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A Comparison of the Structure of Granular Cell Type in the Intestinal Epithelium of the Camel with Paneth Cells of the Rabbit

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Abstract. The epithelium of the small intestine of the camel and rabbit was examined with both the light and electron microscope. Typical short pyramidal Paneth cells were found in the base of the intestinal crypts of the rabbit. They were characterized by apically-situated, large (1.65 cm) spherical, eosinophilic cytoplasmic granules. Electron microscopy revealed electron-dense apical granules and many basally-located cisternae of rough endoplasmic reticulum (rER). In the intestinal epithelium of the camel, typical Paneth cells were not identified in haematoxylin and eosin or safranin-stained sections. Granule-containing cells were, however, easily identified in toluidine blue-stained semi-thin sections, and under the electron microscope. They resembled Paneth cells in that they were confined to the intestinal crypts and characterized by apical cytoplasmic granules. They differed from Paneth cells of the rabbit and other mammals in several aspects. They were tall columnar cells with small (0.44 um) apical cytoplasmic granules and a few basally-situated cisternae of rER.

Introduction

The intestinal tract serves two important functions: on the one hands, it facilitates easy absorption of nutritive materials, on the other it acts as a barrier against micro-organisms, toxins and different antigens [1]. This barrier includes lymphocytes, plasma cells, macrophages and Paneth cells [1]. Paneth cells which are characterized by prominent apical cytoplasmic granules, are confined to the epithelium of the base of the small intestinal crypts of Lieberkühn [2 – 5; 6, pp. 5 – 58]. Many important functions have been attributed to Paneth cells; these include production of lysozyme [7 – 10], phagocytosis of microorganisms [11], elimination of heavy metals [2; 12], secretion of trypsin [13] and production of defensin [14]. Paneth cell secretion is

induced by cholinergic muscarinic secretagogues such as bethanechol and also by neuroblocking agents such as tetrodoxin [15]. Lysozyme, immuno-globulin A [16] and defensin, which are produced by Paneth cells, are considered to play a significant role in the control of the bacterial milieu of the intestine [15], thus reducing infection. In line with this is that equine Paneth cells show severe degranulation during acute alimentary laminitis, which is characterized by dramatic intestinal bacterial proliferation [17].

The structure of Paneth cells has been studied in man [16; 18] and small mammals, such as the mouse [15; 19 – 22] rabbit [23] and rat [24 – 27]. In the domestic animals, Paneth cells are reported to be present in the ox, sheep and goat but are absent in the dog, cat and pig [28, p. 247; 29, p. 205]. Apart from a preliminary report of this study [30], there seems to be no information available on Paneth cells or apical granule-containing cells in the intestinal epithelium of the camel. The purpose of this study is to reveal the structural characteristics of these cells and compare them to Paneth cells of the rabbit.

Materials and Methods

Five camels (4 to 8 months old) and five rabbits (4 to 6 months old) were used for this study. All animals were clinically healthy. Each camel was anaesthetized by injecting 700 to 800 ml of 2.5% chloral hydrate into the jugular vein, whereas the rabbits were anaesthetized by ether inhalation. The abdominal cavity was opened by a midventral incision, about 4-cm long segments of the different parts of the small intestine were ligated at both ends, and 5% glutaraldehyde in cacodylate buffer (pH 7.2) was injected into the lumen of each segment. The segments were excised and immersed in 5% buffered glutaraldehyde (2 h) and then cut into small pieces. For routine light microscopic examination, six tissue blocks from each animal were processed for paraffin sections and stained with haematoxylin and eosin (H & E) or safranin stain. For electron microscopy, nine tissue blocks from each animal were post-fixed in 2% osmium tetroxide (1 h) and processed routinely for epoxy-resin embedding. Semi-thin sections were cut, stained with toluidine blue, and examined with the light microscope. Ultrathin sections of the appropriate areas were cut, stained with uranyl acetate and lead citrate [31, p. 556], and examined with the electron microscope at a voltage of 75 kV.

Results

Light microscopy

In H & E or safranin-statined sections, Paneth cells of the rabbit were easily identified as short pyramidal cells characterized by prominent eosinophilic or safraninophilic granules. They were confined to the base of the crypts of Lieberkühn and were always more numerous in the distal than in the proximal parts of the small

intestine. Their granules were large, spherical and were usually confined to the apical half of the cell (Fig. 1). Paneth cells of the rabbit were also easily recognised in toluidine blue-stained semi-thin sections. On the other hand, typical Paneth cells were not seen in H&E or safranin-stained sections of the small intestine of the camel. Many tall columnar cells with fine, faintly acidophilic granules were seen in the crypts of Lieberkühn of the different parts of the intestine. These granular cells were more easily recongnized in semi-thin sections stained with toluidine blue (Figs 2 and 3). They were tall cells with basally-located oval nuclei and apically-located small, discrete bluish granules. These cells are referred to as granule-containing cells.

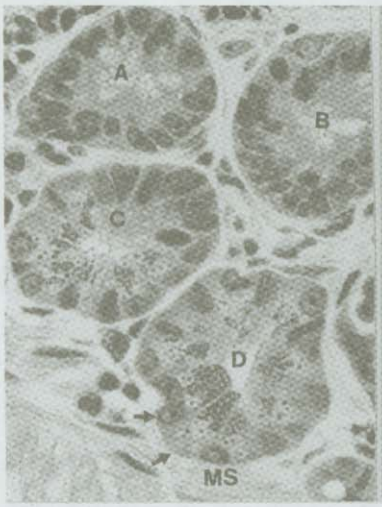

Fig.1. Light micrograph of the basal part of the jejunal mucosa of the rabbit showing cross sections of the basal parts of four intestinal crypts (A,B,C,D). Note that Paneth cells (arrows) which are characterized by apical cytoplasmic granules are seen in the very basal sections (C and D) only. MS, muscularis mucosa. H&E. X 500.

Fig.2. Light micrograph showing part of an intestinal crypt of the camel duodenum. It shows a number of presumptive Paneth cells (P) which are tall columnar cells with small cytoplasmic granules confined to the cell apex. The basal part of the cell appears wider than elsewhere. G, goblet cell. Semithin section stained with toluidine blue. X 1200

Fig. 3. The light micrograph shows longitudenal sections of three intestinal crypts from the jejunum of the camel. The luminal surfaces of these crypts appear finely granular (arrows) due to the presence of numerous presumptive Paneth cells. Note that the presumptive Paneth cells of the middle crypt are seen even as high as the level of the 30th cell from the crypt base. Semithin section stained with toluidine blue. X 250.

Electron microscopy

The nucleus of the Paneth cells of the rabbit showed evenly dispersed fine chromatin; heterochromatin condensations being rarely encountered (Fig. 4). The cytoplasm of the rabbit Paneth cell was rich in organelles. In the basal half, it contained many cisternae of rough endoplasmic reticulum (rER) that were either flattened or moderately distended with a floccular material (Fig. 5). A Golgi complex was occasionally encountered in the supranuclear region. The cytoplasm of the apical half of the Paneth cells of the rabbit showed many large spherical or slightly ovoid granules that were uniformly electron-dense (Fig. 4). A total of 174 granules were measured in three rabbits; their diameter was 1.65 ± 0.25 um (mean$\pm$SD). In addition, the Paneth cell cytoplasm contained a few cisternae of rER and mitochondria. In about 5% of Paneth cell profiles examined in this study, a few small vacuoles and a few small clear vesicles were observed. The apical surface of the Paneth cells was furnished with short microvilli, but otherwise showed no protrusions. Junctional complexes comprising zonulae occludens, zonulae adherens and maculae adherens were usually present in the apical parts of the epithelium, joining the lateral cell membranes of Paneth cells with those of neighbouring cells.

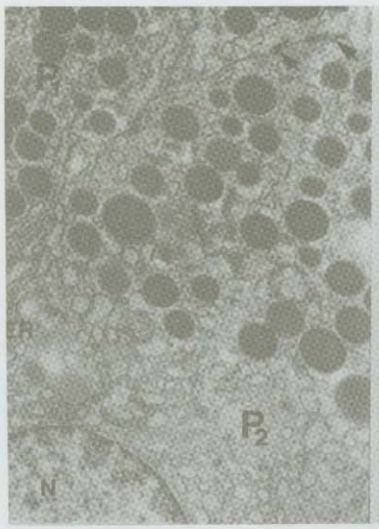

Fig.4. Electron micrograph showing apical parts of two adjacent Paneth cells (P1, P2) from the duodenal crypts of the rabbit. The nucleus (N) of P2 shows finely granular chromatin. The apical region in both cells is characterized by large (1-2 μm) spherical electron dense cytoplasmic granules. rER, rough endoplasmic reticulum; arrows, junctional complex. X 6200.

Fig.5. Electron micrograph showing the basal part of a Paneth cell from a jejunal crypt of the rabbit. The cytoplasm of the very basal part is fully occupied by cisternae of rER; some of which are distended with a floccular material (stars). A few electron dense cytoplasmic granules are also present. Arrows, basal lamina. X 14400.

In the epithelium of the intestinal crypts of the camel tall columnar granule-containing cells showing comparatively small apical granules were present (Fig. 6). A total of 189 granules were measured in three camels; their diameter was 0.44±0.18 um (mean±SD). The basal part of the cell which contained the nucleus was usually wider than the rest of the cell. The nucleus itself was oval and usually showed large peripheral heterochromatin condensations (Fig. 7). The cytopalsm of the basal part of the cell contained a few or moderate amounts of the flattened cisternae of rER, in addition to many polysomes and a few slender mitochondria. The middle part of the cell contained a few cisternae of rER (Fig. 7), many polysomes and occasionally heterophagosomes (Fig. 6) and Golgi complexes. The cytoplasm of the apical part of

Fig.6. The electron micrograph shows two adjacent presumptive Paneth cells (P1, P2) from the ileum of the camel. The cells are tall columnar and contain small cytoplasmic granules in their apices. The basal part of P1 appears wide than the overlying parts. N, nucleus of presumptive Paneth cell; G, Goblet cell, Arrow, heterophagosome. X 3000.

Fig.7. Electron micrograph showing the perinuclear and middle parts of three adjacent presumptive Paneth cells from the jejunum of the camel. The nucleus (N) is oval and shows peripheral heterochromatin condensations. The cytoplasm contains ribosomes and a few cisternae of rER (arrows). X 4400.

the cell was studied with electron-dense granules of varying electron density (Figs 8–10). Most of these granules were spherical, whereas a few were ovoid (Fig. 11). In addition, the cytoplasm of this region contained small amounts of microtubules and free ribosomes. Junctional complexes were usually present in-between the lateral cell membrane of this region and those of adjacent cells. The apical cell surface, which was usually furnished with a few microvilli (Fig. 10), was often irregular, showing apical protrusions or blebs (Figs 8 and 9). The protrusions were usually full of granules and often delineated from the rest of the cell by a double membrane (Figs 8 and 9). Occasionally, the protrusions were devoid of granules but their cytoplasm appeared homogenously electron-dense (Fig. 9). The intestinal lumen usually contained some debris (Figs 8 and 9). The apical surface of the granule – containing cell of the intestinal crypts of the camel was sometimes smooth; in such instances the intestinal lumen was usually devoid of debris (Fig. 10).

Fig. 8. The luminal part of a duodenal crypt of the camel. The electron micrograph shows the apices of about eight presumptive Paneth cells characterized by round or ovoid electron dense cytoplasmic granules (large arrows) about 0.2 to 0.8 μm in diameter. The apical surfaces of the presumptive Paneth cells show cytoplasmic protrusions (PR). One of these protrusions is completely delineated (arrow heads) from the underlying cytoplasm. Small arrows, junctional complexes; D, cytoplasmic debris. X 12000.

Discussion

The observations made in this study on the structural features of paneth cells of the rabbit are in line with those already noted by Pitha [23]. The general structural characteristics of Paneth cells of the rabbit conform to those of other mammals [4; 6, pp. 5–58; 18; 22; 28, p. 247] and need no further discussion.

The epithelium of the small intestine of mammals comprises six different cell types. These are the stem cells, enterocytes, goblet cells, M cells (confined to the dome epithelium overlying Peyer's patches), enteroendocrine cells and paneth cells [1; 6; 28, pp. 244–248; 32, p. 350]. Apart from enteroendocrine cells and Paneth cells,

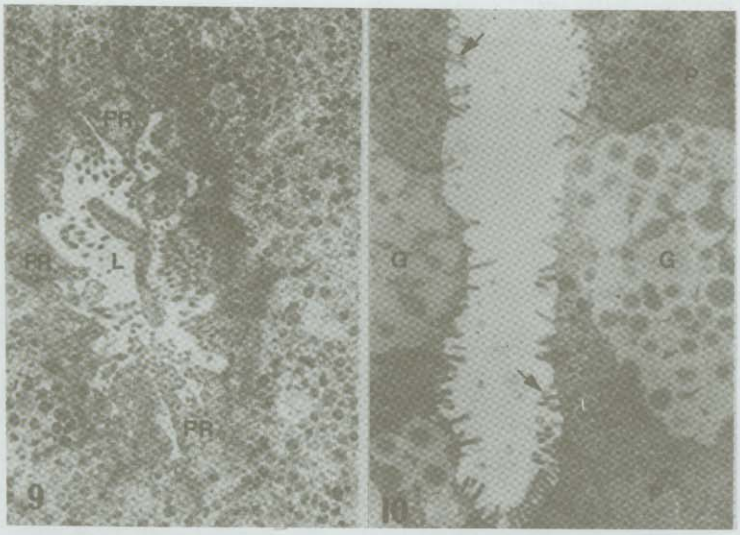

Fig.9. The electron micrograph shows the lumen (L) of an intestinal crypt of the jejunum of the camel in cross section. The apical parts of the numerous presumptive Paneth cells surrounding the lumen are studded with cytoplasmic granules of varying electron density. The apical (luminar) surfaces of the presumptive Paneth cells show many protrusions (PR). Some of the protrusions (Pra) are electron dense. The lumen contains .... debris. X 6200.

Fig. 10. The electro micrograph shows the luminar part of an intestinal crypt of the jejunum of the camel in a longitudenal section. The lumen is surrounded by presumptive Paneth cells (P) and goblet cells (G). The apical surface of the presumptive Paneth cells is furnished with a few microvilli (arrows) but is otherwise smooth. The crypt lumen is free of cytoplasmic debris. X 5000.

all of these cell types are non-granular. Thus, stem cells, enterocytes, goblet cells and M cells are categorically different from the granule-containing cells seen in this study in the intestinal epithelium of the camel.

The most conspicuous feature of enteroendocrine cells of mammals [33], including those of the camel [34], is that their granules are confined to the basal half of the cell. It appears that the Paneth cell is the only cell type that bears resemblance to the granule-containing cells seen in this study in the intestinal epithelium of the camel. Like Paneth cells, the granule-containing cells are confined to the crypts of Lieberkühn, are characterized by apical cytoplasmic granules, bear a few microvilli, have

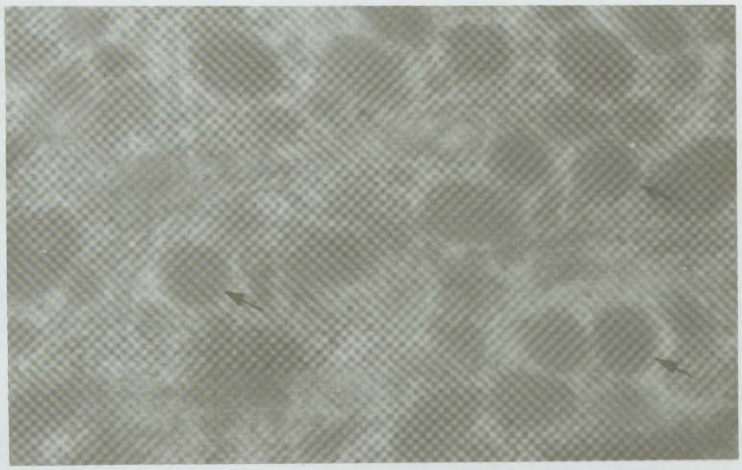

Fig. 11. High magnification electron micrograph showing the electron dense granules of Paneth cells of the camel. The granules are ovoid or spherical and each is delineated by a limiting membrane (arrows). X 35000.

basal rER, and show signs of phagocytic activity (heterophagosomes). They may thus be considered as presumptive Paneth cells. Nevertheless, they display several differences from Paneth cells of the rabbit and other mammalian species.

These difference are summarized in the following points. Firstly, presumptive Paneth cells of the camel are tall columnar cells, whereas those of the rabbit [23] and other mammals [2; 4; 6, pp. 5–58; 28, p. 247] are short pyramidal cells. Secondly, the amount of rER observed in the granule-containing cell of the camel is significantly less than that observed in Paneth cells of the rabbit and other mammals [3; 23; 24]. Thirdly, the secretory granules of these cells of the camel are much smaller, having a diameter of 0.44 um, compared to those of Paneth cells of the rabbit and other mammals, having a diameter of about 1.5 μm [3–5; 23]. As to this point, Satoh and Vollrath [4], and Satoh and Co-workers [5] found that the size of Paneth cells granules is affected by innoculation of bacteria into the intestinal tract, whereas Mathan and Coworkers [3] have demonstrated that it is affected by the stage of maturity of the cell. Nevertheless, the small size of the granules seems to be a characteristic feature of these cells in the camel since all camels used in this study were healthy, and no variations were noticed in the size of the granules of these cells located in different levels of the intestinal crypts.

Finally, the mode of secretion in the granule-containing cells of the camel seems to be different from that of other mammals. The presumptive Paneth cells of the camel show apical cytoplasmic protrusions containing secretory granules. These protruding parts are usually delineated from the underlying cytoplasm. Moreover, cytoplasmic debris is present in the lumen of the intestinal tract of the camel. All these features may be suggestive of an apocrine mode of secretion [32, p. 90; 35, p. 914]. Such features were not observed in Paneth cells of the rabbit (the present study), nor have they been previously reported in Paneth cells of other mammals. In fact, secretion in the mammalian Paneth cells is suggested to take place by the process of exocytosis, *i.e.* merocrine mode of secretion [4; 10].

It is recalled here that the most important function attributed to Paneth cells is their ability to secrete the antibacterial lysozyme [4; 8; 10]. The presence of large numbers of presumptive Paneth cells showing signs of active secretion and phagocytosis of foreign bodies (heterophagosomes), in the intestinal epithelium of the camel, may then indicate a significant role played by these cells in prevention of bacterial invasion through the intestinal tract. However, immunocytochemical studies are needed to confirm whether or not the presumptive Paneth cells of the camel produce lysozyme as do those of other mammals.

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مقارنة بين تركيب نوع من الخلايا الحبيّبية في الطلائية المعويّة للإبل وخلايا بانث في الأرنب

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ملخص البحث. لقد تمّ فحص الأمعاء الدقيقة في كل من الجمل والأرنب بوساطة المجهرين الضوئي والإلكتروني، ووجدت خلايا بانث هرمية الشكل قصيرة نموذجية في قواعد الطويقات المعوية في الأرنب وتميّزت هذه الخلايا بحبيبات هيوليّة قميّة كرويّة كبيرة (١-٢ ميكرون) أليفة للآيوسين، وأوضحت المجهرية الإكترونية أن لهذه الخلايا حبيبات قميّة ذات كثافة إلكترونية عالية إضافة إلى العديد من صهاريج الشبكة الهيولية الداخلية الخشنة (ش هـ خ)، لم يتم العثور على خلايا بانث نموذجية في الطلائية المعويّة للجمل في المقاطع المصبوغة بهيماتوكسلين و أيوسين أو سفرانين ولكن كان من السهل التعرف على خلايا حبيبيّة في المقاطع شبه الرقيقة المصبوغة بأزرق التولويدن كما كان من السهل أيضًا التعرف عليها تحت المجهر الإلكتروني، شابهت هذه الخلايا خلايا بانث في أنها اقتصرت على الطويقات المعوية وفي أنها تميّزت بحبيبات قميّة ولكنها اختلفت عن خلايا بانث في الأرنب والثدييات الأخرى في أوجه عديدة، فلقد كانت عمودية طويلة ذات حبيبات هيوليّة قميّة صغيرة (٠,٢ ـ ٠,٨ ميكرون) وصهاريج ش هـ خ قليلة العدد وقاعديّة الموقع.