

Light and electron microscopy of the pancreas of the Egyptian one-humped camel (*Camelus dromedarius*)

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SUMMARY

The exocrine portion of the pancreas consists of the pancreatic acini and their ducts. Hormones are synthesized by the cells of islets of Langerhans; they include insulin- (β - cells), glucagon- (α -cells), somatostatin- (Δ -cells), and pancreatic polypeptide- (F- or PP-cells) immunoreactive cells. This study discusses the light and electron microscopic architecture of both the exocrine and endocrine pancreas of the dromedary camel.

The cells of the pancreatic acini were pyramidal in shape with large spherical and centrally located heterochromatic nuclei. The basophilic basal and eosinophilic apical regions seen under the light microscope showed concentrated rough endoplasmic reticulum and homogenous electron-dense zymogen granules, respectively, on ultramicroscopy. Abundant elongated numerous mitochondria and Golgi complexes were found in the cytoplasm.

The islets were scattered randomly among the acini and appeared as irregular spherical or oval masses of cells in light microscopy. The islet cells could be identified based on their location, cytological features, density of granules, and the

degree to which the granule matrix was separated from its limiting membrane by an electron-dense area. The peripherally located α -cells had an irregular outline and possessed numerous relatively small membrane-bound granules with a moderate to high density core. Beta cells were larger in size and had fewer granules than of α -cells. Halo areas surrounded the moderate electron-dense granules. The polygonal Δ -cells were found in clumps throughout the islet and in between β -cells. Their granules were of moderate electron density and were tightly enclosed by a limiting membrane. Pancreatic polypeptide cells were seen associated with α and β cells. They were irregular small cells with small granules and dark cytoplasm.

The juxtaposition of the endocrine and exocrine elements of the pancreas can be traced back to their embryological origin and can be also of functional significance. It facilitates the mutual functional interaction of both portions of the pancreas.

Key words: Camel pancreas – Pancreas light microscopy - Pancreas electron microscopy – Pancreatic acinus – Pancreatic islet – Islet of Langerhans cells

INTRODUCTION

The pancreas is a mixed exocrine-endocrine gland that produces digestive enzymes and hormones. The exocrine portion of the pancreas is

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a compound tubuloacinar gland (Frappier, 2007). The secretory acini produce digestive enzymes that are routed to the intestine by a branched ductal network. Hormones are synthesized by the endocrine cells of the pancreatic islets. The histologic and histochemical characteristics of the endocrine part of the dromedary camel's pancreas were discussed elsewhere (Hafez et al., 2015). The islets were identified as compact areas of pale cells with rich vasculature surrounded by more darkly-stained exocrine pancreatic tissue in conventional preparations (Hafez et al., 2015). In the camel, Insulin-immunoreactive cells (β -cells) were seen to be distributed in the center and the periphery of each islet. Beta cells were found in the islets, but also outside the islets between the secretory acini and the interlobular connective tissue. Glucagon-immunoreactive cells (α -cells) were mostly observed as clumps in the peripheral area of the islet. Clumps of small numbers of somatostatin-immunoreactive cells (Δ -cells) were found throughout the islet. A few pancreatic polypeptide-immunoreactive cells (PP-cells) were found throughout the islet.

The rate of glucose clearance in the camel is slower than in other animals (Dahlborn et al., 1992); this may be attributable in part to a weak insulin response to exogenous glucose, which could potentially be a result of a structural difference in insulin-producing cells (Araya et al., 2000; Firshman et al., 2013). Additionally, knowledge of islet microanatomy in diverse mammalian species is of fundamental importance for the evaluation of the general principles underlying the intra-islet regulation of islet hormone secretion (Hafez et al., 2015; Redecker et al., 1992).

The camel's extraordinary tolerance for heat and desiccation may not be directly related to the function of the pancreas, but heat and desiccation can have indirect effects; they are types of stress. Stressors can affect every organ in the body (Bsoul et al., 2013; Pampori et al., 2010). The present study provides baseline morphological information to understanding the physiology of the camel pancreas.

To the best of our knowledge, only one report has dealt with the ultrastructure of the dromedary camel's endocrine pancreas (Bsoul et al., 2013); the ultrastructure of α -, β -, and Δ -cells was discussed in this report, but not that of PP-cells.

The present study discusses the light and electron microscopic architecture of the exocrine and endocrine pancreas of the dromedary camel. The findings presented in this study, together with those presented in our previous study (Hafez et al., 2015), provide a comprehensive report of the light and ultrastructural architecture, as well as the histochemical characteristics, of the pancreas of the single-hump camel.

MATERIALS AND METHODS

Pancreata from five apparently healthy adult dromedaries were collected immediately after slaughter at the Kom Hamada abattoir in El-behera, Egypt. These animals were slaughtered for human food, and consequently had been examined prior to slaughter by the slaughterhouse veterinarian to obtain approval for human consumption. Samples of 1 cm³ were obtained, fixed in 10% buffered formalin and paraffin embedded. Sections 6.0 μ thick were stained with Hematoxylin and Eosin.

For electron microscopy, 1 mm thick samples were obtained from the pancreas, and the specimens were immediately fixed in 6% phosphate buffered glutaraldehyde, pH 7.4 at 4°C; then serially washed in cold (4°C) 0.1 M phosphate buffer, post fixed in 1% osmium tetroxide, processed and then embedded in Araldite epoxy resin (Hayat, 1986). Semithin sections (1 μ m) were cut and stained with toluidine blue, and studied for localization of the pancreatic islets. Ultrathin sections (60-100 nm) were cut and stained with uranyl acetate, followed by lead acetate. The sections were examined and photographed with a JOEL transmission electron microscope at 80 KV.

RESULTS

Light microscopy

The parenchyma of the pancreas was separated into distinct lobules by a connective tissue stroma. Each lobule consisted of numerous tubuloacinar secretory units. Scattered among the secretory units were the functionally distinct pancreatic islets (Fig. 1). The exocrine secretory areas were represented by the pancreatic acini, the basic secretory units, and their duct system. The glandular epithelial cells of the pancreatic acinus were pyramidal in shape with a spherical, more or less centrally located nucleus. The basal basophilic region of the acinar cells was visibly distinct from the apical eosinophilic zymogen granules (Fig. 1).

The cells of each acinus were grouped around a small lumen which connected to an intercalated duct (Fig. 2). This duct began with flattened centroacinar cells. The intercalated duct represented the beginning of a series of increasing caliber ducts which eventually convey their contents to the duodenum via the pancreatic duct. Each intercalated duct led to an intralobular duct, which then led to a larger interlobular duct (Fig. 2). The small intralobular ducts were lined with low simple cuboidal epithelium which transitioned to columnar in the interlobular duct (Fig. 3).

The pancreatic islets were scattered randomly among the acini. They were seen as irregular

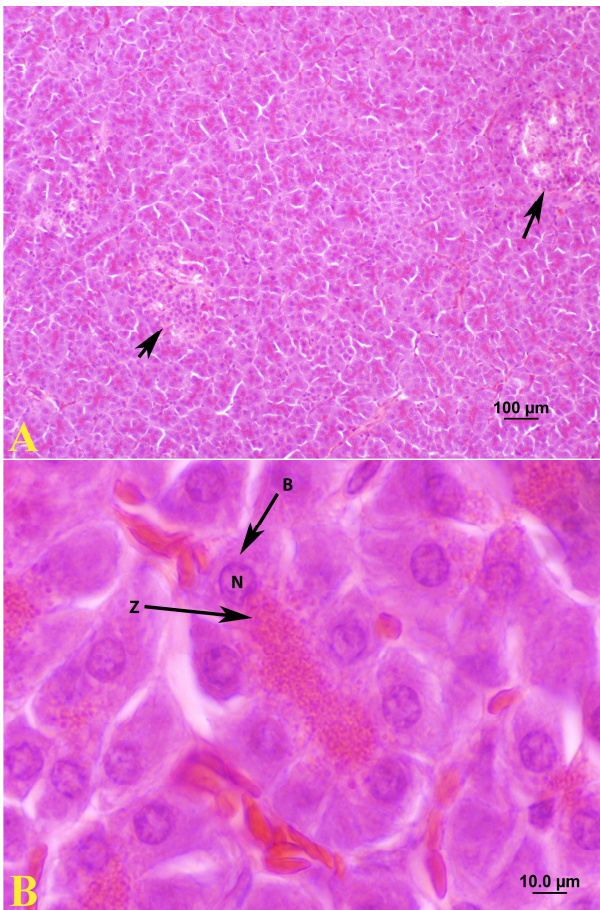


Fig. 1. (A) an overview of the camel's pancreas showing acini forming the exocrine pancreas. Islets of Langerhans (arrows) are scattered among acini. (B) a camel's pancreatic acinus showing at the center of the image with its pyramidal-shaped epithelial cells. The basal basophilic region (B) is distinct from the eosinophilic zymogen granules (Z) at the apical region of the cells. N: nucleus.

spherical or oval masses of cells (Fig. 1).

Electron microscopy

The pyramidal-shaped pancreatic acinar cells had relatively large spherical heterochromatic nuclei with areas of electron density mostly at the periphery (Fig.4). Nucleoli were prominent and occupied an eccentric position in each nucleus. The majority of the cytoplasm was occupied by stacked cisternae of rough endoplasmic reticulum. The apical region of the cells contained zymogen granules which were seen as spherical homogenous electron dense bodies. Abundant elongated mitochondria and Golgi complexes were found in the cytoplasm of each acinar cell.

The acinar lumen was continuous with the intercalated duct, and sometimes it contained amorphous material of moderate electron density, presumably representing the secretory products.

Centroacinar cells could be seen in sections and were continuous with the first order duct sys-

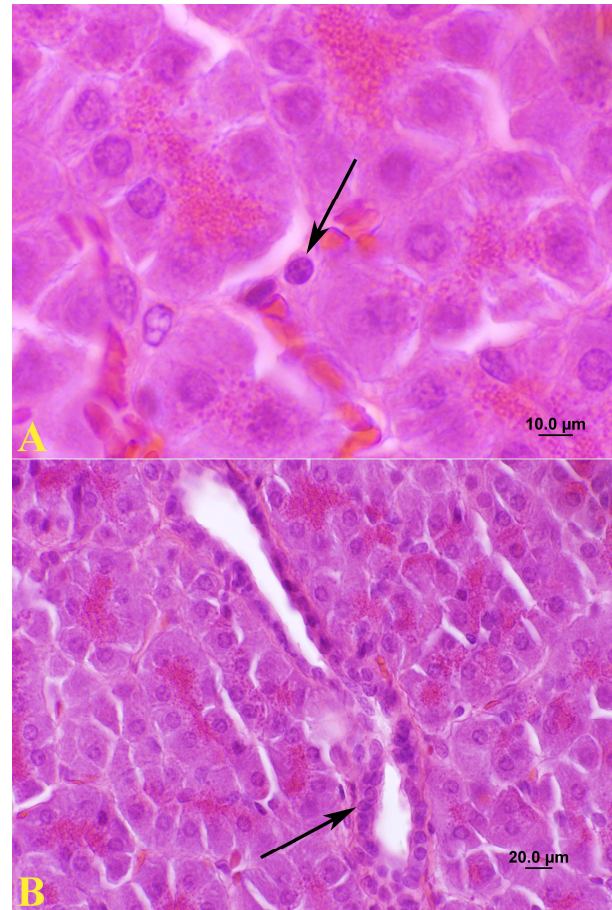


Fig. 2. A camel's exocrine pancreas. (A) the arrow is showing the direction of flow of the enzymatic products from the lumen of an acinus to an intercalated duct. The beginning of this duct (centroacinar cells) is indicated by the tip of the arrow. (B) shows a pancreatic intralobular duct which is lined with low simple cuboidal epithelium (arrow).

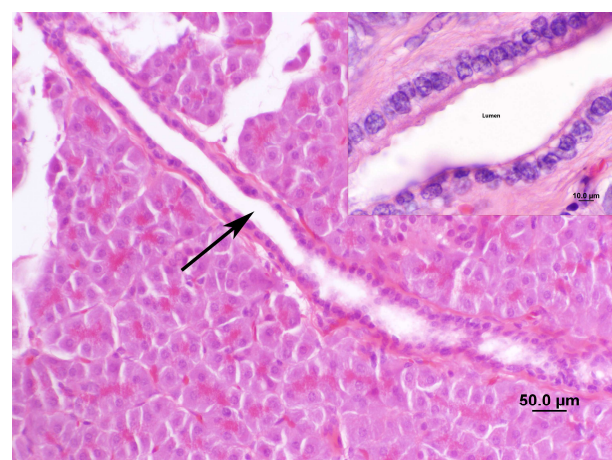


Fig. 3. Portions of two camel pancreatic lobules. An interlobular duct (arrow) is lined with simple columnar epithelium (inset).

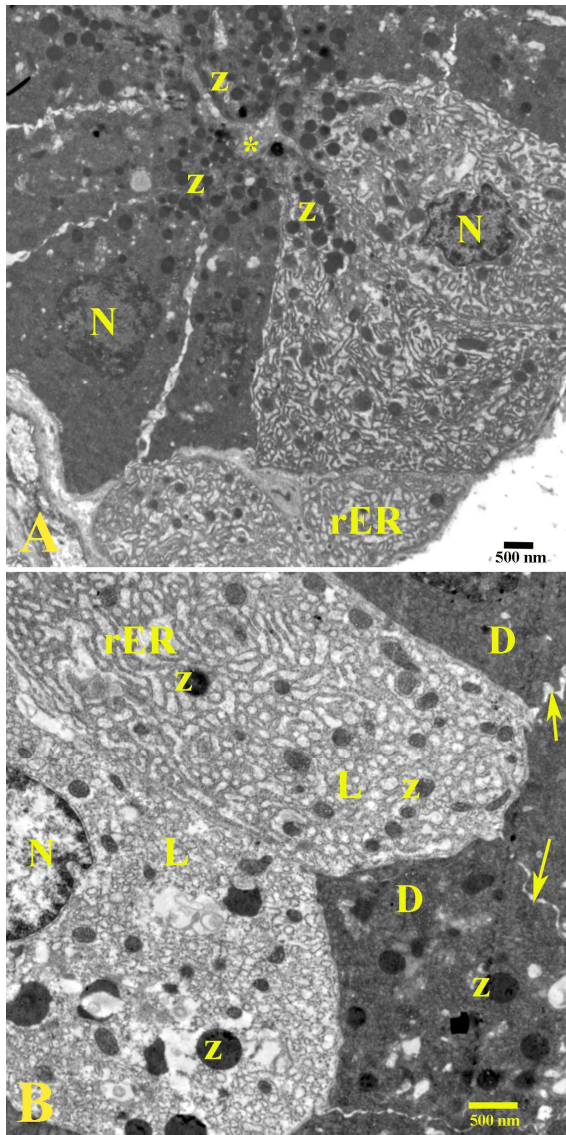


Fig. 4. Transmission electron micrograph of a pancreatic acinus of the dromedary camel showing the light electron-dense (L in B) and dark electron-lucent cells (D in B) that surround the lumen (asterisk) in A. The centrally located nuclei (N) and the apically located zymogen granules (z) are shown in both A and B. Note the abundant rough endoplasmic reticulum (rER) easily visible in the light electron-dense cells. Note the interdigitation between the lateral borders of the dark cells (arrows) in B.

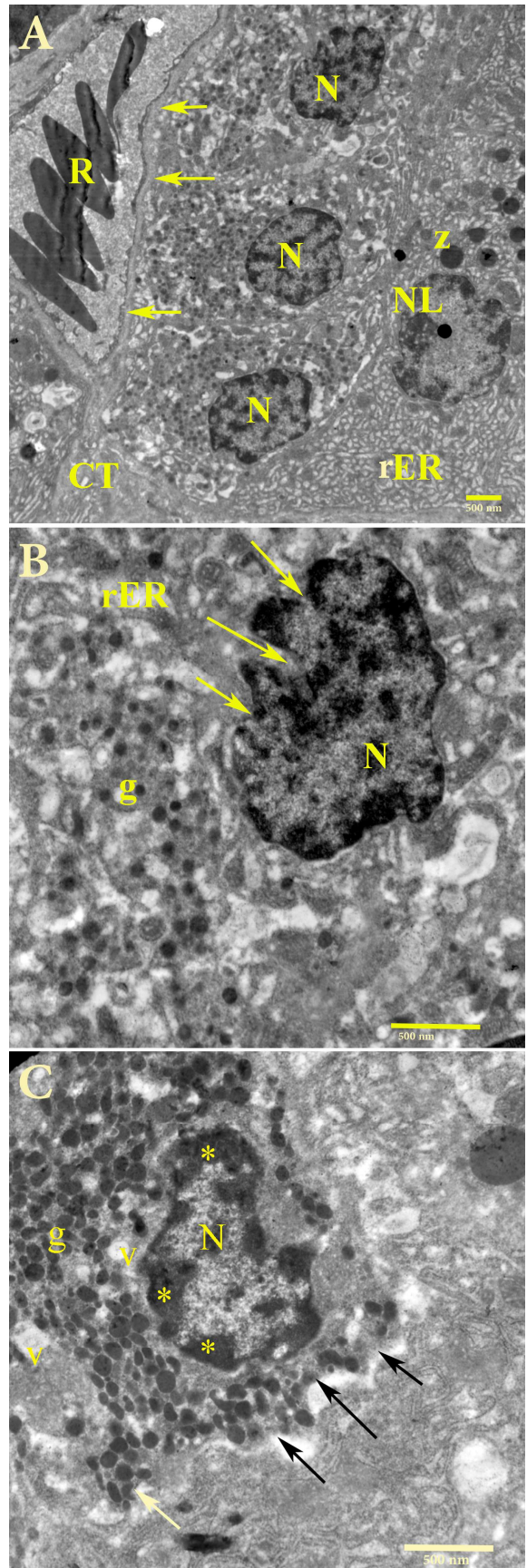


Fig. 5. Transmission electron micrograph of the dromedary camel's pancreas. (A) showing three α -cells with heterochromatic nuclei (N). The cells are surrounded by a layer of connective tissue (CT) separating the islet of Langerhans from the pancreatic acini. The endocrine cells are resting on a basal lamina (arrows) next to a blood vessel with erythrocytes (R). The acinar cell has abundant rough endoplasmic reticulum (rER), zymogen granules (z) and a nucleus with clear nucleolus (NL). (B) alpha cell showing a nucleus (N) with many indentations (arrows) and clumps of condensed chromatin, cytoplasmic granules (g), and rough endoplasmic reticulum (rER). (C) showing the irregular cell outline (arrows) of an α -cell, ovoid nucleus (N) with peripheral clumps of condensed chromatin (asterisks), a number of cytoplasmic granules (g) of moderate to high electron density, and cytoplasmic vacuole (v). The cytoplasm of the cell is of moderate electron density. Bar: 500 nm.

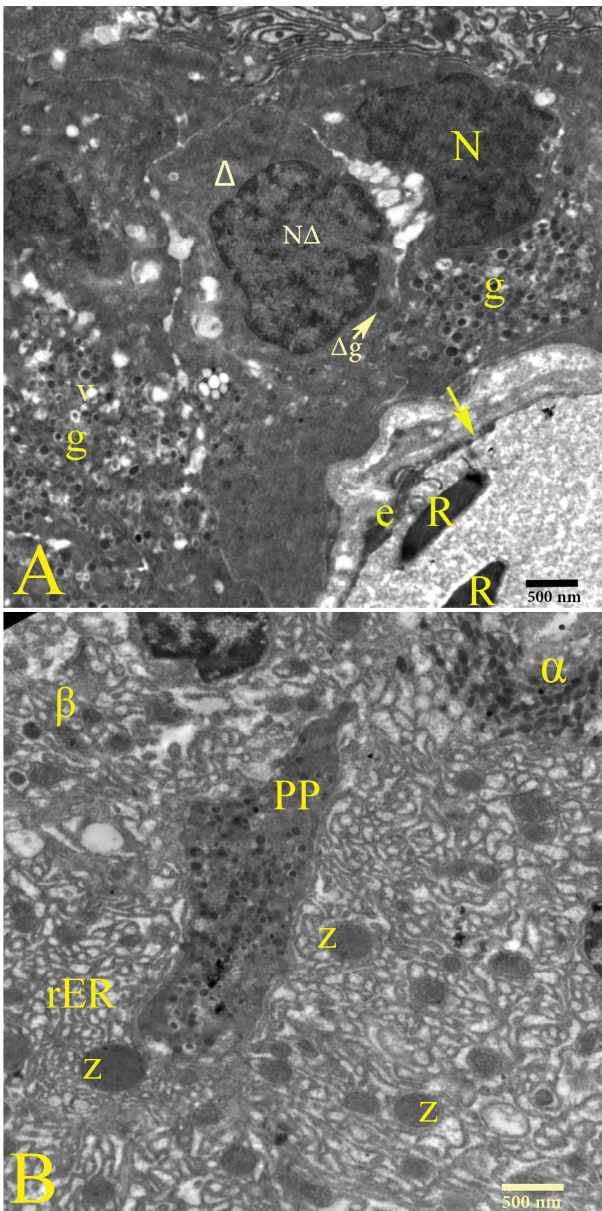


Fig. 6. Transmission electron micrographs of the dromedary camel's pancreas. **A:** β - cells showing dark cytoplasm, with many small cytoplasmic granules (g); each surrounded by a hollow area and enclosed by a limiting membrane. Nucleus (N) with dark well distributed chromatin. The cells are shown next to a blood vessel with fenestrated (arrow) endothelial lining (e). R: Erythrocytes, V: vesicles. A delta cell (Δ) with its granules (Δg) is showing between β -cells in **A**. $N\Delta$: delta cell heterochromatic nucleus. **B:** showing α -cell (α), β -cell (β), and PP-cell (pp) with its small granules and dark cytoplasm associated with them and the acinar cells. Halo areas surrounded some of PP-cells' granules. Acinar cells are shown with abundant rough endoplasmic reticulum (rER) and zymogen granules (z). Bar: 500 nm.

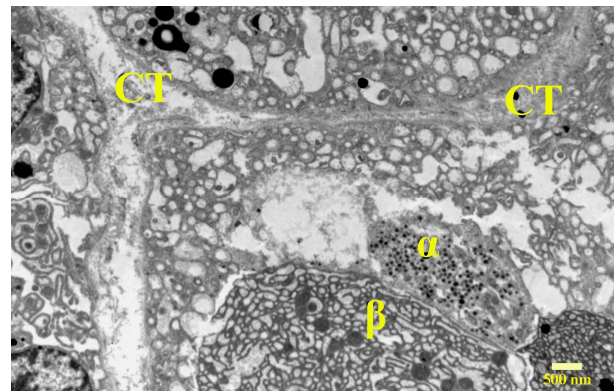


Fig. 7. Transmission electron micrograph showing the pancreatic islet in the dromedary camel surrounded by a connective tissue layer (CT). Alpha cells (α) and β -cell (β) are showing. Bar: 500 nm.

tem. Compared to acinar cells, these cells were characterized by the lack of secretory granules and the presence of little rough endoplasmic reticulum. The cytoplasm of centroacinar cells was of lower electron density compared to that of the acinar cells.

Some acinar cells were electron-lucent and others were electron-dense. Adjacent electron-dense acinar cells were joined by junctional complexes. Electron-lucent acinar cells interdigitated laterally. Electron-dense acinar cells had more prominent rough endoplasmic reticulum than did electron-lucent acinar cells (Fig. 4).

Four types of islet cells could be identified based on their ultrastructural architecture; α -, β -, Δ -, and PP-cells. The peripherally located α -cells (Fig. 5) had an irregular outline and possessed numerous relatively small granules characterized by a moderate to high density core surrounded by a membrane. The granules were mainly found at the poles of the cells. The nuclei of the cells were ovoid with condensed chromatin seen as clumps at the periphery; sometimes they were indented. Some vacuoles were seen in the cytoplasm in addition to the rough endoplasmic reticulum; the latter consisted of narrow tubules dispersed throughout the cytoplasm. Attached and free ribosomes were present. The Golgi apparatus appeared as small irregular lamina and vesicles.

Beta cells were distributed throughout the center and the periphery of the islet; this appearance was consistent with our previous histochemical study (Hafez et al., 2015). Beta cells were larger in size and had fewer granules than α -cells. Their granules were spherical or ellipsoid, were distributed throughout the cytoplasm, and were larger than those of α -cells. Beta cells' granules were membrane-bound with a moderately electron dense core. Some showed a halo area that surrounded the granules and was enclosed by the

limiting membrane. Several vesicles were also observed inside the cytoplasm (Fig. 6).

Delta cells (Fig. 6) were generally polygonal in shape and found in clumps throughout the islet; they were also identified in between β -cells. Their granules were of moderate electron density and were generally larger than β -cells' granules. These granules were tightly enclosed by a limiting membrane, but with no halo areas around them. Delta cells heterochromatic nuclei were irregular in shape.

Pancreatic polypeptide cells (Fig. 6) were seen associated with α and β cells. They were irregular small cells with small granules and dark cytoplasm. Halo areas surrounded some granules.

The highly vascular islets were surrounded by collagen fibers (Fig. 7). The endothelial lining of the blood vessels was fenestrated.

Additional endocrine cells could be seen outside the islets and among the acinar cells (Fig 8). Extra-islet endocrine cells contained special electron-dense granules. Some of these granules were spherical, but others were rod-shaped. Mitochondria and Golgi saccules were prominent in these cells.

DISCUSSION

The ultrastructural architecture of the pancreatic acini explained their light microscopic appearance. The basal basophilia and the apical eosinophilia of the acinar cells seen in the light microscope correlated well with the ultramicroscopic findings. The basal region shown concentrated rough endoplasmic reticulum (basal basophilia), and the apical region contained the zymogen granules (apical eosinophilia). The well-known affinity of the RNA in the rough endoplasmic reticulum for basic stains accounts for the appearance of the basal areas; and the lack of acidic materials in the secretions accounts for the eosinophilia.

The endocrine and exocrine elements of the pancreas are functionally and structurally distinguishable by the presence of the connective tissue capsule (Fig. 7) surrounding the islet cells, which was easily demonstrated by ultramicroscopy. This structural separation could not easily be seen in our study or in a previous study by Khatim et al. (1985) under the light microscope using conventional staining. This structural separation of the pancreatic islets from the exocrine elements of the pancreas appears to be unique to the dromedary camel when compared to other camelids such as llamas and alpacas. Cebra et al. (2006) reported that pancreatic islets in llamas, and alpacas lack such connective tissue capsules.

The structural association of the endocrine and exocrine elements of the pancreas can be traced back to their embryological origin from

the same pancreatic diverticulum. The group of cells that develops isolated from the duct system is that what forms the pancreatic islets (Moore et al., 2015). As the islet cells develop by budding as solid cell columns from the acinus cells (Moore et al., 2015), the most recently formed islet cells retain their direct contact with the acini, while those formed earlier bulge into the inter-acinar space and become more or less isolated cell masses (Ekholm and Edlund, 1959). Those isolated cell masses are the relatively easily distinguished islets seen by light microscopy and by histochemical identification, while the ones that retain their direct contact with acinus cells can be detected only by immunohistochemistry or ultramicroscopy such as the ones detected in this study (Fig. 8).

The juxtaposition of the endocrine and exocrine elements of the pancreas is also of functional significance (Henderson et al., 1981). Diabetic animals are known to have abnormal pancreatic exocrine function (Schapiro et al., 1981). In rats with alloxan-induced diabetes, both the synthesis and secretion of amylase are diminished, but can be restored to normal by giving insulin (Adler and Kern, 1975). Patient with insulin-dependent diabetes have impaired pancreatic exocrine secretory capacity (Frier et al., 1976) and morphological abnormalities of the acinar cells (Klöppel et al., 1978). In the normal pancreas the acinar tissue surrounding the pancreatic islets differs morphologically from the remaining aci-

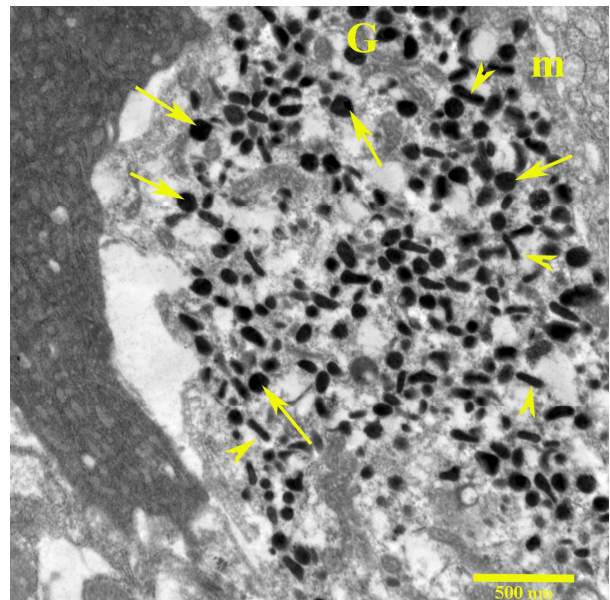


Fig. 8. Transmission electron micrograph of an endocrine cell located among the acinar cells, outside the islets of Langerhans in the dromedary camel's pancreas. Some of the cytoplasmic granules were spherical (arrows) and others were rod-shaped (arrow heads). Numerous mitochondria (m) and Golgi vacuoles (G) were observed. Bar: 500 nm.

nar tissue. The acinar cells in these areas are larger and have a greater content of zymogen granules (Henderson et al., 1981; Malaisse-Lagae et al., 1975).

Blood leaving the islets has also been shown to pass into the capillary meshwork within the exocrine tissue of the pancreas rather than draining directly into the pancreatic veins (Fraser and Henderson, 1980). The capillary shown in Fig. 6A in this study was running next to acinar cells, and therefore might represent a communication channel from the endocrine cells to the acinar cells. Hormones produced by islet cells have been shown to affect acinar cell functions (Saito et al., 1980a, b; Manable and Steer, 1979; Henderson et al., 1981; Singh, 1980).

The functional interaction between exocrine and endocrine elements of the pancreas might work both ways. The exocrine pancreas may, even if indirectly, affect hormonal production of the islet cells. For example, patients with exocrine pancreatic insufficiency have glucose intolerance (Ebert and Creutzfeldt, 1980).

The presence of fenestrated capillaries adjacent to the pancreatic islet correlates well with its endocrine function and matches other endocrine organs (Kleine and Rossmanith, 2016).

The ultrastructural features of the camel pancreas correlated well with those of other species (Caramia et al., 1965; Munger et al., 1965; Lacy, 1957; Legg, 1967). The distribution of the endocrine cells (except Δ -cells) within the islet reported in this study was consistent with what was reported by Bsoul et al. (2013), and Khatim et al. (1985); and to what was reported in our previous study (Hafez et al., 2015) in the dromedary camel. Delta cells were identified throughout the islet in this study, but it was identified only at the periphery in a study by Bsoul et al. (2013). They were also identified in between β -cells in both studies.

Beta cells were easily distinguished by their dark color. This is in agreement with their appearance in some other species such as the guinea pig (Caramia et al., 1965), but not others such as rabbits (Lacy, 1957).

The distribution of β -cells' granules throughout the cytoplasm and their variable size and shape conform well with the observation of Bsoul et al., 2013, and those reported in dogs (Sato et al., 1966) and in humans (Pelletier, 1977). The ultrastructure of the β -cells granules differed among species (Lacy, 1961): it is round and homogenous in rats; rectangular in the dog; irregularly shaped in guinea pigs. This suggests the presence of different mechanisms of insulin storage among species (Lacy, 1961). The morphological features of α -cells reported in this study correlated well with those reported by Bsoul et al. (2013).

The extra-islet endocrine cells observed in the

dromedary camel in this study and in a previous study (Khatim et al., 1985) were also observed in other camelid such as llamas and alpacas (Cebra et al., 2006); in the horse (Helmstaedter et al., 1997); in the cattle and horse (Hafez et al., 2015); in the dog (Redecker et al., 1992); and in laboratory animals (Wieczorek et al., 1998). The regulation of extra-islet endocrine cells is likely to differ from that of their counterparts within the islets with accounting for the differences in their microenvironment (Wieczorek et al., 1998). The regulation of these cells has not been studied in the camel and may need further attention.

However, the camel pancreas still presents salient species-specific features. The mitochondria observed in the acinar cells were elongated or rod-shaped. The mitochondria in the acinar cells of humans were round or oval (Ekholm and Edlund, 1959), but are rod-shaped in the mouse (Sjöstrand and Hanzon, 1954), and guinea pig (Palade and Siekevitz, 1956; Caramia et al., 1965). This provides an example of species variations in ultrastructure of the pancreas.

The acinar lumen was continuous with the intercalated duct, and sometimes it contained materials of moderate electron density which may indicate recently released proteins from the cell by the process of exocytosis.

The granules lacking core material observed in Beta cells suggest that these granules may release their contents via intra-granular dissolution of secretory material followed by passage into the cytoplasm (Orci et al., 1973). These vesicles were also observed by Bsoul et al. (2013).

The ultrastructure of the PP-cells in the dromedary camel was reported in this study for the first time. The features of the PP-cells' granules reported in this study were similar to those in human (Pelletier, 1977). Deconinck et al. (1971) reported the presence of Type III and Type IV endocrine cells in addition to A and B cells in the pancreas of humans, but the ultrastructural characteristics of these cells differed from that of PP-cells reported in the dromedary camel in this study. Pancreatic polypeptide immunoreactive cells were localized immunohistochemically in the dromedary camel (Hafez et al., 2015; Katim et al., 1985) and other related species such as cattle (Hafez et al., 2015; Nakajima et al., 1988; Hiratsuka et al., 1996) and sheep (Larsson et al., 1979). Pancreatic polypeptide-immunoreactive cells were the least numerous in the camel and horse, but they were second numerous cell type after β -cells in cattle (Hafez et al., 2015). It is possible that ruminants might have higher demand for pancreatic polypeptide than camels. Further physiological studies on PP-cells are needed. The ultrastructural characteristics of these cells reported in this study may be helpful as a baseline information for such

studies.

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