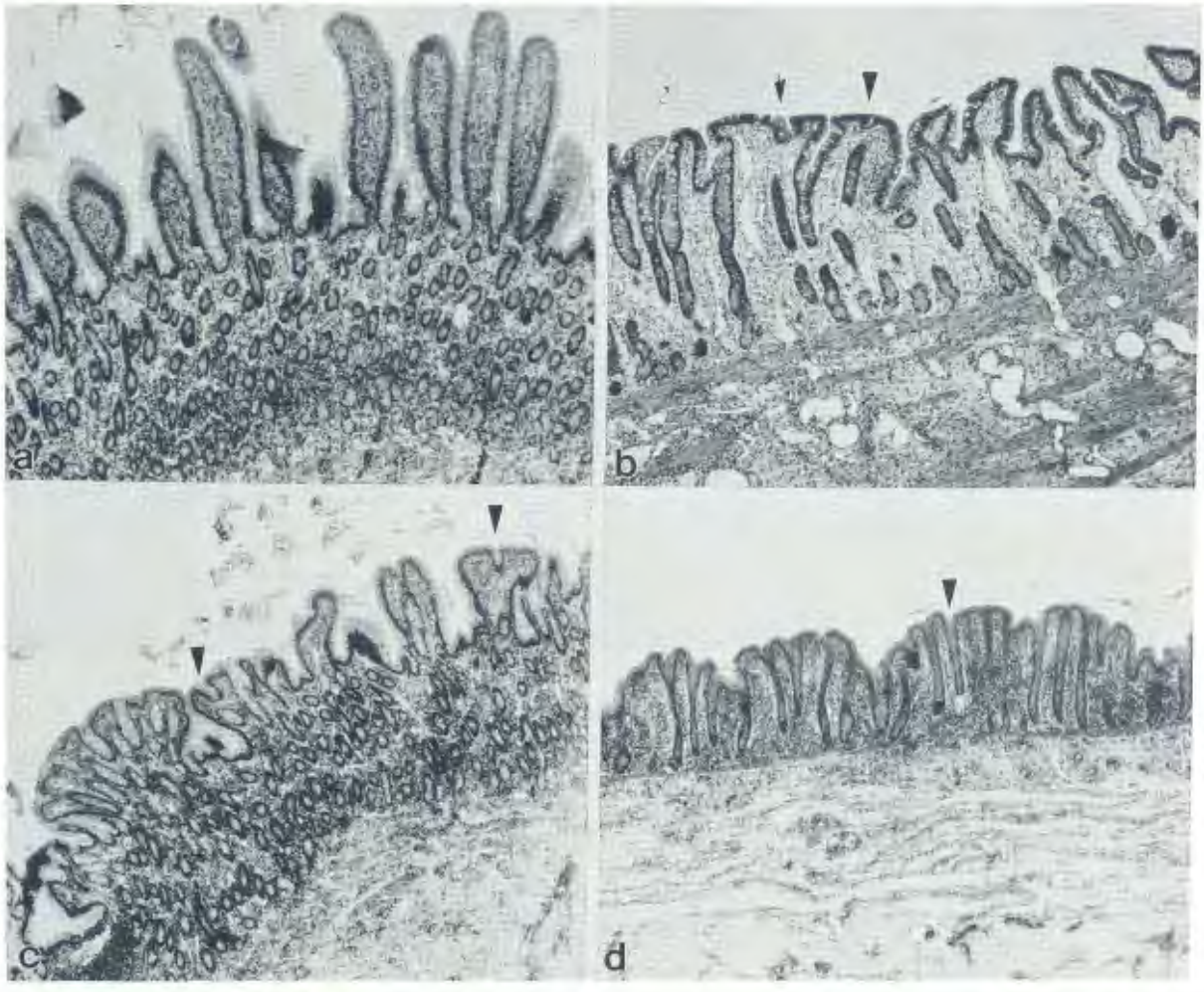

FIG. 3a Finger-like villi of the ileal portion of the ileocaecal junction on the right, changing abruptly to shorter, stubbier villi on the left: ×55.

FIG. 3b The region of short, stubby villi is seen on the right, continuing into the true transitional area on the left with doughnut-like villi (arrows): ×40.

FIG. 3c The true ileocaecal junctional area. Arrows indicate doughnut-like villi: ×40.

FIG. 3d The mucosa of the caecal portion of the ileocaecal junction, showing the characteristic absence of villi and the presence of crypt openings (one of which is indicated with an arrow): ×35.

scribed in the jejunum of the pig (Mouwen, 1971) and bifurcated and bridged villi have been observed in the calf (Pearson, McNulty & Logan, 1978). However, the occurrence of doughnut-like villi in the transitional region in this study, appears to be a unique phenomenon. The shortening and different appearance of villi at the ileocaecal junction can be seen as transitional stages in the gradual disappearance of villi from the ileum to the caecum.

In a SEM study of the horse caecum Wille (1975) notes that the openings of the crypts of Lieberkuhn are equally spaced but he does not described the slightly raised circular mounds around the crypt openings seen in this study. Berry & Evers (1981) describe bulbous nodular projections in the wistar rat colon which, although larger, are of similar design to those seen in this study. The circular grooves found around the cryptal mounds show some similarity to repeating circular units seen around individual crypt openings in a SEM study of cultured rat colon epithelial cells (Schiff, Moore & Ketels, 1980). These grooves were not a constant feature in the present study and may be due to folding, shrinking or compression of the caecal mucosa.

The scanning electron microscope is now widely used in the early diagnosis of diseases that cause changes in the normal mucosal architecture of the intestine (Siew, 1983; Traynor, Costa, Blumgart & Wood, 1981). On SEM and light microscopy, the changes that occur in the mucosa of the normal ileocaecal junction of the horse is described, and an anatomical basis is provided for research in pathology involving this segment of the intestinal tract.

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