Turk J Vet Anim Sci
29 (2005) 581-587

Research Article

An Immunohistochemical Study on the Endocrine Cells in the Gastrointestinal Tract of the Freshwater Turtle, *Mauremys caspica caspica*

Berrin GENÇER TARAKÇI
Department of Histology and Embriology, Faculty of Veterinary Medicine, Fırat University, 23119, Elazığ - TURKEY
E-mail: btarakci@firat.edu.tr.

Sibel ŞİMŞEK KÖPRÜCÜ
Faculty of Aquaculture, Fırat University, 23119, Elazığ - TURKEY

Mine YAMAN
Department of Histology and Embriology, Faculty of Veterinary Medicine, Fırat University, 23119, Elazığ - TURKEY

Received: 23.05.2002

Abstract: The regional distribution of endocrine cells was studied in the gastrointestinal tract of the freshwater turtle, *Mauremys caspica caspica*, by immunohistochemistry using antisera against serotonin, somatostatin, gastrin, insulin, substance P, glucagon and calcitonin gene related peptide (CGRP). The immunoreactive cells were located in the gastric glands of stomach regions and in the intestinal epithelium with variable frequencies. Most of the immunoreactive cells in the intestine were spherical or spindle-like in shape (open-type cells), while round cells (closed-type cells) were occasionally found in the stomach. Serotonin- immunoreactive cells were most commonly found in the pylorus and duodenum. Gastrin- immunoreactive cells were restricted to the ileum and rectum at low frequencies. Insulin-immunoreactive cells were detected from pyloric to rectum. No substance P, glucagon, somatostatin or CGRP-immunoreactive cells were found in this study.

Key Words: *Mauremys caspica caspica*, immunohistochemistry, stomach, intestine, endocrine cells.

Tatlı Su Kaplumbağası, *Mauremys caspica caspica*'nın Gastrointestinal Kanalında Bulunan Endokrin Hücreleri Üzerine İmmünohistokimyasal Çalışma

Özet: *Mauremycs caspica caspica*'nın gastrointestinal kanalındaki endokrin hücrelerinin bölgesel dağılımı serotonin, somatostatin, gastrin, insülin, substance P, glucagon ve calcitonin gene related peptid (CGRP)'e karşı hazırlanmış antiserum kullanılarak immünohistokimyasal metodlar ile araştırıldı. İmmünoreaktif hücrelere midenin gastrik bezlerinde ve bağırsak epitelyumunda değişen oranlarda rastlandı. İmmünoreaktif hücrelerin çoğu bağırsaklarda küremsi veya mekik benzeri görünümdeydi. Midede bazen immünoreaktiviteye yuvarlak hücrelerde de rastlandı. Serotonin immünoreaktif hücreler yoğun bir şekilde pyloris ve duodenumda gözlendi. Gastrin immünoreaktif hücrelere az miktarda ileum ve rectumda rastlandı. İnsülin içeren hücreler gastrointestinal kanal boyunca gözlenirken, substance P, glucagon, somatostatin ve CGRP immünoreaktif hücrelere ise rastlanılmadı.

Anahtar Sözcükler: *Mauremys caspica caspica* , immünohistokimya, mide, bağırsaklar, endokrin hücreler.

Introduction

Gastrointestinal endocrine cells dispersed through the epitelia and gastric glands of the alimentary tract synthesize various kinds of gastrointestinal hormones and play an important role in the physiological functions of the alimentary tract (1).

A large variety of endocrine cells have been described in the gastrointestinal tract in mammals (2-7). However, studies in lower vertebrates are very scarce and few immunohistochemical studies on the reptilia have been performed. Recently a lot of work has been carried out on reptilian species, because they represent an ancient evolutionary line from which the present bird and mammals originated (8).

Identification of regulatory peptides of the alimentary tract in reptilian species has been studied using silver

staining techniques and either radioimmunochemical or immunohistochemical methods (8-15). However, limited data are available on the regional distribution and frequency of endocrine cells along the entire length of the gastrointestinal tract of the Emydidae. The aim of the present study was to clarify the regional distribution and the relative frequency of the endocrine cells, in the gastrointestinal tract of freshwater turtle, *Mauremys caspica caspica* (Emydidae), by immunohistochemistry using 7 types of antisera against gastrin, somatostatin, substance P, serotonin, insulin, glucagon and calcitonin gene related peptide (CGRP).

Materials and Methods

Six adult freshwater turtles, *Mauremys caspica caspica*, of either sex were used in this study. The animals were anesthetized with ethyl ether. After phlebotomizing, tissue samples were taken from stomach, duodenum, ileum and rectum and fixed in 4% neutral-buffered formalin for 24 h. They were then dehydrated through graded ethanol and embedded in paraffin. 7-µm-thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out using the peroxidase-antiperoxidase (PAP) method or the peroxidase linked avidin-biotin complex (ABC) method. Blocking of endogenous peroxidase was carried out with 0.008% hydrogen peroxidase (H_2O_2) in methanol for 5 minutes (16). In order to block unspecific binding, an incubation with (1:10) normal goat serum in 0.1 M phosphate buffered saline (PBS), pH 7.2 was performed.

a. ABC technique. Sections were incubated for 16-20 hours at 4 °C in mouse anti-glucagon (Sigma, G2654, U.S.A) or mouse anti-insulin (Sigma, I2018, USA). Antibodies were diluted to 1:1500 and 1:1000 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in biotinylated goat anti-mouse IgG (Sigma, B7264, USA), followed by streptavidin-biotinylated horseradish peroxidase complex (Dako, P0397, Denmark), both at dilution of 1:50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation. Sections were then immersed in glucose oxidise-DAB-nickel ammonium sulfate (GDN) substrate (17) for 10 minutes, washed in distilled water and counterstained with eosin.

Sections were examined with light microscope (Leitz Dialux 20). Photographs were taken with Kodak film, ASA 50.

b. PAP technique. Sections were incubated for 16-20 hours at 4 °C in rabbit anti-gastrin I (Sigma, G0785, USA), rabbit anti-substance P (Sigma, S8305, USA), rabbit anti-serotonin (Zymed Lab., 18.0077, San Francisco, USA), rabbit-anti somatostatin (Zymed Lab., 18.0078) or rabbit anti-calcitonin gene related peptide (Zymed Lab., 18.0012). Antibodies were diluted to 1:10,000, 1:20,000, 1:200, 1:100 and 1:100 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark), followed by rabbit peroxidase anti-peroxidase complex (Zymed Lab., 61.2003), both at dilution of 1:50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation and finally immersed in glucose oxidise-DAB-nickel ammonium sulfate substrate (17) for 10 minutes. After washing in distilled water and counterstaining with eosin, sections were dehydrated and coverslips mounted with DPX.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (18), including the replacement of specific antiserum pre-incubated with its corresponding antigen.

Sections were examined with light microscope and photographs were taken.

Results

Serotonin, insulin, gastrin-immunoreactive (-IR) cells were observed in the gastrointestinal tract of the freshwater turtle, *Mauremys caspica caspica*. The regional distribution and relative frequency of these immunoreactive cells in the gastrointestinal tract of the *Mauremys caspica caspica* are shown in the Table. Positive immunoreactive of substance P, glucagon, CGRP and somatostatin cells were not observed in any of the areas of the digestive tract of the *Mauremys caspica caspica*.

Serotonin-IR cells were located in the epithelia throughout the gastrointestinal tract at various frequencies. These immunoreactive cells were also found in the gastric gland of the fundus and pylorus with a spherical to round shape (Figure 1). These cells were at

Table. Distribution and frequency of gastrointestinal endocrine cells in the freshwater turtle, *Mauremys caspica caspica*.

	Fundus	Pylorus	Duodenum	Ileum	Rectum
Serotonin	++	+++	+++	-	++
Gastrin I	-	-	-	+	+
Insulin	-	++	++	++	+++
Substance P	-	-	-	-	-
Glucagon	-	-	-	-	-
CGRP	-	-	-	-	-
Somatostatin	-	-	-	-	-

CGRP: Calcitonin gene-related peptide
Relative frequencies: +++ (high), ++ (moderate), + (low), - (not detected).

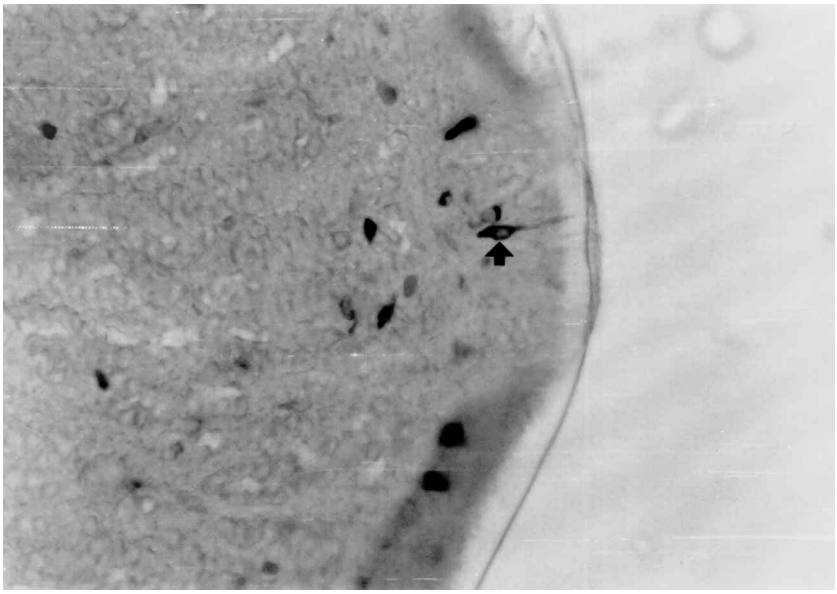

Figure 1. Serotonin containing cells in the stomach regions of freshwater turtle, *Mauremys caspica caspica* (arrows). 400x.

highest frequency in the pylorus and duodenum. No immunoreactive cells were observed in the ileum. However, immunoreactive cells were again increased in the rectum (Figure 2). In the intestinal part of the tract, most of these immunoreactive cells were located in the basal portion of the epithelia with a spindle-like of spherical shape.

Gastrin-IR cells were only detected in the ileum and rectum. These immunoreactive cells were situated in the basal portion of the epithelia in the ileum and rectum at very low frequencies. These cells were spindle shaped (Figure 3).

Insulin-immunoreactive cells were distributed from the pyloric region to the terminal portion of the large intestine (Figures 4, 5 and 6). Most insulin-IR cells were located in the large intestine. Insulin containing cells were usually spindle shaped and of open type.

Discussion

In the present study serotonin, gastrin and insulin-IR cells were identified in the gastrointestinal tract of *Mauremys caspica caspica*.

Serotonin consisted of monoamines and was widely distributed in the nervous system and gastroenteropancreatic endocrine cells (19). The main functions of serotonin were inhibition of gastric acid secretion and contraction of smooth muscle in the

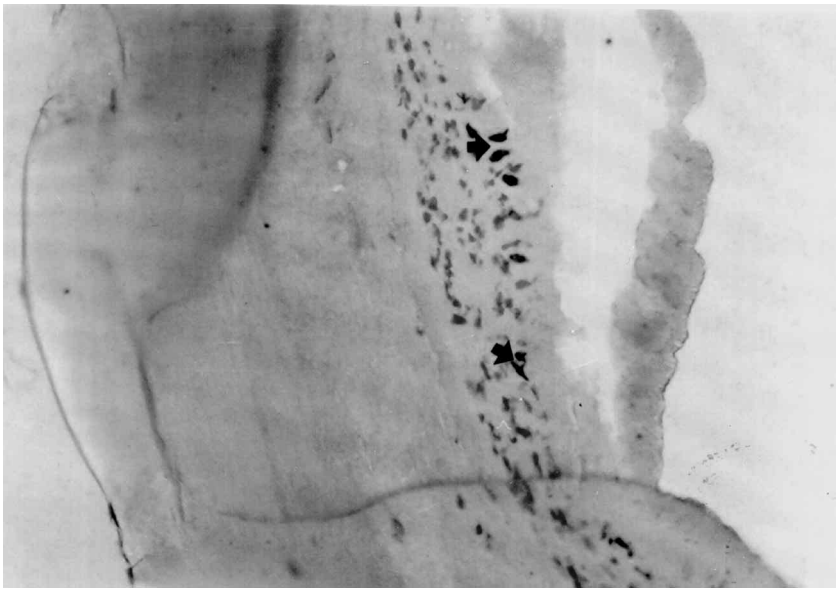

Figure 2. Serotonin immunoreactive cells in the rectum of freshwater turtle, *Mauremys caspica caspica* (arrows). 200x.

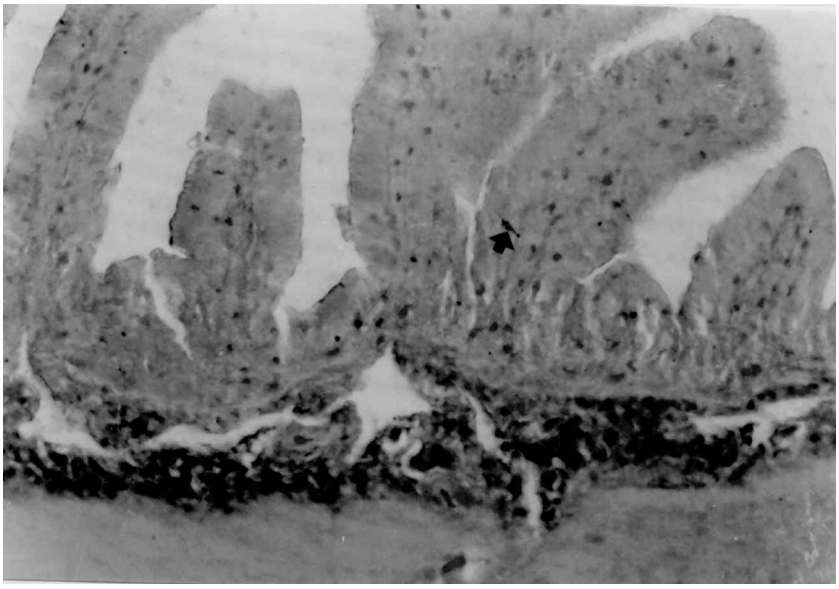

Figure 3. Gastrin containing cell in the ileum of freshwater turtle, *Mauremys caspica caspica* (arrows). 200x.

gastrointestinal tract (20). It has been reported that serotonin immunoreactive cells were detected through the gastrointestinal tract all species, suggesting that they were established in the gastrointestinal tract at an early stage of vertebrate evolution (19). In reptilians, these cells are distributed throughout the alimentary tract (11). In addition, serotonin-IR cells were found through gastrointestinal tract, including esophagus and highest frequency was detected in the pylorus and the proximal part of the small intestine of the Colubridiae (15), similar distribution and frequency in *Kings skink* and *Rhobdophis tigrinus tigrinus* (Colubridae) (11).

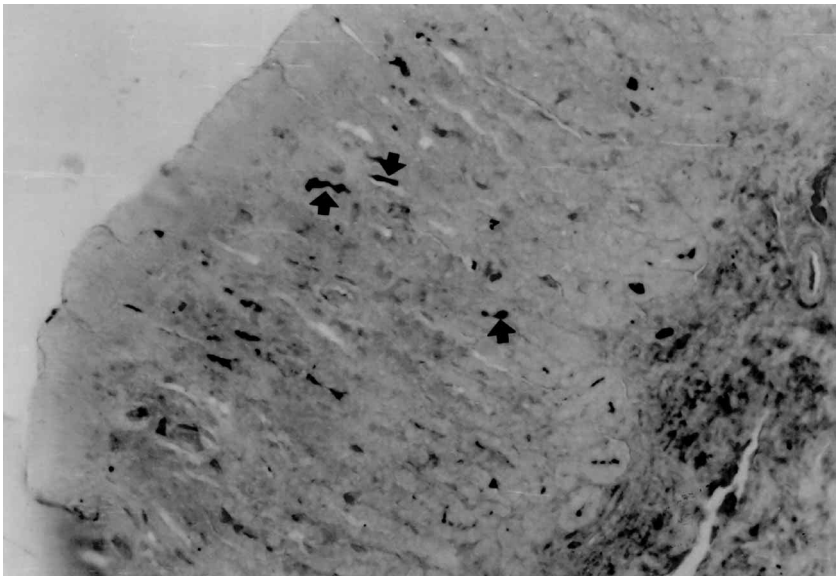

Figure 4. Insulin containing cells in the stomach regions of freshwater turtle, *Mauremys caspica caspica* (arrows). 200x.

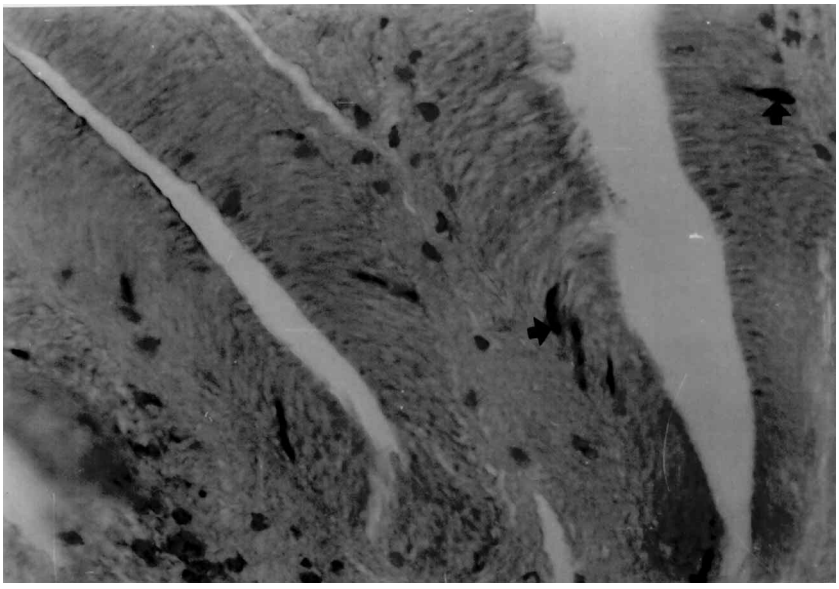

Figure 5. Insulin immunoreactive cells in the duodenum of freshwater turtle, *Mauremys caspica caspica* (arrows). 400x.

In the present study, these cells were observed through gastrointestinal tract except for ileum. Similar distributions were seen in the gastrointestinal tract of *Trachemys scripte elegans* (21). Serotonin-IR cells of large intestine of *Mauremys caspica* and *Lacerta lepida* were located in the surface epithelium and in the small epithelial cell aggregations of lamina propria (22). As pointed out by Wurth and Musacchia (23) in *Chrysemys picta* these aggregations might have regenerative action on the epithelium, with serotonin containing cells having a trophic action.

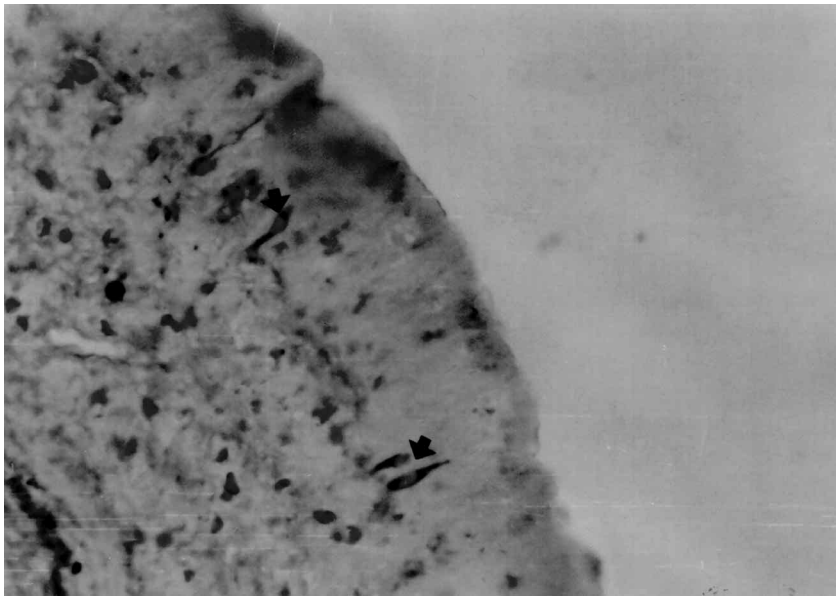

Figure 6. Insulin containing cells in the rectum of freshwater turtle, *Mauremys caspica caspica* (arrows). 400x.

Gastrin was among the first gut peptides to have the structure of its genes determined (24). Gastrin has been localized in G cells of antral glands and small intestine (25). Gastrin secreted by the intestinal G cell promoted gastric acid secretion (26). In this study gastrin-IR cells were detected in ileum and rectum at low frequency. No immunoreactivity were observed in stomach and duodenum. However, gastrin-IR cells were detected from pylorus to the distal intestine of other reptilia such as *Mauremys caspica, Lacerta lepida* and *Testudo gracea* (22). This discrepancy may due to differences in the antisera tested or differences between the species and subspecies. Present study used rabbit anti-gastrin I antiserum which is specific for gastrin I fragment 1-13, whereas Perez-Tomas et al. (22) used rabbit anti-gastrin antiserum, which is specific for whole molecule.

In the present study, insulin-IR cells were found in both the gastric and intestinal mucosa. Similar results have been obtained in *Mauremys caspica* (22). However, in *Chrysemys picta*, another freshwater turtle, insulin-IR cells have only been identified in the intestine (27). In *Lacerta lepida*, these cells were present only in the mucosa of the large intestine (22).

In conclusion, the regional distribution and relative frequency of immunoreactive cells in the *Mauremys caspica caspica* were essentially similar to those of other Reptilia (8,10-13,28-30). However, some characteristic differences were observed in this species, which may due to differences in the antisera tested, the methods used or/and the species investigated in the various studies (30-32).

The absence of positive staining for some of these hormones (e.g., glucagon, substance P, CGRP or somatostatin) does not necessarily indicate their absence, but possibly that the reptilian hormones do not cross react with antibodies of their mammalian counterparts.

References

1. Bell, F.R.: The relevance of the new knowledge of gastrointestinal hormones to veterinary science. Vet. Sci. Commun., 1979; 2: 305- 314.

2. Buchan, A.M.J., Polak, J.M.: The classification of the human gastroentero-pancreatic endocrine cells. Invest. Cell. Pathol., 1980; 3: 51-71.

3. Powell, D., Skrabanek, P.: Substance P. In: Bloom S.R., Polak J.M., Eds. Gut Hormones. 2nd. Ed, Churchill Livingstone, Edinburgh, 1981; 396-401.

4. Ballesta, J., Bloom, S.R., Polak, J.M.: Distribution and localization of regulatory peptides. CRC Crit. Rev. Clin. Lab. Sci., 1985; 22: 185-218.

5. Ohara, N., Kitamura, N., Yamada, J., Yamashita, T.: Immunohistochemical study of gastro-entero-pancreatic endocrine cells of the herbivorous Japanese field vole, *Microtus montebelli*. Res. Vet. Sci., 1986; 41: 21-27.

6. Rombout, S.M.W.M., Van der Grinten, C.P.M., Peeze BinKhorst, F.M., Taverne-Thiele, J.J., Schooneveld, H.: Immunocytochemical identification and localization of peptide hormones in the gastro-entero-pancreatic (GEP) endocrine system of the mouse and a stomachless fish. *Barbus conchonius*. Histochemistry, 1986; 84: 471-483.

7. Weyrauch, K.D., Blahser, S., Perschbacher, J.: Somatostatin cells in the gastric mucosa of small ruminants. Acta Anat., 1987; 128: 188-193.

8. Buchan, V., Lance, J.M., Polak, M.A.: Regulatory peptides in the gastrointestinal tract of alligator mississipiensis. An immunocytochemical study. Cell Tissue Res., 1983; 231: 439-449.

9. Jeon, J.H., Lee, C.E., Lee, A.J.: Electron microscopic study on the endocrine cells in the stomach and duodenum of the pond tortoise (*Amyda sinensis*). Korean J. Electron Microsc., 1986; 16: 25-34.

10. Perez-Tomas, J., Ballesta, L.M., Pastor, J.F., Madrid, J.M., Polak, M.: Ultrastructural study of the endocrine cells of the gut of *Testudo graeca* (Chelonia). Anat. Embryol., 1989; 180: 103-108.

11. Arena, K.C., Richardson, J., Yamada, P.: An immunohistochemical study of endocrine cells of the alimentary tract of the King's shink (*Egernia kingii*). J. Anat., 1990; 170: 73-85.

12. Gapp, J.M., Polak, A.S.: Localization of insulin to gastroentero-pancreatic cells in the turtle gastrointestinal tract. Gen. Comp. Endocrinol., 1990; 78: 48-55.

13. Yamada, M.A., Rodrigues, N, Kitamura, V.D., Pai, T., Yamashita, T., Motizuki N., Yanaihara, J.: Motilin immunoreactive cells in the duodenum, pyloric stomach and pancreas of caimans (*Caiman latirostris* and *Caiman crocodilus,* Alligatorinae): A further comparison using region specific motilin antisera. Arch. Histol. Cytol., 1991; 54: 359-364.

14. Burrel, A.C., Villaro, P., Sesma, G., Rindi, D.V.: Evidence for the colocalization of Gas/CCK- and PYY/PP-immunoreactive substances in the small intestine of the lizard *Podarcis hispanica* immunocytochemical and ultrastructural study. Gen. Comp. Endocrinol., 1992; 88: 40-49.

15. Lee, S.K., Ku, H.S., Lee, A.M.: An immunohistochemical study of endocrine cells in the alimentary tract of the snake, *Rhabdophis tigrinus tigrinus*. Korean J. Vet. Res., 1999; 35: 67-73.

16. Sternberger, L.A.: Immunocytochemistry. 3rd ed., John Wiley, New York, 1986.

17. Shu, S., Ju, G., Fan, L.: The glucose oxidase -DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci. Lett., 1988; 85: 169-171.

18. Sternberger, L.A.: The unlabelled antibody peroxidase-antiperoxidase (PAP) method. In: Sternberger, L.A., Ed. Immunocytochemistry. John Wiley, Sons, New York, 1979; 104-169.

19. El-Salhy, E., Winder, M., Lundqvist, S.: Comparative studies of serotonin-like immunoreactive cells in the digestive tract of vertebrates. Biomed. Res., 1985; 6: 371-375.

20. Guyton, A.C.: Secretory functions of the alimentary tract. In: Textbook of Medical Physiology. 8 th edn, W.B. Saunders, Philadelphia, 1988; 801-815.

21. Ku, S.K., Lee, H.S., Lee J.H., Park, K.D.: An immunohistochemical study on the endocrine cells in the alimentary tract of the Red-Eared Slider (*Trachemys scripta elegans*). Anat. Histol. Embriyol., 2001; 30: 33-39.

22. Perez-Tomas, R., Ballesta, J., Pastor, L.M., Madrid, J.F., Polak, J.M.: Comparative immunohistochemical study of the gastroenteropancreatic endocrine system of three reptiles. Gen. Comp. Endocrinol., 1989; 76: 171-191.

23. Wurth, S.M., Musacchia, W.J.: Renewal of intestinal epithelium in the freshwater turtle. Anat. Rec., 1964; 148: 427-439.

24. Noyes, B., Mearech, M., Stein, R., Agarwal, K.: Detection and partial sequence analysis of gastrin mRNA by using an oligodeoxynucleotide prope. Proc. Natl. Acad. Sci. USA, 1979; 76: 1770.

25. Solcia, E., Capella, C., Buffa, R.: Endocrine cells of the digestive system. In: Johnson, LR., Ed. Physiology of the Gastrointestinal Tract. Raven press, New York, 1987; 111.

26. Solcia, L., Usellini, R., Buffa, G., Rindi, L., Villani, A., Aguzziand E., Silini, J.M.: Endocrine cells producing regulatory peptides. In: Polac, J. Ed. Regulatory Peptides. Birkhauser, Basel, 1989; 220-246.

27. Gapp, D.A., Kenny, M.P., Polak, J.M.: The gastroentero-pancreatic system of the turtle, *Chrysemys picta*. Peptides, 1985; 6: 347-352.

28. Langslow, J.R., Kimmel, H.G., Pollock, H.S.: Studies of distribution of a new avian pancreatic polypeptide and insulin among birds, reptiles, amphibians and mammals. Endocrinology, 1973; 93: 558-565.

29. Giraud, N.D., Yeomans D.J.B., St. John, C.J.: Ultrastructure and cytochemistry of the gastric mucosa of a Reptile, *Tiliquata sincoides*. Cell Tissue Res., 1979; 197: 281-294.

30. El-Salhy, L., Grimelius, M.: The endocrine cells of gastrointestinal mucosa of a Squamata reptile, the grass lizard (*Mabuya quinquetaeniata*). A histological and immunohistological study. Biomed. Res., 1981; 2: 639-658.

31. Dockray, G.J.: Molecular evolution of gut hormones. Application of comparative studies on the regulation of digestion. Gastroenterology, 1977; 72: 344-358.

32. Walsh, J.H., Gastrointestinal hormones. In: Johnson, L.R., Ed. Physiology of the Gastrointestinal Tract. 2nd edn, Raven press, New York, 1987; 181-253.