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# Regional and mucosal distributions of some intestinal immunoreactive endocrine cells in New Zealand white rabbit (*Oryctolagus cuniculus* L.)

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## Summary

The aim of this study was to detect the regional and mucosal distribution of endocrine cells that secrete gulcagon, somatostatin, Chyholecystokinin-8 (CCK-8), serotonin, secretin, substance P (SP) and histamine in the small and large intestine of New Zealand white rabbit (*Oryctolagus cuniculus* L.) using immunohistochemical peroxidase-antiperoxidase (PAP) method. It was found that most of the immunoreactive (IR) endocrine cells, which are oval- or spindle-shaped, are spotted in the basal parts of the relevant glands. It was noticed that cells in the lamina epithelialis of small and large intestine is linked to the lumen and that the cells in their glands cannot reach the lumen. Immunoreactive cells for glucagon, somatostatin, serotonin, secretin and SP were identified in lamina epithelialis of the small and large intestine. It was seen that secretin, SP and histamine-IR cells are rarely deployed throughout the intestinal tract. It was determined that somatostatin-IR cells were identified throughout the intestinal tract. In conclusion, the immunohistochemical study shows that gastrointestinal tract of this species contained different types of endocrine cells similar to those found in other vertebrate species. However, some species-dependent unique distributions and frequencies of endocrine cells were also observed in the present study.

**Key words:** Endocrine cell, Immunohistochemistry, Intestine, Rabbit

## Introduction

Gastrointestinal endocrine cells are spread along the epithelia and glands of the gastrointestinal tract. This cells produce diverse hormones and have regulatory roles in the physiological functioning of the digestive tract (Bell, 1979). Glucagon is secreted from pancreatic A-cells (Holst, 2000) and is responsible for reducing insulin secretion in the digestive tract and inhibiting digestive secretion and motility (Holst and Orskow, 1994). Somatostatin is synthetized from duodenal endocrine cells and from pancreatic D-cells (Brazeau *et al*., 1973). Chyholecystokinin-8 (CCK-8) is conveyed from the duodenum in response to the presence of digested food, particularly fatty acids and amino acids (Wilding, 2002). Serotonin has been proved to influence crypt epithelial secretion and proliferation (Gershon and Tack, 2007). Secretin is found in S cells which gradually decrease from duodenum to jejunum. The free hydrogen ions from duodenum are the strongest secretin stimulators. In addition, salts and alcohol stimulate acid secretion and increase secretin secretion (Dezfuli *et al*., 2000; Dezfuli *et al*., 2002). Substance P (SP) is a neuropeptide that performs some tasks such as neurotransmitter and neuromodulator (Wang *et al*., 1985). Histamine is a decarboxylative form of histidine and a potent vasodilator in animal tissues. Histamine affects 4 types of receptors (H1R, H2R, H3R, H4R) and transmits signal into the cell via G protein (Kalpaklıoğlu *et al*., 2012).

The purpose of this study was to examine regional and mucosal distributions of glucagon, somatostatin, CCK-8, serotonin, secretin, SP and histamine in the intestinal endocrine cells of New Zealand white rabbit (*Oryctolagus cuniculus*) using immunohistochemistry.

## Materials and Methods

Ten adult male New Zealand white rabbits were used in immunohistochemical study. The animals were provided from particular farms and were maintained in proper condition after requisition. The animals were euthanized with the approval of the Animal Experiments Ethics Board of Suleyman Demirel University (SDU-HUDAL B.30.2.SDU.0.05.06.00-186). The animals were anesthetized with intramuscular 35 mg/kg ketamine and 5 mg/kg xylasine (Sanford and Colby, 1980). After anaesthetizing, the abdominal cavities of the animals were opened. Samples harvested from the small (duodenum, jejunum and ileum) and large (cecum, distal colon, proximal colon and rectum) were fixed in Bouin's solution for 15 h. After paraffin embedding, 5-6-µm-thick sections were taken at intervals of every 60 min from 5 pieces of the paraffin blocks. Sections were deparaffinized, rehydrated and stained immuno-histochemically using the PAP method (Sternberger, 1986). According to this method, sections were washed

with phosphate buffered saline solution (PBS; 0.01 M, pH = 7.2) after passing through xylol and alcohol series. Tissue sections were then incubated with 3% hydrogen peroxide ($H_2O_2$) solution for 20 min and 10% normal goat serum for 30 min, respectively, to prevent endogenous peroxidase activity and non-specific reaction. Afterward, sections were incubated for 24 h at 4°C with primary antibodies, whose names and dilution ratios are shown in Table 1. Sections were incubated with secondary antibody (1:10) (goat anti-rabbit IgG) for 30 min in room temperature to visualize the resulting antigen antibody complex after washing with PBS. The sections were taken again with PBS and were incubated with the rabbit peroxidase-antiperoxidase (PAP) complex for 30 min at room temperature and then washed again with PBS. They were treated with 0.05% 3,3´-diaminobenzidine (DAB) for 10 min to visualize the immunoreactivity. The sections were passed through the alcohol and xylen series and were closed with entellan. Sections were examined under light microscope (Olympus CX 41) and photographs were taken with Leica DM 2500.

**Table 1:** Primer antisera used to test immunohistochemical reactions

| Primer antisera | Code No. | Dilution range | Source |
|---|---|---|---|
| Glucagon | SC223 | 1:200 | Santa Cruz |
| Somatostatin | SC13099 | 1:200 | Santa Cruz |
| CCK-8 | C2581 | 1:200 | Sigma |
| Serotonin | S5545 | 1:200 | Sigma |
| Secretin | SC20938 | 1:200 | Santa Cruz |
| SP | S8305 | 1:1000 | Sigma |
| Histamine | H7403 | 1:200 | Sigma |

Negative and positive control sections were included in the staining procedures. Positive controls were carried out using endocrine pancreas, stomach and intestine tissues of mouse, which are known to be immunoreactive (IR) for antiserum. The negative controls were performed by replacing primary antiserum with PBS (pH = 7.4). The application of immunohistochemistry was applied in the same way except this phase.

The event of each type of IR cell was scaled as:
–: No positive cells
1-10: Weak (+)
11-20: Moderate (++)
21-30: Numerous (+++)
>30: Very numerous (++++)

## Results

Regional and mucosal distributions of IR cells in the small and large intestine sections are given in Table 2.

The immunohistochemical results disclosed the regional and mucosal distribution of endocrine cells that secrete glucagon, somatostatin, CCK-8, serotonin, secretin, SP and histamine in the small intestine (duodenum, jejunum, and ileum) and large intestine (cecum, proximal colon, distal colon, and rectum) of New Zealand white rabbit. Cells in the intestines were occasionally spindle shaped with long cytoplasmic extension at the end of the lumen (open-type cells), while generally, cells of oval shape (closed-type cells) were also identified in the intestinal gland areas. Generally, open cell types with apical cytoplasmic processes that attained the glandular or intestinal lumen of the small and large intestinal epitelium were observed. Closed-type cells settle on the basis of the digestive tract epithelium and can not extend to the lümen of the organ. No immunoreactivity was shown in the negative control sections.

Immunoreactive cells for glucagon, somatostatin, serotonin, secretin and SP were found in LE of the small and large intestine.

Glucagon-IR cells were observed in the small and large intestine sections. In the small intestine, few glucagon-IR cells were found in the lamina epithelialis of duodenum, jejunum and ileum (Fig. 1a), but then none were detected in the Brunner's gland. In the large intestine, these cells were moderate in number in the distal (Fig. 1b) and rectal gland regions and then decreased in the intestinal epithelia.

Somatostatin-IR cells were determined throughout small and large intestine. Also, few IR cells were found for somatostatin in the Brunner's glands. The number of somatostatin-IR cells in the duodenum were found to be moderate, but few were observed in LE of jejunum and ileum. A few of these cells were identified in LE and gland of large intestine regions (Figs. 1c-d).

Numerous CCK-8-IR cells were present in the

**Table 2:** Regional distributions and mucosal localizasion of immunoreactive cells in the gastrointestinal tract of the New Zealand white rabbit

| Primer antisera | Small intestine | | | | Large intestine | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Duo[a] | | Jej[a] | Ileum | Cecum | | Dist colon[a] | | Prox colon[a] | | Rectum | |
| | LE[b] | G[b] | LE | LE | LE | G | LE | G | LE | G | LE | G |
| Glucagon | +[c] | –[c] | + | + | + | –/+ | + | ++ | + | + | + | ++ |
| Somatostatin | ++ | + | + | + | + | –/+ | + | + | + | + | + | + |
| CCK-8 | +++ | – | + | + | – | – | + | +++ | – | – | + | +++ |
| Serotonin | ++++ | – | ++++ | ++++ | ++ | + | + | ++++ | + | +++ | + | ++++ |
| Secretin | + | – | –/+ | –/+ | –/+ | – | –/+ | –/+ | –/+ | – | –/+ | – |
| SP | + | – | –/+ | + | –/+ | – | –/+ | –/+ | + | –/+ | –/+ | –/+ |
| Histamine | –/+ | – | – | – | – | – | –/+ | –/+ | – | – | + | + |

[a] Duo: Duodenum; Jej: Jejunum; Dist: Distal; Prox: Proximal, [b] LE: Lamina epithelialis; G: Gland, and [c] –: Not detected; –/+: Rare; +: Few; ++: Moderate; +++: Numerous; ++++: Very numerous

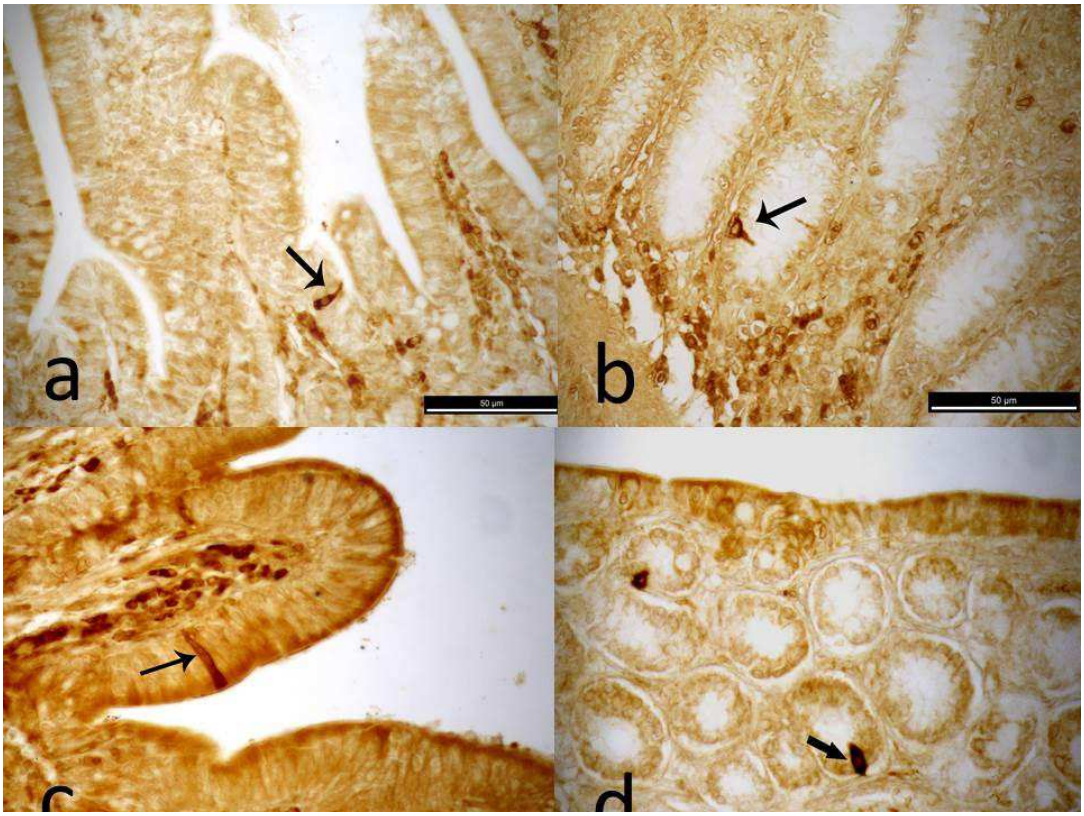


**Fig. 1:** Distribution of glucagon and somatostatin IR cell. (**a**) Ileum. The glucagon-IR cell in the lamina epithelialis (arrow), (PAP, bar: 50 µm). **(b**) Distal colon. The glucagon-IR cell in gland (arrow), (PAP, bar: 50 µm). **(c**) Proximal colon. The somatostatin-IR cell in the lamina epithelialis of the cyrpt (arrow), (PAP, bar: 50 µm). **(d**) Distal colon. The somatostatin-IR cells in glands (arrows), (PAP, bar: 50 µm)

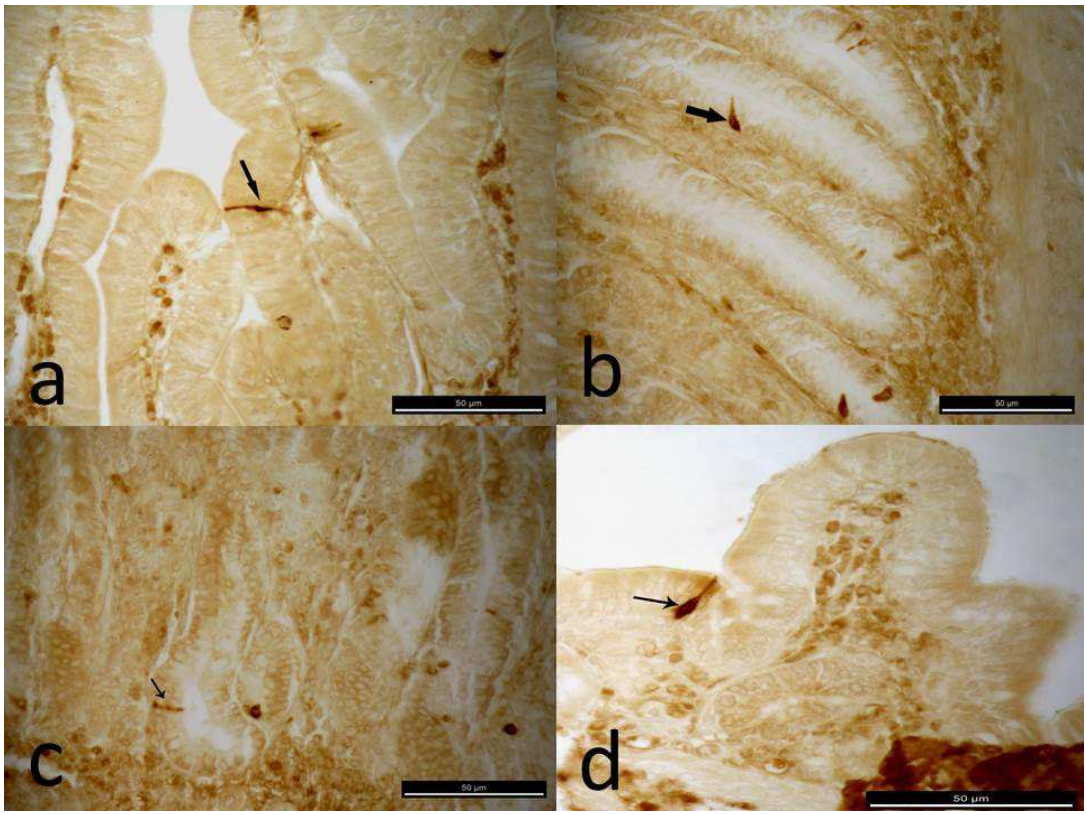


**Fig. 2:** Distribution of the CCK-8-IR cell in the lamina epithelialis of the villus (arrow) (**a**) duodenum and the glands (arrow) of the (**b**) distal colon and (**c**) rectum (PAP, bar: 50 µm). (**d**) Distribution of the serotonin-IR cell in the lamina epithelialis of the cecum crypt (arrow) (PAP, bar: 50 µm)

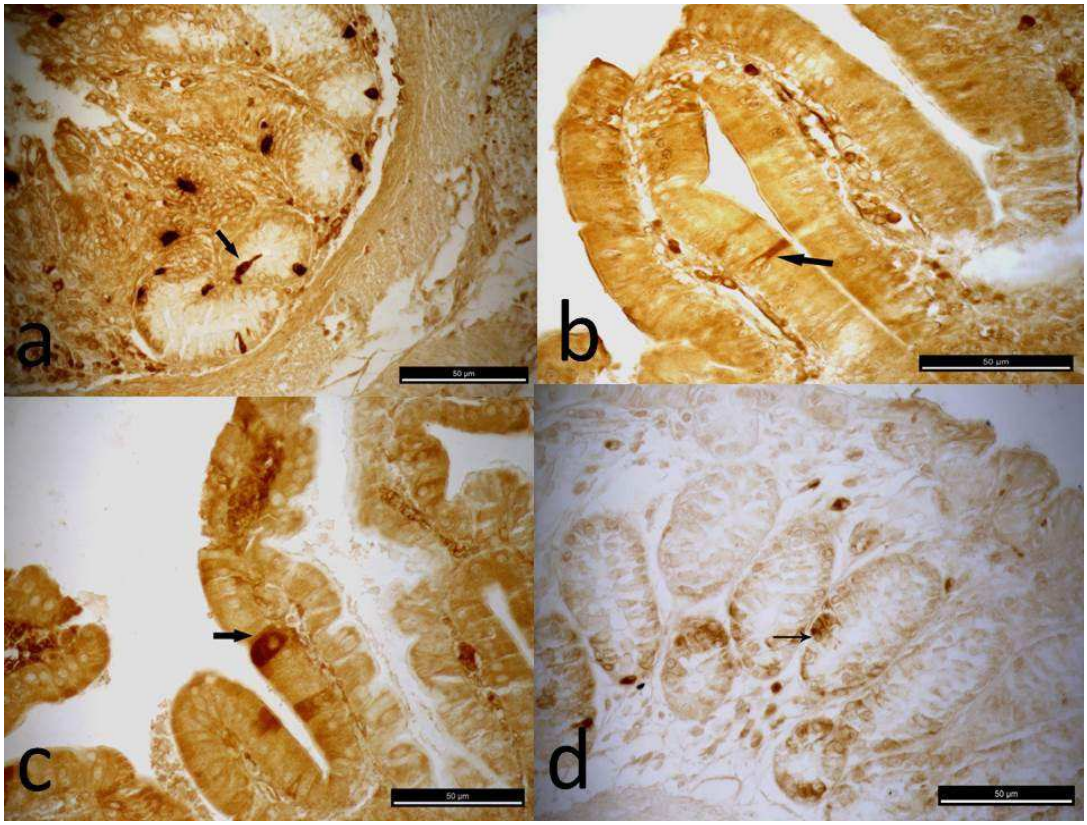


**Fig. 3:** Distribution of positive IR cells in the glands and lamina epithelialis in the rectum and duodenum. (**a**) Rectum. The serotonin-IR cells in the glands (arrow), (PAP, bar: 50 µm). (**b**) Duodenum. The secretin-IR cell in the lamina epithelialis of the cript (arrow), (PAP, bar: 50 µm). (**c**) Duodenum. The SP-IR cell in the lamina epithelialis (arrow), (PAP, bar: 50 µm). (**d**) Rectum. The histamine-IR cell in the lamina epithelialis (arrow), (PAP, bar: 50 µm)

duodenal epithelia (Fig. 2a) but few in the jejunum and ileum regions. These cells were detected numerously in the distal (Fig. 2b) and rectal glands (Fig. 2c), but were absent in the proximal colon and cecum regions. Serotonin-IR cells were detected very numerous throughout the duodenum, jejunum and ileum, although none were found in the Brunner's gland. These cells were observed throughout the whole large intestine regions at various frequencies (Figs. 2d and 3a).

Secretin-IR cells were restricted to the gastro-intestinal tract with rare and few frequancies. These cells were rarely found in the duodenum epitelium (Fig. 3b) but none were found in the Brunner's gland region. These cells were rarely observed in the large intestine regions while the cells were not found in the cecum, proximal colon or rectum glands. It was noticed that cells in the lamina epithelialis of small and large intestine is linked to the lumen.

SP-IR cells were rarely identified in the basal portion of the duodenum (Fig. 3c) but none were defined in the Brunner's gland portion. These cells were also spoted in the middle portion of the jejunum and the ileum. These cells were identified in the large intestine epithelium and glands, but those cells were not observed in the cecum glands.

Histamine-IR cells were only observed in the duodenal section of the small intestine where they were present at a rare frequency in the intestinal epithelia. The cells were not found in the Brunner's glands, jejunum or ileum. No IR cells were observed in the cecum and proximal colon. However, a few number of histamine-IR cells were identified in the rectum (Fig. 3d). Histamine-IR cells in the lamina epithelialis of small and large intestine are linked to the lumen and the histamine-IR ones in rectal glands cannot reach the lumen.

## Discussion

The present study identifies seven types of endocrine cells in the small and large intestine of the New Zealand white rabbit. These cells were IR for glucagon, somatostatin, CCK-8, serotonin, secretin, SP and histamine. In the intestinal regions, the spindle shaped-cells were generally opened-type cells, while generally oval shaped-cells were closed-type cells. The cell types and shapes detected in this study are similar to those reported in other mammals (Baltazar *et al*., 1998; Dall'Aglio *et al*., 1998; Ku *et al*., 2004; Santos *et al*., 2008; Lee *et al*., 2010; Adnyane *et al*., 2011).

Glukagon-IR cells were not found in the small and large intestine regions of SKH-1 hairless mice (Ku *et al*., 2002) and ddN mice (Lee *et al*., 2010), but these cells were determined numerous common tree shrew (Yamada *et al*., 1999). In this study, these cells were identified few in the small intestine regions and moderate number in the large intestine regions.

Our results showed that moderate number of somatostatin-IR cells were present in the epithelium of duodenum. But these IR cells were very numerous in the duodenum of the ostrich (Bezuidenhout and Van Aswegen, 1990) and one-humped camel (Althnaian *et al*., 2012). In accordance with the results of this study, somatostatin-IR cells have been reported few in the large intestine regions of the mush shrew (Kitamura *et al*., 1990), common tree shrew (Yamada *et al*., 1999), ddN mice (Lee *et al*., 2010). Althnaian *et al*. (2012) have been reported that detected very high level of expression at the Brunner's glands thoughout the duodenum. In this study, cells IR for somatostatin were observed few in the Brunner's glands.

Yaman *et al*. (2007) have reported that CCK-8-IR cells were not detected in small intestine regions of porcupine. CCK-8-IR cells were limited to the small istestine of cow, calf (Kitamura *et al*., 1985), lesser mouse deer (Agungpriyono *et al*., 1994). In the present study, these IR cells were numerous in the duodenal epithelia but few in the jejunum and ileum regions.

No CCK-8-IR cell was detected in the large intestine regions of wild boar (Dall'Aglio *et al*., 1998), common tree shrew (Yamada *et al*., 1999), SKH-1 hairless mice (Ku *et al*., 2002), barking deer (Adnyane *et al*., 2011) and New Zealand rabbit except for the distal colon and rectum of this study.

Serotonin-IR cells were detected numerous in duodenum New Zealand rabbit in a similar frequency to other vertebrates (Mimoda *et al*., 1998; Gençer Tarakçı *et al*., 2005; Karadağ Sarı *et al*., 2007; Yaman *et al*., 2007; Althnaian *et al*., 2012).

Numerous serotonin-IR cells were identified distributed in the large intestine regions examined. These results are consistent with previous reports of studies in duck (Gülmez *et al*., 2003), chicks (Rawdon and Andrew, 1994), common tree shrew (Yamada *et al*., 1999) and one-humped camel (Althnaian *et al*., 2012). In addition, these cells were identified moderate in the large intestine regions of the musk shrew (Kitamura *et al*., 1990), Philippine carabao (Baltazar *et al*., 1998), ddN mice (Lee *et al*., 2010). However, serotonin-IR cells were not found in the large intestine regions of the SKH-1 hairless mice (Ku *et al*., 2002).

Our results showed little distribution of secretin in the endocrine cells of the duodenum, similar to that of the musk shrew (Kitamura *et al*., 1990), ruminant (Mimoda *et al*., 1998), SKH-1 hairless mice (Ku *et al*., 2002) and grass lizard (Lee and Ku, 2004). However, the cells are distributed to moderate Philippine carabao (Baltazar *et al*., 1998), sheep (Mimoda *et al*., 1998) and babirusa (Agungpriyono *et al*., 2000). In addition, secretin-IR cells were found numerous in the duodenum of the domestic duck (Castaldo and Lucini, 1991), cow and barbary sheep (Mimoda *et al*., 1998) and common tree shrew (Yamada *et al*., 1999).

In this study, secretin-IR cells were found in the large intestine at low frequency. No immunoreactivity was demonstrated in the large intestine of the ostrich (Bezuidenhout and Van Aswegen, 1990), the King's skink (Arena *et al*., 1990), Philippine carabao (Baltazar *et al*., 1998), the common tree shrew (Yamada *et al*., 1999), the babirusa (Agungpriyono *et al*., 2000), the red-eared slider (Ku *et al*., 2001) and the grass lizard (Lee and Ku, 2004).

SP-IR cells were originally found in the brain and intestinal tract (Otsuka and Yoshioka, 1993). In addition to digestive endocrine cells, it also carries both SP neurotransmitter and endocrine functions isolated in the brain. It has an indirect effect on smooth muscles by stimulating the release of serotonin (5HT) (Creagh *et al*., 1980; Wang *et al*., 1985).

In the present study, SP-IR cells were found in the small intestine regions at rare frequency. Similar results have been obtained in Philippine carabao (Baltazar *et al*., 1998). However, guinea pig, cat, rat and the one-humped camel (Keast *et al*., 1984; Gronstad *et al*., 1985; Lundquist *et al*., 1990; Al Haj Ali *et al*., 2007). SP-IR cells have been identified in the jejunum at moderate frequency. In the ostrich (Bezuidenhout and Van Aswegen, 1990), these cells were present moderate in the mucosa of duodenum. It has also been reported that SP-IR cells were found in the small and large intestine of the cow and calf (Kitamura *et al*., 1985), domestic duck (Castaldo and Lucini, 1991) and one-humped camel (Al Haj Ali *et al*., 2007). But no IR cells were obtained in the these regions of the fresh water turtle (Gençer Tarakçı *et al*., 2005). In this study, SP-IR cells were rarely identified in the small and large intestine.

Histamine is a peptide that provides the vasodilation, stimulates the gastric acid secretion and modulates the gastrointestinal function. Another important site for histamine storage and release is the enterochromaffin-like cell of the stomach (Köse and Hall, 2000; Solcia *et al*., 2000).

This peptide also helps digestion by increasing gastric and intestinal smooth muscle contraction (Bhagavan, 1992). Histamine-IR cells were numerous in the LE and moderate in the intestinal glands of the small and large intestine regions of the guinea pig (Tahara *et al*., 2000). In this study, these cells were only detected in the duodenal section of the small intestine where they were found with rare frequency in the intestinal epithelia. No IR cell was observed in the cecum and proximal colon. However, a small number of histamine-IR cells were identified in the rectum.

In conclusion, the immunohistochemical study shows that gastrointestinal tract of this species contained different types of endocrine cells similar to those found in other vertebrate species. However, some species-dependent unique distributions and frequencies of endocrine cells were also observed in the present study.

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