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Immunohistochemistry of the Pancreatic Endocrine Cells of the Red-eared Slider (*Trachemys scripta elegans*)

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Regional distribution and relative frequency of endocrine cells in the pancreas of the red-eared slider, *Trachemys scripta elegans*, were investigated by immunohistochemical methods. Chromogranin (Cg) A-, serotonin-, insulin-, glucagon-, somatostatin-, bovine pancreatic polypeptide (BPP)- and human pancreatic polypeptide (HPP)-immunoreactive cells were identified in this study. Most of immunoreactive cells in the exocrine and endocrine pancreas (Langerhans islet) were generally spherical or spindle-shaped (open-typed cell), while occasionally cells round in shape (close-typed cell) were found in the basal portion or interepithelial regions of the pancreatic duct. These immunoreactive cells were located in the exocrine, endocrine pancreas and/or basal or interepithelial portion of the pancreatic duct. Serotonin-immunoreactive cells were found in the basal portion of epithelia of the pancreatic duct at a low frequency and interacinar region of the exocrine at a moderate frequency. Insulin-immunoreactive cells were found in the central portion of the endocrine pancreas, interacinar regions of the exocrine pancreas and basal portion of the epithelia of the pancreatic duct at high, moderate and low frequencies, respectively. Glucagon-immunoreactive cells were detected in the periphery of the endocrine pancreas, interacinar region of the exocrine pancreas and basal portion of the epithelia or interepithelia of the pancreatic duct at high, moderate and moderate frequencies, respectively. Somatostatin-immunoreactive cells were dispersed in the whole area of the endocrine pancreas, interacinar regions of exocrine pancreas and basal portion of the epithelia or interepithelia of the pancreatic duct at a moderate frequency. BPP- and HPP-immunoreactive cells were detected in the interacinar region of the exocrine pancreas at moderate and hige frequencies, respectively. However, no Cg A- and motilin-immunoreactive cells were detected in this study.

The red-eared slider, *Trachemys scripta elegans*, belonging to the Emydidae in order Testudines, dwells in the southern part of America and are also called Florida turtle because of their habitation regions. Recently, this slider is being recognized as a pet animal worldwide including Korea. Howerer it is a problem that neglected sliders, after reared as a pet, cause destruction of ecosystem in non-naturally habituated regions of Korea. Gastroenteropancreatic (GEP) endocrine cells, dispersed in the epithelia and gastric glands of the alimentary tract and pancreas, synthesize various kinds of GEP hormones and play an important role in the physiological functions of the alimentary tract and pancreas (Bell, 1979). Until now, investigations on GEP endocrine cells have been considered to be an important part of a phylogenic study (D'Este et al., 1994). In addition, regional distribution and relative frequency of these endocrine cells vary among animal species and feeding habits (Solcia et al., 1975). Although many studies have elucidated regional distribution and relative frequency of different endocrine cells in the pancreas of the various vertebrates, immunohistochemical studies on reptilia have received little attention. Most recently, intensive studies have been done on the reptilian species because their phylogenetical tree is situated at middle in the evolution of vertebrates (Buchan et al., 1983)

Until now, immunohistochemical studies on the pancreatic endocrine cells of turtles were as following. Insulin-, somatostatin-, glucagon-, pancreatic polypeptide (PP)-, peptide tyrosine tyrosine- and neuropeptide tyrosin-like immunoreactivities were identified in the

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pancreas of the red-eared turtle, *Pseudemys scripta elegans* by immunogold labeling and immunohistochemistry (Lozano et al., 2000), and granin proteins (chromogranin (Cg) A and secretogranin)-immunoreactive cells were detected in the pancreas of turtle but not of lizards and snakes (Trandaburu et al., 1999). Ding et al. (1997) reported that neuropeptide Y and peptide YY-immunoreactive endocrine cells in the pancreas of turtle were more numerously detected than in higher vertebrates such as, rat, mouse, human, dog etc. In addition, serotonin-immunoreactive cells found in the exocrine pancreas of the 11 species of the vertebrates including turtle (Ding et al., 1991). In addition, Garcia-Ayala et al. (1987) suggested that the regional distribution and relative frequency of insulin-, glucagon-, somatostatin- and PP-immunoreactive cells varied with season (summer and winter) and that ultra structural morphology of the secretory granules of these immunoreactive cells showed seasonal variation. Agulleiro et al. (1985) reported that the insulin-, glucagon-, somatostatin- and PP-immunoreactive cells were distributed differently from sampled portion of the red-eared turtle, *Pseudemys scripta elegans*. But little data is available on the regional distribution and relative frequency of endocrine cells in the pancreas of the Emydidae.

The purpose of the present study was to clarify the regional distribution and relative frequency of the endocrine cells in the pancreas of the red-eared slider, Emydidae, *Trachemys scripta elegans* by specific immunohistochemistry using 8 types of antisera against Cg A, serotonin, insulin, glucagon, somatostatin, bovine and human PP.

Materials and Methods

Five adult (16-20 cm in diameter) red-ear sliders of the Emydidae, *Trachemys scripta elegans*, without sexual distinction were used in this study. Pancreas samples were prepared from the animals anesthetized with ethyl ether after phlebotomization, and were fixed in Bouin's solution. After paraffin embedding, 3-4 μm serial sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal alimentary architecture.

Each representative sections were deparaffinized, rehydrated and immunostained with the peroxidase antiperoxidase (PAP) methods (Sternberger, 1979). Background blocking was performed with normal goat serum prior to incubation with specific antisera (Table 1). After rinses in phosphate buffered saline (PBS, 0.01 M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS and finally the PAP complex was prepared. The peroxidase reaction was carried out in 3,3-diaminobenzidine tetrahydrochloride solution containing 0.01% H_2O_2 in 0.05 M, Tris-HCl (pH 7.6). Immunostained sections were lightly counterstained with Mayers hematoxylin and the immunoreactive cells were observed under a light microscope.

Table 2. Regional distributions and relative frequencies of the endocrine cells in the pancreas of the red-eared slider, *Trachemys scripta elegans*

Hormone	Pancreatic islets		Exocrine pancreas	Pancreatic duct	
	Central core	Mantle region	Interacinar region	Interepithelium	Basal portion of epithelium
Chromogranin (Cg) A	—	—	—	—	—
Insulin	+++	+	++	±	±
Glucagon	-	+++	++	++	+
Somatostain	+	+	++	++	+
Bovine pancreatic polypeptide (PP)	—	—	++	—	—
Human Pancreatic polypeptide (PP)	—	—	+++	—	—
Motilin	—	—	—	—	—

+++: numerous, ++: moderate, +: a few, ±: rare, —: not detected.

Results

In this study, 6 kinds of immunoreactive cells were detected with antisera against serotonin, insulin, glucagon, somatostatin, bovine and humane PP. The regional distribution and relative frequency of these immunoreactive cells in the pancreas of the red-eared slider are shown in Table 2. Most of immunoreactive cells were generally spherical or spindle-shaped a (open-typed cell), while occasionally round (close-typed cell) cells were found in the basal portion or interepithelia of the pancreatic duct.

Spherical to spindle-shaped serotonin-immunoreactive cells were detected in the interacinar region of the exocrine pancreas at a moderate frequency and in the basal portion of the epithelia of the pancreatic duct at a low frequency. However, no serotonin-immunoreactive cells were found in the pancreatic islets (Fig. 1).

Insulin-immunoreactive cells were found in the pan-

Table 1. Antisera used in this study

Antisera raised*	Code	Source	Diluton
Chromogranin (Cg) A	A430	Incstar Corp., Stillwater.	1 : 1,000
Insulin	PUO290395	BioGenex Lab., San Ramon	1 : 24
Glucagon	PUO391095	BioGenex Lab., San Ramon	1 : 20
Somatostatin	PUO421295	BioGenex Lab., San Ramon.	1 : 20
Bovine pancreatic polypeptide (PP)	1607	UCB bioproducts, Drogenbos	1 : 5,000
Human Pancreatic polypeptide (PP)	PUO660495	BioGenex Lab., San Ramon.	1 : 20
Motilin	AB17	CRB, Billerca	1 : 1,000

*All antisera were raised in rabbits except for insulin which were raised in guinea pigs

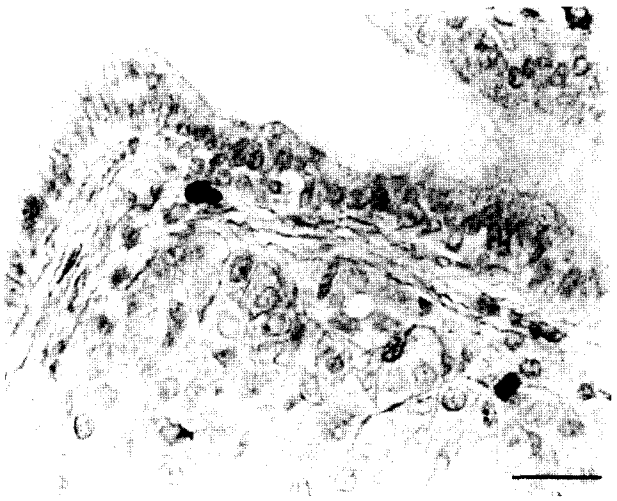

Fig. 1. Serotonin-immunoreactive cells in the pancreas of the red-eared slider, *Trachemys scripta elegans*. Scale bar=100 μm.

creatic duct, exocrine and pancreatic islets. The cells were located in the basal portion of the epithelia in the case of the pancreatic duct at a very low frequency. However, most of the immunoreactive cells having spherical to round shapes were detected in the central parts of the pancreatic islets at a high frequency. In addition, the immunoreactive cells were found at a moderate frequency in the interacinar region of the exocrine pancreas with spherical to spindle shape (Fig. 2).

Glucagon-immunoreactive cells were detected in the basal portion or interepithelial of the duct with spherical to spindle shape and similar to those of insulin- immunoreactive cells. They were also detected in the interacinar region of the exocrine pancreas at high and moderate frequencies, respectively. Spherical to spindle-shaped cells were found at a moderate frequency in the peripheral parts of the pancreatic (Fig. 3)

Somatostatin-immunoreactive cells were found in the exocrine pancreas, basal portion of the epithelia of the pancreatic duct and pancreatic islets at moderate frequencies. They were dispersed in the whole pancreatic islets but were located in the interacinar regions in the case of the exocrine pancreas with spherical to spindle shape. However, occasionally, round or oval-shaped cells were found in the basal portion or interepithelia of the pancreatic duct regions (Fig. 4).

Bovine PP-immunoreactive cells having round, spherical or spindle shape were found in the interacinar regions of the exocrine pancreas at a moderate frequency, and human PP-immunoreactive cells showed similar shape and distribution patterns to bovine PP-immunoreactive cells but at a high frequency (Fig. 5).

However, no Cg A- and motilin-immunoreactive cells were found in this study.

Discussion

In the present study, serotonin-, insulin-, glucagon-, somatostatin-, bovine and human PP-immunoreactive cells were identified in the pancreas of the red-eared slider, *Trachemys scripta elegans*. However, Cg A- and motilin-immunoreactive cells were not found.

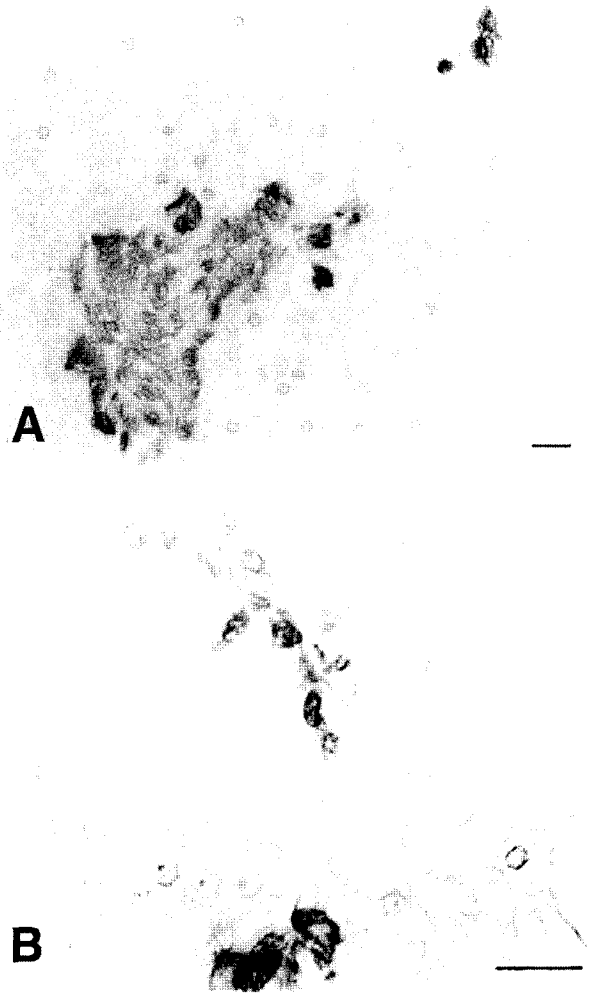

Fig. 2. Insulin-immunoreactive cells in the pancreatic islet (A) and exocrine pancreas (B) of the red-eared slider, *Trachemys scripta elegans*. Scale bars=100 μm.

Cg A-immunoreactive cells are widely distributed in endocrine cells of mammals (Rindi et al., 1986) and have been used as a marker for other endocrine cells (Cohn et al., 1984). In addition, Trandaburu et al. (1999) reported that these immunoreactive cells were found only in the turtle pancreas among the four reptile species belonging to the turtles, lizards and snakes whereas secretogranin II C23-3 immunoreactive cells appeared both in the turtle and snake. They also suggested that, despite the restricted presence in the endocrine pancreas of the reptiles, the Cg As are relatively well-conserved during phylogeny. They did not confirm, however, usefulness of the granin protein family as common markers of neuroendocrine cells as previously accepted. That Cg A-immunoreactive cells

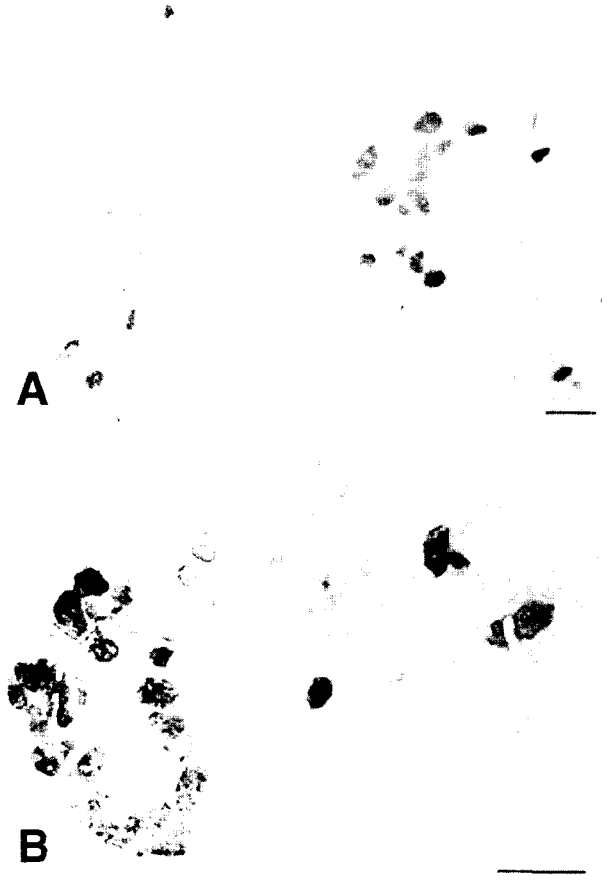

Fig. 3. Glucagon-immunoreactive cells in the exocrine (A), and pancreatic islet and pancreatic duct (B) of the red-eared slider, *Trachemys scripta elegans*. Scale bars=100 μm.

were not found in the present study differed from the previous report (Trandaburu et al., 1999). Based on these results, it is suggested that Cg A is not suitable as a common marker for neuroendocrine cells in the case of the pancreas of the red-eared sliders. Until now, observation of reptilian species were restricted, and the possibility of the usefulness of the Cg A as a common marker for neuroendocrine cells in the pancreas of other reptilian species should be investigated.

Serotonin is a monoamine widely distributed in the nervous system and GEP endocrine cells (El-Salhy et al., 1985). Main functions of serotonin are to inhibit gastric acid secretion and contraction of smooth muscle in the GEP system (Guyton, 1988). El-Salhy et al. (1985) reported that serotonin- immunoreactive cells were detected throughout the GIT of all species and that they are established in the GIT at early stages of the vertebrate evolution. These cells are distributed in the exocrine pancreas and epithelia of the pancreatic duct in the *Testuso graeca*, *Mauremys caspica* and *Lacerta lepida* (Perez-Tomas et al., 1989). In addition, Ding et al. (1991) reported that serotonin- immunoreactive cells were found in the pancreas of the turtle, mainly detected in the exocrine region. Similar to the

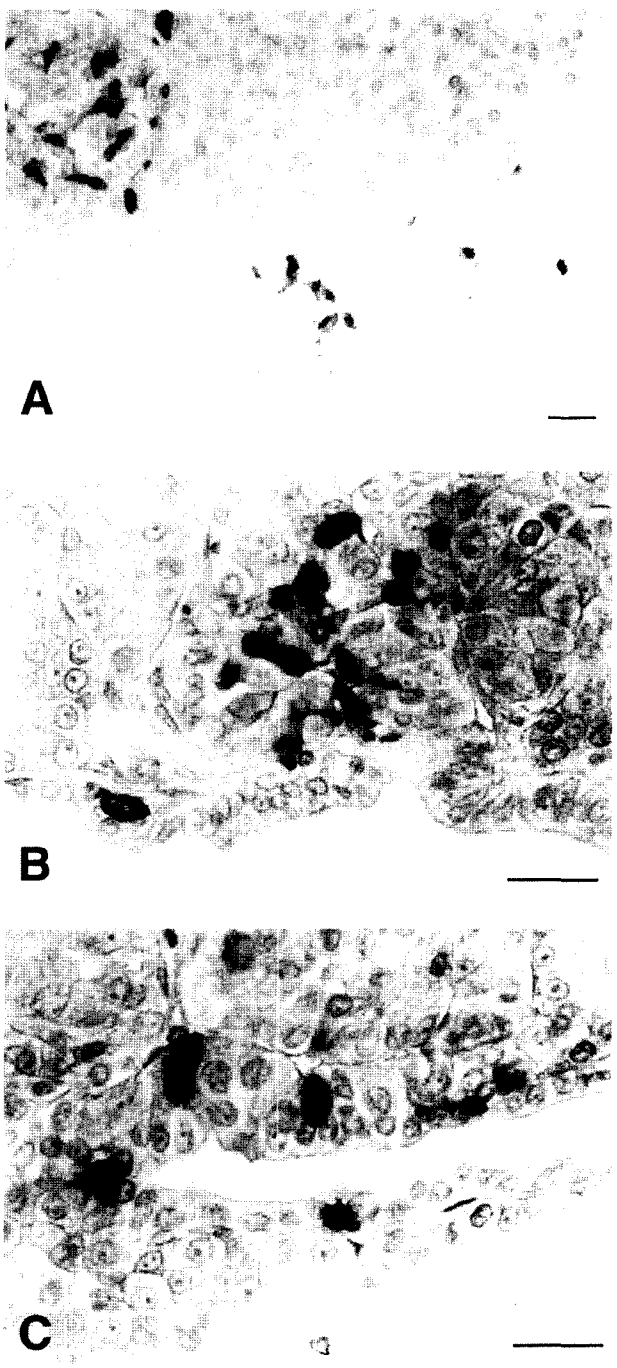

Fig. 4. Somatostatin-immunoreactive cells in the exocrine (A), pancreatic islet (B) and pancreatic duct (C) of the red-eared slider, *Trachemys scripta elegans*. Scale bars=100 μm.

previous reports (Perez-Tomas et al., 1989; Ding et al., 1991), serotonin-immunoreactive cells were demonstrated in the interacinar regions of the exocrine pancreas and basal portion of the pancreatic duct epithelia in the pancreas of the red-eared slider.

Insulin is synthesized in the B cells of the pancreatic islets and regulates serum glucose level (Hsu and

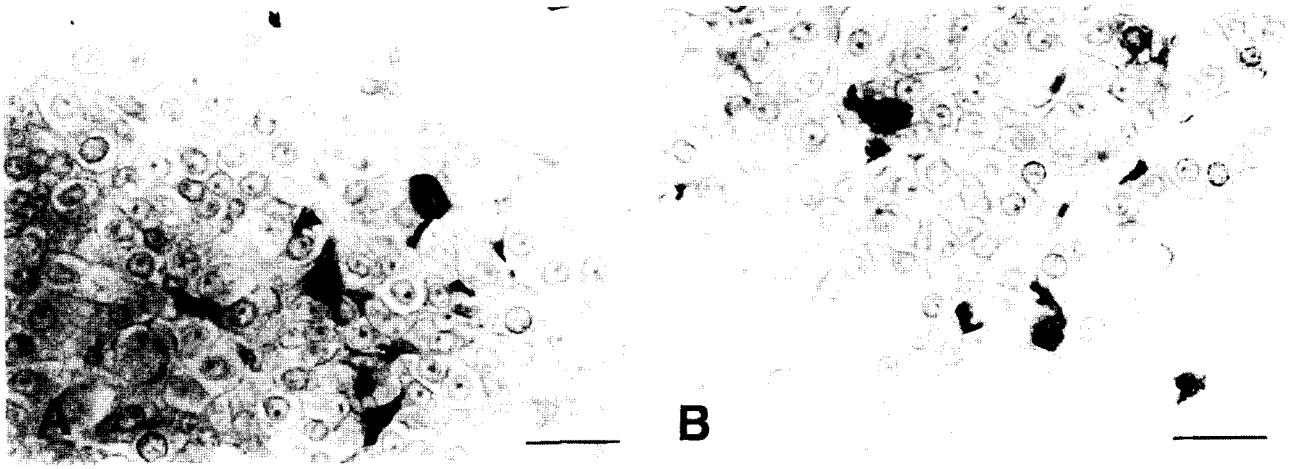

Fig. 5. Bovine (A) and human (B) PP-immunoreactive cells in the pancreas of the red-eared slider, *Trachemys scripta elegans*. Scale bars=100 μm.

Crump, 1989). In the pancreas of the reptilian spices, insulin-immunoreactive cells were present as single elements or grouped in the pancreatic islets of the *Chalcides chalcides* and *Zoonosaurus madascariensis* (Sauridae) (Morescalchi et al., 1997). They were located in the central core of the pancreatic islets in the three reptiles, *Testudo graeca*, *Mauremys caspica* and *Lacerta lepida* (Perez-Tomas et al., 1989). Garcia-Ayala et al. (1989) reported that insulin-immunoreactive cells were more abundant and the cell groups larger in the splenic than in the duodenal region in winter whereas in summer, medium or small cell groups were evenly distributed. These immunoreactive cells were located in the islet center and comprised 3% of dorsal and 0.2% of ventral lobe volume in the squamate reptile, the desert lizard (*Chalcides ocellatus*) (El-Sahly et al., 1983). In addition, Putti et al. (1992) reported that insulin-immunoreactive B cells appeared in 11 species, 3 genera of lacertids. Different distributional patterns were seen in the pancreatic islets but these immunoreactive cells were mainly distributed in the central core of the pancreatic islets with small numbers of glucagon-immunoreactive cells. Consistent with these previous reports (El-Sahly et al., 1983; Garcia-Ayala et al., 1989; Perez-Tomas et al., 1989; Putti et al., 1992; Morescalchi et al., 1997), insulin- immunoreactive cells are located in the central core of the pancreatic islets, some cells scattered in the interacinar regions of the exocrine pancreas and some other cells also found in the basal or interepithelia of the pancreatic duct in a case of the reptilian species. However these distributions and frequencies showed seasonal variation. Similar to those of other reptilian species, insulin-immunoreactive cells were observed in the interacinar regions of the exocrine pancreas, basal portions of the epithelia of the pancreatic ducts, and central regions of the pancreatic islets in the red-eared slider.

Glucagon is synthesized in the A cells of the pancreas and regulates serum glucose level (Hsu and Crump, 1989). Glucagon-immunoreactive cells were distributed in the peripheral mantle zone of the pancreas of 11 species of lacetids (Putti et al., 1992) and some of these immunoreactive cells were located in the central, portions of the pancreatic islets. However, different from this report, no glucagon-immunoreactive cells were detected in the central, portion of the pancreatic islets not in the peripheral, in the present study. But similar to the present study, Perez-Tomas et al. (1989) reported that glucagon- immunoreactive cells were located in the peripheral regions of the pancreatic islets of the three reptiles, *Testudo graeca*, *Mauremys caspica* and *Lacerta lepida*. In addition, these immunoreactive cells were distributed in the peripheral regions of the pancreatic islets, exocrine pancreas and pancreatic duct of the *Testudo graeca* and showed no seasonal variation (Garcia-Ayala et al., 1987).

Somatostatin, consisting of 14 amino acids, was initially isolated from hypothalamus of sheep and it could be divided into straight and cyclic forms (Brazeau et al., 1973). This substance inhibits secretion of other neuroendocrine hormones (Kitamura et al., 1984). It is known that somatostatin- immunoreactive cells show the widest distribution in the whole GEP of all vertebrate species investigated, including the primitive agnathans with serotonin- immunoreactive cells (Falkmer and Van Noorden, 1983). In the present study, the somatostatin-immunoreactive cells were dispersed in the whole pancreatic islets and interacinar regions of the exocrine pancreas. In addition, some of these immunoreactive cells were detected in the basal or interepithelia of the pancreatic duct. Different from the present study, somatostatin- immunoreactive cells in the pancreatic islets of the reptilian species were found in the peripheral region of the 11 species of lacertids (Putti et al., 1992), desert lizard (El-Sahly et al., 1983) and anolian (Rhoten and Smith, 1978). In addition, it was reported that they were dispersed in the exocrine pancreas and pancreatic duct of reptilian species

(Rhoten and Smith, 1978; El-Sahly et al., 1983; Putti et al., 1992) In addition, Garcia-Ayala et al. (1987, 1989) suggested that the somatostatin-immunoreactive cells showed seasonal variation in the pancreas of the *Mauremys caspica* and *Testudo graeca*. El-Sahly et al. (1983) reported that these immunoreactive cells were located at the islet periphery as well as in between the exocrine parenchyma and that they constituted 1 and 0.2% of the volume of the dorsal and ventral lobes, respectively.

Since PP-immunoreactive cells were described for the first time in the lizard pancreas (Rhoten and Smith, 1978), the occurrence of these cells have been demonstrated in the pancreas of the reptiles (Rhoten and Smith, 1978; El-Sahly et al., 1983; Agulleiro et al., 1985; Garca-Ayala et al., 1987, 1989; Putti et al., 1992). From these previous results, the PP-immunoreactive cells were distributed in the interacinar regions of the exocrine pancreas in a case of the reptilian species but they showed different distribution according to sampling portions (El-Sahly et al., 1983). Similar to those of the previous reports, bovine and human PP-immunoreactive cells were found in the interacinar regions of the exocrine pancreas at moderate and high frequencies, respectively. However, somewhat different from the results on the *Mauremys caspica* where PP-immunoreactive cells were demonstrated in the pancreatic duct (Garcia-Ayala et al., 1989), either bovine and humane PP-immunoreactive cells were detected in the pancreatic duct of the red-eared sliders.

Motilin-immunoreactive cells were exclusively present in the small intestine, particularly in its upper part (Pearse et al., 1977). They seem to play an important roh in the modulation of gut motility, especially in the inter-digestive phase (Solcia et al., 1989). In the pancreas, the appearance of motilin-immunoreactive cells were unique characteristics of the alligatorinae, *Caiman latirostris* and *Caiman crocodilus* (Yamada et al., 1991; Ono et al., 1991) and not of other reptilian species (Perez-Tomas et al., 1989). In the present study, similar to those of other repilian species except for alligatorinae, no motilin-immunoreactive cells were observed.

In conclusion, regional distribution and relative frequency of immunoreactive cells in the pancreas of the red-eared slider, *Trachemys scripta elegans*, were essentially similar to those of the other reptilian species. However, some characteristic differences were observed which may have been due to differences in the antisera used, methods used in each study, seasonal variation, sampled regions of the pancreas and/or species differences used in the each study (Dockray, 1977; El-Salhy and Grimelius, 1981; El-Sahly et al., 1983; Walsh, 1987; Garcia-Ayala et al., 1987, 1989).

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