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Immunohistochemical distribution of glucagon -, insulin -, somatostatin -, gastrin-, and serotonin-containing cells in the pancreas of the Van cat

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Abstract: The regional distribution, relative frequency, and appearance of glucagon (A-cell)-, insulin (B-cell)-, somatostatin (D-cell)-, gastrin (G-cell)-, and serotonin (EC-cell)-secreting cells in the endocrine and exocrine pancreas of Van cats were examined using the immunohistochemistry method. Glucagon immunopositive A-cells were principally found in the central region of the islets of Langerhans, while insulin immunopositive B-cells were located in the periphery of the pancreatic islets. Moreover, several A- and B-cells were observed as only single cells or clusters of 2 to 3 immunopositive cells in the exocrine parenchyma and the pancreatic duct epithelium. Somatostatin and gastrin immunopositive reactivities were negligible in the peripheral regions of the pancreatic islets of Langerhans and in the exocrine parenchyma. However, serotonin-immunopositive EC-cells were observed in neither the endocrine islets nor any other sites of the tissue. The existence, regional distribution, and relative frequency of A-, B-, D-, G- and EC-cells in the pancreas of Van cats have been analyzed in this study for the first time. The immunopositivity and distribution of endocrine cells in the Van cat pancreas were determined to be partially different from those of other carnivorous species such as dogs and other cats.

Key words: Endocrine cell, immunohistochemistry, Langerhans islet, Van cat pancreas

1. Introduction

The pancreas comprises exocrine and endocrine sections. These produce digestive enzymes and regulatory hormones for carbohydrate metabolism, respectively. The endocrine sections are the Langerhans islets and distribution randomly into the exocrine sections (1–3). The pancreas of cats is formed of a right and left lobe making a U-shape close to the gastric pylorus, and it consists of the duodenal, gastric, anastomotic, and splenic lobes. The islets of Langerhans in cats contain 4 different endocrine cells, similar to other mammalian species (4–6). These cells release regulatory hormones including glucagon (A, alpha, A_2 cell), insulin (B, beta cell), somatostatin (D, delta, A_1 cell), and pancreatic polypeptide (PP, F cell) (1,2,7–13). Some researchers have also reported that the islets of Langerhans may contain nonspecific endocrine cells such as neuropeptide Y, substance P, galanin, cholecystokinin-8 (11), serotonin (10,14), gastrin, and ghrelin-releasing cells (15).

Islet architecture and composition may differ both among different species and also within the same species (9,16–20). For example, the Langerhans islets in the pancreas of birds comprise alpha islets (A-cell rich), beta islets (B-cell rich), and mixed islets (A-, B-, D-, and PP-cells) (11,17), but are found only as mixed islets in mammalian species (1,9). Many researchers have reported that insulin-immunopositive cells are located in the central zones of Langerhans islets in mammals, and that the glucagon-, somatostatin-, and pancreatic polypeptide-secreting immunopositive cells are located in the mantle zones or periphery (1,2,12). In addition, insulin-immunopositive cells are found in the center of the islets in humans, rats, and mice (9), but in the periphery of the islets in horses (4), monkeys, and kangaroos (18). Some researchers have reported that the distribution of pancreatic endocrine cells in different species might be due to physiological functions, feeding habits, and pathophysiological conditions such as diabetes and obesity rather than the difference in species per se (9,18).

Previous immunohistochemical and electron microscopic studies have suggested heterogeneities of islets in various animals such as nonhuman primates (16),

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dogs (7), and rats (1). Studies of the regional distribution and the relative frequency and appearance of pancreatic endocrine cells among carnivorous species have already been performed on dogs (7,16) and some domestic cats (5,6,8), but Van cats have not yet been studied. The Van cats are a naturally occurring variety of domestic cat, found principally in the Lake Van area of eastern Turkey. They are recognizable by their almond-shaped eyes of mismatched colors, with an amber-green eye and a blue eye. The goal of this study is to investigate, for the first time, the distribution of glucagon-, insulin-, somatostatin-, gastrin-, and serotonin-secreting cells in the Van cat pancreas using immunohistochemical analysis.

2. Materials and methods

2.1. Animals and tissue

Four adult Van cats weighing 2.8–3.3 kg were obtained from the Yüzüncü Yıl University Veterinary Medicine Animal Clinics, Van, Turkey. Tissue samples were taken from cats that had been killed in road traffic accidents.

2.2. Histochemical and immunohistological staining

Pancreatic tissue samples (right and left lobes) were fixed in 10% neutral buffered formalin for 24 h at room temperature. The tissue samples were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Six serial sections of 5–7 μm thick were collected from each lobe of the pancreas. One of 6 serial sections was stained with Crossman modified Mallory's triple stain for examination of the histological structure of the pancreas, and the remaining 5 serial sections were used for immunohistochemical staining in order to identify each of the pancreatic endocrine cells. Langerhans islets in these sections were examined under light microscope, digital images were recorded (Optiphot 2 and i50, Nikon, Japan), and unit area (μm^2) of islets was calculated with a Kameram SLR 1.6.1.0 (Mikro Sistem Ltd. Sti., Turkey).

Pancreatic endocrine cells were detected using the streptavidin–biotin–peroxidase method. The immunohistochemically stained antibodies and chemicals can be seen in Table 1. Each tissue sample was treated with 0.3% H_2O_2 (20 min) for inhibition of endogenous peroxidase activity, and they were incubated in 5% normal bovine serum (1:5 diluted Tris; 20 min) to reduce nonspecific staining. In this study, 5 primary antibodies were used: 1) polyclonal rabbit antiglucagon for A-cells, 2) polyclonal guinea pig antiinsulin for B-cells, 3) polyclonal rabbit antisomatostatin for D-cells, 4) polyclonal rabbit antigastrin for G-cells, and 5) monoclonal mouse antiserotonin for EC-cells. All primary antibodies were incubated at room temperature for 60 min. These sections were incubated with biotinylated secondary antibody and streptavidin-HRP for 30 min, and then 3,3'-diaminobenzidine tetrahydrochloride (DAB) or 3-amino-9-ethylcarbazole (AEC) substrate kits for 3–5 min to obtain immunolabeling. Finally, DAB and AEC studies were performed; nuclei were counterstained with Harry and Mayer's hematoxylin.

Image analysis was performed using a personal computer, a camera, software (Kameram SLR 1.6.1.0), and an optical microscope (Nikon i50). Islets from sections of the head, body, and tail of all lobes were evaluated for concentration and distribution of each of the pancreatic endocrine cell types. Ten endocrine islets from each of the pancreatic lobes were randomly selected. The intensity of staining with antibodies was subjectively scored as follows: A = nonreactivity; B = weak, individualized cell reactivity in ≤25% of islets; C = mild to moderate reactivity in ≤50% of islets; D = strong reactivity in ≤75% of islets; and E = very strong reactivity in >75% of islets. The average staining intensity was calculated by the formula [(A × 1) + (B × 2) + (C × 3) + (D × 4) + (E × 5)] / (A + B + C + D + E) and were reported as follows: i) not detected – = 0.00, ii) rare + = 0.01–1.00, iii) small number ++ = 1.01–2.00, iv)

Table 1. Antibodies and chromogens used in immunohistochemistry.

Antibodies	Product Code	Dilution	Companies
Polyclonal rabbit antiglucagon	NCL-GLUCp	1:50	Leica-Novocastra
Polyclonal guinea pig antiinsulin	A0564	1:50	Dako
Polyclonal rabbit antisomatostatin	332A-18	1:50	Cell Marque
Polyclonal rabbit antigastrin	NCL-GASp	1:50	Leica-Novocastra
Monoclonal mouse antiserotonin	M 0758	1:50	Dako
Biotinylated secondary antibody	K0690	Ready for use	Dako-LSAB kit
Streptavidin-HRP	K 0690	Ready for use	Dako-LSAB kit
3,3'-Diaminobenzidine tetrahydrochloride (DAB)	D5905	Ready for use	Sigma
3-Amino-9-ethylcarbazole (AEC)	HAC15144	Ready for use	Lab-Vision

moderate amount +++ = 2.01–3.00, and v) high number ++++ = 3.01–4.00 (11,21).

3. Results

The pancreas is morphologically located between the ascending and descending loops of the duodenum in Van cats. During histochemical examination, pancreatic sections colored with triple stain displayed a similar appearance in both endocrine and exocrine tissues, as with other mammalian species. The exocrine pancreas was composed of glandular acinar cells and excretory ducts with both small and large diameters. The lumen of the acini was shown to be single or in clusters of 2 to 3 centroacinar cells. In all lobes, the endocrine pancreas was mostly composed of small islets or, more rarely, large islets with oval, round, irregular shapes. Moreover, noticeably small islets of <393 μm^2 and large islets of >4800 μm^2 were found. Semiquantitative analysis of immunopositive endocrine cells in the pancreas of Van cats can be seen in Table 2.

In this study, glucagon-, insulin-, somatostatin-, and gastrin-immunoreactive endocrine cells were detected, whereas serotonin-immunoreactive cells were not observed in the islets of Langerhans and/or exocrine pancreas using immunohistochemical staining. The regional distribution, relative frequency, and appearance of A-, B-, D-, and G-cells within the islets of Langerhans were similar in all pancreatic lobes of the Van cat. In these animals, endocrine islets were observed as 3 different structures: 1) large islets were composed of a combination of A- and B-cells; 2) small islets contained numerous A-cells, a few B-cells, and a minimal number of other endocrine cells; 3) small islets contained primarily B-cells, a few A-cells, and, rarely, other endocrine cells.

Glucagon-immunopositive cells were identified to be most commonly distributed throughout the central area (Figures 1A and 1B) and were rarely observed in the peripheral areas of the Langerhans islets (Figure 1C). In addition, a small number of these cells were also found in the exocrine parenchyma (Figure 1B) as single or small clusters in the ductal epithelium and the connective tissues of the excretory ducts (Figure 1D).

Generally, localizations of insulin immunopositive cells were found surrounding the entire periphery of the Langerhans islets (Figure 2A). However, in some islets these cells were randomly scattered throughout the area (Figure 2B). On rare occasions, these cells were observed in the central region, also. Insulin-immunopositive cells were also found within islets in the interstitial connective tissues (Figure 2C). Single or 2- to 3-cell clusters were identified in the exocrine parenchyma (Figure 2D) and the epithelium of ducts (Figure 2E).

In the analyses of somatostatin- and gastrin-immunopositive cells, they were observed in both the Langerhans islets and the exocrine pancreas (Figures 3A–3D and 4A–4D). D- and G-cells were located, usually as single cells or in 2-cell clusters, in the peripheral regions (Figures 3A and 4A), although a small number was distributed throughout the islets. A small number of D- and G-cells were also found between the acinar cells in the exocrine parenchyma (Figures 3B and 4B), and in the excretory duct epithelium (Figures 3C, 3D, and 4C). A small number of single gastrin-immunopositive cells were also observed in the ganglia of the pancreas (Figure 4D). However, serotonin-immunopositive cells were not identified anywhere in the Van cat pancreas.

4. Discussion

The Van cat pancreas consists of acinar cells, small and large excretory ducts, and round, oval, or irregular Langerhans islets, and it is similar to those of other mammals (1–3,9,18,19). The Langerhans islets contained A-, B-, D-, and G-cells. However, using this technique, EC-cells were not detected in either pancreatic islets or exocrine parenchyma. The Langerhans islets of carnivores are reported as being composed of glucagon-, insulin-, somatostatin-, and pancreatic polypeptide-immunopositive cells in cats (5) and dogs (7,16) along

Table 2. Distribution and localization of immunopositive endocrine cells in pancreas of the Van cats.

Antibodies	Langerhans islet			Exocrine pancreas	Ductal epithelium	Ganglion
	Periphery	Central	Throughout			
Glucagon	++	++++	+++	+	+	-
Insulin	++++	++	+++	++	++	-
Somatostatin	++	+	-	+	+	-
Gastrin	+	-	-	+	+	+
Serotonin	-	-	-	-	-	-

– = not detected, + = rare, ++ = small number, +++ = moderate amount, ++++ = high number.

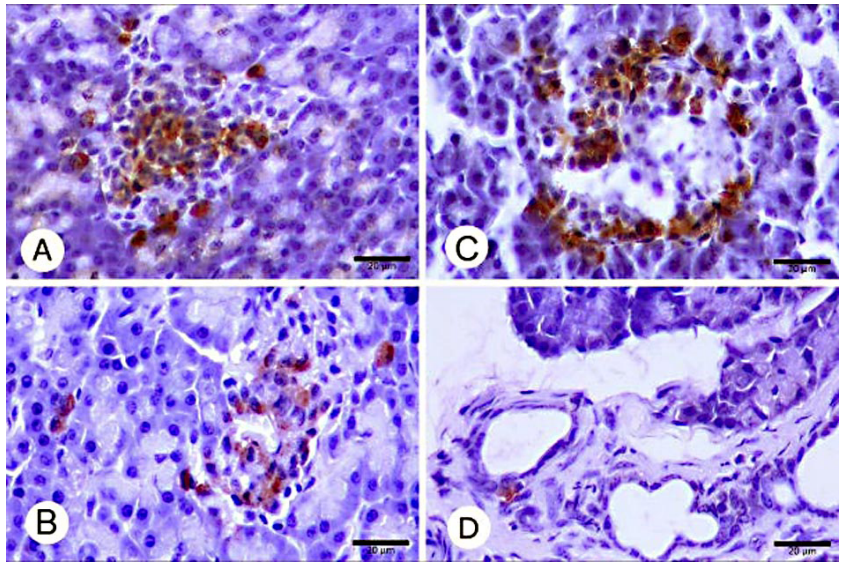

Figure 1. Glucagon immunopositivity in Van cat pancreas. A number of A-cells are located in the central region **(A, B)** and are arranged in the peripheral zone **(C)** of the islets. These cells are also observed both among acini **(B)** and duct epithelium cells **(D)**. Streptavidin–biotin–peroxidase with DAB chromogen. Bar = 20 µm.

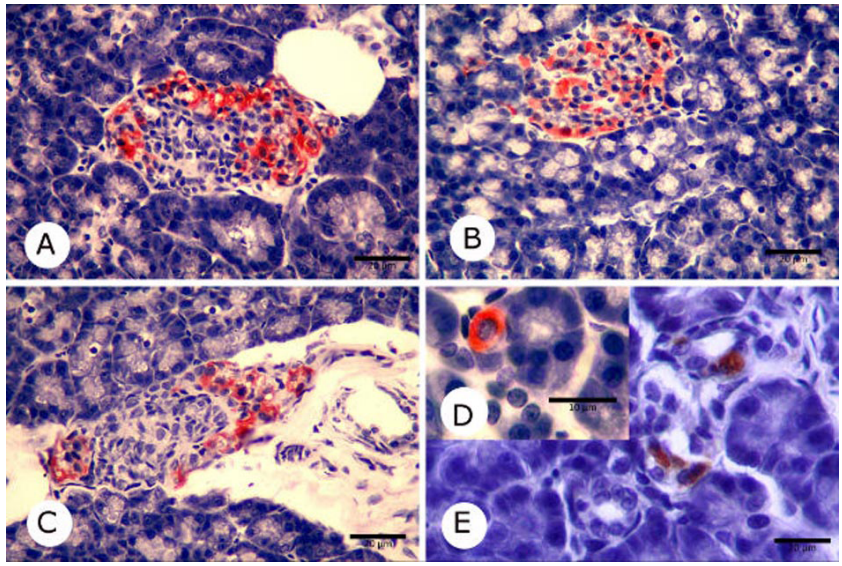

Figure 2. Insulin immunopositivity in Van cat pancreas. A number of B-cells are arranged in the peripheral zone **(A)** and are distributed throughout **(B)** the Langerhans islets. Some Langerhans islets are observed in the interlobular area **(C)**. A few insulin-positive B-cells are shown in the exocrine pancreas area **(D)** and among the duct epithelium **(E)**. Streptavidin–biotin–peroxidase. A, B, C, E: Bar = 20 µm; D: bar = 10 µm. A, B, C, and D with AEC chromogen; E with DAB chromogen.

with chromogranin A, keratin, synaptophysin, Leu-7, and proliferating cell nuclear antigen in the pancreatic islets of dogs, except with gastrin- and bombesin-immunopositive cells (16).

Some studies suggest that, in rats (3), dogs (7), and quails (17), pancreatic endocrine cells might show some differences in regional distribution among species. There are a higher number of islets in the splenic lobe (tail part) of the pancreas compared with the other lobes. The head part of the dog pancreas has smaller islets and single cells, while the tail part contains large islets (16). In this study, it was determined that the size and distribution of A- and

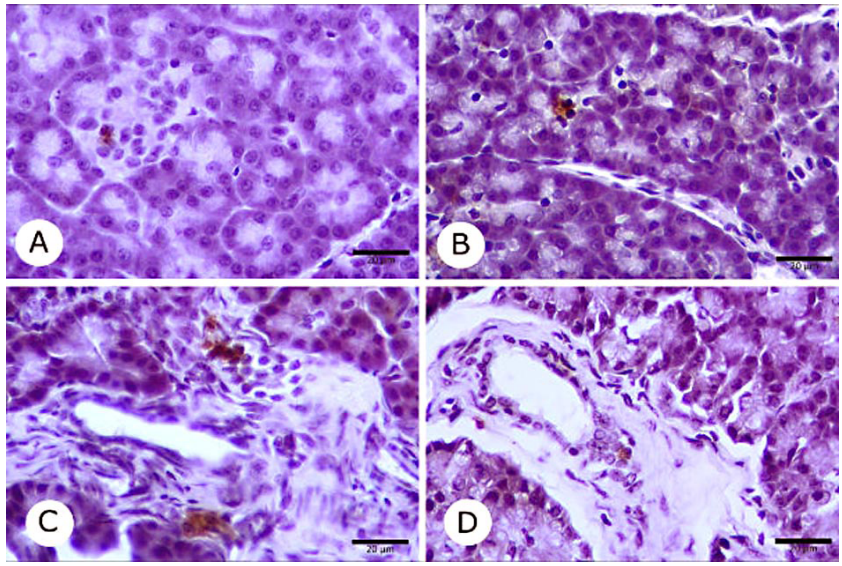

Figure 3. Somatostatin immunopositivity in Van cat pancreas. D-cells are especially arranged as single or 2 cells in the peripheral zone of the islets **(A)**. These cells are also found in the exocrine pancreas **(B)**, in some islets localized in the interlobular connective tissue **(C)**, and among the duct epithelium **(D)**. Streptavidin–biotin–peroxidase with DAB chromogen. Bar = 20 µm.

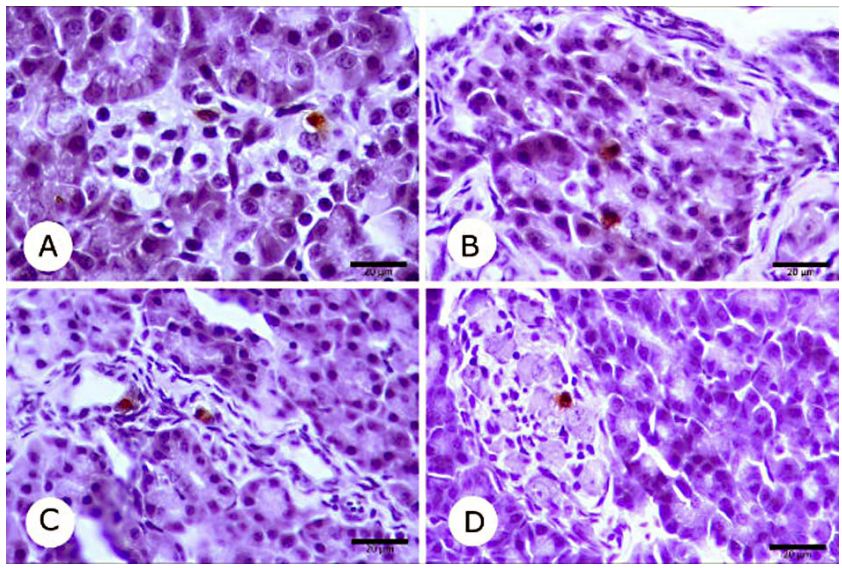

Figure 4. Gastrin immunopositivity in Van cat pancreas. Gastrin positive G-cells were distributed as single cells in the peripheral zone of the islets **(A)**, in the exocrine part **(B)**, among the duct epithelium **(C)**, and in the ganglion **(D)**. Streptavidin–biotin–peroxidase with DAB chromogen. Bar = 20 µm.

B-cells in Langerhans islets were similar in the all lobes of the Van cat pancreas. This study also found that there was a higher proportion of small diameter islets (<393 µm^2) compared to those of a larger diameter (>4800 µm^2).

The glucagon-secreting A-cells are usually found to form a continuous mantle surrounding the B-cells in the islet core for most rodent species (guinea pigs, mice, rats) (2,20). These cells are generally located in the center of islets in horses (4), monkeys (19), and kangaroos (18). Recent research also suggests that the endocrine cell architecture of the pancreas in monkeys and humans shows irregular distribution (9,18). Pancreas islets

generally consist of approximately 40% A-cells in humans (2,9) and 16%–24% in dogs (7), and 30% of A-cells are found in the body and the tail of the cat pancreas rather than in the head (5). Gustavsen et al. (12) reported that the African ice rat (*Otomys sloggetti robertsi*) displays a greater proportion of A- and D-cells than other rat species. A-cells in this species are peripherally located as 2 or 3 cell layers around a core of B-cells. The present study reveals that a higher number of glucagon-immunopositive cells were distributed throughout the central region of the Van cat's endocrine pancreas. Additionally, these cells were found (infrequently) in the periphery of the islets and were found as single or 2-cell clusters in the exocrine parenchyma and ductal epithelium. These results were found to be similar to those of human, monkey (9), and horse pancreatic islets (4), but contradicted previous reports on rodents (2,20).

The insulin-secreting B-cells are commonly localized in the periphery of the pancreatic islets in horses (4), monkeys (19), and kangaroos (8%–50% of islet cells) (18). However, in mice and rats these cells are found most frequently (60%–80% of islet cells) in the central core (2,18). In the adult guinea pig, the Langerhans islets are mostly composed of B-cells localized throughout the endocrine regions (16). The results of this study show that insulin-immunoreactive cells are generally found in both the small and the large islets and are commonly distributed in the peripheral regions or throughout the islets. However, a few of these cells were also restricted to mantle zones, which are similar to previous reports on horses, monkeys, and kangaroos.

Somatostatin-immunopositive cells are found in both the periphery and center of endocrine islets in cattle, sheep, and goats, but only in the periphery of endocrine islets in rabbits, rats, and mice. In cats, dogs, and humans, this distribution is random (18). Muranishi et al. (7) reported that D-cells in the dog pancreas tend to be randomly scattered throughout the Langerhans islets. The D-cells constitute <15% of islet cells in the tail lobe and are found in very small numbers in the head of the pancreas. In domestic cats, solitary D-cells have also been observed in small numbers in the periphery of the Langerhans islets (8). The results from this study are parallel to those from reports on domestic cats (8). Furuzawa et al. (5) reported that the somatostatin-immunopositive D-cell populations appeared to be closely similar to the glucagon-immunopositive A-cell populations in all lobes of the cat pancreas. The somatostatin-immunopositive cells were observed in small numbers as single cells or 2-cell clusters in the periphery of the Langerhans islets in Van cats.

Many researchers suggested that pancreatic endocrine cells might be found in the exocrine parenchyma and the ducts epithelium of the pancreas (5,16,21,22). In the cat pancreas, solitary A-, B-, and D-cells have also been observed in the epithelium of pancreatic ducts (5,8), in nerves, and in the ganglia of interlobular ducts (6). In dogs, islets usually have 20–30 endocrine cells, although small clusters of cells appear in the connective tissues of the excretory ducts (16). The fetal and adult pancreatic endocrine cells are regulated by a balance of endocrine cell regeneration and apoptosis or new islet cells could be formed by stem cells associated with the ductal epithelium (21–23). In this study, A- and B-immunopositive cells were found as single cells or in 2- or 3-cell clusters in the epithelium of ducts, interlobular connective tissues, and exocrine tissues near the acini. These results are parallel to reports on other domestic cats and dogs but contradict the reports of Furuzawa et al. (6). However, somewhat different from the findings of other researchers, we observed B-cells in high numbers as single cells or 2- or 3-cell clusters in the exocrine parenchyma, in the small islets in the connective tissues, and in the epithelium of ducts. Additionally, our findings in the connective tissues and epithelium of ducts, according to some researchers (21–23), may be an indication of islet cell proliferation or neogenesis in the Van cat pancreas.

Gastrin- and serotonin-releasing cells are generally found in the gastrointestinal tract, but are also found in the islets of the developing and adult pancreas. Although the role of gastrin in the pancreas is undefined, some authors suggest it may increase islet development and may be responsible for islet neogenesis in the ductal epithelium (22,23). Wieczorek et al. (16) reported that gastrin-immunopositive cells are not found in the pancreatic islet, parenchyma, and ductular epithelium of dogs. This is the first study to record gastrin-immunopositive cells in the duct epithelium and some intrapancreatic ganglia cells, and in the small islets associated with small and large excretory ducts of the Van cats. According to the above research, these findings may be an indication of continuing islet cell proliferation or islet neogenesis during postnatal periods in Van cats.

Serotonin combines with insulin in granules of B-cells to modulate insulin secretion in islets (14) and regulates zymogen secretion in acinar cells (24). Kim et al. (10) and Schraenen et al. (25) suggested that synthesis, storage, and secretion of serotonin are performed by a subpopulation of beta cells during pregnancy and in newborn mice. Melatonin is formed by conversion of serotonin, which in treated diabetic rats causes B-cell regeneration (13) and neogenesis originating from the endocrine cells associated with the ductal epithelium (21). The appearance of EC-cells on day 10 of gestation in the fetal mouse pancreas was reported by Rodríguez Sánchez et al. (26); in these cells, at day 15 of gestation, they observed a drastic increase in the classic pancreatic endocrine cells but a decrease in the EC-cell numbers in the pancreatic parenchyma. In addition,

the serotonin-immunoreactive cells were very scarcely distributed in the ductal system and endocrine pancreas of the newborn and adult mice. Furuzawa et al. (6) reported that calcitonin gene-related peptide-, galanin-, vasoactive intestinal polypeptide-, cholecystokinin-, bombesin-, and substance P- immunopositive reactions were demonstrated predominantly in intrapancreatic ganglia cells of the cat pancreas. In this study, serotonin-immunopositive cells were not identified in Langerhans islets, ganglia cells, and excretory ducts of the Van cat pancreas.

In conclusion, this is the first study of its kind to record the regional distribution and relative frequency of A-, B-, D-, and G-cells in Van cat pancreas' Langerhans islets, exocrine parenchyma, and excretory duct epithelium, although EC-cells were not reported.

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