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## The Localization of Insulin, Glucagon, Somatostatin Immunoreactive Cells in the Pancreas of the *Martes Foina*

In this study, three kinds of the immunoreactive endocrine cells were detected using to insulin, glucagon and somatostatin antisera in the pancreas of the *Martes foina*. Different regional distributions and relative frequencies of these immunoreactive cells were observed in the different pancreatic regions. Insulin immunoreactive cells were located in the central regions with high frequency, and a few of these cells were also demonstrated in the mantle zones. Glucagon immunoreactive cells were mainly restricted to mantle zones but a few of these cells were also demonstrated in the central zones. However; rare examples were found in the peripheral regions. Somatostatin immunoreactive cells were detected in the mantle zones and peripheral regions with moderate and rare frequencies, respectively. However, somatostatin immunoreactive cells were not demonstrated in the exocrine portions.

**Key words:** Immunohistochemistry; insulin; glucagon; somatostatin; pancreatic islets; *Martes foina*.

### İnsülin, Glukagon ve Somatostatin İmmunoreaktif Hücrelerinin Sansar Pankreas'ındaki Lokalizasyonu

Bu çalışmada, sansar pancreas'ında insülin, glukagon, ve somatostatin antiserumları kullanarak 3 çeşit immunoreaktif hücre saptandı. Bu immunoreaktif hücrelerin, Langerhans adacıklarındaki bölgesel dağılımları ve nisbi sıklıklarının farklı olduğu incelendi. İnsülin'e immunoreaktif hücreler en fazla merkezi bölgelerde olmakla birlikte, birkaç tanesi de ara bölgede görüldü. Glukagon'a immunoreaktif hücreler, başlıca ara bölgeyle sınırlandıysa da, bu hücrelerin birkaç tanesi merkezi kısımlarda görüldü. Bununla birlikte nadir örnekleri periferel bölgede bulundu. Somatostatin immunoreaktif hücreler orta derece ve seyrek olarak ara bölge ile periferel bölgelerde saptandı. Bununla birlikte somatostatin immunoreaktif hücreler ekzokrin kısımlarda saptanmadı.

**Anahtar Kelimeler:** Immunohistokimya, insülin, glukagon, somatostatin, langerhans adacıkları, *Martes foina*.

### Introduction

The marten, which is the subject of this study, belongs to the Mustelidae family, order carnivora. Beech martens or stone martens range in coloration from dark brown to pale grayish brown. The limbs are long, a bushy tail is present (1, 2).

Functionally, the pancreas is divided into 2 portions, the exocrine portion, from which digestive enzymes are released and the endocrine portion, from which regulatory hormones, such as insulin, glucagon, somatostatin and pancreatic polypeptide (PP), are released into the blood circulation. The appearance, regional dispersion and relative frequency of these regulatory hormones secreted by endocrine cells in the pancreas are well recognized by their histochemistry using (3), immunofluorescence method (4) and immunohistochemistry (5,6). In addition, investigations of gastroenteropancreatic (GEP) endocrine cells are considered to be an important part of phylogenetic study (7, 8).

The pancreas has been treated as a valuable organ for endocrine studies and endocrine pancreas has been extensively studied, associated with diabetes (3,9). Until now, the regional distribution and relative frequency of four major immunoreactive cells insulin, glucagon, somatostatin and pancreatic polypeptide (PP) have been reported in the pancreas of the rodents such as hamster (10), sand rat (11), C57BL/6 mouse (9), guinea pig (12), gerbil (13), and wood Mouse (14).

It has been accepted that insulin- immunoreactive cells are located in the central regions and that THY other immunoreactive cells such as glucagon, somatostatin immunoreactive cells are in located in the peripheral or mantle zones. But, many researches suggested that species-dependent characteristic distribution of cells mantle

zones. But, many researches suggested that species-dependent characteristic distribution of cells producing different hormones in the pancreas of each species of animals might due to feeding habits and this is now generally accepted (15).

The pancreatic endocrine system has been widely studied by means of immunocytochemistry (13, 16-18), there have been no reports on immunohistochemical studies into the endocrine cells of the pancreatic islets of the marten. The object of the present study was to clarify the regional distribution and relative frequency of endocrine cells in the pancreatic islets of the marten, *Martes foina*, using an immunohistochemical method (ABC) and three types of specific antibody against insulin, glucagon, somatostatin.

### Materials and Methods

Six adult beech or stone martens (*Martes foina* Erxleben, 1777) were used without sexual distinction in this study. Under penthotal-induced (6 ml/kg) anaesthesia, the pancreas was removed immediately and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 18 hours before paraffin embedding. Tissues were routinely processed through a graded series of alcohols, cleared in xylol and embedded in paraffin. 5µm thick sections were obtained and processed for immunohistochemical staining.

**Immunohistochemistry:** Immunohistochemical staining was carried out by the peroxidase linked avidin-biotin complex (ABC) method. Blocking of endogenous peroxidase activity was carried out with % 0.08 hydrogen peroxidase (H<sub>2</sub>O<sub>2</sub>) in methanol for 5 minutes. In order to the block unspecific binding, an incubation with (1:10) normal goat serum in 0.1 M PBS, pH 7.2 was performed.

**ABC Technique:** Sections were incubated for 16-20 hrs at 4 °C in mouse anti-insulin IgG (Sigma), anti-glucagon IgG (Sigma) and anti-somatostatin IgG. The antibodies were diluted to 1:500 in PBS containing % 0.25 sodium azide and % 2.5 bovine serum albumin. Sections were then incubated in biotinylated sheep anti-mouse IgG (Sigma) and to follow with streptavidin horseradish peroxidase (Dako), both at a dilution of 1:50 in PBS, for 1 hr at room temperature. Sections were washed in PBS for 30 minutes after each incubation. Sections were then immersed in glucose oxidase- DAB-nickel ammonium sulphate (GDN) substrate (19) for 10 minutes, washed in distilled water and counterstained with eosine. Sections were examined with light microscope and photomicrographs were taken.

Controls were made by replacing each specific antiserum with normal rabbit or goat serum. No immunoreactive structures were observed.

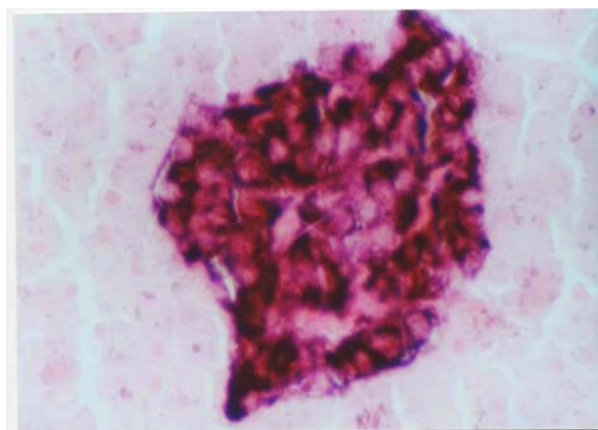
### Results

In this study, three kinds of the immunoreactive endocrine cells were detected using antisera to insulin, glucagon and somatostatin in the pancreas of the marten. The principal islets were often clearly distinguishable in the central, mantle and the peripheral regions by their cellular composition. Different regional distributions and relative frequencies of these immunoreactive cells were observed in the different pancreatic regions, and these differences were shown in table 1. Spherical to spindle or occasionally oval to round-shaped insulin, glucagon and somatostatin immunoreactive cells were observed in this study.

**Table 1.** Regional distributions and relative frequencies of the endocrine cells in the pancreas of marten. --: not detectable, +: rare, ++: a few, +++: numerous.

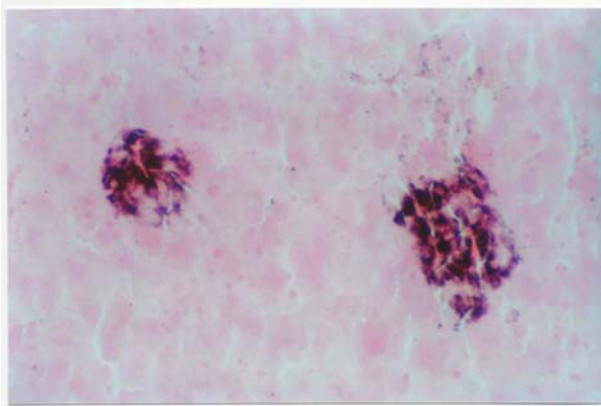
Immunoreactive Cells	Pancreatic islets portion			Exocrine Portion
	Central	Mantle	Peripheral	
Insulin	+++	++	--	--
Glucagon	++	+++	+	--
Somatostatin	+	+++	++	--

Insulin immunoreactive cells were situated in the central regions with high frequency, and a few of these cells were also demonstrated in the mantle zones. Spherical to spindle shaped insulin- immunoreactive cells were located in the central pancreatic islet region with numerous frequency and rarely round to oval shaped cells of variable size were also observed. In addition, a few frequency cells were also observed in the mantle zone intermingled with other immunoreactive cells, especially glucagon and somatostatin immunoreactive cells. However, no insulin- immunoreactive cells were found in the peripheral regions and exocrine portions (Fig 1).



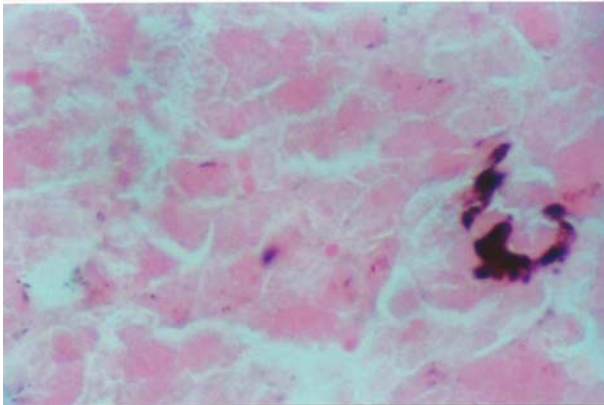
**Figure 1.** Insulin-immunoreactive cells in the pancreatic islets of *Martes foina*. Insulin-positive cells appear as intensely labelled, dark cells. 40 x 5.

Glucagon immunoreactive cells were mainly restricted to the mantle zones. In addition, a few of these cells were also demonstrated in the central zones (Fig 2). However; rare examples were found in the peripheral regions and, no glucagon immunoreactive cells were found in the exocrine portions.



**Figure 2.** Glucagon-immunoreactive cells in the pancreatic islets of *Martes foina*. 20 x 5.

Somatostatin immunoreactive cells were detected in the mantle zones and peripheral regions with moderate and rare frequencies, respectively. However, also a few somatostatin immunoreactive cells were observed in the central regions where numerous insulin immunoreactive cells were found (Fig 3). Somatostatin immunoreactive cells were not demonstrated in the exocrine portions.



**Figure 3.** Cells containing somatostatin were scattered throughout the islets. 40 x 5.

### Discussion

This study revealed that the principal pancreatic islets of the *Martes foina* contained insulin, glucagon and somatostatin immunoreactive-cells. Additionally, somewhat different distributional patterns of these three types of immunoreactive cells were observed in the *Martes foina*.

Insulin is synthesized in the B cells of the pancreatic islets and regulates the serum glucose levels (20). In the mammals, the regional distribution and relative frequency of insulin immunoreactive cells in the pancreas were reported in the hamster (10), C57BL/6 mouse (11), gerbil (13), wood Mouse (14), voles (21), opossum (22), and various laboratory animals (15). From these reports, it is well recognized that insulin immunoreactive cells are situated in the central regions of mammalian pancreatic islets and that other cells, such as glucagon and somatostatin immunoreactive cells, surrounded them. However, somewhat contradicting the finding of other researchers, Redy et al. (23) reported that these immunoreactive cells are observed in the majority of islets where they occur peripherally as groups of cells and within the pancreatic islets of several marsupial species. In the present study, most of the insulin immunoreactive cells were restricted to central regions of islets in the *Martes foina*, which is similar to previous reports on rodents (9,10,14,15,21).

Glucagon is synthesized in the A cells of the pancreas and regulates blood glucose levels (20). Morphologically similar cells are also present in the digestive tract of the dog (15). In the present study, glucagon immunoreactive cells were mainly restricted to the mantle zones but a few of these cells were also demonstrated in the central zones in the pancreas of the marten. This results were found to be similar to that of mammalian pancreatic islets (9,10,15,21,22). Although it is seldom in rodents, cell clusters consisted of glucagon immunoreactive cells located in the connective tissue regions of pancreatic duct portions that are generally detected in higher mammals (24).

Somatostatin which consisted of 14 amino acid, was isolated from hypothalamus of sheep for the first time and it could be divided into straight form and cyclic form (25). This substance inhibits the secretion of the gastrin, cholecystokinin, secretin, glucagon, insulin, motilin and gastric acid (26) and absorption of amino acid, glucose and fatty acids in the gastrointestinal tract (27). In the pancreas of *Martes foina*, somatostatin immunoreactive cells were detected in the mantle zones and peripheral regions with moderate and rare frequencies, respectively. However, somatostatin immunoreactive cells were not demonstrated in the exocrine portions. This results are paralleled that somatostatin immunoreactive cells have been found in the outermost regions of mammalian pancreatic islets (9, 10, 14, 15, 21, 22). However, in the present study, most of these immunoreactive cells were found in the mantle zones and mixed with glucagon immunoreactive cells. These presented topographically different distributional patterns in mammalian species (9, 10, 14, 15, 21, 22).

In conclusion, the regional distribution of endocrine cells in the pancreatic islets of *Martes foina* was found to be similar to that of other mammals, especially rodents, except for the topographically different distribution of somatostatin compared to that of other rodents.

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