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Immunohistochemical identification of the endocrine cells in the pancreatic islets of the camel, horse and cattle

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SUMMARY

This study considers the distribution of various endocrine cells in islets of Langerhans in the pancreas of several species of domestic animal, including the dromedary camel (*Camelus dromedarius*) using immunohistochemistry, and relates our observations with reference to the well-documented general histology of the mammalian pancreas. The pancreatic islets were observed as compact areas of pale cells surrounded by darker presumably exocrine tissue. The most distinct delineation of the islets from the surrounding acini was in the horse and the least was in cattle. Insulin -immunoreactive cells (β-cells) were most abundant followed by glucagon- (α-), somatostatin- (Δ-), and pancreatic polypeptide-immunoreactive (F- or PP-) cells in decreasing order, in all species except cattle where PP-cells were second to β-cells in their distribution. The most prominent special pattern was observed in the distribution of α- and β - cells in the pancreatic islet of the horse where α-cells were located in the center of the islet surrounded by β-cells. In the camel, β-cells were distributed throughout the islet in the center and the periphery. Alpha cells were mostly observed as clumps in the periphery area. Clumps of small number of Δ-cells and a few PP cells were found throughout the islet. In cattle, β-cells were distributed throughout the islets. Other cells occupied a more peripheral location. The physical differences in distribution of endocrine cells might result in differences in the need and interaction of hormones to each other in different species.

Key words: Pancreatic islet cells – Immunohistochemistry – Camel pancreas – Horse pancreas – Cattle pancreas

INTRODUCTION

It is well documented that in mammalians the pancreas is composed of both exocrine and endocrine elements. The bulk of the exocrine pancreas is a compound tubuloacinar gland that synthesizes, stores and secretes digestive enzymes. The endocrine pancreas (*Pars endocrina pancreatica*) is composed of aggregates of cells, known as *islets of Langerhans* (*Insulae pancreaticae*) that are scattered among acini (Gartner, 2006). The endocrine part of the pancreas represents approximately one or two percent of the pancreatic mass (Johnston et al., 1988). The endocrine component of the mammalian pancreas is composed primarily of alpha cells (α-cells or glucagon-producing cells), Beta cells (β-cells, insulin-producing cells), Delta cells (Δ-cells, somatostatin-producing cells), and F-cells (PP-cells, pancreatic polypeptide-producing cells) (Gartner, 2006).

Only a few studies have been performed on the endocrine pancreas of the one-humped camel (Khatim et al., 1985; Adeghate, 1997; Tadjalli and

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Meamary, 1998), despite this species' unique ability to deal with the harshest of environmental conditions. Studying the endocrine pancreas in camels may help to elucidate the physiology behind the camel's amazing adaptation to long periods of water deprivation. This adaptation of the camel to water deprivation causes increased levels of blood sugar but decreased levels of insulin. It has been found that glucose infusion to a dehydrated camel results in a more pronounced hyperglycemia but milder glucosuria than in the hydrated animal. The later observation was attributed to an increased tubular reabsorption of glucose and a decreased glomerular filtration rate (Khatim et al., 1985). But apart from this, little is known about blood sugar regulation and the role the endocrine pancreas plays in this phenomenon in camels.

The distribution of cells of the pancreatic islet might be related the mechanisms that govern the integrated functions of the endocrine pancreas. 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 (Redecker et al., 1992).

This study was undertaken to identify species-specific regional arrangement of islets in the pancreas of camel, and to compare it to those of horses and cattle; and to characterize the distribution of hormone-producing cells in the islet in these species.

MATERIALS AND METHODS

Fresh specimens from pancreata of 3 adult camels were obtained from Kom Hamada Abbatoir in Egypt immediately after slaughter. These animals were slaughtered for human consumption and were examined prior to slaughter by the slaughterhouse veterinarian for approval for human consumption. Specimens from pancreata of 4 adult horses and 6 adult cattle were obtained immediately after laboratory euthanasia. Those animals had been donated to the anatomy lab and they were approved for euthanasia. Pieces of tissues less than 5 cubic mm in size were fixed in 10% buffered formaldehyde.

Table 1. Antibodies used for immunolocalization of hormones produced by pancreatic islet endocrine cells

Antibody	Manufacturer	Concentration
Insulin IHC antibody Cat. # IW-MA1052	IHC WORLD, Woodstock, MD 21163.	Ready to use; as provided by the manufacturer
Glucagon IHC antibody Cat. # IW-MA1047	IHC WORLD, Woodstock, MD 21163.	Ready to use; as provided by the manufacturer
Polyclonal Rabbit Anti-somatostatin	Dako North America, Inc., Carpinteria, CA 93013.	1:1000
Pancreatic Polypeptide Polyclonal Antibody	Thermo Scientific, Rockford, IL 61105.	1:1000

Following fixation and washing, and embedment in paraffin by routine methods, sections of pancreata from all three species were stained with hematoxylin and eosin (H&E) stain. Selected sections were stained with modified Aldehyde-Fuchsin stain using the Halmi (1952b) modification as given in Humason (1979). This stain shows β-cells (deep purple-violet), α-cells (yellow) and ∆-cells (green). Aldehyde-Fuchsin stain was purchased from Electron Microscopy Sciences, Hatfield, PA 19440, catalog number: 26328-01.

Immunohistochemistry

Histochemical labeling was performed using mouse or rabbit polyclonal antibody against insulin, glucagon, somatostatin, or pancreatic polypeptide as shown in Table 1. For Histochemistry, paraffin-embedded sections were deparaffinized in xylene and rehydrated in increasingly dilute ethanol/distilled water baths. For antigen retrieval, slides were trypsinized for 10 minutes at 37°C (Trypsin-EDTA, Media Tech Inc., Manassas, VA, USA). Slides were incubated in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase, then washed with PBS and incubated with serum to prevent non-specific binding in the case of using anti-somatostatin and anti-pancreatic polypeptide antibodies. The serum blocking step was omitted in the case of insulin and glucagon antibodies as per manufacturer's recommendation.

Sections were incubated with the appropriate primary antibody in a humid chamber overnight at 4°C followed by incubation with universal biotinylated anti-mouse/rabbit IgG secondary antibody (Vector Laboratories, Burlingame, CA 94010) at room temperature. A final incubation, after washing, with ABC reagent (avidin: biotinylated horseradish peroxidase complex, Vector Laboratories) lasted 30 minutes under the same conditions. Immunoreactivity was visualized with 3,3'-diaminobenzidine (DAB substrate kit, Vector Laboratories) in a dark place as outlined in the manufacturer's protocol. Slides were counterstained with Hematoxylin, dehydrated, cleared in xylene, and mounted.

RESULTS

A summary of pancreatic islet characteristics and the intra-islet cellular distribution is shown in Table 2.

The size of the islets showed considerable variation among species and within the same species. Based on visual estimates overall islet size tended to be smaller in cattle than horses or camels.

The islets of Langerhans were scattered randomly throughout the pancreas and were seen in our

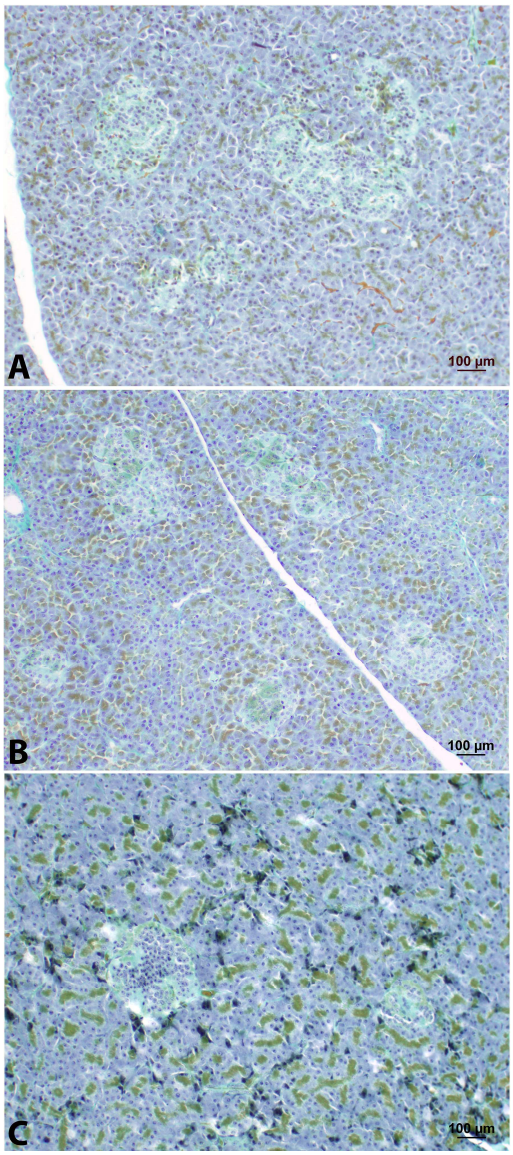

Fig. 1. Modified Aldehyde-Fuchsin staining of sections of the pancreas of **(A)** camel, **(B)** horse and **(C)** cattle. Islets of Langerhans are shown as compact areas of pale cells surrounded by darker exocrine pancreatic tissue.

sections as irregular spherical or oval masses of cells. This shape description coincides with the information on pancreatic islets in general and is the closest possible depiction of the very irregular shapes of islets which are seen in sections as two-dimensional representations of three-dimensional structures. On H&E or modified aldehyde fuchsin stained sections, the islets were seen as compact areas of pale cells infiltrated by rich vasculature and surrounded by darker exocrine pancreatic tissue (Fig. 1).

The islets were delineated from the surrounding acini by delicate connective tissue fibers. The connective tissue demarcation of the islets was visibly more distinct in the horse compared to the camel and cattle. Cattle showed the least distinct connective tissue boundaries.

The islet cells were arranged in such a way as to suggest clumps or cords in three dimensions. Islet cells had round nuclei and granular cytoplasm. The granules were not seen with H&E staining.

Four types of cells could be distinguished based on the immunohistochemical reaction of their produced hormones; α-, β-, ∆-, and PP-cells. Less frequently, what appeared to be single endocrine cells within the exocrine pancreas were observed.

Although the proportions of the various endocrine cells in the islet varied from one species to the next, β-cells showed the widest distribution among other islet cells.

In the camel, β-cells were distributed throughout the islet profile in the center and the periphery. They were also found outside the margins of the islets between the secretory acini and the interlobular connective tissue. Alpha cells were mostly observed as clumps in the periphery area. Clumps of small number of ∆-cells were found throughout the islet. A few PP-cells were found throughout the islet. These cells stained lightly (Fig. 2).

Distribution of cell types in the islets was distinctly different in the horse. In the horse the center of he pancreatic islets was predominantly occupied

Table 2. Relative comparison of the pancreatic islet characteristics and cellular distribution within islet in Camel, horse, and cattle

Criteria	Camel	Horse	Cattle
Size of islet	variable	variable	Smaller than the camel or horse
Islet delineation from the surrounding exocrine tissues	Less distinct than horse and more distinct than cattle	Most distinct	Least distinct
Islet's cell relative distribution (most to least)	β-cells α-cells ∆-cells PP-cells	β-cells α-cells ∆-cells or PP-cells	β-cells PP-cells ∆-cells or α-cells
Regional distribution of cell types within the islet:			
β-cells	Throughout	Periphery	Throughout
α-cells	Periphery	Center	Periphery
∆-cells	Throughout	Throughout	Periphery
PP-cells	Throughout	Throughout	Periphery

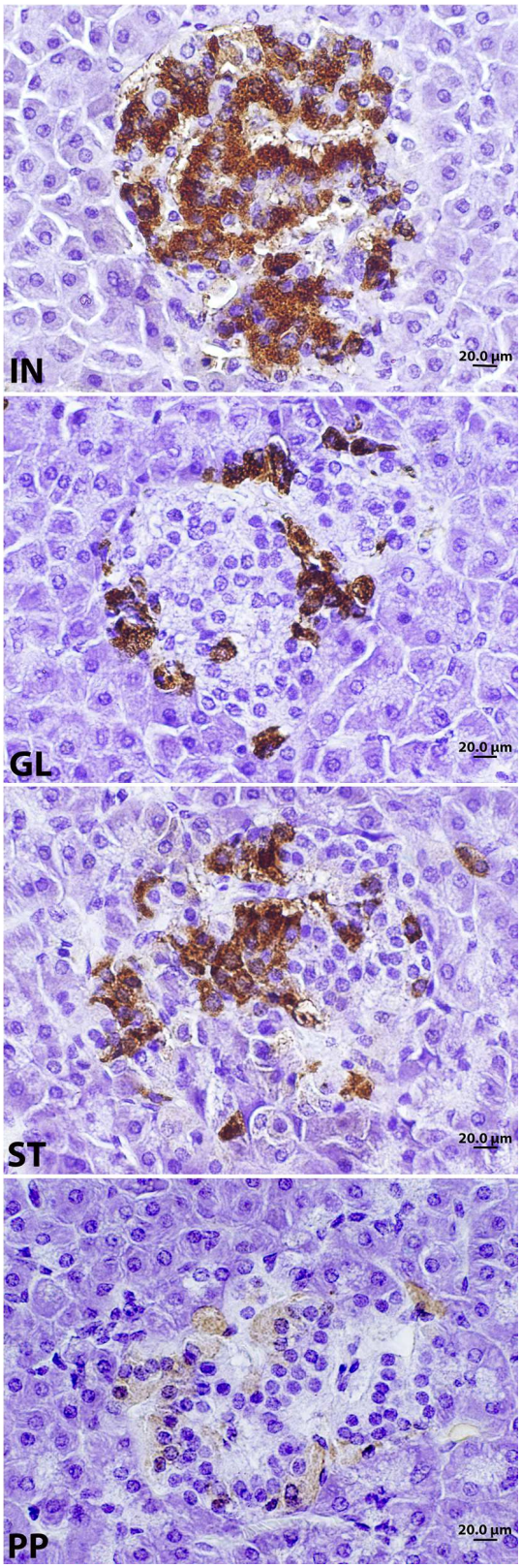

Fig. 2. Light microscopic images of the pancreas of the camel, using immunostaining of insulin-producing cells (IN), glucagon-producing cells (GL), somatostatin-producing cells (ST), and pancreatic polypeptide-producing cells (PP). Beta cells were distributed throughout the islet in the center and the periphery. Alpha cells were mostly observed as clumps in the periphery area. Clumps of small number of Δ-cells and a few PP-cells were found throughout the islet.

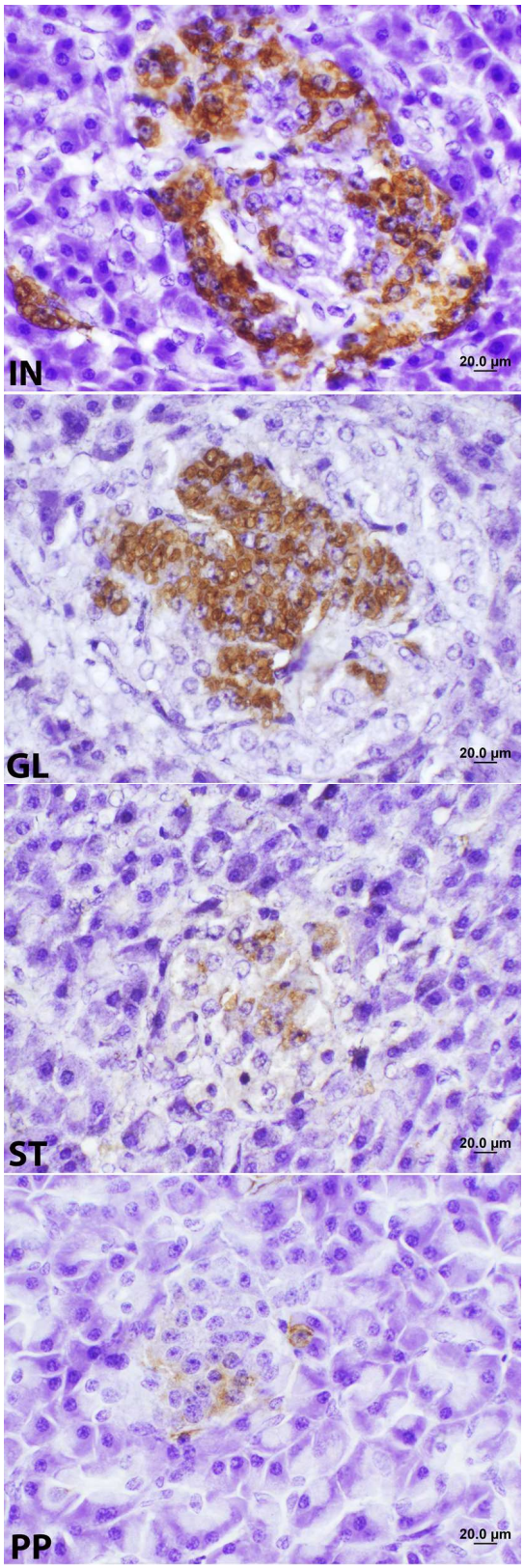

Fig. 3. Light microscopic images of the pancreas of the horse using immunostaining of insulin-producing cells (IN), glucagon-producing cells (GL), somatostatin-producing cells (ST), and pancreatic polypeptide-producing cells (PP).The center of the pancreatic islet was predominantly occupied by α-cells. Beta cells form a more or less complete mantle around alpha cells. Few Δ -cells and PP-cells were located among alpha or beta cells or at the border of these two types of cells.

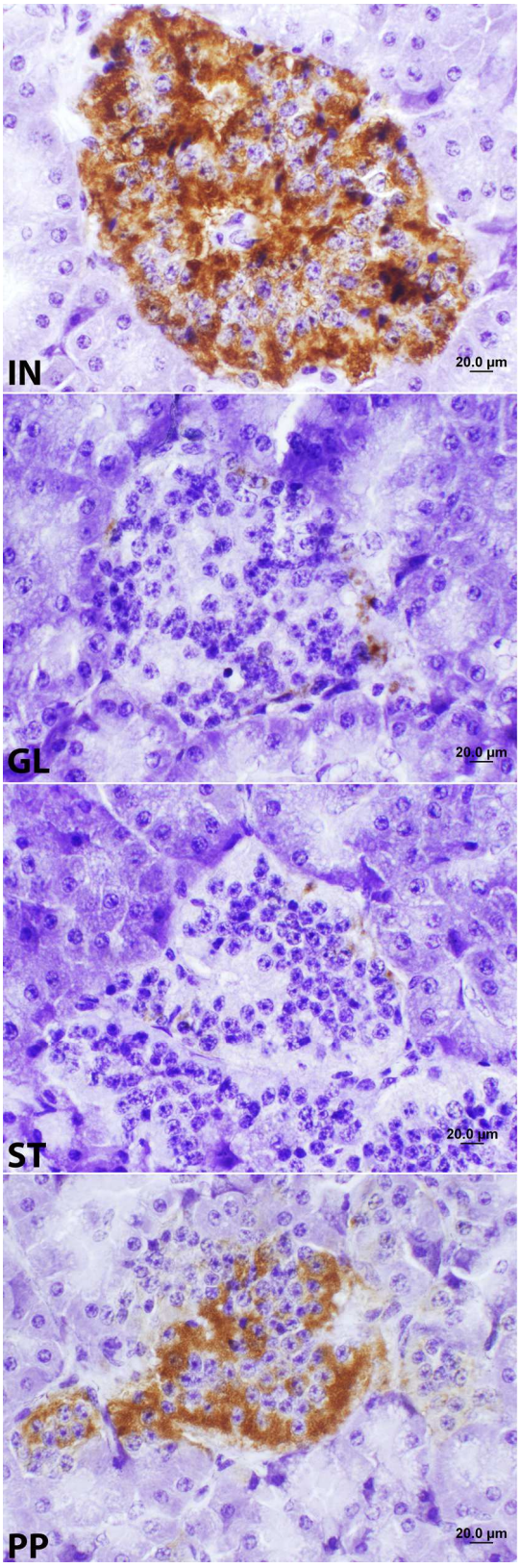

Fig. 4. Light microscopic images of the pancreas of the cattle using immunostaining of insulin-producing cells (IN), glucagon-producing cells (GL), somatostatin-producing cells (ST), and pancreatic polypeptide-producing cells (PP).Beta cells were distributed throughout the islets. Other cells occupied a more peripheral location. Pancreatic polypeptide-immunoreactive cells were well distributed in the form of clusters.

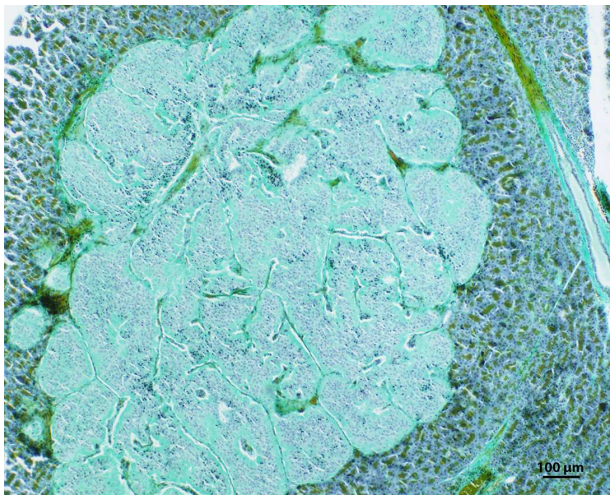

Fig. 5. Modified Aldehyde-Fuchsin staining of sections of the pancreas of cattle. Islets of Langerhans are coalesced into a single big islet surrounded by darker exocrine pancreatic tissue.

by α-cells. A few α-cells were seen outside the islet in the duct system or associated with the acini. Beta cells were usually seen in clumps in the peripheral area of the islet, forming a more or less complete “mantle” around α-cells. As in the camel, β-cells were also detected outside the islets. A few ∆- and PP- cells were located in islets. They were seen among α- or β-cells or at the border of these two types of cells. These cells exhibited extrainsular location in the duct system or between acinar cells (Fig. 3).

In cattle, β-cells were distributed more or less uniformly throughout the islets. Other cells occupied more peripheral locations. Beta cells were the most abundant cell type, as in camels and horses, and they were also detected outside the islets. Glucagon- and somatostatin-immunoreactive cells occupied a peripheral location in the bovine islet. They were the least numerous of all cell types and they tended to have a less intense staining. Pancreatic polypeptide-immunoreactive cells were well distributed in the form of clusters at the periphery of islets or in the exocrine portion adjacent to the islet. They were the most second numerous cell type after β-cells (Fig. 4).

In one cow, the endocrine portion of the pancreas was consolidated in the form of a big islet surrounded by the exocrine pancreas (Fig. 5).

DISCUSSION

The endocrine pancreas of the camel is dispersed into islets of hormone-secreting cells similar to other species. The delineation of these islets from the exocrine portion of the pancreas was most pronounced in the horse. The size of the islets and their cell populations varied significantly in different species. The variations of the size of the islets have been noted before in various species

(Elayat et al., 1995; White et al., 1999; Aizawa et al., 2001; Heller, 2010); including the camel (Adeghate, 1997), the horse (Helmstaedter et al., 1976; Furuoka et al., 1989), and cattle (Bonner-Weir and Like, 1980; Hiratsuka et al., 1996). The exact reasons for these variations are unknown, but it is probably due to changing need in response to metabolic stimulus or the consequence of embryological development (Adeghate, 1997).

Shape variations noted in our study coincided with those reported by other authors (Elvestad et al., 1984; Erasmus and Van Aswegen, 1997; Wieczorek et al., 1998; Larsen and Rolin, 2004; Bellinger et al., 2006; Gustavsen et al., 2008; Heller, 2010). Since morphological and functional differences in cell types have been documented, this is not surprising.

Although the relative proportions of the various endocrine cells in the islet varied from one animal to the next in all species; β-cells were the most abundant type, followed by α, ∆, and PP cells in decreasing order. Pancreatic polypeptide-immunoreactive cells were the least numerous in all species except in cattle where they were second numerous cell type after β- cells.

Furthermore, pp-cells were distinctively more abundant in cattle than in any of the other species. This is in agreement with the findings of Nakajima et al. (1988) and Hiratsuka et al. (1996). The former authors actually named some islets in certain regions as “the so called PP islet” and stated that such made up 40% of the islet cell mass. These authors attributed the presence of abundant PP cells in certain islets to regional variations within the pancreas. Larsson et al. (1979) reported high frequency of PP-cells in sheep. It is possible that ruminants might have higher demand for pancreatic polypeptide than other groups of mammals. Human and canine pancreata share with cattle the presence of considerably more PP-cells at least in some regions (Gersell et al., 1979; Brissova and Powers, 2008; Redecker et al., 1992).

The distribution of α- and β-cells in camels reported here was similar to what was reported in Adeghate, 1997. A different pattern of distribution was reported in Khatim et al. (1985).

The most prominent special pattern we observed was in the distribution of α- and β-cells in the pancreatic islet of the horse. There, α-cells were located in the center of the islet surrounded by β-cells in the periphery. This observation is in agreement with those made in other studies (Helmstaedter et al., 1976; Furuoka et al., 1989). Glucagon-producing cells formed a central core in the pancreatic islet of the horse. The reverse pattern of distribution of endocrine cells has been described in other species; in the mouse (Lee et al., 2010), rat (Wieczorek et al., 1998), Gerbil (Ku et al., 2001), porcine (Vantyghem et al., 1996). However, a similar distribution of α-cells to that in the horse has been seen in monkeys (Wieczorek et al., 1998). Human islets have intermingled endocrine cells as demonstrated by co-localization of α-, β-, and ∆-cells (Samols et al., 1983, 1986, and Brissova and Powers, 2008).

Some authors argue that the regional distribution of endocrine cells in the pancreas is indicative of their embryologic origin (Hiratsuka et al., 1996). The pancreas develops from two separate primordia: the dorsal pancreas appears first, as a bud situated on the dorsal wall of the duodenum, while the ventral pancreatic bud arises, a little later, in the angle below the hepatic anlage. Each of the buds grows rapidly and the duodenum undergoes a rotation so that what was its ventral surface is directed to the right together with the right pancreatic lobe, while the dorsal bud forms the body and the left pancreatic lobe (Dobois, 1989). Speculations on the differences existing between the ventral and dorsal lobes have attributed these regional differences in the cattle pancreatic islets to their development from two different primordia, the ventral and the dorsal (Hiratsuka et al., 1996). It has also been suggested that the differences of endocrine cell patterns could be related to two different arterial systems of irrigation of pancreatic islets for the ventral and dorsal lobes (Wieczorek et al., 1998).

The anatomic juxtaposition of different cell types in the endocrine pancreas may lead to the physiologic influence of one on another. It is likely that these cells in such close proximity communicate by cell-cell interactions, by paracrine mechanisms, and by secretion of hormones and products from one endocrine cell type influencing “downstream” endocrine cells. That is intraislet positive-negative insulin-glucagon feedback or inhibition of somatostatin of α- and β- cells, or glucagon promotes somatostatin release (Samols, et al., 1983, 1986). Considering the islet as a highly sophisticated micro-organ and considering the differences among species in distribution of islet cells observed in this study this putative interaction might have significant physiological consequences. The physical differences in distribution of endocrine cells may reflect differences in the need for and interaction of hormones with each other in different species.

Caution must be practiced when proposing models of intra-islet regulation of hormone secretions given the heterogeneity in the arrangement of various cell types. In addition to our observations, heterogeneity has been demonstrated in several mammalian species such as dogs (Redecker et al., 1992), rabbits (Jorns et al., 1988) and humans (Grube and Bohn, 1983). Inter-islet heterogeneity becomes even more obvious if the size and shape of islets in various species is considered. Differences in islet size have been observed in this study and have been documented in several species; in the camel (Aldeghate, 1997), in the horse (Helmstaedter et al., 1976; Furuoka et al., 1989), and in cattle (Hiratsuka et al., 1996). Hence, it is of

crucial importance to take into account both inter-species differences and inter-islet heterogeneity in the evaluation of concepts of intra-islet regulatory mechanisms. Such concepts, conclusive as they may seem, are definitely limited by islet anatomy (Redecker et al., 1992; Rutter and Hudson, 2013).

In all animals we studied, single scattered endocrine cells were found among the exocrine pancreatic cells and in the vicinity of ducts. This may be accidental occurrences; or it may be that these extra-insular endocrine cells have a function of their own in relation to their location. These arrangements could represent an important component of the gastro-entero-pancreatic system. It is still unknown whether the observed dual distribution of endocrine cells between islets and extrainsular locations reflects a dual function (Wieczorek et al., 1998). Endocrine cells within the epithelium of exocrine acini and that of excretory ducts can also lie in close proximity to surrounding capillaries and, therefore, their hormonal fraction may nevertheless contribute to the total hormonal output of the pancreas. The regulation of extrainsular cells is likely to differ from that of their counterparts within the islets, thereby accounting for the differences in their microenvironment (Wieczorek et al., 1998).

There remains the question of whether—and if so, how—the distribution of cell types in the camel pancreas is related to this species' inherent resistance to the effects of water deprivation. It is possible to speculate on this by considering the discussion by Rutter and Hodson (2013) on the importance of Ca++ and K+ flux in mediating the release of insulin from the beta cells; and the variations observed in murine and human islets with respect to the spatial arrangement of the different cells within an islet.

It is conceivable that the three-dimensional arrangement of cells in the camel islet facilitates an exchange of ions between neighboring cells, via gap junctions (also shown to be an important factor in regulating secretion in mice and humans) and/or by localized exchanges between the intravascular and intracellular compartments. If this is the case, it may be further speculated that the camel's ability to retain water by virtue of its efficient renal dynamics influences pancreatic function rather than the other way around. By tightly controlling the total body load of Ca++ and K+ and/or by somehow "allocating" these ions to specific functions of more immediate importance to survival than serum glucose levels under conditions of water and nutrient stress, the camel may temporarily lower or even cease secretion of insulin and glucagon.

Suggestions such as these obviously require considerably more investigation, and the employment of techniques other than those of histological investigation. In order to determine the likelihood of this "reversal" actually happening, much more has to be known about the normal fluctuation of serum values not only for glucose, but for insulin, in the camel; and extensive probing of the structure of the islet using confocal microscopy, electron microscopy, and perhaps patch-clamping techniques (to determine the state of membrane charge in the islet cells. Once these currently-unknown factors are understood a more comprehensive understanding of the relationship between water balance, ionic regulation, and carbohydrate metabolism in this important desert animal may be forthcoming.

Insulin, glucagon, somatostatin, and pancreatic polypeptide are generally the commonly described hormone products of the pancreatic islet cells; however, other endocrine hormones have been discussed in other species such as ghrelin (Wierrup et al., 2002; Prado et al., 2004; Heller et al., 2005), secretin (Lee et al., 2003), cholecystokinin (CCK) (Shimizu et al., 1998), peptide YY (PYY) (Bottcher et al., 1993), thyrotropin releasing hormone (TRH) (Tsuruo et al., 1988), and cocaine amphetamine regulated transcript (CART) (Wierup et al., 2004). These hormones might be a good subject for further immunohistochemical studies in camels, horses and cattle.

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